Selected drugs in solid matrices :

A review of environmental occurrence

and properties of principal substances

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Acknowledgement

This work has been carried out with financial support from the following EU Member States: UK, Germany, France, Italy, Spain, Nordic countries, Netherlands, Denmark, Austria, EU DG XI and JRC, Ispra.

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SUMMARY

After intake, drugs absorbed by human or animal organism are subject to metabolic reactions, such hydroxylation, cleavage or glucuronation. However, a significant amount of the original or metabolized substance leave the organism via urine or feces. Thanks to the improvement in analytical chemistry, several pharmaceutical compounds and endocrine disrupters are more often observed in water environmental compartment, surface and waste waters, at concentration till ppb.
But what's the contamination of our solid environmental matrices? These substances can be eliminated by sorption or biodegradation but there is not enough data actually permitting an evaluation of the behavior of the substances through solid compartment, as soil, sludge and bio-waste.
The focus of this paper is an overview of occurrence of the pharmaceutical compounds in solid matrices on basis of their high quantity in use, the literature data that indicated the drug potential to persist in sediment, soil or sludge and on basis of their physical and chemical properties.

Keywords : Pharmaceuticals, Antibiotics, steroids, properties, sludge, sediment, soil, environmental occurrence

1. INTRODUCTION

Today 100 000 different chemical substances are recorded in the European union, which 30 000 products marketed in quantity above 1 ton (Giger, 2002).
Among them, Pharmaceutical compounds have become of increasingly concern since recent years as they have been identified as emerging environmental contaminants.
The medicine makes constant progresses thanks to drugs, which active substances always in evolution increase the opportunities to treat human and animal diseases. Human and veterinary pharmaceuticals represent more than 4000 molecules and 10 000 specialties naturally made to be hydro-soluble.

Hydrophilic metabolized or original Contaminants not eliminated completely on WWTP enter the environment, surface water, through industrial, hospital and domestic effluents.
Persistence of the lipophilic pharmaceuticals could present environmental risk, for groundwater, and potential human health effects especially through run-off of sewage sludge used for agriculture after rainfall events, through domestic and farm animal excretions following by wet weather.

Thanks to developed extraction protocols coupled with analytical methods, LC-MS/MS or GC-MS, (Heberer *et al.* 2001, Ternes *et al.* 1998 & 2001, Sacher *et al.* 2001, Petrovic *et al.* 2003), the concentrations of some drugs in water are comparable levels at which pesticides are typically found in environment but with possible environmental different dose/effect. Since the first studies of the ninety years, the active principals of different therapeutic classes were found at concentrations in waste and surface waters around the ng/l and sometimes superior to the µg/L for Salicylic Acid, Diclofenac and Carbamazepine (Heberer *et al.* 2001, Ternes 1998).
Concerning antibiotic classes, The most prevalents found in the environment have been some of Macrolides, Fluoroquinolones and sulfonamides groups (Sacher *et al.* 2001, Daughton and Ternes 1999) whereas Tetracyclines or Penicillins have only been founds in some cases and generally at low concentrations.
But these residual concentrations in the aquatic environment can they have an impact in term of human health? Today, evaluation studies on toxicologic effects at trace levels are scare (Suling and Thiemann 2000, Schulman *et al.*, 2002). In certain case the risk can be associated to the specific effect of the molecule (endocrine disruption, bio activity…..)
In the same way, actually, because of lack of quantitative data concerning the contamination of environmental solid matrices there is not enough eco-toxicological evaluation (Brooks *et al.*, 2003).
Nowadays, Tendency is the development of model for exposure prediction or potential of eco- toxicological effects (Jones *et al.*, 2002, Khan and Ongerth, 2002).

The aim of this review is to identify pharmaceutical compounds presenting an interest to be targeted in term of important quantity use, and persistence according recent data of the literature and physico-chemical properties of the substances with the objective to develop the analytical techniques needed to accurately detect these compounds in complex environmental samples such as sludge, soil or biowaste.

2. PRESCRIPTION INFORMATION : DISPENSED MASS ACCORDING COUNTRIES

There are 3000 new active compounds a year but it is difficult to access the exact figures of consumption or production. The table 1 summarizes for the principal drug groups, the active substances most prescribed, both in drugstore and hospital, via accessible data of different countries. The predominant therapeutic classes according country therapeutic practices are : Analgesics/anti-Inflammatories, lipid regulators, antibiotics, Betablokers and anti-epileptics and Hormones.

Concerning particular case of Steroids, the most common synthetic hormone component is the 17alpha-EthynylEstradiol (EE2). One major medicinal application of estrogenic substances has been the development of contraceptive pills since 1960. The oral contraceptive contains between 30 and 50 µg of EE2 per pill (Desbrow *et al.* 1998). The annual prescription of 17alpha-ethynylestradiol in Germany is approximately 50 kg (Ternes *et al.* 1999b).

Other uses are the improvement of livestock yield and Hormone Replacement Therapies (Estradiol HTR) (Christen, 1998). The table 2 reported the natural and synthetic hormones daily excretion for humans.

