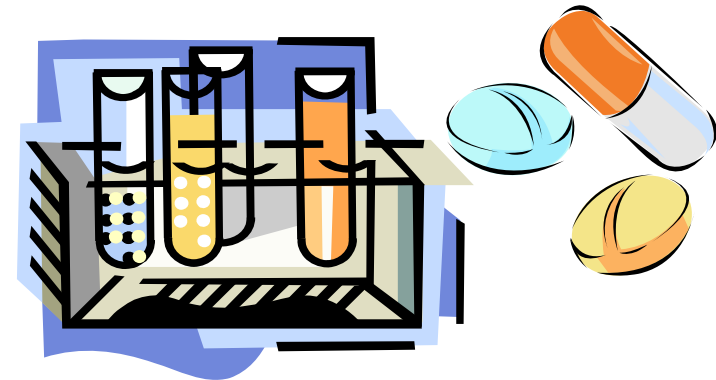




Veterinary Medicines in the Environment

– special emphasis on the fate and transport of antibiotics and antiparasitics with manure and biosolids



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Scope

1. Veterinary medicines (VETmeds), concentration levels in manure and slurry
2. Exposure scenarios for Veterinary medicines
3. Compounds to prioritise: antibiotics, antiparacetics,
4. Fate of compounds
5. Conclusions



- Veterinary medicines are primarily transported to the terrestrial environment with different types of manure, slurries or other types of biosolids
- Manure, slurry and biosolids are very different in water content and constituents and therefore the resulting VETmed concentration can be quite different!



- **When wastes are applied to the land it is assumed that the waste components will move through the soil matrix slowly and therefore:**
- **Pathogens will remain in the soil a long time and will die from starvation, scavenging, cold, ...**
- **Organic components will be digested by microbes**
- **Inorganic components will be adsorbed or taken up by crops or consumed by microbes**

But this is not always the true because we find the VETmeds in the environment



- Why bothers about pharmaceuticals in the environment?



Why are we assessing the potential effects of drugs in the environment?

VICH International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products

VICH GL 38 (ECOTOXICITY PHASE II)
October 2004
For implementation at Step 7

err
The output of these legislative guidance documents:

- Veterinary medicines will be band due to a negative environmental assessment.
- Medicine for human use can NOT be band due to the environmental assessment

Research in Europe has learned us:

If we re-use treated surface water as drinking water - the drinking water will contain pharmaceutical residues in the ng/l level.

Is that a medical problem?

Probably Not/Could be?

Is that an ethical problem? Yes!

•

Why are we concerned?

- Maybe more biosolids applied than land can handle
- Contributes to surface and groundwater contamination
- Increased nutrient runoff into surface waters
- Detectable presence of drugs and resistance determinants in groundwater





Constituents (VETmeds) in manure

Antiparacitics

- Avermectins
 - Ivermectin
- Ecto-parasitics
 - Cypermethrin
- Endo-parasitics
 - Bendimidazol

Antibiotics

- Tetracyclines
- Sulfonamides
- Macrolides
- Penicilliner

Antifungals

Miconazole

Growth promoters

monensin
salinomycin

Coccidiostatics

NSAIDS

Tranquilizers

Hormones

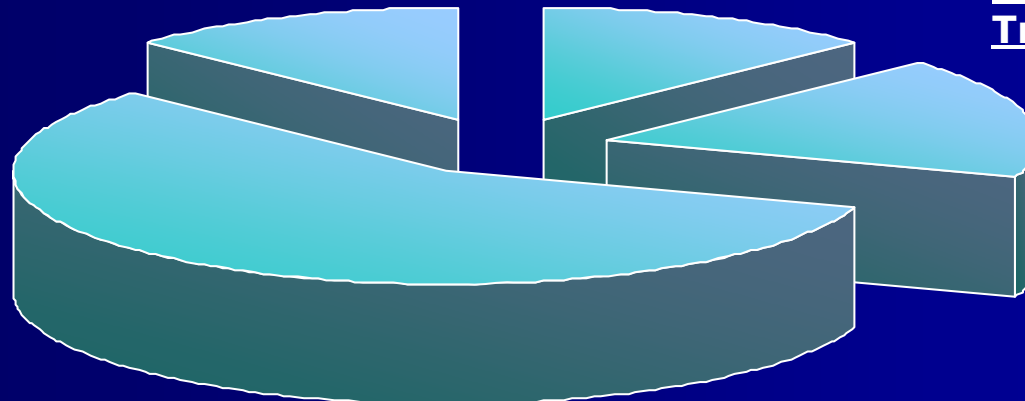
- 17- β - o estradiol
- 17- α - o estradiol
- o stron

Other xenobiotics

- Desinfectans
- LAS
- Metals
- Pesticidresidues
-

- Testosteron
- Androsten
- Cholesterol

More than
500 different
Drugs (bioactive
compounds)





Annual consumption

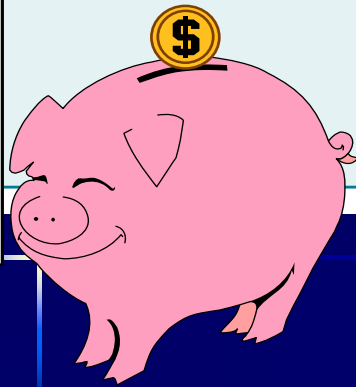
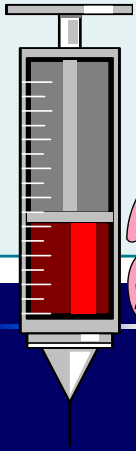
Table 1: Annual consumption of antibiotics for veterinary medicine, especially livestock, in the European Union, European countries and regions of Germany (without coccidiostatics).

Tabelle 1: Jährlicher Verbrauch an Antibiotika in der Veterinärmedizin, v.a. für landwirtschaftliche Nutztiere, in der Europäischen Union, europäischen Staaten und Regionen in Deutschland (ohne Kokzidiostatika).

