



National Reference Laboratories Wageningen Food Safety Research

Annual report 2023

Y. Hoffmans, M. Alewijn, M.D. Klijnstra, G. van der Borg, J.J.P. Lasaroms, J.P. van Dijk, T.W. Prins, J.G.J. Mol,
G.M.H. Brust, M.M. Soped, L.L. Leenders, K.S. Krätschmer, I.L.A. Boxman, N. Sezer, Y. Hogenes



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This report was funded by the Dutch Ministry of Agriculture, Nature and Food Quality and The Netherlands Food and Consumer Product Safety Authority as part of the statutory tasks of food safety (WOT-02 and WOT-NVWA).

Wageningen, November 2024

WFSR Report 2024.008

Y. Hoffmans, M. Alewijn, M.D. Klijnstra, G. van der Borg, J.J.P. Lasaroms, J.P. van Dijk, T.W. Prins, J.G.J. Mol, G.M.H. Brust, M.M. Sopel, L.L. Leenders, K.S. Krätschmer, I.L.A. Boxman, N. Sezer, Y. Hogenes, 2024. *National Reference Laboratories Wageningen Food Safety Research Annual report 2023*. Wageningen, Wageningen Food Safety Research, WFSR Report 2024.008. 62 pp.; 0 fig.; 13 tab.; 0 ref.

BAS-code: WOT-02-001-004, WOT-02-001-005, WOT-02-001-006, WOT-02-001-007, WOT-02-001-008, WOT-02-001-010, WOT-02-003-001, WOT-02-004-001, WOT-02-004-002, WOT-02-004-003, WOT-02-004-053, WOT-02-004-054, WOT-HH-001-001, WOT-HH-002-006
Project title: National Reference Laboratories WFSR – Annual Report 2023
Project leader: Y. Hogenes

This report can be downloaded for free at <https://doi.org/10.18174/685522> or at www.wur.eu/food-safety-research (under WFSR publications).

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WFSR report 2024.008

Distribution list:

- Netherlands Food and Consumer Product Safety Authority (NVWA)
- Ministry of Agriculture, Fisheries, Food Security and Nature (LVVN)
- Ministry of Health, Welfare and Sport (VWS)

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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 intermediary between European Union Reference Laboratories (EURLs) and OLs and provide expertise to the OLs, Ministries, and competent authority.

This annual report details WFSR's execution of its NRL tasks in 2023.



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, which include milk and milk products, marine biotoxins, animal proteins in feedingstuffs, certain substances and residues thereof, 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 in poultry meat. 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 2023.

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 2023, WFSR's NRLs attended all the workshops. They actively participated in the EURL working groups to enhance their analytical methods.

EURLs conduct proficiency tests to assess the analytical capabilities of NRLs. As the scope of these tests is sometimes limited, WFSR's NRLs also participated in proficiency tests organised by other relevant organisations. Most proficiency tests yielded satisfactory results, with only a few instances of 'questionable' or 'unsatisfactory' 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, some OLs were provided with technical support related to their analyses.

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

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*
- Water content 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 water content in poultry meat is appointed under Regulation (EU) No 1308/2013 establishing a common organisation of the markets in agricultural products.

This report aims to provide an overview of activities performed by the NRLs of WFSR in 2023.

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 in poultry meat. 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 LVVN. In 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 non-compliant 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 (EURL-AP)
Foodborne viruses	Livsmedelsverket Sweden
Genetically modified organisms (GMOs)	The Joint Research Centre of the European Commission Italy (EURL-GMFF)
Halogenated persistent organic pollutants (POPs) in feed and food	Chemisches und Veterinäruntersuchungsamt (CVUA) Germany
Marine biotoxins	Agencia Española de Seguridad Alimentaria Nutrición (AESAN) 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
<i>Residues of pesticides:</i>	
Cereals and feedingstuffs	Technical University of Denmark (DTU) 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
<i>Residues of veterinary medicines and contaminants in food of animal origin:</i>	
Mentioned in Annex I, Group A1a, A1b, A1c, A1d, A3e, A3f, A3g, B1c, B1d of Regulation (EU) 2022/1644. Stilbenes, antithyroid agents, steroids, resorcylic acid lactones (including zeranol), peptide protein hormones, sedatives, antivirals	Wageningen Food Safety Research (WFSR) Wageningen The Netherlands
Annex I, Group A2a, A2b, A3a, A3c, B1a, B1b, B3e of Regulation (EU) 2022/1644 Antimicrobials and dyes (A3c, B1a, A3a of Annex I of Regulation (EU) 2022/1644)	Agence nationale de sécurité sanitaire de l'alimentation, de l'environnement et du travail (ANSES) France
Beta-agonists, anthelmintics, anticoccidials, nitroimidazoles, non-steroidal anti-inflammatory drugs and plant protection products (A1e, A3d, B1b, A3d, B2, A2c, A3f, B1d, A3b, B1b of Annex I of Regulation (EU) 2022/1644)	Bundesamt für Verbraucherschutz und Lebensmittelsicherheit (BVL) Germany
<i>Other:</i>	
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 in poultry meat	Board of Experts: JRC (Directorate F – Health, Consumers and Reference Materials), 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. Activity plans to describe the tasks for 2023 were drawn up in 2022. In addition, budgets for staff costs and costs for facility and equipment were prepared. The 2023 activity plans were positively reviewed by the Client Consultation Board (consisting of employees of the NVWA, the Ministry of LNVN (formerly the Ministry of Agriculture, Nature and Food Quality (LNV)) and the Ministry of VWS and approved by the Ministry of LNVN. The activity 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 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 water content in poultry meat (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 in 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	<ul style="list-style-type: none"> • Dioxins, dioxin-like and non-dioxin-like PCBs
SANTE/11312/2021 v2	<ul style="list-style-type: none"> • Residues of plant protection products (all matrices)
Regulation (EU) 2015/705	<ul style="list-style-type: none"> • Plant toxins in food
Regulation (EC) 333/2007	<ul style="list-style-type: none"> • 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	<ul style="list-style-type: none"> • 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	<ul style="list-style-type: none"> • Residues of veterinary drugs and hormones
Regulation (EC) 1829/2003	<ul style="list-style-type: none"> • Genetically modified food and feed
Regulation (EC) 641/2004	<ul style="list-style-type: none"> • GMO
Regulation (EC) 619/2011	<ul style="list-style-type: none"> • GMOs in feed (for GMOs with pending authorisation or expired authorisation)
Regulation (EC) 543/2008	<ul style="list-style-type: none"> • Water content poultry meat

2 National Reference Laboratory Milk and milk products

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 2023. 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. Unfortunately, in 2023, there was no initiative for a workshop with the active NRLs MMP, and communication was limited to a few brief email exchanges. Some additional interaction, especially on method development and implementation, also takes place on and around ISO/IDF activities, where the current NRL MMP methods are discussed and maintained on a global level.

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 2023.

Organiser	Country (code)	Date	Matrix	Analyte ²	Performance ¹			
					z-scores	S	Q	U
ALP, CH (22C)	ALP, CH (22C)	Sep	Stabilised milk	SCC	-0.0 – -0.1	4		
ALP, CH (23B)	ALP, CH (23B)	Jan	Stabilised milk	SCC	-0.6 – 0.8	*		
ALP, CH (23C)	ALP, CH (23C)	May	Stabilised milk	SCC	-0.8 – -0.1	*		
Cecalait, FR	Cecalait, FR	March	Milk	SCC	-7.4 – -2.5	0	2 ^a	8 ^a
Cecalait, FR	Cecalait, FR	June	Milk	SCC	-7.0 – 2.4	4	3 ^a	3 ^a
Cecalait, FR	Cecalait, FR	Sept	Milk	SCC	-3.7 – 1.5	7	1 ^a	2 ^a
Cecalait, FR	Cecalait, FR	Dec	Milk	SCC	-3.5 – 0.1	7	1 ^a	2 ^a
LGC, UK	LGC, UK	Jan	Stabilised milk	ALP	0.7 – 0.8	2		
LGC, UK	LGC, UK	Nov	Stabilised milk	ALP	0.0 – 1.7	2		
Cecalait, FR	Cecalait, FR	Jan	Milk	TF	-0.1 – 1.2	10		
Cecalait, FR	Cecalait, FR	April	Milk	TF	-0.1 – 1.9	8		^b
Cecalait, FR	Cecalait, FR	Sep	Milk	TF	-1.7 – 0.1	9		^c
Cecalait, FR	Cecalait, FR	Dec	Milk	TF	-1.0 – 1.0	10		

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 yet available at the time of writing. The last result from the 2022 edition was reported in 2023 and reported here.

^{a-b}) 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, especially in the March series. In the years before 2021, poor performance was explained because of the inclusion of participants with routine instruments, which have low variance and possibly a bias against the reference method. Since the JRC ERM-BD001 SC reference materials became available, WFSR's reference methods proved fine in terms of trueness 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. However, the better precision of the majority of the participants in the Cecalait trials will still easily lead to large relative deviation

as WFSR's reference method unavoidably has a larger spread. Performance on the ERM and in the Swiss ALP ring trial on SCC suggests WFSR performs well on the SCC count.

WFSR was unable to report two samples in Cecalait's TF round of April ^(b) due to technical problems during the execution of the analysis. In Cecalait's TF round for September, one sample was disqualified from further analysis by the ring trial organiser. All remaining samples in the TF ring trial results were very close to the assigned values, and no further action was deemed necessary.

2.2 Assistance to official laboratories

2.2.1 Quality control

In 2023, 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, 6 series with 6 raw milk samples each were analysed independently by two 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 Kwaliteits Zaken)) and the official laboratory on the process of implementing the reference material on somatic cells (JRC ERM-BD001) into routine analyses. In 2023, the NRL had a meeting with the Netherlands Food and Consumer Product Safety Authority (NVWA) on the need for official control of official routine analyses of dairy products. With 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 2024.

3 National Reference Laboratory Marine biotoxins

Coordinator: Mirjam Klijnsstra

3.1 Activities within the EURL-NRL network

3.1.1 Participation in EURL-NRL workshops

On 5 and 6 October 2023, an EURL workshop was organised in Thessaloniki, Greece. 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;
- Results of the interlaboratory comparative study for cyclic imines (CIs) in bivalve molluscs;
- All NRLs presented their institute, their monitoring programme and, if applicable, some of their research results;
- Status and review of activities in Work Programme EURLMB 2023-2024;
- Update from the EU Commission;
- Issues proposed by the EURL/NRLs were discussed: The NRL proposed to start working on a document on performance criteria.

3.1.2 Participation in a working group

Three working groups have been started: one on phytoplankton detection, one on the microbiological monitoring of harvesting areas, and one on the working group for cell-based assays (CBA) ciguatoxin (CTX) analysis (SOP harmonisation).

On 15th December 2022, a working group meeting on molecular methods for phytoplankton detection was organised. This meeting was held online. The Netherlands was represented by experts from WFSR and Wageningen Marine Research (WMR). There were presentations from invited experts, and the participating NRLs presented their work on molecular detection methods. In 2023, the working group produced a white paper titled "Molecular approaches supporting and facilitating HAB (harmful algal blooms) monitoring programs: Needs, challenges and suggestions".

An expert from the Dutch National Institute for Public Health and the Environment (RIVM) represented the Netherlands in the working group on microbiological monitoring of harvesting areas.

On 14th December 2023, a working group on the SOP harmonisation of the CBA method for CTXs was organised. This meeting was held online. There were presentations from invited experts, and the participating NRLs presented their CBA protocols. This working group will continue in 2024.

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-23-A	Domoic acid	1.5 and 1.7
EURLMB-23-L	Lipophilic toxins	Between -1.5 and 0.6
EURLMB-23-P	Paralytic shellfish toxins	Between -2.0 and 1.7
QUASIMEME BT12	Paralytic shellfish toxins	Between -1.2 and 0.8
QUASIMEME DE16	Tetrodotoxins	0.3 and 7.1

For ASP (domoic acid), two PT samples were analysed with the EU reference method using HPLC-UV. The results of both samples were satisfactory (z-score < |2|).

Two EURL PT samples were analysed for lipophilic marine biotoxins using the EU reference LC-MS/MS method. Samples contained OA group toxins, yessotoxins and azaspiracids. Fourteen 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 samples for PSTs were analysed using the HPLC-FLD method (CEN14526). For the individual toxins, all 8 reported values were satisfactory (z-score < |2|). In one of the samples, 2 individual toxins were not reported because values were below the LOQ of the method. For both samples, the total toxicity gave a satisfactory result (z-score < |2|).

Furthermore, three Quasimeme samples (round 1, 2023) for PSTs were analysed with the HPLC-FLD method (CEN14526). For the individual toxins, all 17 reported values were satisfactory (z-score < |2|). Four individual toxins were not reported. For all samples, the total toxicity gave a satisfactory result (z-score < |2|).

WFSR participated in a development exercise for TTX organised by QUASIMEME. The results of the biota sample were satisfactory (z-score < |2|). The result of the standard was unsatisfactory (z-score < |2|). The cause of the deviation has not yet been determined and will be further investigated in 2024.

WFSR provided sample materials to the EURL for an interlaboratory comparative study on cyclic imines in bivalve molluscs. WFSR participated in the EURL interlaboratory comparative study on cyclic imines.

