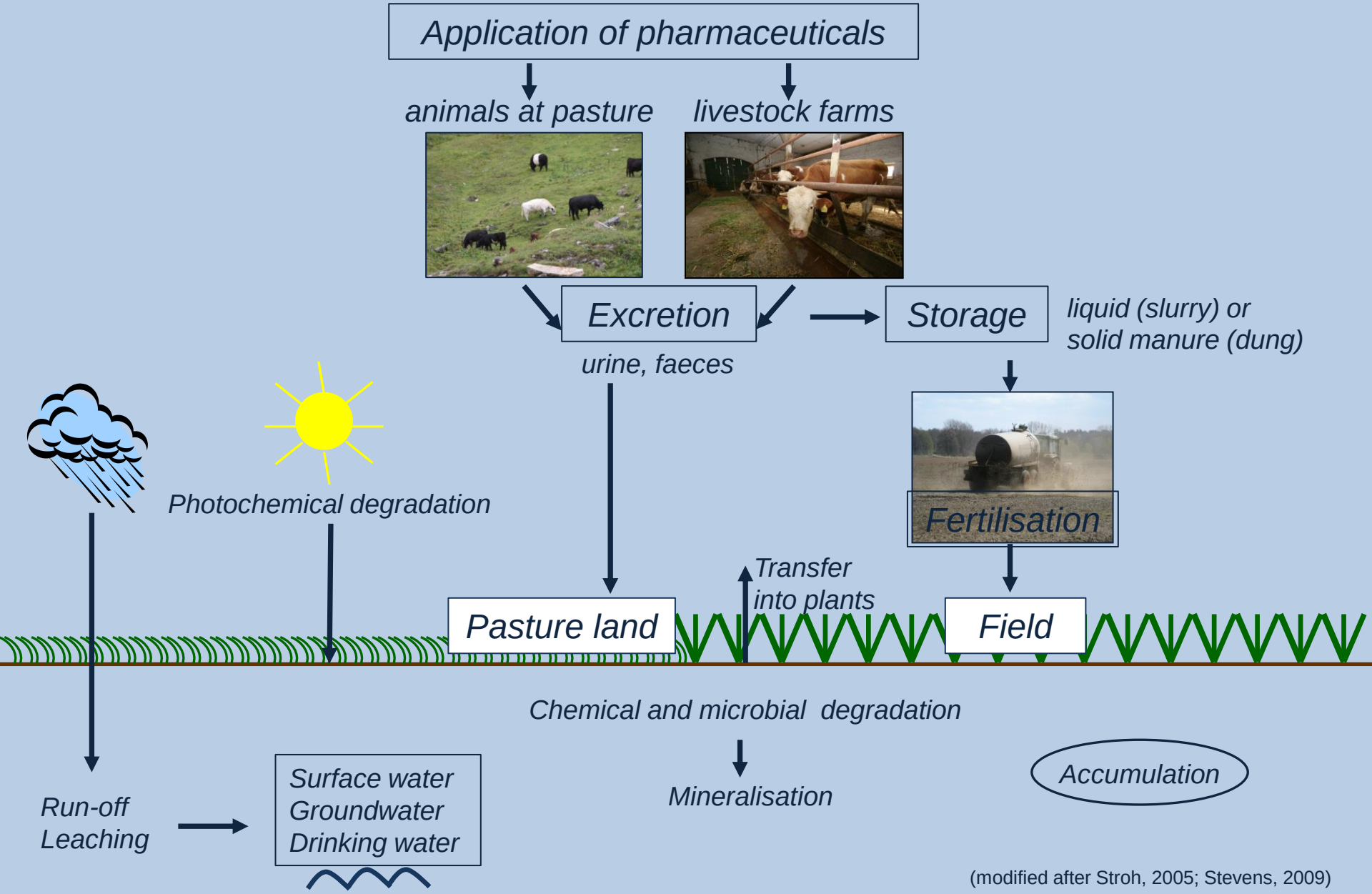
Mixtures of veterinary medicinal compounds in manured soils

Nadine Tauchnitz UNIVERSITÄT LEIPZIG

Daniela Gildemeister, Silvia Berkner Umwelt Bundes Amt Für Mensch und Umwelt

Input of veterinary pharmaceuticals into soil



(modified after Stroh, 2005; Stevens, 2009)

Input of veterinary pharmaceuticals into soil

- ✓kind and amount of pharmaceuticals
- ✓ animal metabolism
- ✓ kind and amount of fertilisers
- ✓ matrix properties of fertilisers
- ✓ storage of fertilisers
- ✓ application techniques
- ✓ application times
- ✓ soil tillage system/incorporation of fertilisers

Transfer into plants

Chemical and microbial degradation

metabolites

Mineralisation

Accumulation

Run-off
Leaching

Surface water
Groundwater
Drinking water

Physicochemical properties of pharmaceuticals
- ✓ degradability
- ✓ adsorption capacity (K_d)
- ✓ lipophilicity (K_{ow}, K_{oc})
- ✓ polarity
- ✓ and others

Soil properties
- ✓mineral content and composition
- ✓ content of organic matter
- ✓ ionic strength
- ✓ pH-value
- ✓ temperature
- ✓ and others

Veterinary pharmaceuticals in environmental samples

Antibiotics
- ✓ tetracyclines (*chlortetracycline*)
- ✓ sulfonamides (*sulfadiazine*)
- ✓ trimethoprim
- ✓ fluoroquinolones (*enrofloxacin*)
- ✓ macrolides (*tylosin*)

Antiparasitics
- ✓ benzimidazoles (*flubendazol*)
- ✓ avermectines (*ivermectin*)

