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DETECTION OF DICLAZURIL, POULTRY FEED ADDITIVE, BY LCMS

Vesna Furtula*, Brad McPherson**

*Aquatic Ecosystem Impacts Research Division, Environment Canada, Vancouver, BC

**Pacific Environmental Science Centre, Environment Canada, Vancouver, BC

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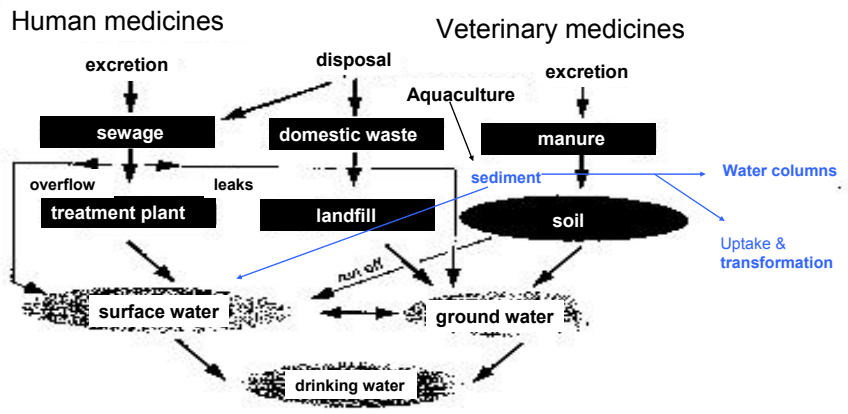


Modified (by PESC) Terns, T. Water Res., 1998, 32, 3245-3260

Veterinary pharmaceuticals as poultry food additives

litter

compost

soil

Effects on plants and terrestrial organisms
(collaboration with University of Aveiro, Portugal)

Antimicrobial resistance
(Collaboration with Agri-Food Canada)

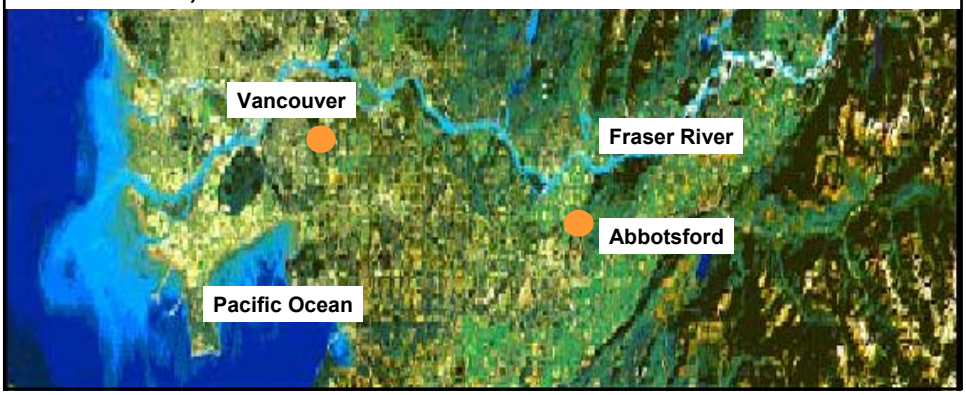
surface water, ground water

Lower Fraser Valley, British Columbia

Estimated waste loading from agriculture entering environment:

811,000 m3/year solid waste and bedding from poultry
368,000 m3/year of manure (including urine, faces and wash water) from swine
200,800m3/year of manure ((including urine, faces and wash water) from dairy

Schmitt O. and Van Kleeck R. (2005 BCMAL personal communication)

