# National Reference Laboratories RIKILT Wageningen University & Research

Annual report 2016

S.P.J. van Leeuwen, J.G.J. Mol, M.K. van der Lee, A. Gerssen, J.J.P. Lasaroms, S.S. Sterk, L. van Raamsdonk, J. de Jong, I.M.J. Scholtens, A. Alewijn, Y. Weesepoel, L. van Ginkel, N. Meijer and M.Y. Noordam

WAGENINGEN
UNIVERSITY & RESEARCH

# National Reference Laboratories
# RIKILT Wageningen University & Research

Annual report 2016

S.P.J. van Leeuwen, J.G.J. Mol, M.K. van der Lee, A. Gerssen, J.J.P. Lasaroms, S.S. Sterk, L. van Raamsdonk, J. de Jong, I.M.J. Scholtens, A. Alewijn, Y. Weesepoel, L. van Ginkel, N. Meijer and M.Y. Noordam

This research has been carried out by RIKILT Wageningen University & Research, institute within the legal entity Wageningen Research Foundation funded by the Dutch Ministry of Economic Affairs as part of the statutory tasks food safety (WOT-02).

Wageningen, June 2017

RIKILT report 2017.007

WAGENINGEN
UNIVERSITY & RESEARCH

S.P.J. van Leeuwen, J.G.J. Mol, M.K. van der Lee, A. Gerssen, J.J.P. Lasaroms, S.S. Sterk, L. van Raamsdonk, J. de Jong, I.M.J. Scholtens, A. Alewijn, Y. Weesepoel, L. van Ginkel, N. Meijer and M.Y. Noordam, 2017. *National Reference Laboratories RIKILT Wageningen University & Research; Annual report 2016.* Wageningen, RIKILT Wageningen University & Research, RIKILT report 2017.007. 50 pp.; 0 fig.; 3 tab.; 0 ref.

BAS-codes: WOT-02-001-004, WOT-02-001-005, WOT-02-001-006, WOT-02-001-007, WOT-02-001-008, WOT-02-001-010, WOT-02-003-001, WOT-02-004-001, WOT-02-004-002, WOT-02-004-003, WOT-02-005-001, WOT-02-005-002

Project title: National Reference Laboratories – Annual Report 2016
Project leader: M.Y. Noordam

This report can be downloaded for free at http://dx.doi.org/10.18174/417476 or at www.wur.eu/rikilt (under RIKILT publications).



P.O. Box 230, 6700 AA Wageningen, The Netherlands, T +31 (0)317 48 02 56, E info.RIKILT@wur.nl, www.wur.eu/rikilt. RIKILT is part of Wageningen University & Research.



RIKILT report 2017.007

Distribution list:

- Netherlands Food and Consumer Product Safety Authority (NVWA): H.N. de Groot, J.M. de Stoppelaar, E. Thomas, R. van Buuren, J.A. van Rhijn, A. Lam, E.A.J. v.d. Made, R.G. Herbes, S.M. Hoffer
- Ministry of Economic Affairs (EZ): E. Pierey, F.B. Leijdekkers, S.J. Beukema, M. Graf, F.W.A. Vink, M.G.M. van Creij, M.J.B.M. Weijtens

# Contents

# Preface

RIKILT Wageningen University & Research has been assigned several reference-tasks in the field of safety and quality of food and feed. The execution of these reference-tasks is performed within RIKILT's role of National Reference Laboratory (NRL) in various fields. NRLs are the link between the European Union Reference Laboratories (EURLs) and Official Laboratories (OLs). NRLs and OLs perform analyses on food and feed in the framework of the national official controls. NRLs are a centre of expertise for the OLs as well as the Ministries and Competent Authority.

In this annual report, RIKILT reports on the execution of its NRL tasks.

# Summary

National Reference Laboratories (NRLs) are part of the system responsible for the control and enforcement of EU food and feed law. RIKILT Wageningen University & Research has been designated as the NRL for twelve subjects. The tasks of a NRL depend on its research fields. This report gives an overview of the activities performed by all of RIKILT's NRLs in 2016. These NRLs are for: dioxins and polychlorinated biphenyls (PCBs), pesticides in products of animal origin, mycotoxins, heavy metals, polycyclic aromatic hydrocarbons (PAHs), marine biotoxins, certain substances and residues thereof as laid down in Directive 96/23/EC, genetically modified organisms (GMOs) in food and feed, animal proteins, feed additives, milk and milk products, and water content of poultry.

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

An important NRL task is to stay up to date with current developments within its NRL domain. Every EURL organizes one or two meetings (workshops) every year for that purpose. Participation in these EURL-NRL workshops is mandatory. In 2016, 18 workshops have been attended by RIKILT's NRLs. Additionally, the NRLs have actively participated in EURL working groups to improve analytical methods. To test the analytical capabilities of NRLs, the EURLs organize proficiency tests. Due to EURL proficiency tests sometimes being limited in their scope, the NRLs have also participated in proficiency tests organized by other organizations if thought to be relevant. Most results (Z-scores) in these proficiency tests were good; only a few 'questionable' and a single 'unsatisfactorily' result were reported. Follow-up actions were implemented in those cases. The performance of the OLs has been assured by checking the results of their performance in proficiency tests (organized by other laboratories or the NRL) or by sending assurance-samples. Some OLs have also received technical support with regard to their analyses.

# 1 Introduction

Food law aims to assure a high level of protection of human life and health and to achieve the free movement of food and feed marketed in the European Union. Food and feed businesses are to ensure that they satisfy the requirements of food law; national authorities are to enforce food law, and monitor and verify that the relevant requirements are fulfilled by food and feed business operators at all stages of production, processing and distribution. The manner in which official controls are carried out is prescribed in European and national rules. In the context of those official controls, samples for testing have to be taken. A large amount of sampling is done in the context of multi-annual national control plans set up by the competent authorities of Member States in accordance with broad guidelines drawn up at the Union level. To ensure the uniformity of analytical results, requirements have been set for laboratories, sampling, and analytical methods. For this purpose, European Union Reference Laboratories (EURLs) are tasked with the support of National Reference Laboratories (NRLs). Pursuant to EU legislation, every Member State is obligated to designate at least one NRL per EURL. NRLs are, *inter alia*, expected to stay up-to-date with scientific advances within their field and are tasked with the support of those laboratories where official samples are tested – the official laboratories (OLs). RIKILT Wageningen University & Research has been designated as the NRL for 12 subjects by the Ministry of Economic Affairs and the Ministry of Health, Welfare and Sport. These subjects are:

- Dioxins and polychlorinated biphenyls (PCBs)
- Pesticides in products of animal origin
- Mycotoxins
- Heavy metals
- Polycyclic aromatic hydrocarbons (PAHs)
- Marine biotoxins
- Certain substances and residues thereof laid down in Directive 96/23/EC
- Genetically modified organisms (GMOs) in food and feed
- Animal proteins
- Feed additives
- Milk and milk products
- Water content of poultry

The objective of this report is to give an overview of activities performed by RIKILT's NRLs in 2016.

## 1.1 EU Legislation

Legislation on official controls has not been changed substantially in 2016. The following overview therefore contains only few adjustments compared to the 2015 report.

The most important legislation in the EU on official controls performed to ensure the verification of compliance with feed and food law, animal health and animal welfare rules is Regulation (EC) No 882/2004. This Regulation mandates that Member States uniformly monitor and verify that at all stages of production, processing and distribution the relevant requirements are fulfilled. In addition to the Official Controls Regulation, more specific legislation may apply to certain parts of the production chain or certain subjects. For instance, additional provisions for the official controls of residues of veterinary medicines and banned substances in the production of animals for food production are laid down in Directive 96/23/EC. Moreover, additional provisions have been laid down for residues of plant protection products in Regulation (EC) No 396/2005; for feed additives in Regulation (EC) No 1831/2003; for genetically modified organisms in Regulation (EC) No 1981/2006 (and recommendation 2004/787/EG); and for animal proteins in Regulation (EC) No 999/2001 and Regulation (EC) No 1069/2009. For the official controls on the water content of poultry, additional

provisions have also been laid down (Regulation (EC) No 543/2008). Those Official Controls are carried out to ensure the functioning of the Single Market.

### 1.1.1 Competent authorities

Member States are to designate competent authorities responsible for official controls. The Netherlands Food and Product Safety Authority (NVWA) is the CA for most products in the Netherlands, but for milk, milk products, and egg and egg products this is the 'Centraal Orgaan voor Kwaliteitsaangelegenheden in de Zuivel' (COKZ). The CA is responsible for designating laboratories where analysis of samples, taken for official control purposes, takes place. In addition, the CA is responsible for making the multi annual national control plan (MANCP) which includes attention to physical controls (sample analysis).

### 1.1.2 European Union Reference Laboratories (EURLs)

EURLs are designated by the European Commission. Laboratories have been invited to become an EURL via a tendering procedure. The list of EURLs is laid down in Annex VII of Regulation (EC) No 882/2004. Table 1 shows the EURLs relevant for the NRLs of RIKILT.

***Table 1*** *List of EURLs relevant for NRLs RIKILT*

| EURL | Substances/products |
|---|---|
| Chemisches und Veterinäruntersuchungsamt (CVUA) Freiburg<br>Freiburg<br>Germany | • Dioxins and PCBs in food and feed<br>• Residues of pesticides in food of animal origin and commodities with high fat content |
| Fødevareinstituttet<br>Danmarks Tekniske Universitet<br>København<br>Denmark | • Residues of pesticides in cereals and feedingstuffs |
| Chemisches und Veterinäruntersuchungsamt (CVUA) Stuttgart<br>Fellbach<br>Germany | • Single residue methods for pesticides |
| The Joint Research Centre of the European Commission<br>Geel<br>Belgium | • Mycotoxins<br>• Heavy metals in food and feed<br>• Polycyclic Aromatic Hydrocarbons (PAH) |
| Agencia Española de Seguridad Alimentaria (AESA)<br>Vigo<br>Spain | • Marine biotoxins |
| RIKILT Institute of Food safety, part of Wageningen UR<br>Wageningen<br>The Netherlands | • Stilbenes, stilbene derivatives, and their salts and esters (A1)*<br>• Antithyroid agents (A2)<br>• Steroids (A3)<br>• Resorcylic acid lactones including zeranol (A4)<br>• Sedatives (B2d)<br>• Mycotoxins in animal products (B3d) |
| ANSES – Laboratoire de Fougères<br>France | • Antibacterial substances, including sulphonamides, quinolones (B1)<br>• Dyes (B3e) |
| Bundesamt für Verbrauchershutz und Lebensmittelsicherheit (BVL)<br>Berlin<br>Germany | • Beta-agonists (A5)<br>• Anthelmintics (B2a)<br>• Anticoccidials, including nitroimidazoles (B2b)<br>• Non-steroidal anti-inflammatory drugs (NSAIDs) (B2e) |
| Instituto Superiore di Sanità<br>Roma<br>Italy | • Chemical elements in animal products (B3c) |
| The Joint Research Centre of the European Commission<br>Geel<br>Belgium | • Additives for use in animal nutrition |

| EURL | Substances/products |
|---|---|
| The Joint Research Centre of the European Commission<br>Ispra<br>Italy | • Genetically modified organisms (GMOs) |
| Centre wallon de recherches agronomiques (CRA-W)<br>Gembloux<br>Belgium | • Animal proteins in feedingstuffs |
| ANSES – Laboratoire de sécurité des aliments<br>Maisons-Alfort<br>France | • Milk and milk products |
| Board of Experts: JRC (IRMM), DG AGRI and the three NRL's** | • Water content poultry meat |

* The compound(groups) followed by () are part of the official controls carried out in the context of Directive 96/23/EC.

** 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.

EURLs designated within the context of Regulation (EC) No 882/2004 are responsible for (Article 32):

a. providing national reference laboratories with details of analytical methods, including reference methods;
b. coordinating application by the national reference laboratories of the methods referred to in (a), in particular by organising comparative testing and by ensuring an appropriate follow-up of such comparative testing in accordance with internationally accepted protocols, when available;
c. coordinating, within their area of competence, practical arrangements needed to apply new analytical methods and informing national reference laboratories of advances in this field;
d. conducting initial and further training courses for the benefit of staff from national reference laboratories and of experts from developing countries;
e. providing scientific and technical assistance to the Commission, especially in cases where Member States contest the results of analyses;
f. collaborating with laboratories responsible for analysing feed and food in third countries.

### 1.1.3 National Reference Laboratories

RIKILT is the designated NRL for many chemical contaminants (see under 1 above), GMOs, animal proteins, milk and poultry meat. A working plan describing the tasks for 2016 has been drafted in 2015. In addition, budgets for personnel, and facility and equipment costs have been drawn up. The working plans for 2016 have been positively reviewed by the Client Consultation Board (consisting of employees of the NVWA, the Ministry of Economic Affairs and the Ministry of Health, Welfare and Sport) and has been presented to the director of the Department Agriculture and Nature Knowledge ('Agro & Natuurkennis' (ANK)) of the Ministry of Economic Affairs. The Ministry has approved of these plans. Working plans are based on NRL tasks as described in various EU legislation.

As laid down in Article 33 of Regulation (EC) No 882/2004, these tasks are:

a. collaborate with the Community reference laboratory in their area of competence;
b. coordinate, for their area of competence, the activities of official laboratories responsible for the analysis of samples in accordance with Article 11;
c. where appropriate, organise comparative tests between the official national laboratories and ensure an appropriate follow-up of such comparative testing;
d. ensure the dissemination to the competent authority and official national laboratories of information that the Community reference laboratory supplies;
e. provide scientific and technical assistance to the competent authority for the implementation of coordinated control plans adopted in accordance with Article 53;
f. be responsible for carrying out other specific duties provided for in accordance with the procedure referred to in Article 62(3), without prejudice to existing additional national duties.

