Dear Feed Microscopy specialists,

Fortunately, our association has "survived" the COVID 19 crisis, and we are now able to resume our activities and even managed to organize - and for the majority of us attend - a wonderful workshop and meeting in the Netherlands in 2022.

We are also happy to publish one more Newsletter with new editor.

Contents

Activity of the Board / Activity of the association

The IAG Board with its two new members has been motivated to support the survival of our association, showing perseverance and endurance. The transition to a younger team has begun as a result of the retirement of Leo van Raamsdonk, and the Board is happy to be able to publish this newsletter, after half a year with no "senior" scientific officer.

Since the last issue of the newsletter a new IAG web page has been established. There, all news about IAG activities, future activities, presentations of previews IAG meetings, last Newsletters, interesting micrographs and other information can be found. We hope to maintain the IAG website as updated as possible and **we encourage you to register, fill in and verify your personal data** (https://www.iag-micro.org/).

Hope you will enjoy reading the IAG newsletter!

Yours sincerely

Geneviève Frick, IAG president

Dear Leo van Raamsdonk, Thank you and Goodbye!

Since 2003, when Leo van Raamsdonk, a scientist from Wageningen University, became a member of IAG, his work was more than fruitful. After almost 20 years he has now retired.

He had always something to say according to the theme and opened interesting discussions. At each IAG meeting, Leo had most of the presentations because he always found between two meetings something interesting to study linked to microscopy and shared his results. In that work, he willingly shared his ideas and knowledge and accepted all the people who have the will to join his research group. Because of all the listed, Leo was elected 2014 to the IAG board as scientific officer. Since then, he organized numerous proficiency testing for IAG in the composition of feeds, processed animal proteins, botanical impurities, and packaging materials. After each proficiency test, he processed all the results and wrote the reports. He also published a high number of scientific articles. It has to be mentioned that he developed an expert system *Determinator*, a useful tool for recognition of different ingredients and contaminants in feed.

The long time prepared *Quality Guidelines* published in 2022 were one of the most important written articles of Leo van Raamsdonk for microscopy – you will find the abstracts separately in this newsletter:

- "Quality assurance and control of methods to examine visually recognisable substances in feed and food", doi 10.18174/580536.
- „Introduction to new guidelines for validation of methods to examine visually recognisable substances", doi: 10.1080/19440049.2022.2135768,

At each IAG meeting, Leo was prepared to share selflessly his knowledge and experience. His tireless spirit was also expressed by writing IAG Newsletters, in which he summarized all the interesting and important activities, which had happened during the year.

In 2022 Leo decided to retire but did not stop working. O the contrary: he has completed his Doctor of Philosophy at Radboud University.

We must say, IAG will miss his energy and enthusiastic work on the promotion of microscopy as a powerful tool for feed fraud investigations, feed contaminations, and feed compositions.

Dear Leo, thank you for all the work you have done for the IAG and also together with the association, your never-ending energy and your great patience.

The IAG wishes that those years in front of you will be fulfilled with health and things which make you happy!

The Annual Meeting 2022

The Annual Meeting and workshop, from 7 to 9 June, was again hosted by Masterlab from Nutreco in Boxmeer, the Netherlands. After an online meeting in 2021 this time it offered the opportunity for almost 40 participants to exchange opinions and experiences face to face, and to enjoy a nice social program in Nijmegen as well.

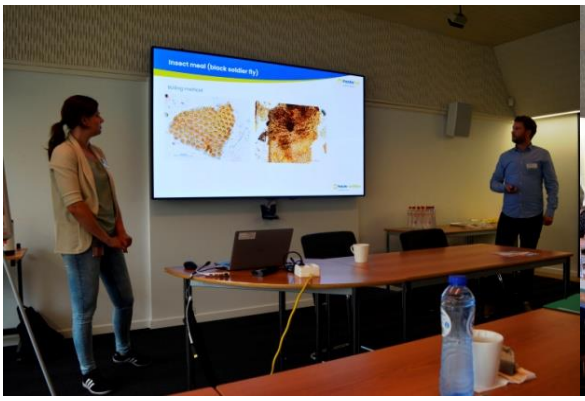
There were nice and interesting workshops on:

