Workshop „Pharmaceuticals in Soil, Sludge and Slurry”

(Dessau, 18th June to 19th June 2013)

JUSTUS-LIEBIG-UNIVERSITÄT GIESSEN

Key Lecture: Entry, occurrence, behavior and effects of pharmaceuticals in the environment

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Contents

- **Introduction**
- Effects and risks of veterinary drugs
- Challenges for food safety
- Strategies for reduction
- Conclusions / Summary

Application of veterinary drugs in livestock farming

- Antibiotics (most important class in UK, Denmark, The Netherlands, USA, China, Germany)
- Endectocides (e. g. ivermectin)
- Coccidiostats (e. g. nicarbazin)
- Antifungals (e. g. chlorhexidine)

Application of veterinary drugs in livestock farming

- Veterinary drugs (administration i. v., i. m., via drinking water or feed)
  - Therapy
  - **Prophylaxis (forbidden in Germany)**
  - Metaphylaxis

- Feed additives („Growth promoters“)
  - *EU ban on this special use since 01.0.1.2006*
  - *Frequently used in USA, Asia*

Use of veterinary antibiotics in Europe

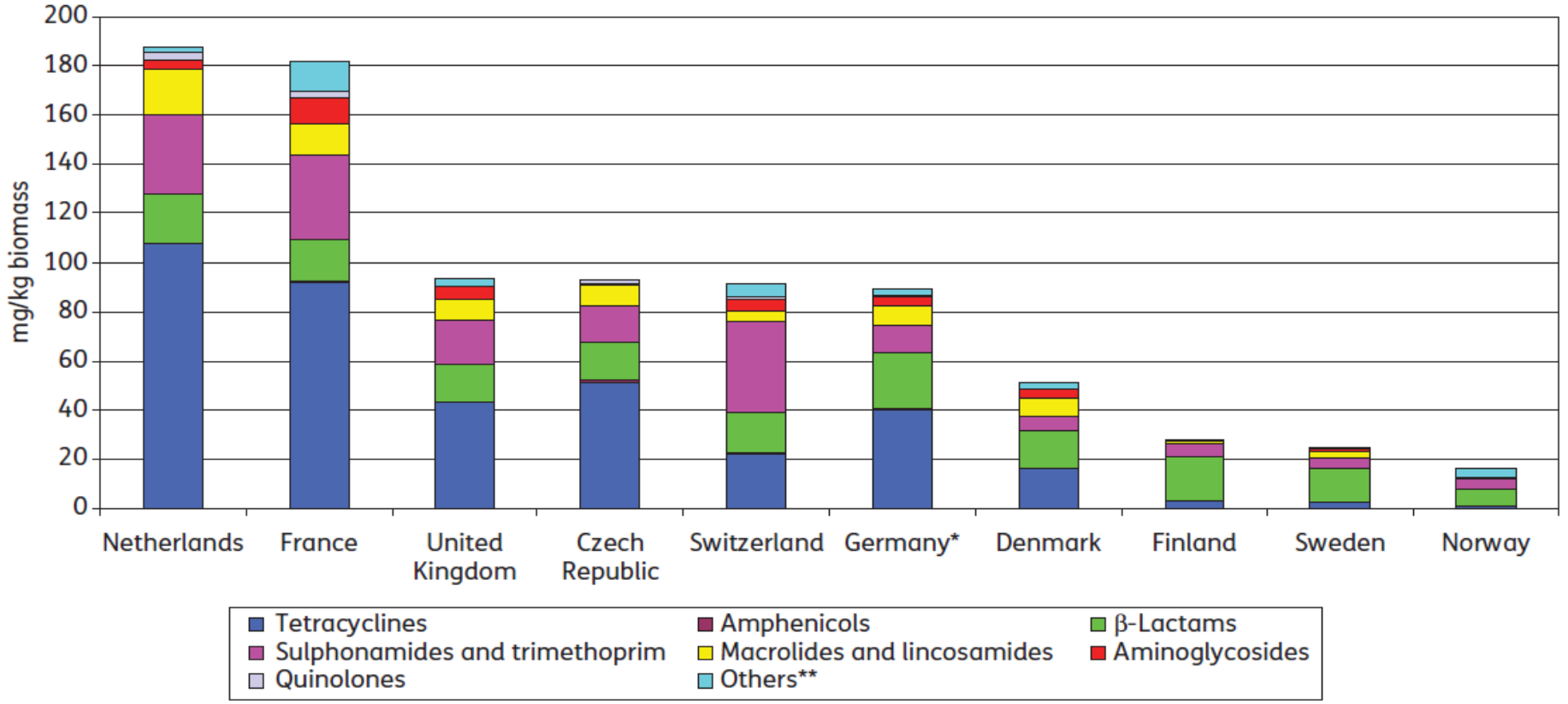
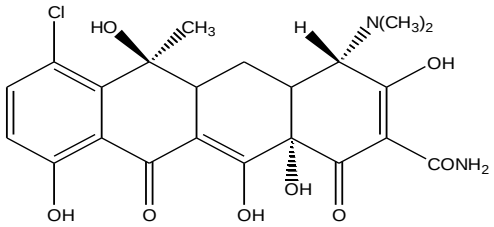


Figure 1. Amounts, in mg, of veterinary antibacterial agents sold in 2007 per kg biomass of pig meat, poultry meat and cattle meat produced plus estimated live weight of dairy cattle. *2005 data. **The substances included vary from country to country.

