Limits of Chemical Safety

Food Safety versus Food Security – A global challenge

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Food Security

FAO, publication 2010:

- 2004: 37.5 million tons of seafood consumed from aquaculture sources
- 2030: 2 billion people more to feed
  → need of 83 million tons of seafood from aquaculture sources!

- Aquaculture: major role in food security

→ need to produce more with increased sustainability

Food Security

<u>2004 – 2030</u>

- Sustainable development of 83 million tons of seafood from aquaculture sources

- Governments & trade organisations: strategy to support long term food production

→Disease control will become very important for the success of aquaculture sources!

  - GAP (Good Aquaculture Practices), probiotics, immunostimulants,…: not sufficient
  - Preventive use of antibiotics = unwanted
  - Pressure on therapeutic use of antibiotics will grow!
  - Availability of safe chemical tools & good regulation about use of antibiotics are crucial!

Food Safety

<u>Residues of antibiotics</u>

- CURRENT EU REGULATION:
  - <u>Principle of precaution</u>:

    substances of Annex IV from Reg. 2377

    (e.g. chlooramphenicol, nitrofurans,…):

    no maximum toxicological levels could be fixed,

    no maximum residue limits are set (<u>lack of scientific data</u>) → <u>zero-tolerance levels</u> are in force ("not allowed in food at all, regardless the concentration" or "dangerous at any dose")
  - Reg. 470/2009: <u>MRPL</u> (Minimum Required Performance Limits) used as targets for regulatory actions

    MRPL = the lowest concentration level regulatory laboratories in the EC can detect and confirm

Food Safety

Residues of antibiotics

- CURRENT EU REGULATION:
  - Zero-tolerance has resulted into ever increasing analytical capabilities of detections (ppm → ppb) & increasing chance on 'false positives' (natural presence in food producing animal, traces from environmental background)
    e.g. chlooramphenicol (CAP) & semicarbazide (SEM)
  - Linear extrapolation of toxicity (linear no-treshold toxicological dose-response model) :
    not reliable estimate of low-dose risk !

Food Safety

Residues of antibiotics

- CURRENT EU REGULATION:

- is not based on toxicological relevance
- does not take into account natural existence of presumed man-made chemicals
- the zero-tolerance precautionary approach to ban certain antibiotics from food has proven unworkable as a result of continuing analytical progress

→ The precautionary principle & linear model needs to be discarded from food safety regulations !!

Food Safety – Hormesis

- Current research: more reliable estimate of low-dose risk for carcinogens: hormesis (U shape): low doses show no linear relation; low doses of a carcinogen may reduce incidence of adverse effects.
- Need for more research on the toxicological implications of unavoidable low-level exposures (environmental traces) to numerous chemicals rather than pursuing the 'next' chemical in some food matrix.

The CAP issue

- **Chloramphenicol (CAP)** (Hanekamp, Kwakman)
  - Despite a ban on animal food production, CAP is still used in human medicine
    (treatment of serious bacterial infections, e.g. eye infections)

The CAP issue

CAP = multi-source issue:

- natural production by *Streptomyces venezuelae*, an ubiquitous soil-bacteria found worldwide

  → natural presence of CAP at very low levels in food, not associated with abuse of antibiotic CAP e.g.:

  - Natural presence of CAP in white wine (2.7 ppb) (2003)
  - Natural presence of CAP in grass and herbs (0.1-450 ppb) (2010)

  →Regulation needs to be revised

The CAP issue

- When considering the difference between therapeutic exposure – as a result of which aplastic anaemia has been observed – and the much lower exposure to food residues, it seems clear that CAP is unlikely to present a substantial risk, if at all.

The CAP issue

- Once a successful antibiotic (late 1940s) curing diseases such as typhoid fever and being effective in animal rearing and aquaculture.
- Now banned for fear of its carcinogenic potential at any exposure level.
- However, apart from the continued efficacy of CAP in human medicine, the potential of CAP in aquaculture is an interesting issue in view of the rising food-requirements in the world.

The CAP issue

- In order to assess the risks and benefits of CAP – in terms of its use in food production such as aquaculture and the resulting exposure to humans through the produced foods – hormesis seems a way forward.

SEM naturally present in shrimp

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Semicarbazide

- Semicarbazide (SEM) = marker currently used to detect nitrofurazone in food
- Nitrofurazone is one of the nitrofurans antibiotics
- Presence of nitrofurans in foods is forbidden in EU since 1993 (Reg. 2377/90)

- Intended use: human medicine for treatment of bacterial infections (e.g. urinary tract)
- Non intended use: veterinary medicine used in aquaculture to prevent or treat bacterial diseases

Dec. 2003/181/EC sets MRPL used to detect Nitrofurans in aquaculture products

Nitrofurans	Metabolites Markers of nitrofurans abuse	MRPL	Effectiveness
furazolidone	AOZ	1 µg/kg (1ppb)	+ (no interferences reported)
furaltadone	AHD	1 µg/kg (1ppb)	+
nitrofurantoin	AMOZ	1 µg/kg (1ppb)	+
nitrofurazone	SEM (semicarbazide)	1 µg/kg (1ppb)	- Poor marker for nitrofurazone abuse

Problems with SEM

- End 2008 to mid 2009:
  - > 90% of all nitrofurans RASSF notifications is due to SEM
  - In total > 80(!) SEM notifications (10 million USD damage), of which 80% notified in Belgium, mainly for freshwater (FW) shrimp (*Macrobrachium rosenbergii*) from Bangladesh, where SEM was tested on the whole shrimp (meat + shell)
  - In other countries SEM only tested on the meat: no significant increase in SEM positive samples

