# National Reference Laboratories Wageningen Food Safety Research

Annual report 2021

Y. Hoffmans, M. Alewijn, M.D. Klijnstra, L.W.D. van Raamsdonk, J.J.P. Lasaroms, A.M. Verschoor, T.W. Prins, J.G.J. Mol, G.M.H. Brust, M.M. Sopel, L.L. Leenders, L.J. de Pagter-de Witte, I.L.A. Boxman, E. Silletti, Y. Hogenes

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# Contents

# Preface

Wageningen Food Safety Research (WFSR) has been assigned several reference tasks in the field of safety and quality of food and feed. These reference tasks are executed in the context of WFSR's role as a National Reference Laboratory (NRL) in various fields. NRLs are the link between the European Union Reference Laboratories (EURLs) and Official Laboratories (OLs). NRLs and OLs perform analyses on food and feed in the framework of the official national controls. NRLs are a centre of expertise for the OLs, Ministries, and Competent Authority.

In this annual report, WFSR reports on the execution of its NRL tasks in 2021.

# Summary

National Reference Laboratories (NRLs) are part of the system responsible for controlling and enforcing the EU food and feed law. Wageningen Food Safety Research (WFSR) has been designated the NRL for thirteen subjects. The tasks of an NRL depend on its research fields. This report gives an overview of the activities performed by all of WFSR's NRLs in 2021. These NRLs are for: milk and milk products, marine biotoxins, animal proteins, certain substances and residues thereof as laid down in Regulation (EU) 2017/625, additives for use in animal nutrition (feed additives), genetically modified organisms (GMOs) in food and feed, pesticides, metals and nitrogenous substances in feed and food, mycotoxins and plant toxins in food and feed, processing contaminants halogenated persistent organic pollutants in food and feed, food-borne viruses and water content of poultry.

This report first gives an overview of relevant legislation and information on the networks of EURLs, NRLs and OLs. For every NRL, a description of all activities performed in the EURL-NRL network, such as participation in EURL-NRL workshops, working groups, and proficiency and comparative tests. This is followed by a description of the assistance provided to OLs, such as a quality check or advice. Finally, the scientific and technical support given to the competent authority is discussed. In some cases, contact with other NRLs is discussed.

An important NRL task is to stay updated with current developments within its NRL domain. Every EURL organises one or two meetings (workshops) every year for that purpose. Participation in these EURL-NRL workshops is mandatory. In 2021, because of COVID-19, the workshops were almost all held online. NRLs of WFSR have attended all workshops and actively participated in EURL working groups to improve analytical methods.

To test the analytical capabilities of NRLs, the EURLs organise proficiency tests. As the scope of the EURL proficiency tests is sometimes limited, the NRLs also participated in proficiency tests organised by other organisations if this was considered relevant. The majority of these proficiency tests' results (z-scores) were satisfactory; only a few 'questionable' and a few 'unsatisfactorily' results were reported. Follow-up actions were implemented in those cases. The performance of the OLs was assured by checking the results of their performance in proficiency tests (organised by other laboratories or the NRL) or by sending them assurance samples. Some OLs also received technical support with regard to their analyses.

# 1 Introduction

Coordinator: Yvette Hoffmans

The main aim of food law is to ensure a high level of protection of human health and life and to achieve the free movement of food and feed marketed in the European Union. Whereas food and feed businesses have to comply with the requirements of food law, the competent authorities (CAs) of Member States are to enforce food law and monitor compliance with it. It is the task and the competence of the CAs 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 CAs 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). These subjects are (ordered as the EURLs in Annex VII of Regulation (EC) No 882/2004):

- Milk and milk products*
- Monitoring of marine biotoxins
- Animal proteins in feedingstuffs
- Residues of veterinary medicines and contaminants in food of animal origin (Regulation (EU) 2019/6)
- Additives for use in animal nutrition and national evaluation dossiers
- Genetically modified organisms (GMOs)
- Residues of pesticides
- Metals and nitrogenous compounds in feed and food
- Mycotoxins and plant toxins in feed and food
- Processing contaminants
- Halogenated persistent organic pollutants (POPs) in feed and food
- Foodborne viruses
- Moisture in poultry meat**

* For ‘Milk and milk products’, the EURL was deemed not required anymore as of 1 January 2018. However, the Dutch ministries decided to keep an NRL for ‘Milk and milk products’.

** The NRL for moisture in poultry meat is appointed under the Single Market Regulation.

This report aims to overview activities performed by the NRLs of WFSR in 2021.

## 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. In addition to this regulation, more specific legislation applies to certain parts of the production chain or particular subjects. For instance, additional provisions for the official controls on the water content of poultry, additional provisions have been laid down in Regulation (EC) No 543/2008. 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 CA assigned by each Member State. The Ministry of LNV assigns the Food and Consumer Product Safety Authority (NVWA) as CA for The Netherlands. In its turn, the NVWA is responsible for assigning official laboratories (OLs) to execute the analysis of samples for the purpose of official controls. Besides that, the NVWA is responsible for making the multi-annual national control plan, which includes sample analysis in the different food and feed supply chains.

### 1.1.2 European Union Reference Laboratories (EURLs)

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

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

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

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

* Note: The board of experts is not referred to in Annex VII of Regulation (EC) No 882/2004 but in Article 19 and Annex XII of Regulation (EC) No 543/2008.

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

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

Furthermore (article 94(3)), the EURLs shall publish the list of NRLs designated by 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 feed, milk and poultry meat. A working plan describing the tasks for 2021 was drafted in 2020. In addition, budgets for personnel, and facility and equipment costs have been drawn up. The working plans for 2021 have been positively reviewed by the Client Consultation Board (consisting of employees of the NVWA, the Ministry of LNV and the Ministry of VWS) and have been approved by the Ministry of LNV. Working plans are based on NRL tasks described in Regulation (EU) 2017/625.

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

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

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

In some cases, the NRLs are mentioned in EU legislation. RIKILT (as was the name of WFSR till June 2019) has been cited as the NRL in: Decision 98/536/EC (residues of veterinary medicine and hormones (Directive 96/23/EC) (both no longer in force)), Regulation (EC) No 378/2005 (feed additives), Regulation (EC) No 1981/2006 (GMOs) and Regulation (EC) No 543/2008 (water content of poultry meat).

### 1.1.4 Official Laboratories

According to Article 37 of Regulation (EU) 2017/625, the competent authorities are to designate 'official laboratories' (OLs) authorised to carry out the analyses on samples taken within the context of official controls. CAs 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 1.2*** *List of documents with requirements for methods of analysis used in the official control.*

| Act | For contaminant/residues/products |
|---|---|
| Regulation (EU) 2017/644 | • Dioxins, dioxin-like and non-dioxin-like PCBs |
| SANTE/11312/2021 | • Residues of plant protection products (all matrices) |
| Regulation (EC) 401/2006 | • Mycotoxins in food |
| Regulation (EC) 333/2007 | • Lead, cadmium, mercury, inorganic tin, inorganic As, 3-MCPD, 3-MCPD fatty acid esters, 3-MCPD glycidyl fatty acid esters, PAHs and acrylamide in food |
| Regulation (EU) 2019/627 | • Marine biotoxins (paralytic and amnesic shellfish poison, lipophilic poisons, emerging marine biotoxins)<br>• Some milk parameters (alkaline phosphatase activity, plate and somatic cell count) |
| Decision 2002/657/EC | • Residues of veterinary drugs and hormones |
| Regulation (EC) 641/2004 | • GMO |
| Regulation (EC) 619/2011 | • GMOs in feed (for GMOs with pending authorisation or expired authorisation) |
| Regulation (EC) 543/2008 | • Water content poultry meat |
| Regulation (EC) 152/2009 | • All parameters in feed (a.o. GMO, animal proteins, feed additives, contaminants) |

# 2 National Reference Laboratory milk and milk products

Coordinator: Martin Alewijn

## 2.1 Activities within the EURL-NRL network

### 2.1.1 Participation in EURL-NRL workshops

As of 1 January 2018, the EURL milk and milk products (MMP) was delisted from the list of EURLs in Annex VII of Regulation (EC) No 882/2004. Without a formal EURL, no EURL-NRL workshops were organised in 2021. However, several member states, including the Netherlands, have expressed the intention to keep their NRL MMP active. NRLs of these states share the belief that the current state of (analytical) harmonisation across Europe will gradually be lost without further interaction and cooperation. After an initiative (and financial support) from the Czech NRL MMP, the Dutch NRL joined meetings of a voluntary cooperative network of NRLs MMP in 2019 and 2020. In 2021, no formal meetings were organised, and activity within the network was limited to a few email exchanges on suitable ring trial organisers and information on national regulations on limits specifically for colostrum. There seems to be no initiative to host or organise a formal meeting in the near future.

### 2.1.2 Participation in proficiency and comparative tests

As no EURL-PTs were organised in 2021, the NRL participated in a number of 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 2021.*

| **Organiser** | | | | **Performance[1]** | | | |
|---|---|---|---|---|---|---|---|
| **Country (code)** | **Date** | **Matrix** | **Analyte[2]** | **z-scores** | **S** | **Q** | **U** |
| ALP, CH (21A) | Jan | Stabilised milk | SCC | -0.2 – 1.6 | 4 | | |
| ALP, CH (21B) | May | Stabilised milk | SCC | -0.6 – 0.8 | 4 | | |
| ALP, CH (21C) | Sept | Stabilised milk | SCC | Not yet reported | | | |
| Cecalait, FR | March | Milk | SCC | -3.8 – -0.1 | 3 | 5[a] | 2[a] |
| Cecalait, FR | June | Milk | SCC | -0.7 – 1.5 | 10 | | |
| Cecalait, FR | Sept | Milk | SCC | -1.5 – 0.8 | 10 | | |
| Cecalait, FR | Dec | Milk | SCC | No participation[b] | | | |
| LGC, UK | Jan | Stabilised milk | ALP | 0.0 – 0.3 | 2 | | |
| LGC, UK | May | Stabilised milk | ALP | 0.6 – 1.2 | 2 | | |
| LGC, UK | Aug | Stabilised milk | ALP | 1.2 – 1.5 | 2 | | |
| LGC, UK | Nov | Stabilised milk | ALP | -0.1 – 0.0 | 2 | | |
| Cecalait, FR | Jan | Milk | TF | -0.3 – 1.6 | 10 | | |
| Cecalait, FR | April | Milk | TF | -4.5 – 4.9 | 7 | 1 | 2[c] |
| Cecalait, FR | Sept | Milk | TF | -1.4 – 5.8 | 9 | | 1[d] |
| Cecalait, FR | Dec | Milk | TF | -0.7 – 1.3 | 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.

a) Results in this round were generally too low compared to the Cecalait assigned value. As a result, all microscopes, slide dimensions, and syringes were re-checked and/or recalibrated. After that, the JRC ERM-BD001 SC reference materials were analysed and proved once more to yield results matching the certified values.

b) Due to the long-term illness of trained technicians for somatic cell count, no capacity could be made available to participate in this planned PT.

c) Most probably the result of two samples being exchanged due to a human mistake.

d) Seemingly a single incident, no traceable problem for this particular sample. The other 9 samples in this round 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 2021, the NRL assisted the Official Laboratory (OL) by providing reference results on the reference material for somatic cell count prepared by the OL, which is used to calibrate the routine equipment at the OL. This year, 7 series with 6 raw milk samples each, were analysed independently by two different technicians using the reference SCC method. The NRL re-analysed two series of routine cheese samples from the OL cheeses to compare the alkaline phosphatase levels obtained by NRL and OL. For total flora, it was arranged that the NRL and OL participated in the same series of Cecalait PTs (paragraph above). Thus the results on the same material could be used for interlaboratory comparison.

### 2.2.2 Advice

The NRL advised the competent authority (COKZ (Controle Orgaan KwaliteitsZaken)) and the official laboratory on the process of implementing the reference material on somatic cells (JRC ERM-BD001) into routine analyses. In 2021 the NRL had meetings with the Netherlands Food and Consumer Product Safety Authority (NVWA) on the need for official control on official routine analyses of dairy products. With the new Control Regulation (EU) 2017/625 in force since December 2019, the current scope of NRL quality control on the dairy routine laboratories needed to be reviewed, and possibly expanded. The competent authority (COKZ) has a wide range of analyses performed at its official laboratories, and the NRL assisted in categorising the different analyses and issues to be resolved. The NRL applied for accreditation for a few extra matrix/product combinations as part of this new work. Discussions on the mode for NRL-quality control of the new tasks were undertaken. Still, some decisions on supporting the competent authority in fulfilling their supervisory task towards the official laboratories remain to be taken further in 2022.

# 3 National Reference Laboratory Marine Biotoxins

Coordinator: Mirjam Klijnstra

## 3.1 Activities within the EURL-NRL network

### 3.1.1 Participation in EURL-NRL workshops

The EURL changed staff on 1 January 2022. In 2021, the former EURL staff did not organise a workshop, as was customary in previous years. Instead, the EURL sent a report on their activities to the NRLs. Some highlights of this report are:

- The EURL organised an online workshop on microbiological control. CEFAS (UK) was the EURL in this field, but due to Brexit, microbiological control has been added to the scope of the EURL for marine biotoxins. The EURL directors for *Salmonella*, *E. Coli* and Foodborne viruses and experts from the network were present at the workshop. One of the outcomes of this workshop was that it was agreed that the Community Guide to the Principles of Good Practice for the Microbiological Classification and Monitoring of Bivalve Mollusc Production and Relaying Areas was successfully updated.
- The EURL online supported a few NRLs and other countries in developing analytical methods.
- The EURL participated in the EU expert group meeting on the EFSA opinions on the shucking of scallops; these opinions were presented and discussed. The possible options of managing *Norovirus* in classified production areas were discussed, but this discussion needs to be followed up during the next expert working group. Finally, the commission announced that Echinoderm species besides Holothuroidea (e.g. sea cucumbers) could be collected outside production areas.
- The EURL participated in the EU working group on Official controls of food of animal origin. The Community Guidelines for microbiological control, phytoplankton control, and marine biotoxins were presented in this working group. Also, the member states gave EFSA permission to proceed with uploading Norovirus genetic data to the European Nucleotide Archive. Finally, the results of a study on Paralytic Shellfish Poisoning toxins (PSP toxins) detoxification were presented.
- The EURL organised a technical working group on the applicability of the PSP method. The results of an intercomparison study in which the Dutch NRL participated are in preparation for publication.
- The EURL organised working groups, which finalised the Guides for phytoplankton control, the Guide for microbiological control and the Guide for marine biotoxins.
- The EURL organised proficiency tests on PSP, ASP and lipophilic toxins. The NRL IT indicated that the main challenges for the future are detecting and monitoring emerging toxins, such as TTX, cyclic imines and palytoxins.

### 3.1.2 Participation in a working group

On 8 and 9 November 2021, a working group on the marine biotoxin guide was organised in Porto, Portugal. WFSR participated in this small working group meeting. The chapter on analytical methods, including the quality control and application of measurement uncertainty, was drafted in this meeting. After the meeting, the completed guide on marine biotoxins was sent to the larger electronic working group for comments before submitting the final draft to the commission in late December.
Furthermore, WFSR participated in the EFSA working group to evaluate the shucking of certain species of scallops contaminated with lipophilic toxins. This focuses on producing edible parts that meet the safety requirements foreseen in the Union legislation[1].

The reference method for PSP analysis is very elaborative; therefore, it is difficult to use this method for routine analysis. The EURL designed a screening version of the reference method. The approach of this

[1] https://doi.org/10.2903/j.efsa.2021.6422

screening version was discussed in an online EURL advisory board meeting on 9 February 2021. With this screening method, samples can be analysed faster. The complete reference method should be performed only when the screening method detects toxin concentrations above a certain threshold. This would make the method more suitable for routine analysis. The NRL participated in a small comparative exercise organised by the EURL. Other participants were the NRLs of France, Portugal and Ireland. The results of this study will be published in a scientific journal. This publication is under preparation by the former staff of the EURL.

### 3.1.3 Participation in proficiency and comparative tests

The NRL participated in the EURL proficiency tests (PTs) on ASP, PSP and lipophilic marine toxins in shellfish, and PTs on PSP and TTX in shellfish organised by QUASIMEME.

For ASP, one PT sample was analysed with the EU reference method using HPLC-UV. The result of the sample was satisfactory (z-score < |2|).

2 EURL PT and 6 QUASIMEME PT samples for PSP toxins were analysed with the EU reference HPLC-FLD method (OMA 2005.06). In the EURL PT for the individual toxins, 6 out of the 11 reported values were unsatisfactory (z-score > |2|, Table 3.1). In the QUASIMEME PT, 21 of the 27 reported values yielded unsatisfactory results (Tables 3.2 and 3.3). All unsatisfactory results can be linked to the recovery correction that WFSR applies. Still, most of the laboratories participating in the PT do not apply a true recovery correction within their analysis. The Dutch NRL believes that a recovery correction should be applied and therefore submitted the recovery-corrected results as they would have been reported in official analyses. However, to evaluate the performance of the Dutch NRL in comparison to the other laboratories, the Dutch NRL also calculated the results without recovery correction. Without applying the recovery correction, all but one of the results would have been satisfactory (Tables 3.1, 3.2 and 3.3). This last unsatisfactory result was due to an incorrect peak integration.