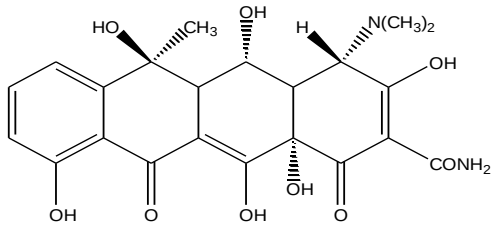
(Grave et al. 2010)

Germany: 1734 tons of antibiotics (BVL, 2012)

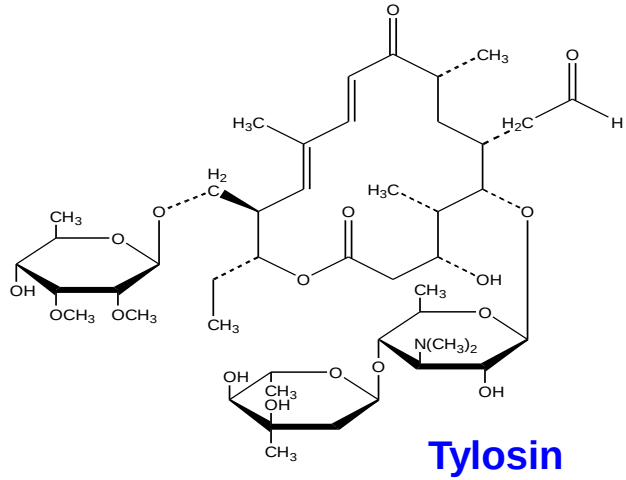
Frequently used veterinary drugs



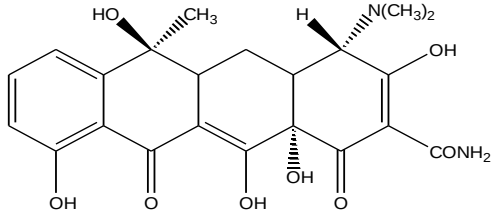
Chlortetracycline



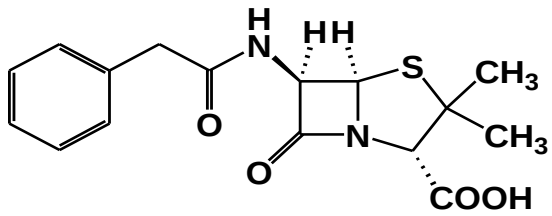
Oxytetracycline



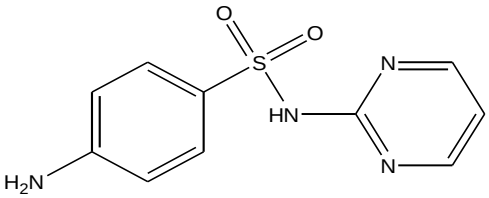
Tylosin



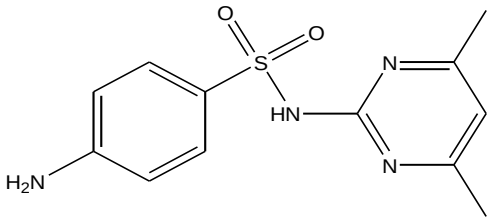
Tetracycline



Benzylpenicilline



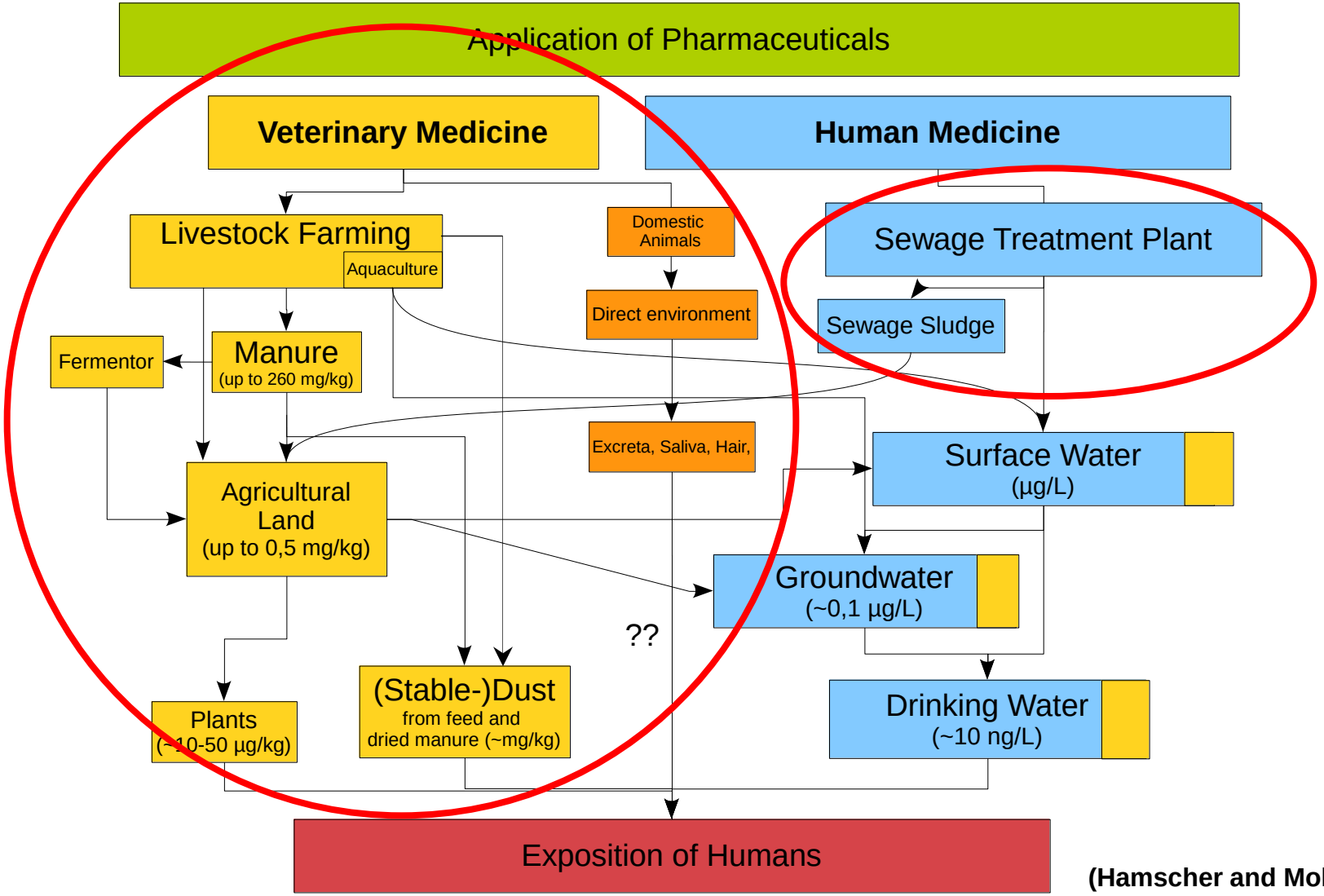
Sulfadiazine



Sulfamethazine

- **Various substance classes**
- **Complex Matrices**
- **Method of choice: LC/MS/MS**

