SYMPOSIUM 2010

On the cross contaminations marks

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March 2010
SYMPOSIUM
March 23rd 2010

TECALIMAN thanks very much all the lecturers
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Coccidiostats: advantages and disadvantages

P. Blahaut
CERAupe
(Laboratoire d'Environnement - Belgique)
Summary

The world population continues to grow rapidly, in particular on the African and Asian continents. Poultry are a quality source of protein that the industrialisation process has enabled us to produce at a low cost. Unfortunately, this situation is favourable to the development of diseases such as coccidiosis. Coccidiosis is a parasitosis which mainly affects young animals. The principal lesions are found in the digestive system and according to the type of coccidia, the symptoms are more or less severe, ranging from wasting to death. The coccidia development cycle is very rapid and exponential.

Today, anti-coccidiostats make it possible to treat and manage the problem effectively. The choice of molecule depends on various factors, among which are effectiveness, side effects and cost.

We began using sulfamides in the 1940s for this purpose. Currently, a number of molecules are available which are active at increasingly lower concentrations. These molecules can, if used incorrectly, leave residues both in the target species and in other species.

A new European Commission directive (2009/08/EC) authorises a maximum cross contamination level of 1 and 3% depending on whether it is found in the target species or not. The previous directive provided for thresholds of 2.5 and 10% respectively. Tightening of requirements poses, on the one hand, problems for feed producers, in particular for factories in which feed is produced for several different animal species, but on the other hand requires the development of a high performance and reliable test method. Mass spectrometry combined with liquid chromatography is currently the method of choice.

The main difficulty encountered by feed manufacturers is that of cross contamination which is alas frequent and this despite the precautions taken by the sector.

In a research project in collaboration with the ILVO, we are seeking to create a model which would make it possible to forecast contamination levels found in eggs and meat. The initial results presented during the talk indicate significant differences depending on the molecule.

In conclusion, coccidiostats are highly useful substances for managing a disease as serious as coccidiosis, but their use must be effectively measured and managed.
Coccidiostats: advantages and disadvantages

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Coccidiostats: advantages and disadvantages

Introduction

- Coccidiosis
  general information
coccidia
coccidiostats

- Legislation - Directive 2009/08/EC
  authorized level
  control

- Cross-contamination
  Respou Project
  results

Conclusion
Advantages of poultry production

- industrialisation and intensification
- cheap
- easy to prepare
- consumption all over the world

Coccidiosis

- parasitic infection
- occurs mainly in young animals
- characterized by an enteritis
- fècal - oral infection
- clinical signs
  - inappetance, weight loss, reduction of productivity
  - diarrhea
  - diphtheroid and hemorrhagic enteritis which can lead to death
Coccidia

- 'little berries' -> coccidia
- protozoal organism -> unicellular organism
- subclass of coccidia with more than 20 genera
  - Eimeria, Isospora, Toxoplasma,...
- in a strict parasitological sense there is no coccidiosis -> eimeriosis

Coccidia: masters of multiplication

- rapid infection in industrial broilers unit
- a sporulated oocyst of Eimeria tenella
  -> 400,000 new offsprings
- life cycle inside the body of its host and outside
- survival in sporulated form
Symptomatology
Aspects of coccidiosis

- infections produced by several species

- management/housing factors
  -> important
    - stocking density
    - period of vacancy
    - quality of litter
    - inadequate cleaning
    - ...

Choice of anti-coccidial drug

- efficacy
- development of resistances
  -> combinations
- growth-promoting effect
- side effects, intoxication
- price...
Coccidiostats

- **sulfamides**: sulfaquinoxaline, sulfaguanidine, ...
- **analogous to thiamine (Vit B₁)**: amprolium
- **quinozolinones**: halofuginone
- **derivative of guanidine**: robenidine
- **ionophorous polyether**: monesin, lasalocid, salinomycin, narasin, maduramicin
- **acetonitrile benzenique**: toltrazuril, diclazuril
**Coccidiostats**: advantages and disadvantages

**Introduction**
- Coccidiosis
  - general information
  - coccidia
  - coccidiostats
- Legislation - Directive 2009/08/EC
  - authorized level
  - control
- Cross-contamination
  - Respou Project
  - results

**Conclusion**

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**Unavoidable carry-over of coccidiostats in feed - legal aspects**

**Commission Directive 2009/8/EC**

<table>
<thead>
<tr>
<th>Allowed carry-over (in % of authorised maximum content)</th>
<th>Type of feed</th>
</tr>
</thead>
<tbody>
<tr>
<td>1%</td>
<td>- food for sensitive non-target animal species</td>
</tr>
<tr>
<td></td>
<td>- withdrawal feed</td>
</tr>
<tr>
<td></td>
<td>- cross-contamination of feed for target species to which no coccidiostats are added</td>
</tr>
<tr>
<td></td>
<td>- non-target feed for continuous food-producing animals (dairy cows or laying hens)</td>
</tr>
<tr>
<td>3%</td>
<td>- food for less sensitive non-target animal species</td>
</tr>
</tbody>
</table>
Unavoidable carry-over of coccidiostats in feed - legal aspects

maximum levels of unavoidable carries-over of coccidiostats feed

<table>
<thead>
<tr>
<th></th>
<th>Target feed</th>
<th>Non-target feed</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>100% (*)</td>
<td>Carry-over 3%</td>
</tr>
<tr>
<td>Lasalocid sodium</td>
<td>125000</td>
<td>3750</td>
</tr>
<tr>
<td>Narasin</td>
<td>7000</td>
<td>2100</td>
</tr>
<tr>
<td>Salinomycin sodium</td>
<td>7000</td>
<td>2100</td>
</tr>
<tr>
<td>Monensin sodium</td>
<td>125000</td>
<td>3750</td>
</tr>
<tr>
<td>Maduramicin ammonium alpha</td>
<td>500</td>
<td>150</td>
</tr>
<tr>
<td>Semduraminic sodium</td>
<td>2500</td>
<td>750</td>
</tr>
<tr>
<td>Nicarbazin</td>
<td>5000</td>
<td>1500</td>
</tr>
<tr>
<td>Dicloranil</td>
<td>100</td>
<td>30</td>
</tr>
<tr>
<td>Decoquinate</td>
<td>4000</td>
<td>1200</td>
</tr>
<tr>
<td>Halofuginone hydrobamide</td>
<td>300</td>
<td>90</td>
</tr>
<tr>
<td>Robenidone hydrochloride</td>
<td>7000</td>
<td>2100</td>
</tr>
</tbody>
</table>

(*) maximum authorized content

Coccidiostats analysis by LC-MS/MS - principle

Sample preparation

- Grinding and homogenization
- Weighting of a lab sample (5 g)
- Addition of a 10% Na2CO3 solution (10 ml)
- Extraction with 10 ml acetonitrile (twice)
- Evaporation of 1 ml extract
- Suspension in 250 µl acetonitrile-water (80-20)

LC-MS/MS analysis

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Coccidiostats in feed: analysis

- Immuno Assay: highly specific
- LC-MS: very selective
- Robustness
- Quantification of all coccidiostats in one analysis