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

| | | | | | | Initial results | | Recalculated results | |
|---|---|---|---|---|---|---|---|---|---|
| **Sample** | **Toxin** | **Assigned value** | **σ** | **Ux** | **Unit** | **Value** | **z-score** | **Value** | **z-score** |
| EURLMB/21/P/01 | Total toxicity | 426 | 78 | 52 | µgSTXdiHCleq./kg | 565 | 1.49 | 416 | -0.11 |
| EURLMB/21/P/01 | C1&2 | 90 | 20 | 7 | µgSTXdiHCleq./kg | 152.8 | 3.02 | 102.4 | 0.61 |
| EURLMB/21/P/01 | dcSTX | 139 | 30 | 12 | µgSTXdiHCleq./kg | 225.3 | 2.67 | 171.2 | 0.99 |
| EURLMB/21/P/01 | GTX5 | 94 | 21 | 4 | µgSTXdiHCleq./kg | 158.2 | 3.11 | 113.9 | 0.96 |
| EURLMB/21/P/01 | GTX6 | 21 | 4 | 3 | µgSTXdiHCleq./kg | 29.0 | 1.55 | 29.0 | 1.55 |
| | | | | | | | | | |
| EURLMB/21/P/02 | Total toxicity | 2886 | 393 | 192 | µgSTXdiHCleq./kg | 3782.0 | 2.05 | 2931.0 | 0.10 |
| EURLMB/21/P/02 | dcGTX2&3 | 286 | 55 | 15 | µgSTXdiHCleq./kg | 354.1 | 1.24 | 262.0 | -0.43 |
| EURLMB/21/P/02 | dcSTX | 2304 | 325 | 161 | µgSTXdiHCleq./kg | 2987.4 | 1.88 | 2270.4 | -0.09 |
| EURLMB/21/P/02 | GTX5 | 8 | 2 | 0 | µgSTXdiHCleq./kg | 16.5 | 4.35 | 11.9 | 1.87 |
| EURLMB/21/P/02 | dcNEO | 175 | 36 | 36 | µgSTXdiHCleq./kg | 336.2 | 3.17 | 299.3 | 2.44 |
| EURLMB/21/P/02 | GTX6 | 64 | 14 | 12 | µgSTXdiHCleq./kg | 87.8 | 1.26 | 87.8 | 1.26 |

***Table 3.2*** *z-scores of the Quasimeme round 1 PSP toxin PT with and without recovery correction.*

| | | | | | Initial results | | Recalculated results | |
|---|---|---|---|---|---|---|---|---|
| **Sample** | **Toxin** | **Assigned value** | **Total error** | **Unit** | **Value** | **z-sore** | **Value** | **z-score** |
| QST302BT | dc-STX | 0.61 | 0.133 | µmol/kg | 0.7 | 0.67 | 0.5 | -0.54 |
| QST302BT | GTX-2,3 | 5.3 | 0.85 | µmol/kg | 8.7 | 4.02 | 6.6 | 1.56 |
| QST302BT | STX | 1.2 | 0.21 | µmol/kg | 1.7 | 2.33 | 1.2 | 0.16 |
| QST302BT | Total toxicity | 1780 | 230 | µgSTXdiHCl-eq./kg | 2836 | 4.60 | 2134 | 1.54 |
| | | | | | | | | |
| QST303BT | GTX-2,3 | 6.9 | 1.09 | µmol/kg | 11 | 4.19 | 8.7 | 1.65 |
| QST303BT | STX | 1.8 | 0.3 | µmol/kg | 2.6 | 2.59 | 1.9 | 0.23 |
| QST303BT | Total toxicity | 2222 | 295 | µgSTXdiHCl-eq./kg | 3550 | 4.50 | 2663 | 1.50 |
| | | | | | | | | |
| QST304BT | C-1,2 | 5.0 | 0.73 | µmol/kg | 8.84 | 5.25 | 5.95 | 1.29 |
| QST304BT | dc-GTX-2,3 | 0.74 | 0.177 | µmol/kg | 1.17 | 2.40 | 0.86 | 0.65 |
| QST304BT | dc-STX | 0.80 | 0.166 | µmol/kg | 1.14 | 2.08 | 0.87 | 0.45 |
| QST304BT | GTX-2,3 | 6.1 | 0.94 | µmol/kg | 9.09 | 3.18 | 6.91 | 0.87 |
| QST304BT | GTX-5 | 5.7 | 0.91 | µmol/kg | 8.12 | 2.68 | 5.81 | 0.14 |
| QST304BT | STX | 1.1 | 0.2 | µmol/kg | 1.51 | 2.30 | 1.09 | 0.20 |
| QST304BT | Total toxicity | 2771 | 390 | µgSTXdiHCl-eq./kg | 3822.5 | 2.70 | 2840 | 0.18 |

***Table 3.3*** *z-scores of the Quasimeme round 2 PSP toxin PT with and without recovery correction.*

| | | | | | Initial results | | Recalculated results | |
|---|---|---|---|---|---|---|---|---|
| **Sample** | **Toxin** | **Assigned value** | **Total error** | **Unit** | **Value** | **z-score** | **Value** | **z-score** |
| QST311BT | GTX-2,3 | 3.0 | 0.51 | µmol/kg | 4.0 | 2.01 | 2.8 | -0.34 |
| QST311BT | STX | 1.4 | 0.22 | µmol/kg | 1.6 | 1.30 | 1.1 | -0.98 |
| QST311BT | Total toxicity | 1158 | 154 | µgSTXdiHCl-eq./kg | 1512 | 2.29 | 1056 | -0.66 |
| | | | | | | | | |
| QST312BT | C-1,2 | 3.2 | 0.50 | µmol/kg | 4.44 | 2.48 | 3.0 | -0.35 |
| QST312BT | dc-GTX-2,3 | 0.39 | 0.109 | µmol/kg | 0.6 | 1.94 | 0.38 | -0.08 |
| QST312BT | dc-STX | 0.35 | 0.097 | µmol/kg | 0.52 | 1.77 | 0.35 | 0.02 |
| QST312BT | GTX-2,3 | 0.94 | 0.181 | µmol/kg | 1.32 | 2.11 | 0.93 | -0.04 |
| QST312BT | GTX-5 | 3.9 | 0.57 | µmol/kg | 5.16 | 2.19 | 3.4 | -0.94 |
| QST312BT | STX | 0.34 | 0.093 | µmol/kg | 0.49 | 1.65 | 0.34 | 0.03 |
| QST312BT | Total toxicity | 791 | 111 | µgSTXdiHCl-eq./kg | 1115 | 2.92 | 759 | -0.29 |
| | | | | | | | | |
| QST313BT | GTX-2,3 | 4.5 | 0.70 | µmol/kg | 6.2 | 2.52 | 4.4 | -0.11 |
| QST313BT | STX | 0.65 | 0.134 | µmol/kg | 0.84 | 1.42 | 0.58 | -0.52 |
| QST313BT | Total toxicity | 1156 | 153 | µgSTXdiHCl-eq./kg | 1706 | 3.59 | 1197 | 0.27 |

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

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

## 3.2 Assistance to official laboratories

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

## 3.3 Scientific and technical support to the competent authority

The NRL assisted the risk assessment department of NVWA (NVWA-BuRO) in the risk assessment of cyanobacterial toxins in food. Also, the NRL gave information to NVWA-BuRO to be used as input for a question submitted by Sweden to the EFSA focal point network. In addition, in another project, WFSR performed a study for NVWA-BuRO on the presence of cyanobacterial toxins in Dutch surface waters. The results of this study were reported to NVWA-BuRO in April 2021. Furthermore, the NRL provided NVWA-BuRO with background information on brevetoxins which are discussed in the EFSA Emerging Risks Exchange Network (EREN).

During the annual NRL meeting, the competent authority (NVWA), the Ministry of VWS, the National Institute for Public Health and the Environment (RIVM) and Wageningen Marine Research (WMR) were updated on the developments in the field of marine and cyanobacterial toxins. These developments include method development on ciguatera toxins in fish, a planned survey on palytoxins in shellfish and the possible transfer of cyanobacterial toxins from irrigation water to edible crops.

## 3.4 Contacts with other NRLs

WFSR has become the NRL for Northern Ireland. CEFAS is the NRL for the UK, but due to Brexit, Northern Ireland had to have its own NRL based within the EU. WFSR started as NRL for Northern Ireland at the end of 2021. The Dutch and Northern Irish NRL tasks will be carried out independently unless it is more efficient to combine tasks, for example when participating in proficiency tests. If tasks of both NRLs are combined, WFSR will ensure that this does not lead to conflicts of interest.

# 4 National Reference Laboratory animal proteins

Coordinator: Leo van Raamsdonk

## 4.1 Activities within the EURL-NRL

### 4.1.1 Participation in EURL-NRL workshops

The annual meeting 2021 of the EURL/NRL network was organised virtually in three sessions due to the COVID-19 pandemic. WFSR participated actively in all three sessions.

### 4.1.2 Participation in Working groups

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

### 4.1.3 Participation in proficiency and comparative tests

The EURL organised an implementation study of the double sedimentation for insect detection (Veys and Fumiere, 2021) during the summer of 2021. The test consisted of eight samples. The preliminary feedback revealed that two samples were blanks (a clean ruminant feed and a clean fish feed), five samples were adulterated with insect material at levels of 0.5% or 1.0%, and an eighth sample consisted of material from practice with a low contamination level of insects. Material of black soldier fly (3 samples), yellow mealworm (1 sample) and lesser mealworm had been used for spiking. The Dutch NRL provided good results.

The results of the insect study 2021 were evaluated by the EURL using three different scenarios: (a) no threshold for the particles over six slides, (b) a threshold of 5 particles over six slides, and (c) a threshold of 5 particles over three slides. A higher threshold will result in higher values for specificity, and higher values for the number of slides will increase the sensitivity. In general, approaches which would increase specificity would simultaneously decrease sensitivity and vice versa. These scenarios resulted in three values for each of the eight samples. Best results for the two blanks were achieved at 100% (fish feed) and 88% (ruminant feed) correct results, respectively, after using scenario (c). Full correct results were found for the three samples adulterated with the black soldier fly and near-optimal results for the two samples with the two species of mealworm after applying scenario (a) in all cases. Applying scenario (c) resulted in lower values for sensitivity, down to 89% for the yellow mealworm at 1%. The best result for the low contaminated sample was achieved after scenario (a), with 71% of the participants detecting insects. Since all samples except the challenger showed to be adulterated at levels of 0.5% or higher, no proof was delivered that the double sedimentation could correctly detect insects at the legal limit of 0.1%. It is recommended to explore alternatives for the detection of insects in feed.

The 2021 proficiency test was organised in Autumn. The test consisted of six samples, including a milk replacer based on skimmed milk powder, a salmon meal with 1% pig Processed Animal Protein (PAP), a pig feed with haemoglobin powder, a mixed ruminant blood meal with traces of porcine material, and a porcine plasma powder with 1% collagen. The Dutch NRL reported correct results with one exception; the collagen particles were not found in the plasma powder. In total, 13 of 26 NRLs did not detect the collagen powder, which resulted in a sensitivity of 50% (Veys et al., 2022). This proficiency test was the last for which only the ruminant PCR method was applied. The protocols for pig and poultry detection have been officially published and will be part of the tests starting in 2022.

The microscopic procedure as currently laid down in the legislation has been validated for processed animal proteins in the legal sense, meaning that the primary markers are bone and fish bone fragments, muscle fibres, fish scales, gill bones and similar materials. Spray-dried globules were not included in the validation

scenarios, and their detection depends on the varying level of expertise of the NRLs and other laboratories applying the method. Spray-dried materials can consist of dairy products, blood meal, blood products, hydrolysed proteins or other types of animal by-products. These ingredients have independent positions in the legal treatment. Because of their different analytical background and legal position, specific detection strategies should be explored for effective monitoring.

## 4.2 Assistance to official laboratories

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

## 4.3 Scientific and technical support to the competent authority

Support to the competent authority was provided whenever appropriate. The following issues and subjects have been communicated with the Dutch Ministry of Agriculture, Nature and Food security and the Dutch Competent Authority.

The EURL distributed a first draft of a combined method for PAP detection and insect detection in early 2021 for inclusion in Annex VI of Regulation (EC) 152/2009. There were several issues in this new version of the method, which continue to require attention. A main issue is the full fixation of the embedding agent for making the microscopic slides. In contrast to chemical methods, where the detection unit is part of the analysing equipment, the microscopic technician visually performs the detection based on experience. The embedding agent used will influence the microscopic appearance of the animal particles and should therefore match the technician's experience. In spite of this argument, the less desirable fixation procedure remained part of the current proposal of the microscopic method. In 2021 WFSR evaluated past results of the annual proficiency test for animal proteins of the IAG section Feed Microscopy on the efficiency of different embedding agents. This evaluation revealed that glycerol, Norland adhesive 65, paraffin oil and immersion oil, all preferred by at least some laboratories as embedding agents, provide equally good results. These results are included in the Quality Guidance for visual research, a forthcoming publication of WFSR in cooperation with a panel of European specialists (van Raamsdonk et al., 2022).

Issues for the collaborative study for validation of the insect detection method were included in the NRL Annual Report of WFSR over the year 2019. These issues appeared to be consistent, as indicated by the results of the implementation study 2021. The Competent Authority (NVWA) and the national representative of the Netherlands were updated on the development of the official method when necessary. The combination of the classic single sedimentation and the new double sedimentation needs further evaluation. The intended publication of the new protocol in Annex VI of Regulation (EC) 152/2009 has been postponed until sometime in 2022.

WFSR, as Dutch NRL, has completed a paper on monitoring methods for hydrolysed proteins in cooperation with the NRLs of France and Poland. The paper for reporting the results has been accepted for publication in the peer reviewed journal Food Additives and Contaminants. Hydrolysed proteins, insects, gelatine and blood products are among the types of material which cannot be characterised by the only currently existing microscopic method: the PAP method. Therefore, it is necessary to develop and maintain a set or toolkit of dedicated methods for animal proteins comparable to the existing situation in the chemical domain. Besides issues related to the doubtful validation status of the insect detection method, it is not recommended to develop a "multi-method" aiming at the detection of different types of animal materials, in the view that separate methods will remain to be applied for a range of other types of material, e.g. hydrolysed proteins and blood products.

The EURL method for detection of poultry using PCR is basically targeting the combination chicken/turkey. The genera of geese and ducks, legally part of the definition of poultry, are not included in the scope. WFSR

has developed a method combining these four species and species groups, which could provide a better fundament for monitoring (Scholtens et al., 2019).

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

## 4.4 Contacts with other NRLs

WFSR serves as a scientific officer and board member of the IAG section for Feed Microscopy. The EURL AP and most NRLs are members of this section. During the IAG annual meeting in June every year and, if necessary, during other meetings, exchanging viewpoints and information is stimulated. WFSR, on behalf of the board, organises the annual IAG proficiency test (PT) on animal proteins in feed, of which the report is published annually. This flow of information is regularly discussed in the meetings and complements the information of the EURL/NRL AP network. The IAG PT for animal proteins was again organised in 2021, and the annual meeting was scheduled virtually in September 2021.

The cooperation with the NRLs of France and Poland for developing monitoring methods for hydrolysed proteins as part of a research project in the WOT programme was continued.

## 4.5 References

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

Raamsdonk, L.W.D. van, Genouel C., Weiner A., Prins T.W., Jardy N., Vonsovic S., Barbu I.M., Bescond M., Paprocka I., Kwiatek K., 2022. Development and application of criteria for classification of hydrolysed proteins in the framework of feed safety. Food Additives and Contaminants (accepted).

Scholtens, I.M.J., T.W. Prins, R.J.C.F. Margy, H. Dahlmans, L.W.D. van Raamsdonk, 2019. Applicability of the poultry qPCR method to detect DNA of poultry processed animal protein materials. Food Control, volume 96: 53-58.

Veys, P., Fumière, O., 2021. Implementation study on the detection of insect PAP in feed by double sedimentation method PE/TCE followed by light microscopy detection. European Union Reference Laboratory for Animal Proteins in feedingstuffs, Walloon Agricultural Research Centre, Gembloux, Belgium.

Veys, P., Fumière, O., Marien, A., 2022. Combined microscopy-PCR EURL-AP Proficiency Test 2021. Final version. European Union Reference Laboratory for Animal Proteins in feedingstuffs, Walloon Agricultural Research Centre, Gembloux, Belgium.

# 5 National Reference Laboratory 96/23/EC

Coordinator: Johan Lasaroms

## 5.1 Activities within the EURL-NRL network

### 5.1.1 Participation in EURL-NRL workshops

Due to the ongoing COVID-19 pandemic and the restrictions on travelling abroad, the EURLs decided again that the Workshops in 2021 would be held as online meetings.