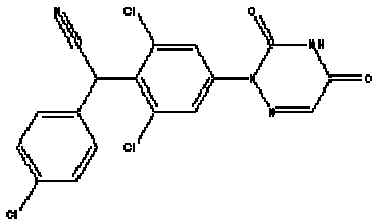


Antimicrobial schedule for farms

Farm 1, 3 and 4		Farm 2	
Ration (days fed)	Antimicrobials	Ration (days fed)	Antimicrobials
Pre-starter (0-8)	Narasin Nicarbazin Oxytetracycline HCl	Pre-starter (0-14)	Naracin Nicarbazin Oxytetracycline HCl
Starter (9-19)	Narasin Nicarbazin Virginiamycin	Starter (15-20)	Naracin Nicarbazin Procaine Penicillin G
Grower 1 (20-26)	Naracin Nicarbazin Virginiamycin Oxytetracycline HCl	Grower 1 (21-27)	Naracin Nicarbazin Bacitracin methylene disalicylate
Grower 2 (27-31)	Narcin Bacitracin methylene disalicyate	Grower 2 (28-35)	Salinomycin sodium Bacitracin methylene disalicyate
Finisher (32-37)	Virginiamycin	Finisher (36-market)	Salinomycin sodium

Type of feed additives	Antimicrobial agents in diet (mg/kg of feed)		
	Starter: Days 0-20	Grower: Days 21-27	Finisher: Days 28-36
I	Narasin (40) Nicarbazin (40) Bacitracin (110)[b]	Narasin (70) Bacitracin (55)	Virginiamycin (22)
II	Narasin (40) Nicarbazin (40)	Narasin (40) Nicarbazin (40) Salinomycin (60) Bacitracin (55)	Salinomycin (60) Bacitracin 55
III	Narasin (40) Nicarbazin (40) Bacitracin (110)	Narasin (40) Nicarbazin (40) Bacitracin (55)	Diclazuril (1) Virginiamycin (22)

Diclazuril



Formulations: Clinacox®
Diclosol

Water soluble

Molecular formula : C17H9Cl3N4O2
MW : No. 407.64
CAS :101831-37-2
Coccidiostat

Banned in EU as of 2005

Agilent 1100 LCMS System

Flow Injection Analysis (FIA)

	Antimicrobial method	Diclazuril
Capillary voltage	4000 V positive	
	3700 V negative	3100 V negative
Drying gas flow	11 L/min	10 L/min
Drying gas temperature	300 ^{0}C	325 $^{\circ}$C
Fragmentor voltage	120 V-180 V	130 V
Gain	1	1
Nebulizer gas pressure	22 psi	22 psi

LC Parameters

Column: ZORBAX 300SB C-18, 100 mm x 2.1 mm, 3.5 µm pore size

Injection Volume: 10 µL

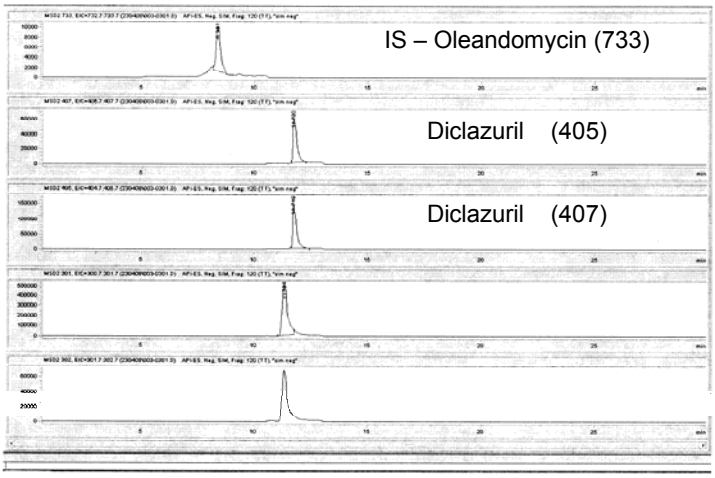
Mobile phase: A (95% H20, 5 %AcN, 0.1% formic acid)
B (100% AcN, 0.1% formic acid)