Analytical Method

LC-MS/MS analysis: chromatogram of the 11 coccidiostats

Symposium Tecaliman - March 23rd 2010
### Analytical Method

**LC-MS/MS analysis:** transition of quantification and confirmation

- F2:MRM of 8 channels, ES- 405.2 > 334.2
- 8.460 ± 0.003

### Coccidiostats in feed: LOD, LOQ, LCL

**Limits of detection (LOD), of quantification (LOQ) and lowest calibration level (LCL)**

<table>
<thead>
<tr>
<th>Coccidiostat</th>
<th>Conc. for carry-over of 1% (ppb)</th>
<th>Limit of detection (ppb)</th>
<th>Limit of quantification (ppb)</th>
<th>Lowest calibration level (ppb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lutialcid sodium</td>
<td>1250</td>
<td>0.5</td>
<td>1.65</td>
<td>625</td>
</tr>
<tr>
<td>Narasin</td>
<td>700</td>
<td>1</td>
<td>3.3</td>
<td>350</td>
</tr>
<tr>
<td>Salinomycin sodium</td>
<td>700</td>
<td>0.2</td>
<td>0.66</td>
<td>350</td>
</tr>
<tr>
<td>Monensin sodium</td>
<td>1250</td>
<td>0.3</td>
<td>0.1</td>
<td>625</td>
</tr>
<tr>
<td>Maduramicin</td>
<td>50</td>
<td>1</td>
<td>3.3</td>
<td>25</td>
</tr>
<tr>
<td>Semduramicin sodium</td>
<td>250</td>
<td>1</td>
<td>3.3</td>
<td>125</td>
</tr>
<tr>
<td>L'nitrocarbendione</td>
<td>500</td>
<td>2</td>
<td>6.6</td>
<td>250</td>
</tr>
<tr>
<td>Dicloraziril</td>
<td>10</td>
<td>0.7</td>
<td>2.3</td>
<td>5</td>
</tr>
<tr>
<td>Decoquinate</td>
<td>400</td>
<td>0.1</td>
<td>0.33</td>
<td>200</td>
</tr>
<tr>
<td>Halofuginone hydrobromide</td>
<td>30</td>
<td>10</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>Robenidine hydrochloride</td>
<td>700</td>
<td>3.3</td>
<td>10</td>
<td>350</td>
</tr>
</tbody>
</table>
**Coccidiostats**: advantages and disadvantages

Introduction

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  general information
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- Legislation - Directive 2009/08/EC
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  Respoul Project
  results

Conclusion

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**Unavoidable carry-over of coccidiostats in feed**

- production of different feed types in the sample production line

- 'carry-over' or 'cross-contamination'
  = unavoidable transfer from one feed batch to another

- contamination during production, processing, storage and transport of feed
Project RESPOUL

Aim
Development of a model for the determination of transfer factors of residues in poultry (eggs and meat)

Tested molecules
- coccidiostats: monensin, lasalocid,...
- antibiotic: sulfadiazine
- anthelmintic: flubendazole

Partners
- ILVO (Institute for Agricultural and Fisheries Research) Technology and Food Science Unit
- CER Groupe

Physicochemical parameters

Different parameters
- CAS number
- Molecular weight
- Log P: partition coefficient
- pKa: acid dissociation constant
- T1/2: half-life
- Plasma protein binding
- Water solubility

References: EFSA reports, internet database, articles,...
Sometimes very different results:
f.e. monensin sodium in EFSA reports: water solubility of 8.78 mg/L log P > 6.3; internet resource: SRC Phys Prop database: water solubility of 1.61 mg/l and log P = 1.62
### Physicochemical parameters

<table>
<thead>
<tr>
<th>Compound</th>
<th>Mol weight</th>
<th>Predicted log P</th>
<th>Predicted pKa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lasalocid</td>
<td>590.8</td>
<td>6.547 ± 0.529</td>
<td>3.15 ± 0.39</td>
</tr>
<tr>
<td>Monensin</td>
<td>670.9</td>
<td>3.716 ± 0.736</td>
<td>4.26 ± 0.27</td>
</tr>
<tr>
<td>Sulfadiazine</td>
<td>250.3</td>
<td>-0.117 ± 0.255</td>
<td>1.64 ± 0.10; 6.50 ± 0.30</td>
</tr>
<tr>
<td>Narasin</td>
<td>765.0</td>
<td>6.591 ± 0.787</td>
<td>4.36 ± 0.10</td>
</tr>
<tr>
<td>Chlortetracycline</td>
<td>478.9</td>
<td>-0.325 ± 0.819</td>
<td>4.50 ± 1.00; 11.01 ± 0.70</td>
</tr>
<tr>
<td>Robenidine hydrochloride</td>
<td>370.7</td>
<td>4.548 ± 0.661</td>
<td>5.75 ± 0.70</td>
</tr>
<tr>
<td>Dicloazuril</td>
<td>407.6</td>
<td>2.699 ± 0.671</td>
<td>5.89 ± 0.20</td>
</tr>
<tr>
<td>Flubendazole</td>
<td>313.3</td>
<td>3.051 ± 0.592</td>
<td>4.76 ± 0.15; ± 10.29 ± 0.68</td>
</tr>
<tr>
<td>Tylosine</td>
<td>916.1</td>
<td>3.407 ± 0.844</td>
<td>7.39 ± 0.70; 13.06 ± 0.70</td>
</tr>
</tbody>
</table>

### Animal experiments

- **different molecules**
  - Lasalocid
  - Monensin
  - Sulfadiazine
- **laying hens**
  - 10 days of acclimatisation period: blanco feed
  - 14 days of treatment period: medicated feed
  - 16 days of depletion period: blanco feed
- **broilers**
  - 12 days of acclimatisation period: blanco feed
  - 14 days of treatment period: medicated feed
  - 16 days of depletion period: blanco feed
Residues in **breast muscle**: lasalocid

Residues in **complete egg**: lasalocid
## LASALOCID

<table>
<thead>
<tr>
<th></th>
<th>Feed</th>
<th>Muscle</th>
<th>Egg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ppm</td>
<td>ppb</td>
<td>%</td>
</tr>
<tr>
<td>Lasalocid 10%</td>
<td>13.45</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td></td>
<td>13.13</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lasalocid 5%</td>
<td>6.55</td>
<td>26</td>
<td>0.39</td>
</tr>
<tr>
<td></td>
<td>6.86</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lasalocid 2.5%</td>
<td>3.35</td>
<td>12</td>
<td>0.36</td>
</tr>
<tr>
<td></td>
<td>2.78</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Albumen-yolk
- Day 10 of the treatment period
  - Yolk: 3712 ppb
  - Albumen: 35 ppb
  - Whole egg: 1278 ppb

=> 0.32*3712+0.68*35 = 1212 ppb

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Breast muscle - thigh muscle **lasalocid**

Day 13 of treatment
- **Breast** muscle
  - Mean 6 x individual animal = 68 ± 27 ppb
  - Mean 6 x analysis of mixed sample = 62.4 ± 11 ppb
- **Thigh** muscle
  - Mean 6 x analysis of mixed sample = 85 ± 30 ppb
# MONENSIN

<table>
<thead>
<tr>
<th></th>
<th>Feed</th>
<th>Muscle</th>
<th>Egg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ppm</td>
<td>ppb</td>
<td>%</td>
</tr>
<tr>
<td>Monensin 10 %</td>
<td>13.49</td>
<td>1.24</td>
<td>0.009</td>
</tr>
<tr>
<td></td>
<td>11.69</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Monensin 5 %</td>
<td>6.62</td>
<td>0.34</td>
<td>0.005</td>
</tr>
<tr>
<td></td>
<td>5.22</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Monensin 2.5 %</td>
<td>3.08</td>
<td>0.12</td>
<td>0.004</td>
</tr>
<tr>
<td></td>
<td>3.13</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

---

**Sulfadiazine** medicated feed

![Graph showing Sulfadiazine medicated feed levels over time.](#)

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*Symposium Tecaliman - March 23rd 2010*
SULFADIAZINE

<table>
<thead>
<tr>
<th></th>
<th>Feed</th>
<th>Muscle</th>
<th>Egg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ppm</td>
<td>ppb</td>
<td>%</td>
</tr>
<tr>
<td>Sulfadiazine 10 %</td>
<td>26.76</td>
<td>202</td>
<td>0.75</td>
</tr>
<tr>
<td></td>
<td>21.18</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sulfadiazine 5 %</td>
<td>13.60</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>12.70</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sulfadiazine 2.5 %</td>
<td>7.37</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>6.50</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Breast muscle - thigh muscle **sulfadiazine**

Day 13 of treatment

- **Breast** muscle
  - Mean 6 x individual animal = 203 ± 50 ppb
  - Mean 6 x analysis of mixed sample = 202 ± 16 ppb
- **Thigh** muscle
  - Mean 6 x individual animal = 173 ± 50 ppb
  - Mean 6 x analysis of mixed sample = 172 ± 8 ppb
General conclusions: laying hens

Zootechnical parameters:
- No influence of the lasalocid, monensin and sulfadiazine medicated on
  the daily amount of eggs.
- No influence of the lasalocid, monensin and sulfadiazine medicated
  feed on the mean weight of the egg.
Feed conversion

General conclusions: laying hens

- Residue concentration in the complete egg
  - very different between the different molecules
  - quick approach of a steady state and a quick decline for all
    molecules
- Residue concentration in yolk and albumen
  - lasalocid: very low concentration in the albumen and very
    high concentration in the yolk.
    Log P = 6.457 ± 0.529
  - sulfadiazine : high concentration in the albumen and low
    concentration in the yolk.
    Log P = -0.117 ± 0.255
General conclusions: broilers

- large difference between the measured residue concentrations of the individual animals of the same concentration group on the same day
- mean of the 6 animal samples = mean of the six analysis of the mixed sample
- different results for the different molecules
  - lasalocid: conc breast muscle < conc thigh muscle
  - sulfadiazine: conc breast muscle > conc thigh muscle

QUESTIONS
Evaluation of the chemical risk of food: methodological approach

PByt
Oniris & Ates
Summary

Animal productions and foodstuffs of animal or plant origin carry the unavoidable risk of containing various chemical substances, either following intentional veterinary treatment, or following accidental exposure of animals or productions to phytosanitary products or environmental contaminants.