The NRL participated in the EURL-Workshop organised by BVL-Berlin, held online on 31 May and 1 June 2021. The Workshop was divided into three sessions, two dealing with ongoing EURL topics and one Special Workshop dealing with the proficiency test (PT) NSAI1120 and consisted only of a theoretical part including various presentations.

Some of the interesting topics of this workshop were:
- News on the substance groups beta-agonists with an interesting presentation about the validation of the analytical method for the detection of 34 different beta-agonists in the matrix lung at low residue levels, which also includes the comparison between different LC-MS/MS systems.
- For the substance group nitroimidazoles, information was given about the method extension with the matrix casing/gut. It could be concluded that the extended method was suitable for confirming all analytes in this substance group.
- An overview was given on the validation of the detection of coccidiostats in poultry liver. This method includes 49 different compounds, not only coccidiostats but also Ionophores, Nitroimidazoles and some insecticides like cyromazine and fluralaner.
- For the substance group NSAIDs an overview was given of the non-compliant results of all species and product categories of the last 15 years. In 2019 diclofenac, meloxicam, metamizole and paracetamol were the most commonly found analytes.

Secondly, the NRL participated in the EURL-Workshop organised by WFSR-Wageningen, held online on 16 and 17 November 2021. This workshop for the control of Growth promoters as Residues in Food from Animal Origin consisted of only a theoretical part, including various presentations. The scope and aims of the workshop were:
- Bringing together NRLs and discussing topics in the field of growth promotors.
- Discuss and give input into new legislation and Guidance Documents.
- Get insight into trends in the field.
- Exchange knowledge between the NRLs.
- Establish and strengthen long-lasting collaboration between the NRLs.

Some interesting topics of this workshop were:
- News on A2 group: Proposal for a strategy on thiouracil, an overview was given of the research for the natural occurrence of 2-thiouracil. Some conclusions of this research were a recommendation for new thresholds for 2-thiouracil in bovine urine (10 µg/L) and porcine urine (30 µg/L).
- The use of the marker 4-thiouracil to determine the natural occurrence of 2-thiouracil and the use of the marker 6-methyl-2-thiouracil to add additional specificity to determine the abuse of 2-thiouracil.

As a follow-up to the research mentioned above, an update was given on the technique LC-Isotope Ratio MS related to the outstanding issues regarding 2-thiouracil. There is a need for further research on this analytical technique and topic, like optimising the clean-up procedure.

Also, during this workshop, insight was given into new direct analytical strategies based on mass spectrometry. An outcome of this research were two successful new development methods:
1. a hand-held diode LDTD-ESI-MS method for the screening of selective androgen receptor modulators and solid samples of powders of illicit drugs and
2. ASAP-MS for the screening of androgen steroid esters in liquid samples such as oily preparations.

Thirdly, the NRL participated in the EURL-Workshop organised by ANSES-Fougeres, held online from 1 December until 3 December 2021. The 'Control of Antimicrobial Residues in Food from Animal Origin' workshop consisted only of a theoretical part, including various presentations.

Some topics of this workshop were:
'Regulatory Issues like News from the Commission; Regulation 2021/808 & State of Play on Technical Guidances.'

Different presentations of new or improved methods for the detection of residues in various matrices; some interesting presentations were:
- Protecting consumer health by developing an electrochemical immunosensor to detect banned antibiotic residues (chloramphenicol) in honey.
- State-of-play on monitoring of fumagillin residues in honey.
- Determination of nitrofurans, including newly identified bound metabolites, by LC-MS/MS.

In the field of high-resolution mass spectrometry (HRMS) and the advances in VMPR multi-residue screening, presentations were given on the following topics:
- Multi antibiotics screening in eggs by IMS-QToF/MS, in combination with a Unispray source.
- Development of a multi-residue method for the screening of antimicrobial and other VMPRs in meat by quadrupole Time of Flight Mass Spectrometer.
- Multi-residue method for screening multi-class VMPRs (more than 150) in meat and milk by Q-Exactive HRMS. Ongoing method performance verification and from the validation to routine application.

Experts from the NRL in the EU Member states and representatives of a candidate or third countries participated in the workshops mentioned above.

### 5.1.2 Participation in proficiency and comparative tests

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

***Table 5.1*** *Overview of proficiency tests.*

| Description | Organising institute | z-score | Assessment |
|---|---|---|---|
| Tetracyclines and macrolides in honey | FAPAS | Between -0.60 and 2.30 | Deviate result *) |
| Chloramphenicol in egg | Progetto | Between 0.28 and 0.32 | Sufficient |
| Tetracyclines in egg | Progetto | z-score is 1.08<br>Concordance 100% | Sufficient |
| Nitrofurans in egg | Progetto | Between -0.12 and -0.01 | Sufficient |
| Tetracyclines and (fluoro)quinolones in bovine milk | Progetto | Between -0.19 and 0.64 | Sufficient |
| Tetracyclines and (fluoro)quinolones in ovine liver | Progetto | Between -1.32 and 0.34<br>Concordance 100% | Sufficient |
| Synthetic steroids in bovine urine | Progetto | Between -1.8 and 1.0 | Sufficient |
| Resorcylic Acid Lactones (RALs) in bovine urine | Progetto | Between -1.59 and 0.97 | Sufficient |
| Stilbenes in porcine urine | Progetto | Between 0.17 and 1.08 | Sufficient |
| Beta-agonists in bovine urine | Progetto | Between -0.96 and +0.23<br>Concordance 100% | Sufficient |
| Corticosteroids in bovine urine | Progetto | Between -0.31 and 1.92 | Sufficient |
| Thyreostats in bovine urine | Progetto | z-score 0.1<br>Concordance 100% | Sufficient |

| Description | Organising institute | z-score | Assessment |
|---|---|---|---|
| Beta-agonists in porcine liver | FAPAS | Between -0.10 and 0.80 | Sufficient |
| Tetracyclines and aminoglycosides in bovine liver | FAPAS | z-score – 4.5<br>Concordance 100% | Deviate result *)<br>Sufficient |
| Nitrofurans in Fish | FAPAS | Between -4.50 and -0.70 | Deviate result *) |
| Tranquilisers in porcine meat | FAPAS | z-score -1.00<br>Concordance 100% | Sufficient |
| Chloramphenicol, florfenicol and thiamphenicol in bovine milk | FAPAS | z-score -0.30<br>Concordance 100% | Sufficient |
| Tetracyclines in bovine and poultry meat | Progetto | Between -0.73 and -0.28 | Sufficient |
| Coccidiostats in egg | Progetto | Between 0.89 and 1.41<br>Concordance 100% | Sufficient |
| Tetracyclines in fish | FAPAS | z-score 0.70<br>Concordance 100% | Sufficient |
| Antibiotics in bovine meat | WFSR | Between 1.17 and 1.34<br>Concordance 100% | Sufficient |
| Vitamin D3 in poultry feed | EURL-JRC | z-score 0.83 | Sufficient |
| Chloramphenicol in turkey meat | EURL-ANSES | z-score -0.14 and 0.59 | Sufficient |
| Sulphonamides and chloramphenicol in honey | Progetto | Between -1.18 and 3.17<br>Concordance 100% | Deviate result *)<br>Sufficient |
| Nitrofurans in honey | Progetto | Between -0.50 and -0.46 | Sufficient |

Cumulative results are presented in Table 5.2 below.

***Table 5.2*** *Cumulative results PTs.*

| Total z-scores | 86 | % of total |
|---|---|---|
| neg z-score | 44 | 51% |
| pos z-score | 42 | 49% |
| z-score <=2 en >=-2 | 82 | 95% |
| z-score <-2 | 2 | 2% |
| z-score > 2 | 2 | 2% |

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

Macrolides in honey; the quantitative result of the compound lincomycin differs from the assigned value.
Corrective action: the confidence interval of the assigned value overlaps the confidence interval of the quantitative result of the applied method. A conclusion was made that the initial result is acceptable.
No further action was necessary.

Tetracyclines and aminoglycosides in bovine urine; Paromomycin was not detected in the PT samples and therefore reported as not detected. The organiser of this proficiency test reported this as a false negative with a quantitative result of 0 µg/kg and a z-score of -4.6.
Corrective action: a check of the raw data was performed, and a peak was detected at the retention time of paromomycin. Only the peak area was far below the peak area of the lowest calibration point. Therefore, it was concluded that paromomycin was not detected in the sample (conform to the reporting rules of the SOP). No further action was necessary.

Nitrofurans in fish; AOZ was reported as 'not detected', and AMOZ was reported as 'detected in the sample'.
Corrective action: after rechecking the results, it became clear that a mistake was made in reporting the results: AOZ and AMOZ were mixed up. In this case, the reporting was not validated by a second person (the procedure).

Sulphonamides and chloramphenicol in honey; the quantitative result of chloramphenicol differs from the assigned value.

Corrective action: after checking the raw data and initial calculations, it seemed that a miscalculation was made. When the correct calculation is applied, the quantitative result of chloramphenicol agrees with the assigned value (the calculated z-score will be 0.12).

## 5.2 Assistance to official laboratories

### 5.2.1 Quality laboratories

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

The NRL task (supervise the analysis of the OLs) is only focused on the analyte/matrix combinations from Group A (prohibited) and Group B (regulated), mentioned in Annex 1 of Directive 96/23/EC. The current control program includes 44 analyte/matrix combinations, and 39 different analytical methods were tested. A monthly evaluation of the outcome of this control program takes place, and an annual trend analysis based on those results is performed and reported separately.

### 5.2.2 Advice

The Q3 meeting (consisting of the NRL, OL and another laboratory) was held twice in 2021, on 12 January and 4 October.

In 2021 the NRL participated in four meetings for the National Plan Residue control working group; these meetings were held on 15 February, 31 May, 6 September and 6 December.

In 2021 the NRL finalised the Factsheet Paracetamol, which was also sent to the Competent Authority (NVWA).

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

Coordinator: Anthony Verschoor

## 6.1 Activities within the EURL – NRL network

### 6.1.1 Participation in EURL-NRL workshops

Wageningen Food Safety Research (WFSR) is the NRL both for Feed Additives (FA) Authorisation (Reg. (EC) No 1831/2003) as well as for Feed Additives Control (Reg. (EC) No 882/2004 and its successor Reg. (EU) 2017/625). WFSR participated in the annual online workshop on 16 and 17 November 2021 by the EURL, JRC-Geel (Belgium). The yearly workshop for the EURLs on Feed Additives was organised as a Webex meeting. It was a combined meeting for both EURLs, the 21$^{st}$ for Authorisation and the 10$^{th}$ for Control.

An overview was given on the EURL-FA Control activities over 2021 by JRC. An interesting detail from this presentation was that WFSR was among the top 6 NRLs (out of 24 NRLs) that reviewed over 50% of the reports received (WFSR: 25/51), even though WFSR never reviews reports on enzymes or bacteria (24/51). Also, the activities of the EURL-FA Control were presented by JRC.

A representative from the European Commission (DG SANTE) gave updates on (revisions of) Regulation (EC) No 1831/2003, which is the regulation on feed additives and, as such, defines all NRL FA activities.

All NRLs discussed the current revision of Regulation (EU) 152/2009 (methods of sampling and analysis for the official control of feed), in which many NRLs are also involved. Furthermore, another DG SANTE representative provided an update on (revision of) Regulation (EU) 152/2009 & Directive 2002/32/EC.

An EFSA representative gave updates on the activities at EFSA in the area of feed additives. Another EFSA representative gave information on the implementation phase of Regulation (EU) 2019/1381 on transparency and sustainability of the EU risk assessment in the food chain ("Transparency Regulation").

Technical presentations were given on Determination of Antioxidants and Related Substances in Animal Feed (DE), Control of feed additives content using a tracer (JRC), EU method for determination of amino acids (FR, JRC), and on Adaptation of the EN 17299:2019 (=HPLC MS/MS method on the determination of the 11 legal coccidiostats) by the Laboratori Agroalimentari (ES).

Finally, challenges encountered in 2021 were discussed by all NRLs, particularly on pitfalls in the analysis of selenium in feed and the determination of vitamin A. Furthermore, the upcoming activities of the EURL-FA Control in 2022 were presented and discussed.

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

In 2021, the NRL commented on 25 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 (antioxidants), coccidiostats, sensory additives (flavouring compounds), zootechnical additives (other zootechnical additives) and nutritional additives (amino acids, their salts and analogues, trace elements and vitamins, provitamins and chemically well-defined substances having a similar effect).

### 6.1.3 Participation in proficiency tests

In 2021, the NRL FA participated in one PT organised by the JRC in May 2021 on vitamin D3 in chicken feed (1 sample). The outcome was satisfactory, with a z-score of 0.83.

## 6.2 Scientific and technical assistance to the competent authority

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

In 2021, 17 national requests for permission to use substances – which are not authorised at Community level – as additives for experiments for scientific purposes (according to Regulation (EC) No 1831/2003, article 3.2) had been assessed regarding the mixing of the additive in feed and possible risks related to cross-contamination to other feeds. The requests concerned, among others, nutritional, sensory and zootechnical additives and coccidiostats.

For these 17 national requests, it was evaluated if they concerned GMOs or additives produced by GMOs. In 7 cases, it was concluded that the applications indeed concerned GMOs or additives produced by GMOs. In those cases, it was evaluated if there were specific concerns related to the safety of humans and animals and if the applicant submitted enough information to assess these aspects. In a limited number of applications, supplementary information was requested. In 2021, no application was rejected due to GMO safety aspects.

### 6.2.2 Other scientific and technical assistance

Assistance was given on request of the contact persons of the Ministry of Agriculture, Nature and Food Quality (LNV) and the Netherlands Food and Consumer Product Safety Authority (NVWA) as support of the Dutch delegation in the Standing Committee on Plants, Animals, Food and Feed of the European Commission (SCoPAFF). Advice was given on the status of certain products concerning the scope of Regulation (EC) No 1831/2003 and feed additives to be withdrawn from the market. Specific advice was given on the status of certain herbs and on the clay minerals attapulgite and chabasite. Furthermore, advice was given on various products such as riboflavin produced by *Bacillus subtilis* KCCM-10445, borderline products such as tracers based on feed additives, and smoke flavourings.

# 7 National Reference Laboratory GM Feed and Food

Coordinator: Theo Prins

## 7.1 Activities within the EURL-NRL network

### 7.1.1 Participation in EURL-NRL workshops

The Dutch NRL participated in the Steering Committee meetings of the European Network of GMO Laboratories (ENGL) in February (web meeting) and June (web meeting). At these meetings, the 17th NRL and the 32nd ENGL Plenary meetings were prepared (18 and 19 November 2021). The 17th NRL workshop consisted of an NRL meeting (half a day).

*NRL meeting.* At the NRL meeting, an update was given on the comparative testing activities in 2021. Fortunately, two proficiency tests (PT) were organised by the EURL in 2021 (Table 7.1). The results of the two PT rounds organised in 2021 were presented and discussed. In the first PT (GMFF-21/01), the EURL GMFF faced difficulties preparing the T1 sample constituted of a rapeseed meal containing oilseed rape event MON88302. Since the material was found to be not homogenous, the performance of the laboratories to identify the GM event was evaluated, but no z-scores were calculated. The T2 sample consisted of mixed seeds powder spiked with soybean event MON87701. Almost all laboratories (95%) obtained z values lower than or equal to two. In the second PT (GMFF-21/02), the EURL GMFF mixed the pate with soybean containing 1.5% (m/m) of GM event MON89788. By optimising both the extraction method and the PCR method, using either ddPCR or hot-start polymerase, results were obtained that confirmed the nominal value of 1.5%. The conclusion is that the validated method is not fit for the purpose of this material and that the method needs to be modified to eliminate the effect of PCR inhibitors. Overall, almost all results reported would be considered acceptable when scored with z-scores; however, it was announced to attribute percentage difference scores instead of performance scores. The T2 sample consisted of T25 maize spiked into non-GM maize powder. Most of the analyses were close to the assigned values, but surprisingly, some participants (15) reported much higher values. The speaker asked whether the laboratories could explain such unexpected results.

The JRC presented the activities of the EURL GMFF. Also, a tour de table was held on NRL issues in 2021. The EURL GMFF has published a report on "NGTs: State of the art review" as part of a more extensive Commission study on New Genomic Techniques (NGTs) and experimentally evaluated, in collaboration with the German NRL, the method for detection of Cibus canola. The latter results will be presented at the ENGL meeting. The JRC summarised the results of a survey on possible training topics previously performed for understanding the NRL's interests. Twenty-eight out of thirty-seven NRLs (76%) from eighteen Member States (MS) responded to the survey. They indicated high interest in subjects such as next-generation sequencing (NGS), bioinformatics, detection of GMM and screening approaches.

*ENGL plenary meeting.* The ENGL plenary meeting was held on one day instead of the usual two days and was held through Webex (online meeting). The meeting was attended by one representative of the Dutch NRL. It consisted mainly of interesting contributions of scientists on topics like (impossibility of) detection of gene-edited plants created with so-called Novel Genomic Techniques (NGTs), using techniques such as ddPCR. There were also presentations on the industrial application of genetically modified microorganisms (GMMs) and on their detection using qPCR methods or whole-genome sequencing.