Within the context of Directive 96/23/EC (Article 14), these tasks are:

- coordinating the work of the other national laboratories responsible for residue analysis, in particular by coordinating the standards and methods of analysis for each residue or residue group concerned;
- assisting the competent authority in organizing the plan for monitoring residues;

- periodically organizing comparative tests for each residue or residue group assigned to them;
- ensuring that national laboratories observe the limits laid down;
- disseminating information supplied by Community reference laboratories;
- ensuring that their staff are able to take part in further training courses organized by the Commission or by Commission reference laboratories.

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

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

### 1.1.4 Official Laboratories

Pursuant to Article 12 of Regulation (EC) No 882/2004, the competent authorities are to designate specific laboratories authorized to perform analyses of samples taken within the context of official controls. These laboratories are termed 'official laboratories' in this Regulation and 'approved laboratories' in Directive 96/23/EC. CAs may only designate laboratories that operate and are assessed and accredited in accordance with the European standards: EN ISO/IEC 17025 on 'General requirements for the competence of testing and calibration laboratories', and EN ISO/IEC 17011 on 'General requirements for accreditation bodies accrediting conformity assessment bodies'. Of course, these accreditation requirements also apply to NRLs.

### 1.1.5 Methods of analysis

The methods of analysis used to test official samples should be (if possible) validated and included in the laboratory's accreditation (Article 11 of Regulation (EC) No 882/2004). For various compounds and products, specific provisions have been laid down in EU legislation concerning sampling and requirements for analytical methods. Table 2 shows an overview of this legislation.

***Table 2*** *List of documents with requirements for methods of analysis used in the official control*

| Act/Document | For contaminant/residues/products |
|---|---|
| Regulation (EU) 2017/644 | • Dioxins, dioxin-like and non dioxin-like PCBs |
| SANCO/11945/2015 | • Residues of plant protection products (all matrices) |
| Regulation (EC) 401/2006 | • Mycotoxins in food |
| Regulation (EC) 333/2007 | • Lead, cadmium, mercury, anorganic tin, 30MCPD, PAHs and melamine in food |
| Regulation (EC) 2074/2005 | • Marine biotoxins<br>• Some milk parameters |
| Decision 2002/657/EC | • Residues of veterinary drugs and hormones (Directive 96/23/EC) |
| Regulation (EC) 641/2004 | • GMO |
| Regulation (EC) 619/2011 | • Low level presence (LLP) of GMOs in feed |
| Regulation (EC) 543/2008 | • Water content poultry meat |
| Regulation (EC) 273/2008 | • Quality parameters milk and milk products |
| Regulation (EC) 152/2009 | • All parameters in feed (a.o. GMO, animal proteins, feed additives, contaminants) |

# 2 National Reference Laboratory Dioxins and PCBs in food and feed

## 2.1 Activities within the EURL-NRL network

### 2.1.1 Participation in EURL-NRL workshops

Two EURL workshops have been organised by the EURL (CVUA, Freiburg), one in Uppsala (Sweden) and the other in Freiburg (Germany). Frans Verstraete (DG SANTE) visited both meetings and elaborated on the RASFF notifications. There was a notification for dioxins (7.9 ng/kg, 9.2 ng/kg) in dried apple pomace from Ukraine, probably caused by an inappropriate drying process and for dioxins (5.4 ng/kg) in complementary feed (mixed bile salts) for fish from Brazil. In the last years, there have been several findings of elevated levels in dried products from Ukraine. The EURL will take a look at the congener patterns in order to check if there is an underlying problem and further follow-up is needed. Verstraete furthermore explained on the need for a monitoring database for fish from the Baltic region, which may contain dioxin levels that exceed the current MLs. Such a database should facilitate a common understanding of the level of contamination of the different fish species from the different ICES zones from the Baltic region.

Verstraete also updated the EURL-NRL network on the progress of the updated documents for analytical criteria for food and feed, for sampling and analysis and for the recast of Regulation (EC) No 1881/2006. The aim of the recast of Regulation (EC) No 1881/2006 is to improve readability, avoid overlaps and introduce a consistent terminology. He also updated the network on Regulation (EU) 2015/786 dealing with criteria for detoxification processes of feed. Updates on the progress of the Core Working Groups (CWGs) were also provided. Details on the CWG activities are provided in paragraph 2.1.2. An important aspect of the workshop is the discussion of the performance of laboratories in the proficiency tests (PTs) organised by the EURL. See below in section 2.1.3 for details regarding the NRL. In response to a discussion on worker safety for those working in the laboratory with high concentration standard solutions, it was concluded that if normal laboratory safety procedures are adhered to, there should be no risk for the worker under normal conditions.

Thorsten Bernsmann (CVUA, Germany) informed the network on elevated concentrations of dioxins and PCBs found in meat and liver of wild animals. Philippe Marchand (LABERCA, France) described the discovery of a contamination case in France. >100 samples were analysed to find the source of contamination, which turned out to be small fragments of PCB containing paint from an old oil tank that polluted a nearby feed supply for pigs. This underlines that contamination sources may impact the feed and food chain on individual farms. Guillaume ten Dam (NRL-NL) informed the network on the pros and cons of atmospheric pressure chemical ionisation (APCI)-MS/MS for official control analysis of dioxins in food and feed. Although the instrument competes with GC-HRMS in terms of sensitivity, selectivity is somewhat worse for APGC-MS/MS, particularly for contaminated samples such as fish and cod livers. For details, see the publication specified below (paragraph 2.4)[1]. The Uppsala workshop was completed with a visit to the laboratories of the NRL of Sweden.

### 2.1.2 Participation in working groups

The NRL participated in three working groups. The core working group (CWG) on measurement uncertainty (CWGMU) finalised their work on a document. The guidance document was distributed in the network. At the Uppsala workshop, the guidance document was unanimously accepted by the EURL/NRL network. The final version of the document became available in the course of 2016, as well as Excel spreadsheets for calculation of MU according to the presented approaches. The conclusions of

[1] http://www.sciencedirect.com/science/article/pii/S0021967316315473

the CWGMU and the main aspects of the guidance document were presented at the dioxin conference. The completion of the work also led to the finalisation of the CWGMU.

The core working group on dioxin patterns (CWGDP) got together in April at the Dutch NRL, in July at the Umweltbundesamt (UBA; Federal Environment Agency), Berlin, and in November at the EURL, in Freiburg. At these meetings, the collection of dioxin patterns and how to make this accessible to stakeholders in the field was discussed. Patterns are very helpful in the elucidation of possible contamination sources of food and feed, via which the contamination source can be removed. The primary focus at the moment is on dioxin patterns which are found as single source in feed (no mixed patterns). For comparison and different purposes, levels are expressed as absolute levels and TEQ levels. A number of different patterns was collected: PCBs (different chlorination); chlorophenols (different chlorination); clays; minerals and burning (drying). A request for additional patterns was distributed in the EURL-NRL network and at the November workshop all collected patterns were categorised and discussed. A draft for a joint paper was discussed at the November meeting. The paper will be finished and submitted in 2017.

The EC has requested the EURL-NRL network to add a new group of persistent organic pollutants to their task, being the chlorinated paraffins (CPs). CPs are high production volume chemicals that find their application in a wide array of consumer and industrial products and processes, resulting in a ubiquitous contamination of the environment, as well as food and feed (ingredients). The way CPs are produced makes the analysis extremely challenging: >100.000 individual compounds may be present in the technical mixtures. The current analytical approaches do not target the individual compounds, but rather the sum of all CPs in the sample, or with more detail, the sum of homologue groups.

To coordinate this task and the required developments in the EURL-NRL network, a core working group on CPs (CWGCP) was initiated in 2016. The NRL participates in this working group, which had its first meeting at 9 November (Freiburg, Germany). The following topics were discussed at this meeting: the scope of the task requested by the EU Commission; getting a first overview on the present status of CP analysis in the EURL/NRL network; presenting the current possibilities of CP analysis including recent efforts at universities (ETH Zurich/Uni Hohenheim) and options for the organisation of the CP PT in 2017.

### 2.1.3 Participation in proficiency and comparative tests

The NRL has participated in all PTs on dioxins and PCBs in food and feed organised by the EURL. In addition, the NRL participated in PTs organised by Folkehelsa (Norway), Ducares and the NRL from their laboratory quality services program. The EURL round 1 samples were fish oil and a halibut filet. The round 2 samples were a grass meal and dried basil. The Z-scores for all samples for the GC-HRMS were excellent with all Z-scores -2<Z<2. The DR-CALUX successfully participated in the PTs organised by the EURL. The Folkehelsa PT comprised a salmon fillet, fish oil and sheep liver sample. The Z-scores for all samples for the GC-HRMS were excellent with all Z-scores -2<Z<2 for most compounds. In a single case, a cross contamination led to Z-scores>2. The source of the cross-contamination was identified and the problem was solved. In a PT organised by Ducares, feed and wheat samples were analysed. Although the values corresponded very well with other laboratories, only 5 laboratories participated. Therefore, the results should be interpreted with caution. In a PT organised by the NRL itself, crude palm oil and palm fatty acid distillate (‘PFAD’) samples were analysed. The Z-scores for all samples for the GC-HRMS were excellent with all Z-scores -2<Z<2.

The NRL also participated in PTs on brominated flame retardants (BFRs) organised by Folkehelsa. In this PT, no Z-scores are provided on a single congener basis, except for the sum of PBDEs. In that case, the Z-score was 2.7 for a cheese sample and 3.3 for a beef sample. This had to do with the fact that the NRL reported <LOQ values, which are somewhat higher than those reported by other laboratories. Following upper bound approaches, these LOQ values resulted in an elevated Z-score. Nevertheless in cases where the NRL reported a real measured value, this corresponded very well with the other laboratories: for the salmon sample, a z-score of -0.55 was obtained, which is well within -2<Z<2.

## 2.2 Assistance to official laboratories

### 2.2.1 Quality control

In two rounds (spring and autumn), dairy samples were exchanged with the OL. Their results were evaluated and feedback was given to the OL. In addition to previous years, the repeatability and reproducibility were evaluated.

### 2.2.2 Advice

Information from the EURL-NRL network was exchanged with the OL.

## 2.3 Scientific and technical support to the competent authority

There has been frequent support of the Ministries of Economic Affairs (EZ), of Health, Welfare and Sport (VWS), NVWA and RIVM with regards to dioxins and PCBs in eel, Chinese mitten crab, and food and feed in general. Support was given on the interpretation of analysis reports of commercial laboratories in specific cases.

## 2.4 Contacts with other NRLs

The NRL visited the NRL of Belgium to evaluate a new automatic system for sample clean-up for confirmatory analysis. In addition, there has been frequent contact with other NRLs to discuss topics on dioxin patterns and novel approaches for confirmatory analysis of dioxins and PCBs in food and feed. The outcomes of these discussions and other activities in the EURL-NRL network were presented at several occasions being, with NRL involvement:

**Dioxin 2016 conference**

- Eppe, G, Scholl, G, Schaechtele, A, Haedrich, J, Ceci, R, Diletti, G, Fernandes, A, Hart, J, Hove, H, Leondiadis, L, Vassiliadou, I, Marchand, P, Moche, W, Scortichini, G, Tondeur, Y, Van Leeuwen, S, Abballe, A, Iamicelli, A, Di Domenico, A, Bernsmann, T, (2016), Measurement uncertainty estimation for laboratories performing PCDD/F and PCB analysis by isotope dilution mass spectrometry.
- Schaechtele, A, Haedrich, J, Ceci, R, Diletti, G, Eppe, G, Fernandes, A, Hart, J, Hove, H, Leondiadis, L, Marchand, P, Moche, W, Scholl, G, Scortichini, G, Tondeur, Y, Van Leeuwen, S, Vassiliadou, I, Malisch, R (2016), Estimation of LOQ for the analysis of persistent organic pollutants, in particular PCDD/Fs and PCBs.
- Hoogenboom, R, Malisch, R, Ten Dam, G, Van Leeuwen, S, Hove, H, Fernandes, A, Schächtele, A, Rose, M (2016), Congener patterns of polychlorinated dibenzo-p-dioxins and dibenzofurans as a useful aid to source identification during food contamination incidents.

**Feed 2016 Conference**

- Ron Hoogenboom, Rainer Malisch, Stefan van Leeuwen, Guillaume ten Dam, Leo van Raamsdonk, Huig Vanderperren, Helge Hove, Alwyn Fernandes, Alexander Schächtele, Martin Rose (2016) A database for dioxin congener profiles for identification of sources *poster presentation*

In addition a joint peer reviewed paper was published jointly with the NRL Belgium and the EURL on the performance of a new analytical approach for the analysis of dioxins and PCBs in food and feed:

- ten Dam, Guillaume; Pussente, Igor; Scholl, Georges; Eppe, Gauthier; Schaechtele, Alexander and van Leeuwen, Stefan (2016) The performance of atmospheric pressure gas chromatography–tandem mass spectrometry compared to gas chromatography–high resolution mass spectrometry for the analysis of polychlorinated dioxins and PCBs in food and feed samples. Journal of Chromatography. A Volume 1477, Pages 76–90.