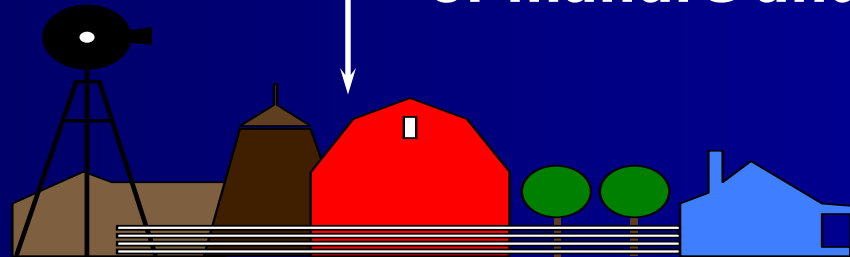
	EU 1999 ^a		France 1980 ^b		Sweden 1996 ^c		Denmark 1997 ^d		Switzerland 1997 ^e		UK 2000 ^f		Weser-Ems 1997 ^g		Brandenburg 1998/99 ^h		Mecklenburg-Vorpommern 2001 ⁱ	
	t	%	t	%	t	%	t	%	t	%	t	%	t	%	t	%	t	%
Therapeutics	3902		625		20		57		14		437		88 ^j		6.6 ⁱ		10 ⁱ	
Tetracyclines	2575	66	117	19	2.7	13	13	23	1.0	7	228	52	40	57	4.6	69	6.4	41
Sulfonamides^k	78	2	139	22	2.2	11	13	23	n.a.		94	22	14	21	0.9	14	2.5	16
Aminoglycosides	1564		57	9	1.1	5	7.7	14	n.a.		12	3	7.1	10	0.2	3	0.2	2
β-Lactams	351	9	50	8	n.a.		15	26	9.2	64	49	11	3.8	5	0.2	3	0.1	1
Macrolides	468	12	37	6	1.5	7	1.7	3	0.3	2	41	9	0.2	0.3	0.01	0.1	0.1	1
others	234	6	226	36	n.a.		6.5	11	3.8	27	12	3	4.5	6	0.7	10	0.8	8
Ergotropics	786		n.a.		n.a.		107		36		24		18		0.1		n.a.	

^a FEDESA (2001), data for percentages of compound classes from 1997; ^b Espinasse (1993); ^c Mudd et al. (1998); ^d Halling-Sørensen et al. (2002a); ^e Swiss Importers of Antibiotics (1998) cited in Alder et al. 2001; ^f NOAH (2002); ^g Winckler and Grafe (2000); ^h Linke and Kratz (2001); ⁱ Thiele-Bruhn et al. (2003a); ^j veterinary prescriptions for feed antibiotics only; ^k incl. trimethoprim; n.a. = no data available

Exposure scenarios

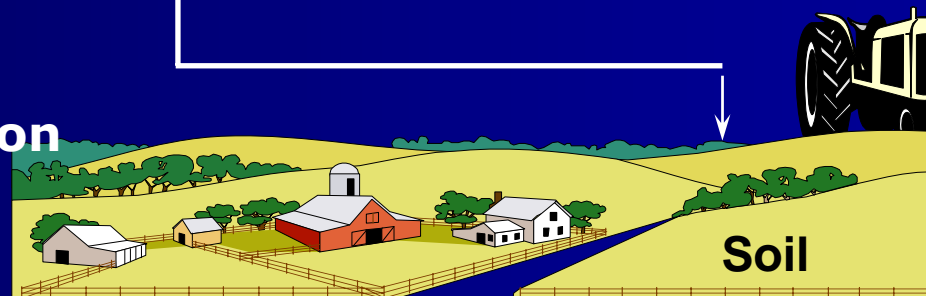


**Fate of Vetmed during storage
of manure and slurry**



- **Aquaculture treatments (receiving water)**
- **Livestock treatment (soil)**
- **Treatment of companion animals (soil)**
- **Inappropriate disposal (soil)**

Manure and slurry spreading



Soil

Leaching

groundwater

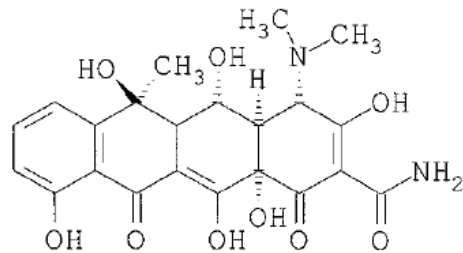


Current state of the problem

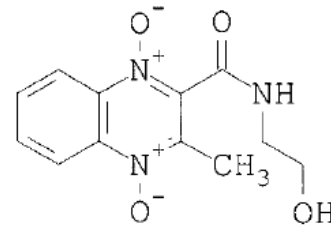
- VETmed designed to be biological active molecules
- Used in high quantities 1-10 tons/year
- Only few and often insufficient informations to perform ERA of most already existing VETmed
- Current ERA methods for drugs only been in operation short time. Presently the only "complete" dataset (ivermectin) that will appear in the open literature is under construction in ERApharm project.



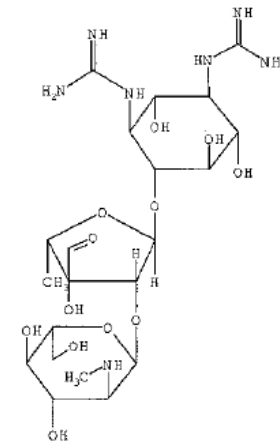
Chemical structure of antibacterial agent



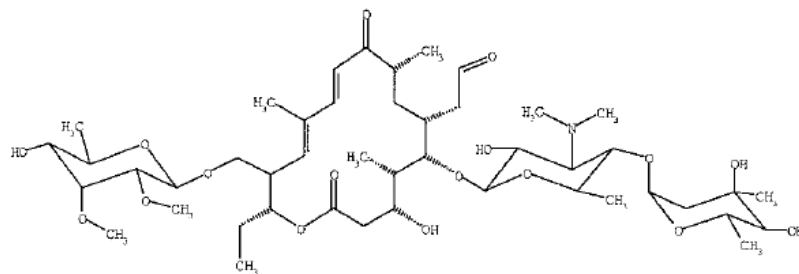
Oxytetracycline (OTC)



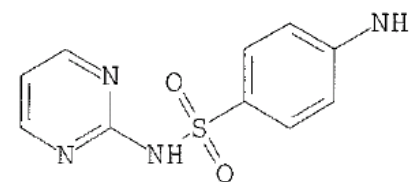
Olaquinox (O)



