

IAG section Feed Microscopy

Newsletter 2014



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The IAG section Feed Microscopy and its board is proud to be able to present you the first newsletter covering all kinds of activities in the area of feed microscopy. In an era where safe and reliable food products, and feed as an intrinsic part of it, we would like to inform you on the highlights and prospects of microscopic research. The abstracts of IAG reports are included, and new ring tests are being announced.

We hope that you will appreciate the contents of this newsletter. In the meantime, our research will continue for collecting new intricate results and getting new highlights.

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Board: dr. I. Paradies-Severin, Germany, Inge.Paradies-Severin@LWK-Niedersachsen.de; dr. G. Frick, Switzerland, genevieve.frick@alp.admin.ch; dr. J. Vancutsem, Belgium, jeroen.vancutsem@favv.be; dr. L. van Raamsdonk, The Netherlands, leo.vanraamsdonk@wur.nl; dr. R. Weiss, Austria, roland.weiss@ages.at.

Editing newsletter: L. van Raamsdonk, RIKILT, Wageningen.

Website: www.iag-micro.org

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Standpoint and mission statement IAG section Feed Microscopy

Aim of feed microscopy

The microscopic examination and evaluation of feeds and feed materials is targeted to the proper identification of ingredients of plant, animal or anorganic (mineral) origin. This is an important discipline in the entire area of feed research and covers for, among others:

- Proof of identity and purity of feed materials,
- Establishment of impurities or prohibited contaminants, and quantification thereof,
- Recognition of undesired elements or plagues, which are unintentional present, such as insects and moulds,
- Indication of the composition of compound feeds, and estimation of the share of the recognisable ingredients.

With these technical possibilities, microscopy is an important legal tool for enforcement of regulations, with the mere notification that some of those regulations can only be enforced exclusively by microscopic inspection. In addition, the results of microscopic investigations are in a range of situations complementary to analytical chemical research. Multidisciplinary research is necessary in most cases in order to clarify and understand obtained results, and to trace causes and origins for proactive and risk-based monitoring.

Specific examples of microscopic, or in broader terms, visual research include detection of animal proteins (Regulation (EC) 999/2001), botanic impurities (Directive 2002/32/EC), and prohibited ingredients such as packaging materials (Regulation (EC) 767/2009).

Basic requirements for feed microscopy

In contrast to the instrumental analysis of analytic chemistry, the visual identification of a large diverse range of materials relies heavily on the knowledge of biology and adjacent disciplines. Also the application of procedures for preparing the sample in such a way that specific fractions can be selected, identified and evaluated needs sufficient experience. The analyst can gain routine in a day-by-day process of investigating sample material, which is a long and intensive process. Sources for expertise can be found among handbooks, training by experienced scientists, collections of reference materials and dedicated expert systems on a computer. Guidance from supervisors with experience in the specific requirements of visual and microscopic investigations is strongly recommended.

IAG section Feed Microscopy

A platform on microscopy of feed materials of German institutes organised in the Association of German Agricultural Analytic and Research Institutes (German abbreviation VDLUFA), together with microscopists from other European countries resulted in the foundation of a section Feed Microscopy in the International Association for Feed Analysis (German abbreviation IAG). The activities of the section include meetings at an annual basis or more frequently if necessary, with the following aims:

- Exchange of information and experiences in the daily practice of sample investigations,
- Presentation and discussion of the results of ring tests, which are meant as necessary support of lab accreditations,
- Development and formalisation of new methods, in the framework of European Union law enforcement, or in other legal contexts,
- Expression of opinions on actual issues in the area of feed research, both for composition and safety, in national or EU framework,
- Training sessions or courses for developing expertise and exchange of experiences.





Current situation and prospects of feed microscopy

The more recent development of highly sophisticated analytical equipment for chemical analysis, high throughput processes, and budget savings resulted in a lower volume of microscopic investigations. The principles of the General Food Law (Regulation (EC) 178/2002), which put the primary responsibility for feed quality and safety in the hands of the producers, resulted generally in a lower public investment in law enforcement. In addition, the shift from ingredient-based to nutrient-based strategies in animal farming, the change from open to half open declaration (Regulation (EC) 767/2009) and optimization of production processes resulted in a lower demand of microscopic and visual investigations.