### 7.1.2 Participation in working groups

The Dutch NRL is chair of the Working Group (WG) DNA. After a slow start in 2021, the WG progress reconvened online again in several sessions, but still no physical meeting was held. Several online conferences were organised. Updates were given to the EURL, and it is the intention to finalise the report of the WG DNAex in 2022.

A physical meeting was foreseen for the WG Minimum Performance Requirements Part 2 (MPR2), but instead, several web meetings were organised in 2021. The Dutch NRL participates in this working group as a subgroup member focusing on detecting genetically modified animals. The WG MPR2 concentrated on the applicability of the guidance set out in the minimum performance requirements document and proposed amendments, when necessary, for digital PCR, detection of gene-edited products, and detection of genetically modified animals. In 2021 a final version of the "Definition of minimum performance requirements Part 2" was prepared. This final version was sent to the steering committee to make it an official ENGL publication.

After the ENGL Kick-off of the WG Genetically Modified Microorganisms in November 2020 (with two representatives of the Dutch NRL), a second meeting of the WG GMM was held in 2021. Contributions were made to a chapter describing GMMs, their host organisms (bacteria, fungi, and yeasts) and selection markers and vectors commonly used. A summary of known GMM products was compiled. Contributions were made to the introduction chapter as well. A first draft of the report, also containing recommendations for detection strategies for GMMs and a description of available tools for detection, was finalised in 2021.

### 7.1.3 Participation in proficiency and comparative tests

Two EURL proficiency tests were organised in 2021. In the first PT(GMFF-21/01), rapeseed meal containing MON88302 soybean and a mixed seed powder containing MON87701 soybean were used as samples (see also 7.1). In the second PT (GMFF-21/02), a feasibility study was performed on the meat pate sample (T1) since it was the first time an animal-based material was used in a PT study. The T2 sample consisted of T25 maize spiked into non-GM maize powder. The Dutch NRL participated in this PT with good results (Table 7.1) except for the GMFF-21/02 T2. This will be investigated further in 2022.

***Table 7.1*** *Overview of PT results GMO detection in 2021.*

| Proficiency test | Analyte | Matrix | z-score |
|---|---|---|---|
| GMFF-21/01 | MON88302 soybean | T1: Rapeseed meal | n.a. |
| GMFF-21/01 | MON87701 soybean | T2: Mixed seed powder | 0.51 |
| GMFF-21/02 | MON89788 | T1: Meat pate | n.a. |
| GMFF-21/02 | T25 maize | T2: Maize flour | -2.02 |

n.a. = not applicable.

## 7.2 Assistance to official laboratories

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

## 7.3 Scientific and technical support to the competent authority

WFSR participated in discussions with the Ministry of Agriculture, Nature and Food Quality (LNV) and the Netherlands Food and Consumer Product Safety Authority (NVWA) to allow animal feed export to the Russian Federation again. Rosselkhoznadzor (The Russian Federal Service for Veterinary and Phytosanitary Supervision) visited the Netherlands to inspect six Netherlands Feed establishments from 14-19 November 2021. WFSR

hosted the meeting on 15 November, where the Dutch authorities gave several presentations on the GMO screening strategy of the Netherlands.

In 2021 a risk-based sampling strategy was used for the Dutch GMO feed monitoring programme. The GMOnitor module, developed by WFSR in 2015 and partially updated in 2018, was used to determine the type of samples for the GMO analyses in feed. This module uses available data on the areas of growth of GMOs that have or have not been approved for the European market to determine the country-crop combinations that are most likely to contain GMOs that are not authorised to enter the EU. This module is used by the Competent Authority and the Human Environment and Transport Inspectorate (ILT) to monitor the presence of non-authorised GMOs in feed.

WFSR was visited as part of a proposed audit of the Netherlands_DG(SANTE) 2022-7397_Fact-finding study/Genetically Modified Organisms, held from 7 to 11 February 2022. In 2021, preparations were made for this fact-finding mission.

## 7.4 Contacts with other NRLs

Contact with other NRLs in the EU occurred during the Steering Committee meetings, the NRL meeting, and the ENGL Plenary Meeting. Since 2017 WFSR is also the NRL GM Food and Feed for Ireland. Ireland finances the NRL activities for Ireland in a separate project. For the 4-year period of 2021-2024, WFSR will continue to be the NRL GM Food.

# 8 National Reference Laboratory Pesticides in food and feed

Coordinator: Hans Mol

## 8.1 Activities within the EURL-NRL network

### 8.1.1 Participation in EURL-NRL workshops

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

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

In 2021, the joint workshop for all four EURLs was held at the EURL-FV in Almeria, Spain, from 20-22 October. Due to COVID-19 restrictions, this was a hybrid workshop. One participant per Member State was allowed to participate in person, and others could join online. WFSR joined both in-person and online.

The purpose of the workshop was to inform the NRL network about relevant matters from the Commission, to exchange technical information (analytical methods, new technologies, issues with certain pesticide/matrix combinations), to present the 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 residues 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 Regulation (EC) No 396/2005 with regard to processing factors (Pfs) and composite food and feed. When assessing compliance with MRLs for processed/composite products, relevant factors included drying, dilution, peeling, pitting, milling, proportions of ingredients and degradation/evaporation during processing. A stepped approach for the use of Pfs was presented. The main points raised during stakeholder consultation included: non-harmonised Pdfs ('hierarchy' of data sources), the definition of processed foods and the use of measurement uncertainty. This will be considered in the next revision of a note dealing with this topic.

- EFSA presented the main results and recommendations from the report on pesticide residues in food as reported by all member states in 2019. Based on the overall data from the EU coordinated program and the national programs, over 96,000 samples were analysed. Residues were reported in 40% of the samples. 27% of the samples contained multiple residues. MRL exceedances occurred in 3.9% of the samples. Several commodity/pesticide combinations were recommended to monitor specifically. Twenty-eight pesticides (including chlorpyrifos, lambda-cyhalothrin, pyraclostrobin, deltamethrin, tebuconazole and acetamiprid) exceeded the ARfD in 170 samples (including apples, lettuce, peaches, tomatoes and spinach).

- Nine technical presentations were given. The Belgian NRL presented the investigation of the transfer of pesticides from tea leaves to tea brew. The EURL-AO shared its experience automatising a modular method (EN 1528) for pesticide residue analysis in food of animal origin. The EURL-FV addressed the automatisation of sample preparation, specifically for complex/dry matrices such as coffee, cocoa, and tea. Another presentation on this topic was given by the EURL-CF, here focussing on micro-SPE and automated preparation of dilutions and calibration standards. The EURL-SRM provided an overview of the ethylene

oxide crisis. The EURL-AO and the EURL-FV discussed a problem in the determination of spinosad, which is due to the fact that not all analytical reference standards have the same composition and that laboratories use different approaches for quantification. The NRL-CF (Italy) shared its experiences with various methods and findings in cereals and feed materials, including insects. The EURL-FV shared the outcome of a comparison of custom-made analytical reference standard solutions. This remains problematic because these are still not always entirely reliable. He also commented on instrumental response differences of specific pesticide isomers. The Dutch NRL presented on updating the scope of pesticide residue analysis in feed, based on a desk study focusing on products from third countries for which local MRLs are higher than EU-MRLs.

- Discussion of EURL-proficiency tests (PTs). In total, six PTs were organised in 2021 by the various EURLs: rapeseed cake (EUPT-CF15) containing 20 pesticides (partially incurred), mainly in the range 0.01-0.10 mg/kg (up to 0.57 mg/kg). Some of the pesticides were only measured by 50% of the participating laboratories. Percentages of questionable or unacceptable results ranged from 5 to 20%. As a measure for the interlaboratory variability, the robust relative standard deviation ($RSD_R$) was used, which varied from 25-38% (average 30%), which was relatively high and also higher than the 25% that is used as a fixed value for expanded measurement uncertainty for enforcement purposes. This indicated that this feed material was a challenging matrix for analysis. In whole egg (EUPT-AO16), 16 pesticides were added, mostly in the 0.01-0.10 mg/kg range. Satisfactory results were obtained by 75-93% of the participants. Also, several pesticides were only measured by part of the laboratories. $RSD_R$s ranged from 9% to 35%, with an average below 25%. In the fruit/vegetable commodities, aubergine was used (EUPT-FV23) spiked with 20 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, except for two pesticides for which determination was voluntary. Satisfactory results were obtained by more than 90% of the participants for most pesticides. $RSD_R$s ranged from 13% to 25% (average 18%). For SRM pesticides, sesame seeds were used as a matrix (EUPT-SRM16), triggered by the ethylene oxide issue. In total, 12 pesticides were spiked, requiring various methods for their determination. The coverage of the pesticide ranged from 23-33% (trimesium, paraquat, ethylene oxide, diquat) up to 88% (glyphosate). Specific attention was given to ethylene oxide. Relatively high variability was observed ($RSD_R$ of 39%, 30% when excluding results from laboratories that used a less suitable extraction solvent). Satisfactory results were obtained by 72%-91% of the participants.

- AQC document. Two 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/12682/2019), moderated by Tuija Pihlstrom. Adjustments to the previous version were modest and included a rewritten section on recovery and bias, a new appendix on options to compensate for method bias, and various minor text modifications. After discussion, the proposed revision was adopted by all NRLs. A new version of the AQC document (SANTE/11312/2021) to be used by 1 January 2022 was issued (but only passed the Standing Committee end of February 2022). https://ec.europa.eu/food/system/files/2022-02/pesticides_mrl_guidelines_wrkdoc_2021-11312.pdf.

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

### 8.1.2 Participation in working groups

WFSR is a member of the advisory group on provision tests organised by the EURLs and the analytical quality control-working group for the bi-annual revision of the AQC document. In 2021, meetings were held and attended on 26-28 January (online), 30 June – 2 July (online), and 19 October (in person, Almeria, Spain). WFSR also participated in the CEN working group on standardising methods for pesticide residue analysis (CEN/TC 275/WG3 Pesticides).

### 8.1.3 Participation in proficiency and comparative tests

The NRL participated in 23 proficiency tests on pesticides in fruits/vegetables, cereals, products of animal origin, feed and other food matrices. An overview is given in Table 8.1. A total of 240 z-scores (indicators for

quantitative performance) were obtained. In general, the performance of the NRL was satisfactory (231 out of 240) and the pesticides were correctly identified and quantified. A questionable result was obtained for two pesticide/matrix combinations (within the statistically expected range). In seven cases, the result was unsatisfactory, triggering a root cause analysis. In five cases, the deviation was due to erroneous reporting (reporting pesticides out of the method's scope, or 0 instead of <LOQ). In one case (DMPF in honey), the variation in the participants' results was very high, and the assigned value chosen by the PT-organiser was considered questionable. Nevertheless, a follow-up was done, but no error in the analysis could be found. Where appropriate, corrective actions were taken.

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

| PT Organiser | | | Performance [1] | | |
|---|---|---|---|---|---|
| **and PT code** | **Matrix** | **Analytes[2]** | **S** | **Q** | **U** |
| EUPT AO16 | whole egg | MRM pesticides | 24 | 0 | 0 |
| EUPT CF15 | rapeseed cake | MRM pesticides | 19 | 0 | 0 |
| EUPT FV-SM13 | aubergine | MRM pesticides, qualitative screening | 100% (18) found | | |
| EUPT FV23 | aubergine | MRM pesticides | 19 | 0 | 0 |
| EUPT-SRM16 | hulled sesame seeds | SRM pesticides (incl. ethylene oxide, glyphosate, glufosinate, trimethylsulfonium, nicotine, matrin, ethephon, paraquat, diquat, chlorate, bromide, phosphoric acid) | 10 | 1 | 1 |
| Fapas 19323 | grapes | MRM pesticides | 13 | 0 | 0 |
| Fapas 09141 | soya bean | MRM pesticides & glyphosate/AMPA | 10 | 0 | 1 |
| Fapas 19306 | melon | MRM pesticides | 23 | 0 | 0 |
| Fapas 19308 | pear | SRM (dithiocarbamates) | 0 | 0 | 1 |
| Fapas 19312 | chili | MRM pesticides | 14 | 0 | 0 |
| Fapas 19314 | Lettuce | MRM pesticides | 13 | 0 | 0 |
| Fapas 19319 | herb (coriander) | MRM pesticides | 13 | 1 | 0 |
| Fapas 19326 | honey | MRM pesticides | 9 | 0 | 1 |
| Fapas 05154 | pork fat | MRM pesticides | 9 | 0 | 2 |
| Fapas 05151 | oily fish | MRM pesticides | 9 | 0 | 1 |
| Fapas 09138 | oats | SRM glyphosate/AMPA, mepiquat | 3 | 0 | 0 |
| Fapas 09142 | feed | MRM pesticides | 5 | 0 | 0 |
| Fapas 12106 | water/3% acetic acid | SRM (formaldehyde) | 1 | 0 | 0 |
| Fapas 09135 | Wheat | SRM (glyphosate/ampa, chlormequat, mepiquat) | 4 | 0 | 0 |
| Fapas 09136 | rice | MRM pesticides | 9 | 0 | 0 |
| Fapas 19304 | honey | MRM pesticides | 9 | 0 | 0 |
| Fapas 19303 | Green Tea | MRM pesticides | 11 | 0 | 0 |
| Test Qual153 | Tomato | SRM (ethefon, fosethyl, glyphosate, phosphonic acid) | 4 | 0 | 0 |

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

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

## 8.2 Assistance to official laboratories

### 8.2.1 Quality control

In the Netherlands, besides WFSR, three laboratories perform official analysis. This assignment is for a particular subdomain in all three cases: one for dairy products, already been acting for many years. The other two laboratories were assigned in 2021, specifically for organic products. For the dairy laboratory, in the frame of a quality control program, one sample of milk powder (a blind sample taken from a previous proficiency test) was sent for the determination of organochlorine pesticides. Results were reported to, and evaluated by the NRL. Feedback by the NRL was provided to the OL. An introduction meeting (online) was held for the two new laboratories. Information on their methods and quality control was exchanged. Both laboratories are already participating in a large number of relevant proficiency tests. Therefore the quality control of these laboratories by the NRL was done by reviewing their planned participation in proficiency tests and evaluating their performance and follow-up actions in case of deviating results.

### 8.2.2 Advice

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

## 8.3 Scientific and technical support to the competent authority

The NRL participates in regular (approx. every six weeks) meetings with the competent authority on official analysis (opdrachtgeversoverleg). Additional meetings are held where needed. Specific topics addressed in 2021 were ethylene oxide, the use of PRIMo to assess potential ARfD exceedances for residues between MRL and 2xMRL, and the use of measurement uncertainty in food industry self-control. The competent authority also asked for and will be supported on technical matters in the meetings of the Standing Committee for Plants, Animals, Food and Feed – Section Phytopharmaceuticals, Pesticide Residues (SC PAFF). For feed, advice was given on adjusting the scope of analysis and options to deal with unexpected residues in feed materials from third countries.

## 8.4 Contacts with other NRLs

Contacts with other NRLs were through the EURL workshop and a CEN meeting on pesticides (both online). WFSR participated in the LAPRW 2021 (Latin American Pesticide Residue Workshop, Panama, May 2021, online event). An oral presentation and a poster presentation were given. Due to COVID-19, other meetings and symposia were cancelled.

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

Coordinator: Hanneke Brust

## 9.1 Activities within the EURL-NRL network

### 9.1.1 Participation in EURL-NRL workshops

The DTU in Denmark hosts the EURL for Metals and Nitrogenous compounds. In 2021, the EURL organised the annual workshop and online training on determining inorganic arsenic in feed and food. The online training, held on 14 December 2021, was not attended by the Dutch NRL. The Dutch NRL has extensive experience with the determination of inorganic arsenic, and the training was intended for NRLs with little or no experience.

The annual EURL workshop was held as a hybrid workshop on 9 and 10 November 2021. The meeting was attended by approximately sixty participants representing NRLs from each EU member state, of which about twenty participants were present in person at the DTU. The remaining participants participated in the workshop online. The Dutch NRL attended the workshop in person.

Proficiency tests (PTs) organised by the EURL and issues associated with these PTs are usually extensively discussed during the workshop. In 2021, the EURL organised three different PTs:

- The first PT concerned the determination of total arsenic, cadmium, lead and inorganic arsenic in rice-based baby food. The network's performance was very good for all four parameters, although the number of laboratories (18) that submitted results for inorganic arsenic was relatively low compared to the other parameters (~35). The Dutch NRL submitted results for all four parameters, and the obtained z-scores were all acceptable.
- The second PT covered the determination of nickel, arsenic, cadmium, mercury and lead in insect meal. Insect meal was chosen as a PT matrix because of its emerging use in feed and food. The performance of the network was good for all parameters. The results of the Dutch NRL for this PT are shown in Table 9.1.
- The third PT was organised by the EURL and concerned the determination of arsenic (total and inorganic), cadmium, lead, copper, nickel, nitrite and nitrate in vegetable-based feed. The discussion focussed on nickel because of a relatively large variation in the results submitted by the different laboratories. The EURL concluded that the network's performance could be improved. Still, during the discussion, it was argued that the homogenisation of the test material may have resulted in nickel contamination from the homogenisation equipment. This argument was supported by the observation of outliers in the homogeneity tests. The Dutch NRL also observed contamination of feed samples with nickel from homogenisation equipment in 2021. Furthermore, the nitrite results could not be evaluated because the stability tests showed that nitrite was not stable in the PT material.