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

During the annual NRL meeting on 20th March 2023, the competent authority (NVWA), the Ministry of Health, Welfare and Sport (VWS), RIVM and 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 and enforcement samples, NRL activities, results of method development and questions from third parties. Also, the activity plans for 2023 were presented.

On 4th December 2023, an EC Expert group meeting on food hygiene and control of food of animal origin was organised. The NRL was present (online) to present a proposal to work towards methods based on performance criteria for marine biotoxins. Every country accepted the initiative. A working group on this topic will be included in the EURL work plan for 2025-2027.

3.4 Contacts with other NRLs

Since end 2021, WFSR is also the NRL for Northern Ireland. The Dutch and Northern Irish NRL tasks will be carried out independently unless combining them is more efficient, 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.

The NRL has been in contact with the NRLs of Germany, Belgium, and Sweden to exchange materials or knowledge on the detection of several toxin groups. Training was completed on the analysis of CTX. The availability of CTX standards was discussed and assistance has been provided for the analysis of cyanobacterial toxins.

4 National Reference Laboratory Animal Proteins in feedingstuffs

4.1 Activities within the EURL-NRL

4.1.1 Participation in EURL-NRL workshops

The annual meeting 2023 of the EURL/NRL network was organised online over two sessions on the 23rd and 24th of May. WFSR actively participated in both sessions.

WFSR also participated in training courses offered by the EURL-AP. These training courses were given both online and on-site. Two technicians attended online from March 28th to March 30th, and one technician attended the training on-site at Gembloux, Belgium, from April 18th to 20th.

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 (Veys et al., 2024) during the winter of 2023. The test consisted of six samples. The preliminary feedback revealed that two samples were blank (clean ovine feed), and the other four samples were contaminated with materials of animal origin. Three samples contained particles of terrestrial vertebrate origin, and one sample contained particles of terrestrial invertebrate origin. None of the samples was contaminated with materials of fish origin. Two samples were spiked with bovine Processed Animal Proteins (PAPs), one sample was spiked with pork blood and pet hairs, and one sample was spiked with *T. molitor* PAPs. The Dutch NRL provided unsatisfactory results, as all samples were erroneously marked as containing insect materials, and one of the samples as containing plasma.

As a result, an investigation was launched to determine the cause of the erroneous reports. The investigation concluded that the samples were unlikely to have been contaminated. It was determined that the particles, erroneously identified as plasma or terrestrial invertebrate, were of plant origin. In response, work was accelerated to create an expert system to aid in the recognition of terrestrial invertebrate particles in animal feed. The results of the investigation, along with a proposed improvement plan, were communicated to the EURL.

In agreement with the EURL, WFSR participated in a follow-up test to show the results of the planned improvements. This follow-up test consisted of five samples: two blanks, two of which contained terrestrial vertebrate PAPs, and one contained terrestrial invertebrate PAPs. All samples were correctly identified and reported, and with that, the EURL considered the matter closed.

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

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.

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, the exchange of viewpoints and other information is stimulated. WFSR participated in 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 2023. The meeting was held at the Landwirtschaftszentrum Eichhof in Bad Hersfeld, Germany from the 13th-15th of June.

4.5 References

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5 National Reference Laboratory of Pharmacologically active substances in animal products

Residues of veterinary medicines in food of animal origin for Group A and B substances as stated in Commission Delegated Regulation (EU) 2022/1644

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 held live on the 3rd and the 4th of May 2023 for the theoretical part. Additionally, there was a third day on the 5th of May for the practical part, which was optional to attend. The workshop comprised several sessions, including informative sessions on the work program of the EURL Berlin, news from the Commission, and the legal aspects of conducting animal studies. Another session focused on laboratory findings and recommendations from audits performed in several member states, while a separate session provided background information on NRLs such as NRL Italy, NRL Croatia, NRL Ireland, and NRL France (Laberca). Additionally, there was a session dedicated to discussing the challenges in routine analyses using High-Resolution Mass Spectrometry. Some interesting topics, background information or news on various substance groups were presented during this workshop. In more detail:

- News on coccidiostats:
 - New classification of coccidiostats according to Annex I of CRD (EU) 2022/1644
 - New available substances
 - New legislation for coccidiostats as a feed additive
 - Discrepancy in the determination of salinomycin and narasin in standard solutions and samples
 - NRCP Evaluation (2021) and non-compliant results
- News on NSAIDs:
 - New classification of NSAIDs according to Annex I of CRD (EU) 2022/1644
 - New available substances
 - Determination of salicylic acid in egg and feed
 - NRCP Evaluation (2021) and non-compliant results
- News on beta-agonists:
 - Availability of retina as reference material
 - NRCP Evaluation and an overview of non-compliant results (2004-2021)
- News on anthelmintics:
 - New available substances
 - New analytical methods for the detection of avermectin in milk
 - NRCP Evaluation (2021) and an overview of non-conform results (2015-2021)
- News on nitroimidazoles:
 - Recommendation of substances and preferred matrices (minimum required and recommended)
 - NRCP Evaluation (2021) and an overview of non-compliant results (2004-2021)
 - The second workshop in which the Dutch NRL participated was the EURL workshop organised by ANSES Fougères. This workshop was held live from June 20th to June 22nd and was titled "Control of antimicrobial and dye residues in food from animal origin". The workshop included theoretical presentations on regulatory issues and new regulatory challenges, biological screening methods, multi-residue confirmatory LC-MS/MS methods, multi-residue screening with LC-HRMS(/MS) and LC-LRMS(-MS) methods, and VMPPR reference materials. The workshop also involved evaluating the EURL PT program for 2022-2023.

Some specific topics or presentations of this workshop were:

- Regulation 2018/470 for use of MRL cascade & consequence for the control of group B substances.
- Biocides – state-of-the-art and control as of sub-group A3b according to regulation 2022/1644.
- Challenges in switching from an LC-MSMS control strategy to an LC-HRMS strategy.
- Monitoring of nitrofurans in honey and other bee products.

The third EURL-Workshop that the Dutch NRL participated in was organised by and held at WFSR-Wageningen on the 7th and 8th of November, 2023. This workshop focused on the control of growth promoters residues in food from animal origin and consisted of a theoretical part with several presentations.

The scope and aims of the workshop were:

- Bringing together NRLs and discussing topics in the field of growth promoters, tranquilisers and antivirals.
- Reconnecting with new and already-known colleagues.
- Discuss and give input into new legislation and Guidance Documents.
- Get an insight into trends in the field of growth promoter, sedative and anti-viral research.
- See trends in the laboratory, instruments and techniques.
- Exchanging knowledge between the NRLs.
- Establishing and strengthening long-lasting collaboration between the NRLs.

Some of the interesting topics and presentations of this workshop included:

- An overview of frequent laboratory findings and recommendations in Member States, observed during the auditing of official residue control against EU legislative requirements.
- Updates of rBST, GHRP, anti-viral and Ecdysteroids research at WFSR.
- Use of HRMS in the field of veterinary drug residues (hormones and antibiotics).
- HRMS use in the Dutch Surveillance plan.

Experts from the NRL in the EU Member States and representatives from candidate countries and/or third countries participated in the aforementioned 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
Beta-agonists in urine	EURL-BVL	z-scores between -1.8 and -0.30 Concordance 100%	Sufficient
Nitroimidazoles in meat	EURL-BVL	z-scores between -0.10 and 1.7	Sufficient
Antibiotics in milk	EURL-ANSES	z-scores between -0.20 and 4.4	Deviating result *)
Avermectins in fish	FAPAS	z-score 0.80 Concordance 100%	Sufficient
Beta-agonists in liver	FAPAS	z-scores between - 1.0 and -0.2 Concordance 100%	Sufficient
Synthetic hormones in urine	FAPAS	z-scores between -0.3 and 0.7	Sufficient
Coccidiostats in egg	FAPAS	z-scores between -2.2 and 1.1	Deviating result *)
Beta-lactams in milk	FAPAS	z-scores between -1.4 and -0.4 Concordance 100%	Sufficient
Sulfonamides, trimethoprim and macrolides in meat	FAPAS	z-scores between -0.4 and 1.6	Sufficient
Nitrofurans in shrimp	FAPAS	z-scores between -0.4 and 0.4	Sufficient
Chloramphenicol, florfenicol and thiamphenicol in shrimp	FAPAS	z-score 0.0	Sufficient
Aminoglycosides in meat	Progetto	z-scores between -0.6 and -0.29	Sufficient
Corticosteroids and thyreostats in urine	Progetto	z-scores between -2.32 and 1.16 Concordance 0% and 100%	Deviating result *) Deviating result *)
NSAIDs in milk	Progetto	z-scores between -0.07 and 0.91 Concordance 100%	Sufficient

Description	Organising institute	z-score	Assessment
Dyes in fish	Progetto	z-scores between -2.85 and 0.48 False positive result	Deviating result *)
Sulfonamides and chloramphenicol in honey	Progetto	z-scores between -0.33 and 0.50	Sufficient
Chloramphenicol in intestines	EURL-ANSES	z-score 0.5	Sufficient
Coccidiostats in liver	EURL-BVL	z-scores between -0.6 and 0.5 Concordance 100%	Sufficient
Antibiotics, anthelmintics and coccidiostats in animal feed	WFSR	z-scores between -1.1 and 3.1 Concordance 100%	Deviating result *)
Antibiotics, anthelmintics and coccidiostats in animal feed	WFSR	z-scores between -0.66 and 0.20 Concordance 100%	Sufficient
Gestagens in kidney fat	WFSR	z-scores between -1.0 and 0.17	Sufficient
Antibiotics in meat	WFSR	z-scores between -0.61 and 0.99 Two false positive results	Deviating result *)
Beta-agonists in feed	FAPAS	Concordance 100%	Sufficient
Thyreostats in urine	WFSR	z-scores between -0.36 and 1.30 Concordance 100%	Sufficient
Antibiotics, dyes and parasiticides in salmon	WFSR	z-scores between -1.58 and -0.13 Concordance 100%	Sufficient

Cumulative results are presented in Table 5.2 below.

Table 5.2 Cumulative results PTs.

Total results	102	% of total
Number of z-scores	81	
Number concordance results	21	
neg z-score	39	48%
pos z-score	43	53%
z-score ≤ 2 en ≥ -2	73	90%
z-score < -2	5	6.1%
z-score > 2	3	3.7%
z-score < -3	0	0%
z-score > 3	2	2.4%

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

Antibiotics in milk; the z-score of the compound kanamycin was above 2, and the z-score for the compound neomycin was above 3.

Corrective action taken: The internal standard linked to the components did not correct properly. Octamethylkanamycin was used as the internal standard for both components, and this choice was validated. A reassessment was carried out in which results were evaluated based on two alternative internal standards and even without an internal standard. The results of these three elaborations were all extremely close to each other and close to the assigned values of the PT report. Given these findings, the assigned values for kanamycin and neomycin were found to be correct.

Coccidiostats in egg; the z-score of the compound nicarbazin (DNC) was above 2.

Corrective action taken: Upon investigation, no definitive cause was identified for this deviation. Considering the minimal deviation in the z-score, it was concluded that no corrective action was needed.

Corticosteroids and thyreostats in urine; the z-score for the compound 6-methyl-2-thiouracil was above 2, and the concordance for 2-thiouracil was strongly deviating.

Corrective action taken: The proficiency material provided consisted of freeze-dried urine. After resolving the freeze-dried urine, no stabilisation step was done. Reanalysis was performed with a stabilisation step. Taking this into account, the assigned values were satisfying.

Dyes in fish; the z-scores for the compounds malachite green and brilliant green were above 2. A false positive finding for the compound leuco-crystal-violet was observed.

Corrective action taken: An unambiguous reason for the deviating results was not found. The proficiency samples were reanalysed, taking into account that all of the reagents used were freshly made.

Unfortunately, the results were still unsatisfactory, except that no false positive result was observed for the compound leuco-crystal-violet.

Antibiotics, anthelmintics and coccidiostats in animal feed; the z-score of the compound monensin was above 3.

Corrective action taken: The reason for the deviating result was the overloading of the component at the highest additions of the standard addition, which was not accounted for during processing. This overloading was due to the strong sensitivity of ionophores combined with the existing concentrations. Consequently, the sample should have been repeatedly diluted to achieve optimal results. The standard addition calibration line was corrected for the overloading points. As a result, the recalculated z-score for monensin was 0.6.

Antibiotics in meat; two false positive results for the compounds sulfachloropyridazine and trimethoprim.

Corrective action taken: Instead of using the internal standard trimethoprim-d9, the compound trimethoprim was mistakenly used in the proficiency samples, which led to the false-positive finding. Furthermore, the false-positive finding for sulfachloropyridazine could be attributed to the similarity between the mass-trace of this compound and that of the internal standard sulfadimidine-13C6, as well as the almost similar retention times of these two compounds. These findings explained the false-positive results.

5.2 Assistance to official laboratories

5.2.1 Quality laboratories

Pursuant to the NRL tasks, the OLs are supervised. The NRL ensures the quality of the analyses of the OLs, which are carried out within the National Residue Control Plan framework, through a third-line control program. Additionally, the NRL 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), as stated in Commission Delegated Regulation (EU) 2022/1644.

