Selected drugs in solid matrices :

A review of environmental occurrence, determination and properties of principal substances

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CONTENTS

LIST OF TABLES

SUMMARY

After intake, drugs absorbed by human or animal organisms are subject to metabolic reactions, such as hydroxylation, cleavage or glucuronation. However, a significant amount of the original or metabolized substance leaves the organism via urine or faeces. Thanks to improvements in analytical chemistry, many pharmaceutical compounds and endocrine disrupters are more easily detected in the water environmental compartments, surface and waste waters, at concentrations reaching the ppb.

But what contaminates our solid environmental matrices? These substances can be eliminated by sorption or biodegradation but, at present, there is not enough data to allow an evaluation of the behaviour of the substances through the solid compartment, such as soil, sludge and bio-waste.

The focus of this paper is an overview of occurrence of the pharmaceutical compounds in solid matrices on the basis of their quantities used, their physico-chemical properties and based on data from literature indicating the drug's potential to persist in sediment, soil or sludge.

Keywords : Pharmaceuticals, Antibiotics, Steroids, properties, sludge, sediment, soil, environmental occurrence and determination.

1. INTRODUCTION

Today 100 000 different chemical substances are recorded in the European union, of which 30 000 products marketed in quantities above 1 ton (Giger, 2002).
Among them, Pharmaceutical compounds have become of increasingly concern in recent years as they have been identified as emerging environmental contaminants.
Medicine makes constant progresses thanks to drugs, of 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 completely eliminated on Waste Water Treatment Plant (WWTP) enter environment, surface water, through industrial, hospital and domestic effluents.
Persistence of the lipophilic pharmaceuticals could present environmental risk for groundwater through run-off of sewage sludge used for agriculture after rainfall or through domestic and farm animal excretions followed 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 detected at comparable levels at which pesticides are typically found in environment but with possible environmental different dose/effect. Since the first studies of the nineties years, the active agents of different therapeutic classes were found at concentrations in waste and surface waters around the ng/l and sometimes greater than the µg/L for Salicylic Acid, Diclofenac and Carbamazepine (Heberer *et al.* 2001, Ternes 1998).
Concerning antibiotic classes, the most prevalent found in the environment have been the Macrolide, Fluoroquinolone and sulfonamide 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 can these residual concentrations in the aquatic environment have an impact in terms of human health? Today, evaluation studies on toxicologic effects at trace levels are scarce (Suling and Thiemann 2000, Schulman *et al.*, 2002). In certain case the risk can be associated with the specific effect of the molecule (endocrine disruption, bio activity…..)
In the same way, at present, because of the lack of quantitative data concerning the contamination of environmental solid matrices there is not enough eco-toxicological evaluation (Brooks *et al.*, 2003).
Nowadays, the tendency is to develop models for exposure prediction or potential 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 terms of important quantities used, physico-chemical properties and persistence according to recent data from literature with the future objective to develop the analytical techniques necessary to accurately detect these compounds in complex environmental samples such as sludge, soil or biowaste.