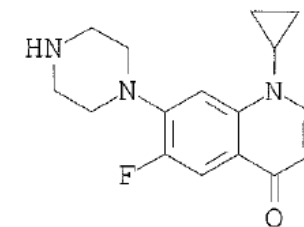
Streptomycin (ST)



Tylosin (TYL)



Sulfadiazine (SDZ)



Ciprofloxacin (CP)

Fig. 1. Chemical structures of the antibacterials tested



Potency (LC-50) of ivermectin degradates formed in aqueous solution at pH=6 to zebra fish

Compound	LC-50 $\mu\text{g/L}$ 95% CI
Ivermectin	26 [9-51]
Mixtures of degradates that has lost 1 sugar moiety	81 [67 – 102]
Mixtures of degradates that lost both sugar moieties	148 [132-175]

Halling-Sørensen B, unpublished results



- Concentration levels of antibiotics in manure and slurry



Conc. levels of antibiotics in manure (mg/kg dwt)

Table 5 Amounts of antibiotics in manure samples from six different farms, quantified by standard addition

Manure #1	Manure #2	Manure #3	Manure #4	Manure #5	Manure #6
Tetracycline					
<LOQ	0.17 [0.11–0.22]	0.091 [0.089–0.094]	1.6 [1.3–1.9]	0.33 [0.31–0.36]	0.32 [0.25–0.39]
Oxytetracycline					
<LOQ	<LOD	1.5 [1.4–1.6]	0.048 [0.037–0.060]	0.94 [0.88–0.99]	<LOQ
Chlortetracycline					
<LOQ	1.1 [0.95–1.3]	1.8 [1.7–1.8]	15.7 [6.9–24.4]	6.2 [4.5–7.9]	7.1 [5.3–8.9]
Doxycycline					
0.76 [0.74–0.79]	0.62 [0.53–0.71]	1.3 [1.3–1.3]	0.55 [0.29–0.82]	0.59 [0.52–0.66]	3.1 [2.7–3.5]
Epi-tetracycline					
<LOD	0.19 [0.15–0.22]	0.061 [0.056–0.067]	0.99 [0.84–1.2]	0.22 [0.20–0.25]	0.21 [0.16–0.27]
Epi-oxytetracycline					
<LOD	<LOD	0.45 [0.42–0.47]	<LOD	0.33 [0.31–0.35]	<LOD
Epi-chlortetracycline					
<LOQ	1.7 [1.3–2.0]	1.3 [1.2–1.3]	14.1 [7.2–20.9]	5.6 [5.4–5.9]	7.5 [5.8–9.3]
Sulfadiazine					
<LOD	<LOD	<LOD	0.63 [0.49–0.76]	<LOQ	2.1 [1.1–3.2]
Sulfamethazine					
<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Sulfadoxine					
<LOD	0.015 [0.009–0.020]	0.22 [0.21–0.22]	<LOD	0.098 [0.070–0.13]	<LOD
Tylosin A					
<LOD	<LOD	<LOD	<LOD	<LOD	<LOD

<LOD: Below limit of detection

<LOQ: Below limit of quantification

Values are mean concentrations from analysis of three replicate samples; 95% confidence intervals are reported in square brackets

Concentration of antibiotics ($\mu\text{g g}^{-1}$ freeze-dried manure)

Source: Jacobsen and Halling-Sørensen (2006) Anal Bioanal Chem. 384: 1164–1174



- Biodegradation in manure (microcosmos)



Antibiotics – manure (batch reactor) - anaerob conditions under entire test period Ct=0 Conc = 2000µg/kg manure (wet weight)

