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Annual report 2006/2007 of the National Reference Laboratory for residues of veterinary drugs in products of animal origin according to 96/23/EC

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Summary

This report of the National Reference Laboratory (NRL) for residues of veterinary drugs in products of animal origin according to 96/23/EC describes the activities employed in 2006 and 2007.

The communication with routine field laboratories (RFL), the preparation of quality control samples for RFL and the advisory function for the competent authority and RFL are the main tasks of the NRL.

Communication and advices

In the period 2006/2007 the NRL organized 5 official meetings with RFL (minutes are available) and several times informal meetings by telephone and e-mails. The competent authority was advised on two different issues viz. the use of hair as a test matrix for unauthorized compounds and (on request of the competent authority) on the method of analysis used by a private lab for the control of milk and milk products. Furthermore the NRL participated in an EU meeting. The objective of the meeting was to collect information on how the control of antibiotics in milk is organized within the different member states.

Coordinating activities

The NRL prepared quality control samples (approximately 155 samples for 11 different analyses). The NRL provided the RFL with 9 new methods of analysis and advised 3 times NRLs outside the Netherlands - on their request - about different methods of analysis for the determination of veterinary drugs.

Participation in workshops and proficiency tests

Employees of the NRL participated in workshops (5 times) and proficiency tests (5 times) organized by community reference laboratories. NRL employees participated also in 11 proficiency tests organized by other organizations like FAPAS. The results of the proficiency tests (except one) were all acceptable (Z-score <2).

In 2006 RIKILT was accredited (according to ILAC G13:2000) for the organization of proficiency tests. Three (inter)national proficiency tests were organized by RIKILT.

Finally, several scientific papers were written, posters presented and lectures given on trends in the analysis of veterinary drugs and growth promoting agents. The construction of a RIKILT-NRL website was initiated. For the coming period this web-site will be extended and collaboration and communication of the NRL and RFLs will be continued. Special attention will be paid to the quality assurance procedures like control/checks of (commercially) available reference standards.

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1 Introduction

The European Commission is committed to protecting consumers from intolerable health hazards, which may be associated with residues of veterinary drugs or even of non-licensed or forbidden substances in animal products intended for human consumption. For this purpose legislation on veterinary drug residue control has been established as the indispensable basis of the consumer protection within the EU. The European residue legislation commits the Member States to establish a national residue control plan and provides for the establishment of a hierarchically structured system of Community reference laboratories (CRLs), national reference laboratory (NRLs) and routine field laboratories (RFLs).

The responsibilities of the NRL are described in 96/23/EC and included the following items:

- coordinating the work of the other NRLs 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 the CRLs,
- ensuring that their staff are able to take part in further training courses organized by the Commission or by Commission reference laboratories.

This report describes the activities of the NRL for veterinary drugs according to EU document 96/23/ EU. It covers the groups of compounds assigned to RIKILT-NRL regarding veterinary health viz. nitrofurans, dapson, nitroimidazoles, chloroform, antibiotics (including sulphonamides, quinolones, tetracyclines), anthelmintics, carbamates, pyrethroids, coccidostats, non-steroidal antiinflammatory drugs (NSAIDs), organochlorine compounds (including PCBs), organophosphorus compounds and dioxins. These groups belong to Group A6, Group B1, B2 (a, b, c, e), B3 (a, b, f) compounds as described in EU document 96/23/EC.

The activities employed in 2007 regarding the Group B2 (c) compounds (carbamates and pyrethroids) and the Group B3 compounds (organochlorine compounds including PCBs, organophosphorus compounds and dioxins) will be reported separately.

2 Communications and advices

One of the tasks of the NRL is to communicate with the Competent Authority, Routine Field Laboratories and other NRL on issues regarding the control of residues of veterinary drugs. Sometimes the communication is on a regular base and sometimes ad hoc. The same is applicable to the advices given by the NRL. Sometimes advice is requested and sometimes advice is given. The communication and advice activities employed by the NRL in 2006/2007 are described below.

2.1 Communications

2.1.1 *With competent authority*

On a regular base there is meeting between the competent authority The Ministry of Agriculture, Nature and Food Quality on the content of the National Monitoring Plans.

In 2006/2007 RIKILT participated in the following national platforms:

- policy working group plan for monitoring residues
- policy working group dioxins (since 2007 different RIKILT-NRL project).

2.1.2 *With Routine Field Laboratory (Food and Consumer Product Safety Authority, Laboratory Region East)*

On a regular base the management of RIKILT communicate with the management of the Food and Consumer Product Safety Authority, Laboratory Region East (in this report referred to as VWA-East).

On a regular base the analytical technicians of RIKILT communicate with the technicians of VWA-East. See also item 3.1.1.

2.1.3 *With National Reference Laboratory*

On a regular base the management of the national reference laboratories in the field of veterinary health viz. RIVM and RIKILT meets with the management of VWA-East in the so called R3 meeting. During these meetings the analytical activities are discussed and there is an exchange of information and experiences. Furthermore within the R3 meeting the requests for proficiency tests are being discussed and proficiency tests planned. Next to the R3 meetings on a regular bases the activities in the field of veterinary drugs analysis regarding the technical innovations and trends are discussed in the Q3 (Quality, Control, Quartet). In the Q3 participate the R3 laboratories and TNO.

In 2007 the R3 group had a meeting on June 26 (meeting No. 72) in Wageningen/RIKILT and on October 31 (meeting No. 73) 2007 in Bilthoven/RIVM. From these meetings minutes were made by respectively RIKILT and RIVM. The participants were RIVM, RIKILT and VWA-East.

In 2006 the Q3 group had a meeting on September 20 (meeting No. 106). From these meeting minutes were made by RIVM and send to the other participants RIKILT, VWA-East and TNO.

In 2007 the Q3 group had a meeting on June 26 (meeting No. 107) in Wageningen and on October 31 (meeting No. 108) 2007 in Bilthoven. From these meetings minutes were made by respectively RIKILT and RIVM. The participants were RIVM, RIKILT, VWA-East and TNO.

2.2 Advices

2.2.1 *To EU regarding the use of hair for control of beta-agonist*

On the occasion of the Residue Expert Meeting in May 2007 a revised document was made available (SANCO 2006/3228) which contains some particular statements regarding the use of hair as a matrix for the analysis of beta-agonist. The statement was that hair can be used for screening purposes only. The Dutch laboratories involved in residue analysis of veterinary drugs in products of animal origin disagreed with this statement. Based on the information from the Dutch NRL/CRL laboratories, the Dutch Competent Authority (VWA) had send a letter to the European Commission informing them about this disagreement. This letter is presented in Annex I.

2.2.2 *To EU regarding monitoring antibiotics in milk*

A meeting/workshop was organized by the European Commission, (DG Health and Consumers Protection E3). The meeting was the last one of a series of discussions that the Commission had organised with different stakeholders involved in the control of residues of antibiotics: i.e. European dairy federations and producers of kits for the screening of antibiotics. The objective of the meeting was to collect information on how the control of antibiotics is performed in the different levels, farm, collecting tanks, dairies industry autocontrols and official controls in the laboratories and to reflect on the different strategies followed.

