

**EUROPEAN COMMUNITY COMMENTS ON**  
**CODEX CIRCULAR LETTER CL 2005-10 RVDF**

**Subject:**           **REQUEST FOR COMMENTS: Methods of analysis  
for veterinary drug residues (CL 2005/10 RVDF)**

The European Community supports the drafting of a list of analytical methods suitable for adoption as CODEX methods for the determination of veterinary drug residues and thanks the Chairperson of the *ad hoc* Working group on Methods of analysis and sampling for having prepared this list including all of the methods recommendations identified from JECFA and CCRVDF Working group. The EC considers that validation of methods according to performance criteria implemented under assurance of quality allows for technical progress whilst ensuring the methods meet the required quality. However, a non-exhaustive list of recommended methods assessed on the basis of a summary supplied by the laboratories can also be useful. In addition, the European Community supports the establishment of a list of contact points responsible for keeping up to date the information on analytical methods on particular substances to complete the list of analytical methods.

In general it must be stated that an evaluation of the methods laid down in Annex 1 of CL 2005-10 RVDF as such could not be carried out since the necessary methodical details were not provided. Therefore the comments refer mainly to the MRLs in comparison to EU MRLs according to Regulation (EC) No 2377/90. The limit of quantification of the analytical methods should not be greater than half the MRL.<sup>1</sup>

The detection techniques could not be checked with regard to their compliance with analytical performance criteria (e.g. that established in the EU by Commission Decision 2002/657/EC) as they were not indicated in most cases.

Finally, the European Community also supports the establishment of a list of international contact points of experts or laboratories in the analysis of particular substances. This was also discussed at the Joint FAO/WHO Technical Workshop on Residues of Veterinary Drugs without ADI/MRL (Bangkok, 24 – 26 August 2004), conclusions and recommendations No 9: *“to facilitate transparency and the sharing of scientific analytical methods for the control of residues, it is recommended that FAO, in cooperation with other international agencies, develop an international network among official residue control laboratories”*.

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<sup>1</sup> As it is laid down in Volume 8, (Notice to applicants and Note for guidance for Veterinary medicinal products) on the establishment of maximum residue limits (MRLs) for residues of veterinary medicinal products in foodstuffs of animal origin, the limit of quantification of the analytical methods should not be greater than half the MRL.

The limit of quantification corresponds to the smallest measured content of an analyte above which a determination of the analyte can be made with a specified degree of accuracy and precision. In its "region of" quantification" (at and above the limit of quantification and over a range of analyte concentration suitable for the enforcement of the MRL) the method has to meet certain requirements of accuracy and precision.

1. ANTHELMINTICS<sup>2</sup>

## 1.1. Avermectines

• **Abamectin**

The MRL values for cattle liver, -kidney und -fat range considerably above those laid down in Council Regulation 2377/90/EC.

| <b>Matrix</b> | <b>MRL Codex [µg/kg]</b> | <b>MRL 2377/90 [µg/kg]</b> |
|---------------|--------------------------|----------------------------|
| liver         | 100                      | <b>20</b>                  |
| kidney        | 50                       | -                          |
| fat           | 100                      | <b>10</b>                  |

Milk as an important matrix is missing.

The LOQs are sufficient for the MRL control.

• **Doramectin**

The MRL values for cattle liver, -kidney, -muscle and –fat are in accordance with CR 2377/90.

However, a few deviations can be stated regarding the values for pigs.

| <b>Matrix</b> | <b>MRL Codex [µg/kg]</b> | <b>MRL 2377/90 [µg/kg]</b> |
|---------------|--------------------------|----------------------------|
| liver         | 100                      | <b>50</b>                  |
| kidney        | 30                       | 30                         |
| muscle        | 5                        | <b>20</b>                  |
| fat           | 150                      | <b>100</b>                 |

There is no European MRL for doramectin in milk (cattle).

| <b>Matrix</b> | <b>MRL Codex [µg/kg]</b> | <b>MRL 2377/90 [µg/kg]</b> |
|---------------|--------------------------|----------------------------|
| milk          | 15                       | -                          |

The LOQs are sufficient for the MRL control.