Table 2 Daily excretion (µg) of estrogenic steroids in humans (Ying *et al.* 2002)

Category	**E2**	**E1**	**E3**	**EE2**
Males	1.6	3.9	1.5	
Menstruating females	3.5	8	4.8	
Menopausal females	2.3	4	1	
Pregnant women	259	600	6000	
Women(Contraceptive)				35

Table 1 Estimated Tons /Year commercialized, prescribed or used in several countries

Therapeutic classes (references)	**Generic name**	**Denmark in 1997/98 (a ; b)**	**UK in 2000 (c / d)**	**Australia in 1998 (e)**	**Germany in 1995 (f ; g)**	**France in 1998 (h)**
Analgesic and anti-inflammatories	Paracetamol	248	2000/390	296		2294
	Acetylsalicylic acid	213	770/18	20		880
	Ibuprofen	34	-/162	14	105	166
	Naproxen		60.6/35	23		39
	Diclofenac		-/26		75	
Lipo-regulators	Fenofibrate				15	86
	Bezafibrate				30	34
	Gemfibrozil			20	6	
Antiepileptics	Carbamazepine		-/40	10	80	38
Beta-bloquants	Metoprolol				50	
	Propranolol		11.8/-		3	
Antidepressants	Fluoxetine		2.0/-			
Hormones	EE2		0.029			
	Estradiol	0.119				
antibiotics						
Beta-lactamides	Amoxicillin		-/71	46	25.5-127.5	438
	Ampicillin				1.8-3.6	
	Pencillin V				140	
Sulfonamides	Sulfametoxazole				16.6-76	
Macrolides	Spiramicin					42
	Erythromycin		67.7/26		3.9-19.8	
	Roxithromycin				3.1-6.2	
	Tylosin	1.08 - 13.15		11		
Tetracyclines	Oxytetracycline	2.66	33.7/27			

References :a : Rabølle and Spliid, 2000 ; b : Stuer-Lauridsen; c : Webb 2001; d : Jones *et al.* 2002 ;
e : Khan and and Ongerth 2002 ; f : Verlag 1996 ; g : Hirsch *et al.* 1999 ; h : Janex *et al.* 2002.

3. PHYSICAL AND CHEMICAL PROPERTIES

By nature, most pharmaceuticals are designed to be water-soluble, biodegradable and have short half-lives. Majority of substances presented in table 3 have acidic properties and the compounds with high log Kow, Gemfibrozil to Estriol, can present affinity to the sludge or soil. Estrogens are as well hydrophobic organic compounds with low volatility. It is expected that their sorption on soil or sediment will be a significant factor in reducing aqueous phase concentrations (Ying *et al.* 2002).

Distribution coefficient, Kd , is define as ratio between concentration adsorbed in solid matrice (soil or sludge) and concentration adsorbed in solution after equilibration. Calculated (theoretical or experimental) values permit to give tendency, according compounds, on adsorption distribution or solid-liquid partition (table 4).
Without to take into account persistence/biodegradability factors, Human and veterinary medecines as Estradiol, Fluoroquinolones, Erythromycin, Ibuprofen in sludge and Tetracyclines, Fluoroquinolones, Tylosin and Avermectin in soil, are expected to be preferentially adsorbed on solid environmental matrices.

Table 4 parameter concerning sorption in L/Kg

Distribution Coefficient (references)	**Kd soil-water** *A / B*	**Kd sludge-Water**
Estradiol		1468(a)
Tetracycline	1140-1620 (b)	
Oxytetracycline	420-1030 (c)	0.02 (d)
Enrofloxacine	260-6310(e)	
Ciprofloxacine	427.0 (e)	416.9 (a)
Ibuprofen		453.79 (d)
Ofloxacin	309 (e)	
Naproxen		217.20(d)
Erythromycin		164.76(d)
Tylosin	8.3 – 128 (c)	
Avermectin	7 – 134 (g)	
Propanolol	*9.6 / 37.6* (f)	
Carbamazepine	1.4 / 4.4 (f)	25.52(d)
Diclofenac	*0.8 / 5.9* (f)	0.72(d)
Sulfathiazole	4.9 (h)	
Sulfamethazine	1 - 3.1 (h)	
Sulfamethoxazole	*0.22 / 1.8* (f)	
Chlofibric acid	- / *3.1*(f)	
Acetylsalicylic acid		2.2(a)
Amoxycillin		1.06(d)
Paracetamol		0.4139(d)

References : a : Stuer-Lauridsen *et al.* ; b : Sithole and Guy 1987 ; c : Rabølle and Spliid 2000 ; d : Jones *et al.* 2002 ; e : Nowara *et al.*; 1997 ; f : Drillia *et al. (A=low organic carbon and high clay content ; B=high organic carbon and low clay content*) ; g : Gruber *et al.* 1990 ; h : Langhammer 1989

Table 3 Log Kow and Pka1 found in literature for some compounds

Compounds	**Log Kow**	**Pka1**	**references**
Gemfibrozil	4.77		m
Diclofenac	4.51 (i);0.70(j)	4.15 (j)	
Bezafibrate	4.25	3.6 (v)	
17alpha-EE2	4.15 (k)	10.4 (l)	
Ibuprofen	3.97 (m)	4.4 (18) ; 4.51 (n)	
17beta-Estradiol	3.94		k
tylosin	3.5	7.1	o
Estrone	3.43		k
Avermectin	3.19		p
Naproxen	3.18	4.2	m ; j
Ketoprofen	3.12-3.16(i)	4.45(q)	
Erythromycin	3.06	8.9	m ; j
Estriol	2.81		k
Roxithromycin	2.75	8.8 (v)	
Clofibric acid	2.57		r
Carbamazepine	2.45 (m);2.25 (j)	13.9 (j)	
Salicylic acid	2.26 (m);1.19(j)	3.5 (j)	
Penicillin	1.87	2.79	
Ampicillin	1.45	2.53	
Acetylsalicylic acid	1.19	3.5	p
Chloramphenicol	1.14		p
Enrofloxacine	1.1 (p)	6.27 (s)	
Primidone	0.91		
Sulfamethazine	0.89 (p)	2.65 (t)	
Sulfamethoxazole	0.89 (u)	5.7 (v)	
Amoxycillin	0.87 (j)	2.4 (m)	
Paracetamol	0.46	9.5	m ; j
Ciprofloxacine	0.4 (w)	6.38 (s) ; 5.9 (w)	
Phenazone	0.38	1.4	
Ofloxacine	0.35	5.97	w
Sulfamerazine	0.21	7.0	
Sulfathiazole	0.05	2	x
Sulfadiazine	-0.09		y
Chlortetracycline	-0.62	6.5	y
Norfloxaxine	-1.0 (w)	6.4 (•)	
Tetracycline	-1.19 (o)	3.30 (z)	
Oxytetracycline	-1.22(o)	3.27 (z)	
Propranolol		9.49	
Trimethoprim		6.6	