In 2023, the control program included 66 analyte/matrix combinations, and 50 different analytical methods were tested. In total, 124 quality control samples were analysed, which resulted in 230 results. 18 results showed a deviate result (7.9% in total), which is higher than in 2022 (4.7%). The overall conclusion is that the assurance of the chemical analyses in 2023 went well, and the results were consistent with previous years. Appropriate actions were taken for the deviate results.

5.2.2 Advice and other activities

The Q3 meeting, which included the NRL, OL, and another Dutch laboratory, took place once in 2023 on June 23rd.

In 2023, the NRL took part in four meetings of the National Plan Residue Control working group: the 20th of March, the 5th of June, the 31st of August, and the 2nd of December.

In 2023, the NRL began participating in a multi-substance-class collaborative study coordinated by the BVL-EURL. In this study, an analytical method for the detection of several classes of compounds (i.e. antibiotics, quinolones, corticosteroids, amphenicols, nitroimidazoles, NSAIDs) will be fully validated according to a provided validation plan. Each participating NRL laboratory has performed this full validation. The BVL-EURL provided a described method for sample preparation, and the NRLs were asked to use LC-hrMS for the detection. The following NRLs are participating in this project: NRL Denmark, NRL Slovenia, NRL Bulgaria, NRL Spain, NRL France, NRL Netherlands, and EURL-Berlin.

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

Coordinator: Jeroen van Dijk

6.1 Activities within the EURL – NRL network

6.1.1 Participation in EURL-NRL workshops

The 12th Annual Workshop of the EURL-FA Control and the 23rd Annual Workshop of the EURL-FA Authorisation were organised as a joint hybrid event, held at JRC-Geel on November 14-15, 2023. A total of 50 participants attended, representing 31 National Reference Laboratories (NRLs) from 22 Member States, including Norway and Switzerland. Representatives from DG SANTE and the EURL-FA were also present. Unfortunately, the Dutch NRL was unable to attend. The proceedings of the event were shared ahead of publication.

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

In 2023, the 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 (binders/anticaking agents), sensory additives (flavouring compounds), zootechnical additives (physiological condition stabilisers, other zootechnical additives) and nutritional additives (amino acids, their salts and analogues, compounds of trace elements and vitamins, provitamins and chemically well-defined substances having a similar effect).

6.1.3 Participation in proficiency tests

The NRL participated in one PT for coccidiostats, antibiotics and anthelmintics at carry-over level in compound feed, also organised by WFSR. This is possible under our ISO 17043:2023 accreditation for the organisation of PTs. Organisation and participation are strictly separated and audited by the Dutch Board of Accreditation. The NRL used LC-MS/MS methods for analysis. Diclazuril, oxytetracycline, erythromycin, and amoxicillin were detected and quantified with sufficient z-scores in the range of -0.66 – 0.2. Ivermectin, salinomycin, and furazolidone were also correctly identified.

6.1.4 Contact on methodological issues

In late 2022, assistance was requested from the EURL regarding two methodological issues: quantification problems with coccidiostats at the additive level and repeatability of the levels of vitamins A, D, and E in animal feed using the CEN 17547 method. Unfortunately, despite contact with the EURL, these issues were not resolved in 2023. As a result, no assistance was needed from the EURL that year. Further investigation and in-house actions are now planned for the second half of 2024 and 2025 under project WOT-02-004-014 (Method development, accreditation maintenance, and inventory research on animal feed).

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, Fisheries, Food Security and Nature (LNVN, former 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 2023, 11 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 the Dutch NRL-FA, which mostly concerned further details on feed processing.

For these 11 national requests, we also evaluated whether they concerned GMOs or additives produced by GMOs. In five 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 2023, 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 provided on the concept of Commission Delegated Regulation (EU) 2024/1229, establishing specific maximum levels of cross-contamination of antimicrobial active substances in non-target feed and methods of analysis for these substances in feed. Furthermore, an update for the Dutch guidance document on the risk of cross-contamination related to 6.2.1 has been discussed with CBG, RIVM, and NVWA, and is to be concluded in 2024.

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 2023, the Dutch NRL participated in the Steering Committee meetings of the European Network of GMO Laboratories (ENGL) in March (web meeting) and June (web meeting): the 44th ENGL Steering Committee on 9 March and the 45th ENGL Steering Committee on 20 June via Webex. At these meetings, the 19th NRL Workshop and the 34th ENGL Plenary meetings were prepared (26-28 September, Seville, Spain). At the NRL meeting, a presentation was given on 'Botanical Impurity'.

44th ENGL Steering Committee Meeting. DG SANTE presented the recent developments on New Genomic Techniques (NGT) policy initiative. JRC analysed some case studies as EFSA is addressing the risk assessment proposal. The legislative proposal was expected by June 2023 (it was published July 5th 2023). GM presence in feed/ food additives/enzymes: The presence of Genetically Modified Microorganisms (GMMs) in feed/food additives and enzymes discussion is still ongoing because different Member States have divergent views on addressing the problem. Update on ENGL Working Groups: WG-MPR published (JRC125975), WG-GMM still on hold.

45th ENGL Steering Committee Meeting. Policy action on New Genomic Techniques (NGTs) will be finalised in early July, according to SANTE. GMM fermentation aspects are still under debate: SANTE organised two working groups. Germany requested a legal clarification on products containing GMM DNA. SANTE explained that "products containing non-authorized GMOs are not compliant while products containing recombinant DNA originating from a GMM that is used as processing aid would be compliant. It further clarified that the legal interpretation concerns use of GMM as processing aids while fermentation products in which the GMM are not removed are considered as food or feed falling under Regulation (EC) No 1829/2003". Update on ENGL Working Groups: WG sequencing, WG DNAex and is published January 2024). WG GMM still on hold. WG New Mutagenesis Techniques published (JRC133689) and mandate expanded to NGT-animals and NGT microorganisms. The current Working Groups gave an update on their work.

NRL meeting. The recent policy action on New Genomic Techniques (NGTs: the COM(2023) 411 final 2023/0226 (COD) Proposal for a REGULATION OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL on plants obtained by certain new genomic techniques and their food and feed, and amending Regulation (EU) 2017/625), Fact-finding studies, and GM presence in feed additives and food enzymes were updated by SANTE. Although the discussion is still ongoing, the Commission suggests two categories in which Cat.I contains NGT-plants that are equivalent to conventional plants, whereas Cat.II contains NGT plants that are not equivalent to conventional plants. These categories have different verification procedures, requirements and demands. Herbicide resistance is excluded from authorised traits.

JRC representatives summarised the activities of the EURL GMFF in 2023 regarding the validation of 5 new maize events: DP23211, DP910521, DP051291, MON94804 and MON94313. Plasmid controls will be distributed with an MTA.

The issue of Botanical Impurity was discussed. This could become a new WG.

The results of PT GMFF-22/02 T1 (Maize DAS59122 in multigrain bread) and T2 (soybean CV127 in soybean seed) were discussed. WFSR received a very good score.

For the PT GMFF-23/01, JRC decided to select outstanding laboratories to quantify the events that were present in the two test items. These were T1 = Popcorn maize containing MIR604 maize and T2 = Soybean powder containing A5547 soybean. By a procedure described as 'Selection of certifier labs', laboratories with a very good score over the past ten years were chosen. WFSR was selected as one of the 4 external labs. GMFF-23/02 T1 contained 3 soybean events in sheep feed. T2 contained one maize event in maize pasta.

ENGL plenary meeting. The ENGL plenary meeting was held on 27-28 September and was attended by two representatives of the Dutch NRL. The meeting started with a JRC-Seville presentation on the NGTs for a sustainable EU food system. Case studies showed the sustainability of NGTs and what they can mean in the Green Deal frame to improve sustainability along the food supply chain. One of the other goals is to contribute to the 'Farm 2 fork' strategy. BVL presented a dPCR detection method for CIBUS gene-edited canola was presented. The WGs DNAex and Sequencing provided a progress update and key outputs. Presentations on GMO detection in several non-EU countries were given by India, Latin America/Caribbean and South-Africa. The representative from South-Africa is a member of the Southern African Network for GM detection Laboratories (SANGL) and stated that they frequently use EUgenius.

The second day started with a presentation by Belgium on the malicious use of GMMs and a JRC presentation on the retrieval of sequences from public databases and the development of a detection method based on these findings. Unfortunately, this detection method was already available. The meeting was closed with the coordinators' presentation of the PlantEd COST action.

7.1.2 Participation in working groups

The Dutch NRL chairs the Working Group (WG) DNA extraction. Several online meetings were organised, and updates were given to the EURL. The WG DNAex report is published in January 2024.

The Dutch NRL participated in the WG Minimum Performance Requirements Part 2 (MPR2), and the final report was published in 2023.

The Dutch NRL participated in the WG Genetically Modified Microorganisms. The final report will probably be finalised before the end of 2024.

The Dutch NRL participated in the WG NGT. The main task of this WG was 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 2023, the report was published.

The Dutch NRL participated in the WG NGT, for which two mandates were extended to NGT animals and NGT microorganisms.

7.1.3 Participation in proficiency and comparative tests

Two EURL proficiency tests were organised in 2023. The Dutch NRL participated in these PTs with good results (Table 7.1). For GMFF-23/01, WFSR was selected as the certifier lab. Therefore, the performance scores were provided in a separate report. For GMFF-23/02, there is currently a preliminary report only.

Table 7.1 Overview of PT results GMO detection in 2023.

Proficiency test	Analyte	Matrix	z-score	ζ score
GMFF-23/01	GM maize MIR604	T1: Popcorn maize	-0.46	-0.70
GMFF-23/01	GM soybean A5547	T2: Soybean seed	-0.83	-1.49
GMFF-23/02	GM soybean event GTS 40-3-2	T1: Sheep feed	n.a.	n.a.
	GM soybean event MON87701	T1: Sheep feed		
	GM soybean event MON89788	T1: Sheep feed		
GMFF-23/02	GM maize event TC1507	T2: maize pasta	-0.06	0.00

n.a. = not applicable (Not provided).

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, Fisheries, Food Security and Nature (LVVN, formerly 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. In 2023 there was an increased involvement due to the "COM(2023) 411 final 2023/0226 (COD) Proposal" of July 5th by the European Commission.

On a monthly basis, bilateral meetings are held with the NVWA regarding GMO food and China rice. Also, 'Domain-meetings' organised by WFSR allow discussions 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 has also been the NRL GM Food and Feed for Ireland. Ireland financed the NRL activities for Ireland in a separate project. For the four-year period of 2021-2024, WFSR continued 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 and maintained since 2014 the GMO database [Euginius](#). The consortium partners are the NRLs AGES (Austria), IHAR (Poland), and IZS (Italy).

7.5 References

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

In 2023, the joint workshop for all four EURLs was organised by the EURL-SRM in Stuttgart, Germany, from 18-21 October. WFSR attended in person with four persons.

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, the bi-annual revision of the "Guidance document on analytical quality control and method validation procedures for pesticide residue analysis in food and feed" was presented and discussed.

The items presented and discussed are briefly summarised below:

- The European Commission (DG SANTE E4) gave a presentation on the role/of way of working of DG SANTE in pesticides regulation (EC) 396/2005 in general, and then addressed specific topics:
 - i) Update on the use of processing factors (PF) in MRL compliance testing of processed food and feed: an updated PF table will be available by 1/1/2024. The use of PFs (and the selection) is the responsibility of the Competent Authorities.
 - ii) Update of guidance document SANTE/2020/12830 rev. 2 'analytical methods for risk assessment and post-approval control and monitoring purposes': note on extraction solvents: n-hexane and toluene are still considered acceptable, chlorinated solvents no longer allowed.
 - iii) Fish: BAC, DDAC, and chlorate are frequently detected, while no MRLs yet exist. Guidance values are proposed for fish for the national authorities. EFSA did not identify intake concerns, however, a narrow margin of acute exposure was identified for chlorate in fish (82% ARfD). The Commission decided no further action was needed.
 - iv) Future challenges: cumulative risk assessment/exposure to mixtures. It is considered not realistic nor economically feasible to specifically assess and regulate an almost infinite number of combinations of chemicals. The proposed approach is to go for Cumulative Assessment Groups (CAGs) for pesticides affecting the nervous system, thyroid, and craniofacial alterations. For the CAGs a probabilistic exposure assessment based on monitoring data will then be performed.
 - v) Sampling directive 2002/63: will be revised to include more products (honey, fish, terrestrial invertebrate animals, amphibians, reptiles). It is also foreseen that text from SANTE/11321/2021 on measurement uncertainty in MRL compliance verification will be included.
 - vi) Pesticides with possible natural background: trimethyl sulfonium cation and matrine residues are not unambiguously originating from use as plant protection products.

