

Project number: 972.036.01
BAS-code: WOT-02-438-III-025
Project title: National Reference Laboratory 96/23/EC

Project leader: Mrs. A.A.M. Stolker

Report 2009.006

June 2009

Veterinary drugs in animal products

Annual report 2008 of the National Reference Laboratory

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The research described in this report was funded by: the Ministry of Agriculture, Nature and Food Quality (project 7203601) and is part of WOT 02 (Food Safety).

Distribution list:

- Food and Consumer Product Safety Authority (VWA), The Hague (A. Lam)
- Food and Consumer Product Authority (VWA-Oost), Zutphen Laboratory (J.A. van Rhijn; H. Keukens)

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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 described the activities employed in 2008.

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 2008 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 national plan activities through the official 'Werkgroep National Plan' meetings. Furthermore the competent authority was advised on one specific issues viz. the draft SANCO document 'Setting maximum levels for coccidiostats or histomonostats in food resulting from the unavoidable carry-over of these substances in non-target feed'. The NRL advised the Community Reference Laboratories (CRLs) regarding the method for validation of screening methods and validation of transferred screening methods.

Coordinating activities

The NRL prepared quality control samples (approximately 149 samples for 12 different analyses). The practical preparation of quality control samples is the aim of a separate project (project no.7203701). The NRL provided the RFL with 3 new methods of analysis and advised 5 times National Food control Laboratories outside the Netherlands - on their request - about different method of analysis for the determination of veterinary drugs.

Participation in workshops and proficiency tests

Employees of the NRL participated in workshops (3 times) and proficiency tests (3 times) organized by CRLs. NRL employees participated also in 8 proficiency tests organized by other organizations like FAPAS. The results of the proficiency tests were all acceptable (Z-score <2).

RIKILT is accredited (according to ILAC G13:2000) for the organization of proficiency tests. In 2008 one (international) proficiency test was 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. For the coming period collaboration and communication of the NRL and RFLs will be continued. Special attention will be paid to the construction of multi-analyte method by combining existing method with minor differences.

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1 Introduction

The European Commission is committed to protecting consumers from intolerable health hazard, 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 members are able to take part in further training courses organized by the Commission or by CRLs.

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

2 Communications and advices

One of the tasks of the NRL is to communicate with the Competent Authority, RFLs 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 2008 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 2008 RIKILT participated in the working group setting up the plan for monitoring residues.

During 2008 there were 3 meetings of this working group.

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.

The NRL had three official management meetings with RFL and five technical meetings (minutes are available) and several times unofficial meetings by telephone and e-mails.

2.1.3 *With National Reference Laboratory*

On a regular base the management of the NRLs 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-assurance, Quality-control, Quartet) meeting. In the Q3 group participate the R3 laboratories and TNO-Ducares.

In 2008 the R3 group had no formal meeting. There were two informal meeting during the Euro Residue VI conference in Egmond aan Zee (May 2008).

- There was communication by e-mail and telephone to discuss and combine the comments on the draft CRL document (version 4) regarding the proposed method for the 'Validation of screenings methods' and the 'Validation of transferred screening method'. See also item 2.2.1.
- There was communication by e-mail and telephone between RIKILT and RFL (parties involved in coccidiostats analysis) to discuss the draft SANCO document 3417/2008 'Setting maximum levels for coccidiostats or histomonostats in food resulting from the unavoidable carry-over of these substances in non-target feed'. See also item 2.2.2.

In 2008 the Q3 group had no official meeting. There were two informal meeting during the Euro Residue VI conference in Egmond aan Zee (May 2008).

2.2 Advices

2.2.1 *To directors of the CRL's regarding validation of screening method*

Two CRL documents describe guidelines for the validation of screening methods and the validation of transferred screening method. The following documents were for discussion:

- 1) Guide for analytical validation of screening methods (Draft 4 Validation_screening-September 2007) by Valerie Gaudin en Pascal Sanders from CRL-Fougeres and Petra Gowik and Steffen Uhlig from CRL-Berlin.
- 2) Guideline for validation of transferred screening methods; Non-Paper 23/6/08 (CRL-Fougeres and CRL-Berlin).

The NRL's were asked for their comments on these documents.

RIKILT gave their opinion on the documents and asked for input from RIVM and RFL.

The final comments (proposed by RIKILT and evaluated by RIVM and RFL) were sent by RIKILT to the directors of the CRLs. See Annex 1.