However, recent incidences in economic fraud and unintentional contamination of feed materials gained new needs for microscopic research.

Recent developments in the area of feed safety and quality are:

- An increasing realization that a range of prohibited components can be managed by controlling their source: plant toxins originate from seeds or whole plants, mycotoxins originate from moulds, etc. Recent developments are the increasing interest in monitoring ragworts (*Senecio*) and autumn crocus (*Colchicum*) at early stages of the feed production chain, monitoring of mould infections in grain batches and in new seed producing species added to Directive 2002/32/EC (*Ambrosia*, *Abrus*).
- There is a slight increase in the interest of monitoring physical contaminants (Regulation (EC) 767/2009 Annex III). New methods for the detection of packaging materials have been published or are in development.
- Composition analysis by visual inspection and microscopic research can support fraud investigations and chemical analysis: microscopic procedures include the possibility to divide a sample in several different fractions, which can be identified and can be the basis for targeted chemical analysis.

Besides the renown disciplines of chemical analysis and DNA analysis, biological and physical contaminants and parameters are evenly important for feed safety, of which monitoring has a legal basis. It is the quest of the future practice of monitoring feed safety to perform smart combinations of multidisciplinary research in order to meet sufficiently the high demands on feed safety and quality. Visual and microscopic research is among the necessary disciplines. Besides this, the microscopic procedures as used in feed safety research can be applied successfully in other areas, such as food safety, economic fraud, customs and forensics.

Aim and scope of IAG section Feed Microscopy

Based on its historic position, current activities and future opportunities, as presented, IAG section Feed Microscopy will state the following aims:

- Promoting the exchange and application of visual and microscopic techniques in the area of feed research, and if possible in other areas of law enforcement.
- Stimulating opportunities to apply established research methods in other areas such as food research, medical research, customs and forensics.
- Promoting the development of visual and microscopic methods for supporting legal monitoring.
- Seeking possibilities to be partner in multidisciplinary research in the mentioned areas.



President's address

Dear colleagues and members,

This is the first newsletter of IAG section Feed Microscopy and it is a pleasure to have this nice opportunity to present our views and activities to a wide audience.

Looking back at our busy and engaged work on important aspects of feedstuff microscopy, it is a great pleasure for mention some of the IAG activities in 2014. The annual IAG meeting took place in June 2014 in Posieux, Switzerland. We were invited by our colleagues from Agroscope, Institute for Livestock Sciences. Focal points of discussion were the detection of animal constituents in feedstuff; changes in Directive (EC) 2003/32 concerning undesired botanical impurities in feed; performed IAG ring tests and improvement of future IAG work.

Special attention should be given to the organisation of the ring tests, organised by RIKILT on behalf of our board. Two of the three ring tests were finalised and reported. The summaries are reproduced in this newsletter. The third one on detection of botanic impurities in bird feed was held in autumn 2014, and the report is expected in Spring 2015. New ring tests are announced, also in this Newsletter.

In Posieux we had a brain storming of IAG work in general. In future we'll work on more information at our website like meeting data, program, publications of the lectures, ring test offer, newsletter, report of other annual activities of interest for microscopists, IAG decisions and statements. Further on we could provide trainings on special microscopic problems. We were also discussing how to gain the interest of students and other scientists from related professions to increase our network. This discussion will continue, of course and all of you are asked to contribute in it.

I would also ask your attention for the new method for detection of animal proteins. This extended and much more detailed method was published in Regulation (EC) 51/2013, amending Regulation (EC) 152/2009 Annex VI. Although in effect from 2013, its implementation is still an issue. The board will refer to the section in this Newsletter dedicated to this topic.

Our annual IAG meeting 2015 will take place in Oldenburg. We are invited by our colleagues from LUFA Nord-West, Institute of Feedstuff Analysis, Germany. The meeting date is June 9 – 11, 2015.

I am looking forward together with you all to have a new fruitful year in 2015.