Entry and exposure routes for pharmaceuticals



(Hamscher and Mohring, 2012)

Fate of antibiotics after application

- Most antibiotics (e. g. tetracycline, macrolids) are only **poorly metabolised** after administration
- **N-acetyl-Sulfonamides are de-acetylated** to the parent compound in manure
- Occurrence of a **cocktail** of persistant antibiotics (e.g. various tetracyclines and sulfonamides) in liquid manure in concentrations up to several 100 mg/kg
- **„Hot spots“** of tetracyclines in dried liquid manure soil aggregates with concentrations up to 1.5 mg/kg

(Langhammer et al. 1988, Winckler et al. 2000, Hamscher et al. 2002, Engels 2004, Boxall 2008)

Fate of pharmaceuticals in soil

- Sorption, transport and degradation processes may occur
- **Many interacting factors** influence the fate of veterinary drugs in soil:
  - Hydrophobic properties, ion exchange, cation bridging at clay surfaces, surface complexation, hydrogen bonding
  - Water solubility, pH value, moisture content, temperature, timing of manure application
  - Problem: wide variety of soil types, behaviour of drugs in the soil-liquid manure matrix

→ **Nearly impossible to predict accurately the environmental fate of a certain drug**

(Tolls 2001, Thiele-Bruhn 2003, Schauss et al. 2009)

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Effects of veterinary antibiotics

- **Remind:** microorganisms are the targets of antibiotics in nature and in medicine
- Manure, marine sediments and dried manured soil aggregates: concentrations **within the minimally inhibitory concentration** (0.5 – 2 mg/L) for various bacteria
- Manure: **reservoir of resistant bacteria and antibiotics**
- Application to agriculturall soils **increases antibiotic resistance genes and the selection of resistant soil bacteria**
- **Changes in microbial communities**
- **Is there a risk for human exposure to soil-borne resistance?**

(Schmitt et al. 2006, Heuer et al. 2010)

Veterinary endectocides – Ivermectin / Moxidectin

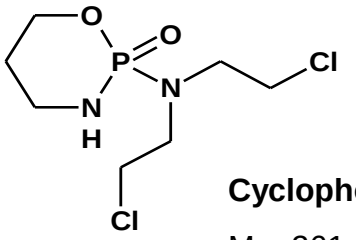
- 1980s: fermentation of soil-derived microbes led to the identification of macrocyclic lactones with strong activity against ectoparasites and endoparasites (**endectocides**)
- Frequent worldwide use in veterinary medicine and agriculture
- Macrocyclic lactones **are substances of high concern** regarding environmental aspects
- **Various environmental consequences on non-target organisms are reported**

Veterinary endectocides – Ivermectin / Moxidectin

- Exposure to ivermectin via dung from treated animals leads to
  - Reduction in growth rate of various dung-inhabiting insects (Wall and Strong, Nature 1987)
  - Increase in adult and larval mortality
  - **Alternative**: repeated treatment with injectable formulations

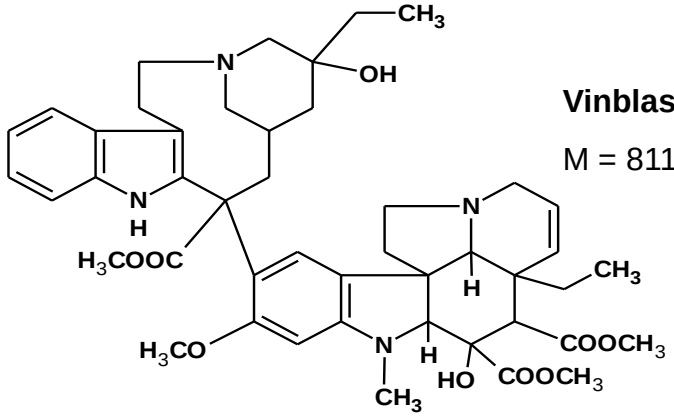
- Replacement of ivermectin by moxidectin
  - Moxidectin less toxic for dung breeding insects
  - **Non-target species toxicity**: more toxic for fish than ivermectin

Veterinary cytostatics



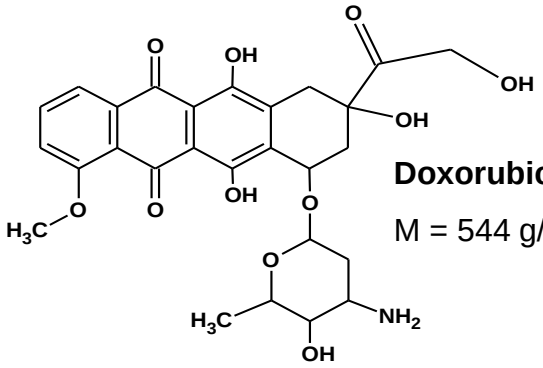
Cyclophosphamide (CPP)

M = 261,1 g/mol



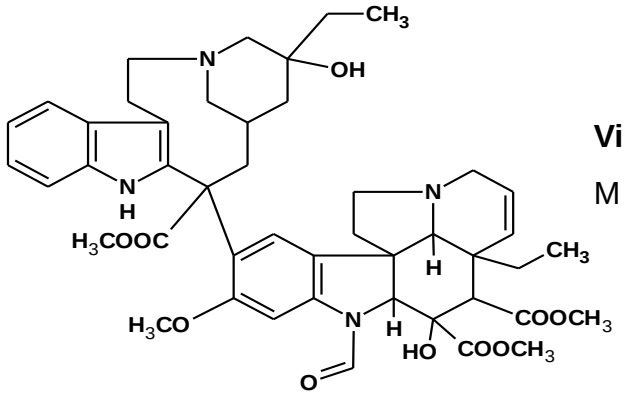
Vinblastine (VIB)

M = 811 g/mol



Doxorubicin (DXR)