Organic fertiliser

Soil

Antibiotics
- ✓ tetracyclines (*chlortetracycline*)
- ✓ sulfonamides (*sulfadimidin*)
- ✓ trimethoprim
- ✓ fluoroquinolones (*enrofloxacin*)
- ✓ macrolides (*tylosin*)

Antiparasitics
- ✓ benzimidazoles (*flubendazol*)
- ✓ avermectines (*ivermectin*)

Antibiotics
- ✓ tetracyclines (*chlortetracycline*)
- ✓ sulfonamides (*sulfadimidin*)

Antiparasitics
- ✓ benzimidazoles (*flubendazol*)

Surface water
Groundwater

Mixtures of veterinary pharmaceuticals

Parent substances + metabolites

=

Mixture of multicomponents

↔ interactions
- synergistic
- additive
- antagonistic

⇒ Increase or decrease of the mixtures toxicity compared to single substances

↷ Underestimation of the environmental risk

✓Requirement of the predicted environmental concentration (PEC_{soil}) to assess mixtures toxicity

⇩

EMA guideline on pharmaceutical fixed combination products:
Consideration of separate active substances (**single substances**) as basis for data on fixed combination products

$PEC_{soil(mix)}$: addition of PEC_{soil} of all single substances in the mixture

EMA: European Medicines Agency

Difficulties in the assessment of environmental risk of veterinary pharmaceuticals mixtures:

- no central registration of the consumption of veterinary pharmaceuticals
- limitation of analytical methods for the quantification of pharmaceutical residues in soils (non-extractable residues, sequestration)

Aim of the present study:

✓Estimation of the exposure of veterinary pharmaceutical mixtures by anonymous questionnaire surveys of farmers

Main objectives:

- quantification of the amount of applied veterinary pharmaceutical agents per farm for the slurry storage period
- calculation of the expected active agent concentration of pharmaceuticals in slurry (worst case, without metabolism in animals)
- exposure assessment of pharmaceutical agents by calculation of predicted environmental concentration in soil (PEC_{soil})

Methods

- anonymous questionnaire surveys of farmers
- duplicates of the regular forms on drug application to agricultural animals for one year (§ 57 AMG, German Drug Law, documentation requirement)
- calculation of the expected agents concentration in slurry without metabolism in animals

$$PEC_{slurry} = \sum \text{agents in slurry storage time/applied slurry amount}$$

- calculation of the predicted environmental concentration in soil (PEC_{soil}) in µg/kg according to the EMA guidelines[1]

$$PEC_{soil} = \frac{N_{max} * PEC_{slurry}}{N_{slurry} * \text{soil depth} * \text{bulk density}}$$

N_{max}: 170 kg N/ha (max. N application according to fertiliser ordinance DüV)
N_{slurry}: 5,3 kg/m³ (N content slurry pig fattening), 3,8 kg/m³ (N content slurry dairy farming)
Soil depth: 0,05 bzw. 0,2 m, Bulk density: 1500 kg/m³

[1] EMEA (European Medicines Agency), CVMP (Committee for Medicinal Products for Veterinary Use). Revised Guideline on Environmental Impact Assessment for Veterinary Medicinal Products. In Support of the VICH-Guidelines GL 6 and GL 38. EMEA/CVMP/ERA/418282/2005-Rev. 1. (2008).

Example

- duplicates of the regular forms on drug application to animals

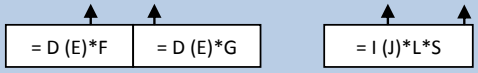
Bestandsbuch Blatt Nr.