• **Eprinomectin**

Deviations can be stated regarding the MRL values for cattle liver and –fat.

| <b>Matrix</b> | <b>MRL Codex [µg/kg]</b> | <b>MRL 2377/90 [µg/kg]</b> |
|---------------|--------------------------|----------------------------|
| liver         | 2000                     | <b>1500</b>                |
| kidney        | 300                      | 300                        |
| muscle        | 100                      | <b>50</b>                  |

<sup>2</sup> CRL Berlin can be contacted for information on anthelmintics, coccidiostats, NSAIDs and Beta-agonists.

|      |     |            |
|------|-----|------------|
| fat  | 250 | <b>250</b> |
| milk | 20  | 20         |

The LOQ are sufficient for the MRL control.

- **Ivermectin**

The MRL-values for cattle and pig liver and –fat are in accordance with CR 2377/90.

There is no European MRL for ivermectin in milk (cattle).

| <b>Matrix</b> | <b>MRL Codex [µg/kg]</b> | <b>MRL 2377/90 [µg/kg]</b> |
|---------------|--------------------------|----------------------------|
| milk          | 10                       | -                          |

The LOQ are sufficient for the MRL control except for ivermectin in milk, where no values were provided.

- **Moxidectin**

Deviations can be stated regarding the MRL values for muscle of cattle and deer as well as for milk.

| <b>Matrix</b>         | <b>MRL Codex [µg/kg]</b> | <b>MRL 2377/90 [µg/kg]</b> |
|-----------------------|--------------------------|----------------------------|
| muscle (cattle, deer) | 20                       | <b>50</b>                  |
| milk                  | -                        | <b>40</b>                  |

The LOQ are sufficient for the MRL control.

## 1.2. Benzimidazoles

- **Albendazole**

Albendazole sulfoxide and albendazole sulfone are not taken into account as marker substances.

Whereas the MRL values for cattle and sheep liver and –kidney are higher by a factor of 5 or 10 respectively, the other values are in accordance with CR 2377/90.

| <b>Matrix</b>         | <b>MRL Codex [µg/kg]</b> | <b>MRL 2377/90 [µg/kg]</b> |
|-----------------------|--------------------------|----------------------------|
| liver (cattle, deer)  | 5000                     | <b>1000</b>                |
| kidney (cattle, deer) | 5000                     | <b>50</b>                  |

The matrix selection is adequate.

The LOQ were not indicated.

- **Febantel/Fenbendazole**

The marker substance is in accordance with CR 2377/90.

Deviations by a factor of 2 can be stated regarding the MRL values for kidney, muscle and fat, deviations by a factor of 10 for milk. The values for liver are in accordance with CR 2377/90.

| <b>Matrix</b>                            | <b>MRL Codex [<math>\mu\text{g}/\text{kg}</math>]</b> | <b>MRL 2377/90 [<math>\mu\text{g}/\text{kg}</math>]</b> |
|--|---|---|
| kidney (cattle, sheep, pig, horse, goat) | 100   | <b>50</b>   |
| muscle (cattle, sheep, pig, horse, goat) | 100   | <b>50</b>   |
| fat (cattle, sheep, pig, horse, goat)    | 100   | <b>50</b>   |
| milk                                     | 100   | <b>10</b>   |

The matrix selection is adequate.

The LOQ are sufficient for the MRL control.

- **Flubendazole**

Deviations can be stated regarding the MRL values for pig and poultry liver and –muscle. The values for egg are in accordance with CR 2377/90.

| <b>Matrix</b>       | <b>MRL Codex [<math>\mu\text{g}/\text{kg}</math>]</b> | <b>MRL 2377/90 [<math>\mu\text{g}/\text{kg}</math>]</b> |
|---------------------|---|---|
| liver (pig)         | 10  | <b>50</b>   |
| liver<br>(poultry)  | 500   | <b>400</b>  |
| muscle<br>(pig)     | 10  | <b>50</b>   |
| muscle<br>(poultry) | 200   | <b>50</b>   |

The matrix selection is adequate.

The LOQ were not indicated.

- **Thiabendazole**

The MRL values for liver, kidney, muscle, fat and milk are in accordance with CR 2377/90.