In the case of veterinary medicinal products, consumer safety is ensured by compulsory evaluation of veterinary medicinal product residues as part of the pharmacotoxicological dossier in marketing authorisation applications. The safety factors retained ensured absolute safety, even where maximum residue limits (MRL) are exceeded. No veterinary medicinal product indicated for use in production animals can be administered if the MRLs have not first been defined. Residue risk assessment involves establishment of a withholding time, which must be observed by farmers and for which farmers and prescribing parties are responsible.

In the case of accidental exposure to phytosanitary products or environmental contaminants or when using medicinal products outside marketing authorisation (MA) recommendations, a chemical risk assessment must be carried out on a case per case basis. This involves identification of the risk followed by establishment of an acceptable daily intake (ADI) and observance of MRLs, or, in the absence of this data, by establishment of theoretical maximum residue limits. By comparing exposure levels, the chemical risk can then be evaluated and a risk management plan established.

The French gFARAD centre provides valuable assistance in chemical risk assessment in the veterinary profession, for animal production professionals, for agri-food industrialists and for State services (Veterinary Services Directorate).
Evaluation of food chemical risk: Methodological approach

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INTRODUCTION

Animal production aims to provide high quality, safe foodstuffs for consumers. Most food consumed by humans actually contains residual traces of various chemical substances. Chemical contamination of food results from the exposure of production animals or crop production under various conditions:

- use of veterinary medicinal products for marketing authorisation (MA) purposes or outside official recommendations in emergency situations, essentially following accidental, incorrect use of veterinary medicinal products (incorrect administration of a veterinary proprietary medicinal product not intended for the given animal species, accidental overdose etc.);
- Application of phytosanitary products on crops or cultures to improve conservation,
- Accidental contamination of animals by phytosanitary products (pesticides, insecticides, molluscicides and herbicides etc.),
- Exposure of animals or crop productions to environmental contaminants (dioxins, PCB etc.).

Current test methods make it possible to detect even the most minute traces of chemical residues in parts per million (ppm), that is to say, one gram per ton, or in parts per billion (ppb), that is to say, one gram per 1,000 tons. Zero tolerance, or “zero residue”, that is to say the absence of all residual traces in foodstuffs, as called for by consumers, makes no scientific sense and cannot be upheld by the public authorities or national agencies (AFSSA). This is why maximum residue limits were established, initially for veterinary medicinal products, but also for a large number of environmental chemical contaminants and phytosanitary products.

The food chemical safety approach shall firstly be addressed within the context of veterinary medicinal products and secondly with respect to accidental exposure of production animals to phytosanitary products. We will however present overall risk assessment first.

1. GENERAL CHEMICAL RISK ASSESSMENT

According to the definition by the WHO (World Health Organisation), the term "residues" describes "any chemical substance persisting in a given medium, in generally low quantities, after it or other components producing it have been voluntarily or involuntarily added to the medium, and of which the presence is qualitatively and quantitatively abnormal". "Veterinary medicinal product residues" are all active principles or their metabolites persisting in meat or other foodstuffs deriving from animals in which the medicinal product was administered.

Chemical risk is the probability of a toxic or negative effect being produced by a given chemical substance, in other words, of a hazard occurring. Chemical risk depends both on the extent of the hazard presented by a substance and on the risk of exposure of animals or animal or crop productions to this substance. To this effect, if a chemical substance does not present a risk, even if the animal or crop is exposed to the substance, it is by definition without risk. On the contrary, if a substance is very hazardous but has no chance of entering into contact with animals or crops, the risk is also nil.

In the first stage of risk assessment, the danger of the substance must be accurately identified. This assessment should first evaluate the intrinsic toxicity of the substance, in other words, its toxic potential. This is the very essence of experimental toxicity assessment… and leads to establishment of the no observed effect level (NOEL).

The results from in vitro tests or tests on laboratory animals are then extrapolated to humans by applying risk factors. The acceptable daily intake is thus determined (ADI).
How to evaluate antibacterial activity. – Bovine antibiotic treatment, PFIZER

In the third stage, the maximum residue limits (MRL) are set by taking into account a standard human diet in order to define the risk of exposure.

1.1. Experimental toxicity assessment (no observed effect level or NOEL)

All these studies have a general underlying principle which is that man is likely to ingest at least one given chemical substance each day of his life. Experimental evaluation is therefore based on repeated administration of the chemical substance tested, by oral route, over several weeks, months or even several years according to the risk studied. The aim is to assess the changes in the most sensitive biological parameters and to identify the specific risk relating to the substance studied.

In practice, experimental evaluation is generally based on the implementation of a certain number of general toxicity tests by repeated administration, special toxicity tests (on reproduction functions, carcinogenesis), mutagenesis and carcinogenesis tests, or even development of bacterial resistance in the case of antibiotics. The substance studied is administered by oral route to several batches of animals at various doses.

Given the costs, these studies are determined according to molecule, their chemical category and the results of the first general toxicity tests.

General toxicity tests by repeated administration last 3 to 6 months. They cost €80,000.00 minimum; carcinogenesis testing lasts 2 years and costs approximately €500,000.00. Complete experimental toxicity evaluation costs around €1,000,000.00!

For each experimental toxicity test, the strongest dose not having any detectable effects in laboratory animals is selected. This is therefore significantly below the minimum toxic dose. The lowest value from all tests is then selected, that is to say the lowest dose likely to affect the most sensitive biological parameter. This is known as the "no observed effect level" (NOEL) according to the Anglo-Saxons.

1.2. Determination of the acceptable daily intake (ADI)

Acceptable Daily Intake (ADI)

In the second stage, the test results on animals are extrapolated to humans by applying safety factors. Arbitrarily, at least, we suspect man to be 10 times more sensitive than laboratory animal species (interspecies factor) and we retain an additional factor of 10 for sensitivity variations in the human species (intraspecies factor). But these safety factors may range up to 1,000 according to the type of experimental effects observed (mutagenic, carcinogenic, teratogenic effects).

By dividing the no observed effect level obtained previously by the safety factor, the acceptable daily intake for man is thus determined (ADI). The ADI therefore represents the quantity of substance that man can ingest each day throughout a lifetime without it having the slightest effect on his health.

1.3. Maximum residue tolerance or limits

In the final stage, the risk of exposure is defined, and more precisely in terms of food safety, the risk of ingestion. To ensure consumer safety and bearing the ADI and food consumption habits in mind, it must be ensured that maximum levels are not exceeded in each major category (meat, milk, liver, kidney and eggs).

In order to determine maximum levels, a typical diet (food consumption standards) was established by the regulatory bodies, on the basis of an individual weighing 60 kg (see table 1). This diet was also established with a significant safety margin, it being impossible to consume such quantities of food daily.
The product of the acceptable daily intake per 60 kg (ADI in mg/kg x 60 kg) gives an overall maximum quantity of residues that a consumer weighing 60 kg may ingest without any problems.