über die Anwendung von Arzneimitteln

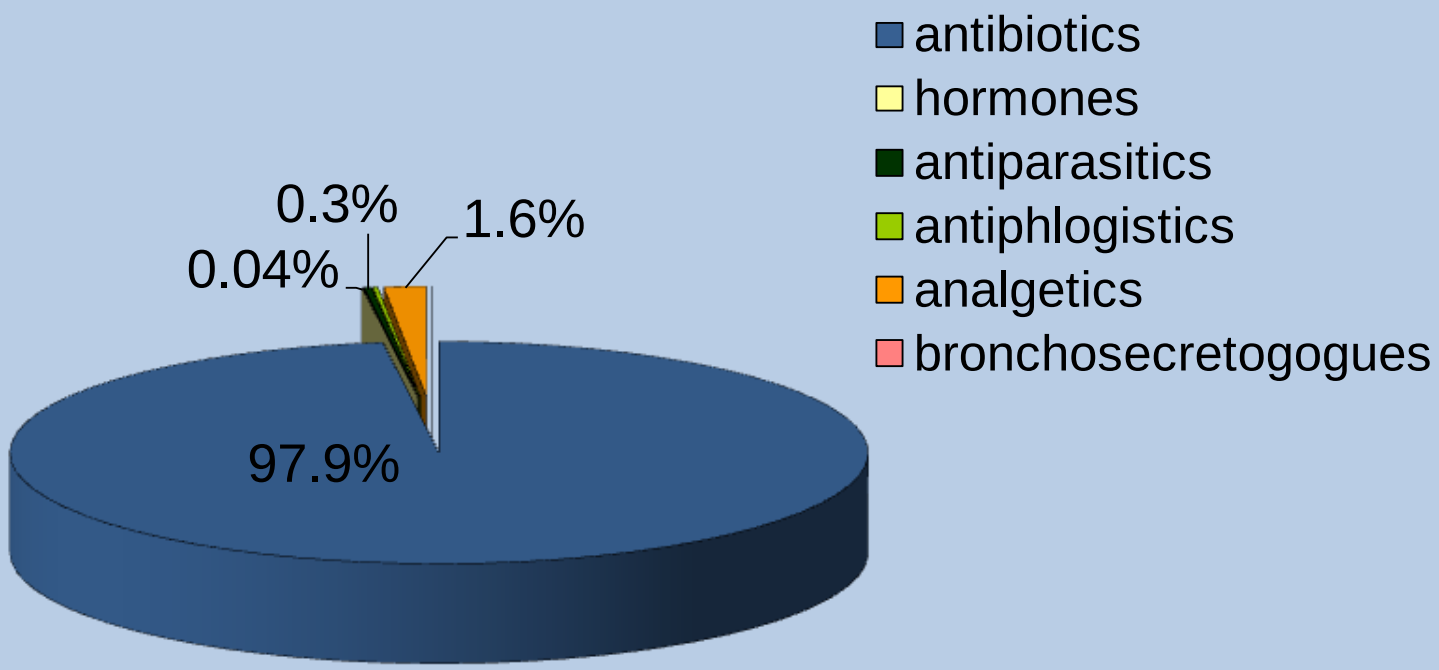
Anzahl / Art der Tiere Identität der Tiere	Standort der Tiere in der Wartezeit	Arzneimittelbezeichnung; Anwendungsmenge; Art der Verabreichung Belegnummer	Wartezeit in Tagen	Datum der Anwendung	anwendende Person
2 Mastschweine Ab-Wegsinfekt.	Mast	Bisolvon 10ml i.m. 5 Tage 1672	2	25.12.10	Pentert
10 Ferkel v. 21.12 Ab-Wegsinfekt.	Abferkel	Amoxycillin Inj. 2ml i.m. 5 Tage 1672	18	25.12.10	Pentert
11 Sauen v. 21.12 Biobetrieb	Warkstall	Rejunate 5ml oral 18 Tage Sau Nr.: 3351, 3877, 3357, 3304, 3875, 3301, 3898, 3364, 3863, 3871 1672	24	26.12 - 12.01.11	Pentert
45 Sauen v. 21.12 MMA	Warkstall	Dinolytic 2ml i.m. 1 Tag 1672	-	27.12.10	Pohl
450 Ferkel Magen-Darm-Infekt.	Flatdeck	Enkroxid N PLV 5 0,0015 kg oral 7 Tage 1653	14	29.12 - 04.01.11	Pohlsch.
450 Ferkel Ab-Wegsinfekt.	Flatdeck	Synuthrin 72% Pulver 0,00032 kg oral 7 Tage 1655	10	29.12. - 04.01.11	Pohlsch.
450 Ferkel Ab-Wegsinfekt.	Flatdeck	Synuthrin 72% Pulver 0,00032 kg oral 7 Tage 1655	10	29.12. - 04.01.11	Pohlsch.