- packaging material in bakery-by-products including presenting the results of the IAG PT packaging material 2021
- the "Boiling Method" to highlight cell structures especially for microscopic examination of animal feed and animal feed raw materials
- the double sedimentation PE / TCE for insect meal detection according to (EU) 2022/893 of 7 June 2022 amending Annex VI to Regulation (EC) No 152/2009


Following topics of some of the presentations during the Annual Meeting show the broad diversity of the field:

- Proficiency Tests (PT) on Animal Proteins (AP), Packaging Material (PM) and Composition
- Unusual matrices as gelatine, liquids or lickstones
- Interlaboratory test about identification of poisonous plants in hay
- E-support: training and micrograph platforms at the EURL-AP
- Update on "Quality Guidelines"

You will find the report and the presentations to all these topics in the member area of the IAG-homepage.




Social life during IAG Meeting

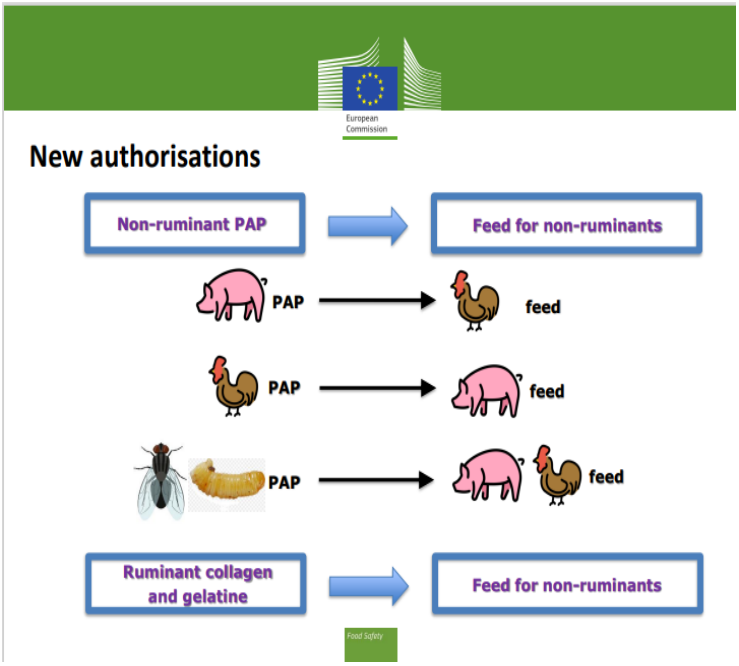
The day before the meeting started, IAG members had a traditional „Come together dinner", where all the tired and hungry participants met each other and the organizers.

Because of bad weather in Nijmegen, the participants were deprived of any guided sightseeing tours. But that did not affect the atmosphere during the official dinner.

Many thanks again to Jos Zegers and his great team for this remarkable meeting, workshop and pleasant time.

Changes in the regulation on animal proteins in the past year

The use of processed animal protein derived from farmed insects has been authorized in the feed of aquaculture animals by Commission Regulation (EU) 2017/893, and in the feed of porcine animals and poultry by Commission Regulation (EU) 2021/1372, but is still prohibited under Regulation (EC) No 999/2001 of the European Parliament and of the Council in certain feed, notably in the feed of ruminants.



As a consequence, (EU) 2022/893 of 7 June 2022 amended Annex VI to Regulation (EC) No 152/2009 and the protocol was adjusted in order to include a double sedimentation step for the preparation of samples to be tested for detecting constituents of terrestrial invertebrates.

At the same time, new authorizations for pig and poultry PAPs were released.

Also, a new EURL-SOP on the combination of light microscopy and PCR was published in 2022 (Method of reference and SOPs – EURL-AP (craw.eu)).
These directives do not solve all questions, because of the variety of ingredients – forbidden and authorized- and the complexity of the matrices we deal with. But they do help to interpret and standardize our results and reports.

Furthermore, we are pleased to announce that the IAG PT on constituents of animal origin will be organized in future by CRA-W Gembloux and certainly address these subjects.

Composition / Contaminations topics

Considering other topics, the 2022 PT on impurities in seed mixes has been sent to 25 participants and will serve to maintain IAG proficiency and fill the Quality Assurance demands. We will still need to organize PTs on composition (verification of the declaration) and on Packaging Material (PM), which gains importance because of the circular economy.

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In this Newsletter, you will find an example of a combined case of contamination with packaging material and traces of terrestrial vertebrate's particles, following the incorporation of recycled food.