Gradient: 0 min 10% B, 8 min 80%B, hold 4 min, 30 min 10% B

Investigated compounds and their ions in API-ES-MS

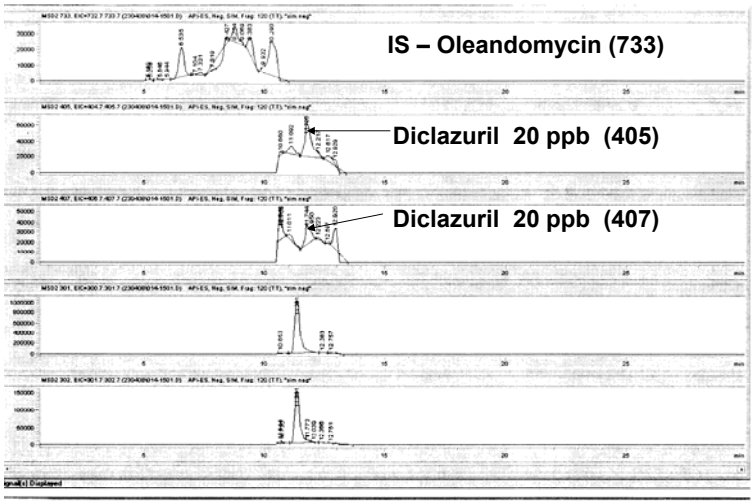
Compound	M	Ionization mode	m/z 100%	Fragment ion	Confirmation ion
Oxytetracycline hydrochloride (OTCHCl)	496.9	Positive	461	[M-Cl]	462
Virginiamycin	525.6	Positive	526	[M+H]	527, 548, 549
Bacitracin	1422.7	Positive	475		470, 711
Nicabrazin	426.4	Negative	301		137
Narasin	764.5	Positive	787	[M + Na]	788,474, 748
Salinomycin	751.0	Positive	774	[M + Na]	775
Monensin	692.9	Positive	693		694
Diclazuril	407.6	Negative	405		407

Single Ion Monitoring (SIM) of Internal Standard (IS) and Diclazuril ions-standard



Treatment		Inclusion Rate*	Birds per Pen	No. of Pens (3/drug)	Total Birds
1	Control	---	150	2, 10, 17	450
2	Bambermycin	2 ppm	150	1, 5, 18	450
3	Penicillin procaine	2.2 ppm	150	5, 9, 15	450
4	Salinomycin	60 ppm	150	3, 4, 14	450
5	Bacitracin (BMD)	55 ppm	150	7, 11, 16	450
6	Salinomycin+BMD	60 / 55 ppm	150	8, 12, 13	450

Single Ion Monitoring (SIM) of Internal Standard (IS) and Diclazuril ions Spike in poultry litter



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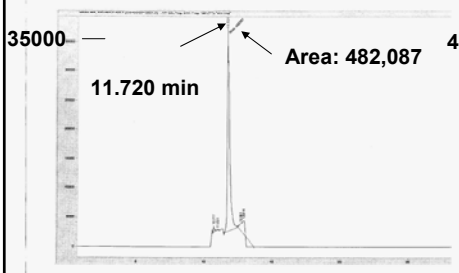
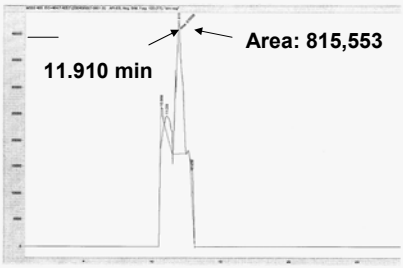
Diclazuril SIM

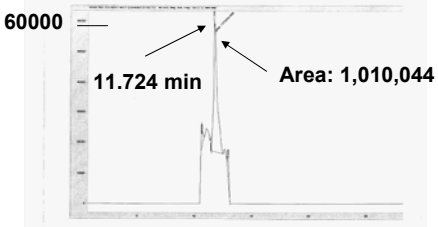
Ion 405 in standard



Ion 405 in matrix blank



Ion 405 in matrix spike



C O N C L U S I O N

Method development still work in progress

Another IS should be added for negative mode

Spikes of diclazuril in water may not have so much interference

LCMS can be used for screening of diclazuril in poultry litter samples