# 3 National Reference Laboratory Pesticides in products of animal origin

## 3.1 Activities within the EURL-NRL network

### 3.1.1 Participation in EURL-NRL workshops

For pesticides there are four EURLs: three covering a type of commodity (fruit & vegetables; cereals & feed; 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. In the EURL-NRL network, one workshop is 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 2016 a meeting organized by the EURL for pesticides in products of animal origin (CVUA, Freiburg) was held at 4-5 October in Freiburg, Germany which was attended by the NRL. RIKILT shares this NRL task with the NVWA that also attended the meeting.

The purpose of the workshop was to: inform the NRL network about relevant matters from the Commission, to exchange technical information (analytical methods, new technologies, issues with certain pesticide/matrix combinations), to present the set up and discuss the outcome of the annual EU proficiency test, and to present the EURL program and activities for the next year.

The items presented and discussed are briefly summarized below:

**From the Commission**
The commodities included in the EU Coordinated Multi-Annual Control Programme for 2017-2019 (Regulation (EU) No 2016/662) were presented. The inclusion in national monitoring programmes of these commodities is mandatory:

- 2017: poultry fat and sheep fat;
- 2018: bovine fat and chicken eggs;
- 2019: cow's milk and swine fat.

In addition to these commodities, the Regulation also specifies the pesticides that need to be included in the analysis. This mandatory list is currently limited to 23 pesticides; mainly organochlorine pesticides, organophosphorus pesticides and pyrethroids. Organochlorine pesticides have been prohibited in the EU but may occur as environmental contaminants and accumulate in animal fat tissue. The other two groups may end up in animal products primarily through feed. Moreover, several organophosphorus pesticides and pyrethroids are used as veterinary drugs, which can result in residues through treatment of the animals.

Besides the 'traditional' pesticides, there are only few modern pesticides included in the programme (famoxadone, indoxacarb). There are multiple reasons for the currently very limited inclusion of other pesticides in the scope of animal products. This is partly because inclusion is not considered relevant because the likelihood of ending up in products of animal origin is considered low (no residues in feed and/or no transfer to animal tissues, milk or eggs). For other pesticides transfer may occur metabolites, rather than in their original form, which are included in the residue definition when they are toxicologically relevant. The problem here is that analytical reference standards of these metabolites are often not available which makes quantification and full identification impossible. The Commission recognises this problem and is increasing pressure on the agrichemical industry to make such standards available to the EURL/NRL/OF network, so far without much success.

Note: the mandatory list of pesticides (including metabolites as far as commonly available) in the EURL proficiency test is 58, in addition, 18 pesticides are included as optional/voluntary pesticides. The current routine scope of the NRL for products of animal origin is approximately 150, prioritised based on inclusion in EURL proficiency tests and data on possible transfer from feed to animal products.

**Presentation on proficiency test organised by the EURL in 2016**
The matrix was lard (pig fat), the target list for analysis was 57 mandatory and 19 voluntary pesticides, of which 12 and 3 turned out to be present, respectively. Concentrations were mostly in the range 0.05-0.10 mg/kg. The proficiency test was mandatory for all NRLs and OLs involved in analysis of animal products. A total of 116 laboratories participated. Overall, the performance of the laboratories for this proficiency test was very good. The inter-laboratory robust relative standard deviations were in the range 14-22%. Of all laboratories, 89.8% obtained acceptable results, 2.5% questionable results, 7.7% unacceptable results. Nevertheless, there were a number of false positives and false negatives: in total 11 false positives (pesticide reported to be present above 0.01 mg/kg, but not present in the sample), and 39 false negatives (pesticide present in the sample above the reporting limit of the laboratory, but not found). This was lower than previous years.

**Exchange of methods and analytical technical details**
Presentations by four NRLs (Italy, Slovenia, Greece, Sweden) on their methods used. Presentation by the EURL on: chemical screening by liquid chromatography with high resolution mass spectrometry, validation of a GC-MS/MS based method, and new options for clean-up in LC-MS-based multi-methods.

**Presentations of methods and surveys for challenging pesticides or pesticides requiring dedicated methods**
- Ethoxyquin is no longer registered as a pesticide but is being used as feed additive and through that route found in animal products (especially salmon). It is an anti-oxidant and challenging in analysis (poor recoveries when no special precautions are taken). Besides ethoxyquin itself, a dimer is found at higher concentrations. Legislation for this is still lacking.
- Chlorate is also not anymore used as pesticide but may end up in dairy products through use as a biocide. It is found in whey products and milk powder.
- Glyphosate: challenging analysis. Issue in 2016 following reports on findings in (human)milk. These where however not confirmed by LC-MS/MS based confirmatory analysis. Also investigated in honey by the EURL.

**EURL work program 2017**
One mandatory proficiency test will be held, the matrix will be egg. The EURL intends to intensify follow up on laboratories with poor performance in the proficiency test. Poor performance include measurement of <90% of the pesticides from the mandatory scope, detection of <90% of the pesticides present in the proficiency test sample, poor quantitative performance in two consecutive proficiency tests. Besides this, a workshop will be organised together with the other EURLs in Freiburg, 27-29 September 2017.

### 3.1.2 Participation in working groups

The NRL is a member of the AQC-working group for the bi-annual revision of the 'Guidance document on analytical quality control and method validation procedures for pesticides residues analysis in food and feed' (current version: SANTE/11945/2015). A first meeting on items to discuss and improve the document was attended July 1$^{st}$ in Madrid. Further meetings are foreseen for 2017, when a revision will be drafted and discussed, adjusted and then presented for endorsement by the EURL-NRL community during the 2017 joint meeting.

### 3.1.3 Participation in proficiency and comparative tests

The NRL participated in two proficiency tests in the domain of pesticides in products of animal origin. The first concerned lard, organised by the EURL (details see 3.1.1). The second concerned the determination of parasiticides in salmon, organised by the RIKILT-PT unit.
In lard, 15 pesticides were present which were all detected and correctly quantified (Z-scores all within ±2). In salmon, three parasiticides/pesticides were present (deltamethrin, cypermethrin, and emamectin) which were all detected and correctly quantified (Z-scores within ±2). No false positives were reported.

## 3.2 Assistance to official laboratories

### 3.2.1 Quality control

In the Netherlands there are two laboratories involved in official analysis of samples of products of animal origin in general: RIKILT and NVWA. Both are also NRL for this domain. In addition, there is one laboratory performing part of the official analysis of dairy products. In the frame of a quality control program, samples of milk powder were sent and analysed for presence of organochlorine pesticides. Results were reported to and evaluated by RIKILT.

### 3.2.2 Advice

Technical advice was provided to the dairy laboratory on technical aspects of an analysis method for organochlorine pesticides in milk fat. The NRL reviewed two reports from proficiency tests on organochlorine pesticides and PCBs organised by Ducares for private laboratories.

## 3.3 Scientific and technical support to the competent authority

Contributions were provided to the competent authority in the frame of Directive 96/23/EC on measures to monitor certain substances and residues thereof in live animals and animal products. This concerned the scope of analysis of pesticides in products of animal origin when monitoring in a risk-based context.

## 3.4 Contacts with other NRLs

Regular ad-hoc contacts took place with the other Dutch NRL on pesticides in animal origin (and other commodities) throughout the year. Furthermore dissemination and discussion of technical aspects (analysis, legislation, analytical quality control) took place during international meetings (see 3.1) and symposia (European Pesticide Residue Workshop, June, Cyprus).

# 4 National Reference Laboratory Mycotoxins in food and feed

## 4.1 Activities within the EURL-NRL network

### 4.1.1 Participation in EURL-NRL workshops

In 2016 a workshop organized by the EURL for mycotoxins in food and feed (JRC, Geel, Belgium) on 5 October in Geel, which was attended by the NRL. The purpose of the workshop was to inform the NRL network about relevant matters from the Commission, to exchange technical information (analytical methods, standardization) and to present the EURL program and activities for the next year. Although formally not within the mandate of the mycotoxin EURL, plant toxins were also discussed within the workshop. Plant toxins are receiving more and more attention and are also included as topic in the program because compound- and matrix-wise it is closest to the mycotoxin domain. In the frame of the 10$^{th}$ anniversary of the EURLs hosted by JRC, a 10th Anniversary event and a science day of the contaminants EURLs was organised in conjunction with the EURL workshop.

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

**Presentation bioassay-based screening methods for mycotoxins**
This year's workshop was preceded by an afternoon session on bioassay-based screening methods for mycotoxins (not part of the formal EURL workshop itself). Eight vendors presented their rapid test kits (ELISAs, lateral flow devices ('dipsticks')) and other rapid bioassay-based methods for detection of mycotoxin in various food and feed commodities. Most of the kits can be used in field or in a basic laboratory setting. The kits come with readers and provide a (semi-)quantitative result in a certain concentration range, typically around the EU maximum limit or guidance values. Some assays can detect multiple mycotoxins at the same time. The kits are mostly used by industry for indicative compliance testing/quality control. In general, the amount of sample extracted and analysed is small, i.e. not compliant with EU legislation for official analysis/enforcement. For that purpose more extensive sampling strategies involving larger sample amounts to be entirely homogenised before analysis are required.

**Presentation of guidance document LOD and LOQ for mycotoxins, heavy metals, PAH, and dioxins**
A guidance document on how to determine the limit of detection (LOD) and the limit of quantification (LOQ) drafted by the EURLs for mycotoxins, heavy metals, PAH, and dioxins was presented (Thomas Wenzl, JRC). At the moment there are several ways of estimating the LOD and LOQ of an analysis method, that provide different values. As a consequence, LODs and LOQs provided by different laboratories are not comparable. Especially for risk assessment purposes this is an issue that may affect the outcome of the risk assessment. For this reason, harmonisation and guidance on how to estimate these method performance parameters is needed. For mycotoxins, three options were presented (which still might result in three different outcomes).

**Presentation overview methods for determination of mycotoxins and plant toxins**
Within the frame of CEN (European Committee for Standardization) a number of methods for determination of mycotoxins in food (11 projects) and mycotoxins and plant toxins in feed (9 projects) are currently under study. An overview of these methods and the progress was presented (H. Mol, NRL-NL). More details on certain CEN studies were given: Alternaria toxins in food (A. Toelgyesi, JRC), Multi-mycotoxin Screening (H. Mol, NRL-NL), and a CEN Technical Report on Performance Criteria (J. Stroka, JRC).

**Presentation results proficiency test determination of tropane alkaloids in cereals and herbal infusions**

The results of a proficiency tests on the determination of tropane alkaloids in cereals and herbal infusions organised by JRC were presented. Since tropane alkaloids are plant toxins, participation of NRLs was on a voluntary basis. One of the aims of the proficiency test was to assess whether laboratories in the EU are capable of determining atropine and scopolamine in cereal-based baby foods down to 1 µg/kg (the legislative limit, Regulation (EC) 1881/2006). The conclusion was that atropine and scopolamine could be reliably determined in cereals at 1 µg/kg, and in herbal teas at 10 µg/kg, or even below. The performance was independent of the origin of standards and method used.

**Presentation final version Guidance document on identification criteria for confirmatory analysis of mycotoxins in food and feed**

The final version of the "Guidance document on identification criteria for confirmatory analysis of mycotoxins in food and feed" drafted by a EURL/NRL working group (chaired by H. Mol, NRL-NL) was discussed and agreed upon. For identification of mycotoxins, chromatography combined with mass spectrometry is the method of choice. The criteria for this are similar to those established earlier for pesticides (SANTE/11945/2015). Alternatively, liquid chromatography with fluorescence detection may be applied, but only when an immuno-affinity based clean-up specific for the targeted mycotoxin(s) has been employed during sample preparation. The use of methods based on UV detection is discouraged; however, already established methods for which adequate selectivity has been demonstrated may continue to be used for now (this typically applies to patulin and deoxynivalenol). The document is expected to be posted on the DG-SANTE website by February 2017.

**Update of legislative provisions on mycotoxins and plant toxins**

This update was given by Frans Verstraete (DG SANTE). Ergot alkaloids: currently regulated as ergot sclerotia, it is foreseen that by July 2017 maximum limits will be established for the alkaloids themselves. Currently, with the Commission and EFSA (European Food Safety Authority), there are concerns on modified forms of mycotoxins (metabolites/conjugates). Modified forms of zearalenone, nivalenol, T2/ HT2 toxins and fumonisins, can add 100%, 30%, 10% and 60%, respectively, to the total exposure. For zearalenone (ZEN) a group TDI (total daily intake) of 0.25 µg/kg bw per day expressed as ZEN equivalents has been set for ZEN and its modified forms. To account for differences in *in-vivo* oestrogenic potency, each metabolite was assigned a potency factor relative to ZEN to be applied to exposure estimates of the respective ZEN metabolites.

Outlook on further developments in the future for mycotoxins in food feed:

- Follow-up on Recommendation 2013/165/EU on T2/HT2 toxin;
- Deoxynivalenol: updated risk assessment from EFSA expected early-2017 in which also acetylated derivatives and deoxynivalenol conjugates will be taken into consideration.
- On-going attention for alternaria toxins and on sterigmatocystin, citrinin (more occurrence data needed, more data on toxicity (genotoxicity and carcinogenicity)), and furthermore on "emerging" mycotoxins: enniatins, beauvericin. For moniliformin and diacetoxyscirpenol, EFSA opinions are awaited.
- Specific issues for feed include gossypol and guidance values for zearalenone, deoxynivalenol and ochratoxin A in pet food (cats and dogs) (Recommendation (EU) 2016/1319).
- Regarding plant toxins, a discussion is on-going on possible regulatory measures as regards the presence of pyrrolizidine alkaloids in honey, tea, herbal infusions and food supplements. Other plant toxins of current concern include: opium alkaloids, tropane alkaloids, THC (food of animal origin, hemp-derived food products), cyanogenic glycosides (apricot kernels). Future developments (requests to EFSA) are on glyco-alkaloids (solanine, chaconine etc) and quinolizidine alkaloids (lupins).