Because PM is found more often and has no proper regulation, you will find, in this Newsletter, a questionnaire on the monitoring of PM in the different European countries. We want to offer you the possibility to contribute to a review publication on the subject (monitoring in the past few years, method, tolerance limit, etc…).

Update on legislation on microscopy: Implementing regulation 2022/893

On June 7th of 2022 the new Implementing Regulation 2022/893 amending Annex VI to Regulation (EC) No 152/2009 as regards the methods of analysis for the detection of constituents of terrestrial invertebrates for the official control of feed was published and came into force on June 28th of 2022.

In the table hereunder the main modifications are summarized:

Annex VI of Commission Regulation (EC) 152/2009 as amended by Implementing Regulation 2020/1560	**Implementing Regulation 2022/893**	**Modification**
These two methods make it possible to detect the presence of constituents of animal origin in feed materials and compound feed.	These two methods make it possible to detect the presence of constituents of animal origin in premixtures, feed materials and compound feed.	Explicit mentioning of premixtures
The constituents of animal origin which (…) are identified on the basis of typical and microscopically identifiable characteristics like (…) blood, (…)	The constituents of animal origin (…) are identified on the basis of typical and microscopically identifiable characteristics such as (…), invertebrates cuticular fragments, insect tracheal structures, blood products, (…)	Addition of insect fragments, blood products instead of blood
/	Double PE/TCE sedimentation for the detection of terrestrial invertebrate constituents. + protocol	A protocol for double PE/TCE sedimentation for the detection of terrestrial invertebrate constituents is added.

Annex VI of Commission Regulation (EC) 152/2009 as amended by Implementing Regulation 2020/1560	**Implementing Regulation 2022/893**	**Modification**
Microscopic slides shall be prepared from the sediment and, depending on the operator's choice, from either the flotation or the raw material.	(…) When appropriate, for the detection of terrestrial invertebrate constituents only, slides shall also be prepared from the final flotation (…). The two resulting fractions (the fine and the coarse one) shall be prepared. Test portions of fractions spread on slides shall be representative of the whole fraction.	Addition of analysis of the flotation for invertebrate constituents and representativeness of the portions on the slides.
/	The additional slides prepared from the final flotation (…), for the detection of terrestrial invertebrate constituents shall not be considered for the identification of other natures (terrestrial vertebrates and fish).	The slides of the flotation for the detection of terrestrial invertebrate constituents shall not be considered for the identification of other natures.
/	Observation flowchart after double PE/TCE sedimentation for the detection of terrestrial invertebrates' constituents (…)	Observation flowchart terrestrial invertebrates
/	Number of determinations: If, following the first determination (…), more than 5 particles of terrestrial invertebrates is detected, no second determination shall be necessary and the result of the analysis shall be reported (…).	Number of determination for of terrestrial invertebrates
/	Expression of the results on terrestrial invertebrates	

Case report

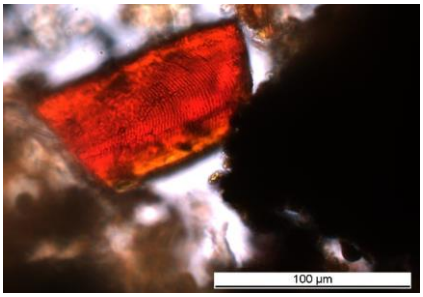
One interesting case with two interrogations occurred in Agroscope, Posieux, Switzerland.

The case is an example of contamination with traces of terrestrial vertebrate particles (AP) and Packaging Material (PM), following the incorporation of recycled food in a pelleted compound feed for cows.

The two interrogations were:

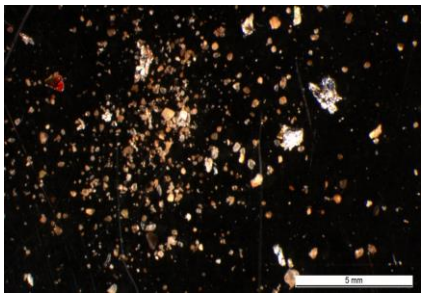
- Animal Proteins:

  → difficulties occurred with the SOP on combination of light microscopy and PCR



- Packaging Material:

  → which kind of method to use? Is there an intervention limit?