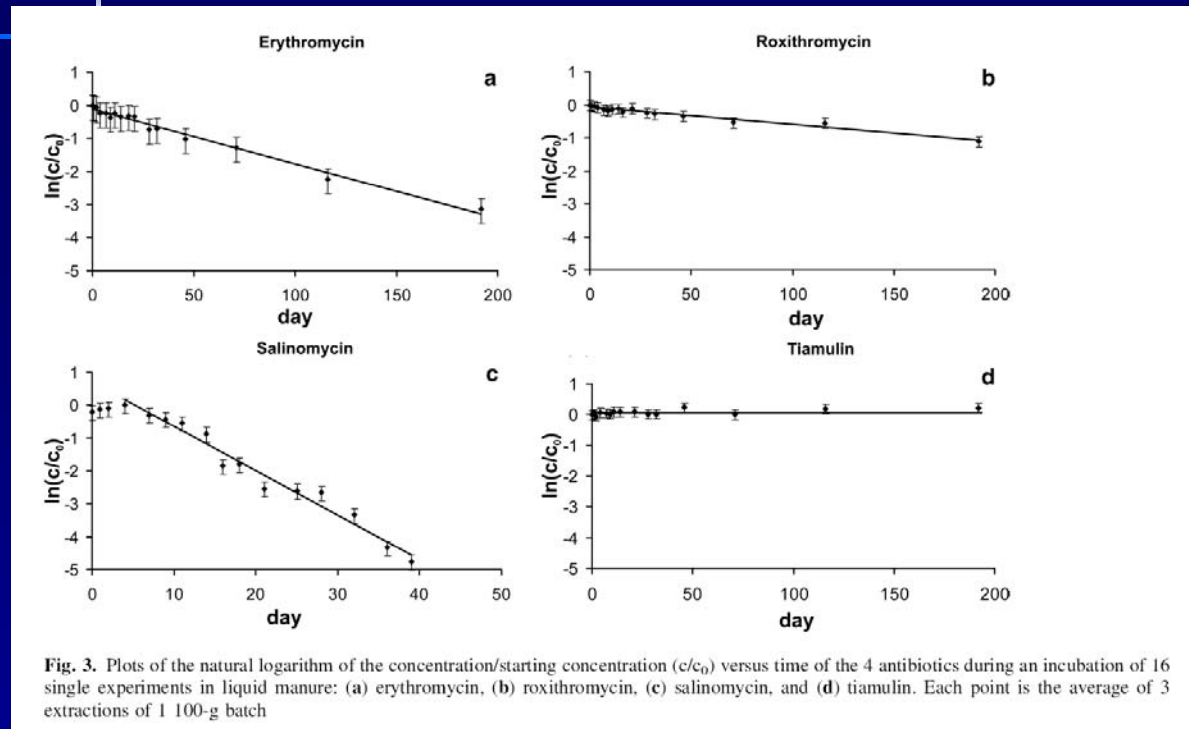


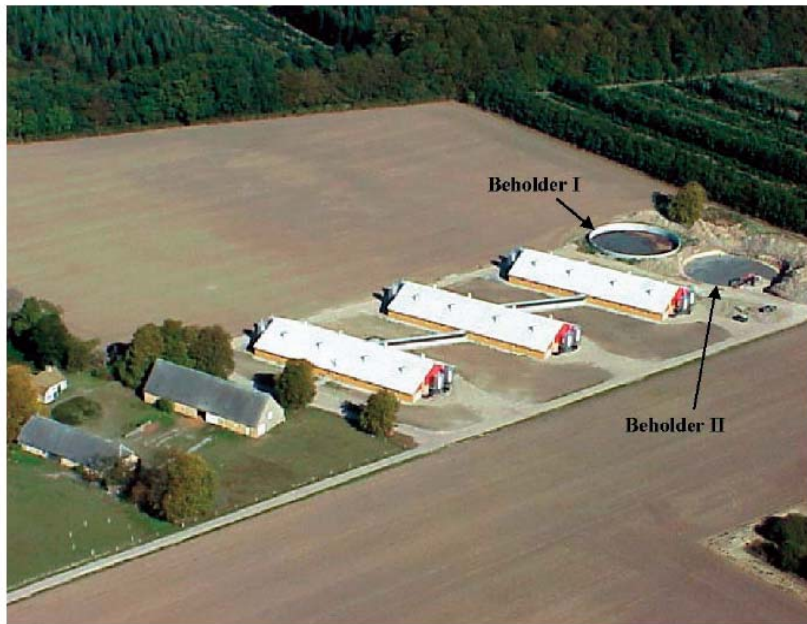
Fig. 3. Plots of the natural logarithm of the concentration/starting concentration (c/c_0) versus time of the 4 antibiotics during an incubation of 16 single experiments in liquid manure: (a) erythromycin, (b) roxithromycin, (c) salinomycin, and (d) tiamulin. Each point is the average of 3 extractions of 1 100-g batch

Antibiotic	Slope	R^2	Half-life (d)
Erythromycin	-0.017	0.98	41 ± 1
Roxithromycin	-0.005	0.95	130 ± 10
Salinomycin	-0.135	0.97	5.1 ± 0.3
Tiamulin	—	—	>200

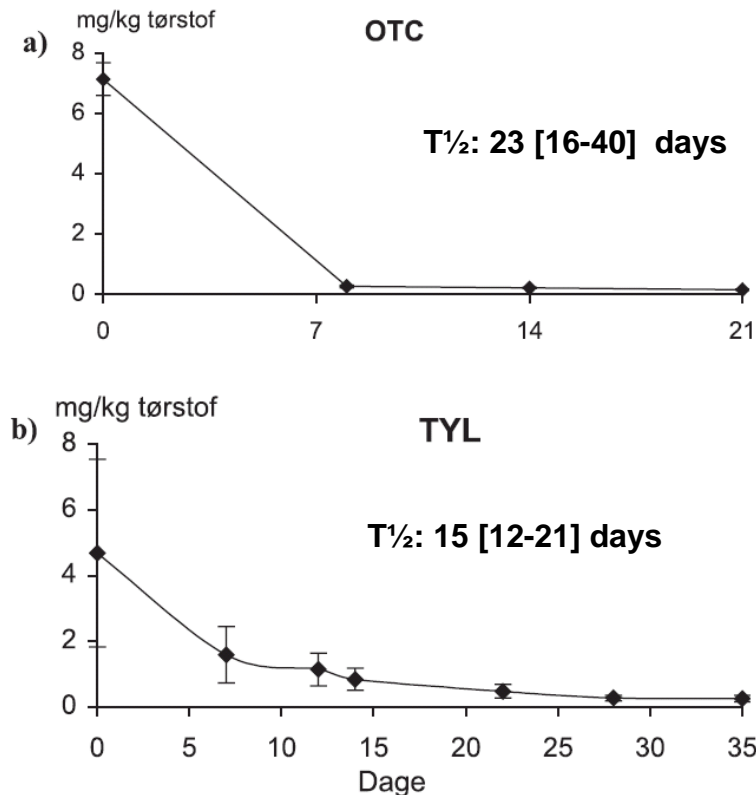
Schlüsener et al (2006) Archives of Environmental Contamination and Toxicology

Manure storage 180 days!