References : i : Avdeef *et al.* 2002 ; j : Jones *et al.* 2002 ; k : Ying *et al.* 2002 ; l : Hurwitz and Lui 1977 ; m : Khan and and Ongerth 2002 ; n : Wan *et al.* 2002 ; o : Wollenberger 2000 ; p : Meylan 1993 ; q : Tixier *et al.* 2003 ; r : Alcock *et al*. 1999 ; s : Nowara *et al.* 1997 ; t : Papastephanou and Frantz 1997 ; u : Kolpin et al 2002 ; v : Huber *et al.* 2003 ; w : Drakopoulos and Ioannou 1997 ; x : Tolls 2001 ; y : Halling-Sørensen 2003 ; z : buser *et al.* 1999 ; • : Takacs-Novak *et al.* 1992

4. METABOLITES OF PHARMACEUTICALS IDENTIFIED IN LITERATURE

A significant amount of the original substance leaving the organism no metabolized via urine or feces and will therefore enter raw sewage or manure. Some of them have yet environmental metabolites identified in literature (table 5).

Photodegradation is often one among several degradation pathways for environmental contaminants, the photolysis experiments and the computer simulation suggested this process to be predominant one for Diclofenac in lake (Buser *et al.* 1998)
From human metabolism of Ibuprofen (Ib), the 3 metabolites Hydroxy Ib, carboxy Ib, carboxy-hydratropic acid could be identified in biodegradation experiments with activated sludge in both biofilm reactor and batch (Zwiener *et al.* 2002). Hydroxy Ib is revealed as the major metabolite under oxic conditions and carboxy-hydratropic acid under anoxic conditions. carboxy Ib was found under oxic and anoxic conditions almost only in batch experiments with activated sludge. The metabolites together do not account for more than 10% of the initial concentration of Ib. In an another way, Buser *et al.* (1999) observed Hydroxy Ib, carboxy Ib in WWTP influents at even higher concentrations than Ib .
The major degradation product of Tylosin A, with half-life found by Loke *et al.* (2000) to be less than two days, in methanogenic as well as aerobic incubation media corresponding to Tylosin B. Furthermore, Tylosin D is believed to be a minor degradation product
The hormones are excreted majoritary (90-95%) under conjugated biologically inactive forms. The hormones can be conjugated with sulfuric or glucuronid acids (Andreolini *et al.* 1987). Du to the common presence of the beta-glucuronidase synthetized by E-Coli in waste waters, some of the excreted metabolites can even be transformed back to the free biologically active drug. It's the case of glucuronide and sulfate conjugated hormones that could be hydrolyzed in sewage increasing contribution of parent drugs in sludge matrice**.** According some data, concentrations of glucuronide conjugates are weak or no detected in WW effluents (Belfroid *et al.* 1999, Huang and Sedlak ,2001).

Table 5 Metabolites identified for selected pharmaceuticals

Selected compounds	**Metabolites identified in literature**
Erythromycin (Hydrolysis)	Dehydro-Erythromycin (Ternes, 1998 ; Sacher *et al.*, 2001)
Acetyl salicylic acid (ASA) (Deacetylation)	Gentisic acid o-Hydroxyhippuric acid (Ternes, 1998) Salicylic acid
Ibuprofen (ib) (Biodegradation)	Hydroxy ib, carboxy ib, carboxy-hydratropic acid (Daughton and Ternes, 1999 ; Zwiener *et al*. 2002)
Carbamazepine (excreted as glucuronides)	10,11 Epoxy-carbamazepine (Ternes, 1998)

5. PERSISTENCE IN SOLID MATRICES

5.1 Sediments

Most of the pharmaceutical data in literature concern occurrence of estrogens on sediment.
Nevertheless, The parasiticide Avermectin, used in human and veterinary medecine, was already found in sediments close to fish farms due to its elevated lipophicity (Löffler and Ternes 2003) and Diclofenac not detected in the sediments of the Greifensee lake showed negligible adsorption onto sediment particles in a laboratory experiments (Buser *et al.* 1998).

Concerning occurrence of estrogens in sediments, the simulation of 17beta-estradiol distribution in rivers shows that the river bed-sediments have the potential to be a environmental reservoir for 17beta-Estradiol (E2), 17alpha-EE2 and Estrone (E1) (Williams *et al.* 1999, Petrovic *et al.* 2001).
Given the relative low polarity of these compounds which present octanol-water partition coefficients mostly between 2.5 and 5 (table 3), sorption to bed sediments appears a quite likely cumulative process from where estrogens can eventually become bio-available specially when they are anaerobic (Williams *et al.* 1999, Petrovic *et al.* 2001). The only way of removal of the chemicals can from the sediments is through scouring or diffusion processes across the sediment water column interface. Degradation in the bed sediment becomes an important consideration in the context of long term accumulation (Williams *et al.* 1999). Nevertheless, with the exception of two high concentrations found for Estrone (11.9ng/g) and EE2 (22.8 ng/g) (Lopez de Alda and Barcelo 2001), the waste waters or river sediments levels monitored were always in the low ng/g range (Ternes *et al.* 2002, Herry and Beausse, 2004, Löffler *et al.* 2003).
On five studied WWTPs, the hormone fraction found in Suspended Matter (SM), table 6, represented less than 10% of total concentration (EE2+E2+E1) of the influent or effluent (Herry and Beausse, 2004).
Over time, seasonal variations, with higher average concentration in the winter months than in summer, were observed (Petrovic *et al.* 2001).