2.2.2 *To competent authority regarding draft SANCO documents*

RIKILT was asked by the competent authority to give their opinion about the draft SANCO document 3417/2008 'Setting maximum levels for coccidiostats or histomonostats in food resulting from the unavoidable carry-over of these substances in non-target feed'. Final comments were sent by the NRL to the competent authority (by e-mail as requested). See Annex II.

3 Coordinating activities

3.1 *Preparation of quality control samples*

The activities employed for the RFL regarding the preparation of quality control samples are described in a separate project (WOT programme on Food Safety - Part 3 Veterinary Drugs; project 7203701 Analytical Chemical Quality Control project for Laboratory VWA-East). In 2008 approximately 149 quality control samples for 12 different analyses were prepared. The details regarding the prepared samples, the analytical results obtained and the discussion/meetings between VWA-East and RIKILT are described in RIKILT Annual report 2008 'De Chemische Borging van Laboratorium VWA door RIKILT ' by B.J.A. Berendsen, status : in preparation.

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 2008 the following methods have been provided to VWA-East:

LC-MS/MS-analysis of Triclabendazol (and metabolites) (RSV A0865);

LC-MS/MS-analysis of Benzimidazoles en triclabendazol in animal tissue (revision of RSV A0768)

LC-MS/MS-analysis of NSAID's (revision of RSV A0702)

LC-MS/MS-analysis of antiviral drugs (draft RSV A1085)

The method for the analysis of quinolones in fish and meat (RSV 0900) and the method for the analysis of coccidiostats in eggs (RSV A1067) were - on their request - also provided to VWA-East.

The RFL (VWA-East) observed problems with the analysis of aminoglycosides and after several meetings and training of personnel by the NRL the VWA-East was able to perform the method at their own lab.

Futhermore methylated internal standards for the analysis of aminoglycosides were provided to the VWA-East.

Next to the national RFLs several national food laboratories from other countries were asking for trainings and advices.

- Training on the analysis of carbadox and olaquinox analysis was given to Zina Theodorou from NRL Cyprus.

- Advices on the analysis of antibiotics were given to Ms. Enktuya National Food Laboratory in Mongolia.

- Information regarding the set up of National Monitoring Plans for residue analysis within the EU was exchanged with Dr. Thomas Korth, manager Residue Chemistry and Laboratory Performance Evaluation from the National Food Laboratory in Canberra (Australie).

- Indonesian delegates visited RIKILT for a training. They were informed about: Setting up National Monitoring Plans for Fish and Fish products; Analysis of Antibiotics and CRL/NRL network in the EU.
- Dr. Hibiru Mandiri (Mutucertification; Indonesia) visited RIKILT and was informed about the accreditation/certification of analytic methods.
- Zahira Herrera from Food Laboratory in Brasil worked as a visiting scientist on methods for antibiotic screening of food.

4 Analytical activities

The activities employed for the RFL 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 7152101 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.

5 Participating in CRL workshops

In 2008 employees of RIKILT participated to the following workshops:

- CRL-Workshop Technical, Analytical and Statistical Issues, June 3-6, BVL Berlin.

T. Zuidema actively contributed to this workshop by means of an oral presentation "The implementation of SPE-LC-MS/MS for veterinary drugs".

- CRL-Workshop antibiotics: Strategies of Confirmation Methods and Proficiency Testing, June 26-27 2008, AFSSA Fougères.

R. Peters actively contributed to this workshop by means of an oral presentation about the organisation of proficiency testing by RIKILT.

- CRL-workshop hormones, 22-24 October 2008, RIVM, Bilthoven. R. Peters actively contributed to this workshop by means of an oral presentation about ToF-MS for multi-analyte screening of veterinary drugs and growth promoting agents. Furthermore J. Lasaroms was an observer in this workshop.

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 2008:

Malachitegreen in fish (FAPAS); z-score 0.3

Tetracyclines in meat (FAPAS); z-score 0.4

Anthelmintica in milk (BVL); not yet reported

Nitroimidazoles in meat (IRMM) used as a quality control sample; good results

Chloroamfenicol in shrimps (FAPAS); z-score 1.2

Nitrofuranes in meat (AFASSA); not yet reported

Tetracyclines en sulfonamides in meat (Progetto Trieste)

Chloroamfenicol in meat (Progetto Trieste); z-score -0.16

Robenidine in animal food (KDLL); no z-score due to limited number of participants n=4

Quinolones in meat (FAPAS); z-score 0

Chloroamfenicol in urine (RIVM); not yet reported

7 Posters, publications and presentations

7.1 Posters

Poster presented at the Euro Residue VI Conference on Residues of Veterinary Drugs in Food; Egmond aan Zee; 19-21 May, 2008. Page numbers refer to the proceedings of the Euro Residue Conference (see 7.2).