2. PRESCRIPTION INFORMATION : DISPENSED MASS ACCORDING TO COUNTRIES

There are 3000 new active compounds a year but it is difficult to obtain to the exact figures for consumption or production. The table 1 the principal drug groups, the active substances most prescribed, both in drugstores and hospitals, via accessible data from different countries. The predominant therapeutic classes according local therapeutic practices are : Analgesics/anti-Inflammatories, Lipo-regulators, Antibiotics, Betablockers, anti-Epileptics and Hormones.
Concerning the particular case of Steroids, the most common synthetic hormone is the 17alpha-EthynylEstradiol (EE2). One major medicinal application of these 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 HRT) (Christen, 1998). The table 2 shows the natural and synthetic daily hormone excretions 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)**
Analgesics 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	
Anti-epileptics	Carbamazepine		-/40	10	80	38
Beta-blockers	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 to have short half-lives. The majority of substances presented in table 3 have acidic properties and the compounds with high log Kow, Gemfibrozil to Estriol, can show affinity to sludge or soil. Estrogens are also 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 matrix (soil or sludge) and concentration adsorbed in solution after equilibration. Calculated (theoretical or experimental) values show a tendency of compounds to adsorption distribution or solid-liquid partition (table 4).
Without to taking into account the persistence/biodegradability factor, human and veterinary medecines such as Estradiol, Ciprofloxacine, Erythromycin, Ibuprofen and Naproxen 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	na	1468(a)
Tetracycline	1140-1620 (b)	na
Oxytetracycline	420-1030 (c)	0.02 (d)
Enrofloxacine	260-6310(e)	na
Ciprofloxacine	427.0 (e)	416.9 (a)
Ibuprofen	na	453.79 (d)
Ofloxacin	309 (e)	na
Naproxen	na	217.20(d)
Erythromycin	na	164.76(d)
Tylosin	8.3 – 128 (c)	na
Avermectin	7 – 134 (g)	na
Propanolol	*9.6 / 37.6* (f)	na
Carbamazepine	1.4 / 4.4 (f)	25.52(d)
Diclofenac	*0.8 / 5.9* (f)	0.72(d)
Sulfathiazole	4.9 (h)	na
Sulfamethazine	1 - 3.1 (h)	na
Sulfamethoxazole	*0.22 / 1.8* (f)	na
Chlofibric acid	- */ 3.1*(f)	na
Acetylsalicylic acid	na	2.2(a)
Amoxycillin	na	1.06(d)
Paracetamol	na	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 ; na : not available

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

Compounds	**Log Kow**	**Pka1**	**references**
Gemfibrozil	4.77	na	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	na	k
tylosin	3.5	7.1	o
Estrone	3.43	na	k
Avermectin	3.19	na	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	na	k
Roxithromycin	2.75	8.8 (v)	
Clofibric acid	2.57	na	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	na	p
Enrofloxacine	1.1 (p)	6.27 (s)	
Primidone	0.91	na	
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	na	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	na	9.49	
Trimethoprim	na	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 ; na : not available

4. METABOLITES OF PHARMACEUTICALS IDENTIFIED IN LITERATURE

A significant amount of the original substance leaves 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 suggesting 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 and 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 in aerobic incubation media corresponds 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 acid (Andreolini *et al.* 1987). Due 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 matrix. 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. ANALYTICAL DETERMINATION IN SOLID MATRICES

5.1 Sample preparation

Prior to analysis, the extraction techniques proposed in the literature are the following : Vortex, Ultra-Sonication (U-S) followed by centrifugation and Accelerated Solid Extraction (ASE). Extraction solvents used are ethyl acetate for the less polar, methanol, acetone or water (table 6).

For efficient Tetracycline extraction, the sample matrix is acidified at pH 4.7 with Citrate buffer (Hamscher *et al.*, 2002, Jacobsen *et al.,* 2004) or EDTA solution, to avoid any complexation of these substances with cations.
Moreover, extraction at room temperature is preferable for Tetracyclines that can be converted to their epi- or anhydroform when they are heated (Jacobsen *et al.,* 2004). The degradation of Macrolides has been observed as well at temperatures higher than 100°C (Thomsen *et al.* 2003).

The clean-up step proceeds on SPE cartridges : Diol, MCX, LiChrolut EN, or SAX+HLB tandem the first to retain humic material and the second antibiotic substances (Jacobsen *et al.*,2004). For the estrogens, Silica gel clean-up is followed by an C18 SPE enrichment (Ternes *et al.*, 2002, Herry and Beausse 2004). The elution of these cartridges, is realized with polar solvents such as acetone, methanol, acetonitril or with ethyl acetate.