M = 544 g/mol



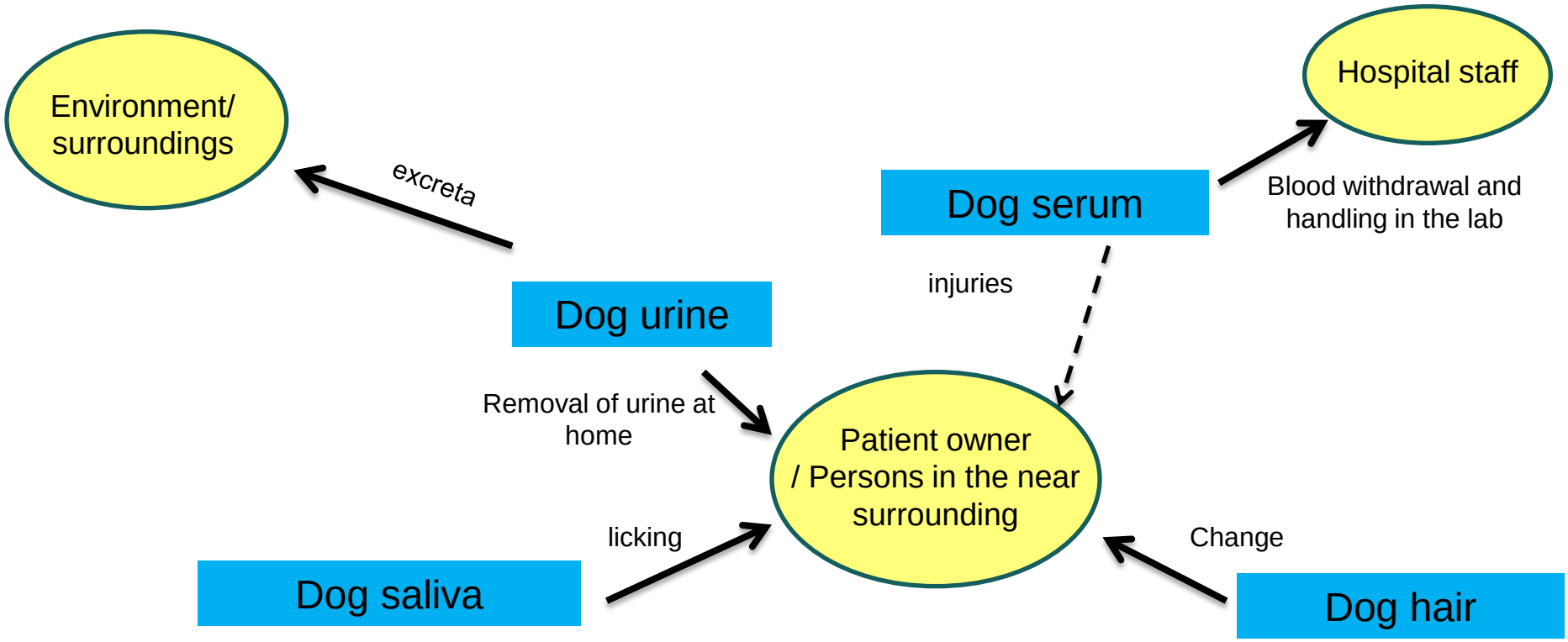
Vincristine (VIC)

M = 825 g/mol

- Domestic animals getting older (nutrition / medical diagnostics)
- **Desire of the owner for “best” medical treatment: chemotherapy**

(Mohring 2011)

Veterinary cytostatics



Is there a risk for the environment/humans in the near surrounding through urine, serum, saliva or hair of treated animals ?

(Mohring 2011)

Veterinary cytostatics – Risk assessment and outlook

Highest risk results from dog urine

Low risk for hospital staff through contact with serum

Considerably lower risk through contact with saliva and hair

Doxorubicin: substance with the most probable risk – excretion up to 21 days with urine

→ Adaption of international guidelines (e.g. ECVIMCA)

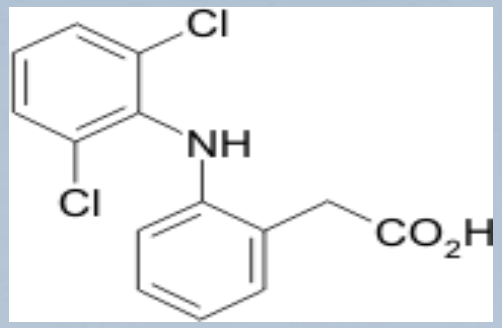
Handout/Suggestions for patient owners

(Hamscher et al. 2010, Knobloch et al. 2010, Mohring 2011)

Dramatic adverse environmental effect of diclofenac

2005: Catastrophic decline (> 95 %) of three vultures species in southeast Asia due to diclofenac poisoning

(lethale dose: 0,1–0,2 mg/kg, < 1 mg / bird)



Diclofenac: Non-steroidal anti-inflammatory drug (NSAID)

Picture: http://www.organische-chemie.ch/chemie/2010/sep/diclofenac.shtm

Dramatic adverse environmental effect of diclofenac

- **Acute kidney failure**
  - Large urate deposits on internal organs
  - Death within a few days

- **Diclofenac residues in animal carcasses of cows and domestic goats**
  - One large “meal” responsible for rapid death of three Gyps vultures, which showed extreme sensitivity to this drug
  - Cows were treated within a day or two before death (Green et al. 2006)
  - Comparable sensitivity in two other vultures species (Gyps africanus and the Eurasian griffon vulture, Gyps fulvus; Swan et al. 2006)