- EFSA presented the main results and recommendations from the report on pesticide residues in food as reported by all member states in 2021. Based on the overall data from the EU-coordinated program and the national programs, almost 87,000 samples were analysed. Residues were reported in 44% of the samples. 26% of the samples contained multiple residues. MRL exceedances occurred in 3.9% of the samples. Several commodity/pesticide combinations were recommended to monitor specifically. An acute deterministic exposure assessment revealed that the health-based guidance value (ARfD) was exceeded in 1.1% of the assessed pesticide/matrix combinations. The pesticides found to contribute most were lambda-cyhalothrin, cypermethrin, acetamiprid, and indoxacarb.
- Eleven technical presentations were given by EURLs, NRLs and OLs. The EURL-AO elaborated on the new regulations for veterinary drugs where pesticides are included as forbidden substances, i.e. two regulatory silos overlap, and the impact on validation and monitoring of pesticides. The EURL-CF presented on transfer of pesticide residues from barley to beer and its by-products. Results from a joint monitoring programme of pesticides in honey were presented by the EURLs AO and SRM. WFSR shared their experiences with import control in the Netherlands. QSI presented on matrine-type alkaloids in honey. Labor Friedle addressed the issue of phthalimide (part of residue definition of folpet) formation from sources other than folpet, and background artefacts of anthraquinone and 1,4-dimethylnaphthalene. Both the EURL-FV and CF presented developments in automated cleanup using μ SPE, with coffee, spices and insects as challenging matrix examples. Experiences of replacement of helium for hydrogen were shared by the EURL-FV. The EURL-SRM presented a variety of challenges for analytically 'difficult' pesticides (captan, folpet, conjugates of acidic pesticides, dithiocarbamates).
- Discussion of EURL-proficiency tests (PTs). In total six PTs were organised in 2023 by the various EURLs. Except for the PT organised by the EURL-SRM, the samples needed to be analysed for 130-212 pesticides. 100-175 NRLs and OLs participated in the PT. EUPT-CF17: wheat kernels contained 20 pesticides (partially incurred) mostly in the range of 0.03-0.10 mg/kg (up to 0.32 mg/kg). One pesticide was only measured by 50% of the participating laboratories. Percentages of questionable or unacceptable results ranged from 3 to 13%. As a measure for the interlaboratory variability, the robust relative standard deviation (RSD_R) was used, which varied from 14-25% (average 18%). This is within the 25% that is used as a fixed value for expanded measurement uncertainty for enforcement purposes. In honey (EUPT-AO18), 19 pesticides were added, mostly in the 0.05-0.10 mg/kg range. Satisfactory results were obtained by 78-97% of the participants. Also here, a number of pesticides were only measured by part of the laboratories. RSD_R s ranged from 12% to 27%, average below 25%. In the fruit/vegetable commodities, melon was used (EUPT-FV25) spiked with 18 pesticides or their formulations at concentrations mostly in the range of 0.1-0.2 mg/kg. Good coverage of the pesticides was observed in the measurements, with the exception of two pesticides for which determination was on a voluntary basis. Satisfactory results were obtained by more than 90% of the participants for most pesticides. RSD_R s ranged from 13% to 22% (average 17%). For SRM pesticides, honey was used as matrix (EUPT-SRM18). In total 12 pesticides were spiked, requiring various methods for their determination. The coverage of the pesticides ranged from 26-64% of the participants. Satisfactory results were obtained by 70%-100% of the participants. RSD_R s ranged from 10% to 25%. As known from earlier PTs, there was no apparent relationship between RSD_R and concentration.
- Besides the four mandatory PTs mentioned above, two additional voluntary PTs were organised. One concerned a 'special commodity' (considered more difficult for analysis), in this case fresh chilli pepper, to be analysed for 211 pesticides. In total, 14 pesticides were present. Levels ranged from 0.018 to 0.89 mg/kg. A total of 37 laboratories participated. Acceptable quantitative performance was obtained by 88%-100% of the laboratories. The RSD_R s varied from 15% to 25% (average 18%), indicating that this matrix was not challenging to the laboratories. The second voluntary PT concerned a rapid screening analysis (qualitative and optionally quantitative) of pesticides in wheat kernels. In this case, no target list was given; laboratories had to detect 'any' pesticide and report within 72 hours. In total, 14 pesticides were present (all between 0.06-0.18 mg/kg), some of which being less common. Seventeen out of 60 laboratories detected all 14 pesticides, indicating that it is still challenging to find 'any' pesticide in a sample. Fluroxypyr and trinexapac-ethyl were only detected by 50% of labs.
- AQC document. Three sessions were devoted to the presentation and discussion of the proposed revision of the analytical quality control (AQC) document: "Guidance document on analytical quality control and method validation procedures for pesticide residues and analysis in food and feed" (existing version: SANTE/11312/2021), moderated by Tuija Pihlstrom. Adjustments to the previous version were modest, including a rewritten section on reporting and interpreting the results, and a clearer distinction between absolute and apparent recovery. After discussion, the proposed revision was adopted by all NRLs. A new

version of the AQC document (SANTE/11312/2021 v2) to be used by 1 January 2024 was issued.
https://food.ec.europa.eu/system/files/2023-11/pesticides_mrl_guidelines_wrkdoc_2021-11312.pdf.

- Various EURL matters: EURL representative shared their activities for the coming year.

In 2023, one co-worker from the pesticides teams of WFSR attended a training course organised by the EURL-AO in Freiburg on 16-17 October 2023.

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 2023, meetings were held on 26-27 January (Malaga), 27-29 June (Madrid), and 17 October (Stuttgart). 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 16 proficiency tests on pesticides in fruits/vegetables, cereals, products of animal origin, feed and other food matrices. This included PTs organised by the EURLs and PTs from commercial suppliers. An overview is given in Table 8.1. A total of 145 z-scores (indicators for quantitative performance) were obtained. In general, the performance of the NRL was satisfactory (136 out of 145 z-scores), and the pesticides were correctly identified and quantified. A questionable result was obtained for two pesticide/matrix combinations (within the statistically expected range, but appropriate follow-up actions were taken). The result was unsatisfactory in seven cases, triggering a root cause analysis. In two cases (EUPT AO18 and Fapas 9153), the deviation was due to erroneous reporting (reporting absence of pesticides that were not in the method's scope). In case of Fapas 19353, analysis was done using a non-validated method, which is not used for official analysis and should not have been used for PT reporting. 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 and PT code		Matrix	Analytes ²⁾	Performance ¹⁾		
				S	Q	U
EUPT AO18		honey	MRM pesticides	18		1
EUPT CF17		wheat kernels	MRM pesticides	20		
EUPT FV25		melon	MRM pesticides	18		
EUPT SC06		chilli pepper	MRM pesticides	13	1	
EUPT SRM18		honey	SRM pesticides (BAC, DDAC, matriline, oxymatrine, nicotine, chlorate, glyphosate, perchlorate, phosphonic acid, 2,4-D, fluazifop)	11		
EUPT SM15		wheat kernels	MRM qualitative screening	100% (14 found)		
Fapas 19353		honey	MRM pesticides	4		5
Fapas 19357		cumin	MRM pesticides	12	1	
Fapas 9152		wheat	SRM (chlormequat, mepiquat, glyphosate, AMPA)	4		
Fapas 9153		rice	MRM pesticides	11		1
Fapas 19362		tea	SRM (perchlorate)	1		
Fapas 19365		grapefruit	MRM pesticides	13		
Fapas 19364		mandarin	SRM (dithiocarbamates)	1		
Fapas 9156		oats	SRM (chlormequat, mepiquat, glyphosate, AMPA)	4		
Fapas 9158		wheat	SRM (ethephon, fosetyl, MPPA, phosphonic acid)	4		
Fapas 5169		fish oil	MRM pesticides	2		

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

²⁾ MRM: pesticides amenable to multi-residue methods; target list typically includes 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, which has 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 2023, representatives from the OLs for organic produce participated in the joint EURL meeting, where experiences of pesticide residue analyses were discussed.

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 (CA) on official analysis. Specific topics addressed in 2023 included the increasing number and variety of import samples, the growing number of complex residue definitions (inclusion of metabolites), and the tendency of lowering the MRL below the default 0.01 mg/kg for specific pesticides. Several ad-hoc questions from the CA were addressed (a.o. disputed results between CA and FBO, ability to measure the full residue definition of acidic herbicides, the feasibility of lowering the MRL for haloxyfop in milk). At the CA's request, WFSR participated in the meeting of the EC Working Group for the Monitoring of Pesticide Residues on 13 October 2023.

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 several conferences where oral presentations were given, and general and technical aspects associated with pesticide residue analysis were exchanged with the experts and other attendees: Latin American Pesticide Residue Workshop (LAPRW 2023, 21-24 May, Panama), 2nd workshop on advances in separation science (28-29 June 2023, Liege, Belgium), AOAC annual meeting (New Orleans, USA, attended online), 12th International Conference 'Pesticide Residues in Food' (Fresenius, 12-13 December 2023, online).

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 2023, the EURL organised the annual workshop. No trainings were organised in 2023 by the EURL.

The annual EURL workshop was held in Copenhagen on 15 and 16 November 2023. Forty NRL representatives attended the workshop in person. Optionally, the workshop could also be attended virtually as “online observers”. This was done by twenty-three participants. The Dutch NRL attended the workshop in person. The workshop was opened with a short presentation of the EURL. Currently, there are 141 maximum limits for metals in food and 76 maximum limits for metals in feed. The large number of maximum limits and the number of notifications in RASFF (Rapid Alert System for Food and Feed) highlights the importance of the official controls on heavy metals in food and feed.

Proficiency tests (PTs) organised by the EURL and issues associated with these PTs are usually extensively discussed during the workshop. In 2023, the EURL organised three different PTs:

- The first PT concerned the determination of aluminium, nickel, cadmium and lead in infant formula. The network’s performance was satisfactory for all parameters. Besides the analytical performance, the NRLs were also requested to assess the compliance of the sample with the EU maximum limits. For lead the majority of the NRLs assessed the compliance correctly. For cadmium, one-third of the laboratories assessed the sample as compliant, whereas two-thirds of the NRLs assessed it as non-compliant. The discussion focussed on the use of the correct maximum limits for certain product types. Due to the large number of maximum limits for metals in food, the network recognised the difficulty of the compliance assessment. The Dutch NRL noted that the EURL itself did not use the correct maximum limit for cadmium. Therefore, this was corrected in the final PT report. All z-scores of the Dutch NRL were acceptable, and the compliance was also correctly assessed.
- The second PT covered the determination of aluminium, nickel, total arsenic, cadmium, lead and nitrate in a root vegetable (carrot powder). For metals, the performance of the network was satisfactory. For nitrate, a large variation in the results from the different laboratories was observed. A relatively large number of laboratories (9 out of 28) did not have an acceptable z-score. The discussion, therefore, focussed on possible reasons for the large variation in nitrate results. Most labs use an HPLC method with either UV or conductivity detection, and some labs noted an interfering peak in their chromatogram. This could be a reason for the scattered results. The Dutch NRL uses a different method (segmented-flow analysis) for the determination of nitrate and achieved an acceptable z-score.
- The third PT organised by the EURL concerned the determination of nickel, total arsenic, cadmium and lead in mineral feed. Mineral feed is considered to be a difficult matrix for the determination of heavy metals. Although the performance of the network was acceptable overall, a larger spread in results was observed than in the food samples. The discussion focussed on the homogeneity of the PT material. Visually, the PT material was inhomogeneous. Some laboratories therefore homogenised the sample themselves by grinding. The homogeneity tests performed by the EURL did not pass the standard criteria for all metals, but the expanded criteria were met. The EURL tested the influence of grinding the sample and fractionating of the sample on the concentrations of the metals. Grinding resulted in an increase of the lead content and, interestingly, in a decrease in the nickel content. Fractionating of the sample into a part with white beads and a part with brown powder resulted in different lead (1.4 and 2.0 mg/kg) and nickel (5 and 22 mg/kg)

contents in the fractions. For arsenic and cadmium these differences were much less evident. The Dutch NRL achieved acceptable z-scores for arsenic, cadmium and lead, but the z-score for nickel was questionable. The Dutch NRL did grind the sample further due to its inhomogeneity.