The meeting took place on April 27, 2007 Brussels Conference Centre Albert Borschette Rue, Froissart 36 (time 10.00 H-18.00 H). Participants of RIKILT M. Pikkemaat (microbiologist) and A.A.M. Stolker (Chemist). Information was given to the EU by the RIKILT participants regarding the approach of the Netherlands regarding the control of milk (based on the questionnaire send by EU to participants see Annex II).

2.2.3 *To competent authority regarding analysis of triclabendazole in milk*

On request of the competent authority RIKILT has audit the activities of QLIP laboratory regarding the analysis of triclabendazole (and metabolites in milk and milk products). The reason for this request was some unexpected 'non-conform' results of milk and milk products for triclabendazole were reported by QLIP. The competent authority (VWA) asked for technical analytical assistance and advice.

In November 2007 employees of RIKILT (W. Traag and T. Zuidema) visited the QLIP facilities and audited the triclabendazole research. Special attention was paid to the activities employed by QLIP to prevent false positive findings and the quality assurance of the method of analysis used. The observations and conclusions are described in a report of RIKILT to VWA of 7 December 2007 (Annex III *in dutch*).

3 Coordinating activities

3.1 Preparation of quality control samples

The activities employed for the routine field laboratory regarding the preparation of quality control samples are described in a separate project (WOT programme on Food Safety - Part 3 Veterinary Drugs; project 672.037.01 Analytical Chemical Quality Control project for Laboratory VWA-East). The details regarding the prepared samples, the analytical results obtained and the discussion/meetings between VWA-East and RIKILT are described in RIKILT report 2007.506 of September 2007 'De Chemische Borging van Laboratorium VWA door RIKILT Jaarrapportage 2006' by B.J.A. Berendsen and in RIKILT report 2008.001 of January 2008 'De Chemische Borging van Laboratorium VWA door RIKILT Jaarrapportage 2007' by B.J.A. Berendsen.

3.2 Providing analytical methods

On request RIKILT will provide the RFLs with methods of analysis and reference materials. Primary this responsibility is focused on the Dutch laboratory (VWA-East), however within the framework of collaboration information has been shared with laboratories in Belgium, UK and Germany also.

In 2006 the following methods have been provided to VWA-East:

- The analysis of nitroimidazoles in plasma using LC-MS/MS (RSV A1006)
- The analysis of stanozolol in urine using EIA (RSV A0962)
- The analysis of aminoglycosides in animal tissues using LC-MS/MS (RSV A1040)
- The analysis of salmeterol in hair using LC-MS/MS (RSV A1041)

The method for the analysis of salmeterol in hair has also been provided to RIVM, Bilthoven (drs. Saskia Sterk) and to FAVV laboratorium, Gent, Belgium (dr. D. Courtheijn).

In 2007 the following (concept) methods have been provided to VWA-East:

- The analysis of steroids in poultry liver by GC-MS/MS (RSV A1050)
- The analysis of coccidiostatics in egg by LC-MS/MS (concept RSV A 1067)
- The analysis of macrolides in meat by LC-MS/MS (RSV A0690)
- The analysis of steroidglucuronides in urine by LC-MS/MS (RSV A1030)
- The analysis of the markers of carbadox and olaquinox in meat by LC-MS/MS (RSV A1063)

Futhermore information regarding the analysis of anabolic steroids by GC-MS was send to Federaal Laboratorium voor Voedselveiligheid, Gentbrugge, Belgie (e-mail van 29 June 2007 to mieke.vandewielc@favv.be)

Information regarding the use of ultrafiltration for sample clean-up during analysis of antibiotics from animal tissues was send to Central Science Laboratory, York, England (e-mail of 4 June 2007 to s.stead@csl.gov.uk).

Information regarding analytical methods available in the Netherlands for thyreostats and beta-agonists in blood was send to Kantonaales Laboratorium Bern, Switzerland (e-mail of 15 November 2007 to susanne.olivera@gef.be.ch).

4 Analytical activities

The activities employed for the routine field laboratory regarding the development of analytical methods to be used in for example the National Monitoring Program residues are described in a separate project (WOT programme on Food Safety - Part 3 Veterinary Drugs; project 671.521.01 Development of methods of analysis for the benefit of the execution of the National Plan according to guideline 96/23/EC). The details regarding the deliverables are described in the annual report of the WOT programme on Food Safety.

Regarding the trends in analysis of veterinary drugs a review paper was published with the title 'Trends in analysis of veterinary drugs and growth-promoting agents' (authors A.A.M. Stolker, T. Zuidema and M.W.F. Nielen). The article was published in the scientific journal TrAC (november 2007). The abstract is presented in Annex IV.

5 Participating in CRL workshops

In 2006/2007 employees of RIKILT participated to the following workshops:

- CRL-Workshop Analytical and Statistical Issues, April 2006, BVL Berlin.
B.J.A. Berendsen actively contributed to this workshop by means of an oral presentation "An improved derivatisation for the analysis of avermectines".
- CRL-Workshop antibiotics: Strategies of Confirmation Methods, September 2006, AFSSA Fougères.
J.J.P. Lasaroms actively contributed to this workshop by means of an oral presentation "Advanced multi-residue screening in food analysis using UPLC-ToF-MS"
- CRL-workshop hormones, October 2006, RIVM, Bilthoven
- CRL-Workshop antibiotics: The Workshop dedicated to a Training Session for the Analysis of Group B1 Antimicrobial Residues by LCMSMS in muscle tissues and in milk was held at CRL-AFSSA-LERMVD Fougères on 10-11 October 2007 for 27 experts from the NRLSs of the 27 EU Member States. Participant of RIKILT: S.J.W. Stappers.
- CRL-workshop hormones, 15-17 October 2007, RIVM, Bilthoven including discussions and presentations on
 - a) new legislation MRL versus MRPL versus RPA
 - b) proficiency testing
 - c) National Plan activities
 - d) analytical methods
 - e) mini-symposium on steroid analysis and trends (including proteomics)Participants of RIKILT: J.J.P. Lasaroms and A.A.M. Stolker

6 Participating in proficiency tests

Due to the scope of the NRL task assigned, RIKILT participated in proficiency tests organized by the CRLs, FAPAS and other international organizations:

In 2006:

- Macrolides in honey (FAPAS)
- Estradiol in urine (CRL-RIVM)
- Quinolones in muscle of broilers (FAPAS)
- Semicarbazide in babyfood and egg (JRC-IRMM)
- Tetracyclines standard comparison (FAPAS)
- Nitroimidazoles in muscle (CRL-BVL, Berlijn)
- Quinolones standard comparison (FAPAS)
- Nitrofuranes in honey (FAPAS)
- Salmeterol in hair (CRL-RIVM)

In 2007:

- Penicillines in meat/liver (RIKILT)
- Sulphonamides in egg (CRL-AFSSA)
- Oxalinic acid in fish (Quasimeme)
- Sulphonamides in milk (Progetto Trieste)
- Corticosteroids in urine (Progetto Trieste)
- Synthetic steroids in urine (Progetto Trieste)
- Nitroimidazoles in egg (FAPAS)
- Nitroimidazoles in egg (CRL-BVL)

For all proficiency tests the Z-scores were acceptable (<2) with the exception of oxalinic acid in fish (Z-score 2.3). The reason for this deviation was that the quantification was not performed on standard addition but by the use of a calibration curve in meat. By using the standard addition method the Z-score was <2 .