Table 6 Mean concentration of steroid hormones in Influent and Effluents Suspended Matters (Herry and Beausse, 2004)

Mean concentration	**In influent**	**In effluent**
[Suspended Matter] mg/L	400mg/L	< 100 mg/l
[EE2] in SM	< 4 ng/g DM	<6 to 20 ng/g DM
[E2] in SM	4 ng/g DM	<3 to 20ng/g DM
[E1] in SM	25 ng/g DM	<3 to 20ng/g DM

5.2 Waste water sludges

The efficiency of the plant can be evaluated thank to the influent /effluent balance. Concerning the removal isn't known whether it 's due to sorption or biodegradation (table 7). Contaminants not eliminated completely on WWTP (WasteWater Treatment Plant) is discharge into receiving water (table 8). Removal by WWTPs differs considerably by individual pharmaceutical and by process conditions.

Table 7 Predominant removal mecanisms following therapeutic classes (Snyder *et al.* 2003)

Group	**Degradation (B/P/AS)**
Antibiotics	B > 90%; P > 70 to 90%
Antidepressants	(B/P/AS) > 70 to 90%
Anti-inflammatory	B> 90%
Lipid regulators	B <20%
Steroïds	B : 20% to > 90%

B : Biodegradation, P : Photodegradation (solar), AS : Active Sludge

5.2.1 Individual compounds

Five sewage treatment plants located in different part of Sweden were investigated by Giger *et al.* 2003 : Amoxicillin, Ampicillin, Metronidazole and erythromycin were not detected in any sample.
The most abundant Macrolide Clarithromycin was detected at 57 to 330 ng/L concentrations in treated wastewater effluents.
The Betalactams, highly important based on the available use data, have not yet been found in wastewater effluents in aquatic environment. Short half-lives in aqueous matrices caused by fast chemical and microbial degradation, such as hydrolysis of the Betalactam ring, is probably the main reason (Giger *et al.* 2003).
In the case of Tetracyclines readily precipitate as complexe forms with cations as calcium and magnesium and accumulate in sludge or sediments (Daughton and Ternes, 1999).

Fluroquinolone Ciprofloxacin and Norfloxacin and, in only one sludge, Ofloxacin were present at concentration between 0.06 and 3 µg/g DM (Lindberg *et al.* 2003). Fluoroquinolone (FQ) eliminations in WWT of 80-90% proceed by sorption transfer to sewage sludge. In digested sludges, the Fluoroquinolones occur at mg/kg levels (Giger *et al.* 2003, Golet *et al.* 2003)

The concentrations of others pharmaceuticals in WWTP effluents range from low µg/L to high ng/L (table 8).

Acid drugs, as Ibuprofen, ASA and their respective metabolites, are easily removed (several µg/L) or completely removed during WWT (Zwiener *et al.* 2002, Tauxe *et al.* 2003, Ternes *et al.*1998). The elimination rate of Diclofenac, partly removed by the biological treatment, depends on the WWTP (17 to75%). Chlofibric acid is not degraded durind water treatment process (6-8%) (Tauxe *et al.* 2003) and more than half of Ketoprofen or Naproxen is removed from the effluents of the plants.

Regarding to the values obtained on WWTPs (Herry and Beausse, 2004), the concentrations in Estradiol and Estrone can reach respectively 40 and 140 ng/l in influents. For these two compounds the efficiency of the plants is above 90%. For EE2, the concentrations in WW influent are more weak (below 10 ng/l) and concerning the efficiency of WWTPs, the elimination of this substance depends of plant treatment caracteristics and can vary in mean between 10 and 75%. The investigated sludges, by Löffler *et al.* 2003, contained up to 30ng/g Estrone, 50 ng/g Estradiol and 10 ng/g 17α•Ethinylestradiol.
According Ternes *et al.* (1999 a & b), if glucuronide conjugated of 17beta-estradiol are mixed with activated sludge, the biologically active forms (17beta-Estradiol and Estrone) are released in 15 min to reach a maximum concentration in 20-30 hours, estrone being the main compound detected (70%).
Neutral substances as Diazepam, Phenazone and Carbamazepine, hardly show any removal during WWT or less than 50%.

Whereas polar substances (antibiotics) or neutral/basic forms are not easily removed on WWTP, acidic forms or more lipophilic compounds, characterized by a log Kow value greater than 2.5, are more effectively eliminated by adsorption on active sludge through hydrophobic interactions.

Table 8 Efficiency and persistence on WWTP

Compounds (references)	**Persistence on WWTP Average values in µg/L**		**Efficiency of WWTP Removal in %**
	Influent	***Effluent***	
Diclofenac (a, b, c, d, e)	0.012, 0.56, 3.02	0.01, 0.365, 0.81, 2.51	17 - 69 - 7 5
Ibuprofen (b, d, e, f, g)	990, 3300	0.1, 0.37, 2, 81	75 - 90 - >90
Ketoprofen (b, e, g)	0.3	0.20, 0.23	69
Naproxen (b, d, e, g)	0.44	0.08, 0.30	66 - 78
Acetylsalicylic~acid (d, e, h)	3.2	0.22, 0.50	77 - 81
salicylic acid (e, g, h)	0.34 , 54	< 0.02, <0.050, 0.04	> 90
Gentitisic acid (h)	4.6	< 0.10	> 90
o-hydroxyhippuric acid (h)	6.8	< 0.10	> 90
Paracetamol (h)	26	< 0.20	> 90
Phenazone(d, e, g)	0.92	0.16, 0.52	33
Estrone (e, i)	0.086, 0.140	0.001, 0.002, 0.005	94 - 98
17beta-estradiol (e, i)	0.006, 0.041	<0.0002, < 0.001	> 95 - >99
17alpha-EE2 (e, i)	0.0009, 0.002	0.0005, 0.0007, <0.001	22 - 75
Carbamazepine(a, d, e)	1.78	1.63, 2.1	7 - 8
Primidone (g)	1.08	0.14	87
Gemfibrozil (b, d, e, g)	0.07, 0.40	46 - 69	
Fenofibrate(b, d, e)		0.38	45 - 64
Bezafibrate (b, d, e)		2.2	50 - 83
Propranolol (d, e)		0.17	96
Metoprolol (d, e)		0.73	83
Roxithromycin (e)		0.68	
Erythromycin-H_2O (e)		2.50	
Sulfamethoxazole (e, g)		0.40, 0.90	
Ciprofloxacin (j)	0.427	0.071	83
Norfloxacin (j)	0.431	0.051	75