5.2 Analytical procedure

Identification and quantification are often realized by liquid chromatography coupled with tandem mass spectrometry with an electrospray ionization source. Two mass technologies are given in literature, Ion Trap (Hamscher *et al*., 2002) and Triple Quadrupole (Löffler and Ternes, 2003, Schlüsener *et al.* 2003, Jacobsen *et al.*,2004). The nature of the chromatographic column is an octadecyl phase. The mobile phase, more often, consists of water/acetonitril mixture acidified with formic acid (pH 2.5, Hamscher *et al*., 2002) or with acetic acid (pH 2.9, Löffler and Ternes, 2003) for pharmaceuticals and with aqueous ammonium, (pH 5.7, Löffler and Ternes, 2003) or acetate buffer (pH 4.0, Löffler and Ternes, 2003, Schlüsener *et al.* 2003) the antibiotics.
In the particular case of Estrogens, the two analytical methods, gas chromatography coupled with ion trap mass spectrometer (Ternes *et al.*, 2002) and liquid chromatography coupled with triple quadrupole mass spectrometer (Herry and Beausse, 2004) are proposed for their determination. The advantage of the second is the absence of a time-consuming derivatization step prior to the analysis.

The difficulty of global analytical development is the wide range of physico-chemical properties of different compounds. The two objectives of the multiresidue determination methods are :

- The optimization of extraction procedure to find the best compromise in term of recovery for all the compounds of interest.
- The quantification protocol has to consider the different matrix effects (according to nature, origin and composition of the sample) for a more exact determination of the sample concentrations. Internal quantification can presented weakness when labelled standards used have different natures compared to the substance to be quantified. Because matrix interference impacts can be different from one substance to another, it

appears to be preferable to use the correction with recoveries obtained in spiked samples with surrogate standards.

Table 6. Pharmaceutical extraction procedures proposed in literature for solid matrices

Substances (Reference)	**Extraction procedure**	**LOQ**
Tetracyclines in fertilized soil (Hamscher *et al.*, 2002)	Vortex with ethyl acetate	5 µg/Kg
Fluoroquinolones in sewage sludge treated soil (Golet *et al.* 2003)	ASE with o-H_3PO_4/CH_3CN	 0.45 mg/Kg 0.18 mg/Kg
Acidic pharmaceuticals & ivermectin in sediments (Löffler and Ternes, 2003)	U-S with acetone/acetic acid then ethyl acetate	0.4 ng/g (exept Diclofenac 8ng/g, Fenoprofen 1ng/g)
sulfonamides & macrolides in sediment (Löffler and Ternes, 2003)	U-S with methanol, acetone and ethyl acetate	20 ng/g
Macrolides in soils (Schlüsener *et al.* 2003)	ASE with 1% aqueous ammonia in methanol	1 - 1.4 µg/Kg
Macrolides, fluoroquinolones, sulfonamides & tetracyclines in soils (Christian *et al.* 2003)	ASE with methanol	na
Sulfonamides & Macrolides in sewage sludge (Thomsen *et al.* 2003)	ASE with methanol/eau	na
Tetracyclines, Macrolides & sulfonamides in agricultural soils (Jacobsen *et al.*,2004)	ASE with methanol/citric acid buffer at pH 4.7	1-13 µg/Kg tetracyclines 1-11 µg/Kg macrolides 1-7 µg/Kg sulfonamides
Hormones in sludge and sediment (Ternes *et al.*, 2002)	U-S with methanol and acetone	0.2 –0.4 ng/g in sediment 2 – 4 ng/g in sludge

6. PERSISTENCE IN SOLID MATRICES

6.1 Sediments

Most of the pharmaceutical data found in literature concerns the occurrence of estrogens in sediment.
Nevertheless, The parasiticide Avermectin, used in human and veterinary medecine, was already found in sediments close to fish farms due to its elevated lipophilicity (Löffler and Ternes 2003) and Diclofenac not detected in the sediments of the Greifensee lake showed negligible adsorption onto sediment particles in 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 an 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 method of chemical removal from the sediments is through scouring or diffusion processes across the sediment water column interface. Degradation in the sediment bed becomes an important consideration in the context of long term accumulation (Williams *et al.* 1999). Nevertheless, the concentrations found for Estrone (11.9ng/g) and EE2 (22.8 ng/g), according to Lopez de Alda and Barcelo (2001), confirm the waste water or river sediment levels identified at 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 7, represented less than 10% of total concentration (EE2+E2+E1) of the influent or effluent (Herry and Beausse, 2004). Sometimes, seasonal variations, with higher average concentration in the winter months than in summer, can be observed (Petrovic *et al.* 2001).