Interrogation 1: Animal Proteins: → difficulties with the SOP in combination of light microscopy and PCR

A feed for ruminants was analyzed by microscopy and a low number of particles of animal origin (between 1 and 5 muscle fibers in the first determination) were detected.

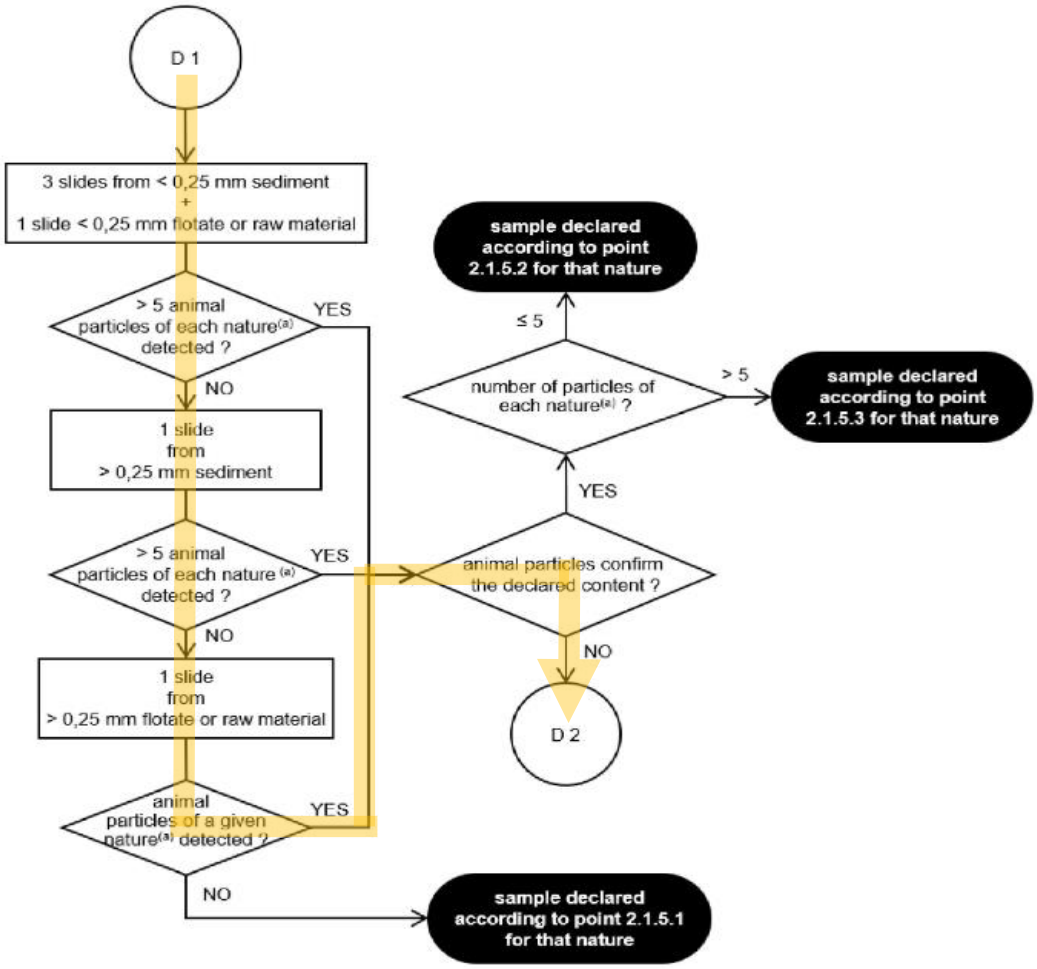
As a consequence, 2 actions were done in parallel:

- Following the Directive, a second determination (D2) was performed by microscopy (see diagram 1)

→ https://www.eurl.craw.eu/wp-content/uploads/2022/07/CELEX-32022R0893-EN-TXT-1-.pdf

Diagram 1

Observation flowchart after single TCE sedimentation for the detection of animal particles other than from terrestrial invertebrates in compound feed, feed materials and premixtures for the first determination



("D1" and "D2" refer to the first and second determinations; (a): terrestrial vertebrates, fish)

- Following the SOP (page 5) the feed was also analyzed with PCR methods (see sentences highlighted) →

As soon as one particle from terrestrial vertebrates PAP and/or blood products is detected by light microscopy, or the presence of such products is indicated from the declaration of content or labelling, the PCR methods shall be applied by following Fig. 1.B

When the feed material is a PAP, without any information on its intended farmed animal category, it shall be analysed both by light microscopy and PCR for each target.