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. In 2024, new maximum limits (MLs) for nickel in food will be published. Currently, there are 141 MLs for heavy metals in food, but not for nickel. More than 30 additional MLs for nickel in different food types are expected. New MLs for inorganic arsenic in different food matrices have been published in 2023. For fish, MLs are still under discussion and are expected to be finalised in 2024. The Dutch NRL's method for inorganic arsenic has been expanded with different food matrices in 2023 and has been used for official controls for the matrices to which the new MLs apply, such as baby food, fruit juice and different rice products. Other topics under discussion are MLs for metals and iodine in seaweed and for nitrate and nitrite in foods of plant origin. For feed, updates on MLs are expected in spring 2024. Similar to food, there are currently no MLs for nickel, but these are expected to be published as well. The Dutch NRL has validated and accredited methods for nickel in food and feed, and has been reporting these levels in samples from the monitoring programs to the competent authority for a few years. The regulation on methods of sampling and analysis for the official control of feed (EC 152/2009) is currently under revision. Some of the analytical methods that should be used for official controls are relatively old and outdated. The revision will include references to EN standards as an alternative to the already described methods. As an example, EC 152/2009 prescribes flame atomic absorption spectrometry for the determination of trace elements in feed. For the determination of trace elements, ICP-MS is currently a more common technique to be used. A European standard (EN 17053) is available since 2018 and will therefore be added to the regulation. The Dutch NRL is still using flame atomic absorption spectrometry for the determination of trace elements in feed, but has an accredited ICP-MS method (based on EN 17053) available as well.
- *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 European Commission now requested the EURL 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. In 2023, EFSA published an opinion on the risk assessment of N-nitrosamines in food. The EURL presented their work so far on the analysis of N-nitrosamines in food. The different NRL discussed their experience with the analysis of N-nitrosamines. The Dutch NRL, the EURL and the Austrian NRL appeared to be the only laboratories working on the analysis of N-nitrosamines. The Dutch NRL has worked on N-nitrosamines in the past and started to work on the development of a new method in 2023. The method development focussed on including the compounds mentioned in the recently published EFSA opinion. In 2024, the Dutch NRL will further develop the method and conduct a survey to study the occurrence of N-nitrosamines in different food matrices.
- *Method validation and limit of quantification.* In 2022, the EURL-MN, together with three other NRLs in the field of contaminants, published an update of the guidance document on the estimation of the limit of quantification (LOQ). The Spanish NRL presented their experience with method validation and the use of the updated approach for estimating the LOQ. The new guidance document focuses on estimating the LOQ for official controls and compliance testing. The LOQ is defined as the lowest validated level for which the criteria for identification, precision and trueness are met. This approach, however, does not yield LOQs as low as possible, which are needed for risk assessment purposes. The new guidance document refers to the old guidance document (2016) for alternative approaches to determine as low as possible LOQs. Because data from official controls is submitted annually to EFSA for risk assessment, the opinion of the Dutch NRL is that LOQs should be as low as possible. Hence, the Dutch NRL is using the approach described in the old guidance document.
- *Groupwork on different topics.* This year's workshop included room for group work. The attendees were divided into smaller groups to discuss different topics. One of the main topics was sample preparation. Labs discussed how they dealt with very large sample sizes and which homogenization equipment was used. The importance of the latter was highlighted by several labs, including the Dutch NRL, because contamination by using steel equipment is a high risk. This is especially the case for nickel, and because of the upcoming MLs it is important to choose the right equipment and to test for contamination issues during

sample homogenization. Other topics discussed were the experiences with the analysis of nitrogenous compounds (nitrate, nitrite and N-nitrosamines), the determination of aluminium and experiences with difficult matrices for which new MLs were published recently, such as edible oil and salt.

9.1.2 Participation in working groups

In 2023, 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 nickel in mineral feed (EURL-MN PT-2023-03). For nickel in mineral feed, a questionable z-score of -2.2 was achieved. As described previously (9.1.1), this questionable z-score was most likely the result of inhomogeneity in the PT material sent out by the EURL. Therefore, 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 07470	Ni, As, Cd, Hg, Pb, iAs	Animal Feed (Fish Meal)	Between -0.4 and 0.8
FAPAS 09153	Br	Basmati rice	-1.8
FAPAS 18113	I, Ca, Cu, Fe, Mg, Mn, Mo, K, P, Se, Na, Zn	Infant formula	Between -1.4 and 0.5
FAPAS 07475	As, Cd, Hg, MeHg	Canned Fish	Between -0.2 and 0.4
FAPAS 07478	Ni, Cd, Pb, Cr, Se, I, Sn	Infant formula	Between -0.3 and 0.7
FAPAS 07485	Ni, As, Cd, Hg, Pb	Vegetable Purée (Root Vegetables)	Between 0.1 and 1.7
FAPAS 10188	Zn	Pig Ration	1.8
FAPAS 07489	Ni, Cu, As, Cd, Hg, Pb, iAs, Co, Fe, Mn, Zn	Powdered Brown Rice	Between -0.1 and 1.2
EURL-MN PT-2023-01	Al, Ni, Cd, Pb	Infant formula based on cows milk protein	Between -0.29 and 0.08
EURL-MN PT-2023-02	As, Ni, As, Cd, Pb	Root vegetable (carrot powder)	Between -0.11 and 1.37
EURL-MN PT-2023-03	Ni, As, Cd, Pb	Mineral feed	Between -1.85 and 1.03; -2.22 for Ni
EURL-MN PT-2023-02	Nitrate (NaNO ₃)	Root vegetable (carrot powder)	0.22
FAPAS 15169	Nitrate (NaNO ₃)	Carrot puree	1.4
FAPAS 015170	Nitrate (NaNO ₃) and Nitrite (NaNO ₂)	Meat	0.8 and 1.0, resp.
FAPAS 30134	Melamine, cyanuric acid	Infant formula	0.7 and 0.4, resp.
FAPAS 30142	Melamine, cyanuric acid	Milk powder	-0.1 and 0.7, 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 organic 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 2023 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 the interpretation of the legislation concerning maximum limits of cadmium in ginger roots. In addition, the other OL was visited by the Dutch NRL on June 7. 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.

9.3 Scientific and technical support to the competent authority

In 2023, 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. Inorganic arsenic levels in fishery products were discussed with the competent authority in relation to the European discussions on maximum limits.

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 workshop

The workshop, organized by WFSR in the Netherlands on 3 and 4 October 2023, brought together a prominent group of attendees, including representatives from the Commission, the Netherlands Food and Consumer Product Safety Authority (NVWA), WFSR scientists of the EURL, and representatives of NRLs. The agenda for the workshop was comprehensive, covering a range of key topics in our field. Attendees were provided with updates on relevant issues, including the latest findings from proficiency tests (PTs) organised by the EURL, invaluable for ensuring the accuracy and reliability of our analytical methods, and discussions on upcoming legislation concerning the sampling and analysis of mycotoxins and plant toxins. This is a critical area that requires continuous refinement to meet evolving regulatory standards and ensure consumer safety. Additionally, there were fruitful exchanges on method development, highlighting the ongoing efforts to advance our analytical capabilities in detecting natural contaminants. In summary, the workshop served as a platform for discussions and knowledge-sharing among experts in the field. The expertise of each participant contributed to a successful and informative event.

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.*** The Commission Regulation (EU) 2023/915 has been issued, marking a meaningful change as it repeals Regulation (EC) No 1881/2006 along with all its numerous amendments, totalling more than 45 revisions over the years. One of the major concerns is the increased prevalence of mycotoxins, worsened by climate change and extreme weather conditions. This poses difficulties in maintaining consistent levels of mycotoxins in agricultural products due to annual and geographical variations. Moreover, achieving the desired levels through common agricultural practices and prevention measures presents its own set of challenges. The discussions also touched upon specific mycotoxins found in food, such as deoxynivalenol (DON), T2, HT2, and Alternaria toxins. Maximum levels or indicative values for these toxins are still largely under review by EFSA, highlighting the ongoing efforts to establish comprehensive guidelines. Furthermore, the replacement of Commission Regulation (EC) 2006/401 brings about notable changes, particularly in sampling methods. The new regulation introduces novel approaches, including the sampling of commodities with a high volume/weight ratio. Additionally, there are updated performance criteria for confirmatory methods, ensuring more accurate and reliable testing processes.
- ***Mycotoxins and plant toxins in feed.*** Guidance levels for feed materials will be reviewed according to the ALARA (As Low As Reasonably Achievable) principle. This principle ensures that levels are set at the lowest possible concentration that still allows for effective control of mycotoxins. Additionally, guidance levels for complete feed will consider reference points for adverse animal health effects, ensuring the well-being of our livestock. Scientific advice has been provided for several key mycotoxins and their impact on specific animal species: Fumonisin impact on horses and pigs, T2 and HT-2 toxin impact on ruminants, DON impact on horses and poultry (excluding laying hens), Ochratoxin A impact on various animal species. In parallel, the Commission is finalising a Recommendation on the monitoring of ergot alkaloids, further enhancing our ability to detect and control these harmful substances. Limits and recommendations for plant toxins in feed have also been carefully listed, with ongoing discussions focusing on cannabinoids, tropane, pyrrolizidine, and quinolizidine alkaloids.

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- Plant toxins in food. A specific focus was placed on plant toxins in food, particularly the monitoring of glycoalkaloids and quinolizidine alkaloids. Both sampling and analytical requirements for these plant toxins were thoroughly discussed, reflecting the ongoing commitment to ensuring the safety and quality of our food supply.
 - Review of Regulation (EC) 152/2009. The review of Regulation (EC) 152/2009 is in its final stages, with publication expected in early 2024. This updated regulation will reflect the latest scientific insights and best practices in mycotoxin management.
 - New risks. This complex issue is influenced by numerous factors, including climate change, evolving dietary patterns, the introduction of new foods, and the persistent challenge of limited data and updated consumption surveys. The emergence of novel contaminants and the ongoing transition towards a circular economy further emphasize the need for comprehensive and adaptable safety measures. It is imperative that we remain vigilant and proactive in our efforts to protect the health and well-being of consumers. Considering these challenges, our commitment to developing robust safety protocols and staying abreast of emerging risks is more crucial than ever. By working together and leveraging our collective expertise, we can effectively address these complexities and ensure the continued safety and integrity of our food supply.
 - Combined exposure. Unfortunately, our progress in both risk assessment and risk management approaches has been limited, presenting numerous hurdles to overcome. A primary concern arises from the lack of sufficient toxicity data for individual compounds within 'similar compound' groups. This often leads to the challenging task of extrapolating the toxicity of one compound to apply to others in the same category. An issue is the unavailability of routine analytical methods to detect some of these compounds, further complicating the ability to assess risks effectively. Additionally, there is a lack of information regarding the presence of these compounds in our food sources. Conversely, when shift focus to combined exposure to 'non-similar' compounds, such as different mycotoxins or various metals, we encounter another set of challenges. The aspects of risk assessment and risk management in these scenarios have yet to be thoroughly addressed, leaving gaps in our understanding of potential risks to human health. To uphold a high standard of human health safety, it is crucial that we devote greater attention to addressing the complexities of combined exposure.
 - Lastly, an overview of EN standards for mycotoxins and plant toxins was provided.

Proficiency tests, based on the presentations given by EURL.

- PT Ergot Alkaloids. In the recent proficiency test on Ergot Alkaloids, involving 33 participating laboratories, the following results were observed: for materials A (wheat) and B (rye), 85% and 94% of the results for 12 epimer pairs (EAs) were satisfactory, respectively. The robust relative standard deviation (RSDR) for the sum of EAs in materials A and B was 19% and 14%, respectively. When combining results for both materials (12 EAs), 90% received satisfactory z-scores ($|z| \leq 2$), 3% were in the questionable range ($2 < |z| < 3$), and 7% were unsatisfactory ($|z| \geq 3$). Compared to the previous proficiency test (EURLPT-MP03), progress has been made with all RSDRs falling below the 25% target. However, some labs faced challenges due to higher limits of quantification (LOQs). Lowering LOQs is crucial for infant and child cereal product analysis, aligning with EFSA's monitoring and exposure recommendations.
- PT Opium Alkaloids. In the recent proficiency test on Opium Alkaloids, involving 31 laboratories, the following was noted: for morphine and codeine in materials A (poppy seeds) and B (bakery product), 81% and 97% of results were satisfactory, respectively. However, RSDR values (24-32%) were mostly higher than the 25% target, except for codeine (97% satisfactory with target RSDR). Despite most labs using isotopically labelled internal standards (IS) to enhance quantification, the expected improvements were not consistently observed. The majority of labs have methods with sufficiently low LOQs, but some need lower LOQs for EU Regulation 2023/915 levels. There is still room for improvement as the result variation remains relatively high.

EURL methods update, based on the presentations given by EURL.

- *Glycoalkaloids in potato products (presented by EURL).* The EURLMP-method_014 Glycoalkaloids in potatoes using LC-MS/MS v1 has been successfully validated to quantify α -solanine, α -chaconine, γ -chaconine, and solanidine in specific potato products like chips, fries, potato powders, and gnocchi. The limit of quantification values obtained are suitable for their purpose. Results show that in chips, glycoalkaloid levels exceeded 12 mg/kg compared to the indicative 100 mg/kg level. However, all other potato products contained glycoalkaloids below 100 mg/kg on average. Among the products surveyed, chips were the most contaminated (112 mg/kg), followed by potato puree powder (90.7 mg/kg), fries and gnocchi (28.6 mg/kg and 28 mg/kg, respectively), pre-processed potato products (21 mg/kg), potato puree (6.9 mg/kg), and potato starch powder (4.1 mg/kg).
- *Ochratoxin A in cured meats (presented by EURL).* The LC-MS/MS method originally developed at WFSR in 2022 for the quantification of OTA in cured meat has been modified and successfully validated in-house for pig edible organ meat - kidney. The limit of quantification (LOQ) achieved with this modification is 0.2 μ g/kg. The survey results (46 samples) have shown that cured meat products are relatively frequently contaminated with OTA, thus at low levels.

High resolution MS and mycotoxins: going deeper, going broader, going further (presented by WUR).

- The presentation highlighted advanced MS methods for mycotoxin analysis, including ion mobility MS, MS imaging, and high resolution MS. Changes in climate, circular economy, and reduced pesticide use are shifting Europe's mycotoxin landscape, making it unpredictable. The evolving mycotoxin patterns due to plant and organism defences add complexity. Being ready for surprises and using cutting-edge analytical tools is crucial to tackle these challenges.

Studies on sample homogeneity.