Disappearance of Tylosin and Oxytetracycline in manure storage tanks



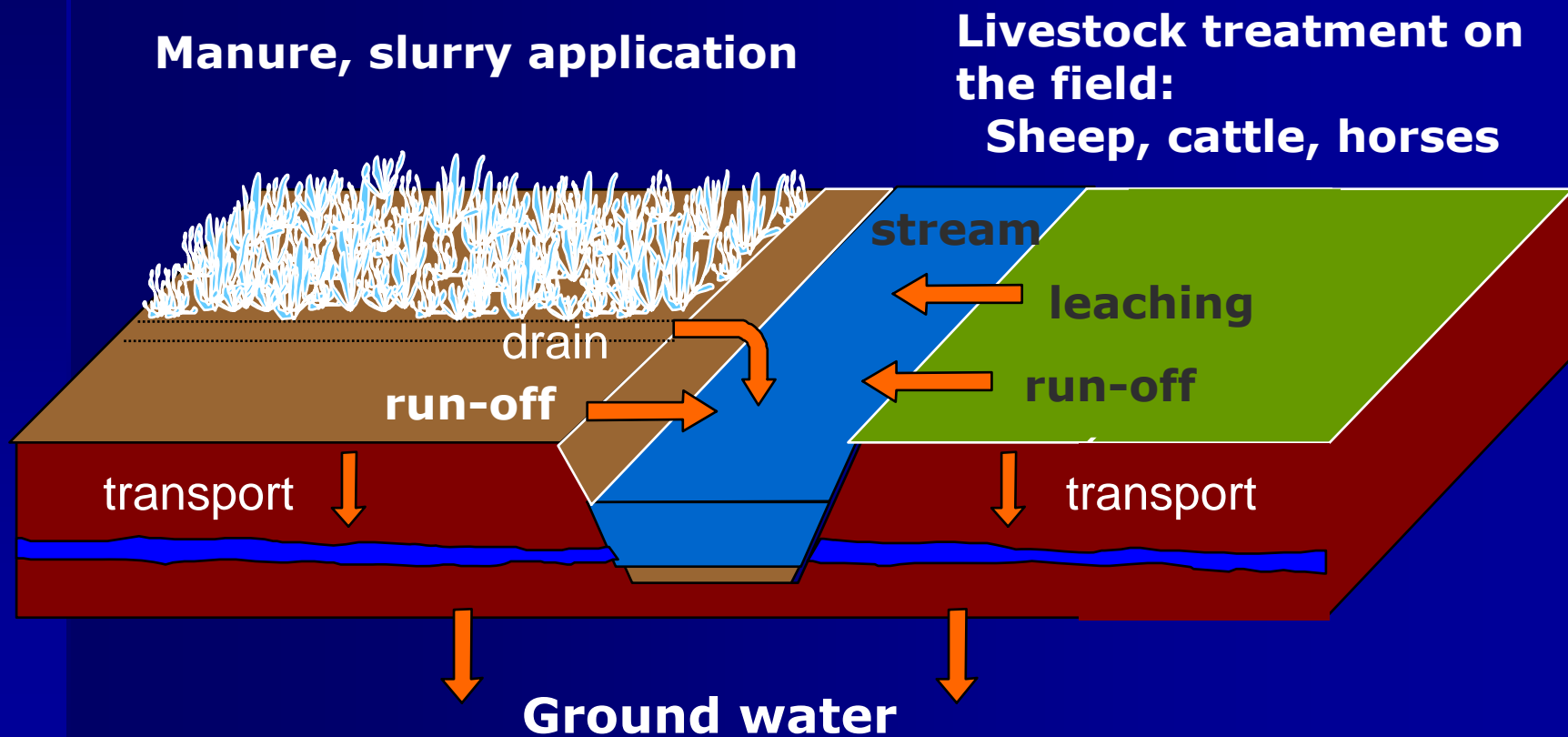
Figur 1. Eksempel på en gård med svinebesætning og to gyllebeholdere.
Foto: Michael Olsen, Danish Airphoto



A field-scale experiment was performed in northern Jutland, Denmark following the disappearance of OTC and TYL in the manure tank. The volume of the tank was 850 m³. The manure tank was kept under methanogenic conditions. Samples were taken at 4 (OTC) and 8 (TYL) occasions after a medication campaign, respectively. Half-lives (days) with corresponding 95% c.i. was found for OTC and TYL as 23 [16-40] and 15 [12-21]. Sorption to the manure could explain the form of the curve of OTC.

Source: Jensen and Halling-Sørensen (unpublished)

Dissipation from soil





Biodegradation of antibiotics in manure/soil mixtures (microcosmos)

TABLE 1
Concentrations of the Major Constituents in the Manure Used in Current Study

Constituent	Concentration (g/liter)
Total N (Kjeldahl)	153.35
Total P	1.7
Total K	68.9
Total C	422.2
Ammonia N	98.1
Dry matter	44.3

TABLE 4
Freundlich Parameters and Standard Errors in Sorption Experiments

	Soil	K_f	n
Olaquinox	Lundgaard	0.007	0.85
	Askov	0.007	0.97
Metronidazole	Lundgaard	0.02	0.55
	Askov	0.009	0.94
Tylosin	Lundgaard	2.03 ± 0.9	0.67 ± 0.0
	Askov	32.1 ± 13.2	0.67 ± 0.0

TABLE 5
Half-Lives (Days) during Primary Degradation of Antibiotics in Soil-Manure Slurries

Content of manure in soil	Olaquinox	Metronidazol	Tylosin	Anilin
Askov				
0%	6.3, 5.9	14.1, 14.3	4.1, 4.2	2.9, 4.8
1%	7.1, 8.8	14.5, 14.7	4.1, ND	2.6, 3.3
10%	8.7, 7.0	13.7, 9.7	ND	1.4, 3.0
Lundgaard				
0%	ND; 5.8	13.1, 16.8	5.2, 6.2	2.1, 4.8
1%	6.3, 6.6	15.3, 14.1	6.7, 5.0	1.1, 3.3
10%	7.5, 6.0	18.0, 26.9	8.1, 3.3	1.6, 2.6

Note. ND, these experiments were not performed. The two numbers indicate results from each replicate degradation experiment. For aniline, the first number indicates results from an experiment conducted in parallel to degradation of olaquinox, whereas the second number indicates results from experiments conducted in parallel with degradation of metronidazol and tylosin.

Source: Ingerslev og Halling-Sørensen (2001) Ecotoxicology and Environmental Safety 48, 311-320.