In absence of any information on the destination of a feed or feed material, as soon as one terrestrial vertebrates' particle is detected by light microscopy it shall be analysed by PCR for each target.

The PCR test for ruminant should NOT be applied in the following specific cases, as it will not provide any useful additional information: if milk or dairy products is detected by microscopy or indicated from the declaration of content or labelling, as such products will often trigger a positive response for ruminant DNA by PCR.

The results were the following:

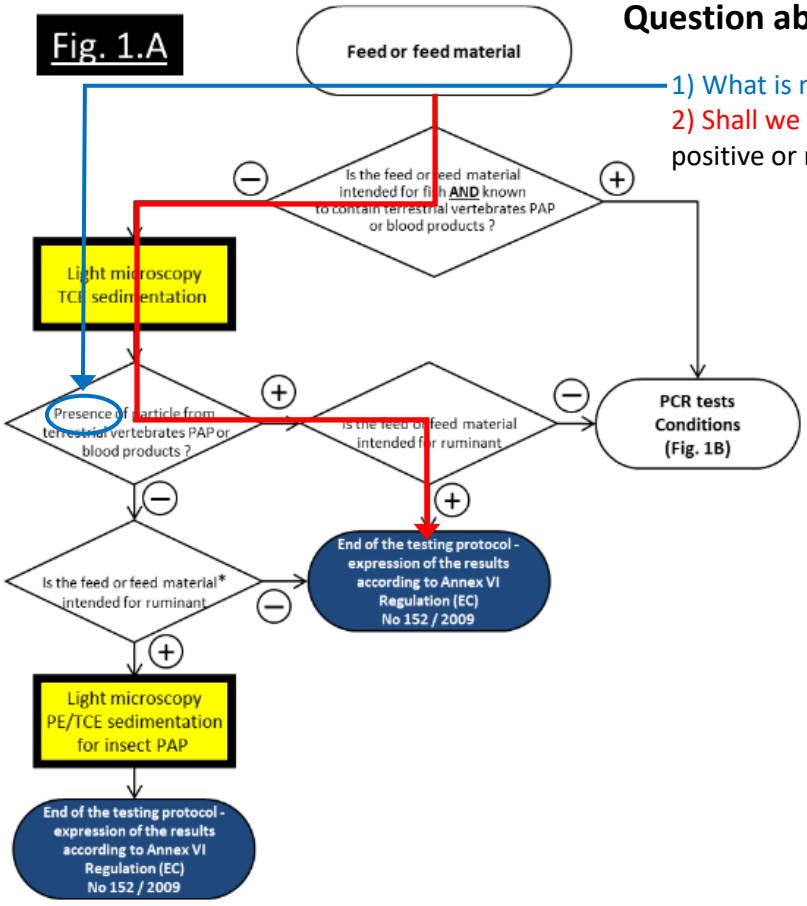
- microscopy = < limit of decision (the second determination confirmed the first one but total number of particles D1 + D2 < 10)
- PCR positive for all three targets (ruminant, porcine and poultry).

Considering the destination and the declaration of the feed (one ingredient was bakery by-products and could contain milk products) made it difficult to decide which PCR target to apply and which results to report.

→ https://www.eurl.craw.eu/wp-content/uploads/2022/05/EURL-AP-SOP-operational-schemes-V5.1.pdf

