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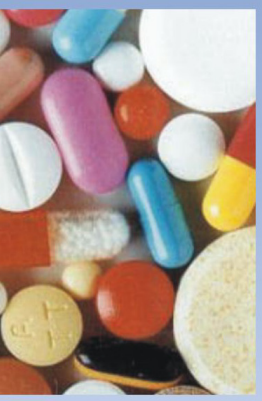
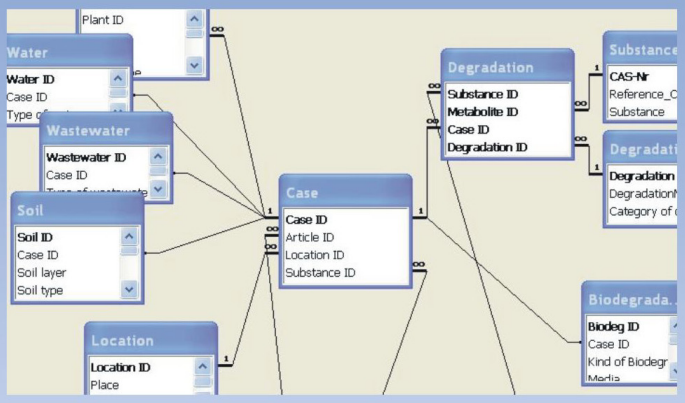
Martina Winker

Pharmaceutical Residues in Urine and Potential Risks related to Usage as Fertiliser in Agriculture





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Pharmaceutical residues in urine and potential risks related to usage as fertiliser in agriculture

Vom Promotionsausschuss der
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Martina Winker

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Not everything that counts can be counted,
and not everything that can be counted counts.

(Reputedly from Albert Einstein,
but probably from an unkown source
according to Calaprice (2000)[1])

[1] Calaprice A. (2000). *The Expanded Quotable Einstein*.
Princeton University Press and The Hebrew University of Jerusalem, USA.

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Abstract

Urine, also called yellowwater, is discussed as an alternative fertiliser for agriculture as it contains relatively high concentrations of the macronutrients nitrogen, phosphorus, and potassium. But this usage of urine includes the risk of spreading pharmaceutical residues on to agricultural fields. Little is known on the fate of pharmaceuticals regarding their accumulation in soils, transfer to groundwater, incorporation by plants, and effects towards microorganisms in soils in the case of fairly high concentrations of pharmaceuticals as expected to be found in urine. As today's wastewater treatment plants are not able to remove pharmaceuticals effectively, source separation of urine would protect surface waters from pharmaceuticals loads to some extent. The study was conducted with means of a database and an additional greenhouse experiment with rye grass.

It was possible to predict the concentrations of 124 active pharmaceutical agents in an average German urine (AGU) by means of pharmacokinetic data and information on pharmaceutical consumption in Germany, although the predicted values were higher than the measured ones. This was shown by a good correlation (R^2 of 0.90 to 0.98) of predicted concentrations with values measured in urine of large communities while correlation of predicted pharmaceutical concentrations in urine and measured ones in raw wastewater was poor.
Evaluation of literature focusing on pharmaceutical behaviour in soils provided poor results. Only for 11 active agents, all related to veterinary usage, was information available. None of them were detected below a soil depth of 90 cm. Screening of literature regarding the effects pharmaceuticals cause on plants resulted in scarce data material focusing on the two aspects of uptake into plants and phytotoxic effects. Finally, information was collected for 39 active agents and 44 plant species, but only 18 datasets were determined involving concentrations which might be relevant for urine fertilisation.
Carbamazepine and ibuprofen uptake by rye grass was investigated within pot experiments. Only carbamazepine was detected in the soil after a period of three months; 49 % of the applied amount was recovered in the soil. Plant matter production was not affected by the applied pharmaceutical concentrations but carbamazepine was taken up into roots (0.2 % of applied amount) and aerial plant parts (30 %). This confirms literature on the biodegradation behaviour of carbamazepine and ibuprofen in soil and leads to the assumption that pharmaceuticals not degraded in soil can be incorporated by plants.

No evaluation of toxic effects of pharmaceuticals ingested by humans through crops is possible at the moment from the findings of this research. However, concerns exist and as long as the concerns are not dispelled, it is recommended that urine from people under medication should not be used for fertilisation of food crops.

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List of Abbreviations

A	Austria
a	artificial
A_B	amount taken up into bloodstream
A_C	amount consumed
A_E	amount excreted
ACA	acetylsalicylic acid
AGU	average German urine
ANOVA	analysis of variance, statistical test
BEZ	bezafibrate
BPI	Bundesverband der Pharmazeutischen Industrie e.V.
C_{H2O}	pharmaceutical concentration in the water phase
C_M	mean concentration of all positively detected samples
C_{MW}	weighted mean concentration
C_P	overall mean concentration in the medium
C_{soil}	pharmaceutical concentration in the soil phase
C_U	concentration of pharmaceutical in urine
C_{UP}	concentration of pharmaceutical in urine of a single person
CH	Switzerland
CI	confidence interval
CZ	carbamazepine
DB	database
DDD	defined daily dose
DDD_G	defined daily dose for Germany
df	detection frequency
DIC	diclofenac
DM	dry matter
DS	datasets
E	excretion
E_R	excretion regarding resorption
E_C	excretion regarding consumption
EC_{50}	half maximal effect concentration
Ecosan	Ecological Sanitation
ecotox	ecotoxicology
EE2	17α-ethinylestradiol
EPA	U.S. Environmental Protection Agency
eq.	equation
E1	estrone
E2	17ß-estradiol
E3	estriol
f_{OC}	fraction of organic carbon

FEN	fenofibrate
FM	fresh matter
G	Germany
GACH	Germany, Austria, and Switzerland
HRT	Hormone Replacement Therapy
IBU	ibuprofen
ID	identification
IND	indomethacine
K	potassium
K_D	sorption coefficient
K_{OC}	sorption distribution coefficient normalized with respect to the fraction of organic carbon
K_{OW}	octanol-water partition coefficient
LOD	limit of detection
LOEC	lowest effect concentration
LOQ	limit of quantification
n	natural
n_i	number of individual concentrations
N^o.	number
$N\text{-}NH_4$	ammonium nitrogen
P	phosphorus
PBT index	Index merging persistence (P), bioaccumulation (B), and toxicity (T)
PEC	predicted environmental concentration
PEN	pentoxifylline
PHE	phenazone
PM	solution of pharmaceutical(s) and methanol
PNEC	predicted no effect concentration
Q_0	fraction eliminated non-renal by human beings
QSAR	qualitative structure-activity relationship
R	resorption
SD	standard deviation
SIT	ß-sitosterol
SMX	sulfamethoxazole
SMZ	sulfamethazine
Susan	Sustainable sanitation
TN	total nitrogen
TOC	total organic carbon
U	Annual amount of urine in Germany
UD	urine diversion
UPmix	Urine containing pharmaceuticals
VFA	Verband forschender Arzneimittelhersteller e.V.
WWTP	Wastewater treatment plant

3a	Combination of CZ, EE2, and IBU at artificial level
3n	Combination of CZ, EE2, and IBU at natural level

Indices

B	bloodstream
C	consumed / consumption
E	excreted / excretion
G	Germany
i	counter
M	mean
MW	weighted mean of one dataset
OC	organic carbon
P	overall weighted mean of all datasets
R	resorbed / resorption
U	urine
UP	urine of one person

1 Introduction

In recent years more and more pharmaceutical residues have been detected in surface, ground, and drinking waters (Daughton and Ternes, 1999). The main reason for this is the limited ability of today's conventional wastewater treatment to work as a barrier for pharmaceuticals (Calmano et al., 2001; Niederste-Hollenberg, 2003). The substances enter wastewater mainly via their usage in households (Heberer, 2002; Kümmerer, 2006). Pharmaceuticals are a very diverse group of chemicals. They vary with respect to types and amounts from year to year. Some substances have been known for over 20 years to enter the environment (Tabak and Bunch, 1970; Norpoth et al., 1973; Garrison et al., 1976; Hignite and Azarnoff, 1977). Therefore, pharmaceuticals dissipated into the environment are not a new phenomenon but became more aware to the public as chemical detection tools improved (Daughton and Ternes, 1999). Hence, it took time until the extent of pollution by these organic micropollutants was realised. Additionally, yearly amounts of prescribed active agents slightly increased in the last decade (29.5 bn DDD (= Defined daily dose, (WHO, 2005) in 1992 via 31.4 bn in 2003) (Arzneiverordnungs-Report 2004, 2004)). Due to this, potential concerns regarding pharmaceutical residues in the environment increased as reviewed by Kolpin et al. (2002): abnormal physiological processes and reproductive impairment, increased incidences of cancer, development of antibiotic-resistant bacteria, potential increased toxicity of chemical mixtures, and potential effects on humans and the aquatic environment not being approximately understood.

In a similar time span as detection of pharmaceuticals in our environment increased, alternative wastewater systems focusing on source control were developed firstly with emphasis on sanitation without water (Winblad and Kilama, 1985). This idea was further developed, the aspect of reuse gained of importance and is now widely known as Ecosan ("ecological sanitation") or Susan ("sustainable sanitation") (Otterpohl et al., 1997; Otterpohl et al., 2003). These concepts allow collection of separated wastewater streams as blackwater (toilet wastewater) and greywater (wastewater from sinks, showers, and washing machines) directly at the source (Otterpohl, 2002). By means of urine-diverting toilets even a separate collection of urine (yellowwater with/without flush water) and brownwater (faeces with/without flush water) became possible offering the advantage of separating the pathogens contained in faeces from the rather hygienically harmless urine (Höglund, 2001). Urine, considered as an excellent liquid plant fertiliser (Vinnerås and Jönsson, 2002; Simons and Clemens, 2003; Ganrot et al., 2007; Muskolus, 2008), contains large amounts of nutrients such as 80 % of nitrogen, 50 % of phosphorus, and 70 % of potassium usually present in domestic wastewater (Ciba Geigy AG, 1977; Larsen and Gujer, 1996; Otterpohl, 2002). Moreover, the recovery of human excreta to close the nutrient cycle can be guaranteed by their use as agricultural fertiliser especially as nutrients in urine are readily available to plants due to their occurrence as soluble ions: nitrogen in the

form of urea degrading to ammonium and nitrate, phosphorus mainly as ortho-phosphate ions; potassium ions; and sulphur as sulphate ions (Schönning, 2006). The best effects for direct application are achieved when the liquid is directly incorporated into the soil to minimise ammonia losses to the air. Another option is extraction of these nutrients through technical treatment (Behrendt et al., 2002; Gulyas et al., 2004; Mauer et al., 2006; Tettenborn et al., 2007). The fertilising effect of urine has been proved (Sasse and Ellmer, 2006; Ganrot et al., 2007). It was already known in the 19th century and discussed as “Latrinendünger” (fertiliser from pit latrines) by Wolff (1868).

Nevertheless, aside from these valuable properties urine also contains pharmaceuticals: around 70 % are excreted via urine accounting for 50 % of the overall ecotoxicological risk (Lienert et al., 2007a; Lienert et al., 2007b). Currently, only little knowledge exists regarding these constituents and their potential effects on our environment upon urine application to agricultural fields. Hence, if urine shall be applied as fertiliser, then more information is needed, i.e. the occurrence and concentrations of pharmaceuticals in urine, behaviour and accumulation of pharmaceuticals in soils and the related ecosystem as e.g. their potential uptake into crops. The “Düngemittelverordnung” (German ordinance on fertiliser, (Bundesministerium der Justiz, 2007)) states that admission of a fertiliser will not be effected “*if containing concentrations of toxic or pharmacologically active substances relevant for the health of people or domestic animals in case of adequate application*”. Therefore, the questions referred to above need urgent answers to be included into the discussion of safe usage of urine in agriculture.

To reduce this knowledge gap and to determine the required research needed, this study tried to find answers on the following questions exploiting the knowledge available in this field and by means of additional experiments:

- Which pharmaceutical residues have been detected in our environment so far?
- Do they originate from the urine discharged to our municipal wastewater and in which concentrations can they be expected in urine?
- What is their impact on the aqueous environment when urine is discharged to sewers?
- What can be concluded from research results already accomplished in the field of pharmaceuticals in soils and their uptake into plants?
- Which are the relevant properties responsible for pharmaceuticals’ behaviour in the ecosystem soil and how far can their behaviour be predicted?

These aims were achieved in two phases (see Figure 1). Phase I contained a theoretical screening of existing literature relevant to the subject. The information was fed into a database. Phase II included practical investigations by plant experiments with three representative pharmaceuticals to evaluate the theoretical

assumptions developed during the screening. Results of both phases were utilised for an analysis and a common evaluation of the situation and our state of knowledge.

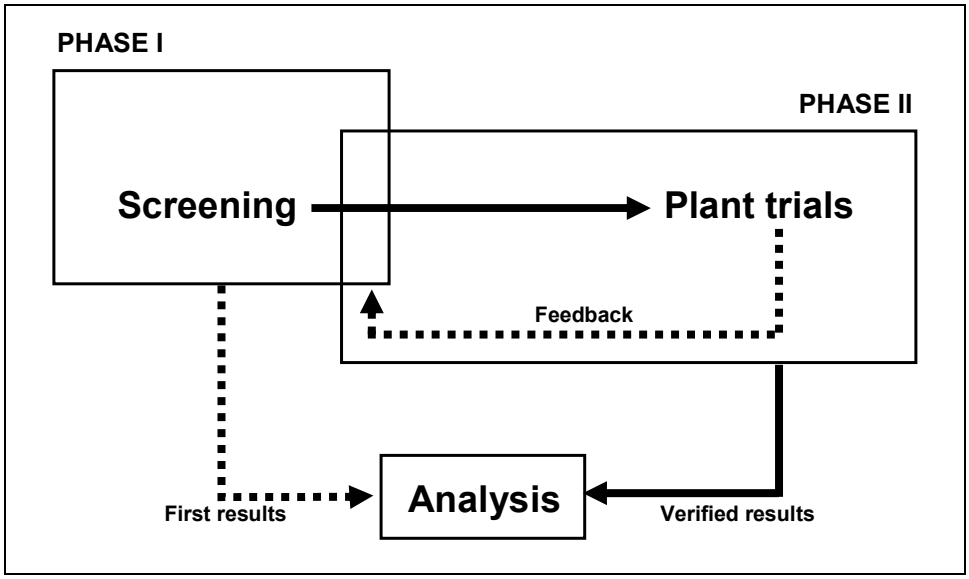

Figure 1: Schematic sketch of the study.

2 Materials and methods

2.1 Screening via database

2.1.1 Database (DB)

Phase I involved a screening of the relevant literature which had reported the detection of pharmaceuticals in the environment. The idea behind this approach was that on one hand an overview of substances would be generated which could be excreted via urine and reach the aqueous environment. On the other hand, information was collected regarding the concentrations of these pharmaceuticals in the ecosystem "soil" to determine its existing load and to learn more about the system's functions with respect to degradation and transport of the pharmaceuticals detected in soil. The study was conducted with emphasis on the German environment. Nevertheless, articles reporting from countries all over the world were included. The gathered information was fed into a database established for this purpose. It was based upon Access 2002 (Microsoft Office). A rough outline of the DB is given in Figure 2.

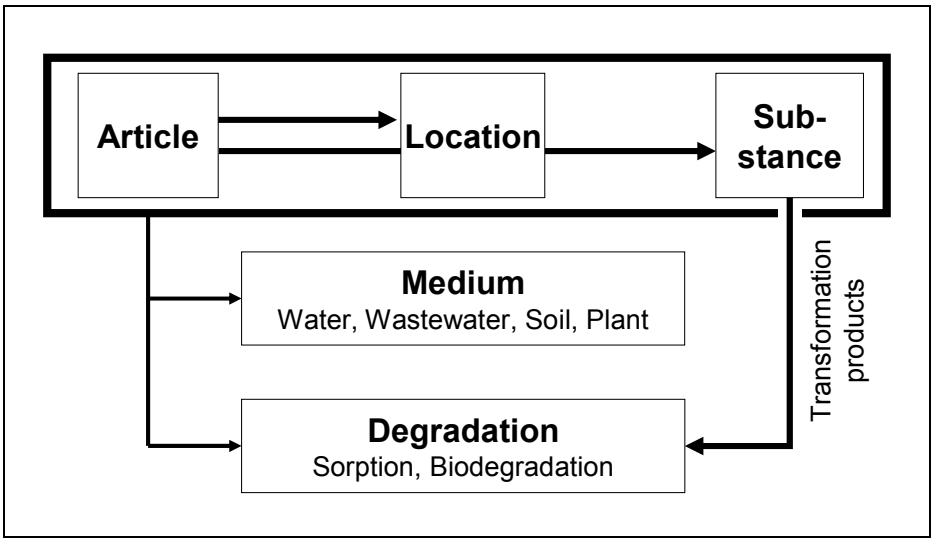

Figure 2: Outline of database structure and its specific sections (in bold letters). Subparts were *Water*, *Wastewater*, *Soil*, *Plant*, *Sorption*, and *Biodegradation*. *Transformation products* belong to the section *Substance* and were determined as *Transformation products* through the link to *Degradation*.

One dataset (also called "case") comprises the sections *Article*, *Location*, *Substance*, *Medium*, *Degradation*, and *Transformation products*. Not all parts were always compulsory due to the fact that some literature did not include information for all of them. As an outcome of this situation the following setup results was founded:

The starting point was always an article to be evaluated representing aspects relevant for being fed into the DB. In a next step the information was analysed and structured for entering into the DB. Each case (i.e. a dataset for one pharmaceutical in a particular medium from one literature source) became a separate identification number based upon the specific *Article* (article ID, name of the author, magazine, year, country), *Location* (location ID, name, county, country), and *Substance* (CAS-N°. (used as ID for each substance), name, properties).
So, it was possible to distinguish a certain case and relate a specific article, location, and substance to its ID number at anytime. Each case was unique in its combination of these three aspects which together caused its specific character. On the other hand, all information from one article regarding a certain location and substance was contained in one case by this procedure.

As a matter of fact these three sections (each enclosed in the database as a table) cannot comprise all of the relevant information. Therefore, the sections *Medium* and *Degradation* as well as the link (between *Substance* and *Degradation*) to determine *Transformation products* were created to save details of each case.
Firstly, the environmental compartment or "medium" (*Water*, *Wastewater*, *Soil*, or *Plant*) was recorded as well as the concentrations of the pharmaceutical and its method of analysis. Moreover, environmental conditions during sampling were added to the dataset. Since all media differed in their characteristic properties, separate entry forms were used. Secondly, often one case contained input information referring to different aspects of the same *Medium* as well as to different subparts. Hence, each case holds various datasets. In the case of *Plant* aside from datasets reporting on measurements in the environment additional datasets on uptake and behaviour in laboratory investigations were included as this was a key aspect of the study.
In *Degradation* the mode of degradation and its efficiency was described, as well as transformation products where applicable. Moreover, two subparts of *Degradation* were available: *Sorption* and *Biodegradation*. As these two processes are of high priority when it comes to activity of pharmaceuticals in soil, they were operated separately with additional entry fields for specific information relevant to these processes.
Pharmaceuticals contained in *Substance* could also be stated as transformation product in any degradation process mentioned under *Degradation*. In such a case, its identity as *Transformation products* was indicated as virtual link from *Substance* to *Degradation*. Hence, each pharmaceutical contained in *Substance* could become a transformation product if stated in the respective article and the link was activated.

The core within the database was *Substance*. This table contained the relevant information of all substances found in the environment as recorded during the review process. The properties were CAS-N°., indication, usage in veterinary and/or human medicine, the octanol-water coefficient, estimated yearly amounts which are

consumed, pharmacokinetic data regarding resorption, excretion and excreted metabolites, and ecotoxicological data referring to three different trophic levels (fish, daphnia, and algae). Most parameters were collected during the literature screening. However, for annual consumption amounts this was impossible because yearly production of pharmaceuticals in Germany is confidential information and reliable data is not published (Heberer, 2002; confirmed by Prof. Eschenhagen, University Medical Center Hamburg-Eppendorf, Germany [personal communication]). A detailed description of how data of the different properties was accomplished is given in chapter 2.2.

2.1.2 Revised literature

A large amount of literature dealing with pharmaceuticals in the environment exists. In addition, the selection of literature has a major input on the results that the database is delivering. Hence, selection of literature used in the DB is of major interest. The goal was to enter as much as possible but at the same time guarantee quality and diversity. A package of strategies was developed which was able to achieve both aims.

Strategy 1: Recommendations
Through experience of researchers working in wastewater management, agriculture, process engineering, environmental research, pharmacology, and pharmaceutics an initial list of interesting literature was provided. Additionally, articles were chosen due to recommendations and by knowledge exchange during the duration of this work.

Strategy 2: Conferences
This strategy worked in a similar fashion to strategy 1. A lot of national as well as international conferences are held in this research field every year. Thus, many interesting and especially more recent research aspects and results are published and conducted through conference proceedings. These proceedings were scanned for the DB. It is kept in mind that these articles are often not peer-reviewed.

Strategy 3: Citation list
In each evaluated article many references were given. Therefore, further articles were easily identified. This information was saved in a list (name of first author, year of publication, start of title) and each time a citation of this article was found again, this was marked in the list. After some time, articles could be chosen with the criterion of the numbers of their citations. Additionally, comments were inserted regarding their topic and subject. This was helpful in their selection referring to a specific topic later on. Finally, around 1200 articles were listed and 700 read.

Strategy 4: Screening of journals
After some time it became very clear which journals publish papers of interest, and these journals were screened systematically. For avoidance of getting too many articles, screening started with the first volume of 2005 and ended in April 2007. These articles document latest research results and could not be found with strategy 3 (reference list) as they were too up-to-date to accumulate larger numbers of citations until now.

2.2 Mode of entry into database

Data had to undergo a certain transformation before it could be entered into the DB. To provide transparency regarding the output of the DB, a careful documentation was indispensable with regards to how data input was deducted.

2.2.1 Amounts of substances consumed

The annually consumed amounts had to be calculated, as they are not available for the public in Germany (Heberer, 2002), before they could be entered in *Substance*.
Theoretical annually consumed amounts (A_C) were calculated using the following equation:

$$A_C = DDD_G \bullet \textit{annually prescribed number of DDDs} \bullet f_C \quad (1)$$

DDD means daily defined dose (WHO, 2005). Contrasting to average DDDs given by WHO (2005), ranges of German DDDs (DDD_G - minimum to maximum daily doses) were available from literature (Therapeutic drugs, 1999; Mutschler et al., 2001; Baselt, 2004; FachInfo-Service, 2005; Scholz and Schwabe, 2005; DIMDI, 2006; Goodman et al., 2006; Arzneimittel-Kompendium der Schweiz, 2007) to verify the applied approach. The annually prescribed numbers of DDDs in Germany were taken from the 2004 edition of an annually published report on prescription: “Arzneiverordnungs-Report 2004” (Arzneiverordnungs-Report 2004, 2004). The 3000 leading pharmaceuticals are listed within this report. These pharmaceuticals contain 466 active substances with the largest amount being reported for diclofenac with 2.6 Mio annual prescriptions and the smallest for vancomycin with 30.000 (Arzneiverordnungs-Report 2004, 2004). f_C is a factor explained in detail in 2.3.1.
Additionally, as Huschek et al. (2003) was the only source of information publicly available, mean calculated amounts were compared with amounts provided in Huschek et al. (2003) (Figure 3). This was done although data reported there focus on A_C data of 1999-2000, while A_C data accomplished within this study are based on 2003. Data of Huschek et al. (2003) were not included into the DB as not for all active agents collected in *Substance*, information was available in that reference. It was considered as important to use only data determined by the same estimation

procedure. Figure 3 shows that A_C datasets determined within this study and by Huschek et al. (2003) correlated, although with an R^2 of 56 %. Poor correlation was obviously due to the long time span. Especially, when looking at A_C of particular years: R^2 improved from 50 % (1999) to 63 % (2001), see Figure 3 right graph.

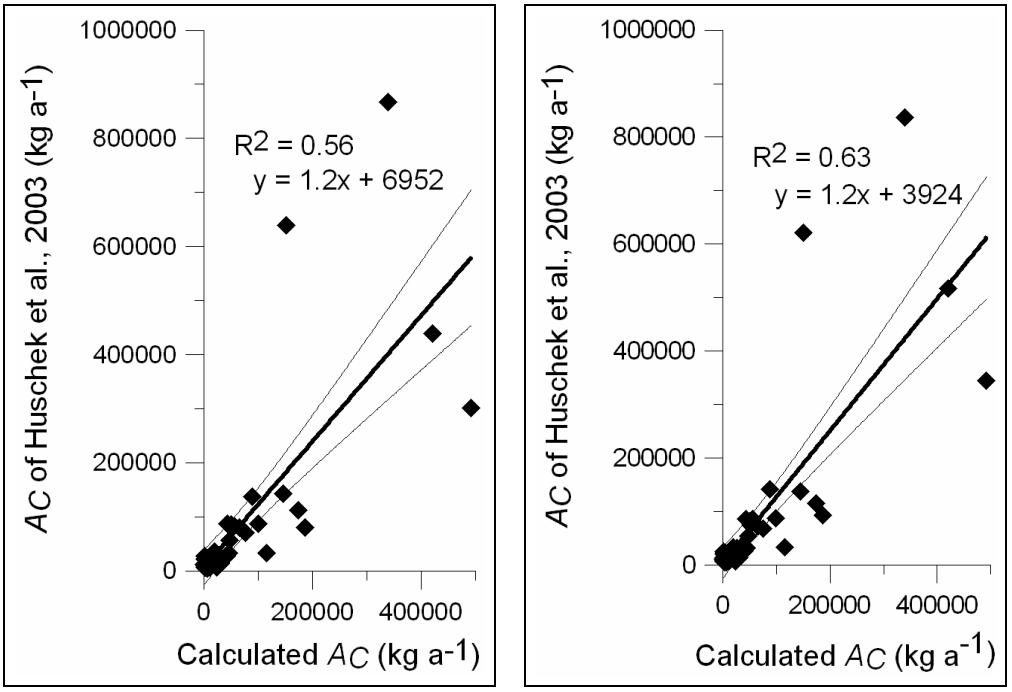

Figure 3: Comparison of A_Cs calculated within this study with those conducted in Huschek et al. (2003). Left graph: mean A_C of the years 1999-2001 were considered from Huschek et al. (2003). Right graph: only A_C reported for 2001 were taken. (April 16, 2008). Thinner lines show 95 % confidence interval.

As stated above, calculation of consumed amounts was based on Arzneiverordnungs-Report 2004 (2004). Hence, the starting point was given by amounts documented which were based on prescriptions. Therefore, pharmaceuticals mainly prescribed in hospitals or sold over the counter – which were not stated in Arzneiverordnungs-Report 2004 (2004) – could not be considered. A_C could thus not be calculated with data derived from Arzneiverordnungs-Report 2004 (2004) for a couple of pharmaceuticals and were based on data of Huschek et al. (2003) during this assessment. Regardless of the earlier stated considerations of equity, this procedure was followed for glutaral, iohexol, iomeprol, iopamidol, iopromide, iothalamic acid, ioxithalamic acid, mezlocillin, and piperacillin. These additional modes of pharmaceutical distribution were taken into account via overall adjustments during the queries (2.3.2). For the comparisons presented in Figure 3, those adjustments were already considered.

Overall, three different types of data were selected for entry into the database (Figure 4): DDD_G (see "German DDD"), annually prescribed number of DDDs, and annually consumed amounts (A_C).