CAMPARISON OF THREE MICROBIAL SCREENING METHDODS FOR ANTIBIOTIC SCREENING OF ROUTINE MONITORING SAMPLES

Mariël G. Pikkemaat, Sabrina Oostra-van Dijk, J. W. Alexander Elferink, Michel Rapallini
419-423

FLOW CYTOMETRIC IMMUNOASSAY FOR SULFONAMIDES IN MILK, BLOOD SERUM, MEAT DRIP AND EGGS.

Monique E. Bienenmann-Ploum, Wouter de Keizer, Henriëtte D.L.M. van Eekelen, Aldert A. Bergwerff, Willem Haasnoot and Michel W.F. Nielen.
425-430

PERFORMANCE EVALUATION OF HORMONE AND VETERINARY DRUG RESIDUE SCREENING BY ULTRA PERFORMANCE CHROMATOGRAPHY COUPLED TO TIME-OF-FLIGHT AND ORBITRAP MASS SPECTROMETRY

Ed van der Heeft, Paul Zomer, Linda A.M. Stolker and Michel W.F. Nielen
565-569

PROFICIENCY TESTING IN THE FIELD OF VETERINARY DRUGS IN FOOD

Bjorn J.A. Berendsen, Linda (A).A.M. Stolker, Hans (J).A. van Rhijn
699-704

THE IMPROVEMENT OF SAMPLE THROUGHPUT IN RESIDUE ANALYSIS USING THE 96-WELL FORMAT

Bjorn J.A. Berendsen, Linda (A).A.M. Stolker, Hans (J).A. van Rhijn
705-710

COMPREHENSIVE SCREENING OF VETERINARY DRUGS IN FOOD USING UPLC COMBINED WITH FULL SCAN MS DETECTION

Linda Stolker, Efraim Oosterink, Paula Rutgers, Johan Lasaroms, Ruud Peters, Hans Mol, Hans van Rhijn, Michel Nielen 717-722

MULTIRESIDUE METHOD FOR THE SIMULTANEOUS DETERMINATION OF BENZIMIDAZOLES IN BOVINE MILK BY ON-LINE SPE-LC-MS/MS

Frédérique L. van Holthoon and Tina Zuidema
849-854

FORMATION OF SEMICARBAZIDE FROM AZODICARBONAMIDE
AND BIUREA DURING BREAD PREPARATION: A MODEL STUDY

Patrick P.J. Mulder, Elodie Mothiron¹, Hans (J).A van Rhijn
1043-1047

7.2 Publications in proceedings of Euro Residue

All presented papers (posters and oral) of the Euro Residue VI Conference on Residues of Veterinary Drugs in Food; Egmond aan Zee; 19-21 May, 2008; are published in the proceedings of the Conference.

Residues of Veterinary Drugs in Food Edited by: LA. Van Ginkel and A.A. Bergwerff, ISBN 978-90-804925-3-0.

7.3 Other publications

The following article was published in a peer reviewed journal:

Title: Comprehensive screening and quantification of veterinary drugs in milk using UPLC–ToF-MS

Authors: A. A. M. Stolker & P. Rutgers & E. Oosterink & J. J. P. Lasaroms & R. J. B. Peters & J. A. van Rhijn & M. W. F. Nielen

Source: Anal Bioanal Chem (2008) 391:2309–2322.

Title: A generic method for the quantitative analysis of aminoglycosides (and spectinomycin) in animal tissue using methylated internal standards and liquid chromatography tandem mass spectrometry

Authors: F.L. van Holthoon, M.L. Essers, P.P.J. Mulder, S.L. Stead, M. Caldwell, H.M. Ashwin, M. Sharman,

Source: Anal. Chim. Acta, In Press, Accepted Manuscript.

Regarding the use of Time-Of-Flight- MS analysis of veterinary drugs a Chapter for a ACS-issue on ToF-MS was submitted.

Author: A.A.M. Stolker;

Title: Application of (UP)LC-ToF-MS for residue analysis of veterinary drugs and growth promoting agents in products of animal origin.

Status: The book is *in press*. Book is edited by Imma Ferrer.