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 2021, maximum limits (MLs) for cadmium and lead were lowered for different food types and new MLs were established. Based on occurrence data collected by EFSA in the past few years, the Commission is considering setting maximum limits for nickel in food and feed and arsenic in different foodstuffs (in addition to rice). New MLs are also drafted for mercury in certain fish species. The Commission plans to publish a recommendation on monitoring inorganic arsenic in feed and methylmercury in fish and fishery products for 2022-2024. Because there is currently no adequate standardised method for determining methylmercury, the Commission has requested CEN TC 275 Working Group 10 to develop a standardised method for the determination of methylmercury. The Dutch

NRL currently has an accredited in-house method for determining methylmercury and is also represented in CEN TC 275 WG 10. For nitrogenous compounds, the current legislation on nitrites in feed was discussed. The Commission discusses the need to keep the current MLs for nitrite and whether new MLs for nitrate should be established.

- *Determination of the moisture content*. In the EURL PT on feed in 2020, a relatively large variation in the results was observed for multiple analytes. Because the results in feed should be expressed on 12% moisture, it was argued that the determination of the moisture content was the reason for the observed variation. The moisture content itself was not a PT parameter. Methods for moisture content determination in feed are laid down in regulation EC 152/2009. As a follow-up, the EURL sent out a survey to all NRLs in 2021. The survey results were discussed, and it was concluded that the moisture content in feed was determined with the same method by most NRLs. In 2021, laboratories were requested to report the moisture content in the feed PT. It was observed that the variation of the moisture content as determined by the different labs was negligible, with only one outlier. EC 152/2009 is currently revised, and the proposed changes were present and discussed.
- *Determination of aluminium.* In 2020 the EURL organised a PT on metals in cocoa powder, including aluminium. A significant variation in the aluminium results was observed. For aluminium, it is known that the digestion parameters are critical. Therefore the EURL sent out a survey to the NRLs and performed a study on the influence of the digestion parameters on the aluminium results in different sample types. The EURL study concluded that the reagents and digestion temperature strongly influenced the measured aluminium content in several matrices. Since the survey showed that the NRLs used a variety of reagents and digestion temperatures, the variation in the PT results was attributed to this. For accurate aluminium determinations, the EURL advised using a high digestion temperature and adding both water and nitric acid to the sample as digestion reagents. The Dutch NRL has an accredited method for determining aluminium in cereal products and herbs. This method uses water and nitric acid as reagents, but according to the advice of the EURL, the digestion temperature of this method should be increased from 210°C to 240°C. The Dutch NRL does not routinely analyse aluminium, but the method will be adapted according to the EURL advice when the determination of aluminium is requested.

### 9.1.2 Participation in working groups

In 2021, 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 (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 calcium in infant formula (FAPAS 18101) and nitrate in vegetable-based feed (EURL-MN PT-2021-03). For calcium in infant formula, the obtained result fitted with the performance characteristics of the method. Reanalysis of the sample yielded acceptable results; hence, no further action was needed. For nitrate in feed, WFSR does not have an accredited method. WFSR has two accredited methods for nitrate, one for nitrate in vegetables and one for sodium nitrate in meat (products). The method for vegetables was used to report the PT results because of the similarity in matrix, but this resulted in an unsatisfactory z-score of +9.56. The sample was later also analysed with the method for sodium nitrate in meat, yielding an acceptable result. Because of the limited amount of PT sample, it was not possible to reanalyse the sample with the method for nitrate in vegetables and to further investigate the unsatisfactory z-score. An apparent reason for the unsatisfactory z-score could not be found.

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

| PT | Analytes | Matrix | z-scores |
|---|---|---|---|
| FAPAS 09136 | Br | Basmati rice | 0.3 |
| FAPAS 10174 | Mn, Zn | Premix (feed) | -0.8 and -0.7, resp. |
| FAPAS 07394 | MeHg | Canned fish | 2.0 |
| FAPAS 07397 | Ni, Cd, I, Sn | Infant formula | Between -0.1 and 0.2 |
| FAPAS 18101 | Na, Mg, P, K, Ca, Mn, Fe, Cu, Zn, Se | Infant formula | Between -0.8 and 2.0; *-2.5 for Ca* |
| FAPAS 07407 | Cu, Cd, Pb | Wine | Between -0.1 and 0.2 |
| FAPAS 07414 | Cd, Pb, Sn | Fruit juice | Between -1.3 and 0.1 |
| FAPAS 07415 | Ni, As, Cd, Hg, Pb, I | Seaweed | Between 0.4 and 1.6 |
| Quasimeme BT1 2020.02 | Ni, As, Cd, Pb, Fe, Cu, Zn, Se, Mo, Mg, V | Mussel tissue | Between -2 and 1.3 |
| Quasimeme BT1 2020.02 | As, Hg, Fe, Zn, Se, Mg | Salmon | Between -0.1 and 0.8 |
| EURL-MN PT-2021-01 | As, Cd, Pb, iAs | Rice-based baby food | Between -0.21 and 0.38 |
| EURL-MN PT-2021-02 | Ni, As, Cd, Hg, Pb | Insect meal | Between -1.16 and 1.71 |
| EURL-MN PT-2021-03 | Ni, Cu, As, Cd, Pb, iAs, nitrate ($NO_3$) | Vegetable-based feed | Between -0.35 and 1.71; *9.56 for $NO_3$* |
| FAPAS 30116 | Melamine, cyanuric acid | Milk powder | -0.1 and 0.0, resp. |
| FAPAS 15154 | Nitrate ($NO_3$) | Lettuce puree | 0.5 |
| FAPAS 15151 | Nitrate (as $NaNO_3$) and nitrite (as $NaNO_2$) | Milk powder | 0.6 and 0.3, resp. |

## 9.2 Assistance to official laboratories

### 9.2.1 Quality control

Analyses for the competent authority (CA) for milk and milk products are carried out by an OL. The quality of the OL assigned by the CA for the analyses of metals and minerals was investigated by the Dutch NRL. 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 NRL reanalysed. The results of the analyses by the OL were discussed with the CA for milk and milk products and reported.

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

### 9.2.2 Advice

Advising the OL for milk and milk products is on an ad-hoc basis and depends on the outcome of the comparison test organised two times a year by the NRL. In 2021 no specific advice was given based on the outcome of the comparison test. On request, one of the OLs for biological products was advised on interpretation of the legislation concerning sample preparation for metal analysis and maximum limits of processed foods.

## 9.3 Scientific and technical support to the competent authority

In 2021, there were several contact moments between WFSR and the competent authority. Specifically, the NRL provided information on the current and future possibilities of arsenic speciation to NVWA and NVWA-BuRO.

## 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

Coordinators: Hans Mol, Marta Sopel

## 10.1 Activities within the EURL-NRL network

### 10.1.1 Participation in EURL-NRL workshops

On 5 and 6 October 2021, a fourth Workshop of the European Union Reference Laboratory (EURL) for mycotoxins & plant toxins in food and feed was organised by WFSR in the Netherlands. Because of COVID-19, the meeting was held in a hybrid format. It was attended in person by the representatives of the Commission, the Netherlands Food and Consumer Product Safety Authority (NVWA) and the researchers from WFSR. The representatives of the NRLs attended online.

The agenda covered three main topic panels: first, an update on EU regulations with related emerging issues and CEN activities on mycotoxins in feed and glucosinolates (GSL). Second, the presentations of results of the proficiency tests (PTs) organised by the EURL and guest presentations of preliminary results of the PT on quinolizidine alkaloids. The last panel focused on method development and research in ochratoxin A, hydrocyanic acid, hydroxyanthracenes, and amendments of the regulation (EC) No 401/2006. The European project HBM4EU was presented by a guest speaker and information on the ongoing work programme for 2021-2022 was introduced. A summary of the topics presented and discussed during the Workshop is given below:

- *European Commission update on legislation and emerging issues, based on the presentation by Frans Verstraete from Directorate-General for Health & Food Safety*.
- *Mycotoxins*. Regulation (EC) No 401/2006 (food) is under revision, and Recommendation 2006/576/EC (feed) is under discussion. Ergot alkaloids and ergot sclerotia in food Commission Regulation (EU) 2021/1399 with ML is applicable from 01/01/2022; maximum levels are lower for ergot sclerotia in unprocessed cereals, except for corn and rice; methods should cover 12 epimers with LOQs for individual epimers at 2 µg/kg for processed cereal-based foods for infants and young children and 4 µg/kg for other cereal-based foods. In feed, it is advised to monitor the presence of ergot alkaloids with the lowered level for ergot sclerotia. Ochratoxin A (OTA) – new EFSA opinion on risk assessment of ochratoxin A in food was published in 2020, and changes in legislation are foreseen as a follow-up. Existing MLs are revised, and limits for foodstuffs which are not yet covered are discussed. It is advised to monitor OTA on cheese and ham and select proper sampling. In feed, current OTA MLs are under discussion. Deoxynivalenol (DON) – current MLs for food and feed are under discussion. In food, the group limit for DON and its modified forms is not set for the time being due to the difficulties related to the analysis of DON-3-glucoside (DON-3-Glc). Modified forms represent on average 10% of 3-Ac-DON, 15% 15-Ac-DON and 20% DON-3-Glc, the ratios reported to EFSA vary considerably. A good correlation is noted between DON and DON-3-Glc, and less consistent between DON and its acetylated forms. T-2 and HT-2 toxin – MLs in feed are under discussion. In food, as a follow-up of the Recommendation 2013/165/EU, tolerable daily intake (TDI) was lowered to 0.02 µg T-2 and HT-2/kg bw. Suggested maximum levels for the sum of T-2 and HT-2 toxins are lower than indicative levels. Modified forms are not considered. Aflatoxins – due to a recent EFSA opinion with regard to the risk assessment of aflatoxins in food, a review/setting of maximum levels for herbs, spices, cocoa, almonds, ices/desserts and gluten is considered. Alternaria toxins – recommendation of monitoring in food, including the setting of indicative levels in certain foods for: alternariol (AOH), alternariol monomethyl ether (AME) and tenuazonic acid (TeA). These are not food safety levels and are proposed for AOH and AME in processed tomato, sesame and sunflower seeds, and sunflower oil at the level of 10-30 µg/kg, and for cereal-based foods for infants and young children at 2 µg/kg. For TeA, an indicative level is also set for paprika powder, tree nuts and dried figs, ranging from 100-10,000 µg/kg. For zearalenon and fumonisins, MLs in feed are under discussion.
- *Plant toxins*. Pyrrolizidine alkaloids (PAs) in food Commission Regulation (EU) 2020/2040 – is applicable from 01/07/2022. MLs refer to the sum of 21 PA's: 10 µg/kg dried product / 0.15 µg/l liquid product. The analytical

requirements are as follows: mean recovery 70-120%, $RSD_r$ and $RSD_{WR}$ ≤ 20%, $RSD_R$: ≤ 25%. Sampling for food supplements: Part M of Annex I to Regulation (EC) 401/2006, for cumin seeds: Part E of Annex I to Regulation (EC) 401/2006. A specific sampling regime is set for tea and herbal infusions and herbs: a sample size of 500 g (smaller amounts for small lots) and incremental samples of 20 g. In feed raw materials, other PAs than in food may be relevant. Tropane alkaloids - Commission Regulation (EU) 2021/1408 is applicable from 01/09/2022. Discussed ML for the sum of atropine and scopolamine are lower bound levels. Provisions on sampling and analysis are under discussion, and the LOQs vary from 1-5 µg/kg, depending on the matrix. Current ML set as 1 g of seeds for Datura sp. should be lowered for horses and pigs. For opium alkaloids - maximum levels in poppy seeds for morphine equivalents (morphine + 0.2 codeine) are considered (1.5 – 20 mg/kg), and sampling and analysis is discussed (LOQ 0.5-2 mg/kg). For the moment, no MLs for thebaine are considered. Tetrahydrocannabinol (THC) - the MLs, referred to as the sum of Δ9-tetrahydrocannabinol (Δ9-THC) and Δ9-tetrahydrocannabinolic acid (Δ9-THCA), are under discussion for hemp seeds, oil and hemp-derived food (3-7.5 mg/kg). The MLs under discussion are between 3-7.5 mg/kg in feed. Cyanogenic glycosides - for hydrocyanic acid (HCN) in food EN 16160 is the preferred method, internally validated for food matrices. Glycoalkaloids - in line with the EFSA opinion, it is recommended to monitor the potato glycoalkaloids, focusing on α-solanine and α-chaconine. Indicative levels are possible for establishment; LC-UV-DAD or LC-MS are recommended analysis methods, with LOQ around 1 mg/kg (not higher than 5 mg/kg). According to the requirements of GMP+: Seeds of Lupinus spp., lupins intended for animal feedingstuffs may contain a maximum of 0.6% alkaloids. However, based on health considerations, the level of quinolizidine alkaloids (QAs) should not be higher than 0.2%. As the transfer from animal feed to food of animal origin can be significant, studies on the transfer are performed in Germany.

- *Control plan*. Regulation (EU) 2017/625 (as of 14/12/2022) repeals the Council Directive 96/23/EC, covering a control plan to monitor residues of pharmacologically active substances, pesticides and contaminants in animals and food of animal origin. However, new regulation refers only to residues of veterinary medicinal products. There are ongoing discussions on the risk-based control plan for contaminants in food of animal origin (for EU production and third countries import) and food other than food of animal origin placed on the market. Contaminants which will be included are those regulated by Regulation (EC) 1881/2006, defined in Council Regulation (EEC) No 315/93, and mercury (also MRL under Regulation (EC) No 396/2005). Criteria for the control plan for EU production are based on: relevant contaminants per commodity group, the selection of establishment, products from specific food business operators, and particular combinations of contaminants/commodities. Criteria for third-country food imports are based on those relevant for EU production and audits. The sampling frequency will be detailed for products of animal origin and general for products of non-animal origin. For food of animal origin equivalent level of control has to be reflected in third country control plans.

- *CEN activities on mycotoxins in feed and glucosinolates (GSL), based on the presentations from the NVWA and WFSR*.
  Within the technical committee TC275, group WG05 on biotoxins in food, current work focused on the method development for ergot alkaloids (EN17425, publication on 2021-04-14), Alternaria toxins in food (EN17521, publication on 2021-08-25), T2-HT2 in food (prEN16923, FV submitted to CCMC on 07/08/2021), and a multimethod for the determination of aflatoxins, deoxynivalenol, fumonisins, ochratoxin A, T-2 toxin, HT-2 toxin and zearalenone by LC-MS/MS (prEN17641, FV to be submitted to CCMC on 10/02/2022). The following methods were planned to be discussed at the next meeting: Alternaria toxins, T2-HT2 and multimethod in foodstuffs. Further developments could be possibly focused on atropine and scopolamine (based on Commission Regulation (EU) 2016/239 and Commission Regulation (EU) 2021/1408 - extension of matrices), hydrocyanic acid (Commission Regulation (EU) 2017/1237), erucic acid (Commission Regulation (EU) No 696/2014), pyrrolizidine alkaloids (Commission Regulation (EU) 2020/2040), quinolizidine alkaloids and glycoalkaloids (EFSA call for data), THC in milk (Recommendation (EU) 2016/2115) and opium alkaloids (Recommendation 2014/662/EU). For natural toxins in feed within the CEN technical working group TC375 WG5, current work in mandate 520 focuses on nine projects: criteria approach document mycotoxin analysis, theobromine LC, and LC-MS methods for ergot alkaloids + tropane alkaloids, free gossypol, pyrrolizidine alkaloids, glucosinolates, multi-mycotoxin method (DON, AB1, FB1&FB2, T2/HT2, ZEN, OTA) and T2/HT2, DON, ZEN. The possible topics for the future mandate are focused on aflatoxins binders, THC in hemp-derived feed materials, scope extension of EN17194 with conjugates and emerging mycotoxins, and the assessment of $RSD_R$s of existing CEN methods. Of lower priority are cyanogenic glucosides, quinolizidine alkaloids (lupin seeds) and Alternaria toxins in feedstuffs.

WFSR presented the preliminary results of the CEN-interlaboratory studies on the determination of glucosinolates from Brassicacea spp using HPLC-MS/MS. A method for the analysis of 21 glucosinolates is based on 70% boiling MeOH extraction, dilution/filtration and quantification using HPLC-MS/MS (C18 column, gradient of water and methanol). In a 2020 pre-trial, 14 participants received feedback on their oil seed and feed material results, and the proposed method was revised.