**EURL work program 2017**

Two proficiency tests for mycotoxins will be organised. One test on deoxynivalenol in a cereal product, and one on ergot alkaloids is foreseen. In addition, a proficiency test on plant toxins (pyrrolizidine alkaloids) will also be organised. Furthermore, the EURL will facilitate expert working groups on analytical topics as well as on plant toxin issues. Other activities include training and support for NRL staff on LC-MS/MS and analytical methodology more generally.

**Change of EURL**
H. Emons (JRC) presented changes that were taking place within the organisation of JRC. One of these changes is that JRC from 2018 on will no longer host the EURL for mycotoxins (and also not for heavy metals and PAHs). In 2017 new EURLs will be appointed and take over the EURL tasks from JRC from January 2018. At the same time, the mandate for the mycotoxin EURL will be formally extended to both mycotoxins and plant toxins.

### 4.1.2 Participation in working groups

The NRL is chairing the working group on identification criteria for mycotoxins in food and feed. This has resulted in a document that was discussed and endorsed at the EURL/NRL meeting (see above) and is expected to be published as a DG-SANTE guidance document by February 2017.

### 4.1.3 Participation in proficiency and comparative tests

In 2016, the NRL participated in four proficiency test on mycotoxins. Two were organised by the EURL (aflatoxins in peanut samples) and multiple mycotoxins in cereal products (aflatoxins, deoxynivalenol, zearalenone, T2/HT2-toxin, fumonisins B1 and B2, and enniatins and beauvericin). The other proficiency tests concerned aflatoxin M1 in milk powder and deoxynivalenol, zearalenone in feed, organised by third parties. For as far the reports on the outcome were available at the time of drafting this report, the mycotoxins were correctly quantified by the NRL (Z-scores within ±2). Besides proficiency tests on mycotoxins, the NRL also participated in one test on plant toxins (tropane alkaloids in cereals and herbal tea, organised by the EURL mycotoxins, see also 4.1.1). Also here Z-scores were within ±2 indicating good laboratory performance.

With respect to the determination of tropane alkaloids (plant toxins), an indirect comparison of the NRL method was done through the proficiency test organised by the mycotoxin EURL. Both the EURL and NRL provided a method for analysis to interested laboratories. In the evaluation of the results, the EURL made an inventory of results obtained by participants using the NRL method, the EURL method and other methods. The results turned out to provide comparable results.

## 4.2 Assistance to official laboratories

### 4.2.1 Quality control

In the Netherlands (besides by the NRL itself) official samples are analysed mainly by one OL, and, specifically for dairy products, by one additional laboratory that analyses aflatoxin M1. Both RIKILT and the main OL analyse most samples using multi-methods also covering many other mycotoxins than the regulated ones. The main OL participates in a number of proficiency test (both EURL and third parties) and quality control by the NRL takes place in the form of reviewing and discussion of the proficiency test results and follow up activities if required. Bilateral quality control of the official laboratories was done through exchange of samples. A set of cocoa powder samples from the main OL in which multiple mycotoxins were detected (including aflatoxins, ochratoxin A, citrinin, sterigmatocystin, beauvericin, and phomopsin A) was reanalysed by the NRL. For the dairy laboratory, quality control was done by sending standard solutions and milk samples containing aflatoxin M1. Results were reported to and evaluated by the NRL.

### 4.2.2 Advice

No specific advice was requested by the official laboratories. There were ad-hoc contacts with the OL in which technical information was exchanged.

## 4.3 Scientific and technical support to the competent authority

There are regular contacts between the NRL and the competent authority, both through bilateral meetings and national meetings of the *Expert working group on agricultural contaminants* in which, besides the competent authority and the NRL, also the Ministry of Health, Welfare and Sport and the National Institute for Public Health and the Environment (RIVM) participate. In these meetings, input is provide on technical aspects (e.g. feasible limits of quantification for certain emerging toxin/matrix combinations), and plans for explorative surveys on emerging mycotoxins and plant toxins are discussed.
In the frame of referee activities of the NRL, confirmatory analyses of aflatoxins and ochratoxin A in spices were done for the official laboratory to settle a dispute on exceedance of the maximum limits.

## 4.4 Contacts with other NRLs

Contacts with other NRLs were through the EURL workshop, through the CEN meetings on mycotoxins in food and mycotoxins/plant toxins in feed, and symposia (World Mycotoxin Forum/World Plant Toxin Forum, 6-10 June, Winnipeg, Canada).

# 5 National Reference Laboratory Heavy Metals

## 5.1 Activities within the EURL-NRL network

### 5.1.1 Participation in EURL-NRL workshops

In 2016 the EURL for Heavy Metals in Feed and Food (IRMM, Geel, Belgium) and the EURL for toxic elements in food form animal origin (ISS, Rome, Italy) organised proficiency tests on the determination of methyl-mercury (meHg), inorganic-arsenic (iAs) and total cadmium (Cd), lead (Pb), arsenic (As), mercury (Hg), tin (Sn), nickel (Ni) and iodine (I) in food and feed. The main objective of this exercise was to evaluate the capabilities of the NRLs in the determination of heavy metals according to Regulation (EC) No 1881/2006 and Directive 2002/32/EC. During the workshops results were discussed and enouncements were made. Furthermore the work programs of 2017 were discussed, and the EURL-NRL network agreed on the PT schedule for the coming year.

The 11th Workshop of the NRL-EURL network on Heavy Metals in Feed and Food was held in Geel, Belgium on 5 October 2016. A total of 52 participants attended the event, representing 27 NRLs. During this workshop, results were discussed on the PTs organized by EURL-IRMM on fish and palm kernal expeller, and information about recent developments of legislation and upcoming new issues about e.g. new legislation was presented. Furthermore, elemental speciation (iAs) was discussed based on the outcome of PT HM-22 and HM-43 and the skills required for elemental speciation of methyl mercury and inorganic arsenic in fish were tested. Finally, Hendrik Emons, Head of the Unit in charge of the EURL-HM, gave an update on the recent JRC developments resulting from the JRC restructuring. A poster session concluded the event, where each NRL presented their main achievements of the last 10 years.

For the NRL-EURL on toxic elements and heavy metals in food of animal origin, a workshop was held in Rome on 28 September. During this workshop, results were discussed on the PTs organized by EURL-ISS on honey and milk; and information and notes about legislation, results interpretation in relation to the maximal limits of metals in food, analytical measurements procedures, and upcoming issues about e.g. new legislation were presented and discussed. Furthermore theoretical exercises were done on: reference materials; result interpretation in relation to limit of detection (LOD), limit of quantification (LOQ) and measurement uncertainty.
The NRLs were requested to disseminate the information received during the EURL/NRL workshops to the official control laboratories and, more generally, to all laboratories performing food analyses.

### 5.1.2 Participation in proficiency and comparative tests

Three PTs were organised by EURL-IRMM (Geel), two by EURL-ISS (Rome). Furthermore, to cover the whole scope of the NRL-task, NRL participated also in six PTs organized by FAPAS:

- EURL-HM-22: total As, Cd, Pb, Hg, meHg and iAs in fish
- EURL-HM-23: total As, Cd, Pb, Hg, and iAs in palm kernel expeller
- EURL-HM-43: total As and iAs in rice
- EURL-ISS-24: total Cd, Pb and Hg in honey
- EURL-ISS-25: total Cd, Pb and As in milk
- FAPAS 07265: total As, Cd, Pb and Hg in offal liver
- FAPAS 07272: total Cd, Pb, Sn and Ni in vegetable puree
- FAPAS 07253: total As, Hg and meHg in canned fish
- FAPAS 07273: total As, Cd, Pb, and Hg in powdered rice
- FAPAS 07260: total I and Cd in infant formula
- FAPAS 01881: total I in milk powder

For the proficiency tests on heavy metals, the participants were requested to perform two or three independent analyses using the method of their choice for the determination of the elements in food and feed. Results of the proficiency tests have been presented and discussed with the NRLs during the EURL-NRL workshops, results from FAPAS PT were reported via FAPAS reports only. Some results were below the limit of quantification, therefore no results were reported to the proficiency test. The results for element speciation on MeHg and iAs as well as the results on total Cd, Pb, As, Hg Sn, Ni and I reported by the NRL were well within the expectable Z-score limits (Z-score should be between -2 and +2).

## 5.2 Assistance to official laboratories

### 5.2.1 Quality control

Since 2016, the OL does not have accreditation for the analysis of heavy metals in food and feed from the Dutch accreditation board (RVA). Since this date, the NRL has taken over the measurements and thus the responsibility for the analysis of the elements in food and feed. The OL works side by side with the NRL, so the information concerning quality control for the determination of heavy metals in food and feed has been discussed on a daily basis. Both the NRL as well as the OL were participating in the EURL PTs and the Z-scores were evaluated by the NRL and OL.

Furthermore, for quality assurance, the NRL has prepared a comparison study based on a the analysis of heavy metals in milk samples (food from animal origin). The samples were handed to OL and results were discussed during a NRL-OL meeting. The NRL has summarised the results in a small report which was sent to the OL.

Analyses for COKZ are carried out by a different OL. Therefore, the quality of that OL was assessed by the NRL. This study included several test rounds based on known samples, CRM materials or spiked samples. The results were discussed with the COKZ and reported. All results were good; Z-scores were within the range of -2 and 2.

### 5.2.2 Advice

Since the merger of the OL and NRL, advice on analytical measurements, quality and measurements strategies is given on a daily basis. Advising the OL COKZ is on ad-hoc base and related to the outcome of the comparison test organized two times a year by the NRL. In 2016 the results were good and no specific advice was given.

## 5.3 Scientific and technical support to the competent authority

Besides the heavy metals in food and feed, also the elemental speciation of arsenic and mercury species is an important topic in the EURL-NRL network. For the speciation of selenium and arsenic in feed, chrome, mercury, and arsenic in food, the NRL has several speciation methods developed in the last years in separate method development projects. These methods are based on HPLC coupled to ICP-MS measurements. In 2016 the NRL together with the OL analysed several fish and algae samples and the results reported to NVWA. These results on methyl mercury were published in a Poster presentation for the NVWA.

## 5.4 Contacts with other NRLs

During the EURL workshops the relationship with other NRLs was maintained. In addition, contact was had with the Danish and German NRLs about speciation via e-mail. In more detail, the speciation of arsenic was discussed with the Danish NRL and selenium speciation with the German NRL representatives.

# 6 National Reference Laboratory Polycyclic Aromatic Hydrocarbons

## 6.1 Activities within the EURL-NRL network

### 6.1.1 Participation in EURL-NRL workshops

In 2016, the NRL participated in the 11th workshop of the EURL for Polycyclic Aromatic Hydrocarbons held on 5 October 2016 at IRMM in Geel (Belgium). Delegates of 23 National Reference Laboratories (NRLs) and DG SANTE were welcomed at the meeting by the EURL director. During the meeting, legislation and the new broadened scope in relation to the EURL/NRL tasks and PT results of 2016 were discussed.

During the 11$^{th}$ workshop in Geel, an overview was given of the amendments of Regulation (EC) No 1881/2006 and Regulation (EC) No 333/2007. Special attention was paid to the new, broadened scope of the EURL and NRLs since January 2016. Work on MCPD, acrylamide, furans and PAH was presented and maximum levels for benzo[a]pyrene (BaP) and the sum of 4 target PAHs: benzo[a]anthracene (BaA), benzo[a]pyrene (BaP), benzo[b]fluoranthene (BbF) and chrysene (CHR) was discussed within the EURL-NRL network. During the meeting also the work program of 2017 was discussed, and the EURL-NRL network agreed to upcoming activities. In more detail also the new document about determination of LOD and LOQ by T. Wenzl was presented.

Results of the EURL PAHs PTs of 2016 on PAHs were discussed. Stefanka Bratinova (EURL) presented the outcome of the PT that was organised. 46 participants reported analysis results. The performance of most participants was satisfactory. More than 66 % of the results reported by NRLs and OLs respectively obtained satisfactory performance ratings. The rather lower rate of successful performance, compared to previous PTs, might be attributed to the complexity of the matrix, and the fact that more than a half of the participants did not have prior experience with it. The NRL reported results for the 4 PAH compounds which resulted in Z-scores between -2 and +2. The reported results were therefore considered as good. For the calculation of the Z-score for the sum of 4 PAHs, EURL used a smaller standard deviation compared to the deviation from PT results on the sum. This resulted in a high number of unsatisfactory results and the NRL scored a Z-score of 2.1 on this which is according to the PT report a "questionable" result.

The NRLs were requested to spread the information received during the EURL/NRL workshops towards the official control laboratories and, more in general, to all laboratories performing analysis of food.

### 6.1.2 Participation in working groups

Within the Netherlands, a workgroup is formed that focuses specially on the difficulties encountered in the analysis of PAH in herbs and food supplements. Delegates of Dutch trade/branch organization of the dried herbs and food supplement producing factories, the OL, contract laboratory, and the NRL are present in this workgroup.
During the first meeting in 2016 the workgroup had a meeting in Wageningen, the Netherlands. The agenda was focused on the differences between results from laboratories which analysed herbs and food supplements. During the second meeting, differences in analytical approaches, results and outcome of PT analyses of laboratories were discussed. In 2017 the workgroup will continue their discussion, the NRL is willing to organise a PT on the analysis of PAH in herbs and food supplements to investigate this issue further.