7 Posters, publications and presentations

7.1 Posters

Posters presented at the 5th International Symposium on Hormone and Veterinary Drug Residue Analysis; Antwerp, Belgium, 16-19 May 2006:

- Residue analysis of tetracyclines in poultry muscle; problems revealed by an inter-laboratory study
Bjorn J.A. Berendsen, Angela de Cocq and Hans (J.) A. van Rhijn
- Can fluorescence detection be considered as a sufficiently conclusive confirmatory method for B-group substances?
Tina Zuidema, Patrick P.J. Mulder, Johan J.P. Lasaroms, Stephan Stappers, Hans (J.) A. van Rhijn
- The occurrence of semicarbazide residues in food products: a novel method to discriminate between nitrofurazone and azodicarbonamide use
Patrick P.J. Mulder, Babette Beumer and Hans (J.) A. van Rhijn
- Direct analysis of endogenous and synthetic steroid glucuronides in bovine urine by LC-MS/MS
Patrick P.J. Mulder, Babette Beumer, Michel W.F. Nielen and Hans (J.) A. van Rhijn
- Investigation of the metabolic path of nitrofurazone in search of an alternative marker residue
Tina Zuidema, Patrick P.J. Mulder, Bjorn J.A. Berendsen, Hans (J.) A. van Rhijn and Ron (L.) A.P. Hoogenboom
- A generic method for the quantitative analysis of penicillins using isotope dilution LC-MS/MS
Patrick P.J. Mulder, Eric O. van Bennekom, Henri H. Heskamp, Tina Zuidema and Hans (J.) A. van Rhijn
- Advanced multi-residue screening in food analysis using UPLC-ToF-MS
Hans (J.) A. van Rhijn, Cedric Bourgeon, Johan J.P. Lasaroms, Efraïm Oosterink and Dan McMillan
- Production and characterization of fluoroquinolone incurred sample materials
M.G. Pikkemaat, J.W.A. Elferink, P.P.J. Mulder, W.M.J. Beek and M.W.F. Nielen.
- The ultimate veal calf reference experiment: histology and chemical analysis
Maria Groot, Michel W.F. Nielen
- Multiplex immunoassay for drug residues using the luminex xMAP technology
M. Bienemann-Ploum, W. Haasnoot, H. Gercek and J. Du Pre
- High throughput screening of hormonal activity in feed, calf urine and sports doping by yeast hormone bioassays

T.F.H. Bovee, R..L.A.P. Hoogenboom, R.J.R. Heldsdingen, H.H. Heskamp, A.R.M. Hamers, G. Bor, M.F.W. Nielen

- Immunological detection of illegally administered growth hormones and - regulator in cattle
T. Heutmekers, M. Bremer, M.W.F. Nielen
- Metalign as a tool for alignment of multiple hyphenated mass spectrometry data sets
A. Lommen
- Biosensor immunoassay-directed identification of fluoroquinolones by liquid chromatography electrospray quadrupole time-of-flight mass spectrometry
G.R. Marchesini, H. Gereck, W. Haasnoot, M.W.F. Nielen
- Metabolic activation and detection of dehydroepiandrosterone (DHEA) using bovine liver S9 in combination with a sensitive yeast androgen assay
J. Rijk, M. Groot, A. Peijnenburg, T. Bovee, M. van Engelen, M.W.F. Nielen
- Changes in gene expression profiles of bovines treated with dehydroepiandrosterone (DHEA) using a bovine DNA micro-array
J. Rijk, M. Groot, A. Peijnenburg, M.W.F. Nielen
- Development of an integrated system for the detection of antimicrobial residues in slaughtered animals on EU MRL level: Nouws antibiotic test (NAT)
H.J. van Egmond, J. Schouten, M. Rapallini, S. van Dijk, L. Kortenoeven, M.G. Pikkemaat, H. Aarts, H. Stegeman

7.2 Publications in proceedings

Publications in proceedings of the 5th International Symposium on Hormone and Veterinary Drug Residue Analysis; Antwerp, Belgium, 16-19 May 2006; *Analytica Chimica Acta* volume 586(2007):

- Screening and confirmation criteria for hormone residue analysis using liquid chromatography accurate mass time-of-flight, Fourier transform ion cyclotron resonance and orbitrap mass spectrometry techniques. M.W.F. Nielen, M.C. van Engelen, R. Zuiderent, R. Ramaker.
- The determination of biurea: A novel method to discriminate between nitrofurazone and azodicarbonamide use in food products. P.P.J. Mulder, B. Beumer and J.A. Van Rhijn.
- Confirmatory analysis of malachite green, leucomalachite green, crystal violet and leucocrystal violet in salmon by liquid chromatography-tandem mass spectrometry. G. Dowling, P.P.J. Mulder, L. Regan, M.R. Smyth.
- The ultimate veal calf reference experiment: Hormone residue analysis data obtained by gas and liquid chromatography tandem mass spectrometry
M.W.F. Nielen, J.J.P. Lasaroms, M.L. Essers, M.B. Sanders, H.H. Heskamp, T.F.H. Bovee, J. van Rhijn, M.J. de Groot.

- Multi-detector of corticosteroids in sports doping and veterinary control using high-resolution liquid chromatography/time-of-flight mass spectrometry.
M.E. Touber, M.C. van Engelen, C. Georgakopoulos, J.A. van Rhijn, M.W.F. Nielen.
- A rapid surface plasmon resonance (SPR) biosensor immunoassay for screening of somatotropins in injection preparations
T.H.J. Heutmekers, M.G.E.G. Bremer, W. Haasnoot and M.W.F. Nielen.
- Dual biosensor immunoassay-directed identification of fluoroquinolones in chicken muscle by liquid chromatography electrospray time-of-flight mass spectrometry
G.R. Marchesini, W. Haasnoot, P. Delahaut, H. Gercek, M.W.F. Nielen.
- Biosensor immunoassay for flumequine in broiler serum and muscle
W. Haasnoot, H. Gercek, G. Cazmier and M.W.F. Nielen.

7.3 Other publications

- Regarding the trends in analysis of veterinary drugs a review paper was published with the title 'Trends in analysis of veterinary drugs and growth-promoting agents'.
Authors: A.A.M. Stolker, T. Zuidema and M.W.F. Nielen
Journal: Trends in Analytical Chemistry (2007) volume 269 pages 67-979.

7.4 Presentations

- In July 2007 a Thai delegation was visiting the Netherlands. The delegation members were interested in the NRL/CRL organization structure. The delegation visited RIKILT and A.A.M. Stolker gave a presentation regarding 'Official control of residues of veterinary drugs in food'.
- In December 2007 a group of Brazilian expert (Brazilian LANAGRO managers and quality managers) visited RIKILT for a training in Quality Assurance, National Monitoring Plan, Quality Audits. During this training A.A.M. Stolker gave a presentation titled: Analysis of antibiotics in animal products; screening and confirmation'.