Possible distribution of the overall maximum quantity is therefore cautiously defined for each food category. Natural distribution of these residues in this food is taken into account. Liver and kidneys often contain higher levels than meat. We can also tolerate significantly higher residue levels in food consumed less regularly (kidneys) than in food consumed regularly (meat).

The maximum residue quantities that do not carry a risk for the consumer on a daily basis are therefore defined per food vector. The ratio of the maximum authorised residue quantity in a given food per quantity of the food ingested daily is equivalent to the maximum residue limits (MRL) (see figure 1). This maximum residue concentration tolerated in food is also known as tolerance. It is expressed in ppm (parts per million) that is to say in micrograms of residue per kilogram of food or in ppb (parts per billion), micrograms of residue per ton of food. It is therefore usual that the ADI as a whole is not divided among the different foods and that the authorities provide for an ADI reserve, this being an additional safety factor.

<table>
<thead>
<tr>
<th></th>
<th>Distribution</th>
<th>MRL</th>
</tr>
</thead>
<tbody>
<tr>
<td>50 kg</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ADI = 0.05 mg/kg</td>
<td>Muscle 300 g</td>
<td>0.15 mg</td>
</tr>
<tr>
<td>ADI x 60 kg = 3 mg</td>
<td>Liver 100 g</td>
<td>1.75 mg</td>
</tr>
<tr>
<td></td>
<td>Kidneys 50 g</td>
<td>0.65 mg</td>
</tr>
<tr>
<td></td>
<td>Milk 1.5 l</td>
<td>0.45 mg</td>
</tr>
<tr>
<td></td>
<td>Eggs 100 g</td>
<td>3.00 mg</td>
</tr>
<tr>
<td></td>
<td>Skin + fat 50 g</td>
<td></td>
</tr>
</tbody>
</table>

Figure 1: Calculation of tolerance

Distribution is arbitrary and may be determined in different ways. This is why the MRLs set by the European Community may be different from those set in the United States.

At all stages of the evaluation, significant risk factors are taken into account, as much when establishing the no observed effect level, as it is far from the minimum toxic level, as when setting the ADI and determining the MRLs. The consumer is safe from all risk. To this effect, and contrary to widespread opinion, the consumer is still far from exposed to any risk even where MRLs are exceeded.

In terms of chemical safety, these MRLs are equivalent to the guide values established for a certain number of environmental contaminants. Where the nitrate limit in drinking water is 50 mg/litre, ingestion of water containing 100 or 200 mg/litre does not necessarily expose the population to a specific risk. However, from a regulatory standpoint this is not the case. Where MRLs are exceeded, the party responsible may incur sanctions ranging from seizure of the foodstuffs to the possibility of legal proceedings.
This is equivalent therefore to one hundredth or one thousandth of the no observed effect level in animals.
Bearing in mind the role of such and such a food category in the daily food ration and the acceptable daily intake, it is possible to calculate the maximum residue quantities that the consumer may ingest daily without risk, for each food category. The product of the acceptable daily intake, the average weight of an individual set at 60 kg, gives the total quantity of residues tolerated each day.

2. VETERINARY MEDICINAL PRODUCTS

In the case of veterinary medicinal products, drug companies must carry out toxicological evaluation of residues, to determine if the residues are hazardous or not, and to determine the maximum residue limits, and on this basis, then establish from which point the concentrations of the medicinal product in the main organs (muscle, liver, kidney and milk etc.) are inferior to the MRLs. This is known as withholding time and should feature on veterinary prescriptions. Farmers must observe withholding time before delivering their production for human consumption.

In legal terms, a veterinary medicinal product for production animals must not be administered if the MRLs have not been previously established. Active substances contained in veterinary medicinal products are registered on a special list (see EU Commission regulation # 37/2010, of 22.12.2009) (1) along with the indication (according to the case):

- MRL (equivalent to the former appendix I),
- temporary MRL (equivalent to the former appendix III), set for a maximum five-year period, and possibly extended once for two years;
- The reference “no MRL required” (equivalent to former appendix II), for chemical substances of which the residues are not hazardous;
- The reference "MRL cannot be established" and which prohibits the administration of any medicinal products containing that substance (equivalent to the former appendix IV), either due to the risk to human health, or due to the fact that it is impossible to conclude on the basis of the data available.

No veterinary medicinal product containing such a substance with the reference "MRL cannot be established" can be prescribed or administered to production animals for human consumption.

Setting withholding time requires leading pharmacokinetic studies in order to monitor changes in the concentration of the active principle and its metabolites in the body’s main organs over time. Withholding time is the time elapsed after the last administration in which residue concentrations must be inferior to the maximum residue limits defined for each food vector (meat, milk and eggs etc.) (See figure 2).
Withholding time is defined by the drug company and validated by the authorities for specific indications and a given treatment protocol (dose, administration time). Extension of the treatment has generally little influence and should not lead to an extension in withholding time, except in rare cases in which the medicinal product tends to accumulate after several doses.

However, the increase in doses extends withholding time. This extension is equivalent to the elimination half life of residues.

Medicinal products may have a set MRL for meat or milk but the authorities prohibit them in certain productions, notably in dairy cows. The main reason for this is that the drug companies, due to the very high cost of these studies, has not carried out additional studies, essential for setting withholding times for milk. Non-observance of this prohibition places the user in an illegal situation.

The safety factors applied mean that the occasional use of a medicinal product outside MA recommendations does not carry a risk for the consumer.

3. ACCIDENTAL EXPOSURE TO PHYTOSANITARY PRODUCTS

During accidental exposure of production animals to phytosanitary products, to environmental contaminants or to use of veterinary medicinal products outside MA recommendations, risk assessment must be conducted in order to protect consumer health. The toxicological or pharmacokinetic data required is not always easily accessible and is often insufficient. This evaluation is not usually practicable by professionals, notably by veterinary surgeons. There is also a problem of legal liability when setting inappropriate withholding times with respect to the medicinal product’s instructions for use, or when setting withdrawal times for foodstuffs from deriving from production animals accidentally contaminated by industrial products or contaminants.

The French gFARAD (Food Animal Residue Avoidance Databank) centre, a university network created in the United States at the beginning of the 1980s, established at the Veterinary School of NANTES, is likely to bring to the veterinary profession, production animal professionals, agri-food industrialists and State services (Veterinary Services directorate) valuable technical assistance. The gFARAD’s expertise is based on four computer data banks:
- A bibliographic data bank including references of over 20,000 articles discussing residues,
How to evaluate antibacterial activity. – Bovine antibiotic treatment, PFIZER

- A pharmacokinetic data bank created by the extraction or generation of data deriving from the analysis of approximately 2,000 articles on several thousand substances,
- A regulatory database containing notably national North-American, European and Asian regulations, and European and North-American maximum residue limits (MRL).

The approach is identical for medicinal products:
- Detection of available toxicological information, notably maximum residue limits (MRL); if they do not exist, detection of ADI or NOEL and calculation of temporary MRL;
- Search for pharmacokinetic data on tissue depletion;
- Evaluation of exposure level and calculation from tissue depletion data of forecast residue concentrations; otherwise, assay in foodstuffs must be carried out;
- Comparison of residue levels to available MRL or theoretical MRL calculated.

Following this evaluation procedure of which the results are included in a written report issued by the French gFARAD, professionals dispose of all the information necessary for risk management, that is to say in practice, observance of withholding or withdrawal time, or removal of any foodstuffs in which the levels exceed the MRL from human consumption. According to the complexity of the question, a response is generally returned within 24 hours or sometimes a few days.

French gFARAD centre
Telephone # (33) 2 40 68 77 41 or (33) 2 40 68 77 40
24hrs a day and 7 days a week
Fax: 02 40 68 77 42
E-mail address: gfarad@oniris-nantes.fr
Postal address: ONIRIS
Pharmacology-Toxicology unit
French gFARAD centre
B.P. 40706 - 44307 NANTES Cedex 03

As an example, a chemical risk assessment is shown below concerning a case of exposure of a herd of dairy cows to metaldehyde.