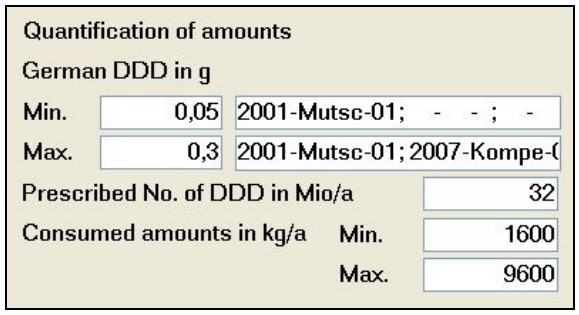

Figure 4: Layout of entry field "Amounts of substances consumed" in DB

Some specific modalities were defined and used:

- For the given DDD_G, only the maximum and minimum doses were considered; certainly also values between these extremes exist. Additionally, it has to be stated that some active agents are either taken as drugs containing only this one active substance or as a combination with other active agents. The latter is often accompanied by a different dosing amount from the DDD of one-agent drugs. To avoid confusions: in the DB only DDD_G were entered for those drugs containing one agent (except benzalkonium chloride and estradiol valerate which are exclusively prescribed as combination products). For "Prescribed N^o. of DDD in Mio a^{-1}" the sum of single and combination products was entered in the DB. In the final calculated amounts, A_C, both aspects of single and combined drugs were included.
- In general, the following rule was always respected: if only one value was available as daily dose or as A_C it was set as the minimum value. By this, the "worst case" was kept in mind assuming that higher values for A_C might always be possible.
- Moreover, the following assumptions were included:
    - creams: 2-10 g was defined as one application to be applied to the skin.
    - eye drops: 0.5-0.7 ml were used within one application corresponding to two drops.

2.2.2 Pharmacokinetic data

Another important aspect in *Substance* was the behaviour of pharmaceuticals in the body. Their modifications in the organism were important to consider especially for estimating the quality and quantity of the final product released via urine particularly as analytical data on pharmaceutical concentrations in urine is only scarcely

available (Winker et al., 2008c). Hence, the theoretical determination was necessary to achieve those values.

The behaviour of pharmaceuticals was split into three consecutive phases during their passage through the human body (Mutschler et al., 2001):

- Pharmaceutical phase: application and break-up of the applied form.
- Pharmacological phase: resorption, distribution, storage, and elimination.
- Pharmacodynamic phase: pharmacological effect (therapeutic or toxic).

The pharmacological phase was in focus of this study as the interest laid on determination of renal excretion rates (E). First, resorption of the substance into the bloodstream was considered. Resorption (R) is the rate of the amount of an active agent consumed (A_C) to the amount taken up into the bloodstream (A_B) and thereby distributed through the body. Only resorbed agents are released via urine (Faika, 2006).

$$R = A_B / A_C \quad (2)$$

Upon resorption, elimination was considered. As it was not possible for all pharmaceuticals to clarify whether excretion data was based upon resorbed (A_B) or on consumed (A_C) amounts of a pharmaceutical (Faika, 2006), both aspects were considered by referring to excretion regarding resorption (E_R) and excretion regarding consumption (E_C) data. A_E represents the amount excreted.

$$E_R = A_E / A_B = 1 - Q_0 \quad (3)$$

$$E_C = A_E / A_C = E_R \bullet A_B / A_C = E_R \bullet R \quad (4)$$

Excretion with regards to resorption (E_R) contains information which was explicitly related to resorption (R) in the sources or was calculated by means of Q_0 values. The Q_0 value is defined by Forth et al. (2001) as the non-renal elimination fraction, i.e. the percentage of the resorbed dose which is either metabolised or excreted unchanged by pathways other than renal. Therefore, the value 1-Q_0 is the fraction which was excreted unchanged via urine. Moreover, it always has to be kept in mind that the specific mode of application (oral, intravenous, rectal, dermal) determinates the resorption rate. Excretion regarding consumption (E_C) contains all data which is stated implicitly to be independent of resorption (R).

Pharmacokinetic data was integrated into the DB in percent (Figure 5). Minimum and maximum values are always given according to values found in different pharmaceutical text books and essays.

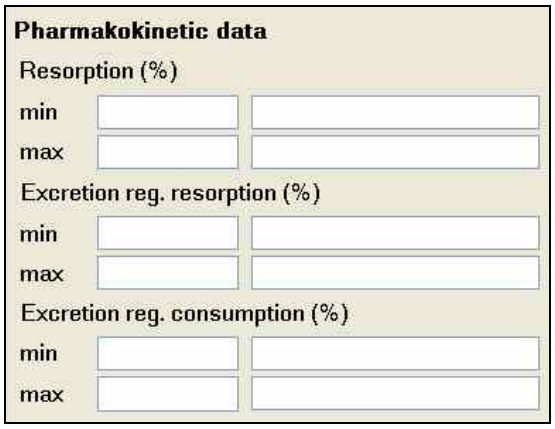

Figure 5: Layout of entry field “pharmacokinetic data” in DB. In the left fields data is fed and in the right one the respective references.

Additionally, the following rules were considered:

- If only on value was available, it was entered as maximum
- Figures were rounded up always at “full percent level”
- Semi-quantitative information was transferred in a quite instinctive way into numbers according to Table 1.

Moreover, it has to be mentioned that excretion rates of human beings are also influenced by age, renal failure, liver disease and other illnesses, as well as by interactions with other drugs (Johnsson and Regårdh, 1967; Faigle and Schenlek, 1998); facts which could not be regarded in these estimations.

Table 1: Overview of how semi-quantitative information for pharmacokinetic data was transferred before entry into DB.

Pharmacokinetic phenomenon	Written information	Equivalent to *R* (%)	Equivalent to E_R or E_C (%)
Resorption (*R*)	none	0	-
	not relevant	2	-
	little/few	15	-
	predominantly	66	-
	good	75	-
	almost completely/ extensive	95	-
	completely	100	-
Excretion (E_R or E_C)	none	-	0
	not relevant	-	2
	in traces	-	5
	little/few	-	15
	predominantly	-	66
	almost completely	-	95
	completely	-	100

2.2.3 Veterinary pharmaceuticals

Many pharmaceuticals are not only used in human medicine but also in animal health care (Díaz-Cruz et al., 2003; Huschek et al., 2004; Hammer and Clemens, 2007). Therefore, it is important when analysing the appearance of pharmaceuticals and their residues in the environment to know which of these substances may result from veterinary activities. The fate in soil of any active substances originating from veterinary medicine was earlier in focus of research than for human substances.
The aspect of veterinary usage is part of *Substance*. Information is entered according to the explanation given in Table 2.

Table 2: Mode of entry and its meaning for the aspect of veterinary usage of pharmaceuticals

Entry mode	Significance
yes	It is used in veterinary medicine.
no	It is not used in veterinary medicine.
both	It is used in both fields.
only	Exclusively used in animal health.
earlier	It was used in the past but is not allowed for medication of animals anymore.

2.2.4 Ecotoxicological data

Another focus was the development of a set of parameters for *Substance* to include possible toxic effects of pharmaceutical residues in the environment and how this aspect can be implemented later on as an indicator for the overall evaluation of the environmental hazards caused by pharmaceuticals. Soil systems and ecotoxicity in soils are only very scarcely investigated. E.g. in the large Ecotox database of the U.S. EPA (2006) ecotox data toward soil organisms for only five pharmaceutical substances is noted: acetylsalicylic acid (2 datasets), etidronic acid (2), formaldehyde (6), hydroquinone (6), phenol (4) and salicylic acid (2). Hence, aquatic toxicity data were used, but only as qualitative parameters for the possible effects which can be expected in soil systems as well. That pharmaceuticals can have an impact on soils is out of question; they can influence the degradation of organic matter as well as the composition of the soil-dwelling biocenosis (Jjemba, 2002; Alexy et al., 2004). Of special concern are antibiotics which might lead to the development of a resistance (Alexy et al., 2004).

EC_{50} values
Different types of toxicity were studied. As various endpoints, differences in time (acute/chronic tests), tested organisms (fish, algae, daphnia, etc.) and two major kinds of tests (in-vitro and in-vivo) exist, it was important to determine a setting with overall availability for as many pharmaceutical residues as possible. Hence, EC_{50} values for daphnia, algae, and fish were selected as appropriate characteristics thus

including representatives of the three trophic levels. EC_{50} denotes a concentration causing adverse effects (with respect to the chosen endpoint) to 50 % of the test population. When more than one value was available for one of the three categories the lowest EC_{50} value was kept in the DB supporting a "worst case" scenario.

Octanol-water partition coefficient

Baseline toxicity is the most general approach. Other terms are nonspecific toxicity (Öberg, 2004) or narcosis (Escher and Hermens, 2002) / non-polar narcosis (Schultz (1989) cited in Schultz, 1989) since it is the minimum toxicity exhibited by any organic compound. Narcosis is related to the hydrophobicity of the compound (Sanderson et al., 2004). It can be understood as a non-specific disturbance/disruption of the function of biological membranes and is a result of partitioning of pollutants into membranes, although the detailed mechanism remains unclear (Escher and Hermens, 2002; Öberg, 2004). Baseline toxicity can be the selected endpoint of an EC_{50} test.

A good indicator for baseline toxicity is the octanol-water partition coefficient (K_{OW}) (Sanderson et al., 2004). Therefore, this indicator was entered as additional parameter to evaluate ecotoxicology of pharmaceutical residues in the environment. Additionally, K_{OW} allows estimations of the sorption ability of pharmaceuticals to organic matter in soil (see 2.2.5) and thus on their potential to reach the groundwater. To gather this information for as many substances as possible, theoretical as well as experimental K_{OW} values were considered due to the fact that measured data was not available for all substances. The widest selection was found in Hansch et al. (1995). This was taken as basic source especially as herein very detailed information including the pH values corresponding to the measured values was provided. This is very important, as K_{OW} of compounds dissociating in water depends on pH (Holten-Lützhøft et al., 2000). K_{OW} of those substances not included in that collection were added from other sources. As a starting basis for the theoretical values, the large dataset provided by Syracure Research Corporation (2004) was used and then completed by others.

K_{OW} data were integrated into the DB as their decadic logarithms (log K_{OW}).

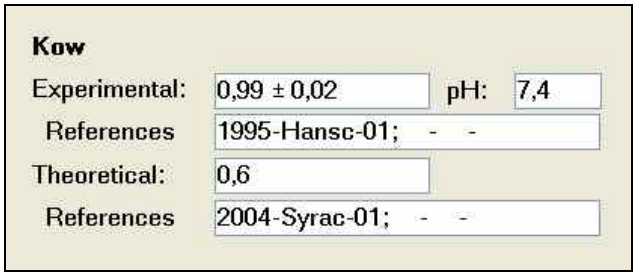

Figure 6: Layout of entry field "Octanol-water partition coefficient (K_{OW})". K_{OW} data were integrated into the DB as their decadic logarithms (log K_{OW}).

2.2.5 Sorption and biodegradation data

Another important aspect is the behaviour of pharmaceutical residues when they come into contact with soil. Organic pollutants can be taken up into the biological cycle and even reach our food (Gisi et al., 1997). This means they are degraded, i.e. transformed or mineralised. Those remaining in solution can be taken up by plants or reach the groundwater (Gisi et al., 1997). The persistence of a drug in soil depends mostly on its photostability (when it is at the surface), its binding and adsorption capacity to soil constituents, and its degradation rate (Díaz-Cruz et al., 2003). As in the case of urine application, its immediate incorporation in soil is suggested (Muskolus, 2008), and decay by irradiation can be excluded.

Sorption is influenced by many chemical and soil properties (Shaw and Chadwick, 1998; OECD, 2000; Rexilius and Blume, 2004). Of major importance are water solubility, pH of the matrix (in case of dissociation in water), volatility and sorption potential of soil (Boxall et al., 2003). This potential is mostly influenced by the organic matter fraction of soil (Thiele-Bruhn, 2003; Thiele-Bruhn et al., 2004). Moreover, sorption is related to K_{OW} (see 2.2.4) which can be used to estimate the sorption capacity of a chemical (Huschek et al., 2003; Jones et al., 2005), but prediction is difficult as information on organic carbon content of soil is missing (Jones et al., 2005). Better parameters to quantify sorption in soil are K_D and K_{OC}. K_D is determined as the ratio between the concentration in soil versus in the aqueous phase. As organic carbon content (f_{OC}) is still included in this relation, K_{OC} factors this out to achieve a soil-independent measure of pharmaceuticals' mobility and sorption (Gustafson, 1989):

$$K_{OC} = K_D / f_{oc} \quad (5)$$

Biodegradation is an important process in the environment (Alexy et al., 2004) and can be defined as "*the biologically catalysed reduction in complexity of chemicals*" (Alexander, 1999). It is influenced by pH, temperature, presence and diversity of microorganisms as well as the presence of manure in agricultural fields (Gavalchin and Katz, 1994; Boxall et al., 2004). The molecular structure determines their biodegradability, e.g. aromatic compounds with sulphate or halogen groups generally show a lower degradation rate (Jones et al., 2005).

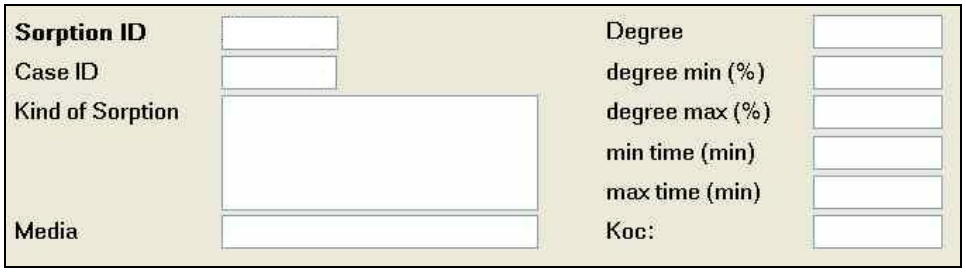

Figure 7: Layout of entry field "Sorption" into DB. "Biodegradation" looks similar. In case of *Sorption*, the entry field additionally contains the K_{OC} parameter.

The information on soil processes was collected in the *Sorption* and *Biodegradation* fields. Both are structured according to Figure 7. Information on details such as process conditions and investigated material were saved in the fields "Kind of Sorption/Biodegradation" and "Media". Details on sorption/biodegradation were saved in "degree min/max" and "min/max time" as far as they were available. The semi-quantitative information was entered in "Degree" as no, low, medium, and high.
For *Sorption*, information derived by K_{OC} was classified into five categories according to Shaw and Chadwick (1998) and Rexilius and Blume (2004) to allow for a more general interpretation of the specific behaviour of the respective pharmaceuticals in soil.

Table 3: Interpretation of K_{OC} values regarding sorption and mobility potential of respective pharmaceuticals (Shaw and Chadwick, 1998; Rexilius and Blume, 2004).

K_{OC}	Sorption	Mobility
<15	no – very low	very mobile
15-74	low	mobile
75-499	medium	moderately mobile
500-4000	high	slightly mobile
>4000	very high	non mobile

In the case of *Biodegradation*, interpretation of collected data was not as easy as for the sorption capacity. Data was collected according the data sheet shown in Figure 7. Aside from the respective degree to which biodegradation occurred, the corresponding time period was also noted. To compare datasets with each other, an overall valid parameter including rate of degradation in correlation with time was needed. As kinetics of degradation were mainly unknown but do not occur in a linear mode, it was not possible to achieve such a parameter. Therefore, as a compromise, data was sorted according their given minimum and maximum biodegradability in %. To relate the developed biodegradation capacity with time, the specific time period was added.

2.3 Analysis of database

The database was analysed by queries. Due to the fact that different quantities and qualities of data exist sometimes for the same pharmaceutical, data queries are described separately for the different key topics. Additionally, many queries evaluated within this work are provided online below https://www.tu-harburg.de/aww/pharma/.

2.3.1 Calculation of pharmaceutical concentrations in urine

For an "average German urine" (AGU)
Experimental data of pharmaceuticals' concentrations in urine derived from large groups are only scarcely available (Strompen et al., 2003; Tettenborn et al., 2007). Most data reports base on limited monitoring campaigns with small numbers of individuals and pharmaceuticals investigated or are clinical investigations of single persons or groups under medication. Hence, they do not represent the real situation concerning average pharmaceutical concentrations in urine of the entire population. This fact made calculations of excreted amounts necessary using pharmacokinetic data from text books. In a first step, total consumed amounts were required (see 2.2.1).

In a second step, the determination of renal excretion rates (E) (for details see 2.2.2) for the calculation of excreted amounts was necessary (eq. 6):

$$E = E_C = R \bullet E_R \quad (6)$$

Moreover, the overall annual amount of urine (U) excreted in Germany was calculated by eq. 7 based on 1.24 l of urine excreted per person and day (Ciba Geigy AG, 1977) and the population figure of Germany (Statistisches Bundesamt, 2006):

$$U = 1.24\ l\ pers^{-1}\ d^{-1} \bullet 365\ d\ a^{-1} \bullet 82.5 \bullet 10^6\ pers = 37.2 \bullet 10^9\ l\ a^{-1} \quad (7)$$

With the calculated data (consumed amounts (A_C - eq. 1, see 2.2.1), excretion rates (E - eq. 6, see 2.2.2), and German amount of urine (U - eq. 7)) the concentrations of the substances in yellowwater (C_U) were estimated:

$$C_U = (A_C \bullet E) \bullet U^{-1} \quad (8)$$

Pharmacokinetics represent very complex processes. It always has to be considered that the mode of application (oral, intravenous, rectal, dermal) determines the resorption rate. As a worst-case scenario was considered here, always the mode with the highest resorption rate was used for calculation. Furthermore, an homogeneous distribution was assumed for the concentration of pharmaceuticals in

urine. Also, the seasonal differences cannot be regarded e.g. a more extensive application caused by an increase of rheumatic diseases due to the cold weather (Heberer et al., 2002). Similarly, interactions with food, age, health conditions, gender, other pharmaceuticals as well as effects of diet and drugs like alcohol, cigarettes, coffee and other caffeine containing drinks (Faigle and Schenlek, 1998; Mutschler et al., 2001) were not reflected upon.

Also, natural hormones were subject of the study. These substances are administered for medical reasons and excreted from particular population groups without any medication at the same time. They had to be calculated differently as their concentration in urine does not only depend on consumed amounts and their respective excretion rate, but also on gender, age of individuals and individual metabolic processes. Therefore, the natural hormones were calculated with respect to the different population groups (Table 4) excreting them naturally. Exemplarily, this method is demonstrated here for the three most popular hormones estrone (E1), 17ß-estradiol (E2), and estriol (E3). Moreover, andorsterone, progesterone, testosterone, and cholesterol were calculated alongside this procedure.

Table 4: Daily concentrations of the natural hormones E1, E2, and E3 excreted in female and male urine for different population groups without any hormone medication and numbers of persons represented by the respective population group.

Substance	Min. excreted concentration (µg l^{-1})	Max. excreted concentration (µg l^{-1})
Women aged between 19 and 64 – 25.1 million* (D'Ascenzo et al., 2003)		
Estrone	5.6	25.8
17ß-Estradiol	1.9	11.3
Estriol	3.7	85.5
Women aged above 64 – 9.9 million* (Key et al., 1996)		
Estrone	-	1.1
17ß-Estradiol	-	0.6
Estriol	-	1.3
Men aged above 18 – 32.9 million* (Dao et al., 1973)		
Estrone	-	2.4
17ß-Estradiol	-	1.3
Estriol	-	2.8

* According to Statistisches Bundesamt (2006).

At first, the naturally occurring mass flows of E1, E2, and E3 annually excreted with urine were estimated based on the respective data in Table 4. As an example the calculation of the amount of E1 is shown for the population group of women aged more than 64 years:

$$\textit{Mass flow} = \textit{Excreted concentration [µg } l^{-1}\textit{]} \bullet \textit{Number of population group [pers]} \bullet 365\ d\ a^{-1} \bullet 1.24\ l\ pers^{-1}\ d^{-1} \quad (9)$$

$$= 1.1\ \mu g\ l^{-1} \bullet 9.9*10^{6}\ pers \bullet 365\ d\ a^{-1} \bullet 1.24\ l\ pers^{-1}\ d^{-1} = 4.93\ kg\ a^{-1}$$

E1, E2, and E3 are natural hormones as well as being synthetic products for use in Hormone Replacement Therapy (HRT) for menopausal women. Their final concentrations in AGU were calculated based on the sum of both prescribed amount and natural excretion. Natural renal hormone excretion of the population group between 0 and 18 years was neglected as no data was available and their contribution is ≤5 % (CBS, 2002). Calculated mass flows of hormones naturally excreted with urine of the three population groups were then added and related to assumed total urine volume flow in Germany resulting in predicted naturally occurring concentrations in AGU.

Moreover, similar calculations were accomplished for ß-sitosterol. Its daily uptake rates with food are between 150 and 300 mg cap^{-1} d^{-1} (Scholz and Schwabe, 2005). Taking into account the overall German population of 82.5 million people (Statistisches Bundesamt, 2006) plus the prescribed amount (2310 to 5005 kg a^{-1}) the consumption resulted in figures between 4.5 million and 9.0 million kg a^{-1}. This shows the contribution of prescribed ß-sitosterol as being negligible.

Aside from these considerations, other important factors, represented in f_C (see also equ. 1) such as consumer behaviour and mechanisms within the German pharmaceutical market influence the annually consumed quantities. Using the Arzneiverordnungs-Report 2004 (2004), only the amounts prescribed by practitioners and sold via pharmacies were included. Therefore, products sold additionally e.g. without prescription (around 19-22 % of the prescribed amounts) and those distributed via hospitals (around 12 % extra) are not contained in Arzneiverordnungs-Report 2004 (2004) according to BPI (2006) and VFA (2006). Even though the annual amounts of pharmaceuticals sold were known exactly, no data exist on amounts actually consumed by people. Therefore, further approximations are necessary. Referring to literature sources, the consumption is between 66 % (Schweim, 2006) and 80 % (Huschek, IUQ-Institut für Umweltschutz und Qualitätssicherung Dr. Krengel GmbH, Germany [personal communication]) of the sold amounts in Germany. Hence, amounts calculated by the Arzneiverordnungs-Report 2004 (2004) need to be multiplied by a factor f_C of 0.86 to 1.07 to reach the final approximation for consumed pharmaceuticals in Germany. This factor range addresses overestimation of consumed drugs and at the same time lack of data concerning pharmaceuticals distributed via pathways alternative to pharmacies and thusly reflects another source of uncertainty.

For one patient

The determination of the pharmaceutical concentration in urine of only one person under medication was also possible. The excretion rate E remains the same as well as the average amount of 1.24 l of urine excreted per person per day (Ciba Geigy AG, 1977). Only the annual consumed amount (A_C) was replaced by the daily dose represented with its minimum and maximum values.

The renal concentration of pharmaceuticals taken by a single person (C_{UP}) was calculated by eq. 10:

$$C_{UP} = (DDD_G \bullet E) \bullet (1.24\ l\ pers^{-1}\ d^{-1})^{-1} \quad (10)$$

Of course, excreted concentrations of hormones and ß-sitosterol can also be determined for a single person (see Table 4 for natural hormones).

2.3.2 Calculation of mean concentrations of pharmaceuticals in environmental compartments

Usually several datasets were available with very diverse numbers of samples as well as different mean concentrations of pharmaceuticals in the respective *Medium*. Hence, a weighted average was calculated. As a first step the mean concentration of all positively detected samples (C_M) from a dataset were determined. Then, the weighted mean (C_{MW}) of each dataset was calculated considering the detection frequency (*df*):

$$C_{MW} = (C_M \bullet df) + (0.5\ LOQ \bullet (100 - df)) \quad (11)$$

In all articles with information concerning the limit of quantification (*LOQ*), LOQ was used directly. In cases where only the limit of detection (LOD) was given, this value was multiplied by a factor of 3 (Funk et al., 2005). If the concentration of pharmaceuticals in a sample was given as “not detected”, meaning the concentration was below the limit of quantification, it was defined as 0.5 *LOQ*. It has to be mentioned that in some articles the mean concentrations already included samples with pharmaceutical concentrations to be “not detected”. Then, detection frequency (*df*) was set at 100 %. Finally, overall mean concentrations in the *Medium* (C_P) were determined from C_{MW} of all datasets for one pharmaceutical.

$$C_P = \Sigma_i (C_{MWi} \bullet n_i) \bullet (\Sigma_i (n_i))^{-1} \quad (12)$$

n_i denotes the number of individual concentrations used to calculate a particular C_{MWi} of a pharmaceutical within one dataset. If n_i was not given, it was set 1.

2.3.3 Are pharmaceutical concentrations applied to plants in other investigations relevant for fertilisation with AGU?

As already mentioned in 2.1.1, datasets reporting on laboratory tests on plant behaviour towards pharmaceuticals were included in *Plant* as well. Although, in all other media only studies reporting on pharmaceutical concentrations reached by today's transmission pathways were regarded. In these tests, pharmaceutical concentrations applied to the plants were often chosen as being higher than those expected in the environment. This "overdosing" was done for several reasons as e.g. to enable the possibility for analytical determination in plant tissue. With the aid of higher concentrations, phytotoxic effects and uptake into plants can be reached which would never occur under natural conditions. Therefore, the applied concentrations in the contained datasets were compared to realistic situations to extract those which would be realistic in case of fertilisation with urine.

Two types of tests had to be distinguished:

- Tests which were conducted in an artificial media as filter paper, saw dust, cotton gauze, and murashige & skoog (an artificial substrate developed by Murashige and Skoog (1962) for plant tests) soaked with water. These tests were mainly performed to investigate germination and effects towards plant seedlings. Additionally, tests in hydrocultures with Hoagland media were added to this category due to their similar set-up, although in the studies longer times spans could be investigated.
- Tests which were accomplished in solid substrates including various soil types.

For tests in an artificial medium it was assumed that in the case of urine application a urine-water mix of 1:20 would be used as this was determined to be the best mix (Schneider, 2005; Ritter, 2008) and pharmaceutical concentrations theoretically predicted for AGU as described in chapter 2.3.1. In case of solid substrates, 25 m^3 ha^{-1} a^{-1} urine are recommended (Hammer and Clemens, 2007). An initial infiltration depth of 0.5 m into soil was estimated and an average mineral soil compaction of 1400 kg m^{-3} DM was taken (Schroeder and Blum, 1992).
Overall, AGU concentration could be calculated for 20 pharmaceuticals mentioned in *Plant*. For the others, their number of DDDs (see 2.2.1) was not listed in Arzneiverordnungs-Report 2004 (2004), indicating either very low annual amounts sold or they were only used in veterinary medicine (enrofloxacin, monensin, sulfamethazine, and tylosin). Nevertheless, those active agents could not be included into the performed comparison.

2.4 Plant experiments

The plant experiments using pharmaceutical-spiked urine for fertilisation were performed in the growth period of 2007. Until then enough data was collected within the database and evaluated for the selection of pharmaceuticals and their concentrations.

2.4.1 Selection of pharmaceuticals

The number of pharmaceuticals selected for these investigations were limited due to the decision that not only the effects of single substances but also of combinations of several substances should be tested. With respect to conditions such as space requirements etc., it was decided to investigate the effects of three pharmaceuticals as well as various combinations of them at two different concentration levels.
The selected pharmaceuticals should fulfil several requirements. First, they should have been detected in German groundwater (so far 21 substances were listed in the database to be found in German groundwater). Additionally, they should differ with respect to log K_{OW}, molecular weight, and their expected concentrations in AGU (see 2.3.1).
Consequently, three pharmaceuticals were selected: carbamazepine (CZ, CAS-N^{o}. 298-46-4), ibuprofen (IBU, CAS-N^{o}. 15687-27-1), and 17α-ethinylestradiol (EE2, CAS-N^{o}. 57-63-6) (for their characteristics see Table 5). All three pharmaceuticals were purchased from Sigma-Aldrich: IBU, minimum 98 % GC; EE2, minimum 96 % HPLC, and CZ (no information on purity given by provider).

Table 5: Properties of the pharmaceuticals used in the plant experiments.

Pharmaceutical	Concentration in AGU[1] (mg l^{-1})			Indication[2]	log K_{OW}[3]	Molecular weight
	min	max	average			
CZ	0.00	0.12	0.058	Anti-epileptic	2.45 (at pH 7.4)	236.3
IBU	n.d.	1.27	0.844	Antiphlogistic, antirheumatic agent	2.41 ± 1.5 (at pH 7.4)	206.3
EE2	0.00	0.000047	0.000024	Sex hormone, Mestranol metabolite	3.67	296.4

n.d. = was not possible to determine this value due to lack of data.
[1] determined as described in 2.3.1, besides the external factors were not considered as they were not available at this time (State: Spring 2007).
[2] Arzneiverordnungs-Report 2004 (2004) and in case of EE2 additionally Orme et al. (1983)
[3] Hansch et al. (1995) and in case of EE2 Syracure Research Corporation (2004)

2.4.2 Preparation of spiked urine

The urine used as fertiliser in the plant experiments was spiked with the average concentration of the three pharmaceuticals calculated for AGU according to chapter 2.3.1 further on referred to as “natural” (n) concentration level. Additionally, a higher

level was used (referred to as “artificial” (a)): the ten-fold concentration in case of CZ and IBU and a 40-fold concentration for EE2 compared to “natural” concentrations in order to ensure analytical detection. Twofold pharmaceutical concentrations compared to AGU were added as pots obtained only half of the liquid planned to add. Nevertheless, the amount of urine guaranteed an optimal supply for the growth period of three months.
Beside the single pharmaceuticals, several combinations of pharmaceuticals were tested at the natural and artificial concentration level as well as a combination of all three substances (Figure 8). Each pharmaceutical in urine mixture was applied to plants in triplicate.