8 Other NRL activities: organisation of proficiency tests

In 2006 RIKILT has obtained accreditation for organizing proficiency tests focusing on veterinary drugs. See Annex IV for press release.

To get answers to the questions 'How do RFL deal with MRL's?' and in particular the question 'How to deal with MRL substances occurring in a none-MRL matrix?' the following proficiency tests were organized by RIKILT:

- The analysis of residues of (fluoro-)quinolones in muscle from broilers. This proficiency test started in 2005 and has been finalized in 2006. Results are described in RIKILT report 2006.003 of May 2006 'Inter-Laboratory Study for Quinolones in Poultry Muscle' by B.J.A. Berendsen, J.A. van Rhijn. The summary is presented in Annex V.
- The analysis of residues of penicillins in muscle and kidney from pigs. Results of this proficiency study are described in RIKILT report 2007.007 of April 2007 'Proficiency study for penicillins in porcine tissues' by B.J.A. Berendsen and T. Zuidema. The summary is presented in Annex VI.
- The analysis of residues of quinolones in egg. This proficiency test started in September 2007 and will be finalized in January 2008. Results of this proficiency study are described in RIKILT report 2008.001 'Proficiency study for quinolones in egg' by B.J.A. Berendsen and A.A.M. Stolker. The summary is presented in Annex VII.

A comparative study between VWA-Oost and RIKILT has been organized ad hoc on request of VWA-Oost for the analysis of residues of dioxins using the CALUX. Results of this study are presented in Annex VIII, *in Dutch*)

Annex I Letter from VWA to EU regarding use of hair as a matrix for the analysis of beta-agonists



voedsel en waren autoriteit

European Commission
DG Health and Consumer Protection
E3 Chemicals, contaminants, pesticides
for the attention of ms. Dr. A.M. Blass-Rico
Rue Belliard 232 4/43
B-10 49 BRUSSELS
Belgium

subject
Doc SANCO 2006/3228

Dear ms. Blass-Rico, Dear Ana Maria,

During the Residues Expert Meeting in June 2006 a list of recommended values was distributed stating the concentrations proposed by the CRLs for control of substances without a MRL. The Netherlands has commented on this document and in particular the proposed values for the A5 group (β -agonists) and the B2(c) group (NSAIDs) were too low in the opinion of the Netherlands to be routinely achievable at acceptable costs.

We regret to note that our comments regarding A5 and B2(e), although shared by several member states, were not adopted by the CRLs.

On the occasion of the Residues Expert Meeting in May 2007 a revised document was made available (SANCO 2006/3228) which contains some particular statements regarding the use of hair as a matrix for the analysis of β -agonists, which had not been included in previous versions of this document. We strongly disagree with the CRLs opinion on the use of hair as a matrix suitable for screening purposes only.

With this letter we want to draw your attention to this issue and we kindly request to be informed of the Commission's position in this matter.

We motivate the reasons for our disagreement below.

The CRLs state, "It is recommendable to use hair for screening purposes only because of the risk of external contamination. When taking hair it is always recommended taking also urine at the same time from the same animal." In the corresponding table the matrix 'hair' is indicated as suitable for screening purposes only.

Many of the substances, which could be abused for growth promoting are rapidly metabolised and excreted via urine and/or faeces. This results in a very short period after administration has been stopped during which residues of those substances can be detected. Unlike urine, hair is a matrix from which the analyses do not rapidly deplete and effectively the active substances are accumulated in hair. As a result, compared to urine, residues remain detectable in hair for a considerably longer period after administration.

date
July 18th, 2007
our reference no.
VWA/2007/31874

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The suggestion of the CRLs to use hair for screening only and sample urine "at the same time from the same animal" for confirmatory purposes, is in fact contra-productive since in many cases urine will no longer contain the residue of interest and hence yield a negative confirmatory result, while screening of hair samples of the same animal could have indicated abuse of growth promoting agents.

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July 16th 2007
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2/2

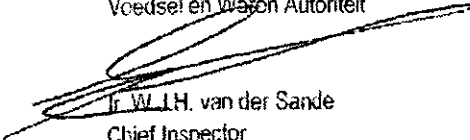
Hair as a matrix for detection of abuse of growth promoting agents has gained considerable interest in recent years. We are convinced that the CRLs are aware of this development. Not only for β -agonists hair has proven a suitable matrix for prolonged detection of abuse, also for detection of application of steroids and especially steroid esters, hair is currently considered an extremely suitable matrix.

We have understood that the Commission intends to publish this document on the SANCO website. We are anxious that the comments of the CRLs with regard to the matrix hair, when included in the published version of this official document, will invalidate previous investigations carried out by the Netherlands in the Salmeterol case of which you have been informed previously. Those investigations have led to still on-going legal follow-up against the owner of the treated animals.

Regarding the judicial acceptability of results obtained from the analysis of hair we emphasise that in the Netherlands we have used hair as the matrix for confirmatory analysis. Those results were considered by the Dutch Court for Appeal for trade and industry as perfectly acceptable legal prove of abuse of illegal substances. We therefore feel that the CRLs unrightfully invalidate hair as a legally acceptable matrix for control of the ban on the use of growth promoting agents and hence, in our view this statement is incorrect.

Last but not least we strongly emphasise that in our opinion it is not within the tasks of any CRL to judge the judicial acceptability of a particular matrix in legal procedures resulting from the detection of illegal use of growth promoting agents. We are therefore of the opinion that the remarks on suitability of the matrix hair should be removed from the document. In our view, the CRLs, by including those statements in an official Commission document, jeopardise the enforcement of the ban on growth promoting agents.

With kind regards,
Voedsel en Waren Autoriteit



J. W. L. H. van der Sande
Chief Inspector

Annex II Questionnaire: control of residues of antibiotics in cow milk

Meeting Brussels 27 April 2007

MEMBER STATE:

1. What antibiotics are authorised for use in milking cows (list) in your country?
2. For what purposes are they authorised in milking cows?
3. Describe briefly the strategy followed in the official laboratories for the control of residue of antibiotics in milk
Example:
 1. Screening microbiological tests (which one, always the same?)
 2. What is the next step in case of suspected non-compliant results obtained with a screening method? Confirmation? Which method?
 3. Are any sample directly analysed by chemical methods (which one?)
4. Which antibiotics-residues are the most frequently found in milk (ranking)?
5. If possible, describe briefly the strategy followed in the dairies, farms or self-checking (autocontrols) laboratories for the control of residue of antibiotics in milk
6. Which antibiotics-residues are the most frequently found in milk (ranking) in the case of self-checking?