### 6.1.3 Participation in proficiency and comparative tests

Since the scope of the EURL and NRL is broadened and besides PAH also mineral oil, MCPD, acrylamide and furans are included; the NRL participated also in PT on these contaminants.

- EURL-IRMM PAH in black pepper
- FAPAS 0665 PAH in olive oil
- DUCARES 627 PAH in Rapeseed oil and Soya bean oil
- DUCARES 450 Mineral oil in Maize oil, Coconut oil and Rapeseed fatty acids

All results of the reported concentrations in above mentioned PTs gave satisfactory results. The sum of PAH in black pepper was questionable, Z-score was expressed on a theoretical calculated standard deviation and not a standard deviation from PT. This particular Z-score was 2.1.

## 6.2 Assistance to official laboratories

### 6.2.1 Quality control

On request by the Dutch controlling authority for milk and milk products (COKZ), the NRL performs analysis for PAHs in milk samples twice a year. In 2016 the OL was contacted to discuss their results on PAH analysis with respect to their participation in PTs and new information from the EURL on e.g. EU legislation.

### 6.2.2 Advice

Advice on PAH analysis, determination of LOD and LOQ procedures and other information from EURL were discussed with representatives of the OL. Since the NRL analysed official control samples milk for COKZ, advising is on ad-hoc basis.

## 6.3 Scientific and technical support to the competent authority

Due to some differences in analytical results reported by contract laboratories in the Netherlands and results reported by the OL; the NRL and OL have started a workgroup on this topic. The focus of the workgroup is to inform as many (Dutch) laboratories on the dos and don’ts in relation to the analysis of PAH in dried herbs and food supplements. Information on the used extraction techniques and the used separation and detection technique is collected by the branch/trade organization. This scientific and technical support is scheduled to continue in 2017.

## 6.4 Contacts with other NRLs

During the EURL workshops, the relationship with other NRLs was maintained. During the year 2016 the NRL has had contact with the EURL about the analysis of PAH in herbs and food supplements.

# 7 National Reference Laboratory marine biotoxins

## 7.1 Activities within the EURL-NRL network

### 7.1.1 Participation in EURL-NRL workshops

During the EURL annual meeting held in October 2016 in Porto, Portugal, results of various proficiency test (PT) studies and other issues were discussed. Paolo Caricato of DG-SANTE attended this meeting to share the vision of the EU Commission on several topics. He stated that discussions on several issues have taken place at Commission level, with the involvement of competent authorities and the EURL, and it was important that the EU laboratory network for marine biotoxins provided an ongoing input into these discussions. Items that were discussed in 2016 in the Commission were on tetrodotoxin (TTX) emergence and the related Dutch authority emergency measures, ciguatoxin in some EU Member States' waters and revision of current legislation.

Examples of these revisions are: possible deregulation of pectenotoxin (PTX), replacement of MBA as the reference method for paralytic shellfish poisoning (PSP) toxin analysis with the 'Lawrence' method (in line with Codex) and the establishment of an EURL guidance document to help clarify legislation for competent authorities and producers on chemical/biological contaminants. Furthermore, the discussions on international trade and 'equivalence' testing between the US and EU are almost finalized.

Next the minutes and conclusions of the previous EURL-NRL (October 2015, Cesenatico, Italy) workshop were reviewed. Some of the issues raised during this meeting were still outstanding and/or ongoing. For example several laboratories are still reporting results in the PT exercise below their limit of quantitation (LOQ). Another important issue was the deregulation of PTX, still no progress was made due to lack of agreement at the competent authority level. The need for the NRLs to provide their opinion to the competent authority was re-emphasized as none of the NRLs present has any objections with the deregulation of PTX. The Dutch NRL highlighted that NRLs should inform their official control laboratories that they should apply the Annex C of the SOP for lipophilic marine biotoxins (correction for losses during processing) in order to have consistent results between Member States. Further, the EURL responsible person for the algae working group (WG) gave an update on the activities of the WG (see participation in working groups) and presented the ideas for 2017. They will focus on population trends and representative testing methods, emerging risks (i.e. sampling methods for epibenthic microalgae and bacterioplankton), species identification and, important for The Netherlands, thresholds and triggers. The EURL indicated that often deadlines of this WG were not met most likely due to the fact that most NRLs have an invited national expert other than the NRL representative in this WG. Therefore, it was recommended to keep the NRLs also in the (e-mail) communication so they can follow up the deadlines with their national expert.

### 7.1.2 Participation in working groups

The NRL participated in several working groups in 2016. At the end of February, the NRL visited the EURL in its role as EURL advisory board member. Several topics were discussed such as decisions foreseen in the CODEX CCMAS to change the mouse bioassay to a type IV method, which implies that the method can't be used as reference method for PSP toxins anymore. Further the possible organisation of an expert meeting on emerging toxins such as TTX, ciguatoxins and brevetoxins was discussed. In April a WG meeting on phytoplankton was convened in Madrid in order to establish guidelines for phytoplankton monitoring. As the NRL has limited experience in phytoplankton monitoring, an expert of Wageningen Marine Research (IMARES) was approached to represent the Dutch NRL during these meetings. Discussions were held on how to sample various water bodies, perform sample preservation and storage, species identification and population trends during this meeting. It became clear that the various Member States have their own preferred approach often

based on historical experience. By the end of 2016 the group reached agreement on the method of sampling (integrated water column) and sampling frequency. Still the progress of the working group is relatively slow due to the complex character of algal monitoring and the differences in the various countries.

In June the LC-MS/MS working group convened in Madrid to discuss several topics. The first discussion was on the improvement and update of the reference SOP for lipophilic marine biotoxins. As this SOP has now already been applied for over 5 years it is suggested to revise the method with new insights due to the experience that all NRLs have nowadays with the method. Examples are: improved LC methods, new LC column technologies, etc. Annex C of the lipophilic marine biotoxin SOP on the analysis of processed samples was discussed. This annex was established in order to apply correction factors to test the compliance of processed shellfish products against the EU established legislation which is set for the edible part of live bivalve shellfish. Although all NRLs accepted the approach when this procedure was established (2015), currently some of the NRLs (DE and DK) suggest that by applying these factors there might be a possible risk for consumers. The discussion is currently ongoing on the level of the EU commission as it is a risk related issue.

The work of the Dutch NRL on TTX was presented and recent findings and developments were shared with the other NRLs. The last issue presented was the outline of an EFSA project on ciguatoxins. This project has a focus on risk characterisation of ciguatera food poisoning in Europe. In September a meeting was organized by the University of Vigo on emerging toxins. Three toxin groups were discussed in depth with experts from around the world including representatives from Europe, Japan, US and New Zealand. The three toxin groups discussed were brevetoxins, ciguatoxins and TTX and the topics discussed were on method development, reference standards and occurrence and legislation.

### 7.1.3 Participation in proficiency and comparative tests

Results of the annual PT on the amnesic shellfish poisoning (ASP) toxin domoic acid in shellfish were discussed during the annual EURL-NRL meeting. There was not much discussion on the ASP group as most laboratories had satisfactory results. The NRL had Z-scores below |2|, which indicates that the method is performing satisfactorily. There was only a short discussion on whether LC-MS/MS methods can be applied during the PT, the EURL re-iterated that the proficiency tests were for evaluation of methods that are included in the legislation. So for domoic acid this is solely the LC-UV method.

Next the results of the PT on PSP toxins in shellfish were discussed. In general with the PSP proficiency test the number of non-satisfactory results are somewhat higher compared to ASP. Again the NRL performed satisfactorily for the total toxicity, for sample 1 a Z-score of 0.9 was obtained, sample 2 was blank and for sample 3 a Z-score of 1.8 was obtained. For the individual toxins only for the toxins C1,2 and C3,4 respectively questionable (Z-score 2.2) and unsatisfactory (Z-score 4.4) were obtained. For the latter most likely the deviation is caused by the lack of a certified standard solution. Analysis of C3,4 is done by an indirect analysis after hydrolysis without the availability of a standard therefore there is no option to correct for hydrolysis efficiency, recovery losses, etc. The contribution of C1,2 and C3,4 to the total toxicity is relatively small due to the low toxicity factors of these toxins. In order to improve the performance and get more experienced, samples displaying a relative unknown toxin profile for the Netherlands, containing C1,2 and C3,4 toxins, will be analysed in 2017. Furthermore, the NRL participated for PSP toxins in the Quasimeme PT with the LC-MS/MS method. This method is applied in our routine control program as screening method. All positive samples analysed with this method were identified as suspect and therefore the performance of the screening method is satisfactory.

In the PT on lipophilic marine biotoxins most laboratories performed satisfactorily. Some laboratories are still reporting low results for toxins <LOQ and participants were reminded to check their reporting criteria and LOQs. In total three samples were analysed by LC-MS/MS. The Dutch NRL performed satisfactory for the total toxicity content in all samples (Z-scores < |2|). Sample 1 was a blank mussel sample, in sample 2 a Z-score of -1.7 was obtained for OA group toxins and 0.3 for the yessotoxin group. For Sample 3 a Z-score of -1.3 was obtained for the OA group toxins and 0.0 for the azaspiracid group toxins. For the individual analogues the NRL obtained good results with the

exception of total content of dinophysistoxin-2 (DTX2), here questionable results were obtained, respectively -2.4 and -2.2 for sample 2 and 3. Cause of the error is most likely related to the preparation of the standard solution of DTX2, re-analysis of PT samples is foreseen for the first quarter 2017.

## 7.2 Assistance to official laboratories

### 7.2.1 Quality control

Besides the NRL, there is one OL. In order to perform quality control, the NRL advised the OL to participate in the Quasimeme PT scheme for all available toxin classes, respectively ASP, PSP and lipophilic toxins. Results of 2016 were not yet available at time of writing.

### 7.2.2 Advice

The results of the 2015 Quasimeme PT assessment of the OL were evaluated by the NRL together with the OL. Based on this evaluation, assistance was given on various aspects. The OL was informed of the activities of the NRL in 2016 during the annual NRL meeting held early 2017.

## 7.3 Scientific and technical support to the competent authority

Over the course of 2016, there was intensive contact between the NRL, NVWA and the Ministry of Health, Welfare and Sport on the TTX issue multiple times. The NRL presented on behalf of the NVWA the results and front-office opinion to the shellfish industry in February. The NRL participated in a bivalve expert meeting in Brussels in may where the Netherlands was requested to present its findings and decisions taken on the presence of TTX. Also in May the NRL contributed to the preparation of a NL request for the preparation of an EFSA scientific opinion on TTX. Finally during the TTX episode in June and July 2017 there was frequent communication between employees of the NVWA and the NRL on the issue.

## 7.4 Contacts with other NRLs

During the TTX episode in June and July, the NRL was contacted by the NRLs of Ireland and the United Kingdom to ask about clarification on the issue. Also the UK NRL was contacted by the NRL to perform some peer review of our findings by analysing samples for the presence of TTX. Further the NRL in Germany send some ciguatoxin research samples which were screened for the presence of a neurotoxin by the Dutch NRL.

# 8 National Reference Laboratory NRL 96/23/EC

## 8.1 Activities within the EURL-NRL network

### 8.1.1 Participation in EURL-NRL workshops

The NRL participated in the EURL-Workshop in Berlin, held from the 14th until the 16th of June and which was organised by German Federal Office of Consumer Protection and Food Safety (Bundesamt für Verbraucherschutz und Lebensmittelsicherheit (BVL)). This workshop consisted of a theoretical part and a practical part. In addition to the regular program (such as discussing the results of organized proficiency testing, developments in the field of residues for which BVL is responsible (nitroimidazoles, coccidiostats, NSAID's, beta-Agonists and anthelmintics)), also the following topics were give attention to: influence of Hydrolysis on the Total Amount of (marker) Residue, discussion on the Revision of Decision 2002/657/EC (in particular the points "decision limit" and "detection capability" as well as the question of the "sum-MRL").
Secondly, the NRL participated in the EURL-Workshop in Fougères, held on the 6th and 7th of October. This workshop for the Control of Antimicrobial Residues in Food from Animal origin Advances in LC-MS/MS method for Screening and Confirming Antibiotics, was organised by ANSES. This workshop consisted of a theoretical part and a practical part. Some topics of this Workshop were: 'Converging method for Screening for more than 70 antibiotics in meat and fish by UHPLC-MS/MS', 'Overview of the Antibiotic Residues PT Program over the period 2015-2016', 'News on the Advances on Screening by UHPLC-MS/MS', 'Testing for Dye Residues in Aquaculture'.

Thirdly, the NRL organized the EURL-Workshop in Wageningen, held from the 14th until the 16th of November. This workshop also consisted of a theoretical part and a practical part. Some topics of this workshop were: 'Update EU-RL activities including Proficiency testing programme', 'Discussion on draft documents revision 2002/657', 'NRL open forum with topics like: - Interfering peaks: any solutions?; - Data mining to find new trends in growth promoter use; - GC-Orbitrap-MS for steroid analysis; - Ambient Ionisation Techniques for MS'.
Experts from the NRL in the EU Member states, but also representatives of candidate or third countries, participated in the above mentioned workshops.