7.4 Presentations

Oral presentations presented at the Euro Residue VI Conference on Residues of Veterinary Drugs in Food; Egmond aan Zee; 19-21 May, 2008:

HERBAL ALTERNATIVES FOR ANTIMICROBIAL GROWTH
PROMOTERS

Maria J. Groot, Tedje van Asseldonk, Johanna Fink, Bart Halkes⁴ and Gerdien Kleijer-
Ligtenberg 53-58

TRENDS IN MONITORING THE USE OF VETERINARY DRUGS
AND GROWTH-PROMOTING AGENTS

Linda Stolker, Marco Blokland, Tina Zuidema, Paul Zoontjes, Saskia Sterk, Michel
Nielen, Leen van Ginkel 81-86

- In August 2008 an Indonesian delegation was visiting the Netherlands. The delegation 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 November 2008 during the WOT Theme 3 Symposium Organized by LNV in Den Haag, a presentation was given regarding the NRL-project. Hand-outs of this presented are presented in Annex III.

8 Other NRL activities

8.1 Organisation of proficiency tests

In 2006 RIKILT has obtained accreditation for organizing proficiency tests focusing on veterinary drugs.

The following proficiency tests were organized by RIKILT:

- The analysis of residues of macrolides in meat. Results of this proficiency study are described in RIKILT report 2009.003 of February 2009 'Proficiency study for macrolides in meat' by B.J.A. Berendsen. The summary is presented in Annex IV.

9 Plan for NRL activities 2009

The quality assurance program for the RFL will be continued as well as the regular meetings between the NRL, RFL and the NRLs within the Netherlands (technical meetings and R3 meeting).

Furthermore the employees of the NRL will participate in the workshops organized by the CRLs and in the organized proficiency tests (for the relevant groups of compounds).

Special attention will be paid to harmonization of analytical methods for the analysis of veterinary drugs in products of animal origin. Nowadays many LC-MS/MS based methods are available for the analysis. Some of them only differs by the composition of the LC mobile phase or by the LC column used or by the SPE material used for the extraction. It is worthwhile to find out it is possible to use one 'standard LC-MS/MS' for the analysis of different classes of veterinary drugs. Due to the fact that most of the analytical methods are fully validated and accredited some minor changes in the LC-MS/MS systems do have influence on the accreditation status. Therefore, a short additional validation procedure has to be developed which can be applied in case an accredited analytical method is slightly changed.

The advance will be that less analytical method will be used which simplifies the control of veterinary drugs in products of animal origin.

10 References

Council Directive 96/23/EC, Official Journal L 125 , 23/05/1996 P. 0010 - 0032

RIKILT report 2009.003, B.J.A. Berendsen, Inter-Laboratory Study for Macrolides in Meat.

RIKILT report 2009. status in preparation, B.J.A. Berendsen, De chemische borging van laboratorium VWA door RIKILT jaarrapportage 2008.

Annex I Comments on CRL proposed guidelines for validation of (transferred) screening methods

From: Stolker, Linda
Sent: woensdag 1 oktober 2008 11:05
To: 'v.gaudin@fougeres.afssa.fr'; 'Dr. Petra Gowik'; 'Leen van Ginkel'
Cc: Peters, Ruud (RIKILT)
Subject: comments on guide for validation of screening methods

Attachments: Comments on screening guidelines.doc

Dear colleagues,

As a follow up of the CRL-workshops in Berlin and Fougeres hereby the requested comments on the guidelines for validation.

From our own experiences with method validation it is concluded that each validation study has its own specific problems, due to analytes (metabolites are included) technique used (microbiology chemical/physical) number of analytes to be detected, number of matrices included etc. One additional (to the attachment) remark is that we think that it is very important that the CRL's advise the NRL/RFL in case they observe any problems or have any questions regarding the set up of the validation study.

In case you have any additional question regarding our comments on the guidelines please do not hesitate to contact me,

Kind regards

Linda Stolker

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1. Guide for Analytical Validation of Screening Methods

Draft document, version 4

The EC Decision 2002/657/EC defines screening methods as: “methods used to detect the presence of a substance or class of substances at the level of interest. Such methods allow for high-throughput screening to identify potential non-compliant samples. They are specifically designed to avoid false compliant results”. For a few years now there is some debate whether, and how, screening methods should be validated. At the NRL workshop of June, 25-27 in Fougeres, France, the latest draft of the

document “Guideline for Analytical Validation of Screening Methods, draft 4, September 2007” was presented. In this document guidelines for the validation of biological, biochemical and physico-chemical methods, qualitative as well as quantitative are given.