Dissipation of steroid hormones in manure/soil mixtures

Source Jacobsen et al.
(2005) JEQ 34:861-71

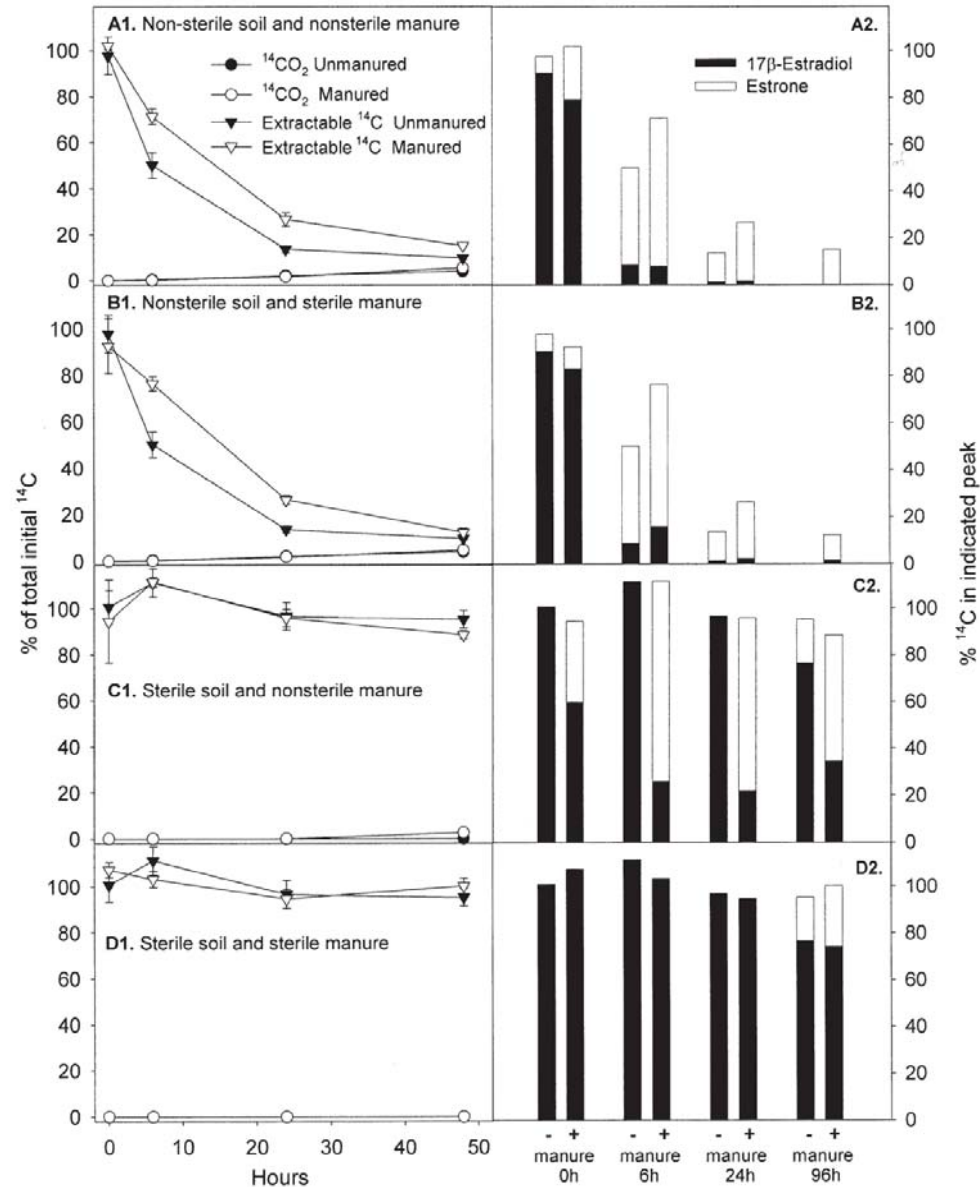


Fig. 7. 17β -Estradiol dissipation in (A) nonsterile soil amended with nonsterile manure, (B) nonsterile soil amended with sterilized manure, (C) sterilized soil amended with nonsterile manure, and (D) sterilized soil amended with sterilized manure. All experiments with loam soil incubated at 30°C . Results are shown for mineralization and total extractable radioactivity in the left panels (manured treatments are shown as open symbols, unmanured treatments as closed symbols). Each point and error bars represent the mean value of three replicate samples and corresponding standard deviation. The relative distribution of 17β -estradiol and estrone in soil extracts is shown in the right panels. The height of the total bars corresponds to total extractable radioactivity and the height for each compound is the mean value of three replicate measurements.



- **Field studies**
 - **Antibiotics**
 - **Estrogens**

Field study from Denmark



▲ Field A (Askov, Denmark)
 ■ Field B (Lundgaard, Denmark)

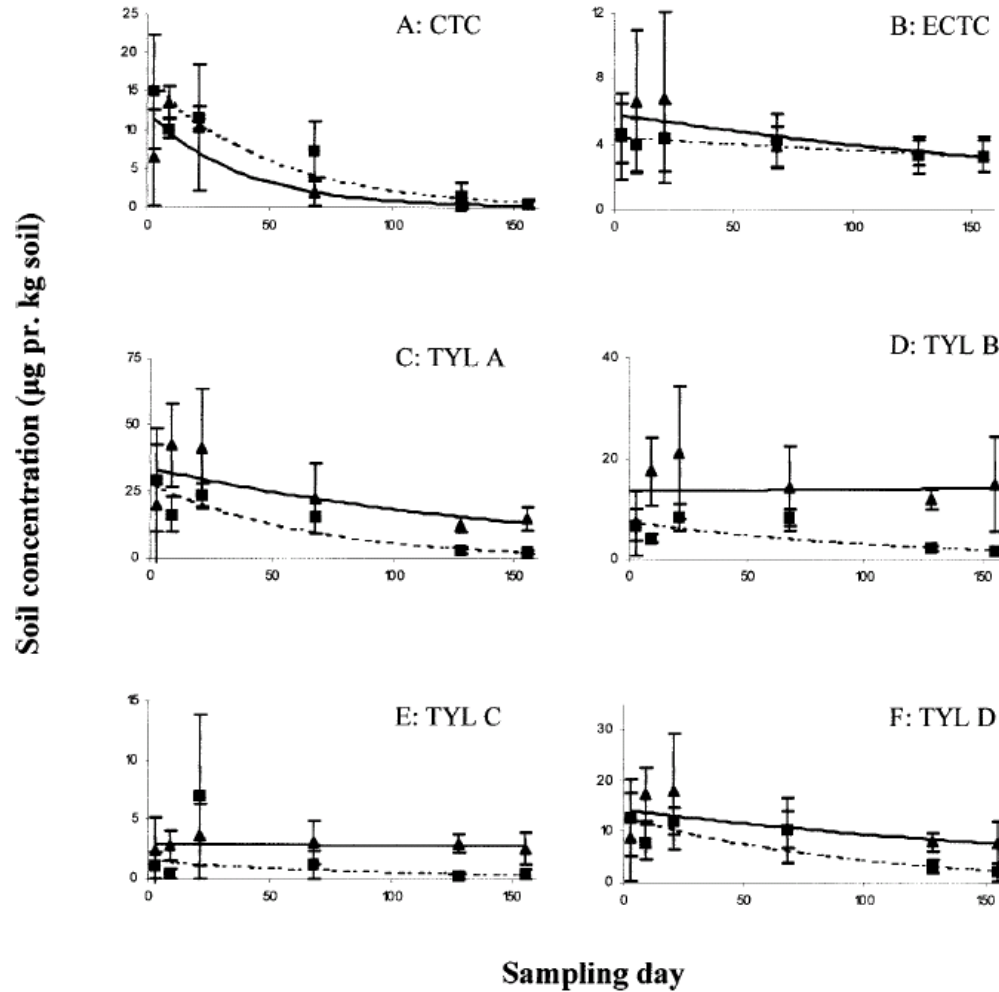


Fig. 2. Soil content of antibacterial agents on the loamy sand soil (▲ field A, Askov, Denmark) and the sandy soil (■ field B, Lundgaard, Denmark) measured during the experimental period of 155 d. For each soil, a point on the curves represents the mean value for replicate measurements from three soil plots and the error bars are the standard deviation. CTC = chlortetracycline; ECTC = 4-epi-chlortetracycline; TYL A = tylosin A; TYL B = tylosin B; TYL C = tylosin C; TYL D = tylosin D.