Table 7 Mean concentration of steroid hormones in five Influent and Effluent Suspended Matter samples (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

6.2 Waste water sludge

The efficiency of the plant can be evaluated from the influent /effluent balance. Concerning the removal, it isn't known whether it 's due to sorption or biodegradation (table 8). Contaminants not eliminated completely on WWTP (WasteWater Treatment Plant) are discharged into receiving water (table 9). Removal on WWTPs differs considerably between individual pharmaceuticals and process conditions.

Table 8 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

6.2.1 Individual compounds

Five sewage treatment plants located in different parts of Sweden were investigated (Giger *et al.* 2003) : Amoxicillin, Ampicillin, Metronidazole and Erythromycin were not detected in any sample.
The short Betalactam half-life in aqueous matrices caused by fast chemical and microbial degradations, such as hydrolysis of the Betalactam ring, is probably the main reason (Giger *et al.* 2003).
The most abundant Macrolide Clarithromycin was detected at 57 to 330 ng/L concentrations in treated wastewater effluents.

The Tetracyclines, readily precipitated as complex forms with cations, as calcium and magnesium were accumulated in sludge or sediment (Daughton and Ternes, 1999).

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

The concentrations of other pharmaceuticals in WWTP effluents range from low µg/L to high ng/L (table 9) :

Acid drugs, such 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%) and more than half of Ketoprofen and Naproxen is removed from the effluents of the plants.
The Chlofibric acid is not degraded durind water treatment process (6-8%) (Tauxe *et al.* 2003).

Regarding to the results obtained on WWTPs (Herry and Beausse, 2004), the concentrations of Estradiol and Estrone can reach 40 and 140 ng/l respectively in influents. For these two compounds, the efficiency of the plants is above 90%. For EE2, the concentrations in WW influent are lower (below 10 ng/l) and concerning the efficiency of WWTPs, the elimination of this substance depends on plant treatment caracteristics and can vary, on overage, between 10 and 75%. The sludges investigated by Löffler *et al.* 2003, contained up to 30ng/g Estrone, 50 ng/g Estradiol and 10 ng/g 17α•Ethinylestradiol.
According to 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 (Betalactam antibiotics) or neutral/basic forms are not easily removed on WWTP, acidic forms or more lipophilic compounds (Hormones, Anti-inflammatories, fluoroquinolones) are more effectively eliminated by adsorption on active sludge through hydrophobic interactions.

Table 9 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)	na	0.07, 0.40	46 - 69
Fenofibrate(b, d, e)	na	0.38	45 - 64
Bezafibrate (b, d, e)	na	2.2	50 - 83
Propranolol (d, e)	na	0.17	96
Metoprolol (d, e)	na	0.73	83
Roxithromycin (e)	na	0.68	na
Erythromycin-H_2O (e)	na	2.50	na
Sulfamethoxazole (e, g)	na	0.40, 0.90	na
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

6.2.2 Process conditions

The biological activity of sewage suggests that organic compounds would 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 sludge samples except the freshest (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, Roxithromycin and Estradiol, 20% for diazepam, Carbamazepine and Ibuprofen, contradictory results are obtained for Diclofenac and EE2. Under anaerobic conditions, E1 is reduced to E2 (Carballa *et al.* 2003).
For E1 and EE2, the maximum removal rate occurs under aerobic conditions. Substrate present in raw water influent competitively inhibits the degradation of E1 and E2, but not for 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 indicates that estrogens can be persistent during sludge digestion (Ternes *et al.* 2002). The activated sludge treatment step removes 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 greater 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), their results suggest sewage sludge as the main reservoir of FQs residues (table 10).
According to 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 10 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 cited 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 11, Khan and Ongerth 2002)

Table 11 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

6.3 Treated soils

Antibiotic pharmaceuticals enter agricultural soils essentially through the use of contaminated manure and sludge as fertilizers (table 12). Sorption and mobility for given compounds seem to be impacted by nature of soils.