References : a : Heberer *et al.* 2002; b : Stumpf *et al.* 1999; c : Koutsouba *et al.* 2003 ; d : 1998 ; e : Ternes 2001 ; f : Buser *et al.* 1999 ; g : Heberer 2002 ; h : Ternes *et al.*; 1998; i : Herry and Beausse 2004 ; j : Golet *et al.* 2003

5.2.2 Process conditions

The biological activity of sewage suggests that organic compounds will be present in inverse proportion to their aerobic and anaerobic degradability. Compounds having relatively short half-lives, as beta-Lactamides or Penicillins, would not be expected to survive in any but the freshest of sludge samples (Khan and Ongerth 2002)

For several pharmaceuticals, Roxithromycin, Sulfamethoxazole, Ibuprofen, Bezafibrate, EE2, an increasing degradation could be shown with higher sludge age (at least 10-15 days). Controversial results are obtained for Diclofenac (McArdell *et al.* 2003).
On Anaerobic digest pilot plant more than 85% removal can be observed for Naproxen, Sulfamethoxazole, Roxitromycin, Estradiol, 20% for diazepam, Carbamazepine and ibuprofen, contradictory results are obtained for Diclofenac and EE2. Under anaerobic conditions E1 was reduced to E2 (Carballa *et al.* 2003).
For E1 and EE2, the maximum removal rate occurring under aerobic conditions. Substrate present in raw water influent competitively inhibits the degradation of E1 and E2, but not EE2 through the influence of diffusive mass transfer inside the flock. More than 90% removal for all estrogens is observed in denitrifying activated sludge processes (Joss *et al.* 2003).
In activated and digested sewage sludge, Estrone and 17beta-Estradiol were detected up to 37 ng/g and 49 ng/g respectively and 17alpha-Ethinylestradiol up to 17 ng/g. The occurrence of estrogens in digested sludge indicated that estrogens can be persistent during sludge digestion (Ternes *et al.* 2002). The activated sludge treatment step removed the estrogens with a higher level of efficiency than the biological filter (Ternes *et al.* 1999 a & b) and 17beta-Estradiol (up to 95% is oxidized in Estrone in 1 to 3 hours which was further eliminated) is removed with a higher efficiency than 17alpha-EE2 (64-78%).

Wastewater treatment resulted in a reduction of Fluoroquinolones mass flow of 88-92%, mainly due to sorption on sewage sludge. No significant removal of FQs occurs under methanogenic conditions of the sludge digesters with concentrations around 3 mg/Kg. According Golet *et al.* (2003), theirs results suggest sewage sludge as the main reservoir of FQs residues (table 9).
According Primary degradation results for sulfonamides obtained after lag phases of 7 to 10 at 20°C from the activated sludge reactors, the biodegradation of sulfonamides is so slow that these compounds may pass the sewage treatment systems because of non sorbing properties (Ingerslev and Halling-Sørensen 2000).

Table 9 Fluoroquinolone Concentrations in Sewage Sludge in mg/kg (Golet *et al.* 2003)

Average ± SD of weekly variation	**Excess Sludge**	**Raw Sludge**	**Digested Sludge**
Ciprofloxacine	2.5±0.1	2.2±0.4	3.1±0.4
Norfloxacine	2.6±0.1	2.1±0.2	2.9±0.4

The different removal processes identified by Thompson *et al.* (2003) are :
- sorption to sewage sludge solids during sedimentation
- sorption to sewage sludge solids during secondary suspended growth or fixed film biological treatment processes
- biodegradation

Hydrophobic medecines are predicted in wet primary sludge at much higher concentrations than in raw influent. Lipophilic compounds clearly have the potential to concentrate in sewage sludge and depending on their anaerobic biodegradability may not be effectively removed during sludge digestion or further treatment (table 10, Khan and Ongerth 2002)

Table 10 Analytical sludge results for different medecines (Khan and Ongerth 2002)

Medecines	Aqueous(µg/L) - dry(µg/Kg) - wet(µg/Kg)	
	in Primary sludge	in Digested sludge
Paracetamol	42 - 4.5 - 178	2 - 0.0006 - na
Naproxen	2 – 1.2 – 33	0.1 - 0.001 - na
Ibuprofen	2 – 3.4 – 121	6 - 0.006 - na
Salicylic acid	11 – 13.7 - 424	1 - 0.002 - na
Gemfibrozil	2 - 1.2 - 37	nd – nd - na
Carbamazepine	3 - 1.7 - 54	6 - 0.01 - na

nd : no Detected ; na : No available

5.3 Treated soils

Antibiotic pharmaceuticals enter agricultural soils essentially through the use of contaminated manure and sludge as fertilizers (table 11). Sorption and mobility for given compounds seem to be impact by nature of soils.

Table 11 Allowed practice according countries for Treatment of the farmland

Allowed practice	**Treatment of the farmland**
Switzerland in 1999 Anae. digested sludge (Golet *et al.* 2003)	5/t/ha/3 years
United Kingdom Manure (Elsom *et al.* 2003)	250 kg N/ha/year
France in 2003 Manure Sludge	170 kg N/ha/year 3/t/ha/year (30t/ha/10 years)
Danemark in 2000 Pig Manure (Sengeløv *et al.* 2003)	20 000-31 000 L/ha

Pharmaceuticals display a wide range of mobility ($0.2<Kd,solid<6000$ L/kg) and variation in Kd,solid for a given compound in different soils can be significant (Tolls 2001). The adsorption of all compounds was generally higher in soil with the higher carbon content (table 4, Drillia *et al.* 2003).