A: loamy sandy soil

B: sandy soil

Halling-Sørensen et al.
 Environ. Toxicol. Chem,
 (2005) 24: 802–810



Half-lives $T_{1/2}$ days

Table 7. Degradation half-lives ($T_{1/2}$) for chlortetracycline (CTC) and tylosin A (TYL A), determined for each soil plot and the complete field, respectively, in the field study, assuming first-order degradation kinetics. CI = Confidence interval

Soil	Soil plot	CTC		TYL A	
		$T_{1/2}$ (days)	95% CIs	$T_{1/2}$ (days)	95% CIs
Field A (Askov, Denmark)	1	26	18–46	58	44–85
	2	25	18–39	76	65–91
	3	58	32–362	ND ^a	NS
	All (-3)	25	20–34	67	54–86
Field B (Lundgaard, Denmark)	1	28	21–40	45	35–62
	2	44	31–77	52	37–88
	3	33	27–42	54	35–113
	All	34	28–42	49	40–64

^a Half-life not determined for this plot.

A: loamy sandy soil; B: sandy soil

Halling-Sørensen et al. *Environ. Toxicol. Chem.*, (2005) 24: 802–810

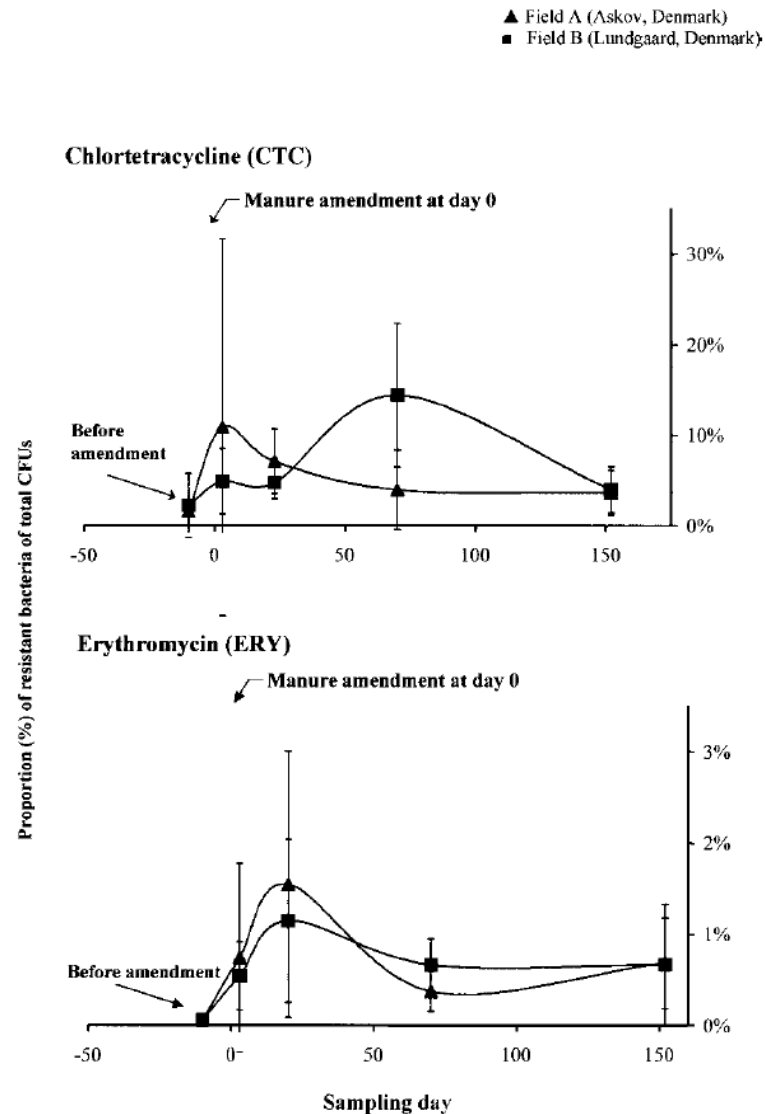


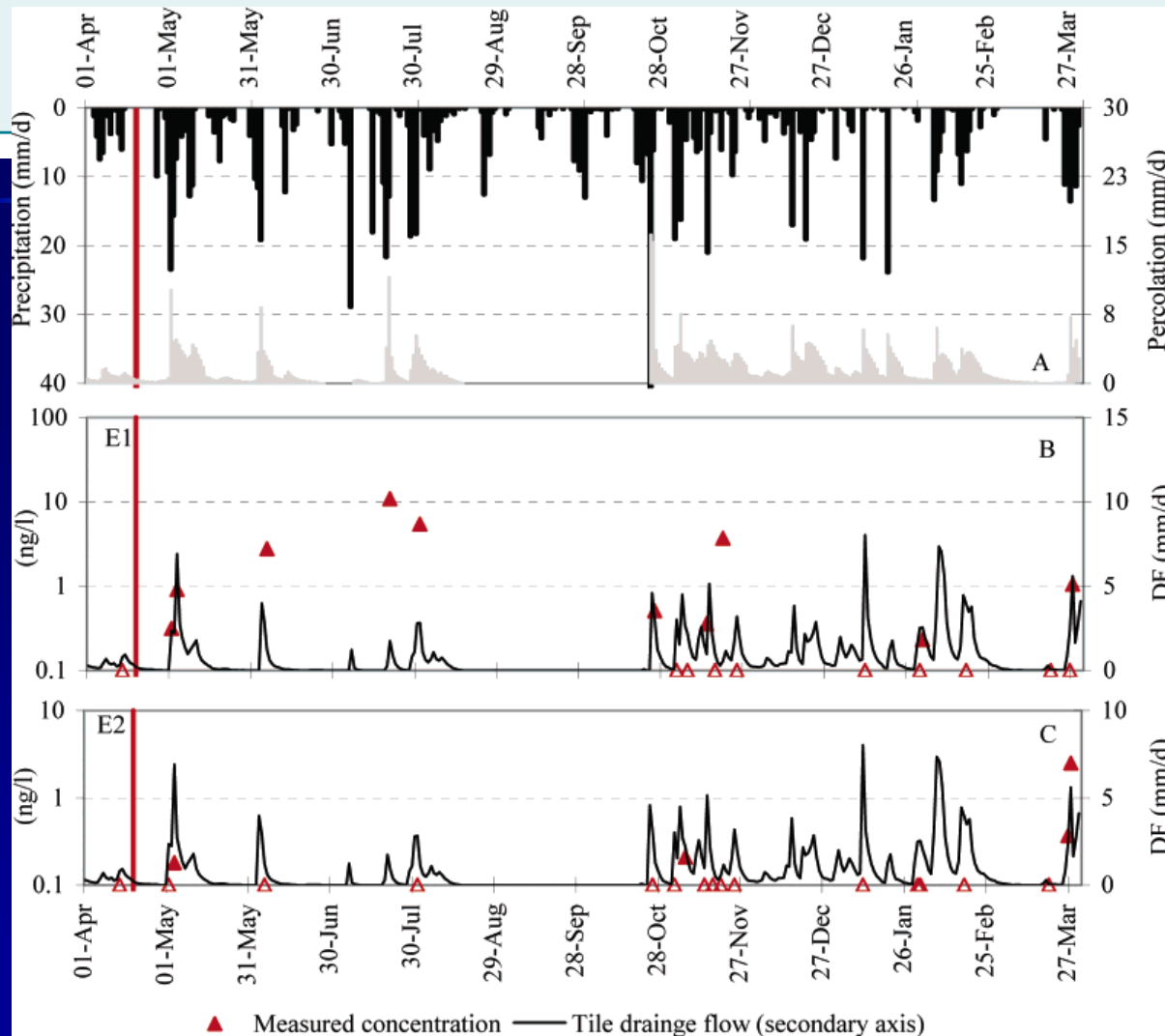
Fig. 3. Proportion of resistant bacteria (%) of total colony-forming units (CFUs) counted at (▲) field A (Askov, Denmark) and (■) field B (Lundgaard, Denmark). Chlortetracycline (CTC) and erythromycin (ERY) as measure for tylosin (TYL). For detailed explanation of statistical model used in this figure, see Sengeløv et al. [12].

Proportions of resistant bacteria (%) of total colony forming units

Much more research to be done in this area

Halling-Sørensen et al. *Environ.Toxicol. Chem.*, (2005) 24: 802–810