A herd of dairy cows gained access to a bag of molluscicide containing metaldehyde 5% (AFFUT) and methiocarb, left carelessly within reach. Three cows died. Test carried out on the tank milk showed metaldehyde levels of 0.25 mg of metaldehyde per litre (0.28 ppm) whereas methiocarb was undetectable. 14.1 ppm was found in the milk of three cows exposed and presenting with symptoms.

WRITTEN RESPONSE FROM THE FRENCH g-FARAD CENTRE

1°) there is no tissue depletion pharmacokinetic study for metaldehyde in milk.

2°) the no observed effect level (NOEL) of metaldehyde is 5 mg/kg and the acceptable daily intake (ADI) retained with a safety factor of 1,000, is 0.005 mg/kg/day.

3°) no MRL exists; we must therefore calculate a “theoretical maximum residue level”; the overall acceptable daily intake for an individual weighing 60 kg is 0.3 mg/day (60 kg x 0.005 mg/kg/day); given the accidental and exceptional nature of this event, we may distribute the totality of the metaldehyde ADI in milk; therefore on the basis of milk consumption of 1.5 litres per day, a theoretical maximum residue level of 0.2 mg/litre of milk, thus 0.2 ppm (parts per million), or 200 ppb can be calculated.

4°) 0.28 mg of metaldehyde/litre (280 ppb) in the milk tank is slightly higher than the theoretical maximum residue limit calculated.

In conclusion, 5 days after exposure, this level is certainly lower than the theoretical maximum residue limit. Furthermore, the tank milk is diluted approximately six times via the transfer of 4,000 litres of milk (capacity of the farmer’s tank) in the 25,000 litre transport tank transferring the milk to the AGRIRIAL dairy. To this effect, the residual level drops to 60 ppb, therefore significantly below the theoretical maximum residue limit calculated of 200 ppb.
BIBLIOGRAPHIC REFERENCES

Updating of the database on the homogeneity and cross contaminations levels in France

M. Btier
Tecaliman
Summary

These results were presented previously at the 2008 symposium. This update concerns the 1999 withdrawal, of which the results were little representative, and entry of the results for 2006 - 2008. The panel of factories in this database is highly representative of French production, with over 80 % of tonnage manufactured by 150 industrial sites in 2008. In terms of tracers used for carrying out the tests, the RF-blue lake micro tracer is now very widely used. In terms of cross contamination, it makes it possible to produce, in most cases, assessments identical to those obtained using internal tracers.

As for homogeneity, 1,579 coefficients of variation were processed. The median over the nine years is around 4.6 % and 3.9 % for 2008. On average, since 2000, the median has decreased by 0.2 % per year and in 2008, 90 % of the tests saw results lower than 10 %. All of these figures show the significant progression in terms of homogeneity. As stated 2 years ago, it will be more difficult to make such progress in the future.

In terms of cross contamination, the improvement in the results was very significant up to 2003. As of this year, improvement continued but at a lower rate. In 2008, 82 % of the results from the first manifold batch were lower than 5 % (target in the best practice guide). Directive 08/09 adds a new challenge: Targets of 1 or 3 % according to the food. In 2008, 60% of the results were lower than 3 % and only 17 % were lower than 1 % for the first manifold batch. 62 % are lower than 1 % for the second manifold batch. Progress therefore needs to be made on processes and on active ingredients in order to reduce cross-contamination. Eliminating it however is illusory.
Updating of the database on the homogeneity and cross contaminations levels in France

F. Putier

Centre technique de la Nutrition Animale

● Previous Presentation in 2008
● Updating
  □ Withdrawal of 1999
  □ Add 3 more years
    ● 2000 to 2008
**Tracers**

- **Homogeneity**
  - Majority of tests with microtracer RF blue lake
  - Percentage growth
    - Internal tracers
    - Oligopeptidase
    - Microtracer RF blue lake
    - Microtracer F

- **Cross contaminations**
  - Majority of tests with microtracer RF blue lake
  - Numbers of tests with internal tracer interne stagnant

---

*Symposium Tecalman - March 23, 2010*
### Homogeneity

- 55% of the tests below 5% and 85 below 10% from 2000 to 2008

<table>
<thead>
<tr>
<th>Periods</th>
<th>Nb tests</th>
<th>&lt; 5%</th>
<th>&lt; 10%</th>
<th>&gt; 16%</th>
<th>Minimum</th>
<th>Median</th>
<th>Maximum</th>
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</thead>
<tbody>
<tr>
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<td>86%</td>
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<td>2008</td>
<td>231</td>
<td>68%</td>
<td>90%</td>
<td>5%</td>
<td>0.9</td>
<td>3.9</td>
<td>55.0</td>
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</tbody>
</table>

### Refocusing of the population

![Graph showing coefficients of variation](image)

Symposium Tecallman - March 23, 2010
- Homogeneity
  - Annual evolution

<table>
<thead>
<tr>
<th>Années</th>
<th>Nb d'individus</th>
<th>Mini</th>
<th>Médiane</th>
<th>Maxi</th>
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<tbody>
<tr>
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<td>66</td>
<td>1.7</td>
<td>5.1</td>
<td>33.9</td>
</tr>
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<td>4.6</td>
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<td>44.2</td>
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<td>2007</td>
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<td>0.9</td>
<td>4.6</td>
<td>39.6</td>
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<td>0.9</td>
<td>3.9</td>
<td>55.0</td>
</tr>
</tbody>
</table>

Symposium Tecaliman – March 23, 2010

- Homogeneity
  - Annual evolution

Overall decrease: - 0.2 % / year

Symposium Tecaliman – March 23, 2010
Cross contaminations

- Method

Collecting batch 2: n + 2
Collecting batch 1: n + 1
Tracer Batch 2: n
Tracer Batch 1: n - 1

---

Cross contaminations

- First collecting batch

Frequency (%) vs. % Contamination

- Medians
  - 2008: 2.7%
  - 2000 to 2008: 3.0%

Symposium Tecaliman - March 23, 2010

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Symposium Tecaliman - march 23rd 2010
Cross contaminations

- **Second collecting batch**
  - Overall 62.0% below 1% and 74% in 2008
  - Problem with detection limits

![Graph showing frequency distribution](image)

<table>
<thead>
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<th>% contamination</th>
<th>Frequency (%)</th>
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<tbody>
<tr>
<td>0</td>
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</tr>
<tr>
<td>1</td>
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<tr>
<td>2</td>
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</tr>
<tr>
<td>3</td>
<td>30%</td>
</tr>
<tr>
<td>4</td>
<td>40%</td>
</tr>
<tr>
<td>5</td>
<td>50%</td>
</tr>
<tr>
<td>6</td>
<td>60%</td>
</tr>
<tr>
<td>7</td>
<td>70%</td>
</tr>
<tr>
<td>8</td>
<td>80%</td>
</tr>
<tr>
<td>9</td>
<td>90%</td>
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</table>

Symposium Tecaliman – March 23, 2010

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Cross contaminations

<table>
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<th>Years</th>
<th>First collecting batch</th>
<th></th>
<th>Second collecting batch</th>
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<tr>
<td></td>
<td>Nb</td>
<td>Median</td>
<td>Nb</td>
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<tr>
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<td>4.1</td>
<td>104</td>
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<td>2003</td>
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<td>3.0</td>
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<tr>
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<td>169</td>
<td>2.8</td>
<td>154</td>
<td>0.8</td>
</tr>
<tr>
<td>2005</td>
<td>168</td>
<td>2.7</td>
<td>160</td>
<td>0.7</td>
</tr>
<tr>
<td>2006</td>
<td>165</td>
<td>2.2</td>
<td>162</td>
<td>0.5</td>
</tr>
<tr>
<td>2007</td>
<td>174</td>
<td>2.5</td>
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<td>0.5</td>
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<tr>
<td>2008</td>
<td>196</td>
<td>2.7</td>
<td>194</td>
<td>0.5</td>
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Symposium Tecaliman – March 23, 2010
Cross contaminations

- First collecting batch

2000 to 2008

<table>
<thead>
<tr>
<th>Year</th>
<th>&lt; 1%</th>
<th>&lt; 3%</th>
<th>&lt; 5%</th>
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<tbody>
<tr>
<td>2000</td>
<td>14%</td>
<td>50%</td>
<td>75%</td>
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<tr>
<td>2008</td>
<td>17%</td>
<td>60%</td>
<td>82%</td>
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</tbody>
</table>

% of the total population

Cross contaminations

- Median evolution

Contamination (%)


| Year | (%)
<table>
<thead>
<tr>
<th></th>
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<tbody>
<tr>
<td>2000</td>
<td>16%</td>
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<td>12%</td>
</tr>
<tr>
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<td>10%</td>
</tr>
<tr>
<td>2004</td>
<td>8%</td>
</tr>
<tr>
<td>2005</td>
<td>6%</td>
</tr>
<tr>
<td>2006</td>
<td>4%</td>
</tr>
<tr>
<td>2007</td>
<td>2%</td>
</tr>
<tr>
<td>2008</td>
<td>0%</td>
</tr>
</tbody>
</table>
Cross contaminations

- Median contamination (C1) according tracer

![Graph showing median contaminations from 2000 to 2003.]