- Studies on the representativeness of sampling large lots of herbs (>15 t) for their PA content and results on the handling of the aggregate sample in the laboratory (Presented by BfR, NRL Germany). In a project by the German Ministry of Food and Agriculture, different sampling techniques were tested on large quantities of herbs (15 to 25 tons). This involved adjusting sample sizes and weights, resulting in varying aggregate sample weights. The study offered insights into these sampling methods and explored different grinding techniques to reduce result variability. Key findings are: 10 kg aggregate samples ensure representativeness for lots >15kg; smaller sample sizes may underestimate PA content and increase result spread; increasing the aggregate sample to 4 kg is recommended, if feasible for lab processing; lab homogenization should ensure particle sizes below 500 μ m, with a minimum 10 g sample weight for PA content analysis.
- Sampling of cumin and culinary herbs contaminated with pyrrolizidine alkaloids (Presented by the EURL). In the EURL project on contamination distribution in laboratory samples of co-harvested plant parts, the homogeneity of cumin seeds and culinary herbs was assessed with various sample sizes and techniques. Slurry mixing of 0.5 kg cumin batches showed less than 5% RSDinter-portion, while dry milling had over 40% RSDinter-portion. Highly contaminated cumin (1032 μ g/kg) with slurry mixing (0.5 kg) had 48% RSDinter-portion, versus 14% for 2 kg portions, following contamination level correlation. To achieve \leq 20% RSD at the maximum limit (400 μ g/kg) for PAs in cumin, a 4 kg portion size is suggested. Herb homogeneity testing found milling effective, unlike in cumin. Dry and cryo milling showed similar efficiency for herbs. Slurry mixing was effective but may increase PA conversion in savory, posing stability concerns.

Recent changes in Reference Points for adverse animal health effects for a number of mycotoxins, as established by EFSA (Presented by WFSR).

- Mycotoxins can contaminate food via feed, posing risks to both humans and animals. This presentation discussed the ongoing EFSA assessment on mycotoxins in animal feed, emphasizing their toxic effects on various animal species. Updated recommendations target reducing mycotoxin levels, including DON in poultry feed and updates on T2/HT2 for ruminants, and fumonisins for poultry, pigs, and horses, ensuring animal health and well-being.

Analytical quality control (Presented by the EURL).

- Updates were given on activities of the EURL AQC group, CEN activities, EURL contaminants joint initiative, and revision of 401/2006. Some changes were made to the revision after discussions in the EC Working Group on Agricultural Contaminants in Food. The revised documents, including an analogue document on plant toxins, were approved in the SCoPAFF meeting (Sept 2023) and published at the end of 2023. Laboratories will have a transition period to adjust to new performance criteria. During breakout sessions, varying practices in using expanded measurement uncertainty for compliance testing were discussed, revealing significant deviations from revised 401/2006 among laboratories. This diversity underlines the need for alignment and harmonization where applicable.

Dietary exposure to mycotoxins of 1- and 2-year-old children from a Dutch Total Diet Study (Presented by WFSR).

- The presentation discussed a total diet study in the Netherlands focusing on mycotoxin levels in foods and beverages consumed by 1- and 2-year-old children. Mycotoxins included were aflatoxins, *Alternaria* toxins, citrinin, ergot alkaloids, fumonisins, ochratoxin A, patulin, sterigmatocystin, T-2/HT-2 toxins, and zearalenone. Concentrations were combined with consumption amounts for long-term exposure assessment, with foods below the detection limit assigned half this limit. The study highlighted potential health concerns regarding aflatoxins, *Alternaria* toxins, ochratoxin A, and T2/HT-2 sum, with significant contributions from bread, biscuits, breakfast cereals, chocolates, dried fruit, follow-on formula, and fruit juices.

Natural and synthetic cannabinoids in food on Czech market (Presented by CAFIA, NRL Czech Republic).

- An informative presentation discussed the challenges of cannabinoid (mis)use in the Czech market. The market has seen a rise in various natural and synthetic products containing cannabinoids. The CAFIA laboratory analysed over 50 samples of products like gummies, cookies, jellies, and shots collected in the past year. Results showed delta-9-tetrahydrocannabinol (Δ^9 -THC) and/or hexahydrocannabinol (HHC) as primary constituents, with smaller amounts of other cannabinoids like tetrahydrocannabinolic acid (THCA). HHC was also found, likely from synthetic routes. The presentation summarised these findings and discussed the analytical methods used.

Grayanotoxins in honey: EFSA opinion and analytical challenges (Presented by the EURL).

- The presentation outlined the work of the EFSA working group on Grayanotoxins (GTXs), neurotoxic compounds found in *Rhododendron* species. Species like *R. ponticum*, *R. flavum*, and *R. luteum* are known to have high GTX levels, found in regions like Nepal and bordering the Black Sea in Turkey. These plants are also cultivated in Europe for ornamental purposes. Since GTXs are found in the nectar of some flowering species, they can be present in honey. The recent EFSA Opinion recommends developing an LC-MS/MS method for GTX analysis in honey and gathering more analytical data. This will be a focus of an EURL MP scientific project in 2024.

Updates on the work program EURL MP 2023/2024 (Presented by the EURL).

- The EURL activities overview covered scientific projects, proficiency tests, trainings, and upcoming annual workshops, along with administrative aspects. Interaction within the NRL-EURL network throughout the year will be enhanced. The AQC group's work will continue in 2024 to address lab discrepancies and other issues. The next EURL MP workshop is reserved for October 1st and 2nd, 2024.

10.1.2 Participation in working groups

As a result of discussions within the working group on analytical quality control 'Guidance document on identification of mycotoxins in food and feed' (SANTE/12089/2016) was revised and new regulations for mycotoxins (Regulation (EU) 2023/2782) and plant toxins (Regulation (EU) 2023/2783) are referring to this guidance for identification of toxins in the samples (Guidance document on identification of mycotoxins and plant toxins in food and feed).

10.1.3 Participation in proficiency and comparative tests

In 2023, the Dutch NRL participated in 14 proficiency tests (PTs): 12 PTs on mycotoxins, and 2 PTs on plant toxins (quinolizidine and glyco- alkaloids), of which the overview is presented in Table 10.1. For the 63 toxin/matrix combinations, 52 satisfactory z-scores were obtained. Six questionable results were obtained in two PTs: multimycotoxins in maize flour and aflatoxins in rice. Five unsatisfactory results were obtained for a PT on aflatoxins in peanut butter. All deviations were analysed using a root cause analysis. For peanut butter, a mistake was made during weighing of the sample for analysis. After recalculation of the results with the correct dilution factor, the results fall within the range for this edition of PT. For maize flour, the standard addition procedure was not executed accurately, and the LC-MS/MS system's linear range was insufficient for this sample. Unfortunately, this oversight was not detected during the analysis, consequently leading to results that yielded a questionable z-scores. For the rice sample, comparing the new and old calibration standards, aflatoxin G1 exhibited a difference of more than 28%. This variance explained the systematic deviation observed in the analysis results.

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 17234	Mixed spice	OTA	1	0	0
FAPAS 04482	Maize flour	AFLA B1, OTA, DON, FB1, FB2, FB sum, ZEN	2	5	0
FAPAS 1683	Apple juice	PAT	1	0	0
FAPAS 04474	Infant food	AFLA B1, B2, G1, G2, OTA, DON, ZEN, FB1, FB2, T-2, HT-2	11	0	0
FAPAS 04459	Rice (powder)	AFLA B1, B2, G1, G2, AFLA sum	4	1	0
FAPAS 04474	Infant food	AFLA B1, B2, G1, G2, OTA, DON, ZEN, FB1, FB2, T-2, HT-2	11	0	0
FAPAS 04497	Peanut butter	AFLA B1, B2, G1, G2, AFLA sum	0	0	5
Progetto Trieste VF3607A	Tomato sauce	Alternaria toxins: AOH, AME, TEN, TeA	3	0	0
FAPAS 04478	Milk powder	AFLA M1	1	0	0
Progetto Trieste HC3605 A	Cured ham	OTA	1	0	0
FAPAS 04471	Animal feed	AFLA B1, B2, G1, G2, AFLA sum	5	0	0
FAPAS 04498	Animal feed	AFLA B1, DON, ZEN, OTA, B1, FB2, FB sum	2	0	0
PROOF-ACS P2320-RT	Potatoe	GA: a-Solanine, a-Chaconine	2	0	0
POOF-ACS P2309-RT	Lupin flour and yoghurt	QA: Angustifoline, 13-a-OH lupanine, Lupanine (sum), Multiflorine	8	0	0

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, QA (Quinolizidine Alkaloids) = quinolizidine alkaloids, GA = glycoalkaloids, AOH = Alternariol, AME = Alternariol monomethyl ether, TEN = Tentoxin, TeA = Tenuazonic acid.

10.2 Assistance to official laboratories

10.2.1 Quality control

In addition to the NRL, which also serves as an Official Laboratory (OL), there is another OL in the Netherlands responsible for analysing milk and dairy products for the presence of aflatoxin M1. Quality control measures for this OL in 2023 involved two rounds of testing. Samples of standard solutions, quality control materials, blank milk powder, and milk samples containing aflatoxin M1 were prepared and sent to the OL for comparative measurements. This approach ensures the accuracy and reliability of the laboratory's analyses.

10.2.2 Advise

Following the recent quality control assessment conducted by the Dutch NRL for aflatoxin M1 analysis, feedback was provided to the OL based on the reported results. The results were found to be satisfactory, indicating the effectiveness of the OL's current quality control measures. It is recommended that the OL continues its quality control procedures in their current form, as they have demonstrated reliability and accuracy in their analyses.

10.3 Scientific and technical support to the competent authority

Regular contact is maintained with the competent authorities through bilateral and national meetings, ensuring a collaborative approach to addressing important issues in food safety. An expert meeting focusing on natural contaminants provides a valuable platform for knowledge exchange and collaboration. The meeting was attended by esteemed representatives, including 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). Together, the technical aspects of detecting emerging contaminants and the advances in analytical capabilities in this area were discussed.

10.4 Contacts with other NRLs

The collaboration with other NRLs remains strong, facilitated through engagement in events such as the EURL-NRL workshop, training sessions, and discussions within the working group on analytical quality control. These interactions are important for sharing best practices, aligning methodologies, and advancing our collective knowledge. In addition to these regular engagements, the Dutch NRL actively participated in several symposia and conferences focusing on mycotoxins and plant toxins. In 2023, the events included the 14th conference of the World Mycotoxin Forum in Antwerp, Belgium (9-11 October), the 44th Mycotoxin-Workshop in Celle, Germany (5-7 June), and the 41st International Conference on Environmental & Food Monitoring in Amsterdam (20-24 November). During these meetings, the Dutch NRL contributed significantly by delivering oral presentations and presenting posters, fostering valuable discussions and knowledge exchange with experts from around the world. These meetings have not only provided opportunities to demonstrate Dutch NRL research but have also offered a chance to learn from the latest advancements in the field.

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 for Processing Contaminants (EURL-PC). In 2023, the Dutch NRL participated in the annual workshop of the EURL hosted in two sessions on 19 and 20 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 2023. The first session ended with a discussion concerning the performance criteria for analytical methods. On the second day, the first part of the scientific programme focused on mineral oil hydrocarbons (MOH), a new scientific task within the processing contaminants domain since the beginning of 2023. The session continued with presentations by the representatives of the EURL-PC regarding the analysis of polycyclic aromatic hydrocarbons (PAHs), followed by a session in which the future needs for NRLs were discussed. The workshop ended with a presentation of the proposed PTs for 2024.

- *Update from the Commission.* DG SANTE presented recent and future developments of the EU policy and regulations for processing contaminants, including the new Regulation (EU) 2023/915 replacing Regulation (EC) 1881/2006. In the new Regulation, harmonised terminology has been applied, and footnotes are replaced by comments in an additional comment box in most cases. Discussions on the setting and reviewing of maximum levels (MLs) for 3-monochloropropane-1,2-diol (MCPD) esters and glycidyl esters (GE) are still ongoing. The MLs for 3-MCPD esters in infant formula, follow-up formula and foods for special medical purposes intended for infants and young children and young child formula (powder and liquid) are reviewed; based on recent occurrence data, the MLs should be lowered. Several Rapid Alert System for Food and Feed (RASFF) notifications have indicated very high levels of 3-MCPD esters in certain foods such as biscuits. It seems that the regulation of ingredients in compound foods is not protective enough. As a result, there is some discussion about the need to regulate the presence of 3-MCPD esters and GE in certain foods. Possible MLs complementary to the MLs established by the Commission Regulation (EU) 2022/1322 are considered. These are based on the available data in the European Food Safety Authority (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 those expected, taking into account fat content. MLs are considered for a wide range of foods and are based on the whole weight. The specific food categories have yet to be decided. Also, the food categories for which MLs will be set need to be well described. For foods for which no ML has been set, there should be guidance for the application of Article 3 of Commission Regulation (EU) 2023/915 to ensure, as much as possible, uniform application of Article 3 across the EU and to ensure effective enforcement of legislation. Due to findings of high levels of 3-MCPD esters and GE in mono- and di-glycerides of fatty acids (in food additives E471, E422, E475 and E476), MLs were set for 3-MCPD esters and GE in Commission Regulation (EU) 2023/1329 and 2023/1428 for emulsifiers. 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. It is expected that these discussions will be finalised in the first half of 2024. 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 is no sufficient occurrence data on alkylfurans (methylfurans, 2-ethylfuran and 2-pentylfuran) in certain foods to perform a reliable exposure assessment. It is vital to collect more data from the NRLs; the recommendation should

help the NRLs to set up a reliable method. Next, a short discussion of N-nitrosamines was presented. Currently, there are some knowledge gaps about the presence of N-nitrosamines in specific food categories. EFSA has identified a health concern related to N-nitrosamines being carcinogens. First, more data needs to be collected regarding these N-nitrosamines in food before MLs could be established. Lastly, the Commission presented a new compound class which was included in the EURL-PC scope in 2023: mineral oil hydrocarbons (MOH). In 2023, EFSA published an updated risk assessment regarding MOH. This assessment stated that it is likely to very likely that the present dietary exposure to mineral oil saturated hydrocarbons (MOSH) does not raise concerns for human health. However, it is extremely likely for toddlers and likely for other age groups that present dietary exposure to 3- or more aromatic ring mineral oil aromatic hydrocarbons (MOAH), which are associated with DNA damage and potentially causing cancer, raises a possible concern for human health. Recommendations related to the analysis of MOSH and MOAH are to determine the concentrations, improve the analytical methodology for better characterisation and consistent reporting and to improve research on the sources of hydrocarbons in food. Furthermore, for MOAH, collecting more data on the composition by aromatic ring number, mainly levels of 3- or more ring MOAH present in food, is suggested. In 2021 and 2022, in view of several findings of MOAH in various foods and in view of the fact that the occurrence of MOAH in food is avoidable, the Member States agreed upon a common enforcement approach of concentrations of MOAH above the limit of quantification in formulae for infants and young children. Taking into account the 2023 updated EFSA risk assessment, discussions will now be started with the Member States on the appropriate regulatory follow-up, including a possible update and integration of the abovementioned enforcement approach into EU legislation.