Example
- excel sheet for data evaluation

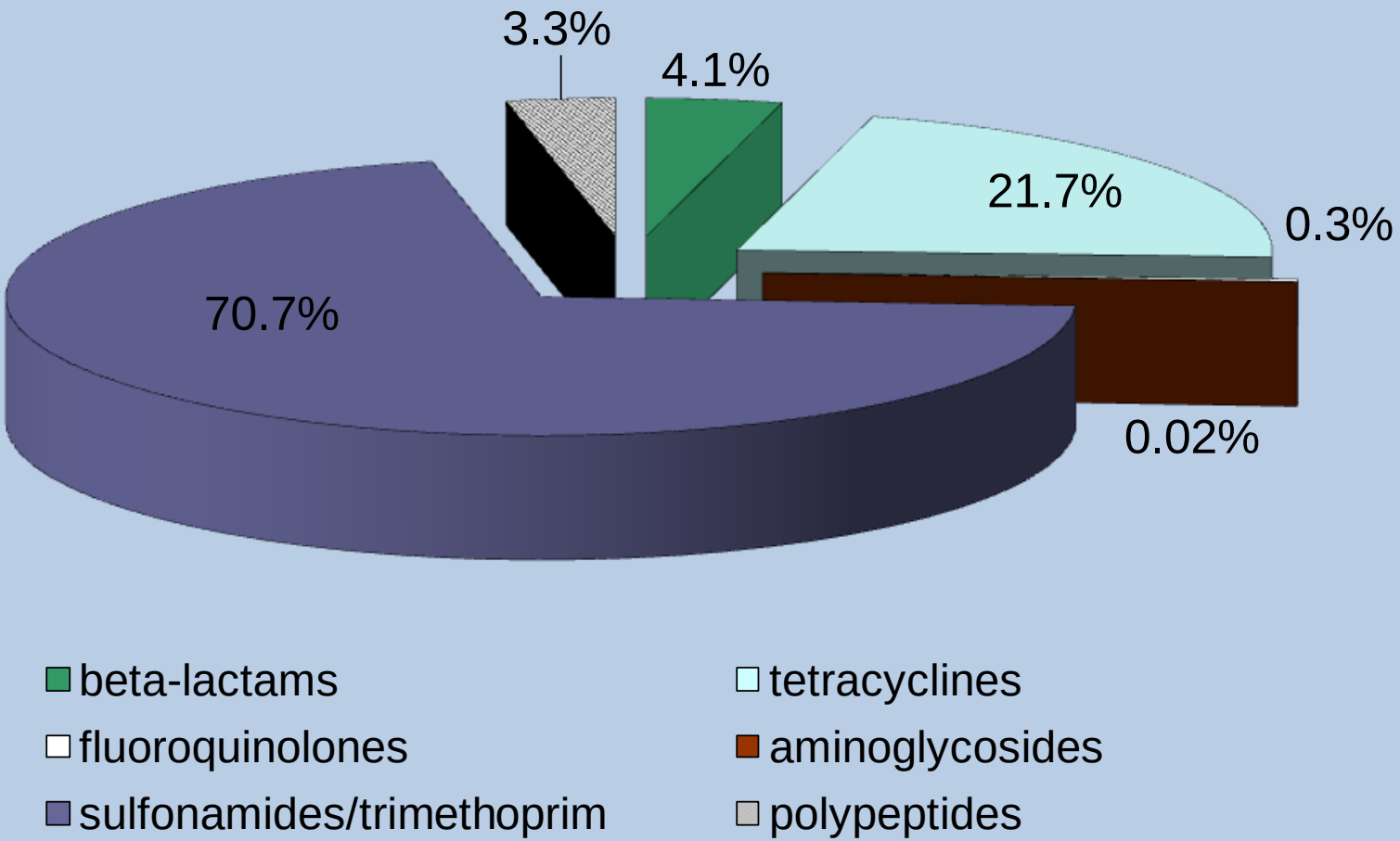
Time period 01.01.-31.12.2010																		
Columns																		
A	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**	**J**	**K**	**L**	**M**	**N**	**O**	**P**	**Q**	**R**	**S**
identification	**classification**	**agent**	**amount ml**	**amount g**	**C_{w1} mg/ml* mg/kg**	**C_{w2} mg/ml mg/kg**	**C_{w3} mg/ml mg/kg**	**C_{w1} mg**	**C_{w2} mg**	**C_{w3} mg**	**animals amount**	**C_{Gw1} mg**	**C_{Gw2} mg**	**C_{Gw3} mg**	**date**	**application**	**animals category**	**duration days**
Amoxycilllin	ß-Lactam	Amoxycilllin	2		150			300			10	3000			25.12.2010	i.m.	F	5
Amoxycilllin	ß-Lactam	Amoxycilllin	2		150			300			20	30000			10.12.2010	i.m.	F	5
Amoxycilllin	ß-Lactam	Amoxycilllin	2		150			300			20	6000			17.10.2010	i.m.	F	5
Amoxycilllin	ß-Lactam	Amoxycilllin	2		150			300			20	6000			16.09.2010	i.m.	F	4
Amoxycilllin	ß-Lactam	Amoxycilllin	2		150			300			20	6000			14.09.2010	i.m.	F	4
Amoxycilllin	ß-Lactam	Amoxycilllin	2		150			300			20	6000			09.09.2010	i.m.	F	4
Amoxycilllin	ß-Lactam	Amoxycilllin	2		150			300			10	18000			18.08.2010	i.m.	F	6
Baycox (5 %)	Kokzidienmittel	Toltrazurilum	1		50			50			250	12500			17.12.2010	oral	F	2
Baycox (5 %)	Kokzidienmittel	Toltrazurilum	1		50			50			250	12500			25.11.2010	oral	F	2
Baycox (5 %)	Kokzidienmittel	Toltrazurilum	1		50			50			250	12500			05.11.2010	oral	F	2
Baycox (5 %)	Kokzidienmittel	Toltrazurilum	1		50			50			250	12500			23.09.2010	oral	F	2
Baycox (5 %)	Kokzidienmittel	Toltrazurilum	1		50			50			250	12500			02.09.2010	oral	F	2
Bisolvon	Bronchosekretolytika	Bromhexinhydrochlorid	10		3			30			2	60			25.12.2010	oral	MS	5
Bisolvon	Bronchosekretolytika	Bromhexinhydrochlorid	10		3			30			20	4200			26.05.2010	oral	JS	5
Cobactan (2,5 %)	ß-Lactam	Cefquinonum	2		25			50			25	1250			22.07.2010	i.m.	F	2
Cobactan (2,5 %)	ß-Lactam	Cefquinonum	2		25			50			25	1250			10.02.2010	i.m.	F	2
Synutrim	Sulfonamid	SDZ, Trim.		0,32	600	120		192	38,4		450	6E+05	120960		29.12.2010	oral	F	7
Synutrim	Sulfonamid	SDZ, Trim.		0,65	600	120		390	78		440	1E+06	240240		21.12.2010	oral	F	7
Synutrim	Sulfonamid	SDZ, Trim.		0,712	600	120		427,2	85,44		800	2E+06	478464		15.12.2010	oral	F	7
Synutrim	Sulfonamid	SDZ, Trim.		0,63	600	120		378	75,6		450	1E+06	238140		02.12.2010	oral	F	7
Synutrim	Sulfonamid	SDZ, Trim.		0,63	600	120		378	75,6		450	1E+06	238140		10.12.2010	oral	F	7
Marbocyl 10 %	Fluorquinolon	Marbofloxacinum	5		100			500			20	10000			29.12.2010	i.m.	MS	2
Marbocyl 10 %	Fluorquinolon	Marbofloxacinum	5		100			500			6	6000			17.12.2010	i.m.	MS	2
Marbocyl 10 %	Fluorquinolon	Marbofloxacinum	5		100			500			20	10000			25.11.2010	i.m.	MS	2
Marbocyl 10 %	Fluorquinolon	Marbofloxacinum	5		100			500			10	5000			06.11.2010	i.m.	MS	2
Marbocyl 10 %	Fluorquinolon	Marbofloxacinum	5		100			500			20	10000			24.09.2010	i.m.	MS	2
Powerflox	Fluorquinolon	Enrofloxacin	2		100			200			16	3200			29.10.2010	i.m.	F	3
Powerflox	Fluorquinolon	Enrofloxacin	2		100			200			16	3200			10.04.2010	i.m.	F	3
Powerflox	Fluorquinolon	Enrofloxacin	2		100			200			16	3200			03.03.2010	i.m.	F	3