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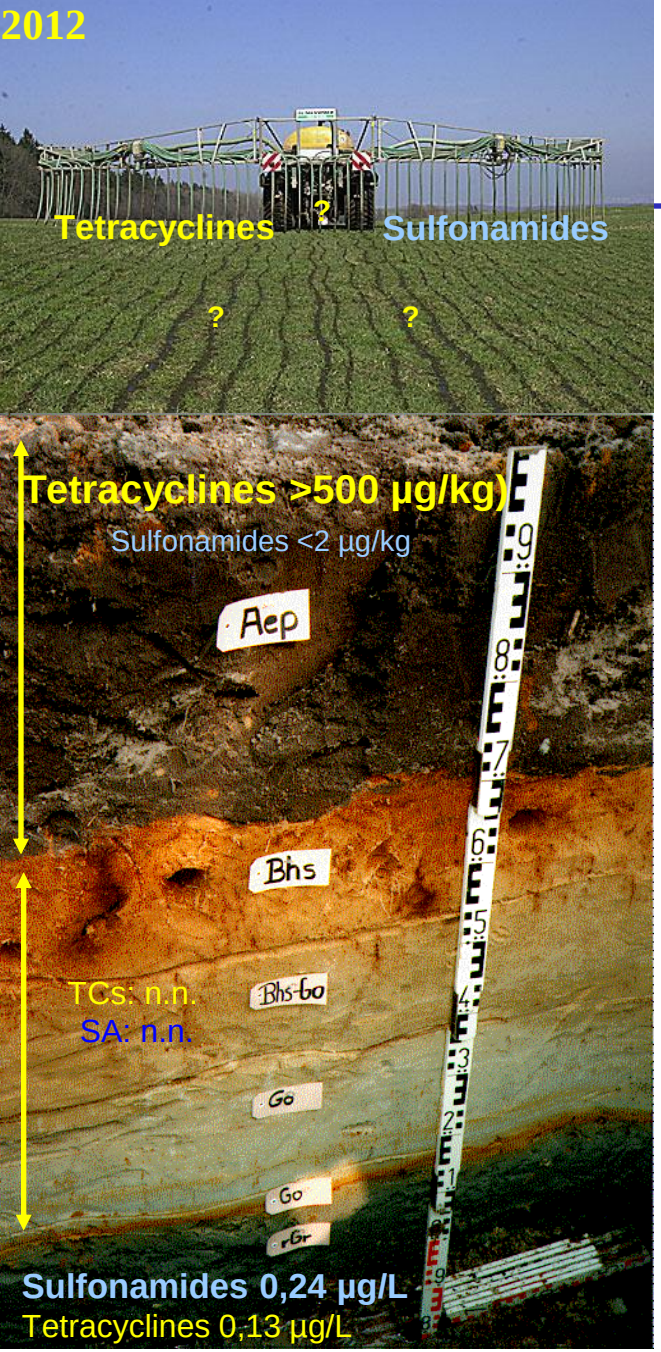
Field and lysimeter studies 2000 – 2012



- Substantial amounts of antibiotics occur in liquid manure, in soil and in dust (mg/kg)
- Strong sorption in soil may reduce bioavailibility but also degradation
- Sulfonamides are reaching our groundwater resources
- Dust may also contribute to the spread of antibiotics and antibiotic resistance

(Hamscher et al. 2002, 2003, 2005, 2008 and current investigations)

Mineral water – Legal aspects (Germany, „AVV Anl. 1a“)

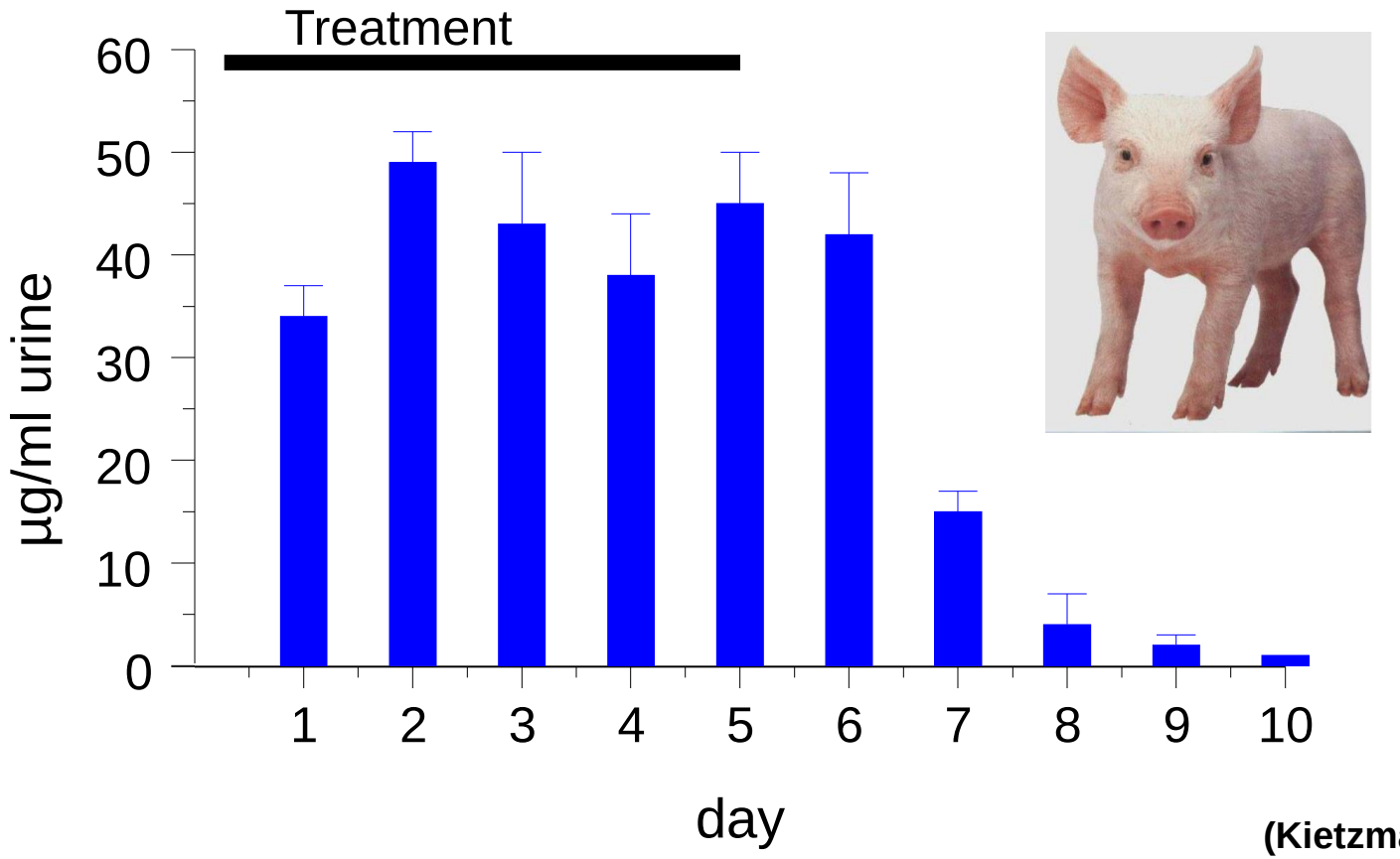
Orientierungswerte für Belastungsstoffe in natürlichen Mineralwässern als Kriterien für die ursprüngliche Reinheit