	CZ	IBU	EE2
CZ	x	x	x
IBU		x	x
EE2			x

3
x

Figure 8: Single substances and combinations of pharmaceuticals investigated in the pot experiments. “x” marks the realised cases, “3” stands for combination of all three substances CZ, IBU, and EE2.

Urine was collected from healthy males in bottles in the two weeks prior to application of urine (week 25) spiked with pharmaceuticals (2.4.1) designated as “UPmix”. It was decided to use male urine to keep the hormone level as low as possible. None of the donors were under any medication.
Urine used for the experiments was analysed for the components as described in Table 6. TOC and TN were performed according the manual of the analyser, a multi N/C 3000 of the Analytik Jena AG. In case of K, the sample was sent for analysis to the central laboratory of TUHH. The sample was solubilised by nitrohydrochloric acid and analysed by a PE-Optima 2000 DV OES with ICP. Conductivity was determined by a conductivity meter LF 191 (WTW) and pH by a pH probe connected to a pH meter pH 196 of the same company.

Table 6: Conditions and concentrations of mixed male urine used for fertilisation in plant experiments.

Parameter	Concentration	Unit	Method
TOC	4310	mg l^{-1}	see text above
TN	4670	mg l^{-1}	see text above
N-NH_4	263	mg l^{-1}	photometric measurement according DIN 38406
Total P	404	mg l^{-1}l	photometric measurement with Küvettentest LCK350 (Hach Lange)
K	1100	mg l^{-1}	see text above
Conductivity	12.2	mS cm^{-1}	EN 27888
pH	6.83		DIN 38404, pH probe

The mixed urine (Table 6) showed average concentrations of macronutrients and TOC as well as average conductivity and pH (Tettenborn et al., 2007). Moreover, a pH of 6.8 indicated that urine was freshly collected as its pH rises with storage time due to urea hydrolysis (Udert et al., 2003a).

Preparation and application of UPmix was carried out in three steps. First, pharmaceutical concentrates were prepared in the laboratory. The required concentration for the three repetitions was achieved by dissolving the solid pharmaceutical in 500 µl methanol. The pharmaceutical-methanol (PM) solution was stored in the fridge at 5°C and then transported in a cooling box to Bonn. In the second step the PM solution was added to 750 ml of urine. The vial containing the PM solution was rinsed with 100 µl distilled water which was added to the UPmix as well. In the case of blanks, the same procedure was executed with the only difference being that the vial contained just 500 µl methanol without any pharmaceuticals. Afterwards, the UPmix was stirred with a glass rod, divided into three equal portions of 250 ml and applied to the pots. After each application, the full equipment was washed with distilled water before the next UPmix was prepared.

2.4.3 Application of pharmaceuticals

The plant experiments were accomplished in cooperation with the Institute of Plant Nutrition of the University of Bonn from June to September 2007 in the greenhouse of the institute. A number of 64 “Kick-Brauckmann-pots” (height: 26 cm, diameter: 20 cm (Kick and Große-Brauckmann, 1961)) were filled with 9 kg air-dried soil (type: Meckenheimer Krume; luvisol: 16 % clay, 77 % silt, 7 % sand (Schneider, 2005)). The pots contained a bottomless inner pot in a planter with drainage for leachate. The drainage was closed and all the water remained within the pot. Pots were connected to a micro-irrigation system (drop irrigation; Blunat) and adjusted to keep the soil moisture at 80 %. Soil moisture was controlled from time to time by weighing the pots. In week 23 (June 4-8, 2007), seeds of ryegrass (*Lolium perenne*) were seeded in rows into the pots (0.95 g of seeds per pot). Germination and development of seedlings was regular.
After establishment and initial growth for a period of 2 weeks, the seedlings were treated with different UPmix as direct application on seeds may lead to delay of germination (Simons and Clemens, 2005). 250 ml liquid were added to each pot.

To keep pots with equal pharmaceutical additions as far away as possible from each other, the experiment was parted into three series: pot N^{o}. 1-20, No. 21-40, and N^{o}. 41-60. Pots N^{o}. 61-64 were additional ones. In each section the same UPmix was only once applied. The experimental setup contained 2 blanks (pots only treated with urine and methanol) in each series as well as 4 pots completely untreated (no

pharmaceuticals, no urine). The following morning plants of pots treated with UPmix did not show any differences to the untreated ones.
Plants were cut seven times until harvest, the last time at day 92, the last day of the experiment. Fresh and dry weight of the aerial plant parts cut was determined on each occasion.

2.4.4 Final harvest and sample preparation

The experiment was terminated after 92 days. After the last cutting of the grass, soil samples from top to bottom of each pot were taken using a mechanical soil corer. Approximately 150 g DM were taken from each pot resulting in 5 to 8 holes per pot. The samples were dried at 40°C until constant weight and then ground to small pieces by mechanical pressure exaggerated by a wooden cylinder (specifically used for soil samples). The low drying temperature was used to prevent pharmaceuticals from eventual destruction. After this procedure, soil was sieved by a certified sieve with a pore size of 2 mm. During sieving, grit, gravel and plant parts were removed manually from the sieve. The fine fractions passing the sieve were collected on a foil. After each sieving, the four endings were lifted; the sample was moved mechanically to the centre and divided into two final samples. Subsequently to processing of each soil sample all tools were cleaned carefully.

After soil samples were taken all pots were completely emptied, and the root-soil mixture was cut into several parts. From this mixture roots were collected manually for further analysis. Roots as well as plant parts close to soil, leftovers from cuttings, were washed with tap water and distilled water in sieves of pore sizes of 0.25 and 0.49 mm, separated from each other and air dried. Aerial plant parts were dried at 40°C for approximately three days until constant weight.

2.4.5 Analytical determination of pharmaceuticals

All analyses were performed by Mrs. Reich and Mrs. Engel, central laboratory of Hamburg University of Technology except analysis of urine (2.4.3).

Soil
Prior to analyses, soil samples were dried once more at 50°C and ground. Samples of 10 g soil were shaken with 50 ml methanol for 2 hours. Subsequently, the suspension was filtered over a paper filter. The extract was concentrated to 1 ml in a rotary evaporator and stored in 2 ml vials until further processing.
For silylation, 800 µl of the concentrated extract were pipetted into a compression-proved vial, evaporated to dryness by a gentle stream of nitrogen, and silylated with 200 µl MSHFBA (N-methyl-N-trimethylsilylhepta-fluorobutyramide) at 70°C for 1 hour.

The residue was dissolved in a small volume of acetonitrile which was transferred to a graduated flask adjusted to a final volume of 1 ml.
The silylated extracts were analysed by GC/MS (GC: Agilent 6890N; column: HP 5ms, ID 0.25mm, film thickness 0.25µm; MS: Agilent MSD 5975B, SIM, MS quadrupol temperature 150°C, MS source 230°C). Each sample was analysed in duplicate, for recovery rates (derived from pharmaceutical-spiked blank soil analysed as described), LOD and LOQ see Table 7.

Table 7: Recovery rates, limits of detection and quantification of the three investigated pharmaceuticals in soil.

Pharmaceutical	Recovery rate (%)	Limit of detection (µg kg^{-1} DM)*	Limit of quantification (µg kg^{-1} DM)*
CZ	90 - 120	0.2	0.6
IBU	30 - 60	1	2
EE2	50 - 60	1	2

* Lowest recovery rate considered.

Roots and aerial plant parts
Roots were cut with a cutting machine into fine parts and further ground in a coffee grinder. As only small amounts of root material were available (1.5 – 4 g DM), the entire material from one plant was used to prepare one sample extract.
The ground material was shaken for 2 h in a buffer solution of HCl and KCl (6.5 ml 0.2 M HCl and 25 ml 0.2 M KCl) at pH 2. Solid parts were filtered out by a fluted paper filter and the extract was subjected to solid-phase extraction with abselut Nexus cartridges (500 mg/12 ml, VARIAN). After washing the cartridges with rinsing water of the extraction bottles and subsequently with little H_2O of analytical grade, the analytes were eluted with 5 ml methanol and the eluate was concentrated to a volume of 1 or 2 ml (roots) and 2 ml (aerial plant parts).

The solutions were analysed by GC/MS as described for soil samples. The retention times of each substance were verified by spiking one part of each sample extract with the respective pharmaceutical standards as the matrix of the extracts was very complex. Out of the three substances only carbamazepine could be determined, albeit as its decomposition product iminostilbene (CAS-N°. 256-96-2). For recovery rate, LOD, and LOQ see Table 8.

Table 8: Recovery rates, limit of detection and quantification of carbamazepine** and ibuprofen in roots and aerial plant parts.

Pharmaceutical	Recovery rate (%)	Limit of detection (µg kg^{-1} DM)*	Limit of quantification (µg kg^{-1} DM)*
	Roots		
CZ**	56 - 61	10	20
IBU	67 - 98	20	30
	Aerial plant parts		
CZ**	15 - 20	20	75
IBU	Not determined		

* Lowest recovery rate considered. ** Detected in form of iminostilbene.

EE2 was not detected. The reason being that uptake rates of the investigated plants were much lower than expected from results and reported by Schneider (2005). So, even the chosen artificial concentration for EE2 was selected as too low.

2.4.6 Statistical evaluation of experimental results

Results of the pot experiments on rye grass were statistically evaluated with SPSS 15. A one-way ANOVA was accomplished as a one-way descriptive method in cooperation with a Student-Newman-Keuls procedure. α was set to be 0.05 to determine a significant difference between various treatments.

3 Results & Discussion

3.1 General overview of pharmaceuticals in DB datasets

The database contained 330 pharmaceutical substances which were entered during the literature screening into the section *Substance* (see Figure 9). Details regarding the different indications were not included as a lot of active substances are used for two or even more indications. This leads to many duplicate nominations and no longer allows for a clear and representative overview. Beside the various typical types of active agents, 17 hormones and 13 disinfectants were listed. Another 61 substances were identified as metabolites, although some of these as e.g. salicylic acid, metabolite of acetylsalicylic acid (Daughton and Ternes, 1999), are used for different purposes at the same time: for example, salicylic acid is also used as dermatic and food preservative (Daughton and Ternes, 1999). Additionally, one phyto-sterol and one faecal sterol as well as one surrogate and two pro-drugs were included in the DB.

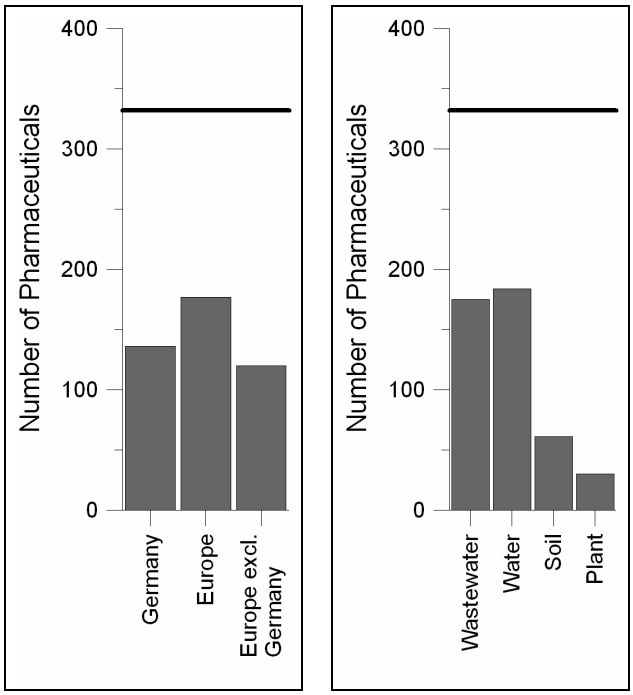

Figure 9: Overview of number of pharmaceutical residues related to the different regions (left) and environmental compartments (right) of datasets collected in DB. Black line shows overall number of pharmaceuticals represented in DB. (January 18, 2008)

As some parts of the DB were still under revision, the evaluation of this database was a dynamic process. This might imply that numbers of datasets or pharmaceuticals presented changed slightly later on. As it was impossible to recheck and overwork the whole results and discussion part after each change, the date of evaluation is

given for each graph and table in brackets to provide the reader with a better understanding.

136 of the pharmaceuticals related to datasets reporting on the German environment while 177 are belonging to datasets of European countries. These numbers show that the main focus was the German environment as only 41 additional substances were counted when widening the scope from Germany towards Europe (Figure 9). However, datasets containing European countries excluding Germany contain information for 120 pharmaceuticals (Figure 9). Hence, diversity is given but information density is much lower then for Germany as can be seen in Figure 10 where percentages of datasets for the specific countries are indicated. The overall number of datasets, 2427, was set as 100 %. Datasets for Baltic Sea (1) and North Sea (11) were excluded although these regions belong to Europe.

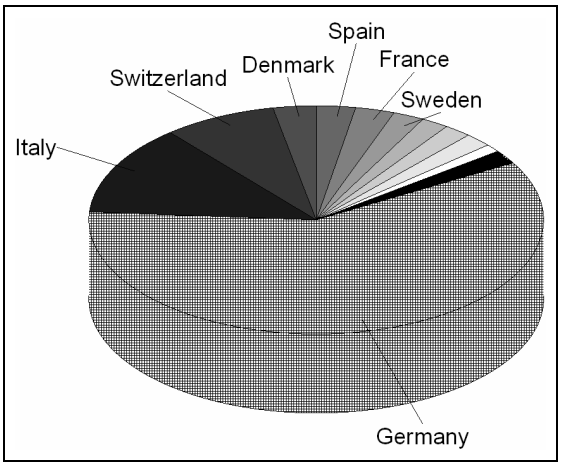

Figure 10: Overview of number of datasets related to the different European countries. Labelled are only these countries for which the contribution to DB was >2 %. The black field anticlockwise from Germany summarises all countries with dataset contributions <1 %. (January 18, 2008)

Figure 9 contains information about the numbers of pharmaceutical datasets created for the various environmental media represented in database sections *Wastewater*, *Water*, *Soil*, and *Plant*. For the two aqueous compartments *Wastewater* and *Water* nearly identical numbers of pharmaceuticals were present, while for *Soil* and *Plant* numbers were much lower (Soil: 61 pharmaceuticals; Plant: 30). In the following discussion, only German, Austrian, and Swiss datasets were used (as long as not explicitly stated elsewhere) in order to avoid variations in location with completely different conditions.

3.2 Detailed overview of pharmaceuticals in *Wastewater* and *Water*

As is known, households are major sources of environmental pollution by pharmaceuticals (Heberer, 2002; Kümmerer, 2006). They enter wastewater by human excreta. Pharmaceutical pollution pathways are well known (Kümmerer, 2001) but only few investigations report on pharmaceuticals' concentrations in yellowwater directly. A major purpose of *Wastewater* and *Water* was to collect information about pharmaceuticals detected in the environment. Hence, if active substances were conducted in the environment and entered the DB, their concentrations in urine could be determined (2.3.1). With help of the calculated concentrations, further considerations regarding their presence in the environment due to application of human urine as agricultural fertiliser could be considered further.

The two categories *Wastewater* and *Water* were containing a wide range of different wastewater and water types. The various categories of wastewater summarised under this heading are raw wastewater, effluent of WWTP (wastewater treatment plants), yellowwater, landfill leachate, as well as influent and effluent of wetlands (Figure 11). The largest number of datasets was collected for "effluent of WWTP" followed by "raw wastewater". This focus of research activities resulted from recent discussions and investigations on contamination of wastewater with pharmaceutical residues and eventual consequences with respect to requirements for WWTPs operation. Additionally, as loading of yellowwater was of major concern, a high number of datasets on this particular wastewater stream was in the centre of this research. Nevertheless, it is clearly seen that this aim was hardly achieved. Only little research was carried out in this field (see 3.3) especially when looking at the number of pharmaceuticals investigated. Only 22 pharmaceuticals were found for yellowwater, while 67 and 108 were counted for raw wastewater and WWTP effluent respectively. Even landfill leachate was investigated for more than 32 pharmaceuticals. Yellowwater datasets were found in 7 different articles.

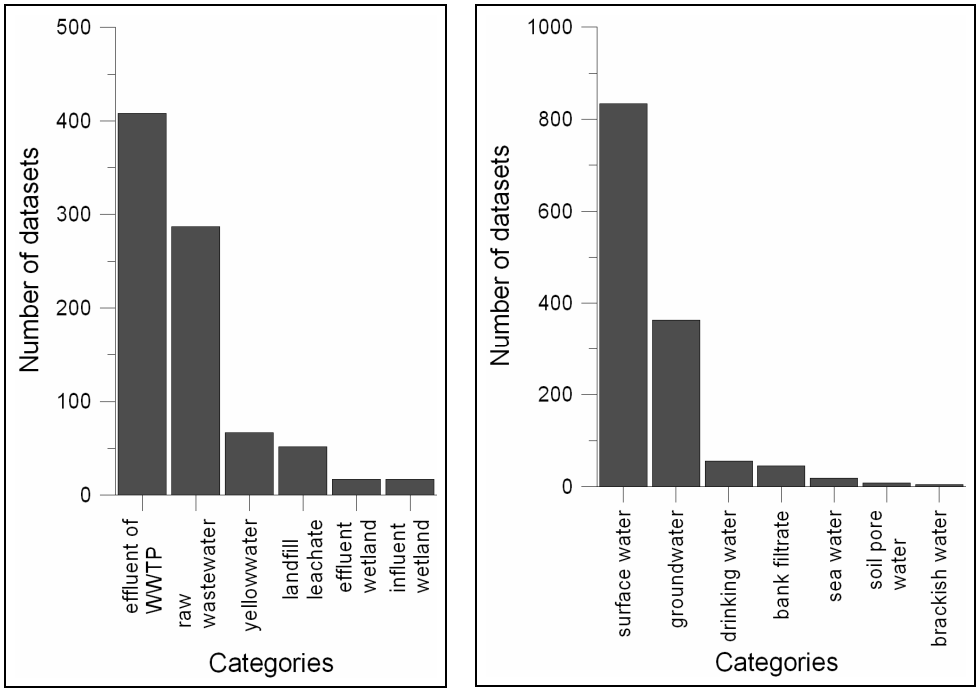

Figure 11: Number of datasets for the different categories of wastewater (left graph) as well as water (right graph). Additionally to German, Austrian, and Swiss sources, sea water from the Baltic and North Sea is contained in “sea water”. (January 18, 2008)

Within the category *Water*, various water sources were collected. For surface water representing mainly river and lake water, most of the datasets were available within *Water* compared to other water and wastewater types (Figure 11). Additionally, groundwater has many datasets while drinking water and bank filtrate exhibit similar numbers as yellowwater and landfill leachate. Bank filtrate was only reported in three articles (Brauch et al., 2000; Heberer et al., 2001; BLAC, 2003) fed to the DB. The tendency of dataset numbers was also reflected in numbers of pharmaceuticals in the different water types: surface water (111), groundwater (93), drinking water (43), and bank filtrate (38). An additional reason for the reducing numbers of datasets could be found in biological degradation and sorption occurring along the pathway (2.2.5; 3.5.3). Sea water was not included in surface water as its characteristics differ largely and its concentrations of pharmaceuticals are much smaller then in other surface waters (Weigel, 2003; see also https://www.tu-harburg.de/aww/pharma/).

To get a closer view of the occurrence of pharmaceuticals and especially their concentrations, the four categories with the largest number of datasets (DS) of Figure 11, raw wastewater, effluent of WWTP, surface water, and groundwater, were investigated in more detail with respect to groups of their indication for Germany. In Table 9 and Table 10 each dataset represents the summary of single datasets belonging to the different active substances detected in the respective media for an indication (for details see 2.3.2). As pharmaceuticals often belong to different

indication groups, multiple nominations were unavoidable. E.g. acetylsalicylic acid belongs to the group of antibiotics as well as thrombocyte blockers (Arzneiverordnungs-Report 2004, 2004).
This way of data presentation (referring to indications) was chosen to provide a general overview of concentrations of groups of pharmaceuticals belonging to one indication expected in the different environmental compartments. It was impossible to provide more details, i.e. concentrations for single substances, within the scope of the study. Of course, this has to be taken as a first impression neglecting the differences between particular pharmaceuticals belonging to one indication group. More details for the single substances are additionally available at https://www.tu-harburg.de/aww/pharma/.

Pharmaceuticals in raw wastewater and effluent of WWTP

152 DS representing 25 pharmaceuticals were found for raw wastewater (Table 9). They belong to 16 different indications. Due to multiple nominations of particular pharmaceuticals in different indication groups, 232 DS were counted in raw wastewater instead of 152. The average pharmaceutical concentrations were slightly higher as in effluents of WWTP, although the overall mean (2195 ng l^{-1}) was significantly above that for treated wastewater (417 ng l^{-1}). In the effluents of WWTP 44 pharmaceuticals were identified belonging to 19 indication groups. Overall, 253 DS were counted for effluent.
In raw wastewater a large range of pharmaceutical concentrations was detected. 10 of the indications showed deviations >10 % from the average for the confidence interval (CI) of 95 %, 8 indications even >20 %. The large variation is also visible in high standard deviations (SD, up to 4-5 µg l^{-1} for analgesics and diagnostic agents). Dermatics even showed an SD of 23 µg l^{-1}.
Values were less varying for effluents of WWTP. Only 7 indications had deviations >10 % from CI 95% and not more than 5 groups >20 %. Accordingly, also standard deviations were smaller. Only antibiotics exhibited an SD of 17.3 µg l^{-1} (Winker et al., 2008a).

Table 9: Summarised German concentrations (C_P) as well as standard deviations (SD) and confidence intervals (CI) of groups of pharmaceuticals with respect to their indication in raw wastewater and effluent of WWTP. (April 02, 2008)

	Raw wastewater (ng l^{-1})						Effluent of WWTP (ng l^{-1})					
Indication	**Min**	**C_P**	**Max**	**SD**	**CI 95% in %**	**N°. of DS**	**Min**	**C_P**	**Max**	**SD**	**CI 95% in %**	**N°. of DS**
analgesic	10	3.390	26.000	4.180	10	18	10	1.810	5.500	2.540	10	20
antibiotic	1	295	1.800	395	23	19	1	944	340.000	17.300	75	62
anti-epileptic	150	1.570	3.000	468	3	15	46	1.220	2.100	635	4	20
antiphlogistic	1	1.860	5.300	1.400	10	34	1	355	1.760	503	7	45
antirheumatic agent	1.347	3.120	5.300	1.180	9	13	13	108	329	96	8	17
antitussiva	-	-	-	-	-	-	25	602	1.391	563	17	5
beta blocker	13	1.380	10.000	2.480	29	26	13	216	1.700	290	8	34
bronchospasmolytic drug	13	82	370	117	39	7	13	32	75	19	4	12
cytostatic agent	1	134	1.521	416	66	9	1	3	27	5	17	10
dermatic	1	12.600	54.000	23.100	74	4	25	175	2.500	441	28	9
diagnostic agent	1	4.030	18.000	5.350	38	9	1	598	8.000	1.380	29	18
disinfection	-	-	-	-	-	-	30	71	75	14	5	3
gynecologic drug	-	-	-	-	-	-	1	4	9	4	37	3
lipid regulation drug	33	1.200	5.600	1.440	19	28	15	450	2.535	657	10	41
ophtalmologic drug	25	1.480	4.000	877	12	17	25	630	1.760	598	7	24
otologic preparations	22	452	1.910	330	8	14	25	171	320	137	7	15
sex hormone	1	83	3.300	301	33	9	1	8	1.100	66	52	27
thrombocyte blocker	210	1.250	3.200	1.050	21	10	13	114	182	58	7	12
Total N°. of DS						**232**						**377**

Pharmaceuticals in surface water and groundwater

The largest number of datasets (more then 1000) was given for surface water (Figure 11), originally 723 DS for particular pharmaceuticals discussed in the included articles (Table 10). The additional ones resulted from multiple nominations for different indications. 57 pharmaceuticals belonging to 23 indications could be determined in surface waters, i.e. the largest number of indications found in the different compartments of the aquatic environment. Concentrations were clearly smaller than in effluents of WWTP and their means were between 4 ng l^{-1} (sex hormones) and 275 ng l^{-1} (stimulants). The reason for the lower concentration is the dilution of the WWTP's effluents with surface water. Standard deviations and confidence intervals were much smaller; only stimulants exhibited with 28 % a CI >20 %. The other 6 indications with values >10 % were between 11 and 15 %. A reason for these results might be the large number of datasets.

Table 10: Mean concentrations (C_P) as well as standard deviations (SD) and confidence intervals (CI) of pharmaceuticals with respect to their indication in surface water and groundwater in Germany. (April 02, 2008)

	Surface water (ng l^{-1})						Groundwater (ng l^{-1})					
Indication	**Min**	**C_P**	**Max**	**SD**	**CI 95% (%)**	**N°. of DS**	**Min**	**C_P**	**Max**	**SD**	**CI 95% (%)**	**N°. Of DS**
analgesic	4	64	630	105	12	43	3	16	1.000	59	39	21
antibiotic	1	17	485	34	7	340	5	13	850	46	10	93
anti-epileptic	3	139	570	123	7	33	5	66	3.600	301	42	25
antiphlogistic	1	32	380	47	8	125	3	40	3.400	262	35	52
antirheumatic agent	3	21	92	17	6	39	3	30	520	89	32	20
antitussiva	25	24	25	5	8	3	25	19	25	13	106	3
beta blocker	2	22	44	10	2	23	5	10	25	8	4	29
bronchospasmolytic drug	2	22	75	14	4	12	5	12	25	10	7	7
corticosteroid	1	5	10	3	7	26	5	9	14	4	6	4
cytostatic agent	1	6	15	4	5	30	5	7	14	3	4	8
dermatic	1	35	192	41	13	31	5	30	850	121	42	10
diagnostic agent	10	116	841	140	13	15	5	157	5.583	882	36	20
feed supplement	25	25	25	3	2	3	5	9	50	8	11	6
gastrointestinal drug	3	16	25	11	15	17	-	-	-	-	-	-
gynecologic drug	1	21	25	9	11	13	5	7	25	6	16	5
lipid regulation drug	2	47	350	70	8	97	2	29	1.000	86	15	52
ophtalmologic drug	3	47	329	63	9	46	3	57	3.400	354	45	30
otologic preparations	3	28	260	40	12	51	5	25	3.000	165	72	18
psychopharmacologic agent	8	28	45	7	5	3	-	-	-	-	-	-
sex hormone	1	4	25	4	9	59	5	6	25	5	27	5
stimulant	8	275	430	171	28	5	-	-	-	-	-	-
thrombocyte blocker	5	22	30	8	6	18	3	4	11	3	35	12
Total N°. of DS						**1.032**						**420**

With 43 pharmaceuticals (representing 20 indications) in 278 DS, an unexpected variety was determined for groundwater (Table 10). Moreover, with 4 ng l^{-1} (thrombocyte blockers) to 157 ng l^{-1} (diagnostic agents) the average concentrations were in a similar range as for surface waters. Although the overall mean concentration of 29 ng l^{-1} was lower than in surface water (41 ng l^{-1}). An explanation for high concentrations, indicated especially by the maximum values, might be the selection of measuring points. Samples were taken very often below waste dumps, wastewater irrigation fields, and similar locations where a contamination of groundwater by pharmaceuticals is likely to occur. This selection might have increased the average values in groundwater artificially. An indication for this assumption are high standard deviations (up to 882 ng l^{-1}) as well as that confidence intervals were >10 % for 14 indications. In no other media was such a high number of deviations listed. Additionally, concentrations in groundwater might be an indicator of penetration through soils, therefore (for further details see 3.5.2).