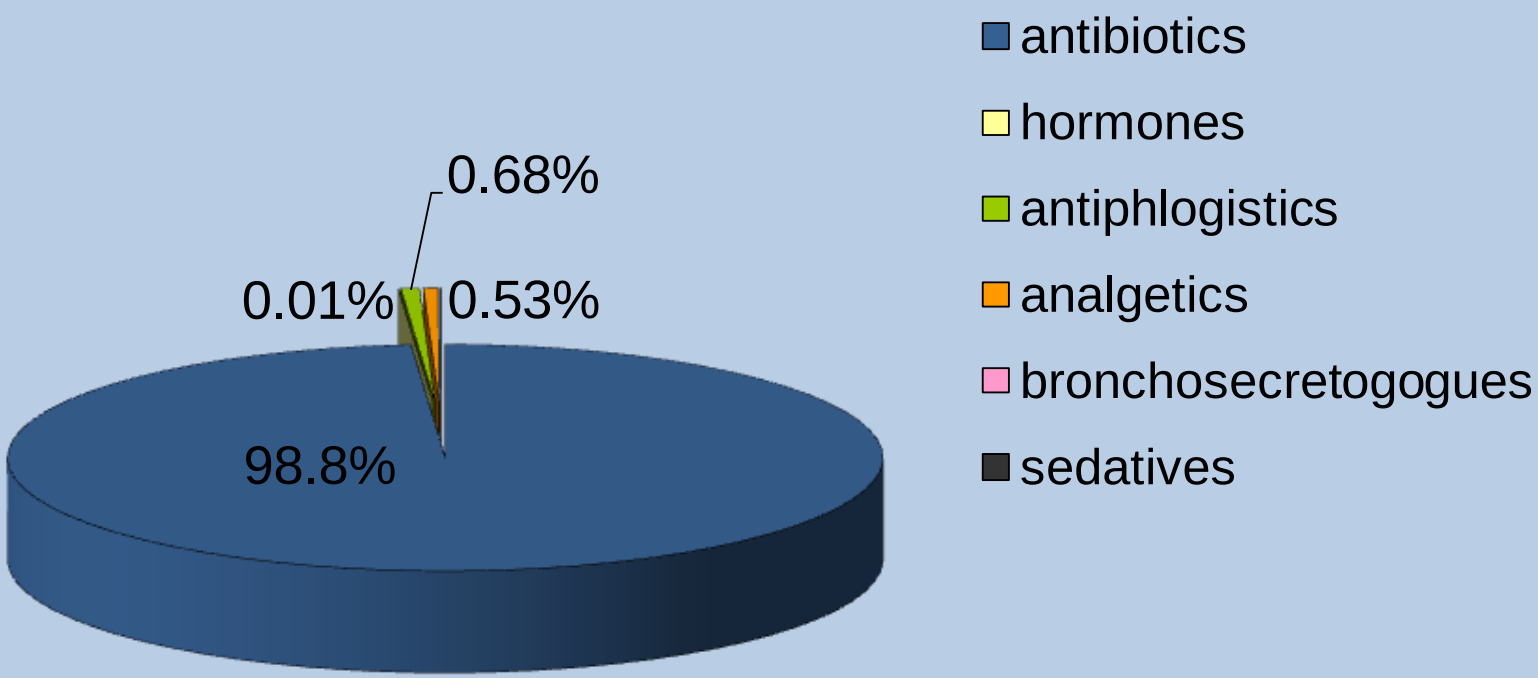
Application of veterinary pharmaceuticals in a pig fattening farm (1173 animals)



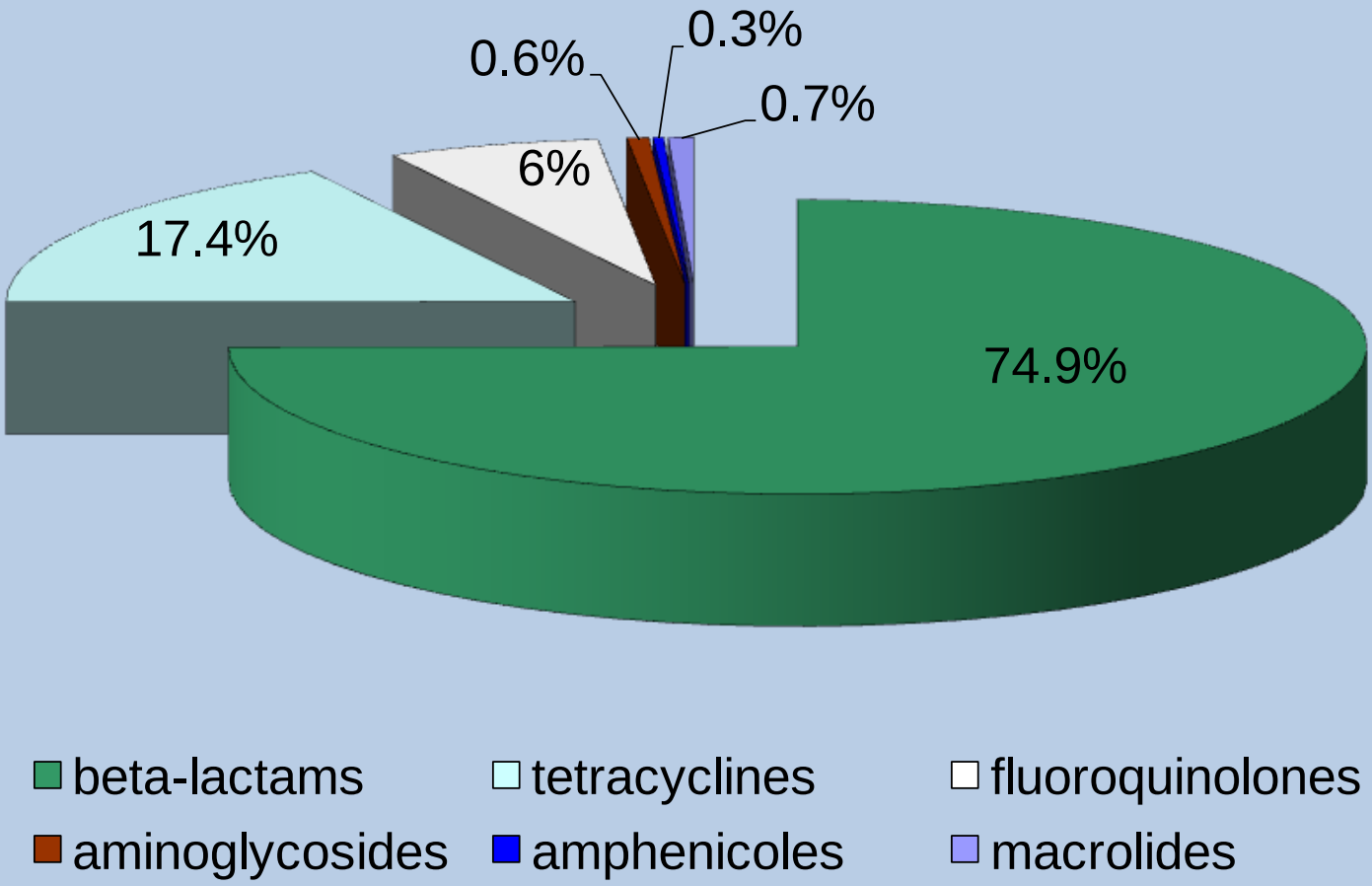
Application of antibiotic agents in a pig fattening farm (1173 animals)