„Natural pure mineral water“

Lfd. Nr.	Parameter	Orientierungswerte für Höchstkonzentrationen
	A. Einzelbestimmungen	
1.	Polycyclische aromatische Kohlenwasserstoffe (mit Ausnahme von Fluoranthen) (Summe)	0,02 µg/l
2.	Flüchtige organische Halogenverbindungen (mit Ausnahme von Trihalogenmethanen) (Summe)	5 µg/l
3.	Trihalogenmethane (Summe)	5 µg/l
4.	Phenole (gesamt)	2 µg/l
5.	Pflanzenschutzmittel, Arzneimittel (Drugs)	0,05 µg/l

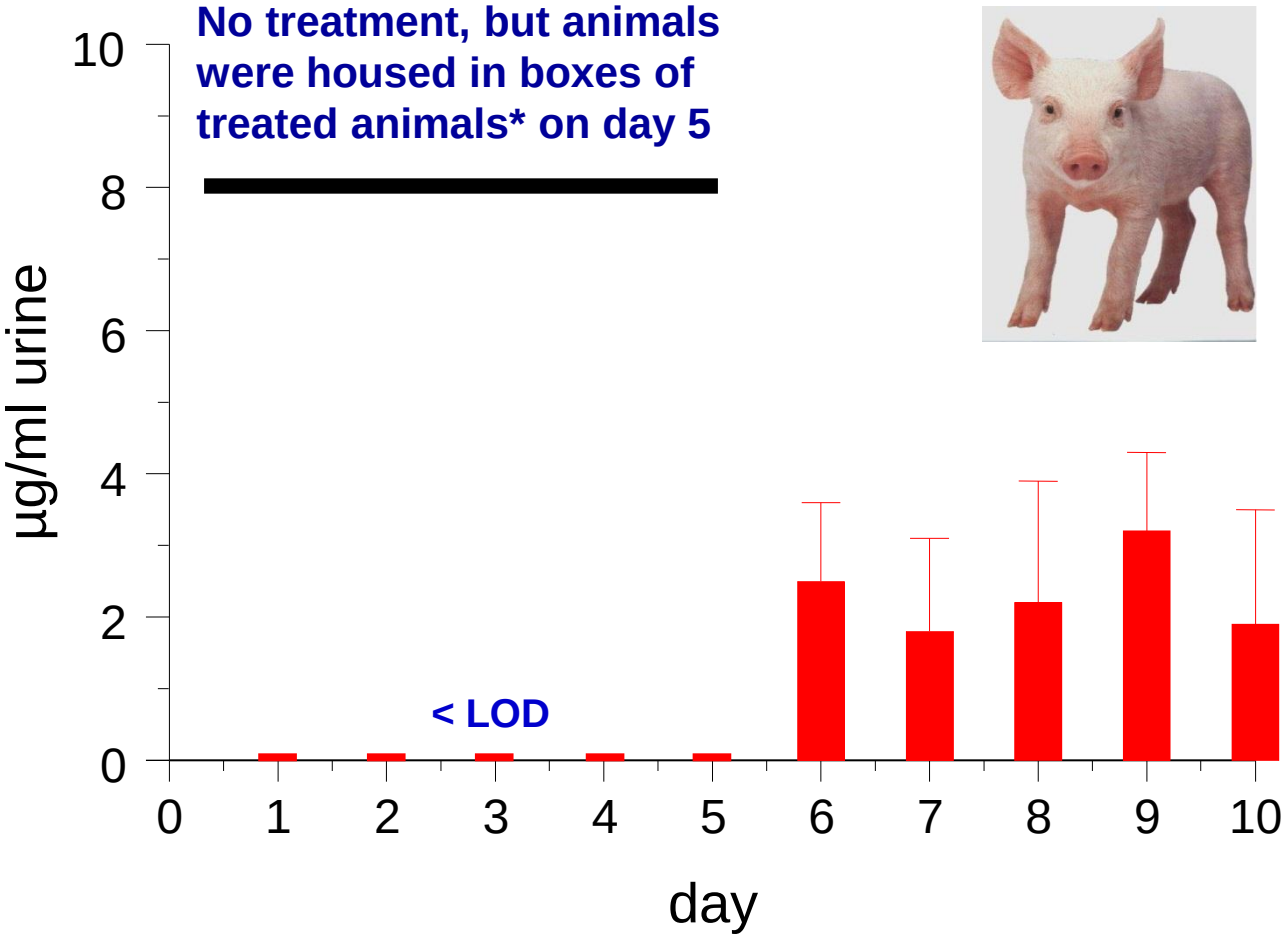
Carry-Over of sulfamethazine

100 mg/kg oral via feed, 5 days of treatment



(Kietzmann et al. 1995)

Carry-Over of sulfamethazine



***100 mg/kg oral via feed, 5 days of treatment**

(Kietzmann et al. 1995)

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The 3R-concept

- **Refinement** of techniques, able to minimize the entry and effects of active substances on the environment as well as reduction of consumption of resources and energy during production and use of pharmaceuticals.
- **Reduction** of pharmaceuticals, e.g. with optimised management, diagnostic approaches, legislative actions or trade restrictions.
- **Replacement** of harmful substances with environmentally benign ingredients.