More than 90% of applied Enrofloxacin (Kd =260-5612, table 4) are adsorbed on five soils from different geographic and cultivation areas (clay and organic carbon). Ciprofloxacin and Ofloxacin showed a similar adsorption (Kd =285-496). At clay mineral, Enrofloxacine removal was above 98% (Nowara *et al.* 1997).
Tylosin sorption seems to correlate positively with the soil clay content (Kd values = 8 and 128 for Tylosin). Oxytetracycline was particularly strongly sorbed in all soils investigated, with Kd values between 417 in sand soil and 1026 in sandy loam. Oxytetracycline and Tylosin, strongly adsorbed, show much lower mobility in any of soil types : 60-80% of the Tylosin added had been leached to a depth of 5cm in the sandy loam and 25 cm in the sand soil (Rabølle and Suter 2000).

According the results on soil-column experiments conducted by Thurman and Lindsey 2000, it is hypothesized that the Sulfamethazine (acidic antibiotics) are transported more rapidly through soil to ground water than the Tetracyclines (basic antibiotics) both present in swine wastewater applied to soil leading to potential infiltration of these compounds. Hypothesis confirmed by experiments of Alonso *et al.* (2003) showing high retention on soil for Oxytetracycline and tetracycline in contrast to low retention for Sulfachlopyridazine : Tetracycline and Sulfachlorpyridazine both showed a 50% approximate dissipation in 21 days at 20°C on soil.

Many antibiotic compounds (Tetracyclines, Sulfonamides and Fluoroquinolones) are photodegraded in liquids (Halling-Sørensen *et al.* 2003). Consequently, photodegradation in soil can also operated in first millimeters and at surface of liquid manure. But under field conditions, photodecomposition is negligible compared to other processes for detoxification of antibiotics as abiotic ageing for tetracycline (Thiele-Bruhn *et al.* 2003). In these results (Elsom *et al.* 2003), it was demonstrated the relatively Penicillins lability according the lower soil half life of degradation in soil found below 6.5 days.

5.3.1 Sludge treated soils

Pharmaceuticals reach the terrestrial environment via disposal of enriched sewage sludge to agricultural soils where traces persist after application.
Field experiments of sludge-application to agricultural land confirmed the long-term persistence of trace amounts of FQs in sludge-treated soils and indicated a limited mobility of FQs into the subsoil: After sludge disposal, Ciprofloxacine and Norfloxacine persisted with residual concentration around 0.25-0.30 mg/kg at topsoil (0 – 2.5 cm) (Rabølle and Spiid 2000). 5 months after sludge disposal (Golet *et al.* 2003, application of sewage sludge : 50t/ha), Ciprofloxacine and Norfloxacine were accumulated in topsoil (0-2.5 cm) at concentrations resp. of 0.45 and 0.35 mg/kg and no mobility to the subsoil was observed (<0.05 mg/kg between 2.5 and 20 cm). Because of no completed biodegradation (or photo-transformation) in soils, important residual of Fluoroquinolones (around 0.3 mg/Kg 21 month after application) can persist in agriculture soils several months after application (Table 12, Golet *et al.* 2002).

Table 12 Persistence of Fluoroquinolones in Sludge-treated soil (Golet *et al.* 2002)

Sample type	**Mean Concentration (mg/kg of dm)** ***Ciprofloxacine***	***Ofloxacine***
Untreated raw sludge	1.40-2.03	1.54-1.96
Digested sludge	2.42-2.27	2.37-2.13
Sludge-treated soil (25t/ha) :		
8 months after application	0.35-0.40	0.32-0.29
21 months after application	0.28-0.27	0.27-0.30

5.3.2 Manure treated soils

A plot study was conducted by Burkhardt *et al.* (2003) on a loamy soil to investigate two factors affecting the transport of sulfonamides : application with manure (3L/m2 ;150 mg/m2) and without (water solution ; 30 mm in 1.5 hours) and contact time in soil (1 and 3 days). Manure application lead to more surface water runoff and highter concentrations for studied Sulfadiazine and Sulfathiazole.
With manure slurry (>20 mg/Kg) being applied onto fields as fertilizer with maximum of 50m3/ha, sulfonamide residues (Sulfamethazine + Sulfathiazole) spread on fields could reach up to 1 kg/ha, a value comparable to application doses of modern pesticides (Haller *et al.* 2002).

During 6 months, an experiment simulating the anaerobic degradation of Oxytetracycline in manure tank was set up and free concentration of the four antibiotics (4-epi-Oxytetracycline, alpha-apo- Oxytetracycline and beta-apo- Oxytetracycline) were determinated : Oxytetracycline was observed up till 6 months after spiking. No important increase in free concentrations of the degradation products was observed (Loke *et al.* 2003)

Resistance to Tetracyclines, erythromycin and streptomycin was measured for a period of 8 months on farmland treated with pig manure slurry coming from 3 farms with Tetracyclines concentrations of 42, 81 and 698 µg/L, respectively. Results obtained in this study thus indicate that tetracycline resistance levels in soil are temporarily influenced by the increasing addition of pig manure slurry (Hamscher *et al.* 2002, Sengeløv *et al.* 2003). For Streptomycin and erythromycin, only minor variations in resistance levels were detected (Sengeløv *et al.* 2003). According Christian *et al.*, Tetracyclines could not be found in manures and soils in quantifiable concentrations. Only in one pure cattle manure there seemed to be chlortetracycline (0.1 mg/kg). Tetracyclines are known to bind strongly to soil particles, due to their ability to form complexes with doubly charged cations (e.g. Ca^{2+}). If Oxytetracycline and Tylosin were not detected in any depth manure fertilized soil, Hamscher *et al.* 2002 observed the highest average concentrations of 198.7 µg/Kg (10-20 cm) for Tetracycline and 4.6-7.3 µg/Kg (0-30 cm) for Chlortetracycline (Table 13).