Other workshop-related activities were:

- The NRL has presented a lecture entitled 'RIKILT and Research on antibiotics' during a study day for Inspectors of the Government of Netherlands and Belgium; May 11, Barneveld.
  The NRL had presented a lecture entitled 'RIKILT and Research on antibiotics' during a study day for Inspectors of the EU countries; May 24, Den Haag.
- The NRL was invited to participate in the '2016 International Symposium and Annual Meeting of the Korean Society of Environmental Agriculture; Integrated Management of Agriculture Environment for Food Security' from 7-9 July in Busan, Korea and presented a lecture with the title 'Zero-tolerance policy what does it mean for food safety and food security'.
- The NRL participated in the Global Action Summit in Nashville, Tennessee USA 14&15 November and was a speaker in the session 'Food Safety: Threats, traceability and Forensics.
- The NRL participated in the Workshop "Detection of NSAIDs in Mild/Meat by LC-MS/MS' and the communication group, NRL Chemistry, 96/23 and Marine Biotoxines, which was held on the 25th of November and was organised by ILVO-T&V (Melle, Belgium).

### 8.1.2 Participation in proficiency and comparative tests

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

| Description | Organizing institute | Z-score | Assessment |
|---|---|---|---|
| Quinolones and macrolides in Honey, quantitative confirmation | FAPAS | Between -0.5 and 1.2 | Sufficient |
| Coccidiostats in poultry meat, quantitative confirmation | Progetto Trieste | Z-score: -4 | Deviate result |
| Antibiotics, Anthelmentica and coccidiostats in feed, (quantitative) confirmation | RIKILT | Between 0.14 and 1.39 | Sufficient |
| A3 steroids in bovine urine, (quantitative) confirmation | RIKILT | 1.72<br>2.88 | Sufficient<br>Deviate result |
| Beta-Agonists in bovine urine, (quantitave) confirmation | EURL-BVL | Between -1.2 and 0.1 | Sufficient |
| Antibiotics in porcine muscle, quantitative confirmation | EURL ANSES | Between 0.34 and 1.26 | Sufficient |
| NSAIDs in milk, quantitative confirmation | EURL-BVL | Between 0 and 0.3 | Sufficient |
| Veterinary drugs low level – Feed (quantitative) confirmation | Ducares | Between -0.1 and 1 | Sufficient |
| Nitrofurans in shrimp, confirmation | FAPAS | Identity of the compound was confirmed | Sufficient |
| Coccidiostats in feed, quantitative confirmation | FAPAS | Between -0.5 and 1.2 | Sufficient |
| Tetracyclines in shrimp, confirmation | FAPAS | Identity of the compounds were confirmed | Sufficient |
| Sulfonamides in honey, quantitative confirmation | FAPAS | –2.1<br>–1.6 | Deviate result<br>Sufficient |
| Nitrofurans in shrimp, (quantitative) confirmation | FAPAS | 0.9 and identity of the compound was confirmed | Sufficient |
| Chloramphecol in honey, quantitative confirmation | FAPAS | 2.8 | Deviate result |
| Quinolones and avermectins in Fish, (quantitative) confirmation | FAPAS | 5.4<br>between – 1.8 and -1.2, identity of the compound was confirmed | Deviate result<br>Sufficient |
| Beta-Agonists in liver, quantitative confirmation | FAPAS | Between -1.4 and - 0.6 | Sufficient |
| Steroids in bovine urine, quantitative confirmation | FAPAS | Between 0.1 and 0.8 | Sufficient |
| Tetracyclines in porcine meat, quantitative confirmation | FAPAS | Between 1.1 and 2.0 | Sufficient |
| Coccidiostats in egg, (quantitative) confirmation | FAPAS | 1.1<br>The identity of the compound was confirmed (sufficient result) | Sufficient<br>Sufficient |
| Sulfonamides in porcine meat, (quantitative) confirmation | FAPAS | Identity of the compound was confirmed and/or between 0.2 and 1.4 | Sufficient |
| Beta-Agonists in porcine urine, quantitative confirmation | Progetto Trieste | Between -1.52 and -0.45 | Sufficient |
| Corticosteroids in bovine urine, quantitative confirmation | Progetto Trieste | 1.68 | Sufficient |
| Tylosine and streptomycine in honey, (quantitative) confirmation | Progetto Trieste | -0.62 and identity of the compound was confirmed | Sufficient |
| Stilbenes in bovine or swine urine, quantitative confirmation | Progetto Trieste | 0.23 | Sufficient |
| Steroids in bovine liver, confirmation | Progetto Trieste | Identity of the compound was confirmed | Sufficient |
| Antibiotics in honey, (quantitative) confirmation | EURL-ANSES | -0.9 and identity of the compounds was confirmed | Sufficient |
| Melengesterol acetate in meat, quantitative confirmation | RIKILT | Between 0.09 and 0.47 | Sufficient |
| Antibiotics in bovine muscle, quantitative confirmation | RIKILT | -0.28 and 0.95<br>3.48 | Sufficient<br>Deviate result |
| Parasiticides and antibiotics in salmon, quantitative confirmation | RIKILT | 0.22 and 1.52<br>3.03 | Sufficient<br>Deviate result |

Corrective actions taken in response to the deviate results:

- Coccidiostats in poultry meat; quantitative result differs from the assigned value. Corrective action: Proficiency sample is reanalysed using a different analytical method and different analytical equipment. New value gives a sufficient z-score.
- Antibiotics in bovine muscle; quantitative result differs from the assigned value. Corrective action: a new reference standard was obtained and the sample was reanalysed. The obtained z-score now was < 2.
- Chloramphenicol in Honey; z-score was 2.8. Corrective action: sample is reanalysed and the outcome gives a z-score of 1.4 (probably in the first analysis an incorrect standard solution was used).
- Quinolones and avermectins in Fish; a z-score of 5.4 was obtained. Corrective action: sample is reanalysed and the outcome gives a z-score of – 0.75 (probably in the first analyses something went wrong in the sample preparation).
- Parasiticides and antibiotics in salmon; a z-score of 3.03 was obtained. Corrective action: used method was suitable for the confirmation of the identity of the compound but not for a quantitative result. No further action will be taken.
- Sulfonamides in honey; a z-score of -2.1 was obtained. Corrective action: problem was probably caused by the hydrolysis step in the sample clean up. Sample is reanalysed using a different hydrolysis step. A z-score of -1.7 was now obtained.
- A3 steroids in bovine urine; a z-score of 2.88 was obtained. Corrective action: sample is reanalysed with a new developed method, the z-score obtained with this method was 0.86.

## 8.2 Assistance to official laboratories

### 8.2.1 Quality control

Pursuant to the NRL tasks, the OLs are supervised. The NRL assures the analyses of the OL which are carried out within the framework of the National Sampling Plan, through a third-line control program. The NRL also supports the OL 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 Directive 96/23/EC. The current control program (third-line control program) includes 39 analyte/matrix combinations. A monthly evaluation of the outcome of this control program takes place and an annual trend analysis based on those results is performed and reported separately.
Five Technical meeting between the NRL and the OL were held in 2016 to inform one another of developments, discuss analytical issues, and establish corrective actions. This meeting also includes discussing the third-line control program.

### 8.2.2 Advice

The Q3 meeting (consisting of the NRL, OL and Ducares) was held once in 2016 (2$^{nd}$ of November). In addition to this Q3 meeting, there is also the so-called Technical Q3 meeting. This meeting is between the NRL and OLs and also the laboratory involved in the company self-control (Ducares and Qlip). In 2016 there were no subjects to discuss, therefor there was no Technical Q3 meeting in 2016.
The NRL discussed the problems or the origin of the impurities which were found in Tilmicosin formulations with Ducares and Elanco in 2016.
In 2016, contra-expertise was performed six times for the OL. These were for Trenbolone in porcine urine, Semicarbazide in liver originating from calf, goat and sheep, Semicarbazide in meat from pigeon, and Ponazuril in egg.
According to the request of the OL and the Competent Authority, the NRL has prepared a recommendation about the finding of Semicarbazide in liver originating from calf, goat and sheep.

## 8.3 Scientific and technical support to the competent authority

The NRL participated in the meeting of the policy working group "National Plan residues", this meeting was held on the 9th of June 2016.
On the 16th of February and 13th of September, two meetings were held with the NRL and the OL about the implementation of antibiotic analysis in dairy product which are exported to Russia.

The NRL participated in the mini-symposium 'Verboden Stoffen' (Illegal substances), held on the 24th of November which was organised by RIKILT and NVWA.
The NRL has presented the following topics: 'Nieuwe oplossingen voor oude en nieuwe vragen – effectieve controle op natuurlijke hormonen' (New solutions for old and new questions - Effective control of natural hormones); 'Opsporing rundergroeihormoon in melk, een decennium lang een uitdaging' (Detection of bovine growth hormone in milk, a decade long a challenge); 'Surveillance en opsporingsonderzoek op verboden stoffen; de praktijk' (Surveillance and detection of illegal substances: in practice); 'Doorkijk naar de toekomst, nieuwe wetgeving en opsporingsmethodieken' (Looking forward to the future, new legislation and detection methods).
This symposium was organised for the stakeholders (NVWA, Ministry of Economic Affairs) and took place at the Ministry of Economic Affairs.

# 9 NRL animal proteins

## 9.1 Activities within the EURL–NRL network

### 9.1.1 Participation in EURL-NRL workshops

The annual meeting of the EURL and the NRLs was organised in September in Namur (Belgium), also to celebrate the tenth anniversary of the EURL AP. In one and a half day a range of topics was addressed, including the results of the grinding study, first attempts to monitor the presence of insects in feed, the results of the annual proficiency test, a survey on pig and poultry feed, the announcement of a GTH proficiency test, the implementation of the pig PCR, the development of the poultry PCR calibrants, and the EURL working programme. In addition to this annual meeting, a half-day symposium was organised with presentations of European Commission, a competent authority from a Member State (Belgium), private companies (Darling Ingredients) and from the EURL.

The issues for recognition of insects by means of microscopy, PCR and mass spectroscopy were presented and first results were discussed. Insects belong to the large phylum of Arthropods, including crab, lobsters, shrimps and krill, which are look-alikes at the microscopic level. Despite this, preliminary information was gathered on discriminating features. Using a set of primers and two different probes, 37 different insects were identified by PCR. The preliminary results for MS indicated that the databases to be used for matching need extension.

Several parameters have been tested in the framework of the grinding study. These include the type of equipment, the duration and the rounds per minute of the grinding process and the effect of staining. The Alizarin staining as included in the EU legislation generally causes a higher number of bones to be detected, which could influence the evaluation of the result in the view of the legal LOD. Other results are difficult to interpret at this time.
So far, analyses of 14 samples of pig feed and eight samples of poultry feed gave preliminary results of absence of bone fragments of land animals. In several samples whey powder, blood products and pig fat were found however.
The NRL was present with experts on light microscopy and PCR. They participated actively in the discussions and in the informal lobby contacts. NRLs were invited to present posters. The NRL presented a poster about the PPS project on the development of a poultry duo-test with PCR and immunoassay.

### 9.1.2 Participation in Working groups

The official advisory groups were not continued in 2015. The NRL maintains active communication with the EURL AP and stimulates cooperation.

### 9.1.3 Participation in proficiency and comparative tests

In January 2016, the NRL performed the experiments and reported the results of an EURL-Animal Proteins (AP) pig PCR implementation test that was started at the end of 2015. In this implementation study, nine samples were analysed for the presence or absence of pig DNA. The NRL was informed by email by the EURL AP that all results were correct. A report has not yet been published.

At the same time a ruminant proficiency test was finished and reported to the EURL-AP. This test was a combined microscopy and PCR proficiency test. The test comprised a total of 10 samples of which seven samples had to be analysed with light-microscopy and six with PCR. This means that three of these samples had to be analysed both with light-microscopy and PCR. The final request in the scheme was to analyse two samples in duplicate by PCR. In May 2016 the final report of this proficiency test was issued. The NRL reported only one error for the microscopic detection of fish

meal. All results for PCR results were correct. One sample contained ruminant blood meal. This was microscopically detected by 10 out of 27 participants, the NRL among them.

The samples for the annual proficiency test 2016 have been sent around in November. This test combined (again) microscopy and PCR analyses. The proficiency test of 2016 comprised a total of eight samples, all of them to be analysed by microscopy and five by PCR (ruminant). Results were send in before the deadline of 9th December. Preliminary information revealed afterwards indicated the presence of feather meal in one of the samples. The NRL found shell fish scales in two other samples without any other presence of fish meal.

## 9.2 Assistance to official laboratories

The Netherlands do not maintain a national network of official control laboratories within this NRL field. Therefore no official activities were performed for this task.

## 9.3 Scientific and technical support to the competent authority

The NRL has provided support to the NVWA and the Ministry of Economic Affairs (EZ). Most prominent activity was a round table discussion organised by the NRL with participation of NVWA, Ministry of EZ and the Ministry of Health, Welfare and Sport. The main reason for this discussion was because PCR based identification methods for ruminants, pigs and horses had been validated and that a PCR method for chicken and turkey was on its way. As an alternative, in order to avoid unwanted detection of milk and its products, pig gelatine and egg materials, respectively, tissue specific identification by means of immunoassay tests is under development. New processing techniques in the feed industry, such as hydrolyzation and the use of novel feed ingredients, require an evaluation of the current mix of detection and identification methods.
The round table discussion was aimed at providing all relevant information to the participants, and at finding the need for future method development. Therefore, the meeting was a product of the NRL AP project and a project for development of methods. The outcome was directed towards a research agenda for 2017-2019 and to support the national competent authority.