Replacement of veterinary drugs – Pitfalls

- Environmentally friendly drugs may be **less active**
- In the case of antibiotics and endectocides: limitation to a small number of compounds may result in an **increase in the development of resistant strains**
- **New and unexpected risks for non-target organisms**

Replacement of diclofenac by meloxicam

- **2006: Ban of diclofenac** in veterinary medicine in India, Pakistan and Nepal
- Diclofenac was replaced by the NSAID **meloxicam**
- Toxicology and pharmacology of meloxicam (Cuthbert et al. 2007):
  - **No association with any toxicity in birds**
  - Investigations in > 60 species without any obvious adverse effects
  - Studies in different vulture species demonstrated a short half-life of elimination, accumulation of the drug unlikely
- **Productivity of Indian vultures (G. indicus) in southeast Pakistan and in some Indian states has increased**

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LBEG Landesamt für Bergbau, Energie und Geologie
GEOZENTRUM HANNOVER

LEUPHANA UNIVERSITÄT LÜNEBURG

DAS TIERARZT-UNTERNEHMEN

Developing environmentally sound sulfonamides

Lysimeter studies

- Risk of groundwater contamination
- All compounds in one cocktail

Drug formulation

- Granulation, micro-encapsulation, nanoparticles
- Higher plasma levels – lower dosing

Ranking of com-pounds and QSAR

- Identification of structural properties
- Recommendations for veterinarians
- Synthesis of new compounds

Photooxidation studies

- Aquaculture: rapid degradation important
- Toxicity / antimicrobial activity testing

Anaerobic fermentation

- Single compound testing
- Toxicity / antimicrobial activity testing
- Identification of degradation products

gefördert durch

DBU Deutsche Bundesstiftung Umwelt

www.dbu.de

General remarks

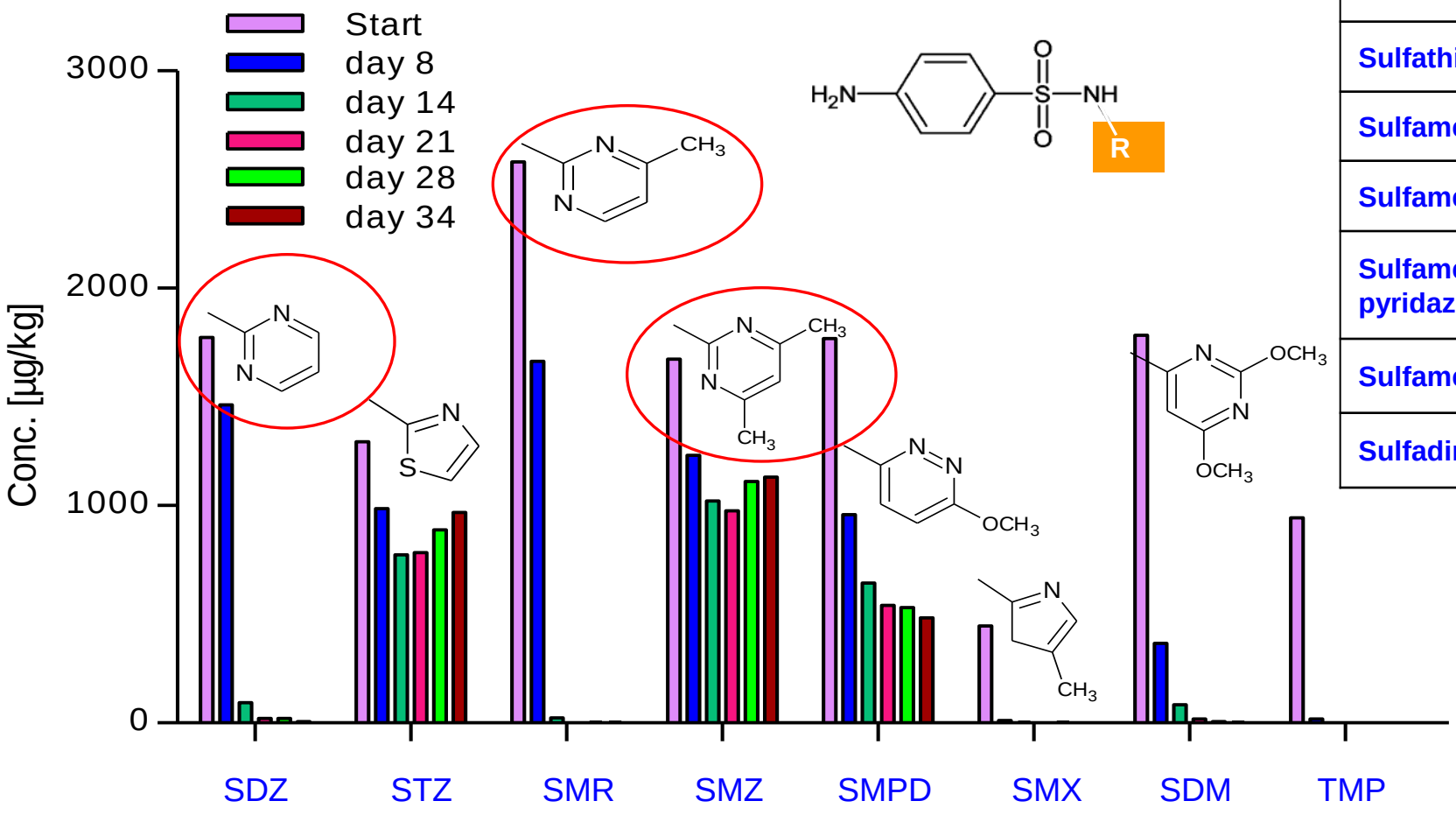
What does „environmentally sound“ imply?

- After therapeutic use, a drug is metabolized or transformed to a biologically inactive or non-toxic product (= elimination)
- In the case of antibiotics: loss of antimicrobial activity

Consequences for research:

- The non-toxicity and / or loss of biological / antimicrobial activity has to be demonstrated
- Degradation products have to be identified and characterised