- *Presentations on method development EURL-PC and NRLs.* The EURL-PC opened this session with a presentation concerning insects as a present and future food and ingredient product, that might accumulate and have residue contents and contents of contaminants. Therefore, a properly validated method is essential for the determination of processing contaminants. Next, a representative from a Belgian NRL gave a presentation regarding grouping of foodstuffs for validation, followed by a discussion on how to choose food items for validation within the processing contaminants area. It was not possible to find a uniform solution across the processing contaminant area due to the variability in the relevant processed foods for the different compound classes. This presentation and discussion were followed by a series of presentations by the EURL-PC concerning a systematic discussion of the PAH CEN methods, the determination of PAH in baby food and infant formula and the new wording in Commission Regulation (EU) 2023/915 for PAH in infant formula, revealing a missing clarification between the ML for liquid and powder infant formula. There was discussion about the addition of water to powder infant formula before extraction and extraction solvents, without a clear conclusion, but a recommendation from the EURL-PC to reconstitute or use water in the extraction of powder infant formula. This session was followed by a presentation by a representative of a Swiss NRL about the development of a novel certified reference material for the determination of PAH in whey protein powder, including possibilities to use this within the NRL network. Furthermore, a representative from a German NRL presented their recent work concerning a study (/proficiency test) of processing contaminants in baby food. The study included MCPD esters and GE, acrylamide and furans and benzene, in which homemade baby food was spiked with the contaminants. Most of the laboratories were able to determine the content of the contaminants in the baby food. However, the study revealed different challenges including the production of the material. Finally, the EURL-PC presented acrylamide method considerations as scientific input in relation to the analysis of acrylamide in cacao with recommendations for the use of an appropriate LC column for the separation of acrylamide and impurities.

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- *Discussion of the EURL PTs performed in 2023.* The workshop continued with a discussion on the three PTs that the EURL organised in 2023. The first PT concerned the determination of furan and alkylated furans in salty baked cereal product. Since the analysis of furans is a relatively new subject, not all NRLs participated in this PT. However, compared to last year, more labs handed in results. Overall, the analysis of furan and 2-methylfuran in cereal product showed good results for most laboratories. However, reporting of 3-methylfuran, 2-ethylfuran and 2-pentylfuran was voluntary, and because not many labs reported results, z-scores could not be defined. Also, the EURL-PC pointed out that many labs reported LOQs higher than 5 µg/kg for the individual furans, although in Commission Recommendation (EU) 2022/495 it was stated that the LOQs should not be higher than 5 µg/kg. The EURL-PC is thinking about a way to make sure all labs are able to report all furans with decent LOQs. The second PT concerned the determination of acrylamide in cacao. Overall, the analysis of acrylamide in cacao showed really good results. Out of the 40 participating labs, 34 provided acceptable z-scores and most labs met the criteria for acrylamide performance set by the Commission Regulation (EU) 2017/2158. Lastly, the EURL-PC PT for free and bound 2- and 3-MCPD and GE in 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. However, there seem to be more issues with free forms of MCPDs (more questionable z-scores). Compared to last year's PTs, the percentage of labs reporting acceptable results was higher in 2023. Future PTs need to confirm whether this upward trend will continue.
 - *Performance criteria for analytical methods.* At the end of the first day of the workshop, performance criteria for analytical methods according to Commission Regulation (EC) 333/2007 within the processing contaminants area were discussed. It is suggested to simplify and align the performance criteria for processing contaminants due to the following: (i) the NRL network for processing contaminants has suggested at previous EURL/NRL-PC workshops to align and, where possible, simplify the performance criteria for the different processing contaminants, (ii) the new joint 'Guidance document on the estimation of LOQ for measurements in the field of contaminants in feed and food' has been adopted and (iii) the performance criteria for 3-MCPD, 3-MCPD esters and GE were mainly based in the analytical method from the EURL-PAH, JRC Geel (the method's extraction of fat from infant formula was not successful under the specified extraction conditions) and the analytical method has been further improved by the EURL-PC. The EURL-PC presented possible changes to the performance criteria, which were discussed by the EURL/NRL network. Furthermore, considerations to simplify or align the performance criteria further were discussed. A small working group was appointed to discuss this in the coming year and will provide updates to the rest of the network.
 - *MOH: the newest addition to the scope of the NRLs.* 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. Since the beginning of 2023 MOH is added to the scope of the EURL-PC. During this session, MOH and the current status and activities in the EURL/NRL network were discussed, including a presentation concerning the possible impact of the upcoming EFSA opinion on the methodology of the analysis of MOSH-MOAH. Also, a MOH core working group/task force was announced to start in November 2023 to harmonise the analytical method.
 - *EURL-PC PTs in 2024 and NRLs' future needs.* A discussion session was held on the future needs of NRLs. The outcome was a request 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-19 pandemic were recognised as helpful by many NRLs, although hands-on training is still preferable. Making alkylated furans obligatory in PTs was discussed. At the moment, a z-score often cannot be defined for these compounds due to the low number of participants. However, alkylated furans are part of the latest EFSA opinion on furans, so labs should be able to analyse them. Proposed PTs for 2024 were presented and are expected to include (1) PAH in baby food, (2) MOH in solvent, (3) Furan and alkylated furans (both obligatory) in biscuits, with higher levels of alkylated furans (4) 3-MCPD, 3-MCPD-esters and GE in a baby food, and (5) MOH in rice. Training to determine processing contaminants (for instance MOH) will be offered to the NRLs in 2024. Also, training for quality control procedures for both quantitative and qualitative methods and screening methods will be part of the workshop in 2024.

11.1.2 Participation in working groups

In 2023, the EURL started a working group for the harmonisation of the analysis of MOSH-MOAH, WFSR has become a member of this group. The first meeting was held online on 21 November. The EURL is discussing setting up more of these focused working groups in the near future.

11.1.3 Participation in proficiency and comparative tests

The scope of the EURL and NRL in 2023 included 2- and 3-MCPD esters and GE, acrylamide, (alkylated) furans, MOH and PAHs. The Dutch NRL participated in PTs for all these processing contaminants. In 2023, the NRL participated in three PTs organised by the EURL. In addition, the NRL participated in several other international PTs organised by other organisations, as shown in Table 11.1.

Table 11.1 Overview of proficiency tests and results for NRL Processing contaminants.

PT	Analytes	Matrix	z-scores
EURL-PC 2023-13	Furan and alkylated furans	Salty baked cereal product	Between 0.5 and 0.9
EURL-PC 2023-14	Acrylamide	Cacao	0.2
EURL-PC 2023-15	Free and bound 2- and 3-MCPD and GE	Baby biscuit	Between 0.1 and 1.6
FAPAS 2673	3-MCPD esters	Soy sauce	-0.3
FAPAS 2674	2- and 3-MCPD esters and GE	Vegetable oil	0.6 and 0.7, resp.; 4.4 for GE
FAPAS 2676	2- and 3-MCPD esters and GE	Infant formula	0.6 and 0.4, resp.; 9.7 for GE
FAPAS 30136	Acrylamide	Vegetable chips	0.4
FAPAS 30137	Acrylamide	Infant food (baby biscuits)	0.3
FAPAS 30144	Acrylamide	Coffee (instant)	1.1
Test Veritas RC3508	Acrylamide	Coffee	-0.3
FAPAS 30132	Furan and alkylated furans	Coffee	Between 0.4 and 0.9
FAPAS 30143	Furan and alkylated furans	Baby food	Between 0.0 and 1.4
Proof-ACS P2301	MOSH-MOAH	Edible oils	Between 0.1 and 1.8; 3.4 for total-MOSH
Proof-ACS P2302	MOSH-MOAH	Veggie and non-veggie sausages	Between -0.1 and 1.8
Proof-ACS P2303	MOSH-MOAH by GCxGC-TOF-MS	Coconut oil	All compounds correctly identified
FAPAS 06113X (HPLC-FL method)	PAHs (PAH4: benzo[a]anthracene, chrysene, benzo[b]fluoranthene and benzo[a]pyrene)	Olive oil	Between 0.6 and 0.7; 2.2 for benzo[a]pyrene
FAPAS 06123 (HPLC-FL method)	PAHs (PAH4: benzo[a]anthracene, chrysene, benzo[b]fluoranthene and benzo[a]pyrene)	Palm oil	Between 0.1 and 1.6
FAPAS 06127 (GC-HRMS method)	PAHs (PAH15+1)	Olive oil	Between 0.0 and 0.7

Most of the results listed in the table above were satisfactory (z-scores between -2.0 and 2.0), yet four were above 2.0. The first unsatisfactory result was a z-score of 4.4 for GE in the FAPAS 2674 PT. As stated by FAPAS, the mode was set as the assigned value for GE because the distribution of the results was skewed. Multiple labs received a z-score higher than 2.0, meaning they all overestimated the level of GE. The raw data and initial calculations were checked, and no errors could be found. Extended research was done to figure out why we (as well as all other labs) overestimated GE in certain samples. The sample was re-analysed multiple times, all leading to the same level of GE as reported. The overestimation of GE could be originating from the fact that, in certain samples, the recovery of the internal standard is sometimes very poor. This does not seem to be matrix-dependent but rather very random, and this was not the case in the analysis of this PT sample (internal standard recovery of 82%). WFSR has started a method development project to address this problem and seek improvements to the method. This work is still ongoing.

The second unsatisfactory result was a z-score of 9.7 for GE in the FAPAS PT 2676. As stated by FAPAS, the distribution of all submitted results was bi-modal. The major mode was set as the assigned value. However, as the associated uncertainty was high, the assigned value was given for information only. No further action was taken on this deviation.

The third unsatisfactory result was a z-score of 3.5 for total-MOSH in a sunflower oil sample in the Proof-ACS P2301 PT. The raw data and initial calculations were checked, revealing some interferences in the chromatogram. Re-analysis of the sample and re-integration of the peaks in the chromatogram yielded acceptable results with a z-score of 0.4.

The last unsatisfactory result was a z-score of 2.2 for benzo[a]pyrene in the FAPAS PT 06113X. The raw data and initial calculations were checked, and it seemed that the HPLC was not equilibrated enough before analysis, leading to an unstable measurement and, therefore, a deviation z-score for benzo[a]pyrene. Also, the wrong calibration file was used for the measurement. Re-analysis of the sample and using the correct calibration file yielded an acceptable result with a z-score of 1.5.

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-FL 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.

11.3 Scientific and technical support to the competent authority

In 2023, 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 2023, two EURL-NRL workshops were held: a two-day hybrid workshop at the Bundesinstitut für Risikobewertung (BfR) in Berlin, Germany, on 16 and 17 May and a two-day online workshop on 23 and 24 November. The representative 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 2022, there have been sixteen RASFF notifications under the scope of the EURL POPs, with the exceptions of the PFOS notifications, all in feed.

Notifications of dioxins and dioxin-like compounds or indicator PCBs:

- Palm fatty acid distillate (PFAD) from Malaysia
- Premixtures from Thailand
- Fish meal from Estonia
- Soy protein hydrolysate for feed from India
- Palm fatty acid distillate (PFAD) from Malaysia
- Copper chloride dihydrate from UK organic alfalfa meal pellets from Italy
- Kaolinitic clay from Spain
- Compound feed from France
- Copper chloride dihydrate from Poland
- Palm fatty acid distillate (PFAD) from France
- Animal meal powder from France

Notifications of other POPs:

- Twice: bovine meat from Belgium (PFOS)
- Twice: skim milk concentrate from UK (PFOS)

This is the first year with RASFF notifications pertaining to PFASs. Interestingly, half of the notifications are about products currently not regulated under Commission Regulation (EU) 2023/915 and were made based on the indicative levels stated in the PFAS monitoring Commission Recommendation (EU) 2022/1431. All PFAS notifications were for information and follow-up only, and not considered to be a serious risk.