## 9.4 Contacts with other NRLs

The NRL serves as scientific officer and qq. as board member of the IAG section for Feed Microscopy. The EURL AP and most NRLs are member of this section. During the IAG annual meeting in June every year and if necessary during other meetings, exchange of viewpoints and information is stimulated. On behalf of the board, the NRL organises the annual IAG proficiency test on animal proteins in feed, the report of which is published annually. This flow of information is regularly discussed in the meetings and complements the exchange of information of the EURL/NRL AP network.

# 10 NRL Feed Additives and national evaluation of dossiers

## 10.1 Activities within the EURL – NRL network

### 10.1.1 Participation in EURL-NRL workshops

The annual workshop for Feed Additive Authorization was organized by the EURL, JRC-Geel, 21-22 November 2016 in Brussels, Belgium. The problems related to the presence of the mutagenic compound p-phenetidine in the antioxidant ethoxyquin were discussed in presentations from DG SANTE and FEFANA. For fishmeal no alternatives are available for the use of this antioxidant. For this reason DG SANTE requires that the content of p-phenetidine will be reduced to 2.5 mg/kg or less. In the presentation of FEFANA, the results of the development of methods for p-phenetidine in feed and animal tissues (muscle, liver and fat) were reported.

In a presentation of EFSA, among others it was reported that some applications potentially concern nanoparticles in the range of 1 – 100 nm. It is not always clear which percentage of the additive consists of nanoparticles. EFSA also reported that an application for authorization of a feed additive had been submitted for selenised yeast that contains selenocysteine, for which EFSA was asked to perform an assessment. It was concluded, *inter alia*, that an analytical method should be submitted to determine the selenocysteine content.

The EURL reported that the methods of analysis that are submitted in the dossiers will be made public on the EURL website. This concerns the methods for the active substance in the additive, in pre-mixtures and in feed but also, if applicable, the methods for residues in animal tissues. Exceptions are made if the methods are under copyright, e.g. CEN- or VDLUFA-methods, or if the methods are available elsewhere, e.g. official EU-methods.

In a combined presentation from DG SANTE and the EURL, the issues regarding the presence of traces of GMO-organisms in vitamin B2-preparations were reported. Worldwide, for the production of vitamin B2 GMOs are applied. Applicants have to indicate which GMO has been used, also for products that have been produced outside Europe. Producers have to purify the additive to such an extent that the GMO is no longer present. A German OL has detected a GMO in reference samples obtained from the EURL and the GMO proved to be viable because it could still produce vitamin B2. Moreover, the GMO contained resistance genes against certain antibiotics.

### 10.1.2 Dossier evaluation on the request of the EURL

In 2016 the NRL commented on 12 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 (anti-caking agents, binders), sensory additives, coccidiostats, nutritional additives (amino acids, trace elements) and zoo-technical additives.

### 10.1.3 Participation in proficiency and comparative tests

The EURL has organised a proficiency test (PT) for vitamins A en E. The Dutch NRL has not participated in this PT because a method was not yet implemented in the laboratory for this group of additives.

## 10.2 Scientific and technical assistance to the competent authority

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

In 2009 a total number of 29 national requests for permission to use substances – which have not been authorized at Community level – as additives for experiments for scientific purposes (in the frame of Regulation (EC) No 1831/2003, article 3.2) have been assessed. This assessment concerned the mixing of the additive in feed and possible risks related to cross-contamination to other feeds. The requests concerned, among others, enzymes, probiotics, amino acids, vitamins and herbal preparations.

Starting from November 2014, the evaluation of GMO aspects is also performed by the Dutch NRL. In 2016, for 26 national requests it was evaluated whether it concerned GMOs or additives produced by GMOs. This was a bit less than in 2015, but there were more applications with combinations of GMOs. In many cases it was concluded that the applications indeed contained additives produced by GMOs. In those cases it was evaluated if there were specific concerns related to the safety for humans and animals and whether the applicant submitted enough information to assess these aspects. In a limited number of applications, supplementary information was requested. In 2016 no application was rejected due to GMO safety aspects.

### 10.2.2 Other scientific and technical assistance

Among others the NVWA was advised about the interpretation of the statement "99 % purity" for choline chloride in additives that contain much less than 99 % choline chloride and about possible risks of the presence of impurities of biphenyl in additives that contain benzoic acid. Biphenyl is a substance that is registered as a pesticide but it is also an impurity in the production of benzoic acid.

# 11 National Reference Laboratory for GM food and feed

## 11.1 Activities within the EURL–NRL network

### 11.1.1 Participation in EURL-NRL workshops

In 2016, the NRL participated in two European NRL meetings: the combined 25th NRL/ENGL (European Network of GMO Laboratories) meeting in April 2016 and the 12th NRL 882 2004 Workshop with 25th ENGL meeting in September 2016, both in Ispra, Italy. The NRL also participated in the Steering Committee meeting in February 2016 that was organised to prepare the NRL and ENGL Workshops.

At the 25th ENGL meeting the following topics were addressed: the outcome of the 30th ENGL SC meeting (February 2016), updates of SANTE, unauthorised rapeseed Oxy-235 (EURL GMFF and SANTE), the possibility of a Network for Species Identification (JRC), CEN and ISO activities (Dr. L. Grohmann, BVL, Germany). Also the progress in the ongoing working groups was presented (AG SMV (Advisory Group on Selection of Methods for Validation), WG on unit of measurement, WG on digital PCR, WG on update of methods). In the scientific and technical session there were presentations on the activities of the Custom Laboratories European Network (CLEN) (Dr M. F. Filippi, Italian Customs Agency-Chemical laboratory of Turin, Italy), recent developments under the Cartagena Protocol on Biosafety and the Biosafety-Clearing House (Dr A. Bowers, Dr M. Pessoa de Miranda, Biosafety Clearing House), single lab validation of qualitative qPCR methods on the basis of POD modelling (Dr L. Grohmann, BVL, Germany). There were also two breakout discussion groups: one on NGS data quality control and regulatory use and the other on GM animals. During this session the members of the WG on ENGL Procedures met for the WG kick-off meeting.

At the 12th NRL882 2004 workshop a Tour de table was held. The issues/opinions/training needs of NRLs were expressed: the participating NRLs summarised their control activities in the past year. On average, 200-300 official control samples of food/feed/seed were analysed per year. Most, if not all of the food and seed samples analysed were found to be negative for unauthorised GMO presence, while most of the feed samples were only containing authorised GMOs. There were a few GM-positive papaya food samples and some incidences of GM rice.
Many NRLs reported the increasing workload due to the accumulating number of GM events to be tested. This requires a need for multiplexing approaches. Several NRLs are also active in other domains, e.g. allergen screening, developing a method for GM salmon, or detection of GMM (Genetically Modified Micro-organisms).
Some NRLs requested training on digital PCR. Further suggestions included NGS, implementation of multiplex PCR, DNA extraction, use (and production) of PSPs, and the use of CRMs at 0.1% (m/m). The JRC concluded that a new training on droplet PCR will be organised in 2017, as well as a workshop / training on DNA extraction experiences.

The JRC gave a summary of Comparative Testing (CT) round CT 01/16 and the observed reasons for obtaining an unsatisfactory performance by some of the laboratories. There was a good balance between food and feed samples and between more complex and easier matrices. It was suggested that the number of GM events to be tested should be limited and the type of crops and events should be in line with market prevalence. Events falling under Regulation (EU) No 619/2011 should not be included too often and too soon after validation; these events are rarely found in control samples.
JRC presented the status of the Pre-Spotted Plates (PSP) project. There is a large interest among NRLs for implementation in the routine control activities, so DG SANTE continues to support the project.
At the 26th ENGL session updates were given on the same topics as mentioned above in the text on the 25th ENGL Workshop. The WG on Method Verification reported that it has finished the revised document. It will first be reviewed by the Steering Committee and then presented to the ENGL Plenary Meeting approval.

In the scientific and technical session there were the following presentations:
- Update from the JRC Advisor for the Bioeconomy (Dr J. Kreysa);
- Technical study to assess the need for harmonisation of sampling and analysis methods for GM material in food (Dr I. Ciabatti);
- Enlargement and capacity building project, the West-African Economic and Monetary Union (WAEMU)
- Regional biosafety program and the Community regulation project, discussion on a training workshop on DNA extraction, high-tech strategies for monitoring of unauthorised GMO on the EU market (NGS and genome walking);
- Multiplex digital PCR method for GM soybean, validation of digital PCR methods.

Finally, there were three break-out discussion groups: on synthetic biology, strategies for handling of increasing numbers of GMOs and conversion of mass-mass percentages to copy numbers.

### 11.1.2 Participation in Working groups

The NRL participated in the Working Group Update of the Method Verification document. This document gives guidance how to verify qualitative screening methods and quantitative GMO detection methods. Several web meetings were attended for this purpose. This Working Group was financed as part of the GMO method validation project WOT-02-004-005 ('Validatie en accreditatie van detectiemethoden voor genetisch gemodificeerde organismen').

The NRL also participates in the WG on ENGL Procedures. The goal of this Working Group is to review the ENGL procedures and where needed propose modifications where needed. A first tele-meeting has been convened on the topic. The ENGL secretariat is compiling an overview of current procedures as a basis for further steps.

### 11.1.3 Participation in proficiency and comparative tests

Participation in the two EURL GMFF proficiency tests each year is mandatory for the NRL 882 2004 laboratories. In these proficiency tests food and feed samples are tested for GMOs and detected GMOs are quantified. The tests contain unprocessed samples as well as more difficult processed matrices. The 2016 proficiency tests included detection and quantification of several GMOs in Mexican tortilla, maize flour, rapeseed cake and soybean flour. The NRL obtained good Z-scores for all quantifications. In one proficiency test a sample was tested twice by accident. As a follow up the EURL GMFF has advised to double check if the right sample is taken for DNA extraction.

## 11.2 Assistance to official laboratories

### 11.2.1 Quality control

Both the NRL and the OL participated in the same two comparative testing rounds organised by the EURL-GMFF. Good Z-scores were obtained by the OL, but not all GMOs were quantified. After the report was received the data and the results for the first EURL GMFF 2016 comparative testing round of the NRL and the OL were discussed in a joint meeting.

### 11.2.2 Advice

The OL has implemented an element screening strategy for screening of non-GMO labelled food samples, based on a set of GMO elements proposed by the NRL. In 2016, the NRL advised the OL on the implementation of a suitable endogenous PCR method for tomato detection.

## 11.3 Scientific and technical support to the competent authority

In 2016 the NRL advised the competent authority with regard to a more risk-based sampling strategy for the Dutch GMO monitoring program. To this end a module GMonitor has been developed in 2015: the module uses e.g. 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 EU-unauthorised GMOs. Also information was shared on GMO related topics and recent developments, such as developments linked to Next Generation Sequencing in the EU Decathlon project, coordinated by the NRL, and with relation to the EUginius database, as has been constructed by RIKILT and BVL (Bundesamt für Verbrauchersschutz und Lebensmittelsicherheit) in Germany.

## 11.4 Contacts with other NRLs

Contacts with other NRLs mainly took place during the two NRL/ENGL meetings, the Working Group meetings and the ENGL Steering Committee meetings. The Hungarian NRL was assisted by email with interpretation of their screening data. For data interpretation the EUginius database was used http://www.euginius.eu/euginius/pages/home.jsf.

# 12 National Reference Laboratory Milk and Milk products

## 12.1 Activities within the EURL-NRL network

### 12.1.1 Participation in EURL-NRL workshops

The NRL participated in the annual EURL workshop, held on 5-6 October 2016 in Paris (Maisons-Alfort). This annual workshop dealt with all sub-topics of this EURL/NRL domain, being hygiene of raw milk (total flora and somatic cell count), and heat tracers (alkaline phosphatase).
DG Agri was represented, and presented the draft version of a revised version of Regulation (EC) No 882/2004, which is expected to be published early 2017. For this NRL, the effects of the update are expected to be minor, but the EURL, for example, will be obliged to provide reference materials to the NRLs, which is considered a key issue and an improvement for this field.

**Hygiene of raw milk, total flora**
The total flora represents the number of microorganisms present in a certain product. Regulation (EG) No 853/2004 lays down limits for this number, which is $<1 \cdot 10^5$ colony forming units (CFU) for raw cow's milk, for example. The EU limits are based on reference methods to determine the number of bacteria present, but most EU Member States, including the Netherlands, employ routine methods to determine the number in official control samples. During the workshop, the implementation of a harmonized conversion factor from routine measurements to the reference units was debated. It was agreed that the decision whether to use this harmonized equation will be up to the national Competent Authority. Furthermore, the work on the procedure to establish a conversion factor (ISO 21187/IDF 196) was presented, as well as the work done to certify (MicroVal) two types of flow cytometers used in routine measurements for total flora (and somatic cell count). The EURL also announced a new study to the time-temperature conditions during transport of raw milk samples to laboratories, as the 2016 enquiry showed a lot of heterogeneity in Europe.

**Hygiene of raw milk, somatic cell count**
Somatic cell count is the determination of somatic cells in milk, which are a measure of udder health and (absence of) mastitis. For this parameter also criteria are laid down in Regulation (EG) No 853/2004. The EURL is working on a document that identifies the critical factors, for a method which is recognised to be a difficult analysis with a tendency for large inter-laboratory bias. One item that is reported to be non-critical during the workshop is the magnification. The current reference method (ISO 13366-1) states a minimal magnification of 500x, but for a number of reasons, 400x magnification is frequently used in practice (including in the Netherlands). The EURL did not find any differences between the magnifications, now allows the use of 400x for future EURL performance tests, and submits its finding to ISO to be reflected in the next revision of the reference method's protocol. JRC (Geel, Belgium) is working on a SCC reference material, and presented the progress.