Annex III (in Dutch) Report from RIKILT to VWA regarding triclabendazole in milk

Verslag van de toetsing door RIKILT van de door QLIP gebruikte methode voor de bepaling van Triclabendazole, Triclabendazole-sulfon en Triclabendazole-sulfoxide in zuivelproducten

Inleiding

In opdracht van de VWA is door RIKILT in het kader van de NRL taak residuen 96/23/EC de methode voor de bepaling van Triclabendazole, Triclabendazole-sulfon en Triclabendazole-sulfoxide in zuivelproducten getoetst. Het bezoek hiertoe door Tina Zuidema en Wim Traag vond plaats op donderdag 29 november (10.30- 15.30 h) bij het laboratorium van QLIP te Leusden. De gesprekspartners bij QLIP waren Dr. P. Steketec en Dhr. R. de Knecht.

De aanleiding van deze opdracht was dat het COKZ in het kader van haar publieke taak ook officiële monsters voor analyse uitbesteedt cq. gaat uitbesteden aan QLIP. Ter voorbereiding op het bezoek is door RIKILT 6 dagen vóór het bezoek bij QLIP schriftelijk de gebruikte methode en relevante informatie opgevraagd. Helaas heeft QLIP niet aan dit verzoek gehoor gegeven, hierdoor was een goede voorbereiding door RIKILT niet mogelijk.

Door de terughoudendheid van QLIP, zowel voorafgaand aan als tijdens het bezoek, en het ontbreken van de voor een goede beoordeling noodzakelijke details is het lastig om een goed gefundeerd oordeel met betrekking tot de gebruikte methode te geven.

Observaties

De stukken betreffende de gebruikte methode werden pas tijdens het bezoek ter inzage gegeven. De stukken bestonden uit:

Algemene omschrijving van de toegepaste methode, zonder details betreffende gebruikte extractiemiddelen, procedure etc.

Resultaten van een beperkte set (n=11) geanalyseerde monsters kaas, room en boter.

Beperkte validatie gegevens. Deze gegevens zijn gegenereerd na rapportage van de monsters kaas, room en boter, in de periode tussen de vooraankondiging van het bezoek en het feitelijke bezoek.

Na bestudering van de ter inzage verkregen stukken is een bezoek aan het laboratorium gebracht waar de metingen werden verricht. Het verzoek om ook het laboratorium te bezoeken waar de feitelijke opwerking (voorafgaand aan de meting) plaats vond werd geweigerd.

Wel werden voorbeelden van extracten opgehaald en getoond. Inzage in labjournaals werd niet toegestaan. Er werd slechts informatie ter beoordeling beschikbaar gesteld betreffende de meting, chromatogrammen, ijklijnen en berekening.

Ad 1:

Omdat het complete voorschrift niet ter beschikking werd gesteld kan er over de gevolgde procedure slechts een globaal oordeel gegeven worden. Het principe van de gevolgde methode lijkt goed.

Ad 2:

De resultaten van twee willekeurig door RIKILT gekozen positieve monsters kaas en boter zijn bestudeerd.

De bijbehorende blanco controle monsters waren beiden positief (circa 25% van de gerapporteerd gehalten). Mogelijke oorzaken:

Aanwezigheid van de component in het betreffende monsters (verkeerde keus blanco).

Cross contaminatie (beide monsters werden vooraf gegaan door een hoge standaard of een monster met toevoeging).

In geen van de gevallen werd aantoonbaar gemaakt dat dit tijdens de analyse was opgevallen en /of er acties waren ondernomen.

De gepresenteerde resultaten van de twee monsters laten zien dat in de extracten van deze monsters de identiteit van Triclabendazol-sulfon bevestigd werd (conform de EU criteria, zoals beschreven in 2002/657/EU).

Monsters worden gerapporteerd via e-mail zonder disclaimers.

Ad 3:

De beperkte validatie betreft het analyseren van een beperkte set blanco monsters en blanco monsters met toevoeging en de resultaten van de duplo analyse van een beperkte set positieve monsters.

De beperkte validatie is gericht op een kwantitatieve bevestigingsmethode, voornamelijk omvattend juistheid, herhaalbaarheid, binnenlab reproduceerbaarheid en lineariteit.

De resultaten van de beperkte validatie geven onvoldoende basis voor het betrouwbaar kunnen kwantificeren (o.a. enorme spreiding in juistheid, lineariteit voldoet niet). Wel lijkt de methode geschikt om de identiteit, conform geldende EU criteria, van Triclabendazole en de twee metabolieten te bevestigen.

De spreiding van de duplo analyses van de positieve monsters lijkt goed.

Conclusie

Door de terughoudendheid van QLIP, zowel voorafgaand aan als tijdens het bezoek, en het ontbreken van de, voor een goede beoordeling noodzakelijke details, is het lastig om een goed gefundeerd oordeel met betrekking tot de gebruikte methode te geven.

Er kan niet geconcludeerd worden dat er een goed beschreven methode op het lab gebruikt wordt.

De monsters zijn gerapporteerd met een niet uitontwikkelde en niet gevalideerde methode.

Over de extractie, de bereiding van de standaarden en de kwaliteitscontrole monsters kan geen oordeel gegeven worden omdat verzoek van RIKILT om dit te kunnen waarmemen, werd afgewezen.

Cross contaminatie tijdens opwerking/meting/analyse kan niet uitgesloten worden.

De identiteit van Triclabendazol-sulfon is in de extracten van de twee nader door RIKILT bekeken monsters bevestigd.

03-12-2007, Tina Zuidema, Wim Traag

Annex IV Press release regarding RIKILT accredited for organization of proficiency tests

Persbericht RIKILT - Instituut voor Voedselveiligheid, Wageningen UR

-

10 januari 2007

RIKILT ontvangt accreditatie voor ringonderzoeken dierbehandelingsmiddelen

Onlangs heeft RIKILT – Instituut voor Voedselveiligheid (onderdeel van Wageningen UR) een accreditatie ontvangen voor het uitvoeren van ringonderzoeken voor de analyse van dierbehandelingsmiddelen. RIKILT is daarmee het eerste instituut in Nederland dat op dit gebied een accreditatie voor ringonderzoeken ontvangt.

Een accreditatie is een onafhankelijk getoetst "bewijs van goed kunnen" voor een organisatie. In dit specifieke geval krijgt RIKILT een formele erkenning voor de uitvoering van ringtesten op het gebied van dierbehandelingsmiddelen (onder ILAC G13:2000) zoals bijvoorbeeld diergeneesmiddelen, groeibevorderaars en antibiotica. Een ringtest is een belangrijk middel om de prestaties van laboratoria met elkaar te vergelijken en daarmee gezamenlijk op een hoger plan te krijgen. RIKILT was al geaccrediteerd voor de analyse van een groot aantal stoffen uit deze groep. De accreditatie sluit goed aan op de nieuwe rolverdeling tussen de overheid en het bedrijfsleven. Deze houdt in dat niet de overheid producten in de markt controleert, maar dat bedrijven zelf de verantwoordelijkheid nemen voor het voldoen aan normen en regels. De overheid zal zich meer toeleggen op het toezicht op deze controlerol van het bedrijfsleven, het zogenaamde 'Toezicht op Controle'. Doordat RIKILT de accreditatie op ringonderzoeken heeft ontvangen, wordt het voor de laboratoria die meedoen aan de ringtesten mogelijk om hun prestatieniveau *aantoonbaar* te waarborgen.