Intermediate conclusion

- Pharmaceuticals were detected in different wastewater types; i.e. raw wastewater, WWTP's effluent, source separated streams as yellowwater and greywater. Also, they are present in many of our natural water bodies as surface waters and groundwater.
- Various indication groups and pharmaceuticals were detected. The concentrations vary widely between different pharmaceuticals and the therapeutic groups as well as amongst the types of wastewater and water.
- Nevertheless, overall concentrations for different types of media can be determined by means of statistical evaluations. These generalisations show that concentrations decrease along the pathway of pharmaceuticals through the environment; from raw wastewater, via effluent of WWTP and surface waters to groundwater.

3.3 Pharmaceuticals in urine

3.3.1 Predicted concentrations of pharmaceuticals in urine

For 124 pharmaceuticals in the database, concentrations in an average German urine (AGU) as described in chapter 2.2.1 and 2.3.1 could be determined. Also pharmaceutical concentrations in the urine of only one person under medication were calculated for 173 of the 330 pharmaceuticals collected in the DB. The higher number of data available for one person's urine is explained by the listing in Arzneiverordnungs-Report 2004 (2004) as mentioned in chapter 2.2.1. Details for the single substances regarding AGU and one person's urine can be found below https://www.tu-harburg.de/aww/pharma/.

As already stated before, renal concentrations for only a few pharmaceuticals have been determined so far. A larger investigation was carried out during the EU-project "SCST" (Tettenborn et al., 2007; Winker et al., 2008b). Therefore, the results of this project were compared with the calculated concentrations to determine the quality of the prediction method (for details as origin and sampling of yellowwater, analytical details, and detected concentrations see Tettenborn et al. (2007) and Winker et al. (2008b)): during the measuring campaign, 7 of the 20 analysed pharmaceuticals were detected. Ibuprofen, bezafibrate, ß-sitosterol, diclofenac, and carbamazepine were detected in all six analyses accomplished between March 2005 and May 2006, pentoxifylline in five and phenazone in three analyses. The 13 pharmaceuticals not detected are stated in Table 11.

By means of the complex procedure to theoretically calculated concentrations of pharmaceuticals in yellowwater – based on amount sold, amount consumed (A_C), resorption (R), excretion (E) (see chapter 2.2.1 and 2.3.1) – the results are

considerably uncertain (Figure 12). This is due to the fact that data retrieved from literature for each aspect of pharmacokinetics give a certain range which is amplified by the combination of data exhibiting ranges themselves. This amplification varies from substance to substance as demonstrated by error bars in Figure 12.

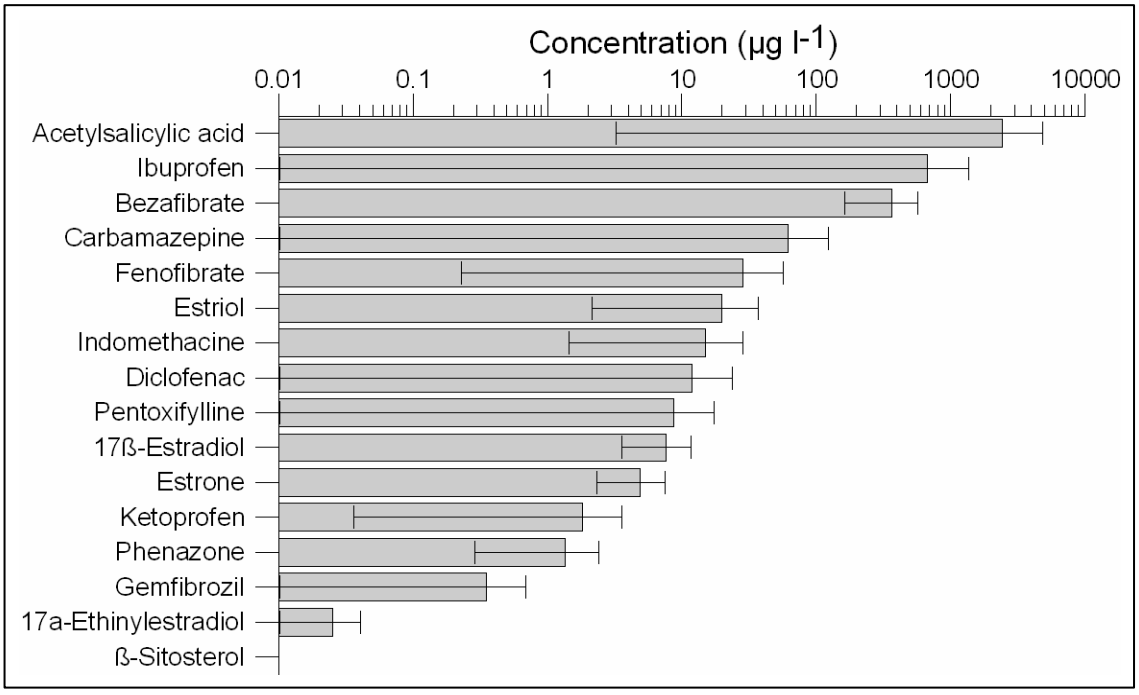

Figure 12: Mean concentrations of pharmaceuticals in urine calculated from consumption of pharmaceuticals in Germany and from pharmacokinetic data. Error bars indicate the range between minimum and maximum concentrations calculated for German yellowwater. (December 13, 2007)

Clofibric acid, fenoprofen, and mestranol were not considered for the theoretical calculations as their amounts sold in Germany were not possible to determine from the Arzneiverordnungs-Report 2004 (2004). This implies that they were not among the pharmaceutical substances sold in amounts high enough to be registered in this annual overview for Germany. Clofibric acid is the active metabolite of the three pro-drugs etofibrate, clofibrate, and etofylline fibrate (Ternes, 2001). None of these three were listed. Mestranol is a pro-drug which undergoes a rapid demethylation in the liver forming 17α-ethinylestradiol (Stanczyk, 1998). Hence, if anything related to mestranol is detected in urine then it will be its metabolite ethinylestradiol.
External factors such as consumed amounts and individual consumer behaviour, prescriptions in hospitals and spreading via manufacturers' agents all have an additional influence on calculations as outlined in the methodology chapter (2.3.1).

3.3.2 Comparison of calculated with measured renal concentrations

As mentioned above, the measurement of pharmaceuticals in urine is costly and time consuming. Therefore, an approximation by a calculation method would be very helpful. Hence, the method to predict concentrations of pharmaceuticals in urine was developed and compared to the values measured during the sampling campaign. Some certainty was given by the fact that within the sampling campaign no large deviations between the two origins of the yellowwater, Hamburg and Berlin (Winker et al., 2008b) were discovered. In Figure 13, the analysed concentrations of pharmaceuticals (carbamazepine (CZ), bezafibrate (BEZ), diclofenac (DIC), ibuprofen (IBU), phenazone (PHE), pentoxifylline (PEN)) are plotted versus the calculated concentrations. The yellowwater collected in Hamburg, resulting from a larger user group (200-500 daily disposals vs. only 25-30 users in Berlin (Winker et al., 2008b)), achieved a better coefficient of determination, R^2, of 0.98, while R^2 for Berlin was only 0.90. This might be explained by the fact that the regression fits were strongly dominated by BEZ and IBU: both exhibited one very large value (out of 3) for Berlin (BEZ: 846 µg l^{-1} and IBU: 794 µg l^{-1}) which resulted in large 95 % confidence intervals (BEZ: 362 µg l^{-1} for the Berlin data versus 22 µg l^{-1} determined for Hamburg; IBU: 246 µg l^{-1} for Berlin versus 63 µg l^{-1} for Hamburg). This is obviously due to the larger donor group, in Hamburg resulting in less outliner concentrations.

From these findings it may be assumed that a donor group of ≥100 people is needed to measure pharmaceutical concentrations which are close to average concentrations with reasonable statistical certainty.
In the comparison between calculations and measurements, ß-sitosterol (SIT) was also included although its appearance in urine probably does not originate from its renal excretion according to literature. But this could not be excluded completely. Nevertheless, R^2 was hardly affected by inclusion of SIT. R^2 improved slightly to 0.99 in the case of Hamburg, but decreased to 0.89 for Berlin when SIT was not considered.

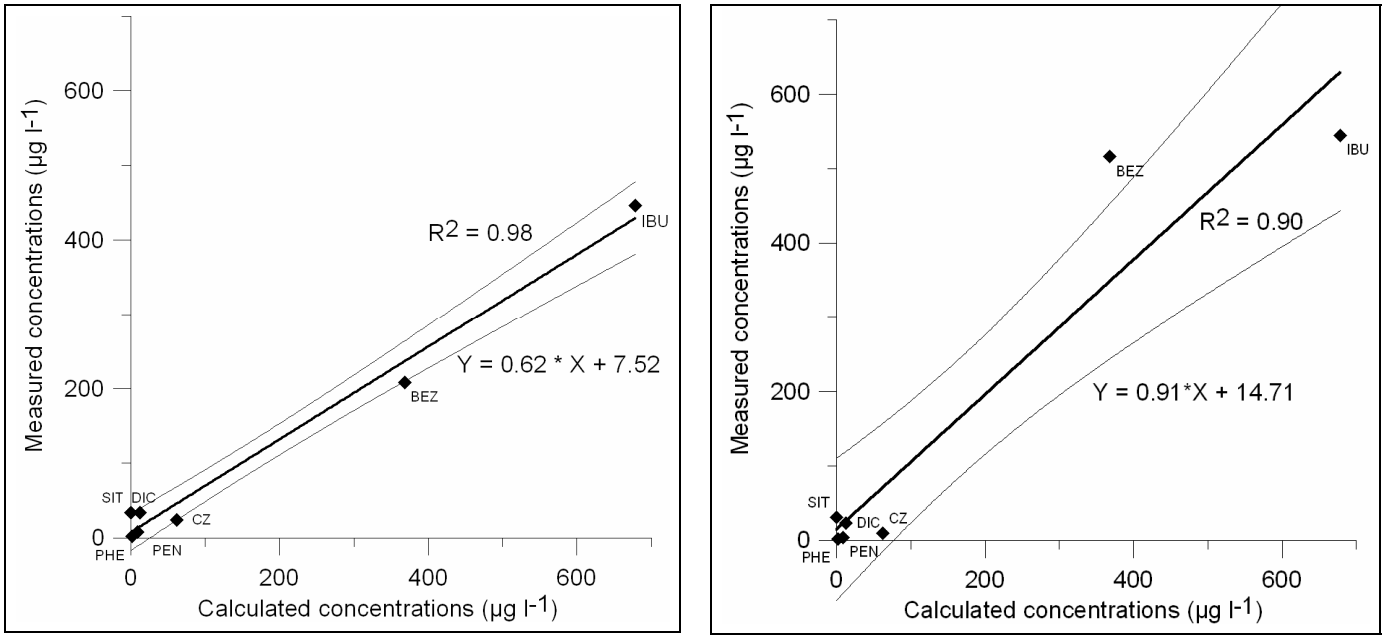

Figure 13: Comparison of mean calculated and analysed concentrations of pharmaceuticals in urine; left: comparison of concentrations in Hamburg and right: in Berlin. Additionally, the confidence range at 95 % for both straight lines is shown. For abbreviations of pharmaceuticals see text. (December 13, 2007)

Overall, it can be concluded that in all cases the calculated concentrations exceeded the measured ones (Figure 13). This indicates that the calculating model includes a safety margin and generally overestimates the expected concentration. Additionally, degradation processes might occur for some pharmaceuticals during storage.
Nevertheless, the comparison shows that approximations by calculations are possible for bezafibrate, carbamazepine, diclofenac, ibuprofen, phenazone, and pentoxifylline and measurements in urine are not necessarily required for these substances for predicting at least an order of magnitude of concentrations in AGU. All analysed concentrations were within the calculated min/max range except for two pharmaceuticals: In one of the six samples, phenazone (4.4 µg l^{-1} in a Hamburg sample) and bezafibrate (846 µg l^{-1} in a Berlin sample) concentrations exceeded the calculated maximum concentrations (2.4 µg l^{-1} for PHE and 573 µg l^{-1} for BEZ).

Aside from those pharmaceuticals for which the measured concentrations in yellowwater could be directly compared to their calculated values, there were also those of interest which could not be quantified by analyses as their concentrations were below LOQs. The prediction model seems to be valid to some extent, as at least the calculated minimum concentrations of seven of the thirteen substances listed in Table 11 were indeed lower than their respective LOQs.
The reason for neither detecting clofibric acid nor fenoprofen nor mestranol was assumed to be the fact that their annual prescribed amounts were too low. Also the

calculated concentrations of the hormones 16α-hydroxyestrone, 17α-ethinylestradiol, estrone, and estriol were below their respective LOQs, thus explaining the lack of detection of these substances in yellowwater. Only the calculated maximum concentration of 17ß-estradiol (13.0 µg l^{-1}) slightly exceeded the LOQ.

Table 11: Comparison of LOQs and calculated concentrations of pharmaceuticals (December 13, 2007) which were not detected in yellowwater during the sampling campaign of Tettenborn et al. (2007).

Substance	LOQ (µg l^{-1})	Calculated mean concentration A_E (µg l^{-1})	Calculated range A_E (min. to max. conc.) (µg l^{-1})
Acetylsalicylic acid	0.5	2430	3.3 – 4860
Clofibric acid	0.5	n.p.	n.p.
Fenofibrate	0.5	28.5	0.2 – 56.8
Fenoprofen	0.5	n.p.	n.p.
Gemfibrozil	0.5	0.4	0 – 0.7
Indomethacine	0.5	15.0	1.4 – 28.6
Ketoprofen	0.5	1.8	0.04 – 3.6
16α-Hydroxyestrone	10	Between estrone & 17ß-estradiol, i.e. 2.9 – 13.0*	
17α-Ethinylestradiol	50	0.03	0 – 0.05
Mestranol	5	n.p.	n.p.
Estrone	10	6.1	2.9 – 9.2
17ß-Estradiol	10	8.7	4.4 – 13.0
Estriol	100	22.6	2.5 – 42.7

n.p. = not possible to calculate concentration in yellowwater because of lack of prescription data.
* According to Xu et al. (1999) and Naganuma et al. (1989).

It has to be mentioned that the calculated mean concentrations of acetylsalicylic acid, fenofibrate, and indomethacine were above their LOQs of 0.5 µg l^{-1} although they were not detected during the measuring campaign. An explanation for not detecting acetylsalicylic acid (ACA) is that its excretion depends on the pH of urine (Therapeutic drugs, 1999) which is also reflected by the large range between calculated minimum and maximum concentrations. Fenofibrate (FEN) is mentioned as a pro-drug completely transformed to fenofibric acid (Therapeutic drugs, 1999; Haefeli, 2004; Arzneimittel-Kompendium der Schweiz, 2007). Only Goodman et al. (2006) state that up to 10 % of FEN is excreted via urine without metabolisation. This value influenced the calculation of the maximum and thus also of the average concentration. The calculated minimum of FEN (0.2 µg l^{-1}) was below the LOQ. The concentrations of the substances listed in Table 11 in urine might also be affected by degradation during storage of the urine. Strompen et al. (2003) found that ACA disappeared completely from spiked urine at pH levels of 2, 7, and 9 within 3 months, while FEN and indomethacine (IND) concentrations only decreased at pH 2 by 30 % (IND) and 40 % (FEN) within 6 months (initial concentrations: 0.1 mg l^{-1}).

Moreover, it has to be pointed out that excreted metabolites as well as pharmaceutical transformation products generated during storage were not included.

They were not analysed and literature lacks comprehensive information concerning metabolites for some of the pharmaceuticals discussed. Especially, quantification of these transformation products was often impossible. A very promising approach to include them was made by Lienert et al. (2007b). Here missing information was estimated by modelling it by means of quantitative structure-activity relationships (QSAR). Thereby, the authors were able to include them in ecotoxicological hazard predictions.

3.3.3 Comparison to pharmaceutical concentrations in yellowwater derived from literature

In some other projects with established source-separation of urine, sporadic pharmaceutical measurements have been conducted: Solarcity, a settlement in Linz, Austria, (Steinmüller, 2006), Kantonsbibliothek Liestal, a library close to Basel, Switzerland, (Pronk et al., 2007), three locations in Danish harbours, mainly public toilets, (Wrisberg et al., 2001) and the already mentioned German project Lambertsmühle (Strompen et al., 2003).

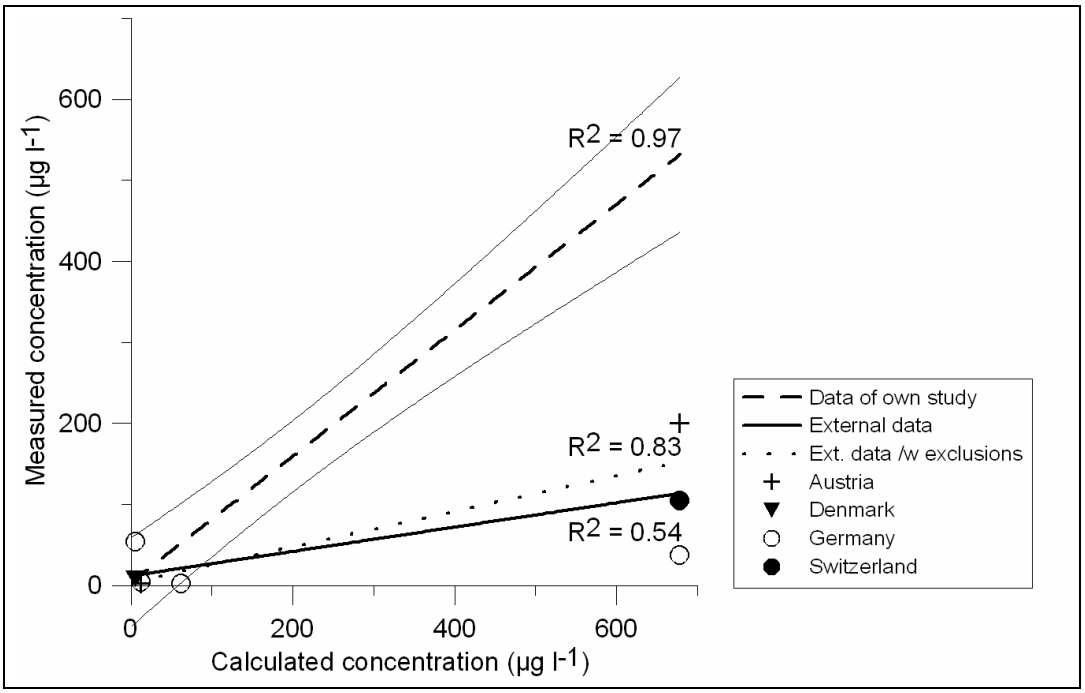

Figure 14: Comparison of mean calculated concentrations of excreted pharmaceuticals in urine and concentrations measured in other projects (mean values; “external data”). “External data /w exclusions”: pharmaceutical concentrations measured in other projects (“external data”) with exception of Strompen et al. (2003). “Data of own study”: data points of this study (mean values of Hamburg and Berlin, Winker et al., 2008b).

When comparing these summarized analytical results to the calculations of this study, R^2 is as low as 0.54 (Figure 14). It has to be mentioned, however, that the pilot project Lambertsmühle, a museum in the Western part of Germany, had only very few donors during the period of measurements. Measurements in Hamburg and Berlin corroborate the assumptions that a comparison to certain number of people are necessary to obtain reasonable values where aberrant behaviour of one person is no longer visible due to the large volume collected. Consequently, R^2 increased to 0.83 when data of Lambertsmühle were neglected, although only four data points remained for comparison.

Another reason for such poor correlations might be that the other measurements were mainly performed outside Germany. Although the pharmaceutical consumption in Austria and Switzerland is quite similar to Germany (Österreichische Apothekenkammer, 2006), this might be an indicator of the sensitivity of the pharmaceutical concentrations and their variability between the different countries caused by even small differences in consumer behaviour. Unfortunately, this assumption could not be evaluated within this research project. Nevertheless, a certain ranking of pharmaceuticals in yellowwater with respect to their concentrations was consistently found in the different projects:

carbamazepine < diclofenac < ibuprofen.

Due to a lack of data, this observation could not be extended to other substances and therefore remains an interesting aspect which should be investigated in more depth.

Overall, it has to be pointed out that (as already indicated for ACA, FEN, and IND) the effect of storage, induced by pH augmentation due to ureolysis (Udert et al., 2003b), remains uncertain and was not considered within these calculations. The reason was lacking data certainty. Butzen et al. (2005) detected efficient removal for DIC after six month; for further pharmaceuticals partial removal during eleven months of storage at pH 2; in the neutral pH range, only tetracycline was reduced by about 20 %; and at pH 9, which represents self-establishing pH in urine after six months storage, IBU, CZ, and FEP by about 15 % as well as two antibiotics by up to 35 %. The initial concentrations of pharmaceuticals were 0.1 mg l^{-1}. In contradiction to these findings, Gajurel (2007) did not find any decay of clofibric acid, CZ, DIC, and IBU in spiked yellow water (initial pharmaceutical concentration: 10 mg l^{-1}) during a one year storage period under all investigated storage conditions: presence/absence of light; various temperatures; with and without agitation; and at three pH levels (4, 7, and 10). Due to these different results, for this study absence of degradation was assumed as a worst case scenario although this might not be true for all pharmaceuticals. Further research is urgently needed in this field.

Looking at urine as a potential fertiliser in agriculture, the application rate is limited by nitrogen (Hammer and Clemens, 2007). For this macro nutrient, the threshold value

is reached first. When comparing yellowwater with cattle and pig slurry, two organic fertilisers already in practice since many centuries, its load on pollutants is much lower. Heavy metals' input would be only 10 % or less then that of the two animal slurries (Hammer and Clemens, 2007). This is similar to antibiotics and hormones. For antibiotics a comparison of the group of tetracyclines was possible. Oxytetracycline had the highest flux with 0.43 g ha^{-1} a^{-1} and was equivalent to 4 % of the flux of cattle and only 0.3 % of pig slurry. A decrease of loads with ranking pig slurry > cattle slurry > human urine (AGU) was found for hormones as well. Although, for other groups of pharmaceuticals calculated and measured to be present in urine (3.3) a comparison was impossible as many human pharmaceuticals are not applied in veterinary medicine. Therefore, it is of tremendous importance to gain more knowledge on the behaviour in soil of these pharmaceuticals (3.5.3) and their potential uptake by plants (3.6) possible risks could not be determined basing on the present situation: The largest use of yellowwater in European agriculture is found in Sweden. However, no detailed investigation and monitoring of the impact of pharmaceutical substances contained in this urine used as agricultural fertiliser was accomplished so far (Vinnerås, Swedish University of Agricultural Sciences, Sweden [personal communication]).

Intermediate conclusion

- The concentrations of bezafibrate, carbamazepine, diclofenac, ibuprofen, phenazone, and pentoxifylline in urine can be determined by calculations in a way that measurements are no longer required when a donor group of ≥100 people is connected to the respective collection system. In all cases the calculated concentrations exceeded the measured ones.
- The theoretical model showed very good correlations with the analytical data obtained for the public waterless urinal in Hamburg (R^2: 0.98) and for the waterless urinals and source separating toilets in offices and flats in Berlin (R^2: 0.90).
- The comparison showed the importance of a large user group of ≥100 people for measuring average values close to those predicted by the calculation for mean German concentrations.
- This result was also verified by using additional datasets for pharmaceuticals' concentrations in yellowwater from other studies although the correlation was worse (R^2: 0.54). As reasons for weak correlation, smaller groups of donors in most cases and differences in pharmaceutical consumption between the countries are assumed.
- Overall, the presented calculations are a good way to receive an overview of potential concentrations of pharmaceuticals in yellowwater before starting an expensive measuring campaign.
- Impacts of these pharmaceuticals concentrations in urine for the environment cannot be determined so far.

3.4 Comparison of pharmaceutical concentrations in urine and raw municipal wastewater

Raw municipal wastewater is a mixture of the wastewater streams blackwater, greywater, industrial wastewater, infiltration water and eventually storm water (in the case of combined sewers). With respect to pharmaceuticals, greywater contributes mainly chemicals used in creams, detergents and other products applied to the skin as cosmetics (Eriksson et al., 2003). As greywater is a highly diluted stream, concentrations are rather low (Otterpohl et al., 1999). Blackwater, equivalent to toilet wastewater and containing urine and faecal matter, is expected to contribute larger amounts of pharmaceuticals to domestic wastewater. As the correlation is unclear so far, a comparison is made between data of raw wastewater collected in the database (and already presented shortly in 3.2), and data on calculated pharmaceutical concentrations in yellowwater. Consequently, the focus of this chapter is to clarify the correlation between pharmaceutical concentrations in yellowwater and raw wastewater.

A query within the database was carried out in order to gather information regarding concentrations of pharmaceuticals determined in raw domestic wastewaters in Germany, Austria, and Switzerland according to the method described in 2.3.2. Limits of quantification were between 10 ng l^{-1} (Düring et al., 2006) and 200 ng l^{-1} (Ternes et al., 2007). Moreover, in some datasets (number of datasets for each pharmaceutical given in brackets: betaxolol (1), bezafibrate (1), carbamazepine (1), cyclophosphamide (1), diclofenac (4), fenofibrate (1), ibuprofen (1), pentoxifylline (1) and phenazone (1)) numbers of samples were not reported in the original article. In these cases n_i was set to 7.93. This was the average of the given numbers of samples from all literature sources displaying this information.

3.4.1 Predicted excreted amounts of pharmaceuticals in urine and faeces

Out of the 332 pharmaceutical substances recorded in the DB, 33 were quantified in urine as well as in raw sewage and are discussed in more detail. The restriction is caused by the availability of data for raw sewage and determination of renal excretion of the respective pharmaceuticals (see 2.3.2 and 2.3.1). The major part of non-metabolised parent compounds are excreted via urine (Figure 15), although some substances also show high faecal excretion rates as erythromycin (50 %) and terbutaline (60 %) (FachInfo-Service, 2005). Besides, the faecally excreted concentration of ciprofloxacin is relatively high with 0.75 g $pers^{-1}$ d^{-1} compared to a consumed dose (A_C) of 3.0 g $pers^{-1}$ d^{-1}. A main reason for this is low intestinal resorption. Although amounts of pharmaceuticals not resorbed are assumed to occur completely in faecal matter (Lüllmann et al., 2003), in the study presented only faecal excretion rates were used which had been reported in literature. As well it has to be considered that data are from different literature sources. Consequently the expected

total of 100 % which should be the maximum achieved when summing up renal and faecal excretion might be exceeded. This was the case for terbutaline (130 %).