Table 12 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 is generally higher in soil with 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 show a similar adsorption (Kd =285-496). In mineral clay, 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 added Tylosin had been leached to a depth of 5cm in the sandy loam and 25 cm in the sand soil (Rabølle and Suter 2000).

According to the results on soil-column experiments conducted by Thurman and Lindsey (2000), it is hypothesized that the Sulfamethazine is transported more rapidly through soil to ground water than the Tetracyclines, 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 occur in the first millimeters and at surface of liquid manure. But, under field conditions, photodecomposition is negligible compared to other processes for detoxification of antibiotics such as abiotic ageing for tetracycline (Thiele-Bruhn *et al.* 2003). In some results (Elsom *et al.* 2003), it was demonstrated the relatively Penicillin lability according to the lower soil half life degradation found below 6.5 days.

6.3.1 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 traces 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 respectively 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 8 and 21 months after application) can persist in agricultural soils several months after application (Table 13, Golet *et al.* 2002).

Table 13 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

6.3.2 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 manure (water solution ; 30 mm in 1.5 hours) and contact time in soil (1 and 3 days). Manure application leads to more surface water runoff and highter concentrations for studied Sulfadiazine and Sulfathiazole than water solution.
With manure slurry (>20 mg/Kg) 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, exept Chlortetracycline at 0.1 mg/kg in only one pure cattle manure. Tetracyclines are known to bind strongly to soil particles, due to their ability to form complexes with double 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 14).

Table 14 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 increased with increased 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, administrated to the pigs, is easily degradable and therefore not detectable as the parent compound in manure (Christian *et al.* 2003)

In liquid manure and treated soil, some antibiotics (Fluoroquinolones) could be found, respectively, at mg/kg and µg/kg level which indicated a stability of some pharmaceuticals, especially by considering, that the soil was manured at least several months before sampling, whereas other administered antibiotics (Betalactamides, Macrolides, Oxytetracycline, Tylosin) are degraded within a short time. In absence of accumulation in soil, a potential environmental risk is limited.

7. CONCLUSION

Since their identification in water, pharmaceutical compounds have been targeted as emerging environmental contaminants. Their physico-chemical properties (Log Kow, pKa, polarity….) show tendency towards 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 (Fluoroquinolones, Hormones, Avermectin, Tetracyclines). For some of them (Diclofenac, Oxytetracycline, Tylosin, Ibuprofen, Macrolides), partial or total biodegradation, may occur, possibly producing unknown metabolites more or less active than initial form.

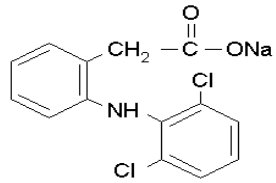
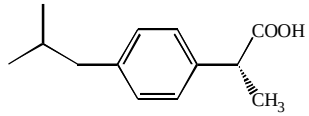
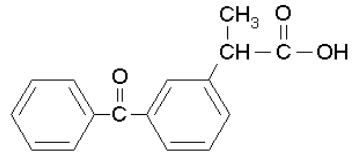
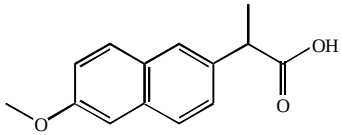
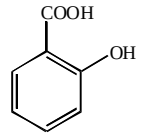
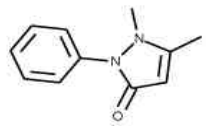
Regarding to this literatture review, amongst the different classes, the therapeutic groups for environmental solid matrices most concerned seem to be Steroid Hormones, Fluoroquinolones, Tetracyclines, Analgesics/Anti-Inflammatories and Avermectin (table15).