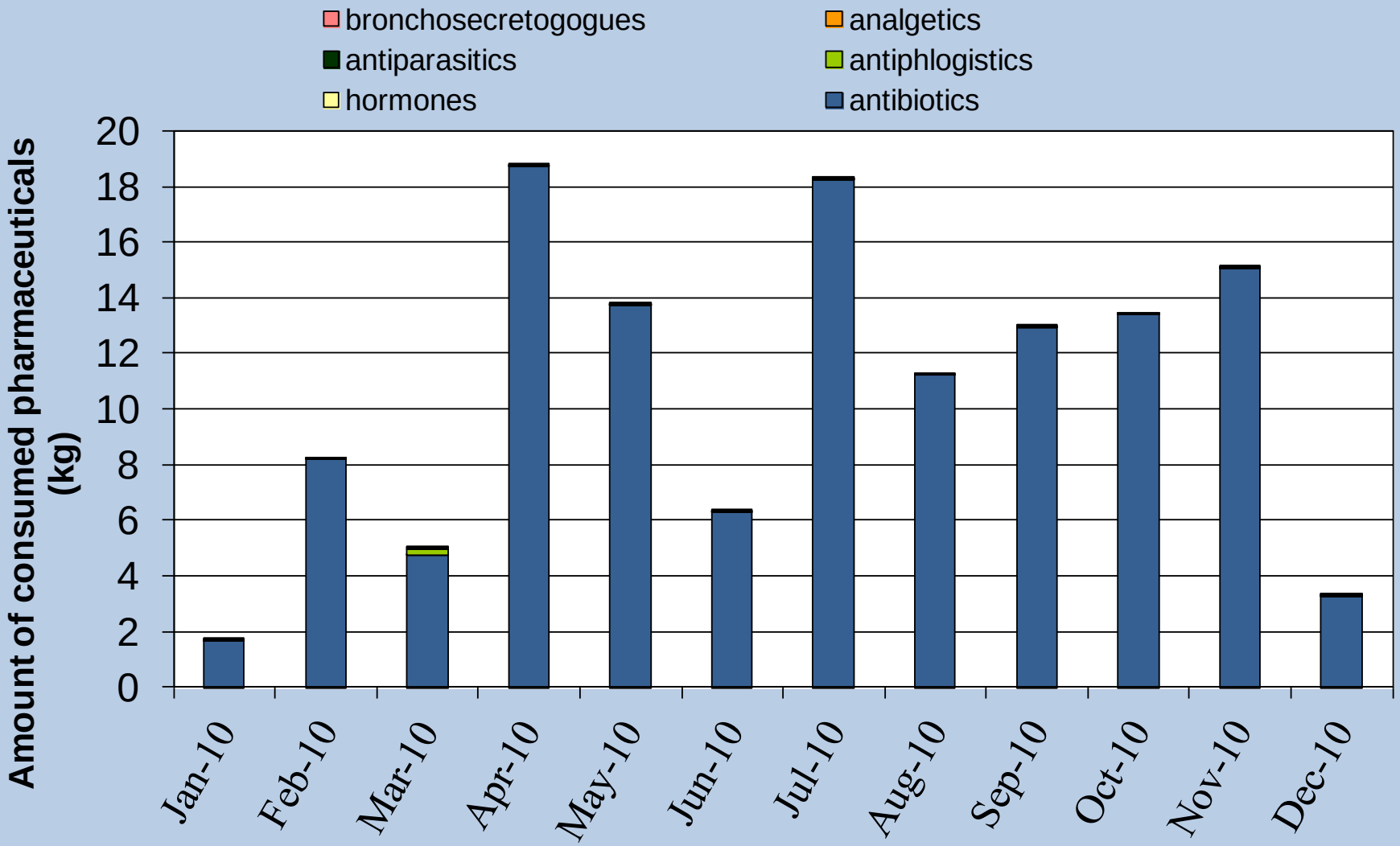
Application of veterinary pharmaceuticals in a dairy farm (750 animals)