RIKILT had al een accreditatie op basis van ISO 17025 voor testlaboratoria.

De testen die hieronder vallen, staan vermeld op de website van de Raad voor Accreditatie.

Noot voor de redactie:

Voor informatie over dit bericht kunt u contact opnemen met Jeannette Leenders van RIKILT, telefoon 0317-475402.

RIKILT – Instituut voor Voedselveiligheid is onderdeel van Wageningen Universiteit &

Annex V Summary report 2006.003: Inter-Laboratory study for quinolones in poultry muscle

The inter-laboratory study for quinolones in poultry muscle was performed in accordance with ISO/IEC Guide 43-1 and 43-2 and ILAC-G13.

For this inter-laboratory study, three test materials were prepared:

- A blank material (A);
- A material containing ciprofloxacin and enrofloxacin, the sum of both being just below the MRL, danofloxacin and difloxacin both at levels of about the MRL (B);
- A material containing ciprofloxacin and enrofloxacin, the sum of both being just above the MRL, danofloxacin and difloxacin at levels of approximately 0.5*MRL (C);

Homogeneity and satisfactory stability of the materials was demonstrated.

Thirty four laboratories were invited to participate in the inter-laboratory study for quinolones in poultry muscle of which seventeen laboratories, i.e. 50%, subscribed. Each laboratory received six randomly coded samples including one sample of material A, three samples of material B and two samples of material C. The laboratories were asked to analyze the samples in duplicate.

Fifteen laboratories managed to submit results that could be included in the evaluation. The majority of those laboratories applied a validated and accredited method for the analysis of quinolones in poultry muscle.

Three laboratories used a method that did not include all the quinolones that are registered for medication in poultry in the EU.

The laboratories applied different methodologies. Four different sample clean-up procedures can be distinguished:

- Solid Phase Extraction (SPE): using reversed phase (C18 or OASIS® HLB) or reversed phase combined with cation exchange interaction (SDB-RPS);
- Filtration (0.45 µm) without any further purification;
- Ultrafiltration (30 kD) without any further purification;
- Partial evaporation of the solvent followed by dilution without further purification.

Two detection techniques were applied for the quantitative and confirmatory analysis of quinolones in poultry muscle: LC-MS/MS and LC-(UV)-FLU.

In accordance with the definition of the MRL, all laboratories considered the sum of enrofloxacin and ciprofloxacin in classifying the results as either compliant or non-compliant.

Most participating laboratories determined values for CC α and CC β and, hence, the majority already complies with the requirements of Commission Decision 2002/657/EC regarding CC α and CC β that apply for registered veterinary drugs as from the 1st of August 2007.

Some laboratories reported values for CC α and CC β below the MRL. This is not in compliance with the definition of CC α and CC β for compounds for which a permitted limit is established. For some laboratories the reported values for CC α and CC β are not in agreement with the reproducibility of the analysis calculated from the reported results in this inter-laboratory study. In both cases, reconsideration of the value of CC α and CC β could be necessary.

4 RIKILT Report number 2006.003

No false positive or false negative results occurred in this inter-laboratory study.

For all compounds and materials a considerable variation of the reported results is observed. In some cases the lowest and the highest value reported differ by a factor 40. In this inter-laboratory study a considerable number of results is classified as questionable or unsatisfactory. Those results could not be

explained based on the applied detection technique. However, it is observed that filtration (0,45 µm) as sample preparation technique, without any further purification is not suitable for the analysis of quinolones in poultry muscle.

For each laboratory, the performance with respect to accuracy, reproducibility, false positive and false negative results was expressed in a laboratory performance score. Only 60% of the laboratories obtained the maximum score.

Based on the results, it is concluded that extra effort in the optimization of analytical methods for the analysis of quinolones in poultry muscle is urgently required:

- Danofloxacin, difloxacin and sarafloxacin should be included in the methods of analysis of quinolones by all laboratories, because those compounds are registered for medication in poultry in the EU;
- Reconsideration of numerical values determined for CC α and CC β may be necessary in some cases;
- An effort should be made regarding the quantitative analysis of all quinolones in poultry muscle with respect to the accuracy and the reproducibility.

Annex VI Summary of report 2007.007: Proficiency study for penicillins in porcine tissues

The proficiency study for penicillins in porcine tissues was organized in accordance with ISO/IEC Guide 43-1 and 43-2 and ILAC-G13, and performed under accreditation.

For this proficiency study, four test materials were prepared:

- A blank porcine muscle material (M-A);
- A porcine muscle material containing about 200 µg/kg cloxacillin and a trace of ampicillin (M-B);
- A blank porcine kidney material (K-A);
- A porcine kidney material containing about 100 µg/kg cloxacillin, 30 µg/kg ampicillin and about 20 µg/kg penicillin G (K-B).

During homogeneity testing, all materials proved to obtain sufficient homogeneity for proficiency testing.

Forty-four laboratories were invited to participate in the proficiency study for penicillins in porcine tissues of which 21 laboratories, i.e. 48%, subscribed. Each laboratory received six randomly coded samples. The laboratories were asked to analyze the samples in duplicate according to their own laboratory procedures. It was mentioned that maintaining the stability of the samples (storage and pretreatment) was part of the proficiency test.

Eighteen laboratories managed to submit results within the timeframe of the study of which 15 reported quantitative results for both the muscle and the kidney samples. Two laboratories reported only results for the muscle samples and one laboratory only reported screening results. The majority of the laboratories applied a validated and accredited method for the analyses.

The stability of penicillins can, according to literature, be maintained by storing the samples below -70 °C. Within this proficiency study a stability experiment at -20 °C was carried out. This stability study showed a degradation of ampicillin, cloxacillin and penicillin G above 75 % in the kidney material during the timeframe of the proficiency study. The penicillins in kidney proved to be instable even after stabilization by buffering the material at pH=6. Therefore, the kidney samples are not suited for evaluation purposes.

During storage at -20 °C the muscle material showed a degradation of 31 % for ampicillin and 27% for cloxacillin was observed. However, the penicillins in the muscle material showed to be stable at -20 °C after buffering at pH=6. Therefore, the muscle materials are suitable for this proficiency study, because maintaining the stability was mentioned as a part of this proficiency study.

In the stability study a degradation of penicillins during storage at -20 °C was observed, which is in agreement with literature. According to the information supplied by the participants, four laboratories stored their samples at -20 °C. Therefore it can be assumed that the samples of these laboratories were instable during the timeframe of the proficiency study. However, no statistically significant difference was observed between the results of the laboratories that stored the samples at -20 °C and the results of the laboratories that stored the samples at a temperature below -70 °C. Therefore, also the stability of the samples, even if stored at -70°C can be questioned. Probably, other factors than storage temperature are of influence on the stability. Based on these observations, the correctness of the calculated assigned values (consensus values) can be questioned. Therefore, this proficiency study is evaluated for information only.