- *Proficiency tests, based on the presentations given by WFSR and BfR Germany*.
  Germany decided to start a monitoring program on quinolizidine alkaloids (QAs) in food, and thus the harmonised method was tested in a ring trial. Nine QAs in wet and dry matrices were validated with an LOQ of 0.3 mg/kg, range of 0.5-5.5 mg/kg, and the recovery and expanded MU were determined. The preliminary results of the combined ring trial and proficiency test indicated that lupanine and iso-lupanine could not be separated using the proposed method. Also, the quality of the commercial analytical standards did not meet the expectations. The participants' remarks will be included to improve the method, including the extension of the scope and the evaluation of HPLC columns. The results of EURL mycotoxins & plant toxins PT on tropane alkaloids (TAs) in cereal-based food and feed submitted by 38 participants are published in WFSR report 2021.005. Buckwheat flour and maize flour were analysed each for 2 TAs. Most of the laboratories met the LOQ of 0.5 µg/kg, with 77-92% of the satisfactory z-scores achieved for the individual compounds, and 83-92% of the satisfactory z-scores for the sum concentration of the TAs. Four NRLs reported that they did not have a method available. The results from 47 participants on a EURL mycotoxins & plant toxins PT on ergot sclerotia in oats using a visual method are published in WFSR report 2021.002. The participants reported the number and weight of the ergot sclerotia present in each of four quarters of the 2 kg sample; 40 out of the 47 participants reported satisfactory results for the whole sample. Not all the invited NRLs could participate in the PT, since they had no method available because their Competent Authority does not request the analysis. The EURL mycotoxins & plant toxins also organised a PT on multiple mycotoxins in cereal-based food and feed. The preliminary results submitted by 45 participants were presented during the Workshop. Two naturally contaminated samples of oats flour and maize flour were sent out for the studies. In addition, the maize flour was spiked with deoxynivalenol (DON), fumonisin B1 (FB1), fumonisin B2 (FB2), T-2 toxin, HT-2 toxin and zearalenon (ZEN). When possible, z-scores were calculated in one or both samples for: deoxynivalenol (DON), T-2 toxin, HT-2 toxin, zearalenone (ZEN), fumonisin B1 (FB1) and fumonisin B2 (FB2), nivalenol (NIV), 3- acetyldeoxynivalenol (3AcDON), 15-acetyldeoxynivalenol (15AcDON), deoxynivalenol-3-glucoside (D3G), alternariol (AOH), alternariol monomethyl ether (AME), enniatin A (ENNA), enniatin A1 (ENNA1), enniatin B (ENNB) and enniatin B1 (ENNB1). Preliminary results for the performance of the regulated mycotoxins were satisfactory. However, 5 false-negative and 14 false-positive results were reposted. The voluntary mycotoxins in the two samples were quantified only by 25-50% of the participants, complicating the evaluation. In 2022. results will be published in a WFSR report. All NRLs were asked to participate in EURLPT-MP07 on pyrrolizidine alkaloids in two samples, tea and herbs, to assess their performance on these compounds that will be regulated as from July 2022 on.

- *Method development and research projects were presented by WFSR; the HBM4EU European project was presented by INSA Portugal*.
  The EFSA opinion on ochratoxin A (OTA) from 2020 states that OTA is a carcinogenic mutagenic mycotoxin for which the MOE approach should be applied. Furthermore, more information is needed on the route of exposure via cured meat and cheese. Apart from the transfer to tissues from contaminated feed, OTA can contaminate cured meat and cheese from the environment during the ripening and ageing process. In 2022, the EURL will update the method for the analysis of OTA in meat and cheese matrixes, including the subsampling to study the migration into the depth of the products. Hydrocyanic acid (HCN) in feed samples linseed and linseed cake are analysed using the CEN16160:2012 method. The enzyme used for hydrolysis is a β-glucosidase from almonds, with an average HCN detected in linseed cake at the level of 20-30 mg/kg. However, when the β-glucosidase enzyme of Aspergillus niger was used, HCN detected in linseed was about 300 mg/kg and even exceeded the legal limit of 350 mg/kg. The β-glucosidase from almonds cannot completely hydrolyse the CNGs in linseed. The inherent enzymes in linseed are inactivated during processing and can therefore no longer release HCN from linseed cake. The β-glucosidase from A. niger can release HCN from the CNG present in linseed. Hydroxyanthracenes (HADs) present in certain plants and their parts are considered, as published in 2018 EFSA opinion, as compounds increasing the risk of colon cancer linked to the use of laxatives containing HADs particularly based on Aloe preparations. In March 2021, Aloe-emodin, emodin, danthron and all preparations in which these substances are present, as well as the preparations of

leaves of Aloe species that contain HAD, were included in Regulation (EU) 2021/46813, amending Part A (prohibited substances) of Annex III of Regulation (EC) No 1925/2006. In Part C (Substances under Community scrutiny), preparations containing HAD derived from bark, leaves, fruits or root of some Rheum and Rhamnus species or Cassia senna are added. On request of the Commission, the EURL designed an LC-MS/MS based method and performed a pilot study on 23 HADs in ten food supplement samples. The first results showed that HADs could be present in high amounts in some food supplements. In 2022, the method will be further improved and used for a survey. The revision of Regulation (EC) No 401/2006 was discussed within the last two years with the NRLs, and suggestions were submitted in 2020 to the Commission. A draft of a guidance document was sent to the NRLs, and the document itself can be found on the EURL MP website and CIRCABC. The project HBM4EU aimed to "coordinate and advance human biomonitoring in Europe to provide evidence for chemical policymakers" and includes 120 partner organisations from 30 countries. Analytical methods were harmonised, 18 individual, or groups of, substances were determined and the results of the exposure will now be linked to health surveys.

- The EURL Work programme for 2021-2022 focuses on research projects on hydrocyanic acid (HCN) - ISO16160:2012 enzymes & processed linseed, Alternaria toxins - lowered LOQ tree nuts, quinolizidine alkaloids - inventory methods & EURLMP method, distribution of co-harvested plant parts in laboratory samples, OTA in cured meat and cheese including subsampling and method development and validation for hydroxyanthracenes in food. Four PTs were planned: EURLPT-MP06 on multi-method regulated mycotoxins (reporting ongoing), EURLPT-MP07 on pyrrolizidine alkaloids: tea and herbs, EURLPT-MP08 on ergot alkaloids in cereals and EURLPT-MP09 on opium alkaloids in poppy seed and bakery products.

### 10.1.2 Participation in working groups

As part of the EURL task, WFSR has initiated a working group on analytical quality control (AQC) in which NRL representatives participate. Discussions were online by commenting on various revisions of a guidance document drafted by the EURL.

### 10.1.3 Participation in proficiency and comparative tests

In 2021, the NRL participated in 13 PTs for mycotoxins and 1 PT for pyrrolizidine alkaloids, of which the overview is presented in Table 10.1. For the 86 toxin/matrix combinations, 83 z-scores were obtained. The assigned values were below the methods reporting limits for the remaining three: Ochratoxin A in roasted coffee, HT-2 toxin in pasta, and ergometrinine in rye flour. Seven questionable results were obtained for Aflatoxin B1, B2 and total Aflatoxins in peanuts, Aflatoxin B1 in sesame pasta, T-2 toxin in pasta, and Ergocornine in pasta rye flower and Deoxynivalenol in maize flour. All deviations were analysed using root cause analysis. This did not always lead to a clear explanation of the cause of the deviation. In some cases, no certified calibration standards are available for specific mycotoxins, leading to a *z*-score between |2| and |3|. However, the results are always within the 50% measuring uncertainty applied by the NVWA.

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

| PT Organiser and PT code | Matrix | Analytes[2)] | Performance [1)] | | |
|---|---|---|---|---|---|
| | | | S | Q | U |
| FAPAS 1677 | Apple juice | Patuline | 1 | 0 | 0 |
| FAPAS 4407 | Black pepper | Aflatoxins, OTA | 6 | 0 | 0 |
| FAPAS 4408 | Peanut | Aflatoxins | 2 | 3 | 0 |
| FAPAS 4410 | Animal feed | Aflatoxins | 5 | 0 | 0 |
| FAPAS 4411 | Maize flour | Aflatoxin B1, OTA, FB, DON, ZEN, T2, HT-2 | 9 | 1 | 0 |
| FAPAS 4412 | Baby Food | Aflatoxins, OTA, DON | 4 | 0 | 0 |
| FAPAS 4418 | Maize | Aflatoxin B1, OTA, FB, DON, ZEN, T2, HT-2 | 10 | 0 | 0 |
| FAPAS 4420 | Sesam pasta | Aflatoxins, OTA | 5 | 1 | 0 |
| FAPAS 4427 | Ginger | Aflatoxins, OTA | 6 | 0 | 0 |
| FAPAS 4430 | Animal feed | Aflatoxin B1, OTA, ZEN, DON, FB | 7 | 0 | 0 |
| FAPAS 17219 C | Roasted coffee | OTA | 0 | 0 | 0 |
| FAPAS 22180 | Rye flour | Ergot alkaloids | 7 | 1 | 0 |
| FAPAS 22181 | Pasta | DON, ZEN, T-2, HT-2 | 2 | 1 | 0 |
| PROOF-ACS | Cumin | PA | 12 | 0 | 0 |

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

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

## 10.2 Assistance to official laboratories

### 10.2.1 Quality control

Besides the NRL, which is also an Official Laboratory (OL), there is another OL in The Netherlands, which conducts the analysis of milk and dairy products for the presence of Aflatoxin M1. The quality control of this laboratory was done by sending standard solutions, PT material and milk samples containing Aflatoxin M1 in two rounds, on 1 June (whole milk) and on 5 October (powdered milk). The results were reported to the Dutch NRL, evaluated, and feedback was provided to the OL. In past years, a trend was observed, where OL reported on average 80% recoveries compared to WFSR. In October, this trend was not observed. For spiked samples, OL reported 97-102% of the recoveries of WFSR (99% on average), and for standards solutions the reported recoveries were at 98 and 99% to the prepared concentrations.

### 10.2.2 Advise

Based on the reported results, the NRL provided feedback to the OL. The results were satisfactory, and OL's quality control should be continued in its current form. It was noted that attention should be paid to preventing any disrupting events.

## 10.3 Scientific and technical support to the competent authority

Contacts are kept regularly through bilateral and national meetings. In the working group meetings of Experts within the agricultural contaminants, competent authority, WFSR, Ministry of Health (VWS), and the National Institute for Public Health and the Environment (RIVM) participate. Technical aspects of detecting emerging contaminants regarding statutory enforcement were discussed. Insight into the past and upcoming activities was given, focusing on the presence of Ochratoxin A in cured meat and cheese.

## 10.4 Contacts with other NRLs

Contacts with other NRLs were through the EURL-NRL workshop, CEN meetings on mycotoxins and plant toxins in food and feed and discussions of working groups on analytical quality control. Due to COVID-19, these meetings and discussions were held in hybrid versions or online.

# 11 National Reference Laboratory for Processing Contaminants

Coordinator: Liz Leenders

## 11.1 Activities within the EURL-NRL network

### 11.1.1 Participation in EURL-NRL workshops

The DTU in Denmark hosts the European Reference Laboratory for Processing Contaminants (EURL-PC). In 2021, the Dutch National Reference Laboratory (NRL) participated in the annual workshop of the EURL hosted in two hybrid sessions on 29 and 30 September. Over 50 participants attended the event, representing the NRLs, the Directorate General Health and Food Safety (DG SANTE) and staff from the EURL-PC. Representatives of six NRLs attended on-site, and all others attended online. All delegates of the NRLs and the Directorate General were welcomed at the meeting by the EURL Director. The first part of the workshop focused on furan and alkylated furans. Representatives of a couple of the NRLs shared some interesting presentations regarding the analysis of furan and alkylated furans in various food products. The session continued with an update from the Commission, some presentations from representatives of the EURL-PC regarding their recent method development and discussions about the results of the PT organised in 2021. The first session ended with a discussion concerning LOQ determination in the analysis of processing contaminants. The second session comprised presentations about various techniques for the analysis of processing contaminants, followed by a quality control training.

- *Analysis of furan and alkylated furans in a variety of food products*. The workshop started with several presentations from representatives of the NRLs regarding the analysis of furan and alkylated furans in a variety of food products. CART, Belgium, presented information on the optimisation and validation of an SPME-GC-MS method to analyse furan and alkylated furans in various matrices and showed fit-for-purpose validation results. BLV, Germany, presented new findings on the analysis of furan and alkylated furans using grinding balls in the analytical procedure and the implementation of this method in the production of PT and reference material. Finally, the Greek NRL completed the furan part with a presentation on furans in coffee samples. It discussed the occurrence, type dependence, concentrations in actual coffee in a cup, and analytical facts, including a comparison of six different coffee preparation approaches.
- *Update from the Commission*. DG SANTE presented recent and future developments of the EU policy and regulations for processing contaminants. Discussions on the setting of maximum levels (MLs) for 3-monochloroprane-1,2-diol (MCPD) esters and glycidyl esters (GE) are still ongoing. Possible MLs are taken into consideration and are based on the available data in the EFSA database and other factors such as specific vegetable oil content. In certain cases, significant divergences are observed among levels found in food and expected levels, taking the fat content into account. Two approaches are under consideration: (I) no specific ML (except for baby food and cereal-based foods for infants and young children), but specific provision to make Article 2 of Regulation (EC) 1881/2006 applicable in terms of mentioning the specific vegetable origin of oils and fats; and (II) specific MLs for certain compound foods such as margarine, cereal-based products, baby food, biscuits, potato chips and others. 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. Existing benchmark levels for several food products are considered to be lowered, and a list of suggested MLs for these food products is shared. Suggested benchmarks for new food products such as rösti, vegetable fries and cocoa powder are also presented. Furthermore, the work in relation to an adaptation of the recommendation for analysis of furan and alkylated furans was presented. Member States and food business operators should use this recommendation for the analysis of furans and alkylfurans. EFSA has identified a health concern related to furans and methylfurans (2-methylfuran, 3-methylfuran and 2,5-dimethylfuran) in food. Still, it is acknowledged that there are no sufficient occurrence data on methylfurans in certain foods to perform a reliable exposure assessment. It is vital that more data are collected from the NRLs; the recommendation could help the NRLs to set up a reliable

method. Lastly, the Commission has asked EFSA for a scientific opinion on the risks for human health related to the presence of N-nitrosamines in food, with a deadline of 30 June 2022.

- *Presentations on method development EURL-PC*. A presentation followed the series of presentations by the EURL-PC concerning 3-MCPD esters and GE in E471. The analytical challenges during method development and the validation of the method were discussed, followed by an overview of the presence of these contaminants in the food additive E471. Some work performed by the EURL-PC on the determination of ethyl carbamate in beer and the effects of beer storage on the levels of ethyl carbamate were shared. It was stated that beer storage and higher temperatures of beer lead to higher amounts of ethyl carbamate.
- *Discussion of the EURL PTs performed in 2021*. The workshop continued with a discussion on the two PTs that the EURL organised in 2021. The first PT concerned the determination of furan and alkylated furans in cereal products. Since the analysis of furans is a relatively new subject, not all NRLs participated in this PT. Overall, the analysis of furans in cereal products showed good results for most laboratories. The low content of 3-methylfuran in the sample material resulted in a broad range of levels reported and thus a very high uncertainty of the assigned values, although outlying results were compensated for. In the draft report, z-scores were determined for 3-methylfuran, but conclusions regarding the performance of the participating laboratories were considered unreliable, and 3-methylfuran was excluded from further statistical assessment in the final report. The second PT concerned the determination of polycyclic aromatic hydrocarbons (PAHs) in plant material. The analysis of PAHs in plant material overall showed excellent results. Most laboratories only reported PAH4 and the sum, but there was still sufficient data to calculate z-scores for the other PAHs. Lastly, the EURL-PC discussed the future PTs and other activities within the network for 2022, which included PTs of acrylamide in biscuits or rusks for infants or young children, as MLs of acrylamide in biscuits and rusks for infants and young children are expected, a PT for 3-MCPD, 3-MCPD esters and GE in a relevant matrix (e.g. infant formula, potato crisps or cookies) and finally a PT in furan and alkylated furans in crisps or bakery goods. Training to determine 3-MCPD and 3-MCPD esters, the analysis of furans, alkylated furans, and acrylamide will be offered to the NRLs in 2022.
- *Discussion on the guidance document for the estimation of LOD/LOQ*. At the end of the first day of the workshop, a draft paper for estimation of the LOQ was presented and discussed. This document was written by the joint EURL for contaminants, consisting of the EURL-PC, the EURL for Persistent Organic Pollutants (POPs), the EURL for Metals and Nitrogenous compounds (MN) and the EURL for Mycotoxins and Plant toxins (MP). The work started in 2019 and was already discussed in the EURL-PC workshop in 2020. Before this workshop, the draft paper was sent out to all participants. There were no objections against the new lowest validated level approach for the LOQ determination from the NRLs, however questions were raised concerning the estimation of the LOQ for matrices with a 'natural' high level of contaminants (for example, acrylamide or furan in roasted coffee), and the estimation of a 'sum-LOQ' in relation to PAH4 was discussed.
- *Analysis of processing contaminants*. The second day of the workshop started with a presentation from Kit Granby of the EURL-PC concerning acrylamide in baby food and the exposure from these products to infants. Data showed that levels of acrylamide in baby food are low, which makes it a complex matrix to analyse. Next, Dimitrios Chrysafidis from the Greek NRL presented the findings of acrylamide levels in canned table olives from the Greek market. The different steps and processes in the production of these olives were investigated, but no correlation between sugar and protein content and the formation of acrylamide was found. The sterilisation step is not necessarily the step where acrylamide is formed; acrylamide is already formed after the immersion in the lye solution and the oxidation. The last presentation on acrylamide was from Sciensano in Belgium and focussed on data for monitoring acrylamide in Belgium, including levels of acrylamide compared to cooking habits. The Croatian NRL gave a presentation in which PAH levels in traditional Croatian meat products were discussed. This included dry-cured hams produced at four different processing methods with differences in primary leg treatment, salting and smoking. The study showed that processed foodstuffs were more severely contaminated with PAHs than food contaminated from environmental sources. Findings in the study pointed out that lower levels of PAHs could be obtained if the smoking of the meat is carried out under controlled conditions and if good manufacturing practice was applied. This overview was followed by the EURL-PC, presenting PAH solvent extraction efficiency tests using four different solvent solutions. Challenges with extracting PAHs by different solvents seem matrix-dependent, and no clear efficiency trend for any of the four solvents used was shown. The session ended with a presentation by the EURL-PC regarding the potential health concerns of contaminants in caramel colours, including 4-methylimidazole (4-MI), 2-acetyl-4-tetrahydroxy-

butylimidazole (THI), hydroxymethylfurfural (HMF) and furan, and an explanation of the analysis of these contaminants at the EURL-PC.
- *Training Quality Assurance of analytical methods*. To produce reliable results, laboratories should have a Quality Assurance (QA) programme. The EURL-PC gave an overview of different factors influencing the analytical quality. These factors included the proficiency of personnel, the quality of reagents and chemicals used throughout the analytical procedure, and the quality and maintenance rate of the used equipment and instruments, including functional checks. The performance criteria for selected contaminants in foodstuffs in relation to the Commission Regulation 333/2007 were discussed, and the use of control charts was presented and discussed using different examples. At the end of the training, the calibration of samples and the criteria for identifying compounds were discussed.