In contrast to CODEX, CR 2377/90 does not lay down any MRL values for pig, sheep and goat.

The marker substances are in accordance.

The matrix selection is adequate.

- **Mebendazole, Oxibendazole and Triclabendazole**: No data provided.

### 1.3. Others

- **Closantel**

The MRL values for cattle matrices are in accordance with CR 2377/90, while for sheep deviations can be stated.

| <b>Matrix</b> | <b>MRL Codex [<math>\mu\text{g}/\text{kg}</math>]</b> | <b>MRL 2377/90 [<math>\mu\text{g}/\text{kg}</math>]</b> |
|---------------|---|---|
| fat (sheep)   | 3000  | <b>2000</b>   |

|                |      |      |
|----------------|------|------|
| kidney (sheep) | 5000 | 3000 |
|----------------|------|------|

The LOQ were not indicated.

- **Levamisole**

The MRL values for cattle liver, -kidney, -muscle and -fat are in accordance with CR 2377/90. For pig, sheep and poultry no MRLs have been laid down in the EU.

The LOQ were not indicated.

## 2. COCCIDIOSTATS

### 2.1. Ionophores

No data were provided for ionophores. In CR 2377/90 an MRL for lasalocid can be found.

### 2.2. Chemical Coccidiostats

- **Clopidol**

No MRL has been laid down, but there is a high LOQ of 100 µg/kg.

LC-UV is not suitable for confirmatory purposes.

- **Diclazuril**

In the EU diclazuril has been rated as ANNEX II substance..

The MRL values for sheep, poultry und rabbit liver, -kidney, -muscle and – fat are very high.

| Matrix                          | MRL Codex [µg/kg] | MRL 2377, [µg/kg] |
|---------------------------------|-------------------|-------------------|
| liver (sheep, poultry, rabbit)  | 3000              | -                 |
| kidney (sheep, poultry, rabbit) | 2000              | -                 |
| muscle (sheep, poultry, rabbit) | 500               | -                 |
| fat (sheep, poultry, rabbit)    | 1000              | -                 |

No data were provided for egg (poultry).

- **Imidocarb**

The MRL values for cattle liver, -kidney, -muscle, -fat and -milk are in accordance with CR 2377/90.

The LOQ are sufficient for the MRL control.

- **Nicarbazin**

In the EU no MRL has been laid down for nicarbazin; it is exclusively used together with narasin.

The MRL values for chicken liver, -kidney, -muscle and -skin seems relatively high (200 µg/kg).

No data were provided for egg.

- Apart from the ionophores, other important analytes like halofuginone, toltrazuril and amprolium are also missing.

### 3. BETA-AGONISTS

- **Clenbuterol**

The MRL values deviate from those of CR 2377/90.

| Matrix          | MRL Codex [ $\mu\text{g}/\text{kg}$ ] | MRL<br>[ $\mu\text{g}/\text{kg}$ ] | 2377/90     |
|-----------------|---------------------------------------|------------------------------------|-------------|
| liver (cattle)  | 0,6                                   |                                    | <i>0,5</i>  |
| kidney (cattle) | 0,6                                   |                                    | <i>0,5</i>  |
| muscle (cattle) | 0,2                                   |                                    | <i>0,1</i>  |
| milk (cattle)   | 0,05                                  |                                    | <i>0,05</i> |

The LOQ were not indicated.

GC-MS is suitable for confirmatory purposes.

- **Ractopamine**

Ractopamine is a banned substance in the EU.

High MRL values are stated.

| Matrix          | MRL Codex [ $\mu\text{g}/\text{kg}$ ] | MRL<br>[ $\mu\text{g}/\text{kg}$ ] | 2377/90 |
|-----------------|---------------------------------------|------------------------------------|---------|
| liver (cattle)  | 40                                    |                                    | -       |
| kidney (cattle) | 90                                    |                                    | -       |
| muscle (cattle) | 10                                    |                                    | -       |
| fat             | 10                                    |                                    | -       |

### 4. NITROIMIDAZOLES

- **Dimetridazole**

Dimetridazole is classified as a banned substance in the EU (Annex IV of CR 2377/90/EC).