In our opinion this guideline, although it is a draft 4 version, still appears fairly complicated and seems unable to really choose between qualitative and quantitative methods. In the end the document distinguishes between 6 procedures; biological, biochemical and physico-chemical, both qualitative as well as quantitative. However, we think that it should be possible to have no more than two procedures, one for qualitative and one for quantitative methods, without further differentiation. In addition we notice that the recommended number of samples in some cases is very high and that up to four concentration levels are used, even for qualitative methods. In this way especially the validation of qualitative screening methods becomes as complicated, and time and money consuming, as a quantitative confirmation method. For that reason, and a broader acceptance by users, we propose a more straightforward guideline for validation that gives a strategy for a qualitative and a quantitative screening. With regard to the strategy we suggest the following approach:

Qualitative methods give a simple “yes” or “no” answer whether this is by a chromatographic peak (physiochemical methods), a color reaction (biochemical methods) or an inhibition zone (biological methods). To differentiate between compliant and non-compliant samples a “matrix-matched control point” can be employed. Such a control point may be considered as a cutoff concentration at some percentage below “The level of Interest”. The level of interest is usually either the Regulatory Limit (MRL, MRPL) or an “Action Level/Limit”. We consider such a methodology particularly useful when screening large batches of samples which are likely to be compliant. False negative results are of the greatest concern in screening procedures since suspected samples are subsequently checked using a confirmation method. By setting the “matrix-matched control point” at 50% of the level of interest, methods generally produce less than 5% false negatives. During validation this can be confirmed by analyzing 20 samples spiked at the level of this “control point”. Only if the sensitivity of the method is such that the “matrix matched control point” is close to the level of interest, a higher number of samples may be required to demonstrate less than 5% false negatives. The false positive ratio can be determined in the same manner by analyzing 20 blank samples.

As an example of this approach we included table 1 that contains data from a validation of a screening method for veterinary drugs in milk where 28 spiked samples were analyzed over 4 different days. By using a matrix matched control point at 50% of the level of interest, the false negative rates were below 5% for all substances with the exception of phenylbutazone. The latter probably results from the poor sensitivity of this substance in this method. As expected the false negative rate increases when the matrix matched control point is chosen at 75% of the level of interest, especially for compounds with a poor sensitivity such chlorotetracycline and phenylbutazone. Therefore, the “safe” level for the matrix matched control point will depend on the sensitivity and with a poor sensitivity more control sample should be analyzed. In addition, if the level of interest is set much lower than the MRL, fewer replicates will be needed during validation where we consider 20 as a minimum.

Table 1. False negative rates with a matrix matched control point at 50% and 75% of the level of interest

	MR(P/L)	level of interest	No. of false negatives	
			control point at 50% of level of interest	control point at 75% of level of interest
Benzimidazoles				
albendazole	100	50	0 (28)	0 (28)
fenbendazole	10	10	0 (28)	0 (28)
flubendazole	-	2	0 (28)	1 (28)
oxfendazole	10	10	0 (28)	0 (28)
Macrolides				
erythromycine	40	40	0 (28)	0 (28)
joramycin	-	50	0 (28)	0 (28)
spiramycine	200	50	0 (28)	0 (28)
tylosin	50	50	0 (28)	0 (28)
Quinolones				
difloxacin	-	2	0 (28)	3 (28)
flumequine	50	50	0 (28)	2 (28)
nalidixic acid	-	2	0 (28)	0 (28)
norfloxacin	-	25	0 (28)	0 (28)
oxolinic acid	-	2	0 (28)	1 (28)
Sulphonamides				
dapsone	5	5	0 (28)	2 (28)
sulphadiazine	100	50	0 (28)	0 (28)
sulphadimethoxine	100	50	0 (28)	0 (28)
sulphadoxine	100	50	0 (28)	0 (28)
Tetracyclines				
chlorotetracycline	100	100	0 (28)	11 (28)*
doxycycline	-	100	0 (28)	0 (28)
oxytetracycline	100	100	0 (28)	1 (28)
tetracycline	100	100	0 (28)	2 (28)
NSAIDs				
diclofenac	5	50	0 (28)	0 (28)
fenbufen	-	50	0 (28)	1 (28)
ketoprofen	-	50	0 (28)	0 (28)
mefanamic acid	10	50	0 (28)	4 (28)
phenylbutazone	5	50	3 (28)*	10 (28)*
piroxicam	-	50	0 (28)	0 (28)
propyphenazone	-	50	0 (28)	0 (28)

*; The screening method had a low sensitivity for these substances

A quantitative method, also a quantitative screening method, is expected to produce an accurate concentration. In our opinion this means that with respect to dynamic range, accuracy and precision, such a method meets the same requirements as a true quantitative confirmatory test, e.g. 2002/657/EC can be followed.