### 11.1.2 Participation in working groups

There were no working groups on EURL-NRL issues related to processing contaminants in food in 2021 to participate in. The EURL is discussing the installation of such focussed working groups in the future.

### 11.1.3 Participation in proficiency and comparative tests

The scope of the EURL and NRL includes PAHs, 2- and 3-MCPD esters and GE esters, acrylamide and furan/methylfurans. The Dutch NRL participated in PTs for all of these processing contaminants. In 2021, the NRL participated in two 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. The EURL planned to organise three PTs but had some trouble preparing the PT material for the EURL PT 2021-08 (free and bound 3-MCPD in liquid infant formula). The EURL cancelled this PT.

***Table 11.1*** *Overview of proficiency tests for NRL processing contaminants.*

| PT | Analytes | Matrix | z-scores |
|---|---|---|---|
| EURL 2021-06 | Furan and alkylated furans | Cereal product | Between -2.0 and -1.8 |
| EURL 2021-07 | PAH | Plant material | Between 0.8 and 3.0 |
| FAPAS 2664 | 2- and 3-MCPD esters and glycidyl esters | Vegetable oil | Between -2.7 and 4.0 |
| FAPAS 2665 | 2- and 3-MCPD esters and glycidyl esters | Potato Crisps | Between 0.8 and 0.9 |
| FAPAS 2666 | 2- and 3-MCPD esters and glycidyl esters | Infant formula | Between -1.2 and 1.8 |
| FAPAS 30108 | Furan and alkylated furans | Coffee | Between -0.5 and 4.7 |
| FAPAS 30111 | Acrylamide | Vegetable Crisps | -0.3 |
| FAPAS 30111X | Acrylamide | Vegetable Crisps | -1.3 |
| FAPAS 30117 | Acrylamide | Coffee | 0.5 |
| Testveritas B1507 | Acrylamide | Bread | 0.1 |

Most of the results mentioned in the table above were satisfactory (z-scores between -2.0 and 2.0), yet four results were either above 2.0 or below -2.0. The first unsatisfactory result was a z-score of 4.7 for 2-ethylfuran in the FAPAS PT 30108. However, FAPAS stated that the analyte level was low, and for some laboratories, this level was on or below their limit of quantification. Eleven laboratories reported values, and the distribution of these values was bimodal. The major mode in the kernel density plot could be seen as the most appropriate measure of consensus, but this parameter had high associated uncertainty. In such a case, FAPAS would not normally set an assigned value. However, because this was the first time 2-ethylfuran had been offered in a FAPAS PT, the major mode has been set as the assigned value to enable the calculation of z-scores for information only. No further action was taken on this deviation.

The following two unsatisfactory results were a z-score of 4.0 for glycidyl esters and a z-score of -2.7 for 2-MCPD esters in the FAPAS PT 2664. While evaluating the PT results, it was noticed that the values of 2-MCPD esters and glycidyl esters were switched during the data input. Recalculating the z-scores would have led to a z-score of 1.1 for 2-MCPD esters and -1.1 for glycidyl esters. As it was a human error, no further action was taken regarding this deviation.

The last unsatisfactory result was a z-score of 3.0 for benzo(k)fluoranthene in the EURL PT 2021-07. This sample was measured in a series in which the peak shape was not good due to a defect in the heater of the transfer line of the Autospec GC-HRMS machine. This heater could not be replaced before the deadline of the PT. Although the data did not look optimal, the results obtained using the broken heater were reported to lead to an unsatisfactory result for one of the PAHs. After replacing the defective heater, the sample was analysed again, and the problem did not appear again in samples measured after the replacement.

## 11.2 Assistance to official laboratories

### 11.2.1 Quality control

The laboratories of the OL and the NRL merged in 2019. Before the merger, the two laboratories used different analysis techniques to analyse PAHs (HPLC-fluorescence vs GC-HRMS). Quality assurance was established by confirming PAH concentrations in several samples analysed by the former OL.

### 11.2.2 Advice

Since the merger of the OL and the NRL laboratories, advice on analytical measurements, quality, and measurement strategies is given regularly.

## 11.3 Scientific and technical support to the competent authority

In 2021, there were several contact moments between the NRL and the ministries of Agriculture, Nature and Food Quality (LNV), Health, Welfare and Sport (VWS), NVWA and RIVM concerning processing contaminants in food.

## 11.4 Contacts with other NRLs

During the hybrid 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 for halogenated persistent organic pollutants (POPs)

Coordinator: Leontien de Pagter-de Witte

## 12.1 Activities within the EURL-NRL network

### 12.1.1 Participation in EURL-NRL workshops

In 2021 two EURL-NRL online workshops were held, a one-day workshop on 20 May and a two-day workshop on 23 and 24 November.

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

*RASFF notifications*
A summary of RASFF notifications related to dioxins, dioxin-like polychlorinated biphenyls (dl-PCBs) and non-dioxin-like PCBs (ndl-PCBs) since the last EURL/NRL meeting in 2020 was given during the first workshop in May:

- The first notification was related to mackerel from Belgium with an elevated level of dioxins of 13 ng WHO-Toxic Equivalence Quantity (TEQ)/kg.
- The second notification was on ndl-PCBs in eel from France, in which a concentration of 566 µg/kg ndl-PCBs was found.

Four additional RASFF notifications were given during the workshop in November:

1. Apple pomace from Poland: an elevated level of dioxins of 6.65 ng WHO-TEQ/kg and the sum of dioxins and dl-PCBs of 7.02 ng WHO-TEQ/kg.
2. Fish meal from Latvia: an elevated level of dioxins of 1.6 ng WHO-TEQ/kg.
3. An elevated level of dioxins in calcium butyrate from India of 3.45 ng WHO TEQ/kg and the sum of dioxins and dl-PCBs of 3.58 ng WHO-TEQ/kg.
4. ndl-PCBs in vegetable fatty acids from Ukraine, a concentration of 15.4 µg/kg ndl-PCBs was found.

*EFSA opinions*
The following EFSA opinions related to halogenated POPs were either adopted or are in the process of adoption:

- PFASs in food and feed: Following last year's EFSA Scientific Opinion on four PFASs in food (the final opinion was adopted on 17 September 2020). Evaluating the 4 PFASs: perfluorooctanesulfonic acid (PFOS), perfluorooctanoic acid (PFOA), perfluorohexanesulfonic acid (PFHxS) and perfluorononanoic acid (PFNA), (a group Tolerable Weekly Intake (TWI) of 4.4 ng/kg bw/week was established), maximum levels for the individual compounds and a sum parameter are currently under consideration for eggs, fish meat, crustacea and molluscs, meat/edible offal and milk. The first draft of maximum levels for PFOA, PFNA, PFHxS, PFOS and the sum of these compounds in several food products was presented in November and discussed within the European Commission at the end of 2021. The vote for implementation is planned for the course of 2022.
  Additionally, a monitoring recommendation is planned for PFASs in food with little occurrence data (e.g. fruit and vegetables, and food for children). Furthermore, the EURL POPs was asked to provide a report on the status of PFASs analysis in feedstuff throughout the EU, with special attention to LOQs, the ability to analyse mineral compound feed, and similar potential challenges. EU-wide monitoring of PFASs in feed is under consideration, as it might help with the investigation of sources of contamination (e.g. fish meal and minerals).

- Hexabromocyclododecanes (HBCDDs): In May 2021, the new EFSA Scientific Opinion on HBCDDs in food was presented to country representatives and the European Commission. As there was no health concern, no maximum levels will be considered for the foreseeable future. There might be a Commission Recommendation to collect more data on the occurrence of HBCDDs in food and feed for future considerations.
- Maximum levels (MLs) Polychlorinated dibenzo(p)dioxin and furans (PCDD/Fs) and Polychlorinated Biphenyls (PCBs): A limited review of MLs was presented by the European Commission during the meeting in November; even though a full review is still depending on the publication of new Toxic Equivalence Factor (TEF) values by the WHO.
  - Feed: lowering of MLs in:
    - Animal fat (including milk fat and egg fat) from 1.5 to 1.0 ng/kg for dioxins and no change in ML for the sum of dioxins and dl-PCBs (2.0 ng/kg).
    - Fish oil from 5.0 to 2.5 ng/kg for dioxins and from 20 to 10 ng/kg for the sum of dioxins and dl-PCBs.
    - For fish meal, no change in ML for dioxins (1.25 ng/kg), but the ML for the sum of dioxins and dl-PCBs is lowered from 4.0 to 3.0 ng/kg.
    - Fishprotein >20% fat and crustacean meal from 1.75 to 1.5 ng/kg for dioxins and from 9.0 to 6.0 ng/kg for the sum of dioxins and dl-PCBs.
    - Compound feed for fish from 1.75 to 1.0 ng/kg for dioxins and from 5.5 to 2.0 ng/kg for the sum of dioxins and dl-PCBs.
  - Food: new MLs for:
    - The existing ML for dioxins (2.5 pg/g fat); the sum of dioxins and dl-PCBs (4.0 pg/g fat) for bovine/sheep meat will be extended to goat meat.
    - The existing ML for dioxins (2.5 pg/g fat); the sum of dioxins and dl-PCBs (5.0 pg/g fat) for hen eggs will be extended to poultry eggs except for goose eggs.
    - Horse meat: 5.0 pg/g fat for dioxins; 10 pg/g fat for the sum of dioxins and dl-PCBs.
    - Boar meat: 5.0 pg/g fat for dioxins; 10 pg/g fat for the sum of dioxins and dl-PCBs.
    - Venison meat: 3.0 pg/g fat for dioxins; 7.5 pg/g fat for the sum of dioxins and dl-PCBs.
    - Rabbit meat: 1.0 pg/g fat for dioxins; 1.5 pg/g fat for the sum of dioxins and dl-PCBs.
    - Farmed game: 2.0 pg/g fat for dioxins; 4.0 pg/g fat for the sum of dioxins and dl-PCBs.
    - Farmed game liver: 2.5 pg/g wet weight (ww) for dioxins; 5.0 pg/g ww for the sum of dioxins and dl-PCBs.
    - Goat liver and horse liver: 0.30 pg/g ww for dioxins; 0.50 pg/g ww for the sum of dioxins and dl-PCBs.

    Besides the new MLs, the MLs for milk will be lowered from 2.5 to 2.0 pg/g for dioxins and 5.5 to 4.0 g/g for the sum of dioxins and dl-PCBs. The ML for crustaceans, including crabs (3.5 pg/g ww for dioxins; 6.5 pg/g ww for the sum of dioxins and dl-PCBs), will be applied to the white meat of appendages and abdomen. Therefore the note 'In case of crabs and crab-like crustaceans (*Brachyura and Anomura*) it applies to muscle meat from the appendages' is to be deleted.
- Chlorinated paraffins (CPs): A follow-up to the EFSA opinion on CPs was given by the European Commission during the meeting in November. A general methodology to analyse CPs is available; this will be a guide to ensure comparable results among labs in the EURL/NRL network and will be published in the course of 2022. Further actions by the Commission still need to be decided; probably, there will be a recommendation to monitor the presence of CPs in food soon. Further, follow-up actions and involvement of the EURL/NRL network are necessary to continue working on CPs.

*LOD/LOQ Guidance Document:*
An update on the harmonisation of the estimation of LOD/LOQ and the corresponding guidance document in the analysis of contaminants in food and feed was given. The four EURLs working in the field of contaminants concluded to develop one joint document for all four EURLs with a focus on one recommended and generally described approach. The original LOD/LOQ guidance document of 2016 will remain unchanged, but a reference or link to the new guidance document will be included. The new document will prioritise LOQs for official controls in compliance testing. It will secondarily focus on LOQs for compounds without legal limits set in legislation or LOQs for monitoring or risk assessment. Still, the guidance document of 2016 may be followed if the criteria of the new document are met or if the estimation of an LOD is necessary. The general approach described in the new guidance document is based on the lowest successfully validated level, meeting respective criteria for identification, precision and trueness. If this general approach is unsuitable for certain analytes, other approaches may be applied for sound reasons. Specific alternative approaches will be

defined in the new version of the guidance document separately for the different fields of the four EURLs for contaminants. For the EURL for halogenated POPs in feed and food, the specific approaches are summarised in the following table:

***Table 12.1*** *Approaches for estimation of LOD/LOQ.*

| Approach | Applicable to the following groups of halogenated POPs | Justification / Reference |
|---|---|---|
| Lowest validated level (LOQ) *(Chapter 3.1 in guidance document)* | PFASs, CPs, HBCDDs and others | General approach |
| Procedural blanks (LOD/LOQ) *(Chapter 7.1 in guidance document)* | CPs | |
| S/N (LOQ) *(Chapter 7.2 in guidance document)* | PCDD/Fs, PCBs, PBDEs* and PCNs* (with internal standard) | Commission Regulation (EU) 2017/644, Commission Regulation (EC) No 152/2009 |
| Estimation from Calibration Standards (LOQ) *(Chapter 7.3 in guidance document)* | PCDD/Fs, PCBs, PBDEs* and PCNs* (with internal standard) | Commission Regulation (EU) 2017/644, Commission Regulation (EC) No 152/2009 |
| Sum parameters *(Chapter 7.4 in guidance document)* | PCDD/Fs and PCBs | Commission Regulation (EU) 2017/644, Commission Regulation (EC) No 152/2009 |

* Similar analysis to PCDD/Fs and PCBs.

The new guidance document will be further discussed in future cluster meetings of the four EURLs for contaminants.

*Core Working Group (CWG):*
The Dutch NRL for halogenated POPs participated in three CWGs: PFASs, chlorinated paraffins (CPs) and brominated flame retardants (BFRs).

*Update of CWG BFRs:*
- Annex A of the guidance document on BFRs, which details examples of methodologies for PBDE analysis in feed and food, is close to finalisation. Annex B, which is about the analysis of HBCDDs, was distributed within the EURL/NRL network for discussion. Annex B is intended for laboratories with no existing methods and plan to start HBCDD analysis or have issues with their current methods. The design is modular for maximum flexibility regarding the laboratory equipment and should cover the most common analytical techniques. At this stage, some sections containing information on e.g. analytical standards, sample pre-treatment, extraction and purification techniques, measurement and reporting of results have been drafted, but details on procedures and information on the other sections are required. The discussions covered some of the issues encountered during the analysis of HBCDDs and how the remaining sections (other measurement techniques) would be added. Automated purification methods do not appear to be used by the CWG members. Annex A will be published on the EURL homepage in March 2022. Any further comments and suggestions regarding Annex B will be discussed in the next CWG meeting in May/June 2022.
- A summary of the current analysis status of other brominated contaminants in some of the network's laboratories was presented during the CWG meeting in October 2021. The possible combination and grouping of brominated contaminants in multi-analyte methods is seen as an efficient way of reducing the number of new methods required. The experiences of CWG members suggested the following grouping: (I) PBB, HBBz, PBT, TBX, BTBPE and DBDPE, integrated into the PBDE method (GC-HRMS or GC-MS/MS); (II) PBDD/Fs integrated into PCDD/F analysis; (III) TBBPA integrated into the HBCDD method; and (IV) BEH-TEBP, EH-TBB, DBE-DBCH and (V) bromophenols possibly in a new method. It was concluded that integrating the analysis of the groups I, II, and maybe III in existing methods for PBDEs, PCDD/Fs and HBCDDs is feasible, and the resulting data would allow a better overview of the POP contamination, which is necessary to refine human exposure estimates. In the next CWG meeting in 2022, this will be discussed further.
- Due to the low number of participants and reported results (some labs are still in the process of setting-up methods) in the interlaboratory studies (ILS) for BFRs organised in 2021, it was suggested that instead of organising another ILS, the EURL could prepare a quality control material in spring 2022, containing all analytes of interest (PBDEs, HBCDDs, TBBPA, eBFR group I, PCNs) and also additional analytes such as

PBDD/Fs. This QC material could then be distributed within the EURL/NRL/OFL network, and laboratories could use it for method development and validation purpose over a period of 6 to 9 months or even longer. Results can and should be submitted several times, and the EURL will collect all data and evaluate them as soon as possible. There was general agreement that this would be a good way for laboratories to try out (or improve) their newly developed methods and to give other laboratories time to develop their capabilities. The QC material could also be used for a range of other analytes.