Conclusion

- Broad use of microtracer RF blue lake
- Homogeneity
  - Good progression
  - Progression still in progress but with a limit related to the analytical variation
- Cross contaminations
  - Significant improvement from 2000 to 2003
  - Thinner progression since 2003
  - Need for new prospects
Feedback on the negotiation having led to the directive 08/09

M. Epishe
Elanco
Summary

Carry over problems in animal feed have become acute since the beginning of 2000, when the Belgian authorities adopted a zero tolerance policy following the dioxins crisis in the country. A certain number of European Union Member States followed suit.
Early on, the various sectors of the industry offered pragmatic solutions based on food contaminant regulations, but we had to wait until 2009 to find a solution for coccidiostats. The solution adopted is smart but does not fully meet all expectations. It must be understood that this is a compromise made between the 27 Member States and a certain number of federations.
Revision of the directive and of the regulation in 2011 may lead to a possible improvement in the texts and authorised levels, but it is up to the industry to work together within an increasingly difficult and complex political context.
Carry-over
Short history of Europe

Dr Olivier Espeisse

Plan

- Zero Tolerance, a little background
- A few years later
- What has happened?
- Quid nunc?
CARRY OVER
HI. PP consult

Health issues and Public Policy

Johan Vanhemelrijck

Food and Pharmaceuticals Public Affairs

Setting threshold levels in feed for non-target species of coccidiostats authorized as feed additives and medicinal substances used in medicated feed

IFAH-Europe – FEFAC – BEMEFA, 27th July 2005
CONCLUSIONI

Le contaminazioni da carry-over e cross-contamination possono essere ridotte in vari modi, ma non è possibile eliminarle completamente.
Management of the risk of the residual presence of coccidiostats in feed for non target species as a result of carry-over in feed mills and of the presence of such residues in animal products from non target species.

Request for meeting

A few years later

Regulation 183/2003
Feed Hygiene

Regulation 2377/90
Maximum Residue Limits

Directive 315/93
Contaminants in Foodstuffs

Directive 2002/32
Contaminants in animal feed

Regulation 124/2009

Directive 2009/8
What is a directive?

- A directive is a legislative act of the European Union, aiming to encourage harmonization of European Law
- A directive obliges Member States to achieve a certain objective, but they are free to choose the means to do so.
- Directives require transposition.

EU Directive 2009/8

- Recitals
  - Inevitable
  - Without prejudice to regulation 183/2005 (Feed Hygiene)
    - HACCP
  - ALARA
  - Harmonization
  - Directive 2002/32 on contaminants in animal feed
  - EFSA Opinion
  - Concomitant establishment of maximum limits in foodstuffs
  - To be revised on 1st July 2011
Directive 2009/8

- Summary
  - 3% non-sensitive species
  - 1% sensitive species and withdrawal feed
  - Including ingredients
  - 50% for pre-mixes
  - Transcription in all Member States

### Directive 2009/8

<table>
<thead>
<tr>
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<td>1.25</td>
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<td>50% Proportional</td>
</tr>
<tr>
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<td>0.7</td>
<td>2.1</td>
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<tr>
<td>Salicylicin</td>
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<td>Semduracin</td>
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<tr>
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<td>0.4</td>
<td>1.2</td>
<td>0.4</td>
<td>50% Proportional</td>
</tr>
<tr>
<td>Habilicin</td>
<td>0.03</td>
<td>0.03</td>
<td>0.09</td>
<td>0.03</td>
<td>50% Proportional</td>
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<tr>
<td>Nisarbazin</td>
<td>0.5</td>
<td>0.5</td>
<td>1.5</td>
<td>0.5</td>
<td>50% Proportional</td>
</tr>
<tr>
<td>Dioctiluril</td>
<td>0.01</td>
<td>0.01</td>
<td>0.03</td>
<td>0.01</td>
<td>50% Proportional</td>
</tr>
</tbody>
</table>

ppm
What about regulations?

- Legislative act of a general scope.
- Regulations are directly enforceable in all Member States, with no transposition. Applies to all persons.

Regulation 124/2009

- Recitals
  - Maximum residue limits apply only to foodstuffs deriving from "target" species
  - Unavoidable contamination
  - Harmonization of the market
  - Maximum concomitant levels established in foodstuffs
  - Based on regulation 315/93
  - EFSA's opinion
  - To be revised on 1st July 2011
Regulation 124/2009

- Essential points
  - Maximum limits (ML) ...coccidiostats....in foodstuffs
  - Foodstuffs cannot be sold if concentrations exceed the maximum limits
    - No mixing with similar foodstuffs
  - However, limits are applied proportionally to dried, diluted and transformed foodstuffs or foodstuffs containing more than one ingredient.
  - Effective on: March 2009
  - Application: 1st July 2009

Regulation 124/2009

<table>
<thead>
<tr>
<th></th>
<th>Eggs</th>
<th>Milk</th>
<th>Liver</th>
<th>Kidney</th>
<th>Skin and fat</th>
<th>Other foodstuffs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lasalocid</td>
<td>150</td>
<td>1</td>
<td>50</td>
<td>50</td>
<td>5</td>
<td>5</td>
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<tr>
<td>Narasin</td>
<td>2</td>
<td>1</td>
<td>50</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Salinomycin</td>
<td>3</td>
<td>2</td>
<td>5</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Monensin</td>
<td>2</td>
<td>2</td>
<td>8</td>
<td>2</td>
<td>2</td>
<td>2</td>
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<tr>
<td>Semduramicin</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
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<td>2</td>
</tr>
<tr>
<td>Maduramicin</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Robenidine</td>
<td>25</td>
<td>5</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>5</td>
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<tr>
<td>Decoquinate</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
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<tr>
<td>Halofuginone</td>
<td>6</td>
<td>1</td>
<td>30</td>
<td>30</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Nicarbazin</td>
<td>100</td>
<td>6</td>
<td>100</td>
<td>100</td>
<td>25</td>
<td>25</td>
</tr>
<tr>
<td>Diclazuril</td>
<td>2</td>
<td>5</td>
<td>40</td>
<td>40</td>
<td>5</td>
<td>5</td>
</tr>
</tbody>
</table>

ppb
What has happened?

- We can tolerate anything as long as it is authorised!

Comitology

- EFSA opinion 10%
- Proposal Commission 3% 1%
- Vote by the Permanent Committee
Quid nunc?

- Transcription
- Control methods
- Controls
- Reconciliation between food and feed
- Medicated feed
- 2011 revision
Control methods

- Screening - simple, rapid, inexpensive
- Education
- Molecules concerned
  - Pesticides
  - Heavy metals
  - Biotoxins
  - Persistent organic pollutants
  - Fluorine compounds
  - Animal health products
    - Coccidiostats
    - Antibiotics
    - Malachite green

http://www.confidence.eu/

Regulation 152/2009

- Article 1:
  - "Sampling for the official control of feed as regards the determination of constituents, additives and undesirable substances, with the exception of pesticides and micro-organisms, shall be carried out in accordance with the methods set out in Annex I."
Maduramicin

- The Slovenian delegation has reported maduramicine levels in eggs exceeding 2 mcg/kg ...
- Controls carried out on the farm and in the factory showed that the feed was within the acceptable limits ...
- The data provided by the Slovenian authorities indicates that maduramicine levels in eggs are not in keeping with those set out for animal feed...