Table 13 Mobility of Tetracyclines and Tylosin in manure treated soil (Hamscher *et al.* 2002)

Concentrations	**In Liquid Manure mg/kg**	**In Fertilized soil µg/kg**			
		0-10 cm	10-20 cm	20-30 cm	30-90 cm
Tetracycline	4.0	86.2	198.7	171.7	ND
Chlortetracycline	0.1	4.6-7.3	4.6-7.3	4.6-7.3	ND
Oxytetracycline	ND	ND	ND	ND	ND
Tylosine	ND	ND	ND	ND	ND

Studies under aerobic conditions showed that the degradation rate found to increase with increasing concentrations of manure particles in the incubation medium. It is, however, not clear whether the decrease in the concentration of Tylosin A is caused by sorption, abiotic or biotic chemical degradation (Loke *et al.* 2003).

Amoxicilline is relatively stable in manure, but obviously not in the environment after some months and Penicillin G, also been administrated to the pigs, is easily degradable and therefore not detectable as the parent compound in manure (Christian *et al.*)

In the investigated liquid manure and soil samples, some antibiotics (Fluoroquinolones) could be found at resp. mg/kg and µg/kg level which indicates a high stability of some pharmaceuticals, especially by considering, that the soil was manured at least several months before sampling (this implies a potential risk of accumulation in soil), whereas other administered antibiotics (Macrolides, Sulfonamides, Tetracyclines and beta-Lactamides) are degraded within a short time and may have hardly an effect on the amount in the environment (Christian *et al.*)

6. CONCLUSION

Since their identification in water, the pharmaceutical compounds have been targeted as emerging environmental contaminants. Their Physico-chemical properties (Log Kow, pKa, polarity….) give tendency concerning environmental persistence in solid environmental matrices.

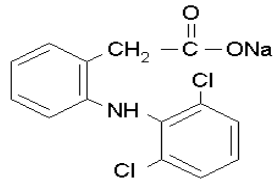
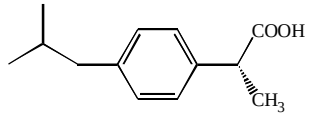
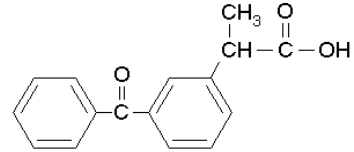
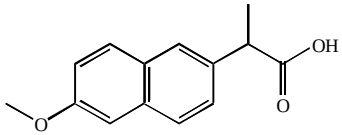
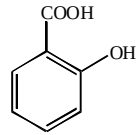
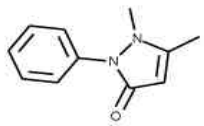
Due to their polarity, persistence and water solubility, some drugs and metabolites are able to pass through the wastewater treatment plants (Sulfonamides, Macrolides, Carbamazepine, Phenazone). Their low adsorption on sludge and soil may cause the contamination of surface and ground water.
The sorption on sludge or soil could let original active substance in hydrophobic links persistent (Cirpofloxacine, Norfloxacine, Hormones, Ivermectin, Tetracyclines, Roxythomycin) . For some of them (Diclofenac, Oxytetracycline, Tylosin, Ibuprofen, Macrolides), partial or total biodegradation, can operate, producing eventually unknown metabolites more or less active than initial form.

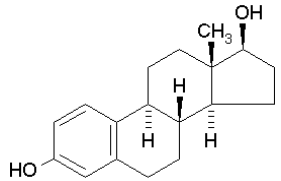
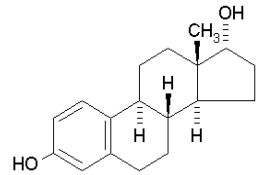
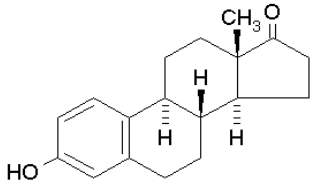
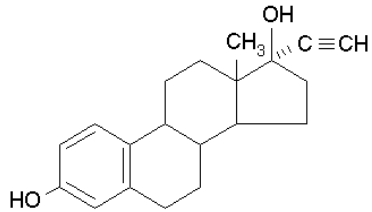
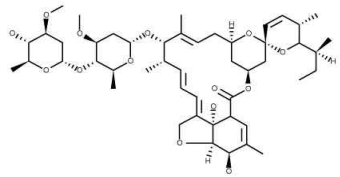
Regarding to this literatture review, amount the different classes, the most concerned therapeutic groups for environment solid matrices seem to be Steroid Hormones, Fluoroquinolones, Macrolides, Tetracyclines, Analgesics/Anti-Inflammatories and Avermectin (table14).

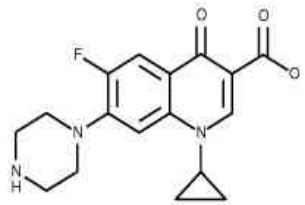
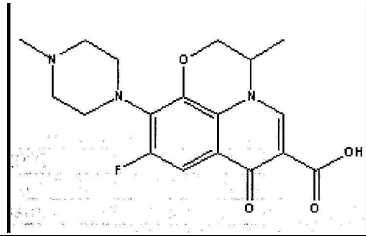
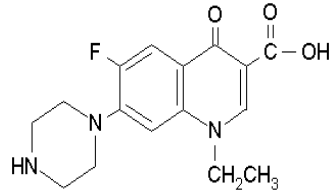
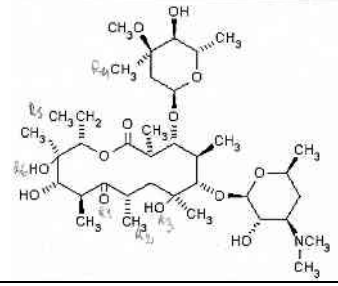
The purpose of next work is to develop analytical methods for trace levels determination in solid matrices of compounds extracted from identified predominant therapeutic classes. Concerning the analytical development, investigation were often limited to the analysis of selected individual compounds, because the diverse range of analytes required highly specialized sample preparation and enrichment procedures.