The participants applied different methodologies for carrying out the analysis of penicillins in porcine tissues. Almost every laboratory applied identical procedures for muscle and kidney.

The mainly applied extraction solvent is a phosphate buffer at pH 8 to 9, sometimes combined with an organic solvent. The majority of laboratories applied Solid Phase Extraction as a sample clean up technique. In all cases a C18 material or OASISTM HLB was used as stationary phase.

Two laboratories applied a derivatization procedure. One laboratory applied a derivatization at the end of the sample clean up procedure using benzoic acid anhydride in combination with triazole and mercury chloride solution. The other laboratory applied a derivatization using piperidin during the extraction.

For all compounds and materials a considerable variation of the reported results is observed, possibly caused by the instability of the materials. No relations were observed between the laboratories results and the storage temperature, storage time (date of analysis) or a combination of these factors.

Based on the results, it is concluded that additional effort is needed to develop a robust method for the analysis of penicillins in porcine tissues. Stability of the compounds during storage seems to be an underestimated factor. Based on the results of the stability study, adequate storage and/or the use of a stabilization procedure at the time of arrival of the samples is required for obtaining reliable results.

Annex VII Summary of report 2007.001: Proficiency study for quinolones in egg

The proficiency study for quinolones in egg was organized in accordance with ISO/IEC Guide 43-1 and 43-2 and ILAC-G13, and under accreditation.

For this proficiency study, four test materials were prepared:

- A blank egg material;
- A blank egg material containing possibly interfering compounds to test the selectivity of the applied methods;
- An egg material containing about 70 µg/kg oxolinic acid (incurred) and about 50 µg/kg of both ciprofloxacin and enrofloxacin (spiked);
- An egg material containing about 125 µg/kg flumequine (incurred).

During homogeneity testing, all materials proved to obtain sufficient homogeneity for proficiency testing. The stability test demonstrated that no significant loss of any of the compounds occurred during the timescale of the proficiency test.

Eighteen laboratories subscribed for participation in the proficiency study quinolones in egg of which three are National Reference Laboratories. Fifteen laboratories managed to submit valid results within the timeframe of the stability study. Five of the participating laboratories applied a validated method which was accredited in all cases.

The minority of participants applied a validated method for the analysis of quinolones in egg. Only three laboratories reported values for $CC\alpha$ and $CC\beta$. It is noted that reported values for $CC\alpha$ and $CC\beta$ severely differ among the laboratories. Most likely these differences exist due to different interpretation of the regulations. From the reported values of $CC\alpha$ it is concluded that some laboratories calculated $CC\alpha$ based on a self set MRL, others applied the zero tolerance approach. From this it is concluded that laboratories cope in very different ways with the non existence of MRL values for quinolones in egg whilst MRLs are set for other matrices. Discussion on this issue resulting in clear legislation is of main importance for obtaining a uniform approach within Europe.

None of the laboratories detected any quinolones neither in the blank material nor in the material containing possibly interfering compounds. It is concluded that the applied methods are all satisfactory specific for the quantitative and confirmative analysis of ciprofloxacin (CIF), enrofloxacin (ENF), oxolinic acid (OXA) and flumequine (FLU) in egg.

One laboratory detected norfloxacin in the two samples that contain quinolones. This is considered as a false positive result. One laboratory did not detect CIF in the material that contains CIF, whilst CIF was included in their method. This is considered as a false negative result.

The laboratory's performance for the materials containing quinolones is summarized in Table 1.

Table 1. Summary of the laboratory's performance of the materials containing quinolones

Compound	Assigned value (X) (µg/kg)	Uncertainty of X (µg/kg)	No. of labs that reported results	No. of satisfactory results Accuracy
CIF	46.4	1.10	13	10
ENF	48.0	1.47	15	14
OXA	73.2	1.99	11	11
FLU	124.9	4.27	13	13

For OXA and FLU all reported results were satisfactory. For CIF and ENF some questionable and unsatisfactory results are observed. The occurrence of questionable or unsatisfactory results could not be explained by the applied detection or sample preparation technique.

However, 75% of the total of calculated z_a -scores is between -0.5 and 0.5 indicating excellent accuracy for most laboratories. Therefore it is concluded that the performance of most laboratories is excellent regarding the quantification of quinolones in egg.

One laboratory detected norfloxacin in one of the duplicate analysis of both samples of material Egg-03. This is considered as a false positive result. The same laboratory did not detect CIF in the samples of material Egg-03. This is considered as a false negative result.

The performance with respect to accuracy, false negative and false positive results was expressed in a laboratory performance score for each laboratory. In this 73% of the laboratories obtained the maximum score.

Based on the results of this proficiency study it is concluded that:

- regarding B group substances for which no MRL is set in a specific matrix, legislation should be clarified to obtain a uniform way for the determination of $CC\alpha$ and $CC\beta$ within the EU and with this a uniform way of characterizing the samples in terms of compliant and non compliant..
- for most laboratories extra effort is needed to validate the analysis of quinolones in egg to be able to report results including a value for measurement uncertainty.
- extra effort is needed by some laboratories to include oxolinic acid in the method of analysis for quinolones in egg because, officially, oxolinic acid is the only quinolone registered for medication of laying hens in the EU and for which an MRL is established.

Annex VIII (in Dutch) Report comparative study between VWA-Oost and RIKILT for the analysis of residues of dioxins using the CALUX

Aanleiding

Op verzoek van de VWA werd op 30-01-2006 op ad hoc basis een rondzendonderzoek voor bepaling van dioxinen met Calux georganiseerd. Er werden een viertal monsters uitgezocht die op basis van de resultaten van de GC-HRMS analyse blanco waren of hoeveelheden dioxinen bevatten die net boven de norm liggen. Het betrof twee monsters varkensvet en twee monsters voer.

Het verzoek aan de VWA is om de verstrekte monsters te onderzoeken op de aanwezigheid van dioxinen met de Calux test en de monsters te classificeren als negatief in geval geen dioxinen werden aangetroffen, of verdacht wanneer de aanwezigheid van dioxinen werd aangetoond.

Methoden

Bereiding monsters

De opzet van de ringtest is beperkt door de geringe hoeveelheid monstermateriaal die beschikbaar was. Het doel was de positieve monsters in duplo te verstrekken en de negatieve monsters in enkelvoud. Voor het varkensvet is dat echter niet mogelijk gebleken omdat er te weinig materiaal voorhanden was, dit monster is dan ook in enkelvoud verstrekt zodat in totaal 5 monsters aan VWA werden aangeboden (tabel 1).

Tabel 1: Monsters dierlijk vet en diervoeder verstrekt aan VWA-Laboratorium Oost ten behoeve van een ad hoc rondzendonderzoek Dioxinen screening met behulp van Calux

Matrix	Gehalte GC-HRMS (ng TEQ/kg)	Origineel RIKILT nr	Nieuw RIKILT nr	Inweeg (g)
Blanko varkensvet		164802	164854	29.9
Varkensvet	1.6	164650	164856	23.5
Blanko diervoeder		164763	164860	19.9
Diervoeder	2.3	164643	164862	21.1
		164643	164864	20.1

Varkensvetten zijn bij 70°C gesmolten, ghomogeniseerd en daarna in de monsterpotten gepipetteerd. Voermonsters zijn met een spatel gedurende ca 5 min geroerd om te homogeniseren en daarna ingewogen in de monsterpotten.