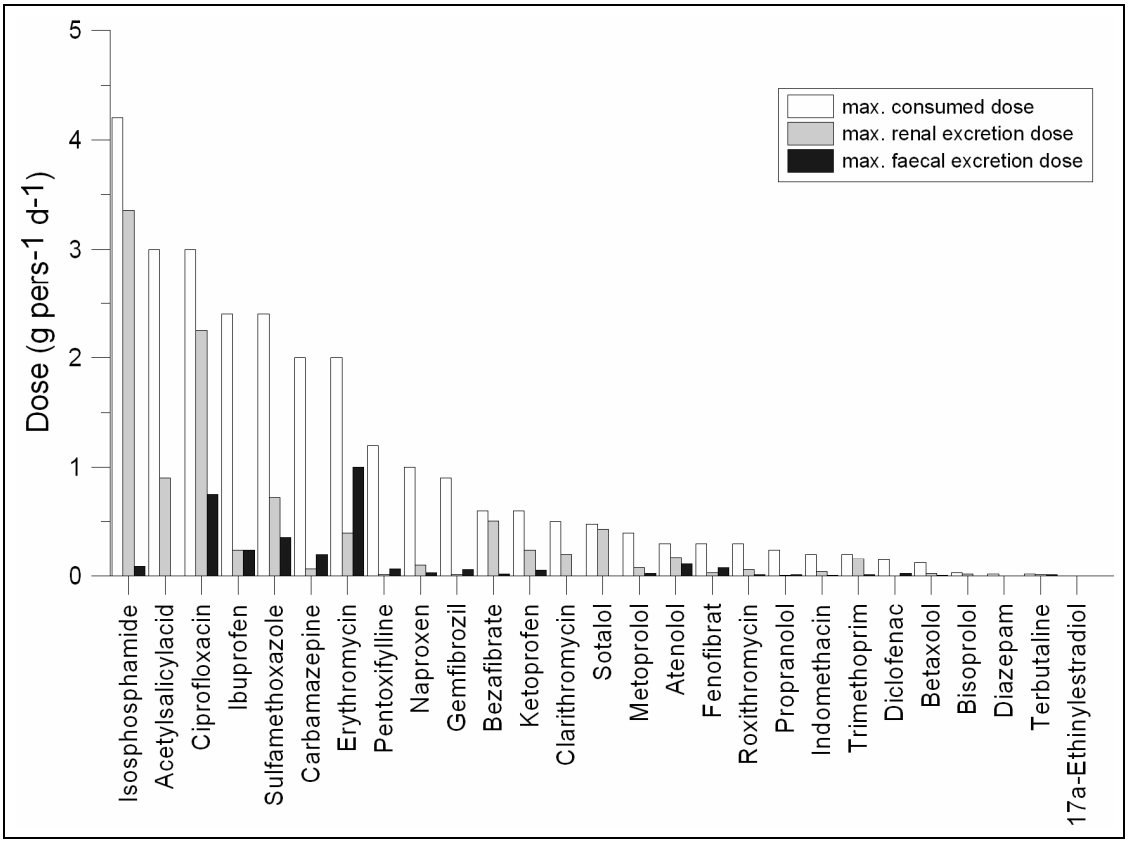

Figure 15: Maximum consumed daily doses for one patient and their related amounts excreted via urine and faeces. (March 15, 2008)

For 6 pharmaceuticals no data of faecal excretion were mentioned in the reviewed literature: albuterol, azithromycin, clenbuterol, cyclophosphamide, paracetamol, and phenazone. This can be looked at as an indicator that this pathway of elimination does not play an important role for them. Hence, these substances are not shown in Figure 15 but presented only with their maximum renal excretion rate in Figure 16. Moreover, in Figure 16 not only the maximum DDD_G are shown but also the minimum ones. In most cases minimum doses are around half of maximum consumed daily doses. Exceptions are albuterol (17 % of max. DDD_G) and clenbuterol (25 %). All drugs are consumed in the range of several mg pers^{-1} d^{-1} to 3 g pers^{-1} d^{-1}. An exceptionally small maximum DDD_G is given for clenbuterol (Figure 15 and Figure 16), a bronchospasmolytic drug: 0.00002-0.00008 g pers^{-1} d^{-1} (FachInfo-Service, 2005) as well as for 17α-ethinylestradiol (0.00005 g pers^{-1} d^{-1}, FachInfo-Service, 2005).

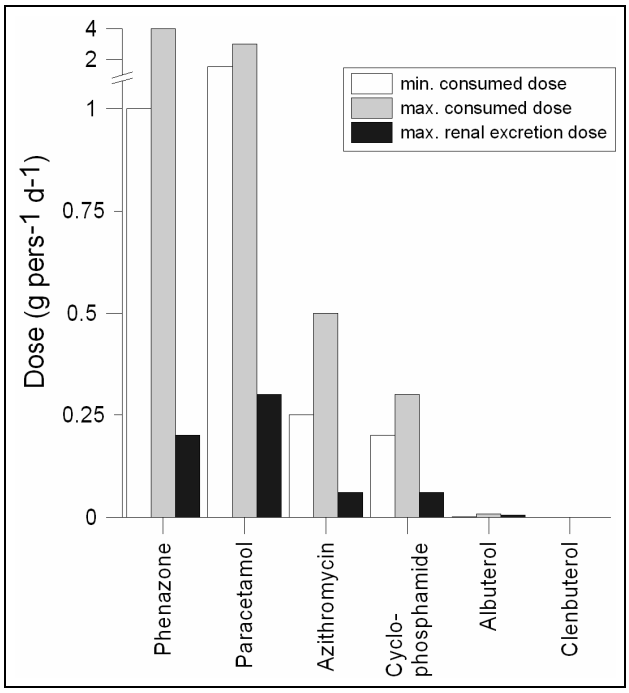

Figure 16: Minimum and maximum consumed daily doses (DDD_G) and equivalent daily renal excretion of those substances without available faecal excretion data. (March 15, 2008)

Figure 17 shows the calculated overall annual amounts of consumed pharmaceuticals in Germany and their excreted amounts via urine in kg a^{-1}. The ratios of consumed to excreted amounts vary widely among the compounds. Only traces of the consumed dose are excreted in case of diclofenac, gemfibrozil, isosphosphamide, and pentoxifylline while 90 % of ingested sotalol are found in urine. Also, clenbuterol (87 %), bezafibrate (85 %), and trimethoprim (80 %) are largely renally excreted.

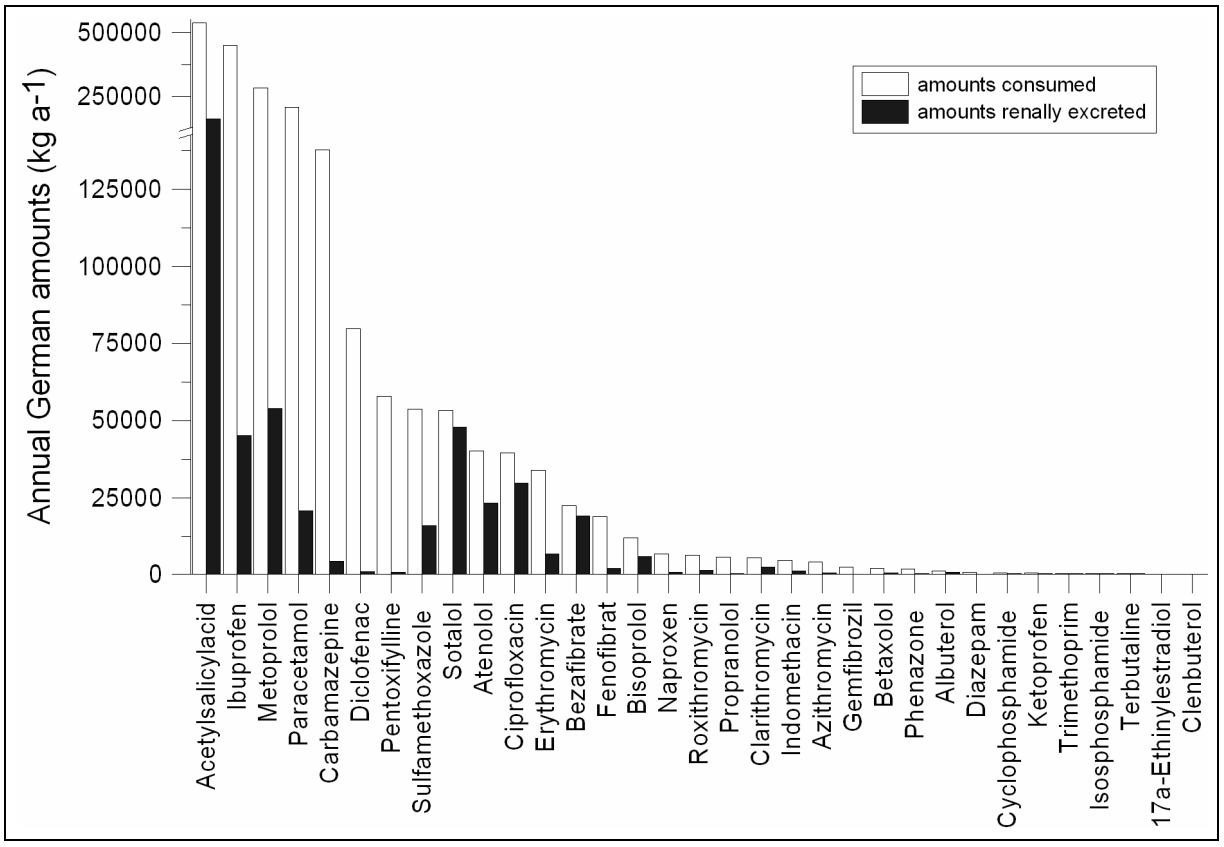

Figure 17: Comparison of maximum consumed pharmaceutical amounts to maximum renally excreted amounts on an annual base for Germany. (March 15, 2008)

The results of comparing consumed with excreted amounts are reflecting the impact of pharmacokinetic activities. Figure 17 indicates that the highest consumed doses do not necessarily indicate the largest amounts discharged to the sewer via urine. Highest annual consumed doses (above 100 t a^{-1}) were calculated for acetylsalicylic acid (539 t a^{-1}), ibuprofen (451 t a^{-1}), metoprolol (283 t a^{-1}), paracetamol (207 t a^{-1}), and carbamazepine (138 t a^{-1}). While among these substances only acetylsalicylacid with 162 t a^{-1}, ibuprofen with 45 t a^{-1}, and metoprolol with 83 t a^{-1} exhibited high renally excreted amounts, other pharmaceuticals which were consumed in far lower amounts are probably discharged to extents of the same order of magnitude as ibuprofen: sotalol (48 t a^{-1}) and ciprofloxacin (30 t a^{-1}). Atenolol (23 t a^{-1}), paracetamol (21 t a^{-1}), bezafibrate (19 t a^{-1}), and sulfamethoxazole (16 t a^{-1}) were calculated to be excreted in reasonably high amounts as well, whereas theoretical discharge of erythromycin to raw municipal wastewater was 7 t a^{-1} and all other investigated substances showed even smaller calculated mass flows for renal excretion.

The influence of pharmacokinetics on discharge to sewerage can be shown very lucidly when considering carbamazepine. Occurrence of carbamazepine in the aquatic environment has been intensely investigated for years. Its high consumption

rate is given as a major reason in the rationales of such research. Additionally, its frequent detection in wastewater, groundwater, and drinking water (Ternes, 1998; Heberer et al., 2001; Roßknecht et al., 2001; Sacher et al., 2001; Ternes, 2001; BLAC, 2003; Wiegel et al., 2004; Zühlke et al., 2004) and its recalcitrance (Strenn et al., 2004) contribute to the significance of this substance. But due to pharmacokinetic effects, only around 4.5 t a^{-1} are considered to be excreted via urine in Germany in a worst case scenario, i.e. taking into account the maximum consumed daily dose. Figure 18 shows very well that maximum renally excreted amounts of two other antiepileptics, gabapentin with 34 t a^{-1} and primidone with 6 t a^{-1}, are much higher although their maximum consumed amounts are only 23 % (gabapentin) and 6 % (primidone) of that of carbamazepine. As Brun et al. (2006) point out that pharmacokinetics are a key issue for excretion of pharmaceuticals and help to predict their mass flow into sewage, the question arises which other pharmaceuticals might be contained in urine, even in higher concentrations than carbamazepine, and are ignored until now as the example shown might not be unique when looking at other indication groups.

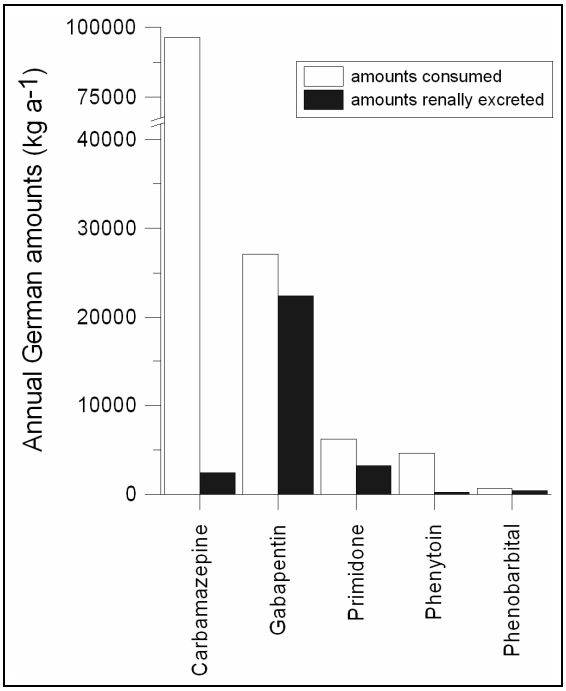

Figure 18: Comparison of mean consumed pharmaceutical amounts with mean renally excreted amounts on an annual basis for Germany for antiepileptics contained in the DB. Only those substances where calculation was possible are shown. (March 15, 2008)

3.4.2 Pharmaceutical concentrations in urine versus raw municipal wastewater

So far it was shown that pharmaceuticals are excreted to a large part via urine and only partially via faeces. As these substances are discharged to raw municipal wastewater, they have been analysed in sewage. So, it can be assumed that there is a correlation between concentrations of pharmaceuticals theoretically calculated in urine and raw wastewater. These concentrations are plotted in Figure 19.

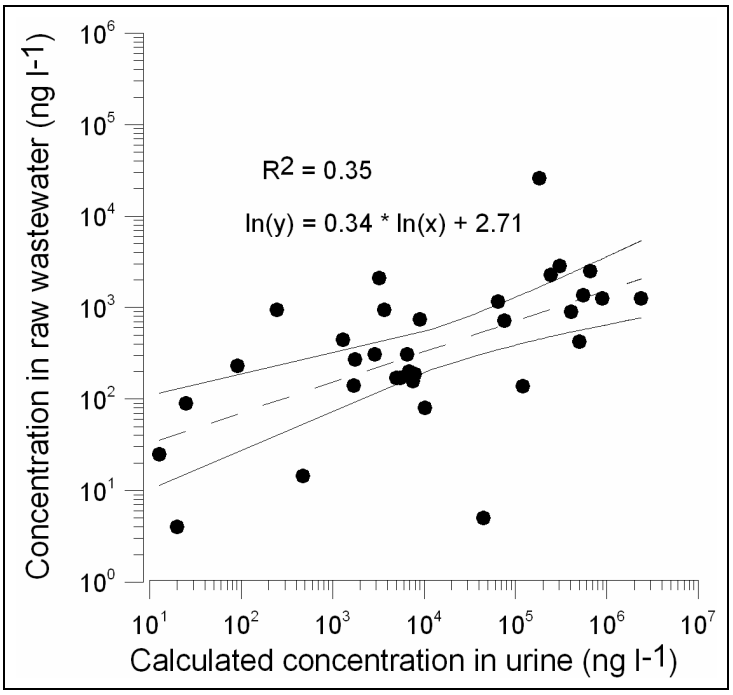

Figure 19: Concentrations of 33 pharmaceuticals in raw domestic wastewater in comparison with their concentrations in urine. Lines show 95 % confidence interval. (March 15, 2008)

The number of available sewage datasets per substance utilized in Figure 19 varied reasonably. For azithromycin, ciprofloxacin, clenbuterol, fenofibrate, gemfibrozil, paracetamol, and pentoxifylline only one dataset was found in the reviewed literature. Furthermore, investigations about betaxolol, ketoprofen, and pentoxifylline occurrence in municipal wastewater were not reporting detection frequency, *df*, in all cases. As the total number of datasets was very small, these particular pharmaceuticals were included (Figure 19) assuming *df* = 100. Albuterol (81 ng l^{-1}), clenbuterol (25 ng l^{-1}), diazepam (14 ng l^{-1}), 17α-ethinylestradiol (90 ng l^{-1}), fenofibrate (10 ng l^{-1}) and isosphosphamide (4 ng l^{-1}) showed very small concentrations in raw wastewater, all <100 ng l^{-1}. This is in accordance with renally excreted mass flows calculated for these substances (Figure 17). Accordingly, those substances detected in wastewater with average concentrations ≥2000 ng l^{-1}, bezafibrate, ibuprofen, metoprolol, paracetamol, and propranolol, exhibit high theoretical concentrations in urine except propranolol. Urine and raw wastewater

data for acetylsalicylic acid and fenofibrate are contradictory. An explanation might be that the excretion of acetylsalicylic acid is related to pH of urine (1999). Nevertheless, an average of 1270 ng l^{-1} was measured in raw domestic wastewater. Fenofibrate is a pro-drug transformed to fenofibric acid (Therapeutic drugs, 1999; Haefeli, 2004). Only Goodman et al. (Goodman et al. (2006)) state in the revised literature that 10 % of fenofibrate are excreted via urine. This rate of 10 % was taken for the worst-case scenario.

However, the ratios of pharmaceutical concentrations calculated for urine to their concentrations detected in raw wastewater show quite wide ranges and vary between 0.3 and 1177 (average: 469) not including acetylsalicylacid and fenofibrate. The ratio of 0.3 was determined for 17α-ethinylestradiol, the ratio of 1177 for ciprofloxacin. A strong correlation was not observed, neither for single fields of indications like e.g. beta blockers nor overall. The determination coefficient is 0.35. Only a slight tendency is visible by means of the confidence interval of 95%. The low extent of correlation is due to the fact that raw domestic wastewater contains approx. 100 times more water than urine (Wendler, 2004) and is additionally diluted by industrial wastewaters, infiltration water and in the case of combined sewers by stormwater. Dilution factors in different catchment areas may differ considerably. Moreover, homogeneous distribution of pharmaceuticals in the entire yellowwater generated in Germany was assumed in the theoretical calculations. Under real conditions this is obviously not the case e.g. when looking at districts with and without hospitals and nursing homes or due to seasonal medication (Heberer, 2002). Also the extent of a sewer catchment area may affect pharmaceutical concentrations in raw wastewater: the smaller the number of inhabitants connected to a treatment plant, the larger the deviation from “average German sewer concentrations” of pharmaceuticals may be.

Additionally, pharmaceuticals entering the sewage via greywater or by pharmaceuticals thrown into toilets augment concentrations in wastewater as well as pharmaceuticals sold over the counter are not included in the Arzneiverordnungs-Report 2004 (Arzneiverordnungs-Report 2004, 2004) and were thus not included in the theoretical calculations. Furthermore, concentrations of pharmaceuticals in faecal matter are not considered in the data of Figure 19 as well. Besides, concentrations in municipal raw wastewater were measured in the influent of WWTPs, effects during transport through the sewerage system most likely altered an unknown part of the original pharmaceutical concentrations. Degradation by microbial activities is such a pharmaceutical sink in sewers. Microbial degradation does not affect all pharmaceuticals to the same extent. E.g. pentoxifylline was degraded by more than 80 % in 7 days in laboratory investigations (Sanofi-Aventis, 2006) while ciprofloxacin shows a very low biodegradation potential (Wetzstein et al., 1999; Kümmerer et al., 2000b; Golet et al., 2003). Therefore, the correlation between raw wastewater and urine was revised for other aspects affecting concentrations of pharmaceuticals in

raw wastewater. No positive response could be found for log K_{OW}, sorption behaviour, biodegradability, or indication. Moreover, seasonal and annual variations can play a role. Seasonal variations can be neglected as average concentrations of raw wastewater originate from samples taken in different seasons. For estimating the scale of annual variations, numbers of DDDs of 1999, 2003, and 2005 were compared (Arzneiverordnungs-Report 2000, 2000; Arzneiverordnungs-Report 2004, 2004; Arzneiverordnungs-Report 2006, 2007). The results showed larger changes of the prescribed amounts (increase of >100 Mio N°s. of DDDs in the period of 1999 to 2006) for the following substances: bisoprolol (+370 Mio N°s. of DDDs), ibuprofen (+100 Mio N°s. of DDDs), and metoprolol (+520 Mio N°s. of DDDs).

Taking into consideration the mentioned reasons for data variation, the results nevertheless suggest that urine is a major source of human pharmaceuticals and their metabolites in wastewater. Due to a much lower dilution factor of pharmaceuticals in urine than in raw domestic wastewater (Wendler, 2004) and the problems of today's wastewater treatment plants as insufficient barriers for protecting the environment from traces of many pharmaceuticals (Paxéus, 2004; Strenn et al., 2004; Castiglioni et al., 2006), separation of yellowwater by urine diverting toilets or urinals seems a promising approach to collect a major part of pharmaceutical pollutants directly at the source. As appropriate source control devices are available (Werner et al., 2005; Otterpohl and Oldenburg, 2007), it is a question to determine effective technologies for yellowwater treatment. Possible techniques are evaporation combined with crystallization and drying, stripping of ammonia, struvite precipitation (Tettenborn et al., 2007), ozonation (Gajurel, 2007), nanofiltration (Pronk et al., 2006c), electrodialysis (Pronk et al., 2006a), and a combination of bipolar electrodialysis with a gas transfer membrane (Pronk et al., 2006b). Due to the fact of lower volumes of the yellowwater stream with higher concentrations, these technologies are expected to work much more effective when adapted to urine than complete wastewater treatment. Aside, recovery of nutrients from urine is accomplished by some of these treatment processes as well (Pronk et al., 2006c; Tettenborn et al., 2007). For the development of promising techniques, the spontaneous processes appearing in separated urine like microbial urea hydrolysis, mineral precipitation, and ammonia volatilisation are important to consider (Udert et al., 2003b). Subsequent to urea hydrolysis leading to elevated pH, very promising results can be achieved e.g. by steam stripping. Only slight traces of ibuprofen (0.4 % of the originally detected concentration of 411 µg l^{-1}) were detected in the condensate, all other analysed pharmaceuticals were not detected (Tettenborn et al., 2007). Another auspicious technology is nanofiltration. Especially the NF270 of Dow-Filmtec achieved a high removal of various pharmaceuticals while recovering nitrogen from urine (Pronk et al., 2006c). These first promising results indicate that further investigations are strongly needed to receive an optimum treatment quality and efficiency for a wide range of pharmaceuticals.

Intermediate conclusion

- Major parts of pharmaceuticals and their metabolites detected in municipal wastewaters originate from urine although some substances show reasonable excretion via faeces.
- Pharmacokinetic data are a key aspect to understand and estimate release of pharmaceuticals to the environment. Although theoretical assumptions on average pharmaceutical concentrations in urine based on these data fit to some extent to levels detected in raw sewerage, more investigation of pharmaceutical residues in urine collected in large communities with regard to consumption of pharmaceuticals within these communities is needed.
- A relation between concentrations of pharmaceuticals in raw domestic wastewater and urine could be conducted in this study which however exhibited only a weak statistical evidence due to environmental effects appearing during the passage from human excretion to influents of wastewater treatment plants.
- Overall, it can be concluded that urine separation and separate handling of this wastewater stream represents a promising approach to lower the pharmaceutical load of raw domestic wastewater, to disburden wastewater treatment plants, and to protect the aquatic environment safely from pharmaceuticals.

3.5 Pharmaceuticals in soil

Halling-Sørensen et al. (2002) stated that concentrations and fate of antibiotics in soil were of major importance to evaluate the role of contaminated manure for water and food crops. Similar accounts for the usage of urine as fertiliser in agriculture. Hence, a major focus is laid on pharmaceuticals detected in soils, their respective concentrations in this medium, pathways of transmission, as well as their behaviour in soil. (Presented data and following discussion includes all datasets regardless of *Location*. Numbers for the area of Germany, Austria, and Switzerland (referred to as GACH) were added in brackets.)

The table *Soil* contains information regarding presence of pharmaceuticals in different solid substrates: soil as well as sewage sludge and manure, plus river sediment and solid waste. Overall, 61 (40) pharmaceuticals were reported in *Soil*. 221 (122) datasets report positive detection ($df \neq 0$) of pharmaceuticals in these substrates. The main interest was in pharmaceuticals detected in soil, but for complementation the other media types were entered, especially as sewage sludge and manure are used as fertiliser in agriculture and are potential transmitters (Hammer and Clemens, 2007). Pharmaceutical concentrations within them reflect the loads that soils already have to deal with now.

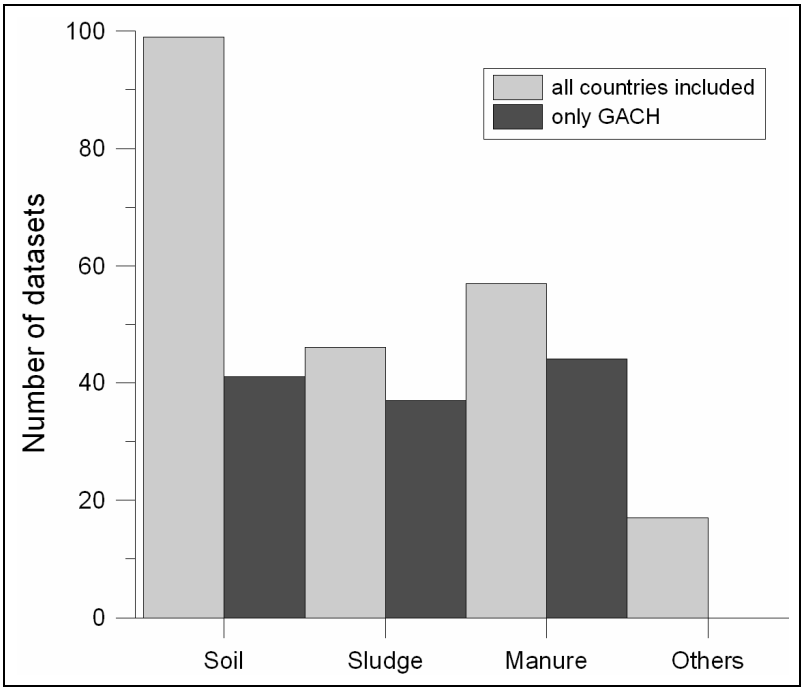

Figure 20: Number of datasets of the different solid substrates summarised under *Soil*. Datasets for the area of Germany, Austria, and Switzerland (GACH) are shown as separate bars. (February 14, 2008)

Figure 20 shows that only 45 % (36 %) of the datasets present actual concentrations measured in soil. Focusing at GACH countries, the data is even poorer: more datasets describe concentrations in manure than in soil. Additionally, the overall number of datasets (99 (44) respectively) reporting on soil is very low compared to other media. Only very little research was carried out in this field contrary to the large number of investigations on pharmaceuticals in wastewater and in water bodies. This lack of data is one of the reasons why prediction of the pharmaceutical impact with respect to fertilising agricultural land with human urine is that difficult to state.
Nevertheless, 61 pharmaceuticals were listed under *Soil* (Figure 9). But compared to the overall number of pharmaceuticals in the DB, this implies only 18 % while *Wastewater* and *Water* hold 53 % and 55 % respectively of all substances listed in the DB. Additionally, as different types of solid substrates were listed below *Soil* (Figure 20), not all pharmaceuticals are just related to datasets reporting concentrations in soils, but in various types of sludge and manure as well. Especially, many pharmaceuticals contained in sludge are expected to be present in urine as well.

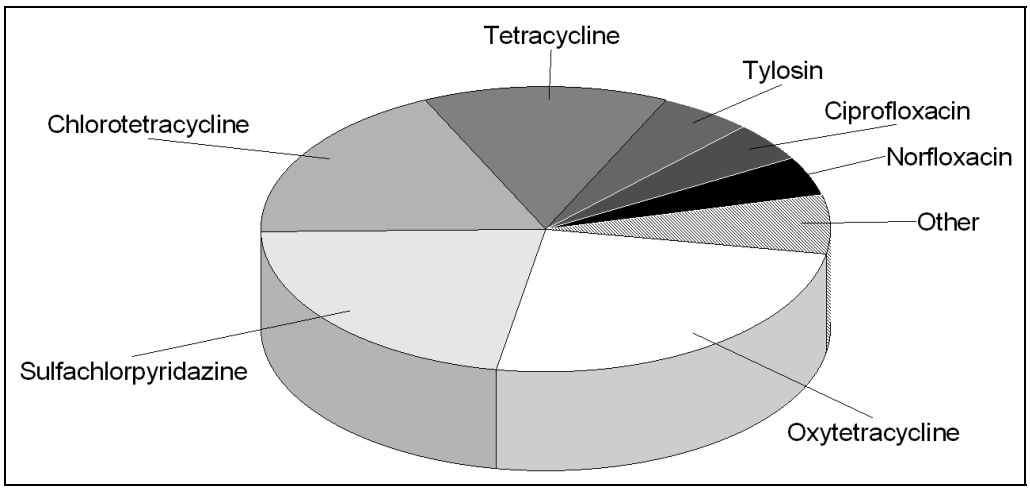

Figure 21: Percentage of pharmaceuticals investigated in soils reflecting the number of datasets related to each substance. Pharmaceuticals <4 % were summarised below "Others". (February 14, 2008)

Only 11 (10) of the DB's pharmaceuticals stated in *Soil* were investigated in "real" soil and listed in one of the 136 (67) related datasets. This does not imply that all of them report detection of pharmaceuticals ($df \neq 0$). All of these 11 (10) substances are used in veterinary medicine. Expect tylosin (feed supplement) all have multiple nominations of indication groups but name "antibiotics" at least as one of the represented groups. This is another indicator for their origin as and usage in veterinary medicine.