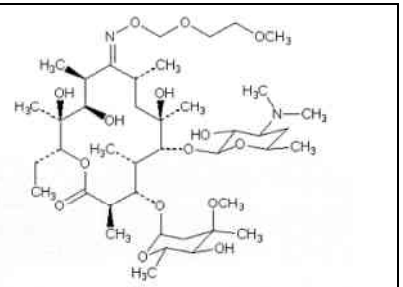
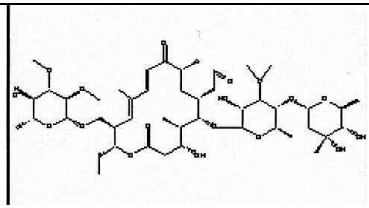
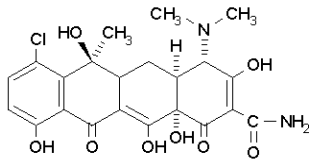
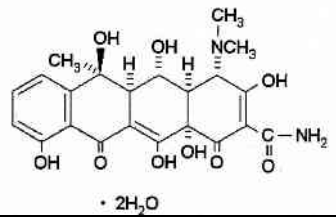
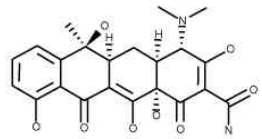
The project could be divided into 2 main parts:
- Development of Extraction procedure for solid matrix (choice of mod and solvent extractions, need of clean-up step optimization),
- Evaluation of the analysis method in term of both sensibility (LOQ) and reproductibility (recovery, standard deviation, need of internal standards…).

Table 14 the most concerned therapeutic groups for environment solid matrices

Generic name	Therapeutic Class	Nomenclature	Chemical Group	Structure
Diclofenac 15307-79-6 (Na)	Analgesic / anti-inflammatories	2-[(2,Dichlorophenyl)amino]benzene acetic acid	Carboxylate Secondary Amine Phenyl acetic acid	
Ibuprofen 15687-27-1	Analgesic / anti-inflammatories	α-Methyl-4-(isobutyl)phenylacetic acid	Arylcarboxylic Propionic acid	
Ketoprofen 22071-15-4	Analgesic / anti-inflammatories	2-(3-Benzoylphenyl)propionic acid	Propionic acid	
Naproxen 22204-53-1	Analgesic / anti-inflammatories	(S)-(+)-6-Methoxy-α-methyl-2-naphthaleneacetic acid	Propionic acid Naphtalene	
Salicylic Acid	Analgesic / anti-inflammatories	Acide hydroxybenzoïque		
Phenazone (Antipyrin) 60-80-0	Analgesic / anti-inflammatories	2,3-Dimethyl-1-phenyl-3-pyrazolin-5-one	Pyrazole Pyrazolinone Tertiary amine	

Estradiol 50-28-2	Hormone	3,17β-Dihydroxy-1,3,5(10)-estratriene 3,17α-Dihydroxy-1,3,5(10)-estratriene	Steroïdes Estratriene	 
Estrone 53-16-7	Hormone	3-Hydroxy-1,3,5(10)-estratrien-17-one	Steroïdes Estratriene	
Ethynylestradiol 57-63-6	Hormone	17α-Ethynyl-1,3,5(10)-estratriene-3,17β-diol	Steroïde Norsteroïde Norpregnatriene	
Avermectin 71751-41-2		Avermectin B1		

Ciprofloxacin	Fluoroquinolone Antibiotic	1-cyclopropyl 6-fluoro 1,4-dihydro 4-oxo 7-(1-piperazinyl)3-quinoleine carboxylique	Derivated Fluor Quinolone	
Ofloxacin 83380-47-6	Fluoroquinolone Antibiotic	acide (RS)-9-fluoro-2,3-dihydro-3-méthyl-10-(4-méthyl-1-pipérazinyl)-7-oxo-7H-pyrido[1,2,3-de]-1,4-benzoxazine-6-carboxylique	Derivated Fluor Quinolone	
Norfloxacin 70458-96-7	Fluoroquinolone Antibiotic	1-Ethyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinolinecarboxylic acid	Derivated Fluor Quinolone	
Erythromycin	Macrolide Antibiotic	(dimethylamino -4 hydroxy-3 methyl-6 tetrahydropyrannyloxy-2)-6ethyl-14 trihydroxy-7,12,13(hydroxy -5 methoxy-4 dimethyl-4,6 tetrahydropyrannyloxy-2)-4 hexamethyl-3,5,7,9,11,13 oxa-1tetradecenedione-2,10.		

Roxythromycin 80214-83-1	Macrolide Antibiotic	erythromycine-(10S)[0[(metoxy-2 ethoxy)methyl]oxime]-9-(E)		
Tylosin	Macrolide Antibiotic	(dimethylamino -4 hydroxy-3 methyl-6 tetrahydropyrannyloxy-2)-6 ethyl-14 trihydroxy-7,12,13(hydroxy -5 methoxy-4 dimethyl-4,6 tetrahydropyrannyloxy-2)-4 hexamethyl-3,5,7,9,11,13 oxa-1tetradecenedione-2,10.		
Chlortetracyclin 64-72-2	Tetracycline Antibiotic	Chloro-7 dimethylamino-4 pentahydroxy-3,6,10,12,12A methyl-6 dioxo- 1,11 octahydro-1,4,4A,5,5A,6,11,12A naphta cenecarboxamide-2chlorydrate	Cycline Naphtacenecarboxamide	
Oxytetracyclin 79-57-2	Tetracycline Antibiotic	4-(dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,5,6,10,12,12a-hexahydroxy-6-methyl-1,11-dioxo-2-naphtacenecarboxamide	Cycline Naphtacenecarboxamide	
Tetracyclin 60-54-8	Tetracycline Antibiotic	4-(dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,6,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-2-naphtacene carboxamide	Cycline Naphtacenecarboxamide	

Veterinary practice ; *Common practice(human & veterinary)*

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