2. Guideline for validation of transferred screening methods

For the procedure necessary to show that a method is “fit for purpose”, the procedure above (item 1) can be used. However by transfer of method not all the analyte-matrix combinations have to be tested but only the most critical (lowest sensitivity, most polar, most non-polar, etc).

More important is that during sample analysis for each series of samples at least 2 additional blank and 2 additional ‘positive samples’ are analyzed.

After ten series the data of the 20 blank and 20 control samples has to be evaluated for containing false non-compliant and false compliant results. These sets of blank and positive samples should preferably consists of a broad range of matrix-analyte combination, representative for the scope of the method.

Ruud Peters, Linda Stolker
RIKILT – Institute of Food Safety
Wageningen
The Netherlands
Date: 30 September 2008

Annex II Comments on draft document SANCO 0324/2008

From: Stolker, Linda
Sent: maandag 17 november 2008 12:04
To: hans.van.rhijn@vwa.nl
Cc: Zuidema, Tina
Subject: comments on draft EU regulation regarding food

Comments on SANCO document 3024/2008

Setting maximum levels for coccidiostats or histomonostats in food resulting from the unavoidable carry-over of these substances in non-target feed

First of all it is good to mention that is an advantage that there is some of a regulation concerning the contamination of food by the unavoidable carry over of histomonostats and coccidiostats during feed production.

However I do have some remarks

The under laying study of EFSA concerning the maximum levels of the compounds in food after administration of contaminated (by unavoidable carry over of histomonostats and coccidiostats) is an alternative approach for setting limits. It differs from the approaches used for setting MRPLs and MRLs. Is it desirable to introduce a third approach? The final proposed Maximum Levels in food are for some of the compounds (due to the used approach) relatively low. I mean by this the levels are relatively low in comparison with some of the existing MRLs for target animal species.

From the analytical part of view it is probably possible to detect the low proposed maximum limits however for some compounds e.g. semduramycin, maduramycin, lasalocid (eggs) and narasin (eggs milk) it will be rather difficult to obtain the proposed low limits. Therefore it is necessary to optimize existing methods and/or develop new methods to finally reach the low detection limits (<5 µg/kg) necessary for monitoring purposes.

It is worthwhile to think about the option to set the limits not lower than 10 µg/kg unless:

- The limit of 10 µg/kg is not a safe limit (based on the toxicological studies)
- There is a MRL for a target species which is below 10 µg/kg.

When the maximum limits are set at 10 µg/kg there is a possibility to use a multi-analyte methods for monitoring purposes. In this way the new limits can probably more easily be included in already developed and validated methods for coccidiostats or histomonostats in target species.

Annex III Hand-out presentation Symposium on WOT Theme 3 (Den Haag, November 2009)

Kwaliteit onder controle ?

Linda Stolker, Bjorn Berendsen




Wat is kwaliteit?



Product of dienst is van goede kwaliteit indien:

- Het voldoet aan de wensen van de klant
- Het voldoet aan onafhankelijk vastgestelde criteria (normen; richtlijnen)



Wensen van de klant



- Wie is de klant?
 - EU → LNV
- Wat vraagt de klant en waarom?
 - Uitvoer van WOT Voedselveiligheid
 - Thema 3 Dierbehandelingsmiddelen – NRL/QA/QC

EU wetgeving

- Richtlijn
 - 96/23/EC (96/22/EC; 2003/74/EC)
- Beschikking 2002/657/EC
- Verordening EEC 2377/90
- Verordening EC 882/2004



Afstemming van activiteiten



RIKILT-projecten binnen WOT Thema 3: NRL/QA/QC

- Nationaal Referentie Laboratorium
- Borging VWA Lab
 - Microbiologisch
 - Histologisch
 - Analytisch-chemisch
- Validatie & accreditatie methoden dierbehandelingsmiddelen