3.5.1 Pharmaceutical concentrations in soil

In the following, concentrations in soil for the above mentioned 11 pharmaceuticals were investigated in more detail regardless of their national origin. The results showed that datasets of one soil type, stagnic luvisol, could not be used any further as not enough details were given to allow for an evaluation of concentrations in soil according 2.3.2. Hence, three pharmaceuticals were excluded as data for them only existed for this soil type: N(4)-acetylsulfadiazine, sulfadiazine, and trimethoprim.
Overall, only ten articles were available. For most datasets information existed for concentrations related to dry matter (DM). This was not the case for Kay et al. (2005b), Stoob et al. (2005) and Stoob et al. (2006). Here, the authors were contacted to allow translation of C_P from fresh matter (FM) into DM.

Table 12: Pharmaceutical concentrations detected in various soil types irrespective of depth in µg kg^{-1} DM. Agricultural land means that the area is under agricultural usage without detailed specification of its respective soil type. DS stands for datasets. (June 13, 2008)

Substance	Soil type	Min	C_P	Max	SD	CI 95% (%)	N°. of DS
Chlorotetracycline	agricultural land	0.5	6.7	13.6	4.0	14	23
Ciprofloxacin	agricultural land	57.5	98.0	157.5	39.5	12	6
Norfloxacin	agricultural land	5.5	106.1	200.0	55.3	15	6
Oxytetracycline	agricultural land	0.5	0.6	2.5	0.6	57	3
Oxytetracycline	clay loam	3.8	116.3	430.0	123.5	28	19
Oxytetracycline	sandy loam	25.0	109.2	526.0	156.8	41	12
Sulfamethazine	agricultural land	15.0	116.2	230.0	125.3	172	3
Sulfamethazine	loam	240.0	327.1	470.0	105.0	12	5
Tetracycline	agricultural land	0.5	31.6	198.7	42.1	39	19
Tylosin	agricultural land	0.5	12.6	423.3	14.8	39	7
Sulfachloropyridiazine	clay loam	3.8	35.2	140.0	34.5	28	18
Sulfachloropyridiazine	sandy loam	18.0	229.6	756.0	269.9	34	12

Data presented in Table 12 is given regardless of the specific soil depths where samples have been taken. Differentiation along depth was not performed within this evaluation (Table 12) as availability of data per substance and soil type was limited. To provide some information on this aspect, pharmaceutical concentrations were evaluated along the soil depth irrespectively of the specific substances in Table 13.

Table 13: Pharmaceutical concentrations in µg kg^{-1} DM detected in soil related to specific depth. Depths overlap due to the varying specifications in the original sources. (June 13, 2008)

Depth in cm	Min	C_P	Max	SD	CI 95% (%)	N°. of DS	N°. of DS > LOQ
0 - 10	4.6	158.1	756.0	161.7	27	54	94
10 - 20	4.7	55.6	470.0	89.6	55	30	87
0 - 20 / 30	0.5	9.2	42.3	12.5	80	11	73
20 - 40	3.6	60.2	619.0	134.3	95	21	76
30 / 40 - 90	1.0	5.0	7.6	2.3	27	11	0
0 - 90	1.0	3.0	5.0	2.3	75	4	0

Pharmaceutical concentrations (C_P) in soil decrease with depth (Table 13) with exception of the dataset representing the layer of 20 to 40 cm. A second indicator for this tendency is the continuously declining frequency of positively detected datasets (DS > LOQ), again showing a deviation for the layer from 20 to 40 cm depth. And last but not least, any pharmaceuticals were detected below 90 cm in the included studies. Moreover, it has to be stated once more that the time of measurement in relation to the potential date of manure spreading was not considered due to the limited number of datasets. Nevertheless, a decrease over time after spreading is reported in literature (Alexy and Kümmerer, 2005; Kay et al., 2005b; Boxall et al.,

2006a) and can be expected at least for medium to highly biodegradable substances. Hamscher et al. (2001) observed an accumulation of tetracyclines between the first months which disappeared in the following year. Furthermore, in the case of antibiotics tillage reduced leaching to deeper soil layers (Kay et al., 2005a). Further details regarding processes in soil are outlined in 3.5.3.

Pharmaceuticals were introduced into soil most likely via application of animal manures (Hammer and Clemens, 2007) and sewage sludge (BLAC, 2003). The database provides information about concentrations in such media within the same section *Soil* (Figure 20). Overall, 30 pharmaceuticals were stated to be detected in various forms of sewage sludge and 11 in different types of animal manure. 3 of these substances detected in sludge and 7 in manure were stated to be found in soil as well. As application to fields occurs, many other insofar undetected substances are likely to reach our agricultural soils.

3.5.2 Presence of pharmaceuticals in groundwater

The contamination of groundwater by pharmaceutical residues has already been roughly stated in 3.2. It was pointed out that measurements were performed by various research groups especially below areas suspected to be polluted with pharmaceuticals such as waste dumps and fields irrigated with wastewater. Although average concentrations were rather low (in the ng l^{-1} range) maximum weighted mean concentrations, C_{MW} (eq. 11), up to 5.6 µg l^{-1} (diatrizoate) were detected. This finding resulted in the assumption of leaching of pharmaceuticals through soil hypothesized by many researchers in this field. This phenomenon must be considered to be also relevant in the case of urine application. Therefore, this aspect is taken up here and analysed in more depth.

According to collected data, 119 (resp. 93 in GACH countries) pharmaceuticals were analysed in groundwater. Only 54 (37) out of this group were positively detected, $df \neq 0$. Positive detection was reported in 216 (124) datasets out of the original number of 527 (363) where $df = 0$ was included (Figure 22).

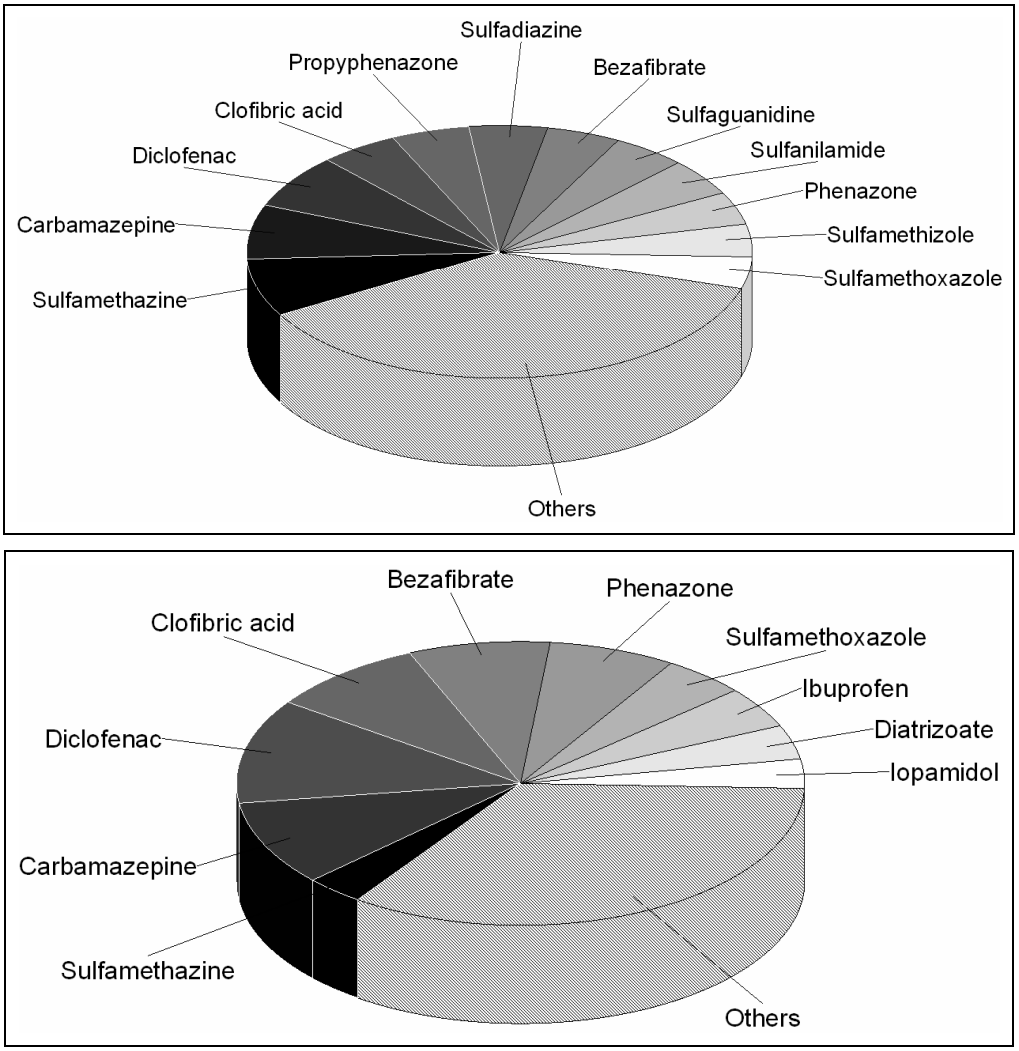

Figure 22: Pharmaceuticals detected in groundwater given as percentage of datasets collected (upper graph) and limited to GACH groundwater (lower graph). Substances nominated in <4 % (top) / <3 % (bottom) of all datasets in this category were summarised under “Others”. (February 14, 2008)

When comparing pharmaceuticals detected in groundwater and holding fractions ≥5 % in the overall dataset to those contained in the restricted dataset for GACH countries, only four pharmaceuticals were among the highest fractions of both groups: diclofenac, carbamazepine, clofibric acid, and bezafibrate (see Figure 22). Only two antibiotics, sulfaguanidine and sulfanilamide, with percentages ≥5 % of all datasets nominated in this category were not detected at all in any groundwater samples taken in Germany, Austria, and Switzerland. Their datasets belong to a Danish investigation of Holm et al. (1995) concerning landfill leachates and the potential of the pollutants to reach groundwater.

In many publications and scientific reports the importance of the octanol-water partition coefficient is discussed to be of major importance when considering the potential of pharmaceuticals to penetrate soils and sediments and reach groundwaters. Therefore, an attempt was made to relate the number of datasets reporting positive detection of pharmaceuticals, or the overall mean concentrations (C_P) in groundwater to the log K_{OW} of the respective pharmaceuticals. A correlation could not be detected at all and values for log K_{OW} between -2.42 (iopamidol; Hansch et al., 1995) and 6.35 (tonalid; Syracure Research Corporation, 2004) were determined. The same picture appeared when considering only those datasets where the origin of contamination such as landfill leachates, leaking septic tanks, wastewater irrigation, or usage of sewage as fertiliser was stated. This demonstrates that as well as the octanol-water partition coefficient, parameters such as amount of pharmaceuticals applied, contact time, type of soil, chemical structure of substance, distance to groundwater, vegetation and many others also interact and have an impact on groundwater contamination by pharmaceuticals.

Overall, 49 % (19 % in GACH countries) of the datasets reporting detection of pharmaceuticals in groundwater ($df \neq 0$) were related to landfill leachates, leaking septic tanks, wastewater irrigation, or usage of sewage as fertiliser as potential sources of pollution. This confirms the aforementioned statement from rough analysis in 3.2 which implies that samples were often taken below locations where a contamination of groundwater by pharmaceuticals is likely to occur. Moreover, Clara et al. (2004) reported that sewage exfiltration could be indicated by measurements of carbamazepine in soil and groundwater. Sacher et al. (2002) investigated the potential of groundwater contamination via agriculture and could not determine a direct link between application of pharmaceuticals with manure and groundwater contamination. This finding is also supported by data contained in Table 13 showing that pharmaceutical concentrations decline with depths between 0-90 cm and number of positively detected samples diminishes in parallel. Boxall et al. (2006a) pointed out that the groundwater level is of importance. Veterinary pharmaceuticals can leach to shallow groundwater from manured fields (Boxall et al., 2006a). An explanation for the decline of pharmaceutical concentrations with depth can be given, as follows: there is a peak of the applied pharmaceutical concentration at the surface during urine fertilisation. The pharmaceuticals then trickle down through the soil (with the liquid phase or simply via rainfall or irrigation). If liquid retention time in a given soil layer is large enough, a partition equilibrium of the pharmaceutical between solid phase (adsorption) and liquid phase (desorption) is more or less realised which can be described by the partition coefficient K_D (eq. 13).

$$K_D = C_{soil} \bullet C_{H2O}^{-1} \quad (13)$$

During this passage (also referred to as chromatographic effect), the peak is widening over a larger soil layer as well as with increasing depth the concentration of

the pharmaceutical in water (C_{H2O}) is decreasing. According to eq. 13, low pharmaceutical concentrations in the water phase result in low pharmaceutical concentrations adsorbed to the soil. This leads to the effect that the respective pharmaceutical finally reaches a concentration below the detection limit. This means that pharmaceuticals may still be present but are no longer detectable in higher depths in soil due to the chromatographic effect and limits of analytical detection.

Hence, a final conclusion regarding the potential pathway via soil cannot be drawn from the database at this point. On one hand, pharmaceuticals listed for groundwater were present in both compartments potentially influencing groundwater: surface waters (3.2) and media applied at soil (Figure 20). On the other hand, research is mainly based on a very limited group of pharmaceuticals used in animal husbandry. Therefore, to learn more about a potential behaviour of pharmaceuticals applied via urine to soil, processes in soil were reviewed in more detail.

3.5.3 Processes pharmaceuticals undergo in soil

Various processes occur in soil. Only few studies on the kinetics for the removal of human pharmaceuticals in soil exist (Richter et al., 2007) and most studies focus solely on percentages of residual pharmaceutical concentrations after a given time span. Information about rate constants is scare in literature. The two investigated processes, sorption and biodegradation, were interpreted separately.

Sorption

483 datasets on sorption of pharmaceuticals in the environment were recorded. This represents a larger number of datasets than those available for pharmaceutical concentrations in soil (337 datasets, Figure 20). 81 active agents for a multitude of indications were contained (Figure 23).

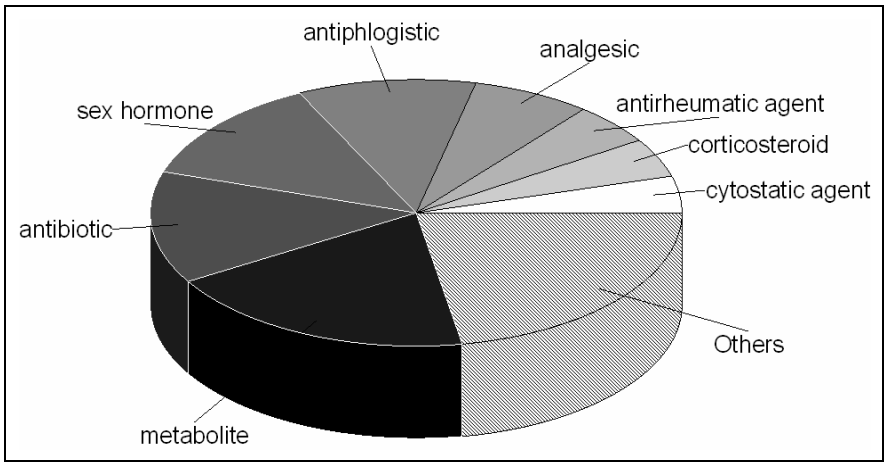

Figure 23: Indications related to sorption given as percentage of datasets collected. All slices shown had values >5 %. The rest are summarised under “Others”. (May 30, 2008)

Although antibiotics exhibit the highest incidence of nominations referring to sorption (14 %) following metabolites (19 %), their frequency of nomination is not that high as is reported for pharmaceutical concentrations in soils (3.5.1). When looking at the pharmaceuticals with the highest percentage of datasets with information on their sorption properties (>4 %), only sulfamethazine represents an antibiotic. Furthermore, many sex hormones are contained in the DB with reference to sorption. They represent the indication group with the highest frequency of nominations following antibiotics and metabolites. Three sex hormones, 17ß-estradiol, estrone, and 17α-ethinylestradiol, are among the group of pharmaceuticals with the highest percentages of datasets related to sorption. Metabolites represent 19 % of the datasets referring to sorption. This is due to the fact that many sex hormones are additionally metabolites. Metabolite's fraction decreased to 7 % when sex hormones were excluded.

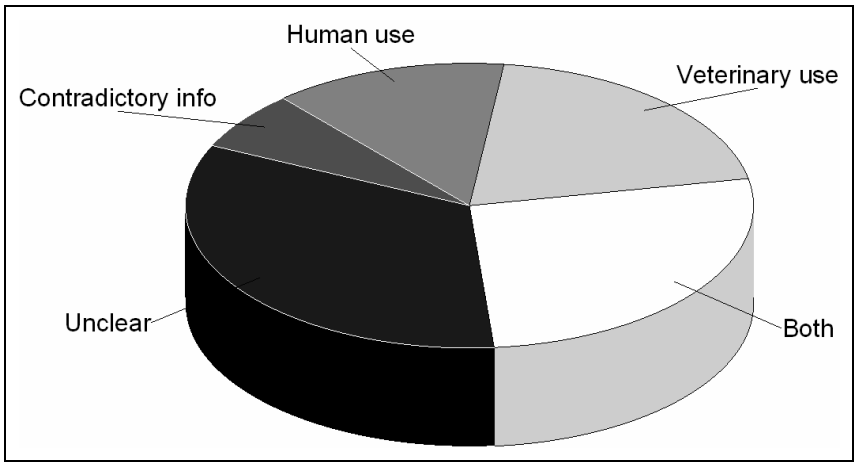

Figure 24: Veterinary usage of pharmaceuticals represented in *Sorption*. "Both" means that pharmaceuticals are used in veterinary and human medicine; "contradictory info": various sources were contradictory to each other with respect to the category; and "unclear" that no information was available. (May 30, 2008)

For one third of the pharmaceuticals with available information on sorption, their usage in human and veterinary medicine was not mentioned. Nevertheless, pharmaceuticals exclusively used for animals (Figure 24, "veterinary use") represent only a fraction (11 %), while 22 % were solely used for humans, and 29 % for both groups. This result underlines the former statement that in *Sorption* use in veterinary medicine was not the major driver for investigations.

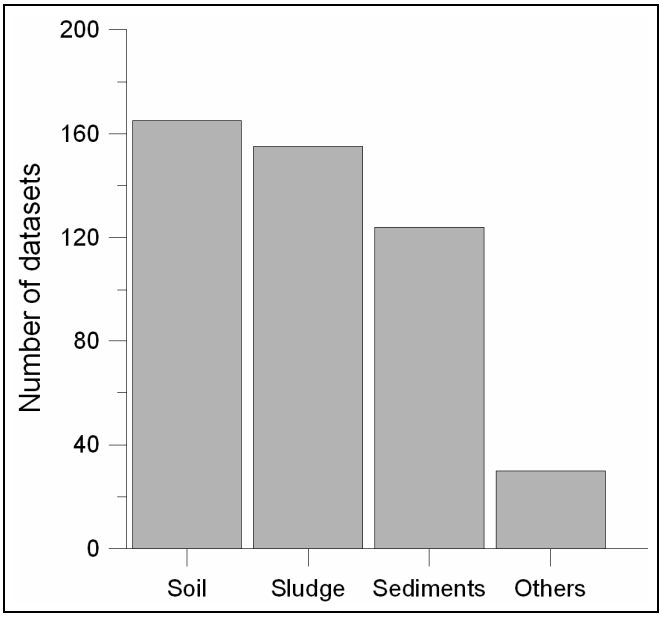

Figure 25: Number of datasets represented in *Sorption* for the various type of media. (May 30, 2008)

Various soil types represent the highest fraction with 35 %, closely followed by sludge (33 %). A major focus was on lake, marine, and river sediments. This might be due to the large amount of investigations focusing on groundwater (3.5.2). Under "Others" various media types were summarised which were interesting to look at but of minor importance to this overall evaluation. Manure was included with only three datasets on pig manure.

It was mentioned in the beginning of the chapter that more then 480 datasets were contained in *Sorption*. Discussions until here referred always to all of these datasets. In the next section only those datasets were evaluated in detail which contained information on K_{OC}, an important parameter indicating sorption capacity of soils for the respective substance. K_{OC} data were translated as sorption potential of the respective pharmaceuticals according to Table 3 (2.2.5). 170 datasets contained information about this parameter for 39 pharmaceuticals. K_{OC} values do not only refer to soil but also towards other media. In Table 14 only datasets with information on K_{OC} values for soils were regarded, therefore, and as a result 5 pharmaceuticals were not listed as their K_{OC} values were only linked to other media types.

Table 14: Pharmaceuticals and their respective sorption potential: their sorption capacity according the classification upon the K_{OC} value, the number of datasets available as well as information whether these pharmaceuticals are expected in AGU are provided. (May 30, 2008)

Substance	Sorption capacity[1]	N°. of DS considered[2]	Contained in *AGU*
Iopromide	very low	1	no
Acetylsalicylacid	low	1	yes
Carbamazepine metabolite[3]	low	1	no
Hydroquinone	low	1	no
Metronidazole	low	4	yes
Sulfamethazine	low - medium	15	no
Sulfamethoxazole	low - high	2	yes
Clofibric acid	medium	2	no
Ketoprofen	medium	2	yes
Phenol	medium	2	no
Salicylic acid	medium	1	yes
Sulfachlorpyridazine	medium	1	no
Sulfadiazine	medium	2	yes
Sulfadimethoxine	medium	2	no
Sulfanilamide	medium	1	no
Sulfapyridine	medium	3	no
Sulfathiazole	medium	2	no
Carbamazepine	medium-high	3	yes
Diclofenac	medium-high	4	yes
Diazepam	medium -high	3	yes
Estriol	high	4	yes
Ibuprofen	high	3	yes
Isobutyric acid	high	1	no
Testosterone	high	2	yes
17ß-Estradiol	high - very high	7	yes
Estrone	high - very high	6	yes
Paracetamol	high - very high	2	yes
Propranolol	high - very high	2	yes
Tylosin	high - very high	4	no
17α-Ethinylestradiol	high - very high	5	yes
Ciprofloxacin	very high	1	yes
Enrofloxacin	very high	11	no
Ofloxacin	very high	4	yes
Oxytetracycline	very high	4	yes
Tetracycline	very high	1	yes

[1] The underlined end of the range was represented by more datasets. The capacity was defined along Table 3.
[2] Only datasets with information on soil type in the entry field “Media” (Figure 7) were considered.
[3] Denotes 10,11-Dihydro-10-hydroxycarbamazepine.

21 pharmaceuticals of those listed in Table 14 can be expected in AGU. Of interest to further considerations regarding urine application are those contained in AGU and with high mobility in soil. Those with a K_{OC} value <500 are considered to be moderately to very mobile in soil and have the potential to reach deeper soil layers, even groundwater. These were acetylsalicylic acid, metronidazole, sulfamethoxazole, ketoprofen, salicylic acid, sulfadiazine, carbamazepine, and diclofenac. Additionally, it became quite obvious that especially antibiotics and sex hormones seemed to have high sorption rates. This might be a further explanation for the fact that until now pharmaceuticals were not detected in deeper soil layers (3.5.1) as almost only sex hormones and antibiotics were investigated. Nevertheless, it has to be mentioned

that the biodegradability of the respective pharmaceuticals has to be determined before final conclusions can be drawn.

Overall, it can be concluded that for sorption behaviour of pharmaceuticals in soil data is more diverse than for other aspects discussed so far. Not all data collected in the DB could be used for the overall assessment due to problems in the comparability (different time spans considered, unknown sorption kinetics, variations in media). Therefore, only data reporting K_{OC} values were evaluated in detail. Additionally, on-going research showed that sorption is influenced by the liquid matrix in which pharmaceuticals are contained. Kujawa-Roeleveld et al. (2008) assume organic matter to be responsible for decreasing the capacity of soil to adsorb pharmaceuticals. While Lucas and Jones (2009) observed that urine is not affecting the sorption rate but enhancing desorption as well as hindering microbial degradation.

Biodegradation

245 datasets for 63 different pharmaceuticals were contained in *Biodegradation*. This is less than datasets available with information on *Sorption* (483 datasets / 81 pharmaceuticals). None of the pharmaceuticals comprise a fraction >5 %. 22 indications (Figure 26) were determined while in *Sorption* 30 different ones were included.

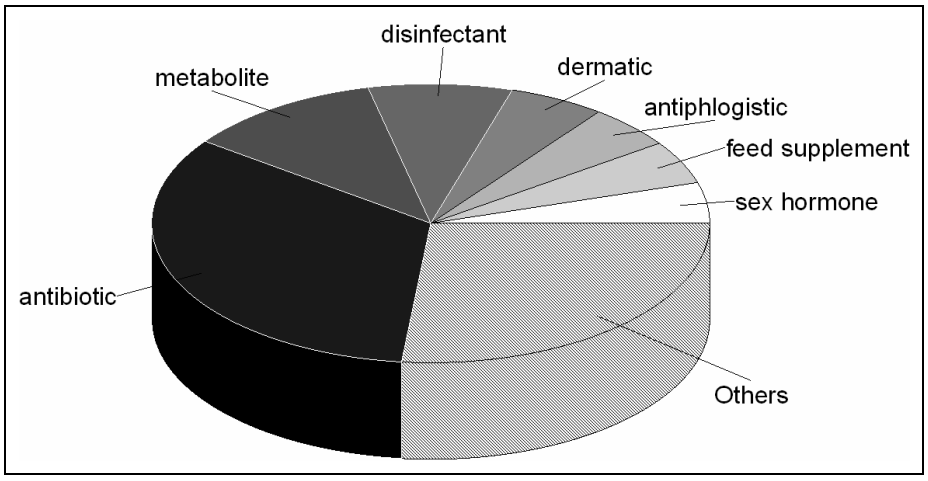

Figure 26: Indications represented in the DB given as percentage of datasets collected. All slices shown had values >4 %. The rest are presented under “Others”. (May 30, 2008)

Figure 26 shows that antibiotics represent 33 % of datasets while sex hormones (which exhibited a large percentage in *Sorption*) with 5 % are of minor importance. Interestingly, disinfectants (8 %) and dermatics (6 %) are among the largest indication groups. While dermatics were always named in combination with antibiotics as additional indication, disinfectants exhibited single-use alone and

included four substances: formaldehyde, glutaral, glyoxal, and phenol. All four have been in use for many years and data on their biodegradation collected in the DB go back to the 1970s (European Commission, 2000).

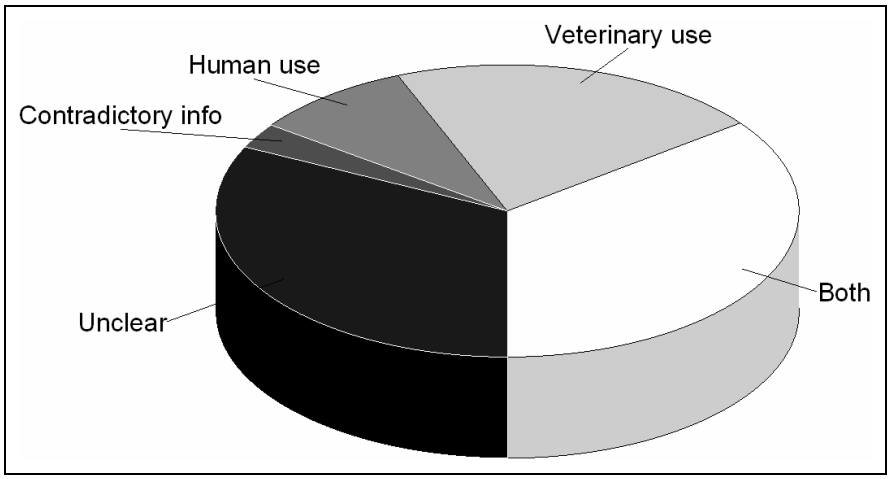

Figure 27: Usage of pharmaceuticals represented in *Biodegradation* in veterinary and human medicine. “Both” means that pharmaceuticals are used in veterinary and human medicine; “contradictory info”: various sources were contradictory to each other regarding their respective use; and “unclear” that no information was available. (May 30, 2008)

Figure 27 shows that the use of pharmaceuticals in veterinary medicine was obviously a major driver for investigations on their biodegradability. While only 9 % were categorised to be of single use for human beings, more than half of the pharmaceuticals recorded in *Biodegradation* in the DB are used in animal husbandry, 21 % of them even exclusively. This aspect is confirmed when the particular media investigated in the category are plotted (Figure 28). While in *Sorption* only pig manure was stated with 3 datasets, in *Biodegradation* manure exhibited 14 datasets as well as 33 additional ones reporting on mixtures of soil and manure. Moreover, Figure 28 represents a large fraction of datasets related to wastewater management: sludge (77 datasets) and wastewater (20). Already in *Sorption* many datasets were related to sludge showing that a high research activity on biodegradability of pharmaceuticals occurs in wastewater management. The main driver for the research activities is to better understand the ongoing processes during wastewater treatment in order to eliminate pharmaceuticals successfully.