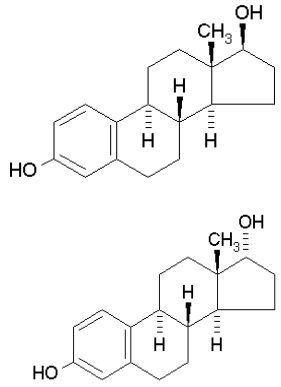
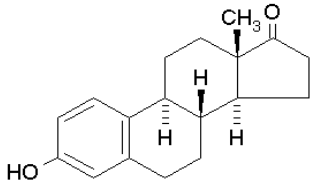
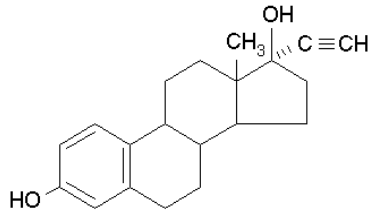
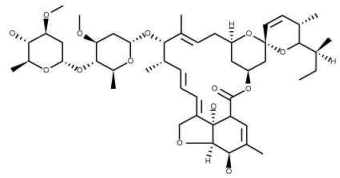
The purpose of the next work phase is to develop analytical methods for trace level determination in solid matrices of compounds extracted from identified predominant therapeutic classes.

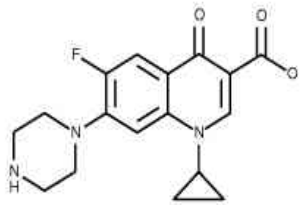
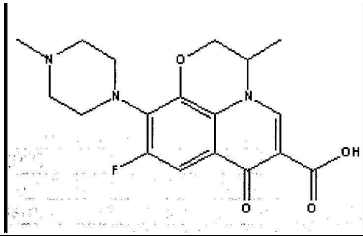
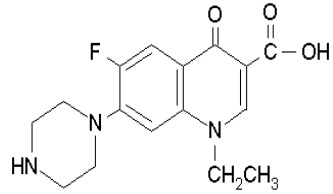
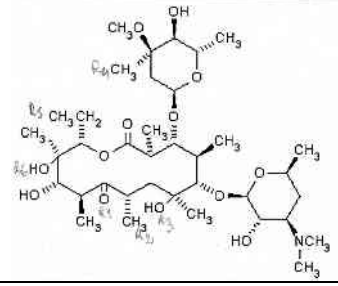
Concerning the analytical development, investigations were often limited to the analysis of selected individual compounds, because the diverse ranges of analytes require highly specialized sample preparations and enrichment procedures.

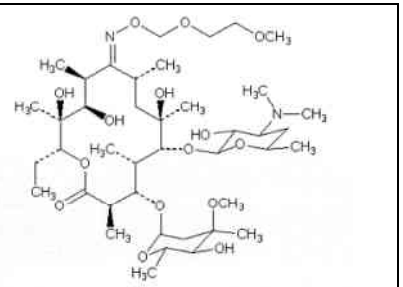
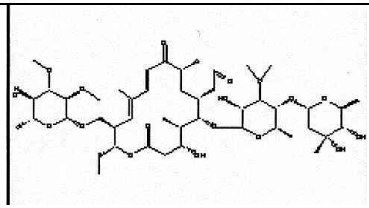
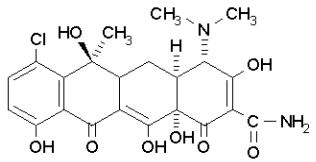
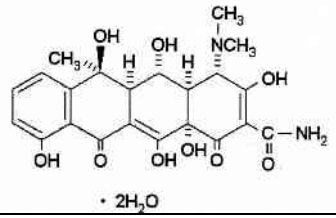
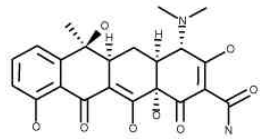
The project could be divided into 2 main parts:
- Development of extraction procedure for solid matrix (choice of mod and solvent extractions, clean-up step optimization),
- Evaluation of the analysis method in term of both sensibility (LOQ) and reproductibility (recovery, standard deviation, need of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

Table 15 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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