Yours sincerely,

I. Paradies-Severin



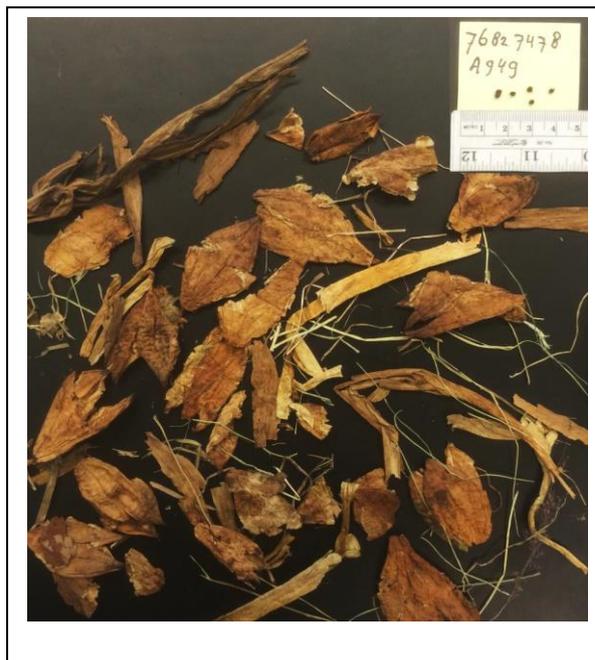
Highlights of 2014

***Colchicum* in fodder.**

In 2013 problems arose in the Netherlands with fodder contaminated with autumn crocus or meadow saffron (*Colchicum*). Several ponies went ill and even died. After that, a range of samples were submitted in 2014 by the official authority, the police and by veterinarians. In most cases after visual inspection the material appeared to belong to *Colchicum*, which was approved by chemical analysis of the toxic compound, colchicine. In some occasional cases the material appeared to belong to other plants, primarily other monocotyledons. Also in these cases this identification was approved by chemical analysis. These recent occasions of an old botanic contaminant prove that visual inspection can offer a serious attribution to feed safety.

Image: dry material of *Colchicum*, selected from hay.

Leo van Raamsdonk, RIKILT, Wageningen



Detection of over-heated maize.

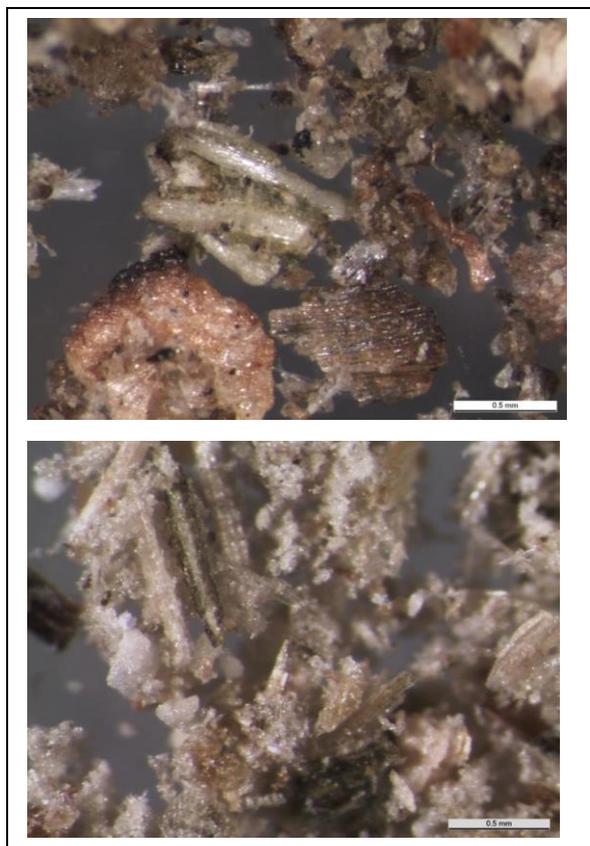
Two samples of maize whole plant material had to be compared by microscopy to attest that one of them had been over-heated.

Observations were made with stereomicroscope and compound microscope. At all magnifications, differences between the two samples were visible. Good pictures could be made at low magnification at the stereomicroscope.

Sample 1 shows black parts that resemble charcoal at the edges of some particles, and small black spots.

Sample 2 had no black parts.

Geneviève Frick, Agroscope, Switzerland.





The here presented two cases are examples of what most of us has on the desk several times a year: interested or odd cases of special samples. These are interesting, sometimes curious and anyway instructive. Knowing that colleagues have some experience with special requests is supportive when you have your own case which might be difficult to figure out. Sharing these examples will turn the group of feed microscopists into a learning and growing community.

The board will encourage you to submit this kind of cases for the next newsletter. Just a few sentences with the problem and your solution together with an image will do.