Transport of steroid hormones to tile drainage



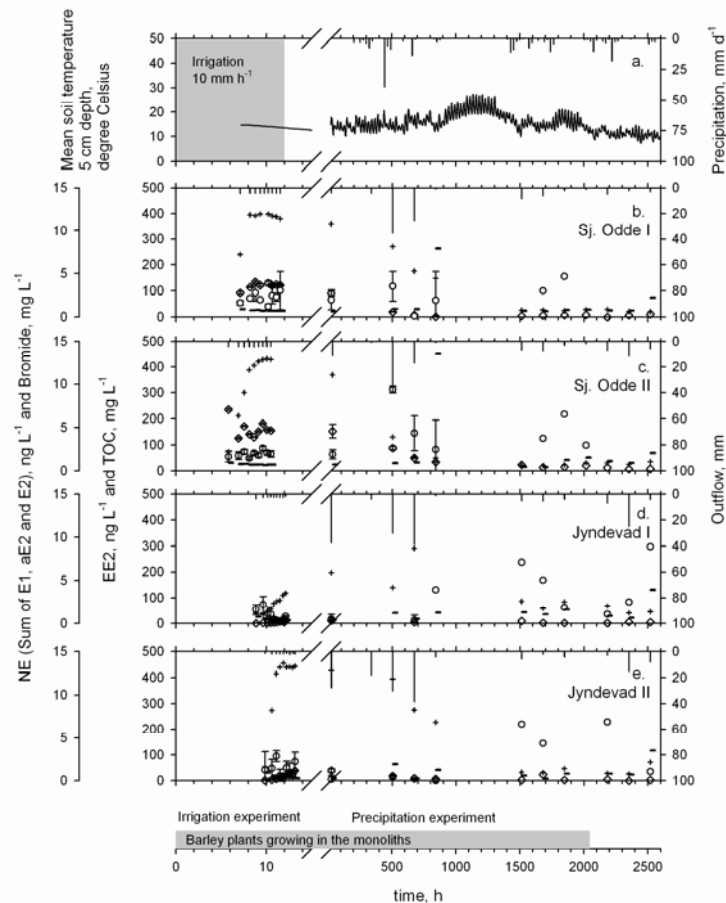
VAP marker Estrup 2005

- Field experiment
- Hormones up to $10 \mu\text{g/l}$ in drainage at heavy rain event mostly as Estron (E1). E2 found at max 1 ng/l .

Kjær J, Halling-Sørensen et al. Environ. Sci. Technol. 2007, 41, 3911-3917



Monolith experiment – facilitated particle bound transport



- Manure at the top
- Two different soils, loamy sandy and sandy soil
- Irrigation study initially then natural precipitation
- Estrogens $\log K_{ow} > 3$
- Transport of estrogens correlated to DOC > particle bound transport
- Mass balance showed less than 1% of E2, E1 in manure transported
- SumE2,E1 up to 15 ng/l in leachate

Lægdsmand M, Halling-Sørensen et al.
Submitted JAQ



Conclusions/Take home messages

- Limited knowledge on the **usage** of certain VETmed
- Limited knowledge on the **fate** of many VETmed
- Limited knowledge on the ecological **effects** of most VETmed
- VETmed are often mobile (probably by particle bound transport) and found in drainage waters
- Limited knowledge on biodegradation i manure and slurry
- More knowledge necessary to predict dissipation/ biodegradation in manure/soil mixtures.
- Priority compounds: certain antibiotics, ivermectins, steroid hormones
- Antibiotic resistance in the environment needs more attention!

The Section of Tox & Environmental Chemistry



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no: SSPI-CT-2003-
511135)