Minutes of the Permanent Committee, July 2009

Medicated feed

- Via contaminants?
- New medicated feed regulation
  - Parliament?
What can be done?

- 2011 Revision
  - Collect economic and technical data within the context of ALARA
  - Transfer studies
  - Mobilise the interprofession and members of the Permanent Committee
  - Take into account revision of directive 90/167

Glossary

- ADI: Acceptable Daily Intake
- ALARA: As Low As Reasonably Achievable
- ML: Maximum Limit
- MRL: Maximum Residue Limit
Feedback on the cross contaminations in the delivery trucks

M. Btier
Tecaliman
Summary

The data presented here was generated during tests carried out in 2002. However it was not published and is today of renewed importance in the light of the new directive, which implies that delivery is a potential source of cross contamination.

The main difficulty in evaluating cross contamination in delivery trucks lies in the many types of trucks used. In France, the large majority of them transfer feed mechanically from their bins into the farmer’s silos. Delivery and loading practices may lead to two types of contamination: Inter-bin contamination between successive deliveries in the same round or intra-bin contamination between successive changes in the same bin during consecutive rounds.

The results were produced under worst-case test conditions: Meal, longest circuit, other bins empty. The measurements were taken on 9 trucks using two types of tracers. These results show that the two types of tracers achieve very similar results, that cross-contamination is very low, that intra-bin cross-contamination is minimal and finally, that the results vary according to the truck. They are more positive for shorter and more modern trucks and less positive for longer, older trucks. In all cases, contamination generated by the delivery trucks is little significant, and more easily manageable with regard to that existing in factories.

The importance of respecting the transfer system purge procedure following delivery of a high risk batch was highlighted. The contamination profiles clearly indicate that contamination takes place within the first ten kilos delivered. Unlike in factories, where contamination involves both mass and dust transfer of a very low quantity from a product of unknown concentrations out of the whole manifold batch, that caused by trucks involves mass, elusive transfer of a low quantity of feed of a known concentration at the start of the manifold batch.
Feedback on the cross contaminations in the delivery trucks

F. Putier

Centre technique de la Nutrition Animale

- A report
  - Implication of the trucks on the aspects of cross contaminations
    - During the cow disease crisis
    - In the delivery of medicamented feed
    - In the 08/2009 European Directive
● Some questions
  - Which practices around the trucks?
  - Which types of contaminations?
  - How to measure these contaminations?
  - Which levels?
  - Which ways for contaminations?
  - Which control?

● Some practices
  - Loading
• Transfer in the truck

Source TSCI Vrac +

• Types of contaminations

Between-boxes
Incide-boxes

Symposium Tecaliman - March 23, 2010
Measuring cross contamination

- **Constraints**
  - 2 types of contaminations: between-boxes or inside-boxes
  - Variation of the trucks types
  - Variation of the physical presentations of the transported products
  - Intervention of the factory (Cross contaminations croisées, loading, ...)
  - Intervention of the delivery practices
Measuring cross contamination

Collecting batch 2

Incide-boxes

30 samples

Symposium Tecaliman - March 23, 2010
Levels of cross contaminations

- Measuring the maximum cross contaminations
- Trials with meal for all batches
- On the boxes furthest away from the exit and thus the longest transfer
- Others boxes empties
- 2 types of tracers
  - Internal: Medicinal substances (OTC ou CTC)
  - External: microtracer RF blue lake (250 ppm)

### Levels of cross contaminations

<table>
<thead>
<tr>
<th>Trucks</th>
<th>Type</th>
<th>Age Tractor/transfer system</th>
<th>Between-boxes (% measured)</th>
<th>Incide-boxes (% measured)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>External</td>
<td>Internal</td>
</tr>
<tr>
<td>A</td>
<td>Long truck, Screw, Gravity drain</td>
<td>14 ans/6 ans</td>
<td>0.55</td>
<td>0.65</td>
</tr>
<tr>
<td>B</td>
<td>Long truck, Chain conveyor, Pneumatic drain</td>
<td>2 ans/2 ans</td>
<td>0.12</td>
<td>0.12</td>
</tr>
<tr>
<td>C</td>
<td>Long truck, Chain conveyor, Blower drain</td>
<td>2 ans/2 ans</td>
<td>0.15</td>
<td>0.05</td>
</tr>
<tr>
<td>D</td>
<td>Long truck, Screw Gravity drain</td>
<td>14 ans/12 ans</td>
<td>0.61</td>
<td>0.45</td>
</tr>
<tr>
<td>E</td>
<td>Long truck, Chain conveyor, Blower drain</td>
<td>4 ans/4 ans</td>
<td>1.41</td>
<td>1.41</td>
</tr>
<tr>
<td>F</td>
<td>Long truck, Screw Blower drain</td>
<td>8 mois/3 mois</td>
<td>0.14</td>
<td>0.18</td>
</tr>
<tr>
<td>G</td>
<td>Long truck, Screw Blower drain</td>
<td>12 ans/3 mois</td>
<td>0.14</td>
<td>0.15</td>
</tr>
<tr>
<td>H</td>
<td>Short truck, Chain conveyor, Blower drain</td>
<td>2 ans/2 ans</td>
<td>0.02</td>
<td>0.04</td>
</tr>
<tr>
<td>I</td>
<td>Short truck, Chain conveyor, Blower drain</td>
<td>1 an/1 an</td>
<td>0.02</td>
<td>0.08</td>
</tr>
</tbody>
</table>
• Levels of cross contaminations
  - Same results with external tracer
  - Low contaminations between boxes
  - Tiny contaminations incide-boxes
  - Evaluated under adverse conditions
  - Variation according trucks

• Contamination ways between-boxes
  - Same scale
    - Truck D
    - Truck F
    - Truck E
    - Truck G
Contamination ways incide-boxes

- Scale / 10

Truck D

Truck E

Truck F

Truck G

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Contamination ways

- Everything in the first 25 to 50 kilos
- Importance of the purgings of the transfer system after delivery of a “risks” feed
  - Possibility of an increase of the contamination from 0.5 to 1 %
  - Record the respect of this practice
- Transfer of a small quantity of a feed of a known concentration

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Controle

- To check the trucks in time
  - Measures contaminations in the first kilogrammes
- To validate the purging practice after the delivery of « risk » feed
- To close the boxes after the delivery
- To show incide the boxes before reloading

Conclusion

Trucks
Mass and fugacious transfer of a small quantity of feed of concentrations known at the beginning of collecting batch

Factories
Mass and dusts transfers of a very small quantity of some unknown concentrations product incide all the collecting batch
Round table to the cross contaminations
Upstream: Account of the industrialist

M. Drel
Ccpa
Management of animal foodstuff quality depends on the control of undesirable substances in animal feed; notion of which is related to cross contamination. The legislator has defined and tightened requirements over 15 years.

Feed factories first of all had to implement measures "to avoid cross contamination as far as possible" and "to bring them to the lowest level possible" (Dir. 95/69/EC and order of 28.02.00).

Secondly, in 2007, Best Manufacturing Practice for medicated feed applied the principles of due care and introduced the obligation to achieve results (limits of 5% and 1% on the first and second manifold batches).

French manufacturers, as seen in the results of the survey carried out by TECALIMAN, acted effectively: Median cross contamination levels in the first and second manifold batches were reduced by half between 2000 and 2008.

In the specific case of medicated feed production, certain sites became specialised while others for which tonnage was low ceased production.

Certain circuits were specialised within individual factories: Either entirely, including the mixer (if there is more than one) or partially (bag tippers: pelleting line, finished products unit etc; delivery trucks).

More generally, we also sought to make handling safer: For example by replacing the mechanical transfer of micro-ingredients by pneumatic transfer to the mixer. The effect of the condition of the screws or conveyor belts on holding is more effectively taken into account. Elevators with a rounded base and that can even be opened and cleaned have appeared.

Investment was also made in industrial data processing in order to optimise flow scheduling. Incompatibilities were managed carefully in terms of the various manufacturing circuit nodes etc.