Microscopy links

Out there on the web lots of interesting information can be found on microscopy. The list below will form a start to share a bit of it.

A very interesting and comprehensive web site on the micro world and microscopy is maintained by Microscopy-UK. There is also e newsletter: <http://www.microscopy-uk.org.uk/>

Microlab Northwest maintains a website with an impressively large gallery of micrographs. Their motto: "sharing our knowledge" !: <http://www.microlabgallery.com/photogalleryname.aspx>

The American Herbal Products Association published a Wiki website with a section on microscopy: <http://www.botanicalauthentication.org/index.php/Category:Microscopy>

Then for your interest just some well-known sites, but any way interesting for supporting your research:

EURL animal proteins with a nice micrograph collection: <http://eurl.craw.eu/>

The expert system Determinator with currently three modules on identifying micro- or macro ingredients in feed: starch, pollen and ragwort: www.determinator.wur.nl



Ring test animal proteins 2014: Abstract of report

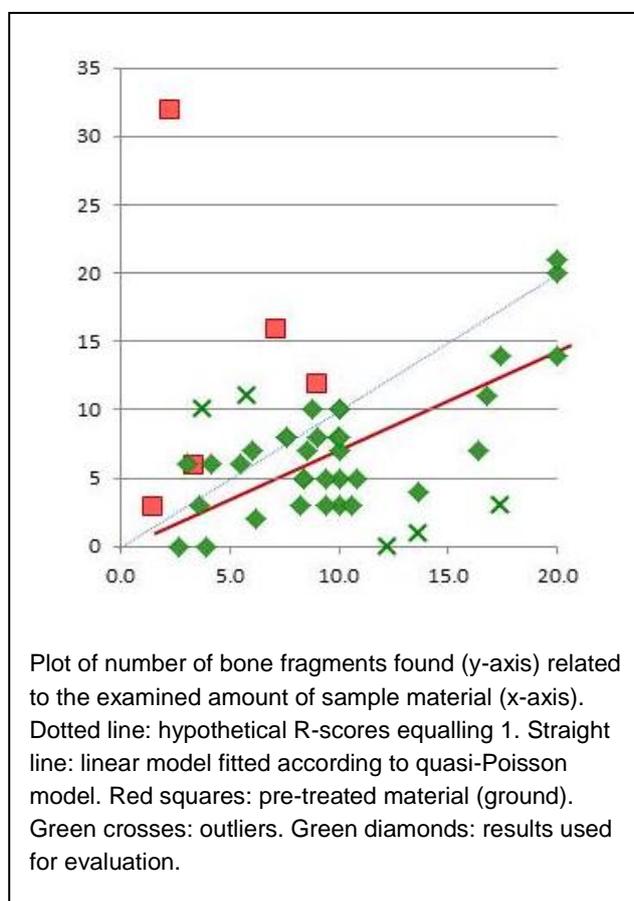
A ring test was organized for the detection of animal proteins in animal feed by microscopy in the framework of the annual ring tests of the IAG - International Association for Feeding stuff Analysis, Section Feeding stuff Microscopy. The organizer of the ring test was RIKILT - Wageningen UR, The Netherlands. The aim of the ring study was to provide the participants information on the performance of the local implementation of the detection method for their local quality systems. A further aim was to gather information about the application of the microscopic method. The current 2014 version of the IAG ring test for animal proteins is the first one in the IAG series of ring tests applying the full new method for microscopy as published in Regulation (EC) 51/2013 amending Annex VI of Regulation (EC) 152/2009.



Three of the four samples used in the ring test were based on an artificial feed with a formulation comparable to that of an average cattle feed. A mix of minerals was included at a level of 1%. The fourth sample was based on a chicken feed produced at a pilot plant dedicated to produce animal protein free test feeds. The contaminations were: no animal proteins (blank), 2% of fish meal and 0.1% of land animal material, 1% of insect meal (*Locusta*), and a special sample with 30 bone fragments (1 bone fragment per 1 gram of sample). All participants were requested to determine the presence or absence of land animal and/or fish and/or protein material of other animal sources (including unidentified muscle fibres and arthropods), and to indicate the type of material found. The participants were asked to report the amount of sediment found (the fraction containing minerals and bones, if present) before and after applying the actual analyses and to answer questions on a series of parameters of the microscopic method. Of the 56 participants 52 sets of results were returned with results using the microscopic method.