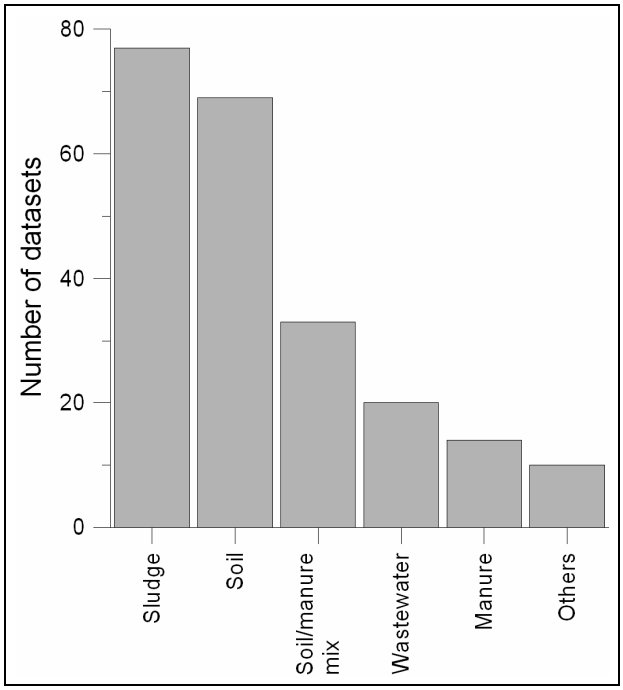

Figure 28: Number of datasets represented in *Biodegradation* for the various media types. Sludge, soil and manure summarise various types of these media, while under "Others" all additional ones are contained. (May 30, 2008)

For 34 pharmaceuticals information on their biodegradation behaviour in soil was available. Datasets of sludge and wastewater were not considered due to the fact that biodegradation varies with media (Weber and Coble (1968) cited in Alexander (1999)). 19 of them are represented by only one dataset and were not further regarded, although this was done in case of sorption capacity (Table 14). This decision was made due to the problems with data analysis as discussed in chapter 2.2.5. For one further pharmaceutical, tetracycline, datasets were very contradictory and were therefore excluded from the overall presentation as well (Table 15). While Höper et al. (2002) states that tetracycline has to be categorised as persistent, Jagnow (1979) determined 100 % degradation within 21 d without stating the applied analytical procedures. This leads to the assumption that it could be an effect of the very high sorption potential of tetracycline (Table 14) which could not be differentiated from biodegradation in that early investigation.

Table 15: Pharmaceuticals and their biodegradability in soil according 2.2.5, the respective time span of biodegradation test, the number of datasets available as well as information on whether these pharmaceuticals are expected in AGU. (May 30, 2008)

Substance	Biodegradation (%)	Time (d)	N°. of DS	Contained in AGU
17α-Ethinylestradiol[1]	20 - 98	70	2	yes
17ß-Estradiol	80 - 100	6 - 70	4	yes
Ciprofloxacin	0 - 33	91	2	yes
Enrofloxacin	0 - 20	56 - 152	2	no
Estrone[1]	55 - 99	70	2	yes
Etidronic acid	7 - 48	80 - 140	3	yes
Metronidazole	50	10 - 27	2	yes
Oxytetracycline	70 - 100	103 - 152	2	yes
p-Chlorophenol	70 - 100	3 - 20	4	no
Phenol[2]	75 - 100	17 - 43	5	no
Sarafloxacin[3]	50	65 - 80	9	no
Testosterone	83 - 87	6	2	yes
Tylosin	50 - 99	3 - 152	3	no
Virginiamycin	50	64	5	no

[1] Difference between the two datasets occurred as in one dataset biodegradability was determined under aerobic and one under anaerobic conditions.

[2] Phenol showed one exception: One dataset reported biodegradability of 20 % within 40 d (European Commission, 2000). This dataset was not included above as biodegradability was determined under anaerobic conditions.

[3] Within the sarafloxacin datasets, three were contained indicating a very low biodegradability of only 1 % within 80 d; however, these data indicate complete mineralisation (measured by $^{14}CO_2$ determined from a radio-labelled antibiotic, Thiele-Bruhn (2003)).

As can be seen in Table 15, the biodegradability is very dependent on time. The best example is p-chlorophenol with a high to very high degradability already after 3 d but an even higher degradability after 20 d, while oxytetracycline showed the same extent in biodegradability at a given time span of 103 to 152 d. Additionally, it has to be stated that the concentration of the respective pharmaceuticals also influence the biodegradation via soil organisms.

Moreover, as molecular structure is of major importance and influences biodegradation extremely (Kümmerer et al., 2000a; Alexy et al., 2006), usage of substances with similar structures as the respective pharmaceuticals is impossible as already minor structural differences can cause large effects in biodegradability. Therefore, further research is highly recommended, especially with a closer focus on pharmaceuticals potentially contained in urine and with high mobility in soil.

3.5.4 Pharmaceutical concentrations in soil determined during pot experiments

IBU and EE2 could not be detected in any soil sample after a three month growth period, although to the "artificial" IBU exposed pots an initial pharmaceutical concentration level of 490 µg kg^{-1} DM soil was added by application of the UPmix (2.4.3). Initial EE2 concentration theoretically reached only 0.053 µg kg^{-1} DM (artificial level) in soil and thus was below the determined limit of quantification in soils. Contrasting to IBU and EE2, CZ was detected in all pots applied except one: one of the two duplicates carried out for pot N°. 44. In this case CZ remained below the LOQ, although traces of CZ which were above LOD were detected. CZ is known to be persistent (Kinney et al., 2006; Ternes et al., 2007 and 3.4.1), and additionally its concentration was higher than in the case of EE2 (2.4.3).

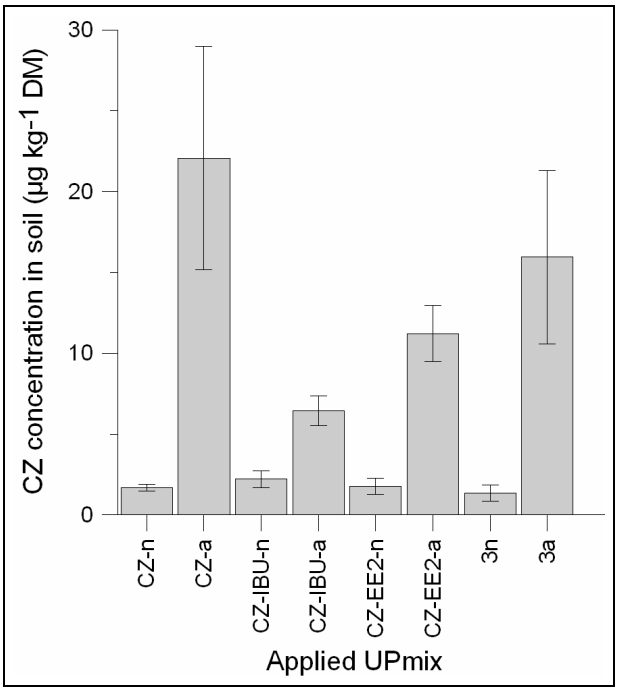

Figure 29: Measured mean concentrations of carbamazepine in soil samples taken at day 92 in µg kg^{-1} DM. By "n" natural and by "a" artificial concentrations applied are indicated. Error bars show standard deviations of the three repetitions receiving the same UPmix.

Concentrations found in soil samples after 92 d (Figure 29) correlate clearly with the applied initial concentrations of 3.2 µg kg^{-1} DM (in the case of natural concentration) and 32 µg kg^{-1} DM (artificial concentration). On average, 49 % of the applied CZ concentrations were recovered 3 months after application varying between 20 % (in CZ-IBU-n) and 69 % (CZ-IBU-a). Additionally, variations between the duplicate analyses for one pot were low. Variations observed between pots with the same treatment were higher, however, as can be especially seen in the case of CZ-a and 3a standard deviation of 13.8 and 10.7 µg kg^{-1} DM soil respectively (Figure 29). In

the case of 3a, concentrations of CZ in one pot were significantly lower with only 4.4 µg kg^{-1} DM, resulting in the large standard deviation.
Via one-way ANOVA the null hypothesis could be clearly rejected: concentrations of CZ applied with the UPmix had clear consequences for concentrations measured in soil. The probability that differences between groups are of coincidental origin was 0.7 %. This result is additionally supported by the Student-Newman-Keuls test partitioning samples into two sub groups confirming that differences were significant.

These findings are concurrent with literature on soils irrigated with treated wastewater. Kinney et al. (2006) report detection and even accumulation of CZ in the upper 30 cm layer of soils irrigated with treated wastewater containing approx. 70 ng l^{-1} of CZ. Concentrations decreased with depth. Additionally, CZ was detected in groundwater wells 12-15 m below fields irrigated with treated wastewater exhibiting 200 ng l^{-1} of CZ, whereas IBU and EE2 were not detected in groundwater below the respective areas (Ternes et al., 2007). This is an additional indicator for the higher stability and higher mobility of CZ compared to IBU and EE2 (Quintana et al., 2005; Ying and Kookana, 2005; Richter et al., 2007).

Intermediate conclusion
Pharmaceuticals are widely investigated in the environment, but information available for soils and agricultural land is limited.

- 11 (10 in GACH countries) pharmaceuticals were listed in the database to be discovered in soil under natural conditions, mainly pharmaceuticals used in veterinary medicine applied to fields via manure and sewage sludge with a main focus on antibiotics.
- Nearly exclusively concentrations of antibiotics were detected in soils in the range of 100 µg kg^{-1} DM regardless of depth. When the relation between concentration and depth was analysed, it became obvious that no detections occurred below 90 cm soil depth, mostly even below 40 cm.
- Groundwater was investigated for 119 (93 for GACH countries) pharmaceuticals of which 45 % (40 %) reported positive detection. Nevertheless, until now no direct pollution by pharmaceuticals from fertilisers applied to agricultural fields to groundwater by leaching through soil could be proven.
- K_{OC} is a very appropriate parameter regarding sorption and mobility of pharmaceuticals in soil. A surprisingly large base of data for many pharmaceuticals was available.
- Evaluation of data on biodegradation is very difficult as kinetics were mostly unknown. Again, mostly data was available especially with focus on soils for pharmaceuticals implemented in animal husbandry. Further research is urgently needed.

- Pot experiments concurred with the findings from literature. IBU and EE2 were not detected in soil anymore, while approx. 49 % of CZ originally applied by urine was recovered in soil samples taken after 3 months.
- At present, statements about behaviour of human pharmaceuticals in soils are only highly speculative. Therefore, further investigations are necessary to determine the causes and effects of pharmaceuticals for agricultural fields.

3.6 Pharmaceuticals in plants

The uptake of pharmaceuticals in plants and the effects they exaggerate on plant physiology and development are of major interest especially for agricultural crops with regards to fertilisation with urine. Pharmaceuticals can affect plant growth (Scheurer (2006) based on U.S. EPA (2006)) when dosed in sufficient concentrations. The question is, whether concentrations applied by urine fertilisation to fields are causing any adverse effects and how these effects would themselves manifest. Are pharmaceuticals taken up by these plants, and in which concentrations, and in which plant parts?
Overall, three pathways exist how pharmaceuticals can enter plants (Fragemann et al., 2006):

- Systematic path – via roots
- Gas path – via stomata and cuticles
- Path of contamination – via contaminated plant surface

In the case of pharmaceuticals, the “systematic path” and additionally the third option of surface contamination e.g. during fertilisation when leaves come into contact with urine are assumed to have a major impact. Literature states that the pharmaceutical uptake of plants is correlated to the molecular weight of the pharmaceutical (Topp et al., 1986). It is assumed that a molecular weight of >1000 makes the absorption by membranes impossible (Sanderson et al., 2004). Moreover, the octanol-water partition coefficient is looked at as a driving force for the uptake. Already Briggs et al. (1982) and (1983) have detected that uptake into shoots was most efficient for chemicals with log K_{OW} = 2. Nevertheless, K_{OW} is of lower importance according to Trapp (2000) and Topp et al. (1986) who found predictions of the membrane permeability of very lipophobic compounds to be very weak (Trapp, 2000). Responsible is an ion-trap mechanism, a process with the chemical being neutral outside and dissociated inside the cell (Briggs et al., 1987; Trapp, 2000).

The database contains two different types of information regarding pharmaceuticals and their effects on plants. On one hand, upakte into plants gives details upon concentrations detected in specific plant parts. On the other hand, plant physiological aspects such as weight or height provide information on phytotoxicity, i.e. on the way how plants react when incorporating pharmaceuticals. Regional and national aspects

were not considered in this part as they are of minor importance. Overall, information was collected within 361 datasets disregarding which of the two described types of effects was included. These datasets contained 39 pharmaceuticals and 44 plant species belonging to 17 different plant families. 40 % of all datasets belong to *Poaceae*, also named *Gramineae* and representing the family of cereals (Diepenbrock et al., 2005).
Before going into detail, it has to be pointed out that not only concentrations measured in outdoor plantations were included but laboratory experiments as well. This decision was taken because of the very limited data availability of outdoor experiments. Nevertheless, this aspect and its consequences for data analysis were discussed in detail in 3.6.3.

3.6.1 Uptake into plants

Here all data is presented where plant parts were investigated for concentrations of pharmaceuticals. Overall, 162 datasets were available. They included 25 plant species representing 16 families.

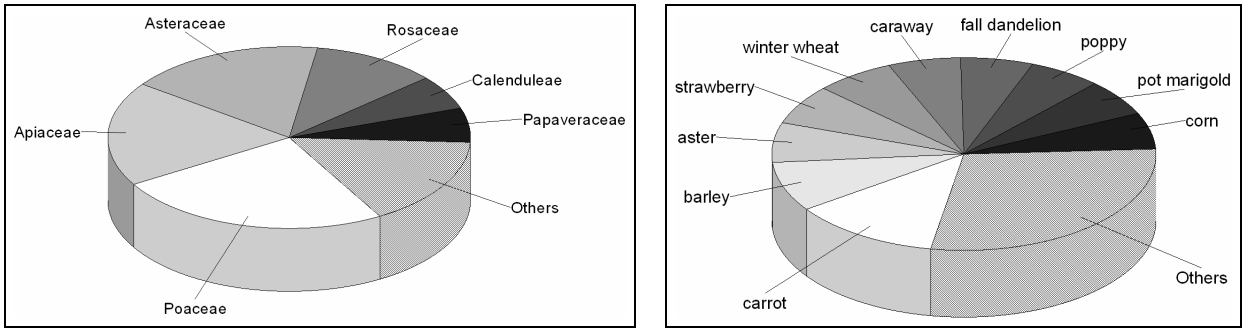

Figure 30: Percentages of plant families (left) and plant species (right) represented in the DB with information regarding concentrations of substances accumulated. All slices shown had fractions >5 %. The rest (≤5 %) is presented in the category “Others”. (March 06, 2008)

A large number of datasets was represented by the studies on the family of *Poaceae*, cereals such as wheat, barley, corn, millet, and rye but also ryegrass. As fertilisation of cereals with urine is an interesting aspect (Hammer and Clemens, 2007), it is convenient that they were present in such high percentage (40 %). The second largest part contains the family of *Apiaceae*, of which carrots and caraway were the only two plant species studied. The large fraction is reached as carrot is the plant species with the major single contribution of 13 % (see Figure 30). The family of *Apiaceae* is followed by *Asteraceae* (with studies on the species aster (7 %), fall dandelion (6 %) and lettuce (5 %)), and by *Rosaceae* (apple and strawberry) (Figure 30). This shows very well the limited number of variety of studied plant families

except *Poaceae*. On a positive side, information on fruit trees and flowers is also available. Fruits such as apples exhibit a long time span between fertilisation and consumption and they never come in direct contact with urine, thereby limiting potential hygienic concerns to a minimum. Flowers on the other hand are not eaten by human beings and therefore, a potential uptake of pharmaceutical residues is unimportant as long as their appearance is not affected.

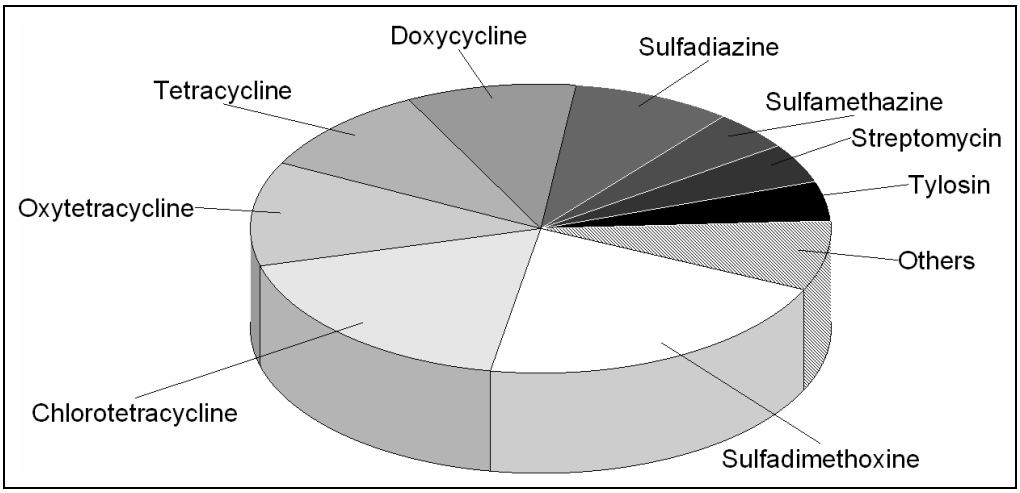

Figure 31: Percentages of pharmaceuticals represented in DB with information regarding their concentrations accumulated in plants. All slices shown had a share of >4 %. Further active substances are summarised under "Others". (March 06, 2008)

Out of the pharmaceuticals investigated for their accumulation in plant parts, 14 substances are used as antibiotic, antiphlogistic, or feed supplement (tylosin). All those apart from diclofenac (which was represented with only one dataset) are used in veterinary medicine; three of them, enrofloxacin, sulfamethazine, and tylosin even exclusively (Huschek et al., 2003; BLAC, 2003; Schüssler and Sengl, 2004). This shows again the restricted availability of data which is mainly linked to the traditional usage of antibiotics in veterinary medicine (Thiele-Bruhn, 2003; Boxall et al., 2004; Boxall et al., 2006b) and as pesticides (Goodman, 1959; Vidaver, 2002).
Pharmaceutical concentrations in plants depend on amounts of pharmaceuticals available in the respective growth media. Therefore, mapping of "real" concentrations in plant parts is nearly impossible. Often very high doses were applied to ensure the possibility of analytical detection (for details see 3.6.3). Although Kumar et al. (2005) states that dependence between provided concentration and uptake was nearly linear, it is impossible to generalise these findings on just one investigation. Especially, as already within this study (Kumar et al., 2005) differences regarding the intensity of pharmaceutical's uptake appeared among the three investigated plant species. An additional challenge was differences in units. Results were presented as related to DM and FM, or kind of matter was not mentioned at all.

Despite these problems, an attempt in a descriptive manner was made for the four most intensively investigated pharmaceuticals (Figure 31) to show the state of knowledge in artificial (filter paper, saw dust, cotton gauze, and murashige & skoog soaked with water) and soil media (2.3.3). Further information for other pharmaceuticals can be found below https://www.tu-harburg.de/aww/pharma/.

Chlorotetracycline

Four articles provided information for this pharmaceutical regarding its uptake potential in plants (Peterson and Sinha, 1977; Grote et al., 2004; Jacobsen et al., 2004; Kumar et al., 2005). 17 datasets reported experiments in artificial media with plants exposed to pharmaceuticals for 1 d. Pharmaceuticals could be detected in roots in all datasets while only in 50 % of the leaves' datasets. Concentrations ranged between 1 and 25 ng kg^{-1}. The other experiments were carried out in pots filled with soil or in agricultural fields and lasted for 7 to 41 d. Concentrations in roots, from 200 to 129,800 µg kg^{-1} FM (fresh matter), were higher then in aerial plant parts. Pharmaceuticals were detected in carrot roots with 200 µg kg^{-1} FM as well as in wheat grain with 49 µg kg^{-1} DM (Grote et al., 2004) but not in barley (Jacobsen et al., 2004). This was even though barley received 10 times higher concentrations (300-500 mg kg^{-1} DM soil) than wheat (50 mg kg^{-1} DM soil) and time of growth was similar (barley: 143 d; wheat: 155 d). Apart from the investigation of grains experiments, growth and exposure periods were rather small. This might be sufficient for field salad (*Valerianella locusta*), but it is questionable whether final uptake in carrots or cabbage already reached a maximum in these short periods.

Oxytetracycline

This pharmaceutical was only investigated in three studies (Peterson and Sinha, 1977; Jacobsen et al., 2004; Boxall et al., 2006b). In experiments on artificial media (Peterson and Sinha, 1977) with exposure to oxytetracycline for one day, higher concentrations were detected in aerial plant parts than in roots under the same experimental conditions. Overall concentrations ranged between 1-122 ng kg^{-1}. In the case of pot and field experiments, only five datasets were available and in none was uptake into plants reported. Four of the datasets investigated uptake into barley grain Jacobsen et al., 2004) and carrot roots and peel (Boxall et al., 2006b) after a growth period of approx. 150 d with LOQs of 5 and 23 µg kg^{-1} DM respectively.

Tetracycline

Only one article presented investigations accomplished in artificial media for five different flowers and strawberries (Peterson and Sinha, 1977). Measured concentrations in leaves and stalks were between 1 and 119 ng kg^{-1}, and in roots between 1 and 24 ng kg^{-1}. Plants were exposed to the substances for one day only. The overall concentrations in plants were between 6-120 ng kg^{-1} in datasets where specific plant parts were not differentiated.

Sulfadimethoxine

Nearly all datasets reported experiments with durations of 8 to 45 d containing information from six different articles (Migliore et al., 1995; Migliore et al., 1996a; Migliore et al., 1996b; Migliore et al., 1997; Migliore et al., 1998; Forni et al., 2002). A major contribution regarding investigations of this chemical was carried out by the workgroup of Migliore resulting in studies on nine different plant species. Concentrations in aerial plant parts (10-2000 ng kg^{-1}) and roots (50-2000 ng kg^{-1}) were in a similar range. Sulfadimethoxine was detected in carrot roots between 126 and 313 ng kg^{-1} (Migliore et al., 1998). This was the sole investigation on uptake of sulfadimethoxine in edible plant parts.

Overall, it became apparent that uptake of pharmaceuticals can occur very differently even within the same group of antibiotics due to large differences in the experimental conditions (plant species, investigated plant parts, applied pharmaceutical concentrations, exposure period, growth period prior to harvesting etc.). Three of the four pharmaceuticals described in detail belong to the group of tetracyclines. While tetracycline was only investigated for a growth period of approx. 40 d, chlorotetracycline and oxytetracycline were tested for 150 d with rather different outcomes. This shows that a generalisation of the uptake potential is currently not possible.

This statement is also supported by further facts. Dolliver et al. (2007) and Boxall et al. (2006b) report that concentrations in tubers of potatoes and roots of carrots were higher in the peel then in the centre, and assume a penetration of the pharmaceuticals through the peel. Additionally, Brian et al. (1951) and Stokes (1954) reported excretion of griseofluvin via guttation drops at leaf apexes of wheat seedlings. The rate of movement in plants was influenced directly by rate of transpiration, being itself affected by both humidity and temperature. This finding leads to two contradictory assumptions. On one side, an accumulation of pharmaceuticals in leaves occurs (Brian et al., 1951; Stokes, 1954). This is supported as higher uptake rates were found in older leaves (Grote et al., 2004). On the other side, leaves are able to secrete pharmaceuticals (Brian et al., 1951; Stokes, 1954) and to degrade uptaken organic chemicals comparable to liver metabolism (Komoßa et al., 1995). The difference to human beings is that instead of excretion in most cases, compartmentation occurs. This means that transformation products are stored inside the plants in lignin or cell walls.

Moreover, it has to be pointed out that many articles were published 20 to 30 years ago and the sensitivity as well as selectivity of chemical analyses at that time was somewhat lower.

3.6.2 Phytotoxicological aspects

In total, 348 datasets collected information on phytotoxicological aspects. As within one dataset information for different plant parts was possible, multiple nominations were included. They contained 30 pharmaceuticals representing 14 indications. The database for this category contained only 4 active substances (clofibric acid, ß-sitosterol, cholesterol, and testosterone) which are not used in veterinary medicine. Besides, theophylline, a bronchospasmolytik drug (Arzneiverordnungs-Report 2004, 2004), is mainly used for humans and only for cats and dogs in veterinary medicine, but not on a large scale (The Merck Veterinary Manual, 2008). Nevertheless, the outcome for phytotoxicity is clearer than for uptake, as apart from the typical group of substances, steroids represented a fraction of 11 %. This is due to the fact that they were investigated for their influence on growth promotion (Kopcewicz, 1969) in the last century.

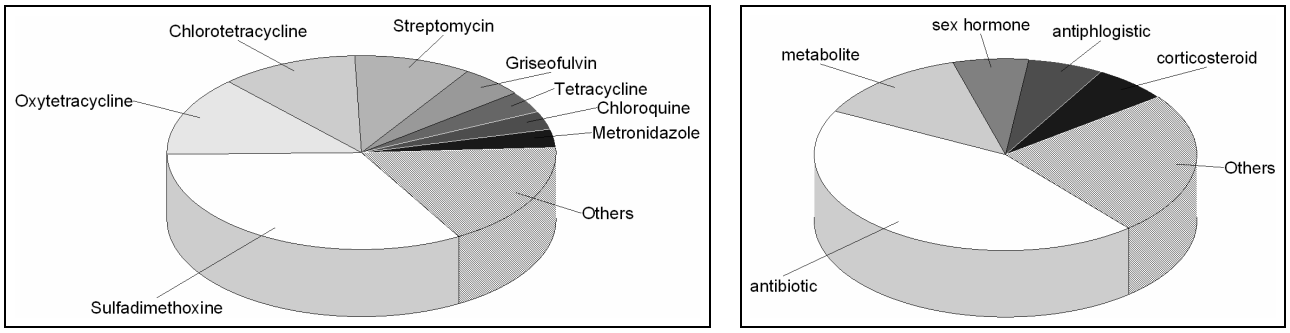

Figure 32: Pharmaceuticals' (left) and indications' (right) phytotoxicity data on plants collected in DB was expressed as percentage of their number of datasets (left) and of active substances (right). Fractions <3 % in the case of pharmaceuticals and <7 % for indications were summarised below as "Others". (March 06, 2008)

Overall, studies on 11 different families exhibited data for phytotoxic effects. As already shown for investigations on bioaccumulation of pharmaceuticals within plants, also in the case of phytotoxicological aspects most data was available for *Poaceae* (50 %). Interestingly, this plant family was followed by *Fabaceae* with 25 %, a family rarely investigated regarding bioaccumulation (2 %). *Fabaceae* represent legumes, which can bind nitrogen from the air by bacterial symbiosis (Diepenbrock et al., 2005) and are of major importance especially to organic agriculture. Pea, soybean, bean, clover and alfalfa were also contained in the database. 30 different plant species were contained in the studies on phytotoxicity of pharmaceuticals (Figure 33). Only plant species of the families of *Poaceae* and *Fabaceae* were represented with fractions >5 % such as corn and pea (Figure 33, right).