De monsters zijn verdeeld in aliquots zoals weergegeven in tabel 1 en voorzien van unieke RIKILT nummers. De monsters zijn op 30-01-2006 aan M. van Brakel VWA overhandigd, een overdrachtsformulier is opgemaakt en getekend. Het mondelinge verzoek aan de heer van Brakel is gedaan om, indien mogelijk, de monsters in duplo te analyseren.

Onderzoeksmethode VWA

De DR-CALUX bioassay van BioDetection Systems (BDS) is toegepast, met enkele modificaties. Het vet wordt geëxtraheerd met hexaan/diethylether, waarna een clean-up volgt over een zwavelzure silica-kolom. De modificaties behelzen een "voorverbranding" met zwavelzuur, wanneer veel vet moet worden ingewogen en langere zwavelzure kolommen dan BDS voorschrijft.

Het verkregen extract wordt vervolgens met een bioassay bepaald, waarbij op iedere plaat een calibratielijn TCDD wordt meegenomen. Aan de hand van deze calibratielijn worden de monsters

middels het gevonden gehalte beoordeeld als negatief of verdacht. Voor de beoordeling van dierlijk vet is uitgegaan van de 0,6 ng TEQ/ g vet (actiedrempel voor varkens) en voor veevoer van 0,5 ng TEQ/g product (actiedrempel veevoer van plantenmateriaal). De monsters zijn in duplo geanalyseerd.

Resultaten

Op 02-02-2006 zijn de resultaten van VWA-Laboratorium Oost gerapporteerd (zie bijlage 1). De resultaten zijn vermeldt in tabel 2.

Tabel 2: Resultaten van de screening op dioxinen met behulp van Calux van de monsters dierlijk vet en diervoeder

Matrix	RIKILT nr	Resultaat GC-HRMS (ng TEQ/kg) (RIKILT)	Resultaat Calux (VWA- Oost)
Blanko varkensvet	164854		Negatief
Varkensvet	164856	1.6	Verdacht
Blanko diervoeder	164860		Negatief
Diervoeder	164862	2.3	Verdacht
	164864	2.3	Verdacht

Conclusie

Er blijkt een goede overeenstemming tussen de resultaten van de GC-HRMS (RIKILT) en de Calux (VWA-Oost).

Bijlage 1

Rapportage van VWA-Oost

Rapportageformulier

Ad hoc inter-laboratoriumonderzoek dioxinen

Contactpersoon VWA-Oost:

E.A.J. van der Made

Datum (dd-mm-yy):

02-02-06

Resultaten

Bij rapportage dienen de geldende normen voor respectievelijk dierlijk vet en diervoeder te worden gehanteerd.

Analysedatum: 2-2-2006

Monsternummer	Matrix	Resultaat		Duploresultaat	
		Negatief	Verdacht	Negatief	Verdacht
164854	dierlijk vet	x		x	
164856	dierlijk vet		x		x
164860	diervoeder	x		x	
164862	diervoeder		x		x
164864	diervoeder		x		x

Opmerkingen:

Duplo: twee aparte analyses, dus twee inwegen per monster.

Welke criteria zijn toegepast op de resultaten bij classificeren als "negatief" of verdacht"?

Voor de beoordeling van dierlijk vet is uitgegaan van de 0,6 ng TEQ/ g vet (actiedrempel voor varkens) en voor veevoer van 0,5 ng TEQ/g product (actiedrempel veevoer van plantenmateriaal).

Informatie over de methode

Welke methode werd toegepast?

Geef svp een korte omschrijving van de methode (analyse inclusief monstervoorbewerkingsprocedure)

De DR-CALUX bioassay van BioDetection Systems (BDS) is toegepast, met enkele modificaties. Het vet wordt geëxtraheerd met hexaan/diethylether, waarna een clean-up volgt over een zwavelzure silica-kolom. De modificaties behelzen een "voorverbranding" met zwavelzuur, wanneer veel vet moet worden ingewogen en langere zwavelzure kolommen dan BDS voorschrijft.

Het verkregen extract wordt vervolgens met een bioassay bepaald, waarbij op iedere plaat een calibratielijn TCDD wordt meegenomen. Aan de hand van deze calibratielijn worden de monsters middels het gevonden gehalte beoordeeld als negatief of verdacht.

Is de methode gevalideerd en zo ja voor welke matrices?

Gebruik s.v.p. tabel 2 voor beantwoording met hieronder eventuele opmerkingen.

De methode voldoet aan de eisen zoals deze in de Richtlijnen 2002/69/EG en 2002/70/EG voor bioassays zijn gesteld, daarnaast heeft er een cross-validatie met BioDetection Systems (BDS) plaatsgevonden. Voor veevoer zijn aanvullende metingen verricht door verschillende analisten op verschillende dagen op drie niveau's (0,375 – 0,75 – 1,5 pg TEQ/g product, n= resp. 8, 11, 6). Uit F- en t-toets bleek dat het laagste niveau niet samengevoegd mocht worden met de twee hogere. De spreiding op een niveau van 0,75 pg TEQ/g product bedraagt 0,14 pg TEQ/ g product. Dit levert een meetonzekerheid (binnenlaboratoriumreproduceerbaarheid) op van 0,39 pg TEQ/g product op een niveau van 0,75 pg TEQ/g product.

Tevens wordt ieder zestiende monster ter controle gecontroleerd door een HR-GCMS-bepaling. Tot op heden zijn geen vals negatieve resultaten na bevestiging met HR-GCMS voorgekomen.

Werd de methode gevalideerd conform Commissie Beschikking 2002/657/EC?

Zo ja, geef s.v.p. de CCB.

Zo nee, welke benadering werd dan toegepast voor validatie en wat is de CCB of LoD?

Nee, de methode is niet gevalideerd conform 2002/657/EC . De LoD is volgens BDS 0,3 pM/well, zodat de methode voor varkensvet een LoD heeft van 0,14 pg TEQ/g vet en 0,07 pg TEQ/g product voor veevoer.

Is de methode geaccrediteerd en zo ja voor welke matrices?

Gebruik s.v.p. tabel 2 voor beantwoording met hieronder eventuele opmerkingen.

De methode is niet geaccrediteerd.

Tabel 2. Informatie betreffende validatie- en accreditatiestatus

Matrix	Gevalideerd		Geaccrediteerd		CCB	LoD
	Ja	Nee	Ja	Nee		
veevoer	x	0	0	x		0,07 pg TEQ/ g product
varkensvet	0	x	0	x		0,14 pg TEQ/ g vet
	0	0	0	0		
	0	0	0	0		
	0	0	0	0		
	0	0	0	0		
	0	0	0	0		
	0	0	0	0		
	0	0	0	0		

Stuur de resultaten zo spoedig mogelijk, bij voorkeur per e-mail naar:

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