Incorrect positive results (positive deviations) were expressed in a specificity score and incorrect negative results (negative deviations) were expressed in a sensitivity score. An optimal score is 1.0. The results are analysed in two ways: numbers below LOD (between 1 and 5 inclusive) have been considered positive and as alternative considered as negative. The choice to consider these number positive was based on the principle that any particle correctly identified as of animal origin is apparently present, and it allows a way to compare the present results with those of previous years.

About one-third (16 out of 52) of the participants applied the wrong number of determinations, although the report form was interactive and guided the participant through the process of choosing the right number of repetitions. Most of the specificity and sensitivity scores were at good levels. The specificity score for incorrect detection of meat and bone meal (MBM) in the blank is good (0.96). The detection of 0.1% of MBM in the presence of 2% fish material appeared to be acceptable (0.94). The detection of animal material of any kind in the sample



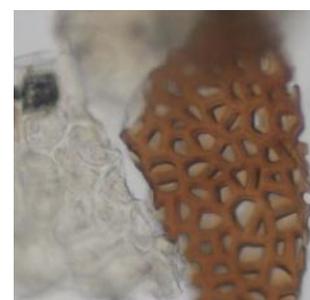


contaminated with insect meal was insufficient (0.69), and the detection of insect fragments and of relatives (arthropods) was very low (0.19). A reasonable number of participants found bone fragments at a contamination level of 1 bone fragment per gram material (0.92), but in the situation that results below LOD were considered negative the sensitivity was very low (0.44). A significant relationship was found between the amount of sediment used for observations and the number of particles found (see figure). Setting a minimum amount of sediment to be used is recommended, in combination with a strategy to quantify the amount of material used.

Full reference: L.W.D. van Raamsdonk, V.G.Z. Pinckaers, I.M. Scholtens, T.W. Prins, H. van der Voet, J.J.M. Vliege, 2014. IAG ring test animal proteins 2014. [Report 2014.011](#). RIKILT, Wageningen, pp. 39.

Ring test botanic composition 2014: Abstract of report

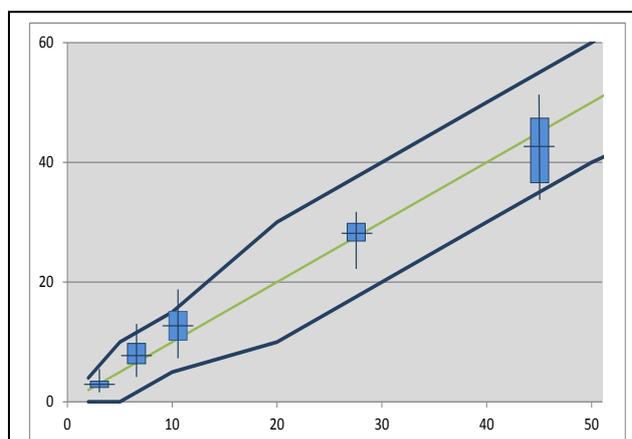
A ring test was organized for the microscopic determination of composition in animal feed in the framework of the annual ring tests of the IAG - International Association for Feeding stuff Analysis, Section Feeding stuff Microscopy. The organizer of the ring test was RIKILT - Wageningen UR, The Netherlands. The aim of the ring study was to provide the participants information on the performance of the local implementation of the method for composition analysis of feed.



The sample was based on a chicken feed produced at a pilot plant dedicated to produce animal protein free test feeds. The sample was contaminated with 1% of insect meal (*Locusta*) and offered with an incorrect declaration. All participants were requested to confirm or reject the declaration and report the correct composition. The results were analysed using the IAG model for uncertainty limits. Shares of ingredients in the feed formulation outside the limits of the model were indicated as “wrong”.

A total of 24 sets of results were returned. Seven participants made one error and two participants made more than one error. One lab reported up to four wrong results. A plot of the results per ingredient in the frame of the IAG estimation model is shown in the figure. Wheat meal at a share of 45.5% in the formulation was underestimated four times, and corn meal with a share of 10.9% was overestimated four times. Results of IAG ring tests in previous years revealed in general underestimation for higher shares and overestimation for lower shares, which is consistent with the current results.