Certain practices were also implemented: Grouping of medicated feed, partial or total rinsing (bag tipper, press dies) of circuits using a raw material that is then destroyed or recycled.

Since Directive 2009/8/CE and its transposition into French Law in September 2009, coccidiostats are now undesirable substances in feed; feed not being intended to contain them. Although the legislator acknowledges the existence of unavoidable transfer, we have progressed from the principle of due care to the strict obligation to achieve results at the delivered finished product stage (lowest concentrations that can be possibly reached).

For the feed factory, these new rules increase the level of control required and awareness of operators must be raised.

As for medicated feed, grouping feed batches containing coccidiostats may be a solution. In this case, industrialists shall pay special attention to feed for turkeys and rabbits that may contain certain coccidiostats while being sensitive to others.

The possibility of cross-contamination beyond the scope of the bag tippers/loading hopper entry, usually evaluated during qualification tests must be considered.

Upstream, for example in feed recycling where feed passes through the pit, grinder etc. Downstream, management of fines at the end of the pelleting batch should probably be as thorough for coccidiostats as for medicated molecules.

The risk of contamination "from the front", that is to say from one batch to a previous batch due to flap leakage (in the mixer for instance), must be systematically anticipated.

These examples must be adapted on a case per case basis and the means implemented must meet the risk analysis requirements in each factory.
The laboratory, the analysis and the cross contamination detection

M. Le Bouquin
Larál
Round table on cross-contamination – “The laboratory, the analysis and the cross contamination detection”
M. Le Bouquin, Laréal

Recent changes in legislation such as Directive 2009/8/CE, legislative changes labelling of food products in both countries of the European Union, the United States, Canada and Australia, forcing manufacturers extra vigilance for the presence of involuntary molecules from cross-contamination in manufacturing.
Starting from the simple fact that it is not possible for most of the industrials (feed and food) to dedicate exclusively one line to one type of product to avoid any risk of cross contamination, Laréal (laboratory group Invivo NSA) was developed especially for the benefit of its internal or external customers some specific analysis in this issue.
In this context, Laréal provides methods for measuring uniformity and cross-contamination in feed, including a method with cobalt and/or yttrium, a method with the anticoccidial residues, a method with a micro tracer (developed and validated by Tecaliman) ...
If we believe it is the responsibility of manufacturers to choose the best method to achieve goals in terms of contamination and management of production, each method involves implemented special techniques for laboratories.

It is therefore essential for them:
- To have analytical tools to achieve performance thresholds compatible with the regulations. Thus, for analysis of derivatives anticoccidial Laréal moved from a model of detection by fluorescence to mass / mass detection as the only way to move from ppm to ppb. The laboratory must during validation of its thresholds allow itself a sufficient margin for security and if the regulations require a determination in ppb, the laboratory will be able to quantify the tenth of ppb.
- To proceed with development and validation of appropriate methods of analysis on all matrices. Thus, for the analysis of Yttrium the use of ICP with optical detection or an atomic absorption spectrometer with Zeeman correction is required.
- Of self control by the analysis of standard samples or references marked where they exist and to calibrate with some other laboratories by ring tests.
- To adapt its methods to train its personnel in relation to official documents that can not describe all the operations in detail and suggest inaccuracies in the methods. Of course, again, the matrix effects must be managed by the laboratories and we know that for example in the RF Blue Lake method (simple theory) the humidity, the nature of the food and the process, particle size, purity and density affect yields and generates bias between laboratories.

It is important to add that the laboratory must have a quality system to assess its measurement uncertainty, but in this context how industrials can interpret the results of cross-contamination testing: each test will give a different result because the molecules (Yttrium, microtracer RF Blue Lake or monensin) do not react the same way during the process. And each test has its own limit of quantification and its own measurement uncertainty. This is what we propose to discuss at the roundtable.
Downstream: quality of meats
and impact on the consumer

Mrs Laal
Oniris
Round table on cross-contamination – “Downstream: Meat quality and impact on the consumer”
Prof. Arlette Laval, ONIRIS

This subject generally covers two aspects:
- Meat microbiological quality and in a more general manner foodstuffs of animal origin,
- Aspects relating to the presence of chemical compounds of various types: Prohibited products, authorised products present in excessive quantities, environmental contaminants. We will focus on this last point specifically, in relation to feed manufacture directly.

According to the point of view of livestock feed manufacturers, the most frequent contamination is caused by additives and medicinal product residues, which in turn bring the responsibility, reputation and credibility of the sectors concerned into question. In meat, this may come from contamination of the feed at the factory, poor management of treatment in the farm or “original” prescriptions from the vet.

In the factory, cross contamination involves the presence of antibiotics in very small quantities, significantly inferior to the treatment doses. This subject is currently well covered thanks to the work carried out by Tecaliman. In the worst case scenario, contamination can be compared to that which was incorporated previously as an additive. The risk is both toxicological and microbiological. Where a molecule has MA and above all an MRL in the species in question, the toxicological risk can be evaluated. It must however be discussed on a case by case basis, according to the molecules used, the accidental nature of the incident and medicinal product withholding time in normal conditions of use. Incidents have been reported with certain sulfonamides (sulphadimidine) of which total elimination takes a fairly long time and which does not so much raise public health concerns but more commercial problems. Microbiological risk, which consists of creating selection pressure for antibiotic-resistant bacteria, is also limited, as the phenomenon is accidental. The subject of growth factor antibiotics has provided substantial information on this question. The risk must be discussed according to the type of target molecules and bacteria. It is obviously less risky to select resistant or less sensitive Pasteurella multocida strains, which is not a major zoonotic bacterium, than salmonellae which may raise serious public health concerns.

It is therefore clear that in general, the accidental or regular nature of the incidents must be underlined, as in the second eventuality; it is difficult to defend the factory. It must however be highlighted that these subjects are of little concern in factories to date: Either factories have been brought up to standard or have closed.

Mixed feed may also constitute a source of undesirable contamination due to the presence of undesirable components in the raw materials, e.g. pesticide residues, mycotoxins, heavy metals, PCB etc. Raw materials may also contain microbial contaminants, in particular salmonellae. The protection of stocks of wild birds, rodents and insects is essential and must be closely monitored, in particular for farm production.

Management of treatment of infectious diseases on farms is generally brought into question when residues are found in meat during controls. Where medicated feed is used, the silo must be fully empty upon delivery, otherwise the treatment is diluted during the first days and it may become less effective. At the end of the treatment, the silo must be emptied completely for the clean feed used subsequently to remain so. A separate silo for medicated feed is ideal. The risk is minor when used in young animals, long before slaughtering for example. However the risk is highly present if the treatment is administered in animals in the finishing stage, in particular in pigs receiving single feed. Let us not forget that treatment administration is the farmer’s or farm workers’ responsibility, and the latter are not always fully aware of such issues.

Another significant source of difficulty is the use of liquid feeding systems. The veterinary profession addressed this subject, as prescribing veterinary surgeons may be held responsible if collective treatment is prescribed although it is impossible to guarantee the later absence of cross contamination. Management methods are proposed, depending on whether the liquid feeding system has a return tank, with in particular prescription for passage from a liquid meal to empty the remaining supplemented preparation.

The third major cause of difficulty concerns the prescription itself. A number of MA are obsolete and the doses no longer adapted to the sensitivity of the pathogenic bacterial strains encountered. Vets are therefore fully responsible for the dose and treatment time proposed. MA withholding time is only defined for a given dose and specific administration time. In most cases, the behaviour of active principles when combined is not known. Any changes in dose and/or treatment time are risky, regardless of the species and administration route. The same applies for medicinal product combinations. This point may concern pigs in the finishing stage, broiler hens and dairy cows receiving intramammary treatments.
It must however be underlined that generally, the impact of meat quality on consumers is above all related to microbial contamination from animals and/or handling during the transformation process. How meat is stored by consumers is in itself the predominant difficulty.

In France, the detection of undesirable residues as outlined in meat, egg and milk monitoring plans is relatively rare. The situation is therefore rather positive, major efforts having been made in feed factories, slaughterhouses and farms.