EU wetgeving

- Richtlijn 96/23/EC
- Beschikking 2002/657/EC



Afstemming activiteiten




Overlegstructuur LNV RIKILT

- Werkgroep Nationaal Plan
 - Aantal en aard van de monster
 - Te meten dierbehandelingsmiddelen
 - NP wordt opgesteld en geëvalueerd



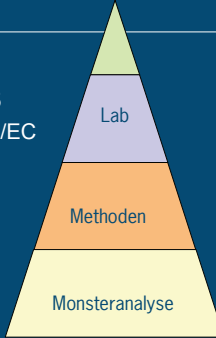

Afstemming activiteiten; overlegstructuur

- CRL/NRL/RFL
 - RIKILT-RIVM-VWA (voorheen CLRVV) vormen R3 overleg; regelmatig overleg
 - Uitwisseling van ervaringen op technisch gebied
 - Bespreken van knelpunten; methoden, standaarden
 - Afstemming ringtesten
 - RIKILT-RIVM-VWA-TNO/Ducares vormen Q3 (Quality-accuracy, Quality control, Quartet) overleg; ad hoc
 - Uitwisseling van ervaringen op technisch gebied
 - RIKILT-VWA
 - Managementniveau
 - Technisch inhoudelijk niveau




Toetsen aan criteria

- Voor laboratorium: ISO 17025
- Gebruikte methode: 2002/657/EC
- Borging van de kwaliteit in de praktijk
 - Referentie standaarden
 - Analyse van borgingsmonsters
 - Deelnemen aan ringtesten



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ISO 17025

Onafhankelijke beoordeling door de Raad voor Accreditatie (RvA)

- Laboratorium organisatiestructuur
- Laboratorium management
- Kwalificatie mensen
- Status apparatuur
- Algemene kwaliteitsborgingssysteem
 - Klachtenprocedures
 - Rapportages
 - Monsterregistratie
 - ARBO.....


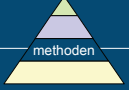



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2002/657/EC Validatiecriteria

FVO inspecties

- Specifiek voor het onderzoek uitgevoerd onder richtlijn 96/23/EU
 - Bevestigings criteria
 - Validatie criteria

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Borging van de kwaliteit in de praktijk

- NRL antibiotica zorgt voor borging van RFL door
 - aanleveren van methoden
 - aanleveren van referentiestandaarden
 - doorgeven van via CRL ontvangen informatie
 - bereiding van borgingsmonsters
 - organiseren van ringtesten





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Aanleveren gevalideerde methoden*

- Nitroimidazoles in plasma
- Stanozolol in plasma
- Aminoglycosiden in vlees
- Salmeterol in haar
- Steroidhormonen in pluimveelever
- Coccidiostatica in eieren
- Macroliden in vlees
- Steroidglucuroniden in urine
- Carbadox en olaquinox in vlees
-

*ook voor uitvoering van AID onderzoek



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Aanleveren van standaarden

- Gezamenlijke aankoop van dure gedeutereerde interne standaarden
- Nieuwe referentie standaarden die moeilijk commercieel verkrijgbaar zijn




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Aanleveren van informatie

monsteranalyse

NRL informeert RFL

- Informatie van CRL's (AFSSA-Fougères, RIVM-Bilthoven, BVL-Berlijn)
- Veranderende regelgeving
- Validatiecriteria...

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Borgingsmonsters

RIKILT/NRL

-Bereiding materiaal
-Meten van concentratie

antibioticum

100x20 µg/kg

VVA/REI

Bepaalt de concentratie

80-120 µg/kg ✓
<90 µg/kg
>120 µg/kg

Optimalisatie/harmonisatie van methoden

RIKILT-VVA: Evaluatie van de resultaten

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Bereiding van borgingsmonsters

- Bereiding van borgingsmonsters
- RIKILT en VVA bepalen in overleg type borgingsmateriaal

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Bereiding borgingsmonsters

- Quinolonen in pluimveevlees
- Dapson in varkensvlees
- Nitroimidazolen in varkensvlees
- Quinolonen in ei
- Avermectines in varkenslever
- Levamisol / thiabendazol in varkenslever
- Sulfonamiden in varkensvlees
- Nitrofuranen in pluimveevlees
- Coccidiostatica in pluimveevlees
- Tetracyclines in pluimveevlees
- Desoxycarbadox in varkensnier