The analysis of composition in terms of ingredients is important for detecting economic fraud and for monitoring feed safety. Composition analysis and label control of feed is regulated in Regulation (EC) 767/2009. In a broader view, composition analysis in the entire food chain can improve the effect of monitoring actions. The new legislation on food labelling



The results of the IAG ring test composition 2014 projected on the uncertainty limits of the IAG estimation model. Ingredients from left to right: rapeseed and rapeseed meal, corn meal, beet pulp, soybean products, partly extracted, wheat meal. Bars: P₂₅ – P₇₅ percentile interval, vertical line: minimum – maximum range, horizontal line: average.



(Regulation (EC) 1169/2011), effective from December 13th 2014, obliges to provide more detailed information to customers on composition and related topics.

The current results indicate that feed ingredients can be identified and shares can be estimated successfully. Besides a proper method, maintenance and dissemination of expertise of analysts are vital for a good performance. An evaluation of the IAG uncertainty model can help to improve its application.

Full reference: L.W.D. van Raamsdonk, V.G.Z. Pinckaers, J.J.M. Vliege. IAG ring test feed composition 2014 . [Report 2014.010](#). RIKILT, Wageningen, pp. 35.



Scheme of ring tests 2015

The IAG section Feeding stuff Microscopy organizes annually several ring tests for the evaluation of composition or detection of prohibited constituents in animal feed. The presidium of the IAG section Feeding stuff Microscopy, RIKILT and Agrosocpe have agreed to organize together the 2015 ring test for the following situations:



- Test IAG-2015-A. Detection of the presence of animal proteins in a set of four samples. This test was already organised by RIKILT in previous years (see abstract in this Newsletter). Targeted protocol: Regulation (EC) 152/2009, consolidated version of February 12, 2013. Cost for participation: € 230.
- Test IAG-2015-B. Declaration of the composition of a compound feed (one sample). This test was organised in 2014 by RIKILT as well (see abstract in this Newsletter). RIKILT will continue the organisation for the year 2015. Targeted protocol: IAG method A2. Cost for participation: € 50.
- Test IAG-2015-C. Detection and quantification of ergot sclerotia in two samples of unground cereals. Targeted protocol: IAG method A4. Cost for participation: € 120.
- Test IAG-2015-D. Detection and quantification of Ambrosia (*Ambrosia artemisiifolia* L.) in non-pelleted Animal Feedingstuff (one sample). Targeted protocol: IAG Method A5. Cost for participation: € 50.

The single sample for the composition test will be part of the animal protein test. On behalf of the IAG section Feeding stuff Microscopy, RIKILT will invite you for participation in these ring tests. RIKILT will encourage you to subscribe to all four tests, although this is not mandatory. Participation in all three test would cost € 450; in this case a discount of 10% will be granted, resulting in a total cost of € 405 for the total set of four tests.

The samples for test IAG-2015-A and IAG-2015-B will be sent around late February or early March 2015. Also a questionnaire will be sent by E-mail, together with instructions and relevant documentation on protocols. A time slot of four weeks is planned for the analyses of the samples by every participant. This means that late March or early April all results are expected to be returned to RIKILT. The samples of test IAG-2015-C and 2015-D will be send late August and results needs to be reported in October. All results are intended to be reported at the annual meeting of the IAG working group Microscopy in Oldenburg (Germany) in June 2015 (tests A and B) or in 2016 (tests C and D). The final reports will be published later in either 2015 or 2016. All communications of the evaluation will be fully anonymous.

If you are interested to participate in one or more ring tests, please return the application form, which accompanies this newsletter, to nastasja.vanderhee@wur.nl or leo.vanraamsdonk@wur.nl. Subscription closes Friday February 27th, 2015. You are requested to make a payment after receiving the invoice from RIKILT. Make sure that the reference number, your name and your institute's name are mentioned upon payment. This information is necessary to avoid loss of payments that cannot be linked to participating institutes.



The new method for detection of animal proteins by microscopic investigation

On February 12, 2013, a new version of the procedure for the detection of animal proteins was published in Regulation (EC) 51/2013, amending Regulation (EC) 152/2009 Annex VI. A lot was changed compared to the previous method and

implementation appeared to be difficult. The board of IAG section Feed microscopy started in 2013 an inventory of issues among the board members. The first results of this inventory were presented during our annual meeting in Vienna in 2013. After that, the board felt that more actions were needed.