**Heat tracers (ALP)**
Alkaline phosphatase (ALP) is a native milk enzyme. While its activity as such is not particularly important for human consumption, its thermal degradation curve coincides with that of a proper pasteurisation treatment. Therefore, the determination of the activity of this enzyme in milk and milk products gives information on whether pasteurisation was performed correctly. A limit of 350mU/l for cow's milk has been laid down in EU legislation. The EURL has been working on establishing a legal limit for pasteurized cheese, and now it is ready to submit a limit of 10mU/g to the EC. At the workshop, the question for a legal limit in goat's and sheep's milk was raised, to be reviewed in 2017. Furthermore, a few initiatives to update this method using microwell-based assays were presented, which look very promising and would speed up the analyses. The Italian NRL and an organisation in Germany (MUVA) presented work on creating reference material for this analysis, which at this point is not available.

### 12.1.2 Participation in working groups, communication with EURL

There were no active working groups in 2016. The NRL answered to all enquires the EURL sent out in 2016, if required with help from National parties, on the following topics:
- Enquiry on transportation conditions of milk samples to laboratories;
- Request to comment on: Critical points for somatic cell counting, microscopic technique;
- Enquiry on the national situation on harmonisation of conversion equations between instrumental methods and reference method for the determination of total flora in raw cow's milk.

### 12.1.3 Participation in proficiency and comparative tests

The NRL participated in the following EURL-proficiency tests:
- PT on ALP activity in cheese (November 2016, 5 samples)
- PT on total flora in milk (December 2016, 3 samples)

Next to the EURL PTs, the NRL also participated to a number of other proficiency tests:
- PT on somatic cell count (stabilised milk): ALP (Switzerland) (January, May, September, 4 samples each).
- PT on somatic cell count (raw milk): Cecalait (France) (March, June, September, December, 10 samples each)
- PT on phosphatase (stabilised milk): LGC (UK) (January, 2 samples)

In 2016, the EURL reported the results on the 2015 EURL PTs:
- In the PT on SCC, the NRL scored well, with Z-scores ranging between -0.9 and -0.3.
- In the PT on ALP, the NRL scored Z-scores between -1.5 and 1.6, which is in the acceptable zone and thus acceptable.

## 12.2 Assistance to official laboratories

### 12.2.1 Quality control

In 2016, the NRL provided assistance to the OL by providing results obtained by the reference method for SCC on the internal reference material prepared by the OL. For ALP, two times 10 comparative samples were prepared by the NRL to the OL. For total flora, four series of samples were analyses simultaneously for level checks.

# 13 National Reference Laboratory water content in poultry meat

## 13.1 Activities within the EURL-NRL network

### 13.1.1 Participation in EURL-NRL workshops

The water content in poultry meat NRL has taken part in two EURL-NRL expert meetings in 2016 together with the other European NRLs and the responsible representatives from Brussels.

*Brussels*
The first expert meeting took place on March 10th 2016 in Brussels. This is the primary annual meeting, aside from the fall meeting held in one of the Member States. The meeting was opened by Kai Uwe Sprenger (DG AGRI), who also introduced the new DG AGRI team in charge of the topic "Water monitoring in poultry meat", Laurence Bonafos and Martin Szentivany. Below are the points raised during the meeting which were of importance to the target groups in The Netherlands (NCAE, COKZ, NVWA, Ministry of Economic affairs and RIKILT, Wageningen Research).

**Collection and presentation of the national control data for 2014**
Alexander Bernreuther (DG JRC) gave an overview on control data obtained by the NRLs for 2014 and reminded the delegates that the NRLs are requested by Regulation (EC) No 543/2008 to provide the results of regular checks as stated in articles 16, 18 and 20. One of the key results shown were the comparison of control data in the period 2011-2014 on controls on poultry carcasses and parts (Table 3). More details can be found in the JRC Technical Report titled "2015 Annual Report of the Board of Experts in Monitoring Water Content in Poultry meat following Regulation (EC) No 543/2008".

***Table 3*** *Comparison of ISAMM control data (2011-2014) – over-the-limit cases (source DG-JRC)*

| | Carcases | | | | Parts | | | |
|---|---|---|---|---|---|---|---|---|
| | **2014** | **2013** | **2012** | **2011** | **2014** | **2013** | **2012** | **2011** |
| **Number of Member States** | 13 | 12 | 12 | 13 | 19 | 20 | 16 | 17 |
| **Number of data sets** | 278 | 294 | 215 | 305 | 1032 | 1013 | 519 | 691 |
| **Number of "Over limit"; reported *(calculated)*** | 20 *(26)* | 13 *(19)* | 20 *(22)* | 16 | 142 *(196)* | 99 *(152)* | 28 *(58)* | 70 |
| *Number of "Not evaluated"* | *0* | *0* | *0* | *0* | *0* | *0* | *3 (0.6 %)* | *0* |
| *Number of "Not reported"* | *0* | *0* | *0* | *0* | *0* | *0* | *35 (6.7 %)* | *0* |
| *Number of "Limit incomputable"* | *0* | *0* | *0* | - | *77 (7.5 %)* | *76 (7.5 %)* | *76 (14.6 %)* | - |
| **Number of "Below limit"; reported *(calculated)*** | 258 *(252)* | 281 *(275)* | 195 *(193)* | 289 | 890 *(759)* | 914 *(785)* | 453 *(385)* | 621 |
| **% of "Over limit"; reported *(calculated)*** | 7.2 % *(9.4 %)* | 4.4 % *(6.5 %)* | 9.3 % *(10.2 %)* | 5.2 % | 13.8 % *(19.0 %)* | 9.8 % *(15.0 %)* | 5.4 % *(11.2 %)* | 10.1 % |

The Netherlands contributed significantly to the quantity of controls by 66 carcass checks and 202 poultry part checks in 2015. Still, considering the amount of slaughter houses present in The Netherlands, the amount of checks performed on carcasses is limited.

**Status of "Study on the impact of sample homogenisation on the water content in poultry meat"**
Yannick Weesepoel (NRL-NL) updated the delegates on the latest modifications of the experimental design of the sample homogenisation study, which also includes now a collaborative trial. The latter is required to cope with a potential laboratory bias, derived from water content and nitrogen content determinations. He prepared an invitation document, which also includes a reply form for interested NRLs. The study was executed amongst 18 NRLs in April and May 2016, and initial results were presented at the expert meeting in Turku (Finland), October 2016.

**Study on state of play of processing technologies and the absorption of water in poultrymeat**
NRLs were informed on the recently launched "Study on state of play of processing technologies and the absorption of water in poultry meat", which was commissioned by DG AGRI and is currently conducted by LGC (UK). Report of the study is expected late 2016.

**Simplification of poultry marketing standards**
Martin Szentivany (DG AGRI) explained that under the simplification agenda of agricultural legislation launched by Commissioner Hogan and the alignment of legislation with the Lisbon Treaty, the Commission has been proposing to bring together provisions of marketing standards in 10 commodity sectors (covered by 15 regulations), including marketing standards in poultry meat. The aim of this exercise was to merge the sectorial marketing standards regulations into one delegated and one implementing act, while streamlining certain parts, such as definitions, marketing and labelling requirements, and harmonising, to the extent possible, provisions on control procedures, reporting obligations.

The approach and structure of such a Commission proposal, in a form of working document, were discussed twice within the Expert Group on Horizontal Issues in January 2016. On those occasions and on SCA meeting on 8 February 2016, a significant number of Member States expressed reservations about the horizontal approach adopted and asked for sectorial approach which would be more understandable by the sectors covered by different standards. Given this reaction, the Commission decided to put on hold this exercise and reflect on another approach.
Consequently, it is likely that external evaluation of marketing standards will need to be undertaken. This process will take more time than a simplification exercise but should allow more significant changes introduced into the marketing standards. All relevant stakeholders will be informed about the final approach taken by the Commission in due time.

The ongoing study of '*Study on state of play of processing technologies and the absorption of water in poultry meat'* will feed into any future review of marketing standards of poultry meat.

*Turku*
The fall meeting of experts was held on October 13 and 14 in Turku Finland on invitation of the Finnish NRL (Evira). On October 13$^{th}$, two Finnish slaughter-houses were visited, organised by Evira. On October 14$^{th}$, the morning session was covered by presentations of Evira on ensuring food safety, animal welfare and plant health in Finland and on the Finnish food control system concerning poultry. Furthermore, there was a presentation of the Finnish Poultry Association (Suomen Siipikarjaliitto ry) on sustainable solutions in Finnish poultry production. In the afternoon session, the following topics of concern were discussed:

**Preliminary results of the study "Impact of homogenisation techniques applied in the NRLs on the water content of poultrymeat"**
Yannick Weesepoel (NRL-NL) and Alexander Bernreuther (JRC-Geel) presented an overview on the status of the study as well as preliminary the results of two collaborative trials.
Reference samples: Set of three types of samples (chicken breast fillets, legs and carcases) with proven homogeneity and stability, which were produced and sent to the participating NRLs by Yannick Weesepoel.

Homogenisation test samples: Set of three types of samples (chicken breast fillets, legs and carcases) sent to the participating NRLs by Yannick Weesepoel for sample homogenisation using the NRL typical routine method.

All samples had to be analysed according to the respective annexes of Regulation (EC) No 543/2008 in conjunction with ISO 1442 (moisture) and ISO 937 (nitrogen). The statistical evaluation was based on ISO 5725. First evaluations indicate a success of both studies, with only few outlying data sets. A correction factor for a potential laboratory bias based on the results of the study with the reference samples was applied to the results of both studies. Nonetheless, after a fruitful discussion with the delegates, it was concluded that a systematic lab correction cannot be applied as currently there are only few data sets per lab available. Finally, a possible correlation between the applied sample homogenisation temperature and the results from moisture and protein determinations was discussed. There was agreement among the participants that the final goal of these studies should be a set of guidelines for sample treatment and homogenisation as well as for the analytical methods to be applied. Last, but not least, it was proposed to launch also in 2017 a study with new reference samples.

**Reflection on the 2016 ISAMM control data**
Alexander Bernreuther (DG-JRC) presented first evaluations of the 2015 control data. A detailed consideration will be included in the 2016 Annual Report, which will be distributed among the NRLs in the beginning of 2017, as well as at the next Annual Expert Group Meeting in Brussels. For The Netherlands there are no urgent matters to be dealt with.

### 13.1.2 Participation in proficiency and comparative tests

The NRL has organised and participated in the study on the impact of sample homogenisation on the water content in poultry meat. The final report is expected after presentation at the experts meeting in Brussels on March 29$^{th}$ 2017. Furthermore, the NRL has participated in the DG-AGRI study on the state of play of processing technologies and the absorption of water in poultry meat, which was conducted by LGC (UK). The NRL performed sampling in Dutch slaughter houses and has performed moisture and protein measurements as a sub-contractor.

## 13.2 Assistance to official laboratories

### 13.2.1 Quality control

The NRL has organised two quality control rounds (April and November) in order to ensure that the OL and the NRL obtain statistically similar results for moisture and protein analysis in poultry meat. The quality controls were comprised of two different steps:

- Inter-laboratory check of the moisture and protein analysis (homogenised reference samples of chicken fillet meat and chicken legs);
- Inter-laboratory check of the influence of homogenisation on the obtained moisture and protein results (intact chicken fillet samples and chicken leg samples).

### 13.2.2 Advice

The results of both rounds resulted in the observations that the quality of the moisture and protein analysis of the reference samples were acceptably reproducible on an inter-laboratory basis. Also the analysis of intact chicken fillet samples were within inter-laboratory specifications. However for the chicken legs (bone containing samples) in many cases the limits of inter-laboratory reproducibility were exceeded in both quality control rounds. Since the NRL organised and launched a study concerning this issue in 2016, homogenisation methods utilized at the NRL and LOVAP will be discussed after the final outcome of the study.

## 13.3 Scientific and technical support to the competent authority

On April 14th 2016 a meeting took place at the Ministry of Economic affairs on invitation of contact person Marijn Graf together with Rini Bouman and Jan Beukens (COKZ) and Yannick Weesepoel and Martin Alewijn (NRL-NL) to discuss a request of the German NRL on Dutch control data. Other points addressed during this meeting concerned the amount of individual carcasses used to produce one control sample by the NRL. It was advised that according to Regulation (EC) No 543/2008 seven carcasses need to be sampled to produce one control sample. Now, only two individual carcasses were sampled. Finally, the NRL raised the issue that samples for contra expertise were not delivered properly sealed with an official NVWA seal any more. The concern is that samples can be tampered with.

In November 2016 the NRL (Yannick Weesepoel) advised COKZ (Esther Heerschop) on the sample procedure for contra expertise practised for this analysis by NVWA. The sampling for contra expertise should not be a separate event, but should take place at the same moment as the initial sample. The NRL advised to organise a meeting on this matter in early 2017.

RIKILT Wageningen University & Research
P.O. Box 230
6700 AE Wageningen
The Netherlands
T +31 (0)317 48 02 56
www.wur.eu/rikilt

RIKILT report 2017.007

To explore the potential of nature to improve the quality of life

RIKILT Wageningen University & Research
P.O. Box 230
6700 AE Wageningen
The Netherlands
T +31 (0)317 48 02 56
www.wur.eu/rikilt

Report 2017.007

The mission of Wageningen University and 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, 5,000 employees and 10,000 students, Wageningen University & Research is one of the leading organisations in its domain. The unique Wageningen approach lies in its integrated approach to issues and the collaboration between different disciplines.