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Resultaten

monsteranalyse

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Organiseren van ringtesten

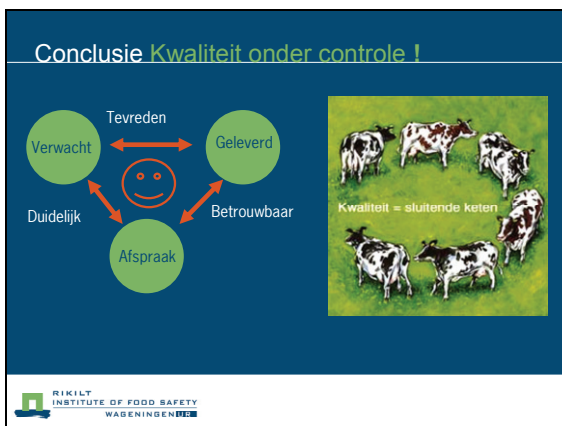
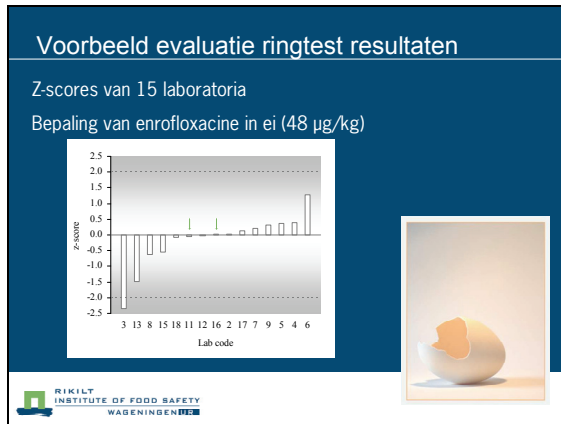
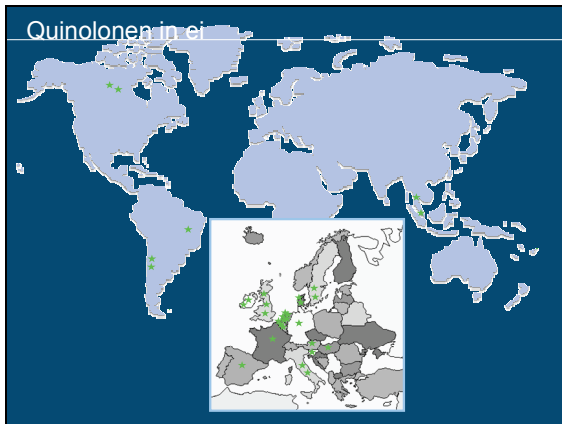
monsteranalyse

RIKILT is geaccrediteerd voor het organiseren van ringtesten (Voglens ILAC/G13 ; ISO/IEC 43-1 en 43-2)

Georganiseerde ringtesten:

- Quinolonen in pluimveevlees (2006)
- Penicillines in varkensvlees (2007)
- Quinolonen in ei (2007/2008)
- Macroliden in vlees en nier (2008)

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Dank voor uw aandacht

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Annex IV Summary report 2009.003: Inter-Laboratory study for macrolides in meat

Thirteen laboratories subscribed for participation in the proficiency study macrolides in porcine tissue. Eleven laboratories managed to submit results for muscle. Ten of them were also able to report results for the kidney samples. Seven of the laboratories that reported results applied a validated method. The majority of labs applied the same method for muscle and kidney. Only one lab carried out an additional extraction for the kidney analysis using hexane.

In this proficiency test three laboratories reported false negative results. These involved tylosin in muscle and tilmicosin in kidney. One of these labs also reported a false positive result: spiramycin in muscle.

Table 9: Overview of the amount of satisfactory results for accuracy

Matrix	Compound	No. of laboratories that reported results	No. of satisfactory results for accuracy	No. of questionable results for accuracy	No. of unsatisfactory results for accuracy
Muscle	Tylosin	10	8	2	0
	Josamycin	8	5	1	2
	Lincomycin	8	8	0	0
	Tulathromycin	5	5	0	0
Kidney	Tylosin	10	7	3	0
	Josamycin	7	5	1	1
	Tilmicosin	6	6	0	0

In all cases $u > 0.3\sigma_p$. This indicates that there is a severe variation among the laboratories. For several compounds the difference between the lowest and the highest reported value is a factor 5. As a result of this variation 6 of the 11 laboratories obtained questionable or unsatisfactory results.

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

- Although regulations for most macrolides are established before 2005, many laboratories do not have a validated and accredited method for the analysis of all relevant macrolides.
- For all compounds in both matrices the variation among the laboratories is severe.