Protocol for the determination of animal constituents in feed or feed material

Fig. 1.A



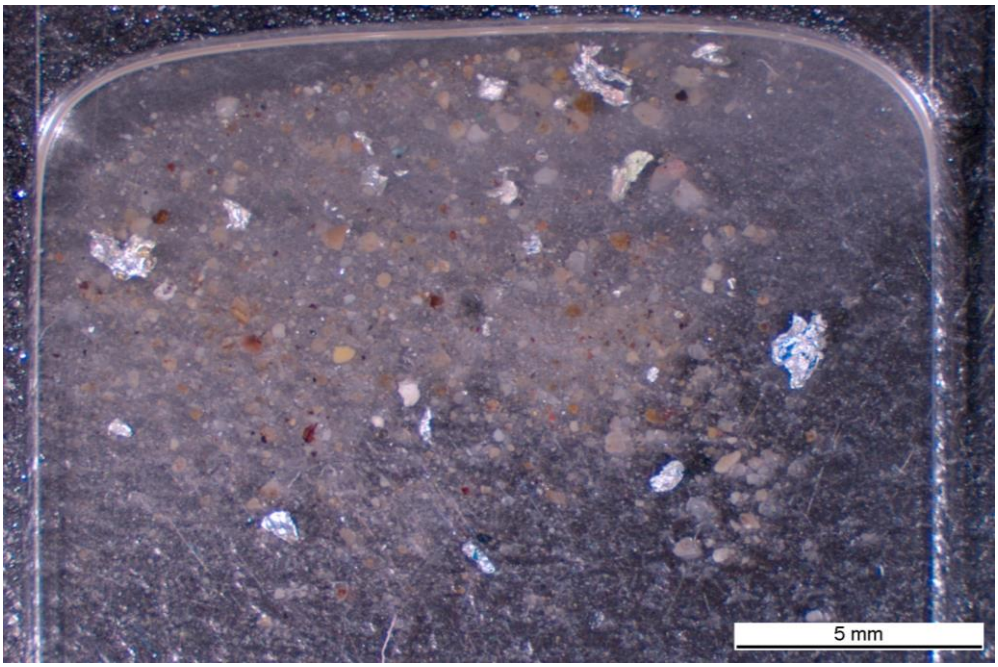
* On the exclusion of whole grains, kernels, oils and fats

Question about the SOP:

1) What is meant by „presence“?
2) Shall we stop the analysis? If yes, declare as positive or negative (below the limit of decision)?

Interrogation 2: Packaging Material → which method to use? Is there an intervention limit?

Performing the sedimentation for AP, Packaging Material (PM) residues were also detected: small pieces of aluminum foil were repeatedly found in the TCE sediments. Aluminum particles are best seen by observation with the stereomicroscope. They have diverse sizes, indicate the presence of PM, but no quantification can be done. Other types of material (plastic and paper) should be detected in the flotation or the original sample material.



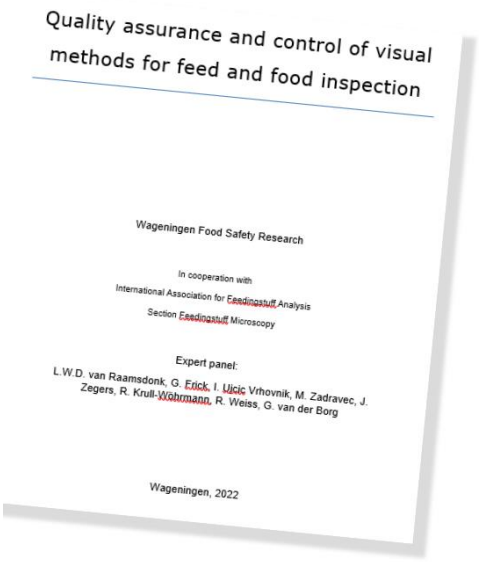
Occuring interrogations:

- Are these few particles problematic?
- Can the contamination be quantified?
- Is there a tolerance limit?
- Which method has to be applied?
- How to analyze pelleted feed?

PLEASE NOTE: All the interrogations of this presentation may be topics for discussion at our next Annual Meeting, the aim of our association being to find solutions, whatever refers to methods and harmonization of results of analysis in the field of microscopy of feeds

Published articles

In last year Leo van Raamsdonk and the authors, all of them IAG members, published a long time prepared article: "**Quality assurance and control of methods to examine visually recognisable substances in feed and food**", doi: 10.18174/580536. It is worth mentioning that the same authors published: „**Introduction to new guidelines for validation of methods to examine visually recognisable substances**", doi: 10.1080/19440049.2022.2135768, which introduces the two parts of guidance, the theoretical part and part two with the validation procedure. In this newsletter an abstract is presented:


Quality assurance and control of visual methods for feed and food inspection

Wageningen Food Safety Research

In cooperation with
International Association for Feedingstuff Analysis
Section Feedingstuff Microscopy

Expert panel:
L.W.D. van Raamsdonk, G. Erick, I. Ujcic Vrhovnik, M. Zadravec, J. Zegers, R. Krull-Wöhrmann, R. Weiss, G. van der Borg