At first the raised issues were discussed further and expressed in a note which was finally send the EURL in April 2014. During the EURL annual meeting in 2014 some of the issues were discussed again. This note was also handed over to the representative of DG-SANCO. This note is distributed and can be send to you upon request.

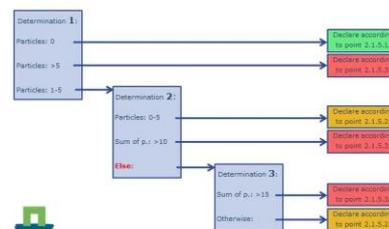
Secondly, after more than a year and a half after its publication, it is very worthwhile to collect the experiences and problems during implementation of the new procedure by the members of our section. Therefore, we would like to invite you cordially to read and respond to the note with your own experiences.

The board decided to distribute the version as discussed during April and May 2014 with the EURL for having a specified time mark. Nevertheless, the board members already encountered additional issues, partly because of the latest IAG ring test for animal proteins, such as:

- The new method does not mention insects as type of animal proteins.
- Usually only one of the mentioned types of animal proteins is found in a sample, either terrestrial animal or fish material. If a second determination is necessary, the procedure does not indicate whether the type not found in the first determination should be included in the search and the report of that second determination.

Besides these procedural issues, the board realises that the current extended procedure might take more time and might be therefore more expensive to carry out. Initial thoughts for optimising the time to do the analysis and the reporting are in consideration.

Finally, based on your responses together with current insights, we will continue the discussion on the optimization and implementation of the new method for detecting animal proteins. Thanks in advance for your contribution.

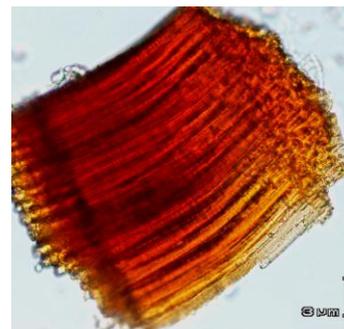




Meaning of traces of botanic impurities

The undesirable substances of botanic origin as listed in Directive 2002/32/EC are regulated with three different types of measures:

- A. Weed seeds and unground and uncrushed fruits at maximum content levels between 50 and 3000 ppm: *Datura*, *Crotalaria*, *Ambrosia* and an unspecified range of seeds containing toxic substances.
- B. Seeds and husks as well as their processed derivatives at a maximum content level of 10 ppm: *Ricinus*, *Croton* and *Abrus*.
- C. Seeds and fruit as well as their processed derivatives in trace amounts not quantitatively determinable: *Fagus*, *Jatropha* and mustard species (*Brassica*).



The indication of trace amounts is only mentioned for the seeds as targeted under C.

The European Commission contacted in Autumn 2014 several members of IAG section Feed microscopy to start a discussion on the interpretation of traces for the mentioned undesirable substances. Some members developed some initial viewpoints to work out and define the concept of “trace”. These viewpoints do not provide a comprehensive answer. So, there is still the desire to raise an expert panel for getting a broader platform and for discussing this issue. In addition, issues on the legislation and enforcement of ergot sclerotia will be put forward by the Commission as well.

The Commission would like to plan an expert meeting in Brussels in February 2015. This is in a few weeks. The Board of IAG section Feed microscopy would like to support this initiative and calls all experienced members of our section to consider to participate in this meeting. It is the intention of the Commission to refund the costs. Further details will be worked out.



Please express your interest to Leo van Raamsdonk, RIKILT, Wageningen or to one of the other board members. We will collect names and interests and facilitate the further communication.

It is great to see that IAG section Feed microscopy is acknowledged and recognised as expert platform. This encourages us to maintain and develop our expertise.



Closing remark.

The board of IAG section Feed Microscopy is looking forward to have a fruitful 2015.

Topics that will be addressed are:

- IAG ring tests 2015
- Botanical impurities
- Forbidden substances
- Implementation of revised Regulation (EC) 152/2009
- Report of the 9th EURL-AP workshop
- IAG future, improvement of our work

To conclude, we wish all members and all colleagues with interest in feed microscopy a healthy and successful New Year.

Board of IAG section Feed Microscopy.