For dimetridazole no CODEX MRL, no LOQ and no method recommendations exist.

- **Ipronidazole**

Ipronidazole is classified as a banned substance in the EU (Annex IV of CR 2377/90/EC).

For ipronidazole no CODEX MRL, no LOQ and no method recommendations exist.

- **Metronidazole**

Metronidazole is classified as a banned substance in the EU (Annex IV of CR 2377/90/EC).

For metronidazole no CODEX MRL, no LOQ and no method recommendations exist.

- **Ronidazole**

Ronidazole is classified as a banned substance in the EU (Annex IV of CR 2377/90/EC). For ronidazole no CODEX MRL, no LOQ and no method recommendations exist.

Due to the missing data it was not possible to carry out an evaluation for this group.

- **Azaperone**

Not fully (multiple laboratory) validated methods currently are included. The references listed are still suitable methods, but relative old. Currently, some methods are published using mass spectrometry. Adam (J. AOAC international (1999), Vol. 82, 815) published a partly validated method for swine liver and Fluchard *et al* (J. Chrom. B 744 (2000) 139) a method for pig muscle and kidney based on LC-MS and Kaufmann *et al.* (rapid comm. Mass spec. (2001) 15 1747) a LC-MS method. These methods can be considered reasonably validated.

The CRL Bilthoven<sup>3</sup> (RIVM) has a method for sedatives in pig kidney, inclusive azaperol and *azaperone*, based on LC-MS, which is not yet published. The method was in house validated. There are no activities know to use with respect to the analyses of kidney-fat. To our knowledge, residues in this matrix are not likely.

- **Bovine and porcine Somatotropin**

In spite of the work ongoing in several research institutes, RIVM<sup>3</sup> inclusive, there currently are no know methods available that can be recommended at this time.

- **Carazolol**

Situation as for Azaperone, with relative old methods provisionally recommended. The methods mentioned above (Fluchard *et al.* and Kaufamnn *et al*) also include carazolol for muscle and/or kidney. Also the CRL Bilthoven<sup>3</sup> -method includes this compound.

- **Chlorpromazine**

Currently Annex I does not contain a recommended method for Chlorpromazine. Of the methods mentioned above only the method by Kaufmann *et al.* and the CRL-method include chlorpromazine. However, additional work still is necessary for full (in house) validation as banned (Group A) compound.

- **Dexamethasone**

No species or matrix indicated in annex I. The technique most frequently used recently is LC-MS. In house validated methods currently are available, amongst others at the CRL Bilthoven<sup>3</sup>.

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<sup>3</sup> CRL RIVM Bilthoven can be contacted for information on this substance.

- **Estradiol 17-β**

No method currently recommended. No species of matrix indicated. Validated methods for urine and muscle tissue are available at the CRL Bilthoven<sup>3</sup>.

- **Melengestrol acetate**

Recommended method fully validated, with reference to CRL Bilthoven<sup>3</sup> method for regulatory purposes.

- **Trenbolone acetate**

The table gives a recommended method. Probably there is an error in the column marker residue ( $\beta$ -Trenbolone should be the marker for muscle,  $\alpha$ -Trenbolone for liver. The method is partly validated. Its suitability for EU regulatory purposes is not clear from the table. Alternative methods are available too. None of these is “fully validated” under the current definition.

- **Zeranol**

Fully validated method recommended. Alternative methods, recently developed, are available too. None of these are “fully validated” under the current definition.

**Antibiotics**<sup>4</sup>:

Certain listed methods are obsolete and should be removed from the list:

- **Chloramphenicol** by GC
- **Carazolol** by LC

Other methods should be introduced to the list

- **Carbadox and Olaquinox** metabolites (QCA, MQCA) in pig muscle by LC/MSMS
- **Chloramphenicol** in animal matrices by GC/MS or LC/MSMS
- **Malachite green** in fish flesh by LC/MSMS
- **(Fluoro)quinolones** in muscle and kidney by LC/fluorimetry or by LC/MSMS
- **Nitrofurans** metabolites by LC/MSMS

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<sup>4</sup> AFSSA-Fougères (Agence Française de sécurité sanitaire des aliments) laboratory can be contacted for information on methods for antibiotics.