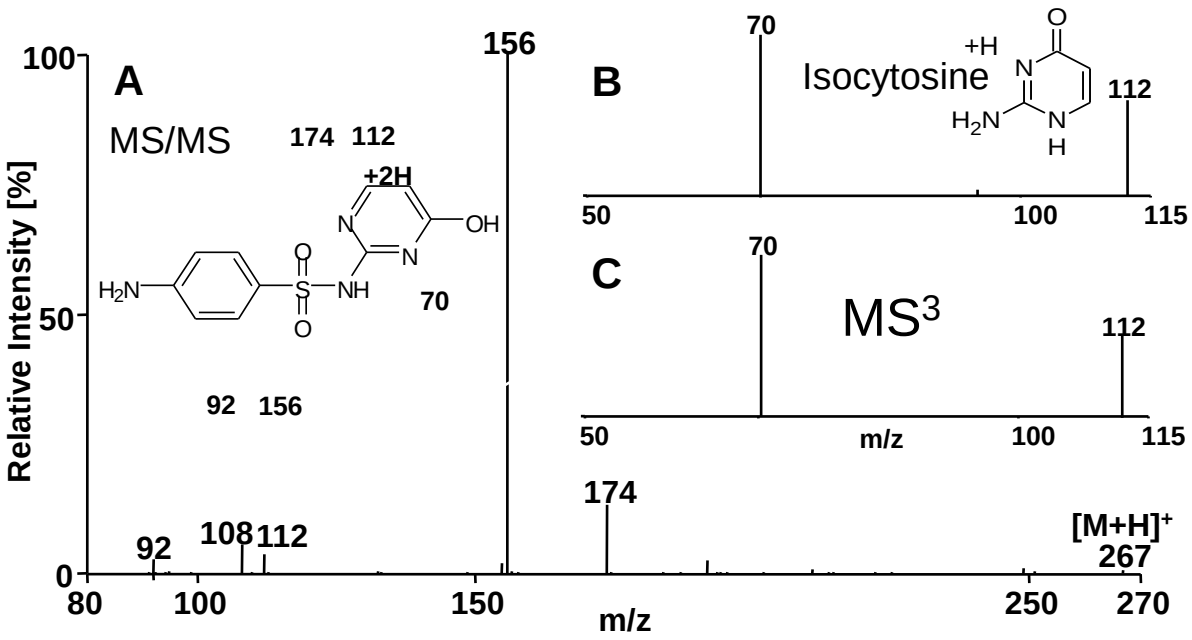
Anaerobic fermentation of various sulfonamides



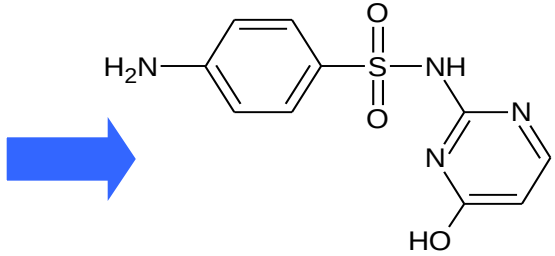
Sulfadiazine	**SDZ**
Sulfathiazole	**STZ**
Sulfamerazine	**SMR**
Sulfamethazine	**SMZ**
Sulfamethoxy-pyridazine	**SMPD**
Sulfamethoxazole	**SMX**
Sulfadimethoxine	**SDM**

(Mohring et al. 2009)

Identification of the metabolite (4-OH-SDZ)

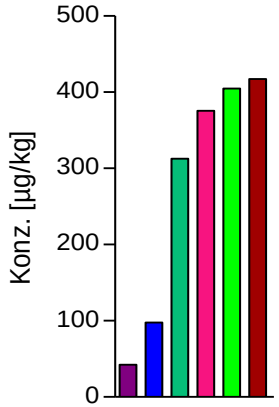


(Mohring et al. 2009)

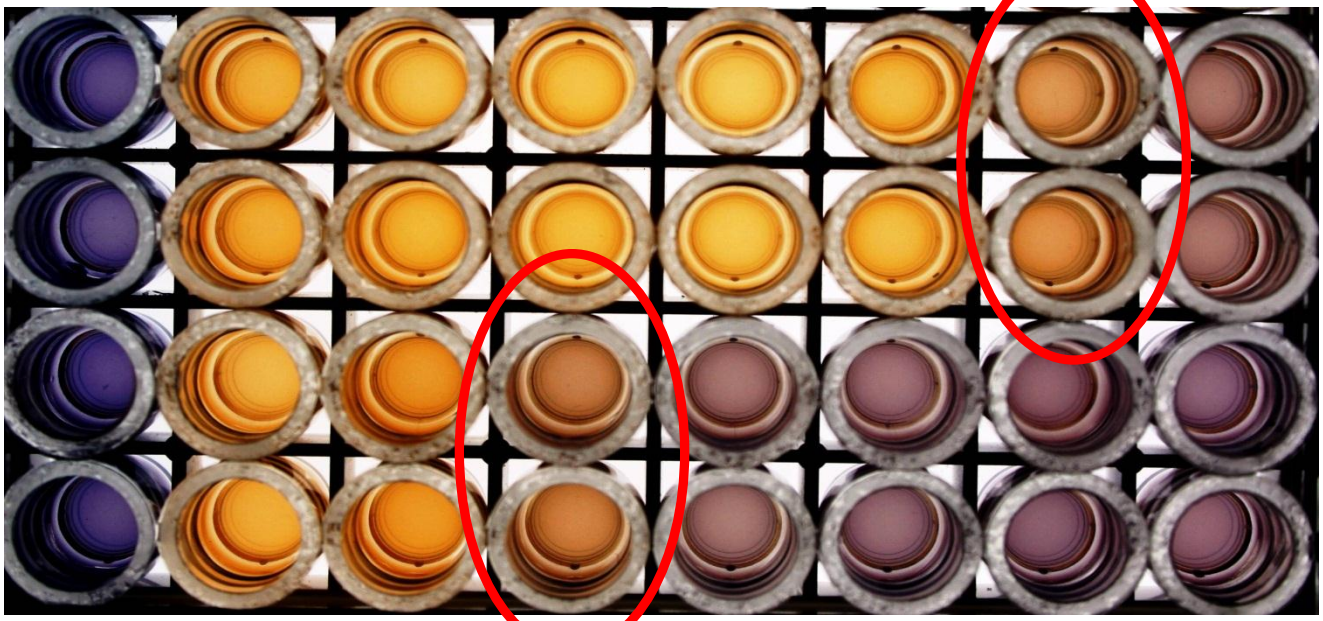


What about the antimicrobial activity?

Half-quantitative detection of the metabolite



Microbial inhibition testing



antimicrobial activity < 10 %

(Mohring et al. 2009)

Conclusions / Summary

There are still a lot of open questions …

- Do we really know all routes of entry for veterinary drugs into the environment?
- Is it really possible to identify and / or synthesise environmentally sound pharmaceuticals without the loss of therapeutic efficacy?

… meanwhile: reduce the entry

- Healthy animals need less treatment
- Prudent use of antibiotics
- Fermentation or (**??**) of liquid manure
- Knowledge transfer to veterinarians, farmers, students and consumers

Acknowledgement

I am grateful to my collegues and co-workers

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AiF

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Thank you for your attention !!!