- During the workshop in October 2021, a full day was dedicated to polychlorinated naphthalenes (PCNs). An introduction to polychlorinated naphthalenes (PCNs) was given. Background information on production, historical applications, sources and the reasons why PCNs are listed by both the Stockholm Convention (Annex A & C) and REACH were shown. A few labs shared their experiences with the analysis of PCNs, including the EURL. A first start was made in the discussion on the congener selection, including what chlorinated groups should be included, what toxic effects should be considered, availability of standards. Since this subject is relatively new to most NRLs, this discussion will continue during the CWG meeting in 2022.

*Update of CWG PFASs:*

- The Dutch NRL announced the two new heads of CWG PFAS during the first CWG meeting in June 2021. Wageningen Food Safety Research (WFSR) focuses on PFAS analysis in food products (milk & egg, vegetables, dairy, muscle, fish, feed), long-chain PFCAs and PFECAs and the detection of 'unknown' PFAS using TOPA and Fragment Ion Flagging. Individual methods, method validation implementation, data for long-chain PFCAs and PFECAs and an overview of strategies for 'unknown' PFAS detection were briefly presented during the meeting.
- The current focus of the CWG PFAS is on establishing a finalised guidance document, which has been under preparation since last year. Various questionnaires were sent around to all NRLs, and approaches to analysing PFASs in feed and food in the network were collected as input for the annexes of the guidance document. The first draft version of the guidance document was distributed to the EURL/NRL network and discussed during the CWG meeting in June 2021. A second draft version was discussed during the CWG meeting in October 2021. One more round for comments by the members of the CWG will be done in 2022, as well as writing annexes regarding modular approaches and reporting of results.

*Update of CWG CPs:*

- The CWG CPs reviewed and discussed the Guidance Document on the analysis of CPs, which was made available for download from the EURL POPs website at the end of 2021.
- A second project the CWG CPs is working on is the set-up of a CP homologue pattern database to better understand the CP patterns predominant in different food groups and maybe to draw some conclusions on connections to patterns in feed and the environment or human tissue. Currently, the database is only available to CWG members; it contains about 200 entries.
- Furthermore, authors from a few NRLs have finished preparing a peer-reviewed publication on shortcomings of commercially available quantification standards and connected analytical considerations, submitted for the Chemosphere Special Issue on PCNs and CPs 2021.
- The ILS on CPs by the EURL started in 2017. Since then, there has been a steady decline in participating laboratories, which makes a statistically sound evaluation of the data very difficult. Due to this, it was decided to pause the dedicated CP PTs until further notice. CPs will be integrated as optional analytes in POP PTs, and the reinstatement of dedicated PTs will only be done when more labs in the network have established CP analysis. Instead, QC material will be distributed so that smaller comparisons between the network labs can be performed on demand.

### 12.1.2 Participation in proficiency and comparative tests

In 2021, the Dutch NRL participated in four PTs organised by the EURL and one PT that FAPAS organised. The results are summarised in Table 12.2.

***Table 12.2*** *Results in PTs.*

| Name of PT | Topic | z-score |
|---|---|---|
| FAPAS | PFAS in fish | -0.4 – 0.2 |
| EURL | PFASs in liquid egg | -3.4 – 1.8 |
| EURL | Dioxins and PCBs, PBDE, HBCDD in baby food | -2.0 – 0.3 (dioxins and PCBs); -1.9 – 0.1 (PBDE); -5.0 – -0.8 (HBCDD) |
| EURL | PBDE, HBCDD in cod liver oil | -0.8 – 0.5 (PBDE); 0.0 – 2.3 (HBCDD) |
| EURL | Dioxins and PCBs, PBDE, HBCDD in dried citrus pulp | -1.0 – 1.5 (dioxins and PCBs); -1.3 – 0.0 (PBDE); 0.0 – 2.9 (HBCDD) |

The majority of the results (97%) were satisfactory (z-scores between -2.0 and +2.0). However, 6 of the 185 z-scores obtained gave unsatisfactory results, with z-scores below -2.0 or above 2.0 (see table above). The first unsatisfactory result was obtained in the EURL PT for liquid egg. This PT material was analysed for PFAS, and the z-scores of the individual components were all satisfactory (z-scores between -0.6 and 0.4). However, the results of the sum of the 4 PFASs were 1.8 (upper bound, ub) and -3.4 (lower bound, lb). This last z-score is unsatisfactory. This is due to the high LOQs of the validated method currently used at WFSR. Higher LOQs may lead to more results <LOQ, which was the case for 3 out of 4 of the components. For the lb scenario, the result is set to 0 when <LOQ should be reported. This has led to the unsatisfactory result for the sum of the 4 PFASs. In 2022, the egg method will be validated again after lowering the LOQ; this has become possible by purchasing more sensitive LC-MS/MS equipment. Implementation of the method for liquid egg on this LC-MS/MS will hopefully lead to better z-scores for the sum of 4 PFASs.
The other five unsatisfactory results originated from the HBCDD analysis. In the PT for baby food, a z-score of -5.0 was established for the sum of α-, β-, and γ-HBCDD (lb); in the PT for cod liver oil, a z-score of 2.3 was established for the sum of α-, β-, and γ-HBCDD (ub); and in the PT for dried citrus pulp an unsatisfactory z-score of 2.4 for γ-HBCDD, a z-score of 2.4 for the sum of α-, β-, and γ-HBCDD (ub) and a z-score of 2.9 for the sum of α-, β- and γ-HBCDD (lb) were established. Most of these unsatisfactory results were caused by the LOQs of the current HBCDD method used at WFSR, which are high compared to other labs. Using these high LOQs, results in z-scores above 2 when calculating the ub of the sum of α-, β- and γ-HBCDD. On the other hand, when all values for α-, β-, and γ-HBCDD are below LOQ, the result is set to 0 when calculating the lb of the sum of α-, β-, and γ-HBCDD. This leads to z-scores below -2. In 2022, the HBCDD analysis will be measured using a different, more sensitive LC-MS/MS-based method, hopefully leading to better z-scores for the sum of α-, β-, and γ-HBCDD. The final unsatisfactory result was established in the PT in dried citrus pulp for γ-HBCDD (z-score of 2.4). No direct cause for this deviating result could be found. In 2022, the sample will be analysed again to determine the z-score for γ-HBCDD once more.

## 12.2 Assistance to Official Laboratories (OLs)

### 12.2.1 Quality control

In 2021 four dairy samples were sent to the OL for analysis on PCDD/Fs and PCBs. Most of the results were comparable to the results obtained by WFSR, but there was a difference in the interpretation of the data. The quantification limits used by the OL differ from those used by WFSR. WFSR determines the LOQs per analysis run, whereas the OL has set fixed LOQs. Therefore, the lower bound reported by WFSR is higher than the one reported by the OL. With this taken into account, the reproducibility for the WHO-PCDD / F-PCB sum-TEQ meets the statutory requirement of 25% (Regulation (EU) 2017/644) for three out of four results. One result was unsatisfactory, but this result was far below the action limit. This was reported back to the OL.

### 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 Health, Welfare and Sport were supported with items mentioned on the agenda of the European Commission, EFSA Opinions, and questions about POPs in food and feed.

## 12.4 Contacts with other NRLs

In 2020 and 2021, the NRL was in contact with the EURL and other NRLs to share data on standards recently put on the market to quantify chlorinated paraffins. The result of this cooperation was published in a scientific paper in 2021. (https://doi.org/10.1016/j.chemosphere.2021.131878).

# 13 National Reference Laboratory Foodborne Viruses

Coordinator: Ingeborg Boxman

## 13.1 Activities within the EURL-NRL network

### 13.1.1 Participation in EURL-NRL workshops

The EURL-NRL network foodborne viruses was started in 2018. The focus of this network is implementing analyses of food for the presence of norovirus (NoV) (as a cause of gastroenteritis) and hepatitis A virus (HAV) (as a cause of hepatitis A) using the ISO 15216-1:2017 (quantitative) or ISO 15216-2:2019 (detection). The EURL is based in Uppsala at the Swedish Food Safety Agency. WFSR was appointed as the NRL for foodborne viruses. RIVM has been appointed as NRL for foodborne viruses in the specific matrix of bivalve molluscs. Knowledge and expertise on this matrix are also present at WFSR, as WFSR has analysed the presence of norovirus and hepatitis A virus under accreditation in this matrix for over 11 years.

The NRL participated in the 4$^{th}$ workshop of the EURL-NRL for foodborne Viruses. The EURL hosted this meeting via a web conference on 2 -3 June 2021. Fifty participants from many countries joined the meeting. An external guest from DG Sante was invited to talk about the Commission's view on the main aims for the EURL and the NRL network for Foodborne Viruses and to get informed whether or not to include work on hepatitis E virus (HEV) detection in food within the EURL. EFSA was invited to present on the monitoring of foodborne outbreaks caused by viruses in the European Union and explain how data is being gathered. Jorvik Food and Environmental Virology, UK, was invited as future convenor of the ISO/CEN activities for the standardisation of a method for HEV detection in food.

The EURL itself presented new work on developing a verification guide for the NRLs who have not yet validated the methods. The EURL verification guide consists of study design and experimental set-up protocols using artificially contaminated samples. The guide covers the matrices oysters and raspberries, artificially contaminated with norovirus genogroup GI and genogroup GII and HAV, whereas other matrices will be added later. The workflow includes calibration, preparation of dilution series, contamination of the samples, down to the data analysis. The performance parameters covered by the guide are LOD95%, linearity, precision, repeatability and LOQ. The protocol is based on the previous version from Cefas, UK, with as few modifications as possible. After the presentation, the guide was discussed in small electronic working groups and suggestions were collected to finalise the verification guide.

Previous Proficiency Tests (PTs) and future PT schemes were also discussed during the meeting. Results for 2020EFV04 (leafy vegetables) and 2020EFV05 and 2021EFV06 (oysters) were presented. While EFV05 and EFV06 consisted of blended digestive tissue of oysters that had been artificially contaminated with NoV GI, NoV GII and HAV. At the workshop, it was decided that future distributions should include virus-bioaccumulated bivalve molluscs. Furthermore, it was decided that PT schemes should cover at least bivalves once a year and seek the possibility to include, besides soft fruit and lettuce, bottled water and food surfaces described in the ISO 15216.

In 2020 the Dutch NRL (WFSR) was appointed as project leader for an interlaboratory study to compare NoV analyses using reverse-transcription digital polymerase chain reaction (RT-dPCR) with analyses using reverse-transcription real-time polymerase chain reaction (RT-qPCR). The EURL provided the reagents and test materials with the EFV06 dispatch. Data analysis is ongoing. The study's outcome will potentially provide results that can lead to more solid conclusions regarding the future direction of using RT-dPCR as an alternative to a reverse-transcription real-time polymerase chain reaction (RT-qPCR).

### 13.1.2 Participation in working groups

The NRL participated in the 2nd meeting of the Next Generation Sequencing (NGS) Working Group for the NRLs for foodborne viruses on 1 June 2021, held as a video conference. The long-term goal for this NRL sub-group is to develop harmonised NGS techniques for typing purposes of foodborne viruses. It was agreed that those with NGS experience should form a Core group of NRLs foodborne viruses on NGS to facilitate a harmonised development. The Dutch NRL (WFSR) will participate in this working group. It was announced that NRL France, Ifremer, is willing to organise a practical course in NGS for a limited number of NRLs in 2022, with a preference for those who participated in the EFSA baseline study for norovirus in oysters.

### 13.1.3 Participation in proficiency and comparative tests

The NRL participated in six PTs for the detection of norovirus genogroup I (NoV GI), norovirus genogroup II (NoV GII) and hepatitis A virus (HAV) in food (oysters, strawberry) or lenticule samples. Two were organised by the EURL, two by Public Health England (PHE), one by Bipea and one by Cefas. Eighteen samples were analysed for three target viruses, resulting in 53/54 (98%) correct detection results and 32/33 (97%) correct quantitative results.

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

| PT (organisation) | Matrix | Number of samples for NoV GI, NoV GII, HAV | Assessment Detection | Assessment Quantification |
|---|---|---|---|---|
| 21EFV06 (EURL, SE) | Oyster 4/20 | 3 | 100% | 100% |
| 21EFV07 (EURL, SE) | Strawberry 9/21 | 3 | 100% | Not included[2] |
| NHV008 (PHE, UK) | Lenticules 1/21 | 2 | 100% | 83%[3] |
| NHV009 (PHE, UK) | Lenticules 11/21 | 2 | 100% | 100% |
| 83a (Bipea, Fr) | Strawberry 11/21 | 4 | 92%[1] | Not included[2] |
| PT82 (Cefas, UK) | Oyster 10/21 | 4 | 100% | 100% |

[1] The artificial contamination level of NoV GII on one sample was chosen below the WFSR LOD.

[2] Quantification was not scored.

[3] Quantification for NoV GI in one sample was too high.

## 13.2 Assistance to official laboratories

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

## 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), October 2021, 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 NRL organised an interlaboratory study to compare digital RT-PCR with the standardised RT-qPCRs on norovirus detection and quantification in oyster PT samples. The EURL provided reagents. NRL foodborne viruses took care of protocols and data analyses. The study will be reported and submitted for publication in 2022.

The Dutch NRL (WFSR) provided dsDNA standards for quantification and EAC RNA as amplification controls for NoV GI, NoV GII and HAV to the Dutch NRL Foodborne viruses in bivalve shellfish (RIVM).

# 14 National Reference Laboratory moisture in poultry meat

Coordinator: Erika Silletti

## 14.1 Activities within the EURL-NRL network

In 2021, the travel restrictions due to the COVID-19 pandemic affected the activities of the EURL-NRL expert group 'Water content in poultry meat'. Usually, this NRL participates in two meetings organised every year together with the other European NRLs and the responsible representatives from Brussels. The first meeting is generally scheduled in Brussels, while the second meeting (two-day meeting) is usually hosted by one of the EU members. No online alternative was provided for the meetings.

### 14.1.1 Participation in proficiency and comparative tests

In June 2021, WFSR organised a proficiency test (PT) among 24 European laboratories, both NRLs and field laboratories. Each laboratory received two chicken fillet samples, which were prepared according to two different procedures. The results were evaluated according to ISO 13528 and reported to the participants in December 2021. Very good results were obtained for both protein and moisture content in both samples. For the long-term, it is recommended to repeat this PT once every two years.

### 14.1.2 Quality control

The Dutch NRL organised two quality control rounds (February/March 2021 and September/October 2021) to ensure that the official laboratory (OL) and NRL obtain statistically comparable results for moisture and protein analysis in poultry meat. The quality controls comprised an interlaboratory check of the moisture and protein analysis conducted on homogenised and non-homogenised samples of chicken fillet meat and chicken legs. From the results of both rounds, it could be concluded that the moisture and protein analyses on an interlaboratory basis were acceptably reproducible. However, for chicken legs (samples containing bone), the interlaboratory reproducibility limits were exceeded in some cases in both quality control rounds. This is in line with the results from previous years.

### 14.1.3 Advice

Common causes for reproducibility issues on poultry cuts containing bones are unavoidable variations in sample material and differences in homogenisation practices. Therefore, it will be advisable to investigate how the differences in homogenisation practices between OL and NRL affect the moisture and protein analysis results in poultry meat.

## 14.2 Scientific and technical support to the competent authority

Apart from the common scientific and technical support to the competent authorities, there were no special events where the NRL experts were consulted.

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WFSR Report 2022.001

The mission of Wageningen University & Research is "To explore the potential of nature to improve the quality of life". Under the banner Wageningen University & Research, Wageningen University and the specialised research institutes of the Wageningen Research Foundation have joined forces in contributing to finding solutions to important questions in the domain of healthy food and living environment. With its roughly 30 branches, 7,200 employees (6,400 fte) and 13,200 students and over 150,000 participants to WUR's Life Long Learning, Wageningen University & Research is one of the leading organisations in its domain. The unique Wageningen approach lies in its integrated approach to issues and the collaboration between different disciplines.

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