- Replacement of Regulation (EC) 1881/2006:

After having been amended more than 45 times since its original issue, the EU regulation on contaminants in food has been recast as Commission Regulation (EU) 2023/915 of 25 April 2023, including all amendments. The regulation now features harmonised terminology and an additional comment box to which most of the previously numerous footnotes have been moved.

- Update of review of WHO-TEFs – follow-up:

The WHO had an expert consultation on updating the 2005 toxic equivalency factors for dioxin-like compounds, including some polychlorinated biphenyls. The resulting updated TEF values with scientific justification were published in a special issue of Regulatory Toxicology and Pharmacology (DeVito et al. (2024), *Reg. Toxicol. Pharm.* 146, 105525, <https://doi.org/10.1016/j.yrtph.2023.105525>).

In brief, consequences and follow-up actions by the Commission include:

- Conversion of existing congener-specific occurrence data in the EFSA database into the new TEF values (in a first survey, human milk and fish data showed roughly a 50% reduction of TEQ when re-calculated with the new TEFs).
- Report on occurrence data and human and animal exposure.
- Update of the risk characterisation part of the EFSA 2018 opinion.
- Comprehensive review of EU legislation/of EU MLs, if needed. This review might also include a regrouping of matrices.

This list of tasks is expected to last until at least 2026.

- Monitoring recommendation for PFAS's in feed:

The monitoring recommendation for PFAS's in feedstuffs is still in preparation, though further details have been discussed. The target LOQ of 0.1 µg/kg that is already mentioned in the monitoring recommendation for PFAS's in food (2022/1431) will be integrated, with some indicative (=threshold) values given for at least five groups of feed:

- Fish, other aquatic animals and products derived thereof
- Seaweed meal and feed materials derived from seaweed
- Feed of mineral origin
- Forage, silage, hay and fresh grass
- Compound feed containing fish, other aquatic animals and products derived thereof and/or seaweed meals and feed materials derived from seaweed.

In addition, soil/bedding and water used for the animals will be named as materials potentially of interest for further monitoring. The monitoring is supposed to be executed in the timeframe of 2024-2026.

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's and CPs). Below is a brief update of new or updated publications in 2023.

- Guidance Document on the Analysis of Organobromine Contaminants

An updated main document was released May 2023, including methods for analysis of PBDEs (Annex A), approaches for the analysis of HBCDDs (Annex B) and performance criteria.

<https://eurl-pops.eu/news/guidance-document-bcon-parameters>

- EURL-NRL POPs Priority List of PCN congeners

This document summarises background information and syntheses of supporting information on the outcome of the discussion of the EURL CWG "BCons & PCNs" meeting held on November 22, 2022. The aim was to provide a priority list of PCN congeners to assist laboratories in method development and also to support future assessments with relevant as well as harmonised data. The guidance in this document is intended for laboratories involved in the official control of contaminants in food.

<https://eurl-pops.eu/news/priority-list-pcns>.

12.1.2 Participation in proficiency tests and interlaboratory studies

In 2023, the Dutch NRL POPs participated in three interlaboratory exercises (two PTs and one interlaboratory study) organised by the EURL POPs, one exercise from Quasimeme 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 2023.

Organiser	Code	Matrix	Analytes	z-scores
EURL POPs	2301-MP	Milk powder	PCDD/Fs, PCBs, (bioassay) PFASs, PBDEs, HBCDDs	-0.6 – 1.3 (0.8) -1.4 – -1.2 -0.7 – 0.2 -2.3 – -0.6
EURL POPs	2302-CF	Compound feed	PCDD/Fs, PCBs, (bioassay) PFASs, PBDEs, HBCDDs	-0.6 – 2.6 (5.0) not reported -2.0 – -0.5 -0.2 – 1.8, outlier 25.5
EURL POPs	(no code)	Pork liver	total PFOS, br-PFOS, l-PFOS	not assigned, exploratory study
FAPAS	6126	Dried egg	PCDD/Fs, PCBs	0.0 – 1.9
FAPAS	06122	Fish	PFASs	0.0 – 0.8
Quasimeme	BT-9	mussel, sole/plaice	HBCDDs	not assigned

Only four results reported by the Dutch NRL POPs in the different interlaboratory exercises were evaluated as questionable or not 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, no results were reported and the sample will be taken into account during further method development. HBCDD results showed strong variability, especially for the alpha-conformer. Troubleshooting revealed expired pre-acidified silica columns used for sample clean-up to be the source of this erratic behaviour. The samples were re-analysed using fresh material and were satisfactory. The one questionable dioxin z-score in compound feed was only present in the lower bound calculations. As such it is likely that an error occurred with one of the LOQs reported to EURL. The investigation on the cause is still ongoing.

12.2 Assistance to official laboratories

12.2.1 Quality control

In 2023, ten specifically prepared milk powder samples were sent twice to the OL for comparative testing. Results for the ten samples were overall acceptable. After questionable results close to LOQs due to underestimation in the previous year, a special focus was on unusual spiking patterns and low levels. While underestimation was still present in some cases, overall performance was suitable for routine controls.

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.

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 human norovirus (HuNoV), as a cause of gastroenteritis and hepatitis A virus (HAV) as a cause of hepatitis A, using ISO 15216-1:2017 (quantitative method) or ISO 15216-2:2019 (detection method). 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 food for the presence of HuNoV and HAV RNA under accreditation for over 13 years.

The NRL at WFSR, further referred to as the Dutch NRL, participated in the 6th workshop of the EURL-NRLs for foodborne viruses. The EURL hosted this meeting in Uppsala, Sweden, 14 -15 June 2023. There were 36 participants from 25 countries at the meeting.

The workshop started with a discussion on the determination of the microbiological criterion for HuNoV in oysters. The network agreed with a proposal from the EURL to provide the EU Commission with documents as a basis to make an informed decision. For this, the EURL, in collaboration with NRLs specialised in bivalve molluscan shellfish, is willing to compile current peer-reviewed literature on the risk of norovirus disease from oyster consumption. NRLs were also requested to provide data on norovirus levels in oysters associated with outbreaks (2016-present) for compilation and assessment. Additionally, the EURL agreed to provide a description of the performance criteria for ISO 15216-1, such as the limit of detection (LOD) and quantification (LOQ) for the EU Commission. Participants preferred the determination of microbiological criteria based on separate results for HuNoV genogroup I and genogroup II over total HuNoV in oysters.

During the workshop, the EURL presented the published verification guide on the EURL website to assist NRLs with in-house verification of the ISO 15216 method. The guide consists of protocols to determine LOD95%, linearity, precision, repeatability and LOQ using artificially contaminated samples with HuNoV and HAV. The Dutch NRL and the EURL presented data on comparisons of detection and quantitation of viral RNA by digital RT-PCR and real-time RT-PCR.

In a separate session on the proficiency testing (PT) scheme, it was found that both the PT schemes 22EFV08 (swabs from bell peppers) and 22EFV09 (oysters) had good overall results among the NRLs. It was decided that PT schemes should cover at least bivalves once a year, and that the EURL would investigate the possibility of including bottled water or food surfaces to the scheme (in 2024). Additionally PT distributions for quantitative detections should always include the possibility to report results from digital RT-PCR alongside the mandatory RT-qPCR results.

In the session on method development and harmonisation, the workshop noted the need for certified double-stranded DNA standards, as demanded in ISO 15216-1, to improve the harmonisation of the quantitative test results. The EURL was asked to investigate the possibility to have the double stranded DNA standards commercially available, also to non-NRL laboratories.

Furthermore, there was a series of presentations by various NRLs. One was on validation according to 16140-4 of a molecular method for the detection of tick-borne encephalitis virus in raw milk products (NRL France). Another presentation was on HuNoV levels in oysters in high-risk contamination areas before, during and after the COVID-19 pandemic. It was observed that during the pandemic, the contamination levels in oysters from these areas were very low, but the post-pandemic contamination levels (2022-23) returned to the pre-pandemic contamination levels. Participants were also updated on the progress of the development of the ISO standard for detecting HEV RNA in meat(products) and liver(products) (WG31) and the standard for validating new virus or parasite methods against reference methods (ISO 16140-8) (presented by German NRL and Dutch NRL).

13.1.2 Participation in working groups

On the 13th of June, the Dutch NRL participated in the meeting of the NRL Core group for Next Generation Sequencing (NGS) of foodborne viruses, held as a satellite meeting prior to the NRL workshop. The group consisted of 15 participants from 8 different countries, all representing laboratories with experience in sequencing viruses with NGS. Here, the Dutch NRL presented methods that were developed for targeted sequencing and non-targeted screening of foodborne viruses.

The NRL Core group's long-term goal is to develop harmonised NGS techniques to determine the NoV diversity in oyster samples. For this, reference material for the typing region of the most relevant norovirus genotypes is required. The EURL will investigate the costs and propose which genotypes to cover. A guidance document on how to perform amplicon sequencing of the capsid-region of norovirus will be produced, followed by a course after the guidance document is released.

13.1.3 Participation in proficiency and comparative tests

The Dutch NRL participated in six PTs for the detection of norovirus genogroup I (GI), genogroup II (GII) and hepatitis A virus (HAV) RNA in food (oysters, raspberry) or lenticule samples. Two were organised by the EURL, two by Public Health England (PHE), one by Bipea and one by FAO/Cefas. Eighteen samples were analysed for three target viruses, resulting in 54/54 (100%) correct detection results. Not all PT organisers could give scores for the quantitative results, as reports are still pending.

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

PT (organisation)	Matrix and Date	Number of samples for GI, GII, and HAV	Assessment Detection	Assessment Quantitation
23EFV10 (EURL, SE)	Raspberry 9/23	3	100%	Not done
23EFV11 (EURL, SE)	Oyster 11/23	3	100%	100%
NHV012 (PHE, UK)	Lenticules 3/23	2	100%	100%
NHV013 (PHE, UK)	Lenticules 11/23	2	100%	100%
PT94 (Cefas, UK)	Oyster 7/23	4	100%	100%

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 2023, 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 the 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 for information exchange during the study. The study is published in 2024 (doi.org/10.1016/j.fm.2024.104478).

In the NRL Core group on NGS, the Dutch NRL worked with other NRLs on the draft guidance document.

14 National Reference Laboratory Water content in poultry meat

Coordinator: Erika Silletti

14.1 Activities within the EURL-NRL network

In 2023, this NRL participated in one meeting, organised as every year, together with the other European NRLs and the responsible representatives from Brussels. This year, the meeting was held online, as described in 14.1.1.

14.1.1 Participation in EURL-NRL workshops

Online expert meeting.

There was one online meeting of the Expert Group "Water in Poultry Meat", held on the 6th of March 2023. During this meeting, several matters were discussed, i.e. the evaluation of national control data from the year 2021, the state of the revision of the marketing standard regulation 543/2008 and the planned physiological water content study. In addition, the German NRL showed the results of their study on homogenisation procedure in chicken filets and drumsticks. The Dutch NRL presented the details of the proficiency test organisation aiming to determine the water- and protein content in chicken breast fillets. The Chair highlighted the importance of participating in proficiency tests for maintaining accreditation and for achieving a high level of quality control. Laboratories participating in the study on physiological water content were encouraged to participate in the proficiency test to demonstrate their competence and to support the credibility of the analytical results on physiological water contents.

14.1.2 Participation in proficiency and comparative tests

A proficiency test, organised by the Dutch NRL, was held for moisture and protein content in poultry meat between May and September 2023. This proficiency test followed the one held in 2021, also organised by the Dutch NRL. Twenty-four European laboratories, i.e. NRLs and field laboratories, subscribed to participate in the proficiency test. Each laboratory received two chicken fillet samples, i.e. sample A and sample B. Both samples were prepared according to an in-house grinding procedure. Participants were asked to determine water content according to ISO 1442, and protein content according to ISO 937. The proficiency test was evaluated according to ISO 13528. Most of the participants obtained satisfactory results for both samples. For water content, all laboratories showed satisfactory results for sample A. For sample B instead, out of 24 laboratories, one participant showed questionable results, and one participant showed unsatisfactory results. In the case of the protein content, most of the laboratories showed satisfactory results for both sample A and B. One laboratory showed questionable results for sample A, while three laboratories showed unsatisfactory results. The same trend was observed for protein content of sample B, where three laboratories obtained questionable results and two laboratories obtained unsatisfactory results. Overall, it is concluded that the performance of most NRLs and field laboratories involved in the analysis of protein and water content in chicken fillets was good. For the long-term monitoring of this quality, it is recommended that this PT be repeated once every two years.

14.1.3 Quality control

The Dutch NRL organised one quality control round (February/March 2023) to ensure that the official laboratory (OL), appointed by the competent authority, 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 homogenised chicken drumsticks (n=4). Both OL and NRL analysed water content according to ISO 1442, and protein content according to ISO 937. For water content, the inter-laboratory reproducibility is determined according to ISO 1442. As ISO 937 does not indicate the inter-laboratory reproducibility criteria, this was calculated for protein content according to Horwitz. For all 8 samples, i.e. 4 filets and 4 drumsticks, the absolute difference between the values obtained by the two laboratories remained within inter-laboratory reproducibility for both water and protein content.

14.2 Scientific and technical support to the competent authority

The NRL experts provided regular scientific and technical support to the competent authorities, but no specific incidents or events required their consultation in 2023.

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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