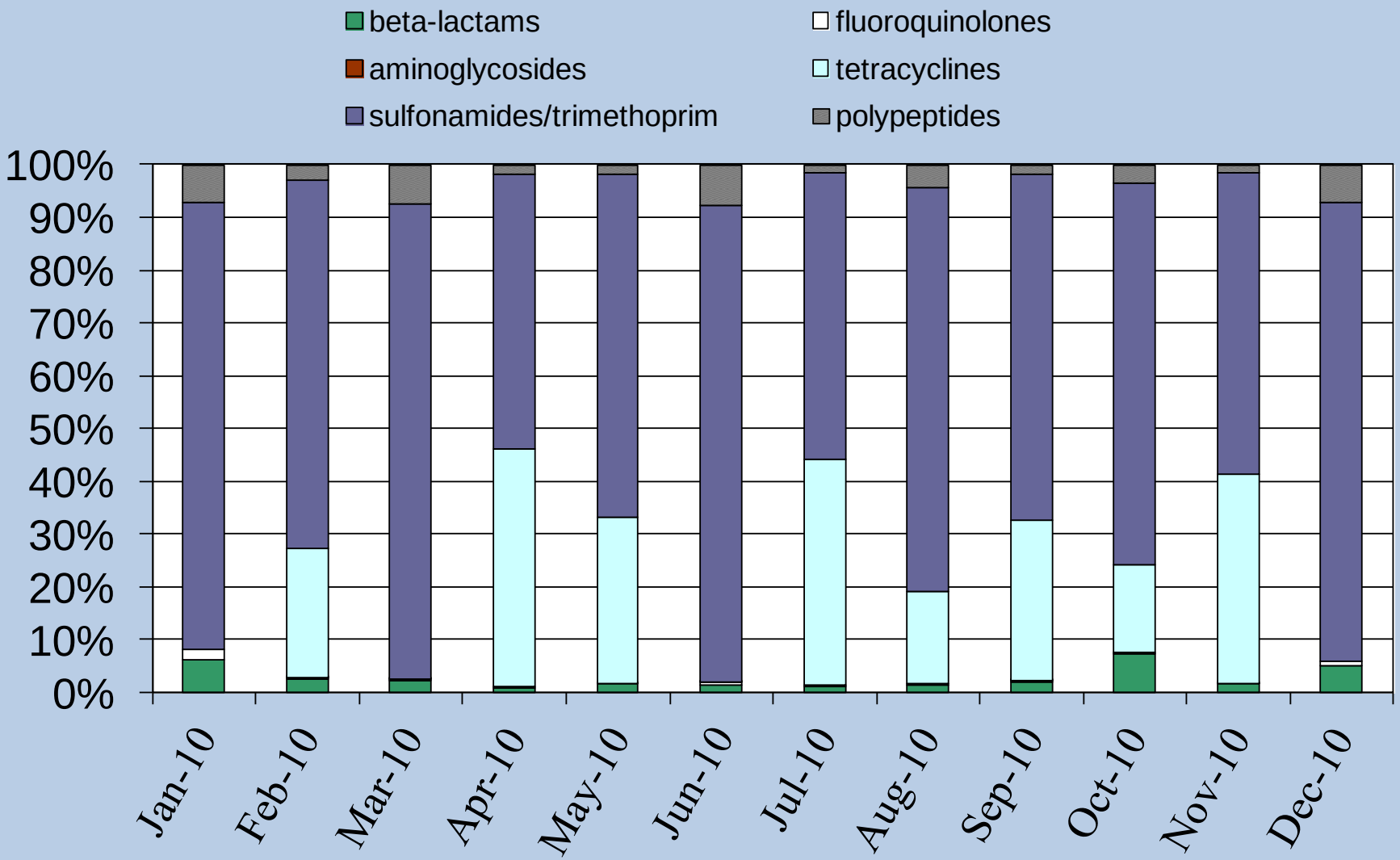
Application of antibiotic agents in a dairy farm (750 animals)



Annual course of the administered veterinary pharmaceuticals (pig fattening farm)



Annual course of the administered antibiotics (pig fattening farm)



Predicted Environmental Concentration in slurry and soil (pig fattening farm)

	PEC_{slurry} [µg L^{-1}] April	PEC_{slurry} [µg L^{-1}] August	PEC_{slurry} [µg L^{-1}] average	$PEC_{soil\ (5cm\ depth)}$ [µg kg^{-1}]	$PEC_{soil\ (20\ cm\ depth)}$ [µg kg^{-1}]
antibiotics					
beta-lactams	350.76	431.67	391.21	16.73	4.18
fluoroquinolones	79.64	79.73	79.69	3.41	0.85
aminoglycosides	4.44	3.33	3.89	0.17	0.04
tetracyclines	1780.60	13758.27	7769.44	332.28	83.07
sulfonamides	8490.72	19076.06	13783.39	589.48	147.37
trimethoprim	1698.14	3815.21	2756.68	117.90	29.47
polypeptides	637.37	911.52	774.45	33.12	8.28
hormones					
oxytocin	0.01	0.01	0.01	0.00	0.00
progestagens	7.04	6.86	6.95	0.30	0.07
gonadotropin	0.03	0.04	0.04	0.00	0.00
prostaglandin agonists	3.36	3.05	3.21		
antiphlogistics					
flunixin	188.47	3.33	95.90		
meloxicam	8.47	4.66	6.57		
dexamethasone	0.00	0.09	0.05		
antiparasitics					
ivermectin	15.36	18.13	16.75		
toltrazurilum	33.33	51.81	42.57		
analgetics					
metamizole	0.28	0.32	0.30		
bronchosecretogogues					
bromhexine hydrochloride	0.00	2.80	1.40	0.06	0.01
PEC_{Mix}				**1100.51**	**275.13**