Wageningen, 2022

The abstract: Visual examination of visually recognisable substances, including microscopy, focus on targets or contaminants such as particles of animal origin, plant seeds, spore bodies of moulds, sclerotia, packaging material, microplastic and 'Besatz' (everything that differs from the norm). The two principal results are counts (numbers) and weights for macroscopic methods, or presence/absence for microscopic methods. The level of detection equals at least the size of one unit, usually with a weight exceeding 1 mg, which is in the range of parts per million (ppm). These parameters do not follow a normal distribution but Poisson (counts), lognormal (weights) or binomial (Booleans) distributions, with effect on the interpretation of validation parameters. As for other domains, examination methods for visual monitoring need to be properly validated and quality control during actual application is needed. In most cases procedures for validation of visual methods are based on principles adopted from other domains, such as chemical analysis. A series of examples from publications show inconsistent or not correct implementations of these validation procedures, which stress the need for dedicated validation procedures. Identification of legal ingredients and composition analysis in the domain of visual examination relies on the expertise of the laboratory staff, therefore validation of a method usually includes the validation of the expert. In the view of these specific circumstances, a Guidance for quality assurance and control of visual methods has been developed, which are being presented and discussed in this paper. The general framework of the Guidance is adopted from ISO standards (17023, 17043, 13528). Part 1 of the Guidance

includes the general background, theory and principles. Part 2 presents the actual validation procedures with experimental designs and equations for calculating the relevant parameters, and can be used as blueprint for a SOP in a quality management system. An EURL and NRL network for physical hazards is strongly recommended.

REMEMBER THE DATE - Next IAG Meeting

Next IAG Meeting will be **Bad Hersfeld** near Kassel, Germany, hosted by Landesbetrieb Hessisches Landeslabor, and which will take place on **13-15 June 2023**.

The invitation, information about the place and location and the registration form will be sent to the IAG Members personally and are also available on the IAG-Homepage (registration necessary!)

-> https://www.iag-micro.org/

Take this great opportunity to meet your colleagues, to participate in an exchange of information and discussions on relevant issues of an important area of monitoring.

Overview of the conference program*

	13.06.2023	14.06.2023	15.06.2023
Morning	Individual arrival, registration	IAG Meeting	IAG Meeting
Lunch	Provided at conference venue	Provided at conference venue	Provided at conference venue
Afternoon	IAG Meeting	IAG Meeting	Individual departure
Social program	Guided city tour in Bad Hersfeld	Excursion by bus to „point alpha“, an important location of German and European history	
Dinner	Restaurant Klosterschänke (self-paying)	Conference dinner at „Hotel am Kurpark“	

*Precise time frame of the conference events will be annouced in a timely manner before the conference to all participants

Feed meetings in 2023

https://www.feedsafety.org/feed-2023-conference/

QUESTIONNAIRE FORM ON PACKAGING MATERIAL (PM) MONITORING

Please fill in and return this form to genevieve.frick@agroscope.admin.ch until **28 APRIL 2023**

NAME OF INSTITUTE and COUNTRY:

CONTACT PERSON and EMAIL ADDRESS:

We have performed a monitoring on PM since YEAR: _____

MATRIX: we analyze SAMPLES of: - BAKERY BY-PRODUCTS ☐
- MIXED FEED (MEAL) ☐
- PELLETED ingredient or mixed feed ☐

NUMBER OF SAMPLES / YEAR: - 1 to 5 ☐
- 6 to 20 ☐
- >20 ☐

DESCRIBE YOU METHOD: - "RIKILT METHOD" ☐
- OWN METHOD ☐
-> Please give a short description:

MATERIAL SORTED and REPORTED: - METAL ☐
- PLASTIC ☐
- PAPER ☐
- OTHERS ☐ Please, describe:

DO YOU APPLY A LIMIT OF INTERVENTION?: - NO ☐
- YES ☐ Please, describe:

I WOULD LIKE TO PARTICIPATE IN PUBLISHING THE COMPILATION OF DATA: - NO ☐
- YES ☐

COMMENTS:

DATE: SIGNATURE:

Closing remarks

Dear reader,

We hope that you enjoyed reading the articles in this Newsletter. Even more, we hope that the included information will be useful for all your activities in the already started year. The Board members, and naturally all other members, of IAG section Feed Microscopy will help to answer your questions. So, you are invited to contact us.

We wish you a fruitful and productive 2023.

Board of IAG section Feed Microscopy

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