Problems with SEM

- Since June 2009, Belgium is no longer testing the whole shrimp, but only meat → number of notifications drastically decreased
- 2010: in total 9 SEM notifications for crustaceans
- 2011 (until 21/09/2011): 2 SEM notifications for crustaceans

Analysis of Nitrofurazone → SEM

- Not possible to test residues (very quick metabolistation); the stable metabolite (SEM) is detectable
- Extraction (hydrolysis with HCl)
- Analysis by LC-MS/MS
- SEM binds easily to the tissue protein; to detect the possible abuse of Nitrofurazone, it is generally accepted to determine tissue-bound SEM: washing step (MeOH, H_2O) needed to remove any free SEM and determine only the tissue-bound portion of the total SEM

Proof that SEM is naturally present in shrimp

Different studies

- Interfield lab, Kochi, India: tests on wild caught FW shrimp from 4 different areas in India and on aquaculture shrimps → all samples, incl. the ones from wild origin (no abuse of antibiotics) positive for SEM in the shell, but not in the meat
- National lab, Bangkok, Thailand: wild caught soft shell crab were found positive for SEM

- Conclusion: as SEM is found in wild caught crustaceans, SEM can have another source than nitrofurazone abuse

Proof that SEM is naturally present in shrimp

Study by University of Ghent, Belgium

- test on aquaculture FW shrimp from Bangladesh shows:
  - whole shrimp: 7 ppb SEM
  - meat: < 0.5 ppb SEM
  - shell: 21 ppb SEM

- other crustacean species, both wild & farmed:
  - meat: < 0.5 ppb SEM
  - shell: 2 – 13 ppb SEM

Proof that SEM is naturally present in shrimp

Study by University of Ghent, Belgium

- study sponsored by SIPA: Natural presence of SEM in lab grown FW shrimp (publication in Journal of Agricultural And Food Chemistry)

  FW shrimp cultivated in 100% controlled conditions
- Exposure study

Treatment	SEM in meat	SEM in shell
Group 1 : no addition of nitrofurazone	Total (free + tissue-bound) 0.8 ppb Bound < 0.5 ppb	Bound 27 ppb
Group 2 : daily 50 mg nitrofurazone per L culture water	Total > 50 ppb Bound > 50 ppb	Bound > 50 ppb

Proof that SEM is naturally present in shrimp

Study by University of Ghent, Belgium

Conclusions:

- SEM is mainly tissue-bound in the shell of FW shrimp
- As tissue-bound SEM is found in the shell in group 1 (no nitrofurazone), tissue-bound SEM can have another source than nitrofurazone abuse: natural presence of SEM in the shell of FW shrimp
- SEM is no good marker molecule for nitrofurazone abuse

Proof that SEM is naturally present in shrimp

<u>Study by Agri-Food & Biosciences Institute, Belfast, UK (Dr. Kennedy)</u>

- Natural presence of SEM in <u>wild caught</u> FW shrimp from Bangladesh:
  - test of wild caught shrimp upstream, midstream and upstream from farms
- Results:
  - meat: no significant differences (< 0.3 ppb SEM)
  - shell: 100 – 200 ppb SEM, no matter what location (about 100x higher than in meat)

Proof that SEM is naturally present in shrimp

<u>Study by Agri-Food & Biosciences Institute, Belfast, UK (Dr. Kennedy)</u>

- Can shell contaminate meat?
  - inner meat (core meat): < 0.2 ppb SEM
  - outer meat: 0.8 ppb SEM
  - soft shell (membrane between meat and hard shell): 10 – 15 ppb SEM
- Moult of shrimp
  - New shell synthesized in epidermal layer (= surface meat)
  - Old shell shed to allow growth → some epidermis stays on the shell which will be shed, some stays on the meat: contamination !

Different sources of SEM

- metabolite of nitrofurazone

BUT many interferences:

- a thermal degradation product of azodicarbonamide (ADC) (= a blowing agent used in plastic gaskets of packaging material)
- a degradation product of ADC, formerly used as an additive in flour (banned now in the EU)
- a reaction product associated with the use of sodium hypochlorite in desinfection processes in food processing
- artifact created during the extraction step (hydrolysis with HCl) in the SEM analysis?
- natural presence in algae (carrageenan)
- an environmental contaminant
- **natural presence in shells of crustaceae, but source is unknown as of yet**

Different sources of SEM

- Before a civil court, there is ample space for discussion about this crucial question: “who can, on the basis of the currently used methodology, prove that the presence of SEM in aquaculture products is NOT due to one of these interferences?”

Conclusion

- SEM naturally present in shells.
- Epidermal SEM contaminates meat.

- Is presence of SEM a risk for health? NO
- Is use of nitrofurazone a risk for health? YES (carcinogenic)
- Is there an alternative for SEM as marker for nitrofurazone? NOT YET

Some suggestions

1) Analysis method
   - Maybe the analysis method for SEM should be amended? Current method is not 100% reliable because the result depends on the variations in the efficiency of the washing step (needed for separation free and tissue-bound SEM)
   - Analysis on tissue-bound SEM of inner meat only, to avoid contamination from shell?
   - Is the MRPL reasonable? Abandon precautionary principle?

2) Maybe there is a need for a different marker to detect nitrofurazone? Albumin is a highly promising candidate (found in many tissues, relatively long elimination half-life)