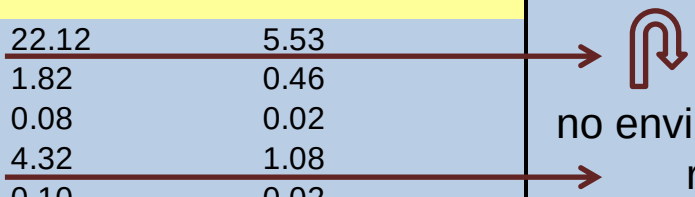
> triggervalue 100 µg/kg

Environmental risk assessment has to proceed to phase II

- ✓ excretion
- ✓ metabolism
- ✓ degradation
- ✓ physical-chemical properties
- ✓ environmental behaviour
- ✓ effects on different aquatic and terestrial test organisms

Predicted Environmental Concentration in slurry and soil (dairy farm)

	PEC_{slurry} [µg L^{-1}] May	PEC_{slurry} [µg L^{-1}] July	PEC_{slurry} [µg L^{-1}] Oct.	PEC_{slurry} [µg L^{-1}] average	$PEC_{soil\ (5cm\ depth)}$ [µg kg^{-1}]	$PEC_{soil\ (20\ cm\ depth)}$ [µg kg^{-1}]
antibiotics						
beta-lactams	299.32	467.10	346.21	370.88	22.12	5.53
fluoroquinolones	3.84	41.53	46.42	30.59	1.82	0.46
aminoglycosides	0.00	0.44	3.42	1.29	0.08	0.02
tetracyclines	0.00	104.21	113.01	72.41	4.32	1.08
amphenicoles	1.04	0.00	3.92	1.65	0.10	0.02
macrolides	0.00	0.00	14.74	4.91	0.29	0.07
hormones						
gonadotropin	0.00	0.00	0.00	0.00	0.00	0.00
prostaglandin agonists	0.00	0.00	0.07	0.02	0.00	0.00
antiphlogistics						
dexamethasone	0.00	0.06	0.19	0.08	0.00	0.00
flunixin	0.00	0.13	9.16	3.09	0.18	0.05
carprofen	0.00	0.00	0.15	0.05	0.00	0.00
meloxicam	0.00	0.00	0.12	0.04	0.00	0.00
analgetics						
metamizole	0.00	0.00	0.00	0.00	0.00	0.00
PEC_{Mix}					**28.93**	**7.23**



no environmental risk

Overview of administered chemical substances

Maximum of 42 chemical substances

chemical substance	substance classes	therapeutic classes
Amoxicillin	beta-lactams	antibiotics
Ampicillin	beta-lactams	antibiotics
Oxacillin	beta-lactams	antibiotics
Penicillin	beta-lactams	antibiotics
Procain-Penicillin	beta-lactams	antibiotics
Benzathin-Benzylpenicillin	beta-lactams	antibiotics
Benzylpenicillin Procain	beta-lactams	antibiotics
Dihydrostreptomycinsulfat	beta-lactams	antibiotics
Benzylpenicillin-Kalium	beta-lactams	antibiotics
Neomycin	beta-lactams	antibiotics
Cefaperazon	beta-lactams	antibiotics
Cefapirin	beta-lactams	antibiotics
Cefquinonum	beta-lactams	antibiotics
Ceftiofurum	beta-lactams	antibiotics
Ceftiofur-Natrium	beta-lactams	antibiotics
Cloxacillin	beta-lactams	antibiotics
Cloxacillin-Natrium	beta-lactams	antibiotics
Cloxacillin-Benzathin	beta-lactams	antibiotics
Oxytetracyclin	tetracyclines	antibiotics
Tylosin	macrolides	antibiotics
Danofloxacin	fluoroquinolones	antibiotics
Enrofloxacin	fluoroquinolones	antibiotics
Gentamycin	aminoglycosides	antibiotics
Lincomycin	aminoglycosides	antibiotics
Neomycin	aminoglycosides	antibiotics
Streptomycin	aminoglycosides	antibiotics
Dihydrostreptomycin	aminoglycosides	antibiotics
Cloprostenol	prostaglandin agonists	hormones
Trometamolum	prostaglandin agonists	hormones
Butylscolopamin	parasympathicolytic	antispasmodics
Buserelin	peptide	hormones
Flunixin	non-steroidal anti inflammatory drugs	antiphlogistics
Carprofen	non-steroidal anti inflammatory drugs	antiphlogistics
Meloxicam	non-steroidal anti inflammatory drugs	antiphlogistics
Dexamethason	glucocorticosteroids	antiphlogistics
Diazepam	benzodiazepine	sedatives
Xylazinhydrochlorid	thiazine amines	sedatives
Embutramid	hydroxybutyrate	narcotics
Florfenicol	amphenicoles	antibiotics
Ketoprofen	non-steroidal anti inflammatory drugs	analgetics
Metamizol	non-opioid analgesics	analgetics
Methylsalicylat	non-opioid analgesics	analgetics

- application of maximal 42 different chemical substances and 16 different substance classes in farms surveyed (primarily antibiotics: 82 to 100 %)
- markedly higher consumption of veterinary drugs in pig fattening compared to dairy farming
- pig fattening farms mainly used not easily degradable, environmentally relevant antibiotics (tetracyclines, sulfonamides/trimethoprim) with PEC_{soil} markedly exceeding trigger value of 100 µg/kg
- for pig slurry calculation of $PEC_{soil(mix)}$ higher than1000 µg/kg and furthermore application of substance classes which was already found in environmental samples suggests:

⇨ a high probability that mixtures of different veterinary drug components may occur in manured soils

- requirement of continued, more representative data collection (central digital registration of drug application)


Thanks for your
attention !