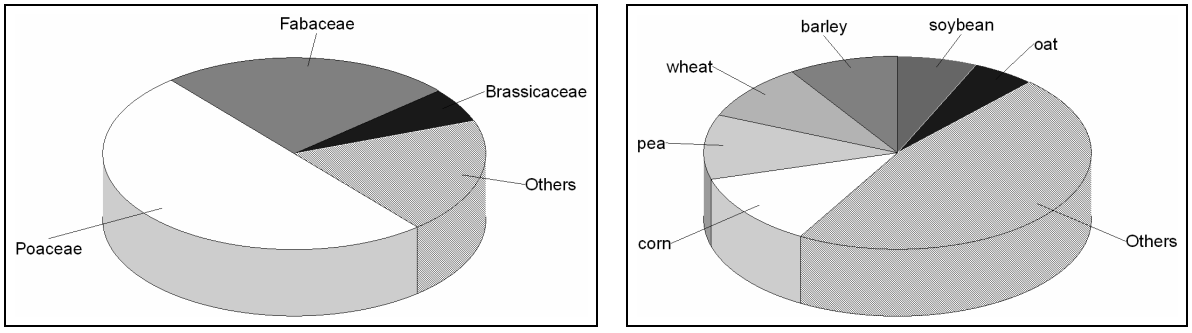

Figure 33: Plant families (left) and plant species (right) investigated for phytotoxicological effects expressed as percentage of their number of datasets. All slices shown exhibited values >5 %. The residue is contained in “Others”. (March 06, 2008)

Most of the phytotoxicological tests focused on height (20 %) and weight (16 %) as well as development of roots (30 %) and leaves (18 %) (Figure 34). This is due to the fact that tests mainly lasted between 5 and 45 d as a result of the test systems used. Three different types of tests can be differentiated:

1) Seeds were soaked with a solution containing pharmaceuticals before germination.

2) Seeds germinate while being in contact with such a solution.

3) Seedlings were brought into contact with pharmaceuticals immediately after germination.

The focus of the tests can be explained by the fact that most tests were done in the years between 1950 and 1970 when the interest was laid mainly on effects of antibiotics and hormones, because these substances were investigated for their use as plant protectants. On one hand, there was hope that these substances would enhance plant development (Goodman, 1959; Kopcewicz, 1969); and on the other hand, these substances disturbed plant development and thus solutions were required (von Euler and Stein, 1955; Goodman, 1959). As it was nearly impossible to measure bioaccumulation in plant tissues, research was emphasised on phytotoxicity.

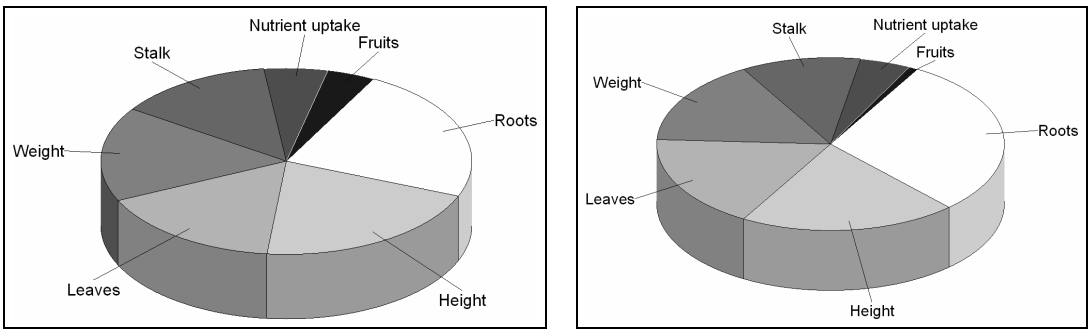

Figure 34: Phytotoxicological aspects collected in DB and presented as percentage of their number of active substances (left) and of datasets (right) reporting the specific effects. (March 06, 2008)

Although fruits and nutrient uptake were considered in some investigations, no information was found concerning development of flowers and influences during flowering, but other additional effects were reported. Change of colour to darker green (Grote et al., 2004) as well as lacking and incomplete colouring (Rosen, 1954) were observed (von Euler, 1948). Moreover, lower chlorophyll content in leaves (von Euler and Stein, 1955) as well as hard and waxy leaves were reported (Rosen, 1954). Germination itself also seems to be affected; speeding up (Barton and MaeNab, 1954) as well as slowing down germination (von Euler, 1948; Ritter, 2008) were observed in certain cases. Moreover, Rosen (1954) reported that no lateral roots were developed subsequent to pharmaceutical exposure and von Euler (1948) found thickened coleoptiles.

3.6.3 Risk determination for plants by database results

As presented so far, effects of pharmaceuticals on plants were investigated. It was expected that in most cases very high pharmaceutical concentrations far from real conditions were chosen in order to visualise any effects. Therefore, concentrations potentially reached in substrate/soil through fertilisation with urine were compared to concentrations reported in the database (see 2.3.3). None of the tests directly investigated the application of pharmaceuticals by urine except Schneider (2005). Within this research an UPmix containing diclofenac (DIC), sulfamethoxazole (SMX), or sulfamethazine (SMZ) was applied but with a concentrations $5*10^5$ (DIC) and $9*10^5$ (SMX) higher than in AGU. Sulfamethazine was not calculable for AGU as it is only applied in veterinary medicine (BLAC, 2003; Schüssler and Sengl, 2004). Schneider (2005) showed that between 15 and 30 % of pharmaceuticals' amounts applied to soil or substrate were taken up by plants. It was important for a meaningful conclusion to identify experiments within the same range of concentration which could be expected in case of urine application. Only such datasets can be used for further predictions regarding the plant uptake of pharmaceuticals and potential consequences for plant development.

204 datasets contained data on tests accomplished in artificial media (filter paper, saw dust, cotton gauze, or murashige & skoog) for 22 different pharmaceuticals. 112 datasets (for 12 pharmaceuticals) could be used for detailed estimation as these datasets represented pharmaceuticals which can be expected to be contained in AGU and were possible to be calculated (2.3.1). Applied concentrations contained in the database were always 10^3 (oxytetracycline (Barton and MaeNab, 1954)) to $6*10^{10}$ (ß-sitosterol (SIT) (Kopcewicz, 1969)) times higher than in AGU. The high DB/AGU ratio of $6*10^6$ for SIT was reached as urinary concentrations of SIT were calculated to be zero (see 3.3.1). If SIT was not considered, the highest fraction was $3*10^9$ for penicillin G (Royse et al., 1975). Only information provided by Peterson and Sinha (1977) showed realistic application rates. Concentrations were 0.01-3.7 times those applied via urine. Peterson and Sinha (1977) investigated effects of doxycycline (with a DB/AGU ratio of 0.01), oxytetracycline (0.1), tetracycline (2), and chlorotetracycline (3.7) on various plant species the roots of which were treated with a pharmaceutical solution for 24 h and sampled subsequently. Concentrations were determined by bioassays with *Arthobacter globiformis* as test organism (Sinha and Peterson, 1972). Antimicrobial activity was tested by a control of juice of untreated plants to exclude effects of the applied plant tissue (Peterson and Sinha, 1977).
Moreover, three datasets of von Euler and Stein (1955) on oxytetracycline with concentrations only 21 times higher than expected by urine application negatively influenced roots of wheat, barley, and oat. As well a defect in chlorophyll production was observed due to a blockage of the succinate dehydrogenase, a catalyst of a redox reaction of the citric acid cycle (Taiz and Zeiger, 2008) resulting in white or only light green leaves. Nevertheless, the test periods were very short (20 d) and did not allow further conclusions apart from that pharmaceuticals were taken up when applied in these concentrations, a fact already stated above (3.6.1).

For solid substrates including various soil types, 98 datasets were available representing 20 pharmaceuticals. Finally only 45 datasets reporting 9 pharmaceuticals could be used for detailed comparison as the others relied on pharmaceuticals the urinary concentrations of which could not be determined due to the reasons mentioned above. In 18 datasets application rates were 2-182 times higher then those expected to be reached by urine fertilisation and for 8 of these datasets bioaccumulation or phytotoxicity was reported (see Table 16). The others showed DB/AGU ratios between $2*10^3$ (chlorotetracylcine (Patten et al., 1980)) and $2*10^8$ (chlorotetracycline (Jacobsen et al., 2004)) and were thus not considered any further.

Table 16: The 8 datasets reporting concentration similar to those in the case of urine fertilisation (DB/AGU ratio <200) which showed phytotoxic or bioaccumulative effects.

Substance	Plant species	Reported impacts[1]	Concentration applied	Ratio DB/AGU[2]	Ref.
Chloroquine	soybean	Phytotoxic: negative impact on w, h, r, s, l (13 d after germination)	8000 ng kg^{-1}	182	Jjemba, 2002
Chlorotetracycline	spring wheat	Phytotoxic: positive impact on h, r (27 d after germination)	160 ng kg^{-1}	82	Batchelder, 1982
Chlorotetracycline	pinto bean	Phytotoxic: negative impact on w, h, r, s, l (45 d after germination)	160 ng kg^{-1}	82	Batchelder, 1982
Chlorotetracycline	green onion	Uptake: 0.013 ng kg^{-1} FW in s and l (42 d after transplantation)	100 ng kg^{-1}	51	Kumar et al., 2005
Chlorotetracycline	cabbage	Uptake: 0.01 ng kg^{-1} FW in s and l (42 d after transplantation)	100 ng kg^{-1}	51	Kumar et al., 2005
Metronidazole	soybean	Phytotoxic: negative impact on w, h, r, s, l (13 d after germination)	2000 ng kg^{-1} DM	67	Jjemba, 2002
Oxytetracycline	spring wheat	Phytotoxic: positive impact on h, r (27 d after germination)	160 ng kg^{-1}	2	Batchelder, 1982
Oxytetracycline	pinto bean	Phytotoxic: negative impact on w, h, r, s, l (45 d after germination)	160 ng kg^{-1}	2	Batchelder, 1982

[1] Letters denote weight (w), height (h), roots (r), stalk (s), and leaves (l).

[2] "Factor DB/AGU" describes the concentration applied in the specific investigation related to the pharmaceutical concentration calculated to be reached in case of urine application. DB/AGU = 1 describes equal conditions, <1/>1 implies that lower/higher concentrations would be applied by a fertilisation with urine under the described conditions. (March 16, 2008).

In none of the cases presented in Table 16 were plants allowed to grow for longer than 45 d. This means longer lasting effects observed after a whole vegetation period, the relevant point in the case of urine fertilisation, were not observed. As plants already germinated in the presence of pharmaceuticals, with exception of the study of Kumar et al. (2005) with pharmaceutical's application one week after germination, these cases do not illustrate a realistic scenario. Additionally, corn and radish treated similarly as spring wheat and pinto beans by Batchelder (1982) but not showing any effect indicated again that different plants have different sensitivity levels towards pharmaceuticals. Nevertheless, these cases show that effects with respect to phytotoxicity and bioaccumulation can occur in the case of fertilisation with urine. More detailed investigations simulating real case scenarios are urgently required. Also, it has to be mentioned that all cases report only upon one pharmaceutical. In the case of AGU, a mix of various pharmaceuticals would be contained – an aspect not investigated so far.

3.6.4 Behaviour of rye grass during pot experiments towards pharmaceuticals

Indirect effects on aerial plant parts

The growth of aerial plant matter (Figure 35) was identified for the entire 3 months experimental period according 2.4.3. No visual effects were observed except Control 2 which received only irrigation water without nutrients and thus showed only about 25 % of the biomass production compared to the fertilised grass. Aerial plant parts were smaller and thinner. The lack of fertilisation led to a large weight reduction. The overall fresh as well as dry matter of all plants fertilised with yellowwater did not show any effect irrespective of the kind and concentrations of added pharmaceuticals (Figure 35).

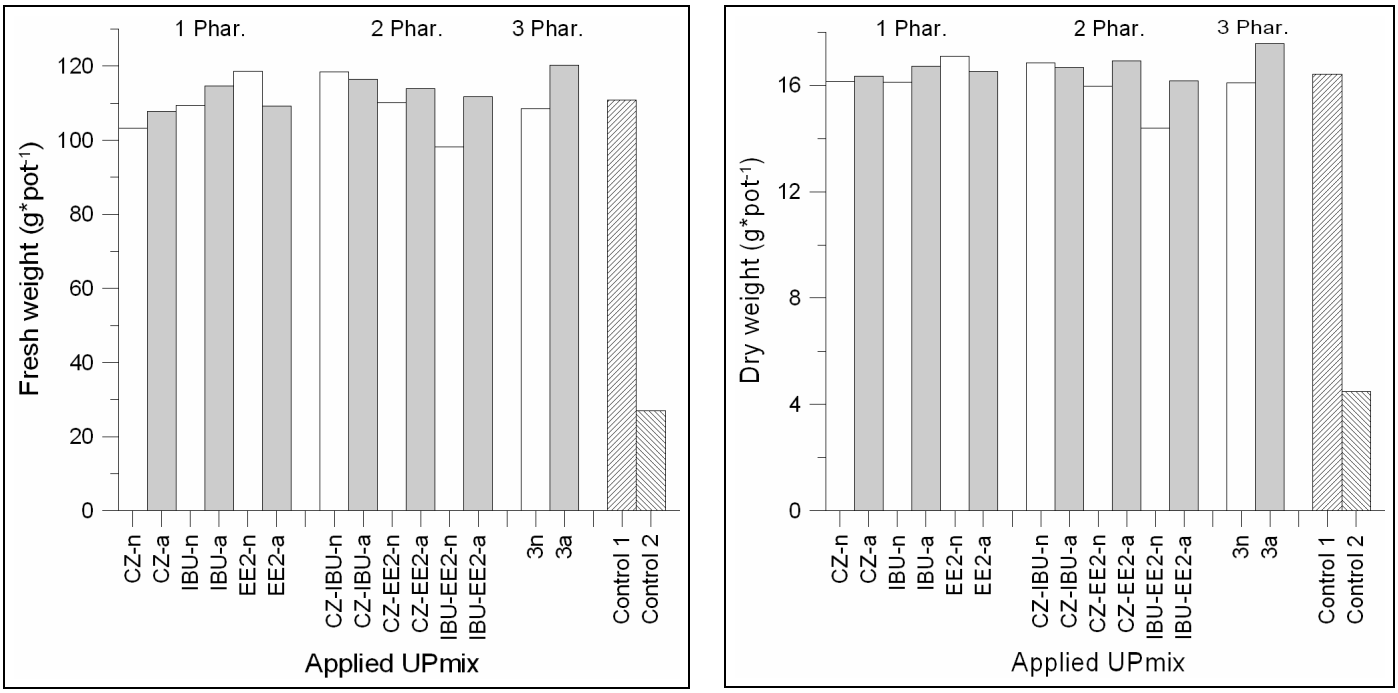

Figure 35: Overall fresh weight (left graph) and dry weight (right) of plant parts of rye grass determined during the full growth period. n = natural concentration (white bars), a = artificial concentration (grey bars). “Control 1” indicates plants treated with MeOH and urine, “Control 2” did not receive any application beside water; “3” is the designation for the combination of CZ, IBU, and EE2.

Statistical analysis confirmed these findings. One-way ANOVA, $P < 0.05$, showed a significant difference for the unfertilised control group for fresh and dry matter. Apart from this result, no significant differences were observed and the null hypothesis cannot be rejected which says that pharmaceutical applications at the tested concentrations do not affect the synthesis of plant matter at the tested concentrations. Moreover, no relationship was detected between the amount of

harvested plant matter and the added doses of the particular pharmaceuticals applied disregarding the specific pharmaceuticals contained in the applied UPmix.
Besides the overall fresh and dry matter production, the dry matter production over the vegetation period was also determined. The factor of fresh weight to dry weight was only slightly varying between 6.01 (Control 2) and 7.03 (CZ-IBU-n). Therefore, the results are presented and discussed only in terms of dry weight in the following:

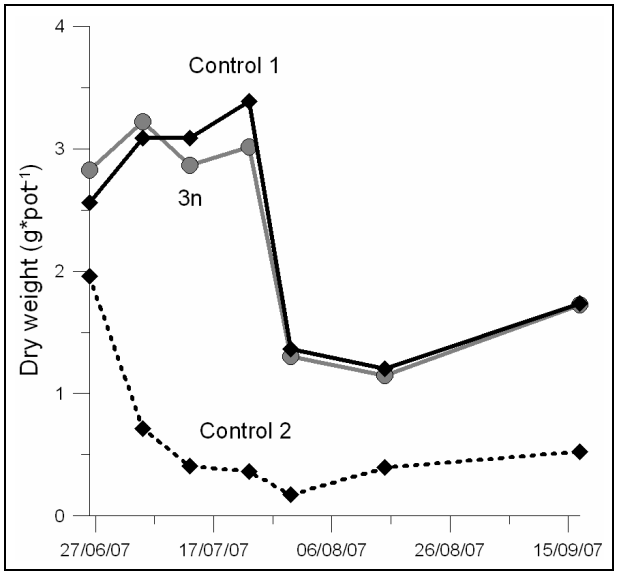

Figure 36: Course of dry matter production from the 1st cut until final harvest (mean concentration of three parallel treatments). “Control 1”: plants treated with MeOH and urine, “Control 2” did not receive any application beside water. “3n”: combination of CZ, IBU, and EE2 at natural concentration levels.

Figure 36 shows that pharmaceuticals did not have any effect on the course of the production of aerial plant parts during the growth period. The combination 3n represents an example for combination of various pharmaceuticals which did not differ much from Control 1. Only Control 2 shows a completely different behaviour as it was not fertilised at all. Its production of organic matter reaches the highest yield at the 1st cutting with 1.96 g DM and afterwards immediately decreased due to the lack of fertiliser. This decline illustrates the fertilising potential of the soil contained in the pots exposed to yellowwater. Temperature was not constant what influenced plant growth (especially yield of 3rd cut).

Uptake of carbamazepine and ibuprofen into roots and aerial plant parts
Due to the matrix of the plant material extract, detection of CZ and IBU was difficult. Only in the plants exposed to artificial concentrations, could CZ be quantified in the roots of rye grass. Similarly, just CZ could be determined in the aerial plant parts exposed to artificial concentration levels. CZ concentrations in the aerial parts of plants exposed to the natural CZ level (58 µg l^{-1} in AGU) were similar to the limit of

quantification (75 µg kg^{-1} DM). IBU was not determined in aerial plant parts due to the problematic matrix.

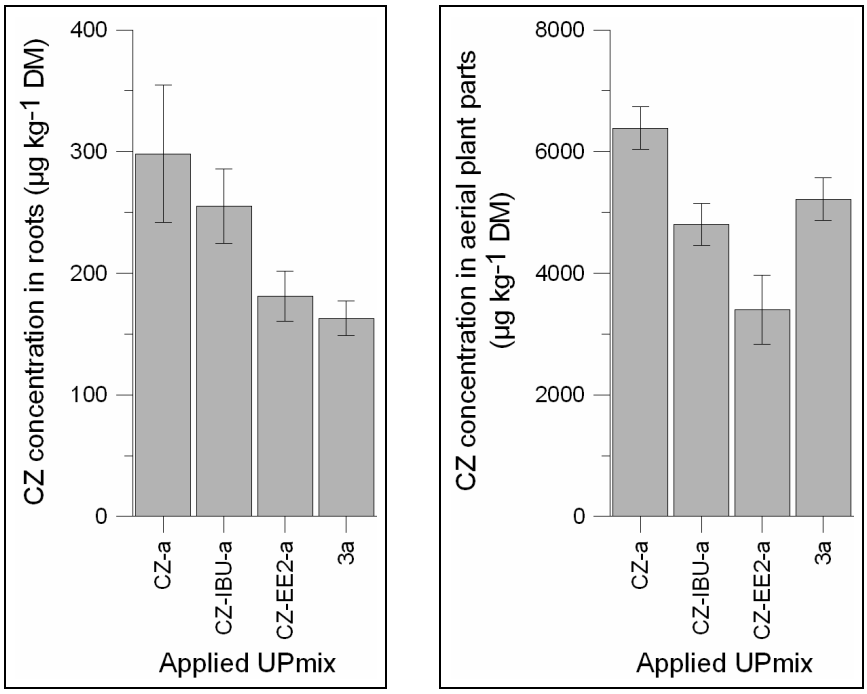

Figure 37: Mean concentrations of carbamazepine measured in roots and aerial plant parts of rye grass after 92 days growth period. Error bars show standard deviations for the three equally exposed pots.

CZ concentrations in roots were between 131 µg kg^{-1} DM (one sample of CZ-EE2-a) and 426 µg kg^{-1} DM (one sample of CZ-a) with a mean concentration of 225 µg kg^{-1} DM while a tenfold concentration was reached in aerial plant parts (mean concentration: 4950 µg kg^{-1} DM; span: 2600 µg kg^{-1} DM (CZ-EE2-a) to 6950 µg kg^{-1} DM (CZ-a)). For completeness it has to be mentioned that instead of measuring CZ concentrations in roots in two pots (exposed to CZ-IBU-a and 3a), the plant crowns were extracted and analysed as the amount of root material was insufficient. As concentrations in the two samples of crowns were within the range of the two analyses of roots of the same series (CZ-IBU-a: 243 µg kg^{-1} DM in the crown, 202 and 321 µg kg^{-1} DM in the roots; 3a: 131 µg kg^{-1} DM, 175 and 184 µg kg^{-1} DM), for these two pots the concentration in crown was assumed to mirror the mean concentration in roots (Figure 37).

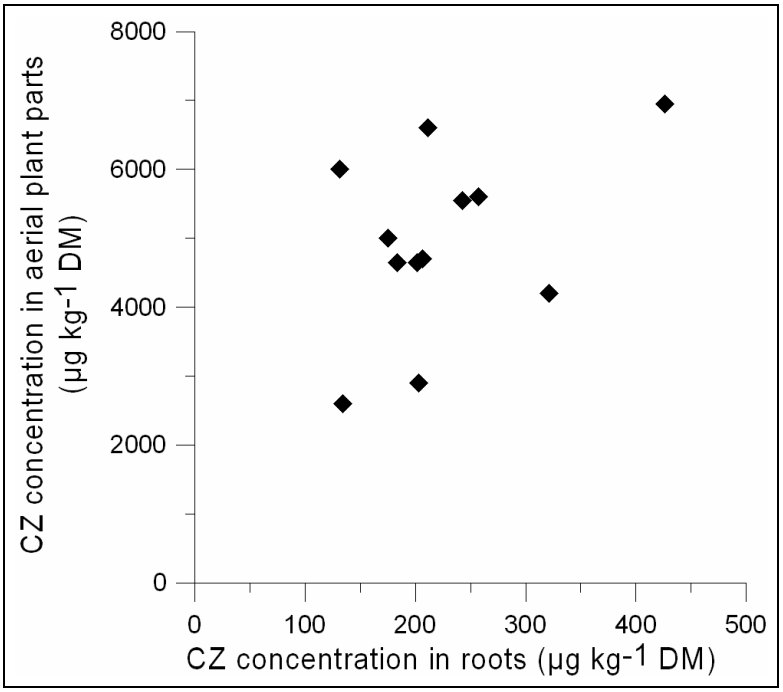

Figure 38: Comparison between incorporation of carbamazepine (applied at artificial level) into aerial plant parts and roots of rye grass.

Statistically relevant correlations between the uptake of CZ into roots and into aerial plant parts could not be determined (Figure 38). This might be a consequence of considerable coefficients of variation in the difficult matrix of plant extracts. Only when comparing the mean concentrations of the three pots of one UPmix, a weak correlation was observed (R^2 = 0.42, Figure 38). In general, CZ concentrations in aerial plant parts were an order of magnitude larger then in roots. An average of 0.21 % (between 0.12 and 0.40 %) of the total amount of CZ applied to each pot (under artificial conditions 290 µg per pot) was found in the roots of rye grass, but 30 % (between 15 and 42 %) in the aerial plant parts.

It can be assumed that IBU (neither detected in soil nor in plants) is not incorporated by plants due to its fast biodegradation in soil, while CZ is present in soil for longer periods due to its recalcitrance. In consequence, CZ remains available for plants for a much longer period and is thus transferred to the plants, especially to the aerial plant parts in the case of rye grass. It has to be pointed out that the uptake rates of the aerial plant parts of natural and artificial concentrations were non-linear. CZ concentrations in the aerial plant parts exposed to the natural CZ level were in the range of the limit of quantification (75 µg kg^{-1} DM) and those exposed to artificial CZ levels showed an average concentration of 4950 µg kg^{-1} DM. While CZ concentrations measured in soil and roots reflected the order of one magnitude (10 fold) which was chosen for the two application regimes (“natural” and “artificial”).

Intermediate conclusion

As has already been concluded for soils, there was only a limited amount of data available from literature with respect to plant uptake and phytotoxic effects. Nevertheless, a large number of laboratory tests were performed in the period between the 1950s and 70s.

- Data from literature show that plants are generally able to take up pharmaceuticals in such a way that they can be detected in roots as well as in aerial plant parts. Concentrations in plant parts detected in other studies were in the range of ng kg^{-1}. Nevertheless, pharmaceuticals were also found in edible plant parts such as carrot roots and cereal grains.
- Pharmaceuticals also cause phytotoxic effects in dependence of the applied pharmaceutical concentration.
- Different plant species have dissimilar sensitivity levels towards the same pharmaceutical as studies have shown.
- It is not possible to extend these conclusions to long term effects in general as most tests described in literature did not last for a whole vegetation period.
- Exposure of rye grass to pharmaceuticals contained in urine at “natural” levels (i.e. real as a consequence of medication calculated for AGU) as well as at higher concentrations did not affect the fresh and dry matter production during the growth period of three months either for single pharmaceuticals, or for the combination of CZ, IBU, and EE2.
- Only CZ was shown to be taken up by roots and aerial plant parts of rye grass. The CZ concentrations in aerial rye grass parts were in the range of 2500 to 7000 µg kg^{-1} DM, and in roots 130 to 430 µg kg^{-1} DM This leads to the conclusion that only pharmaceuticals which are persistent in soil and are not biodegraded are transferred to plants in high concentrations. 30 % of CZ was found in aerial plant parts and 0.2 % in roots while IBU was below the limit of detection in roots (20 µg kg^{-1} DM).

3.7 Extended discussion

3.7.1 Ecotoxicological potential

As already mentioned, the ecotoxicological potential was considered as very important in determining hazards caused by pharmaceuticals in the environment (2.2.4). Nevertheless, a review of the database showed that ecotoxicological data in soil is rare. Investigations on the effects of pharmaceuticals on soil organisms are in their beginnings (Baguer et al., 2000) and none of them considers concentrations and pharmaceutical combinations applied by urine fertilisation. It is assumed that pharmaceuticals cause abnormal physiological processes and influence the reproductivity of microorganisms in soils (Kolpin et al., 2002). An impact on soil dwelling organisms is assumed (Boxall et al., 2003). Van Gool (1993) showed that

pharmaceuticals influence the growth of soil bacteria as well as the establishment of antibiotic-resistant bacteria. However, effects of pharmaceuticals due to urine fertilisation practised in Sweden, the only country applying urine in large areas, are unknown (Vinnerås, Swedish University of Agricultural Sciences, Sweden [personal communication]).

From chapter 2.2.4 “Ecotoxicological data”, the reader would expect an evaluation of the collected data as it was announced: emphasis was set on EC_{50} values of aquatic organisms as well as on baseline toxicity indicated by approximate log K_{OW} values. Nevertheless, data on EC_{50} values could only be collected for 19 % of the pharmaceuticals contained in the DB for algae, 27 % for daphnia, and 6 % for fish. Therefore, an overall evaluation was not possible. Barely the range of EC_{50} values for the three trophic levels is presented in Table 17. Collected data for specific pharmaceuticals is available at https://www.tu-harburg.de/aww/pharma/.

Table 17: Overview of ranges of pharmaceutical concentrations in the different investigated EC_{50} tests (May 09, 2008)

Category of EC_{50} test	Minimum (mg l^{-1})	Maximum (mg l^{-1})
Algae	0.00006 (Penicillin / Penicillin G)	1004.2 (Pentoxifylline)
Daphnia	0.0027 (Propranolol hydrochloride)	10000 (Iopromide)
Fish	0.000001 (17α-Ethinylestradiol)	5174.18 (Nicotine)

The presented data shows a large range of 7 to 9 orders of magnitude of acute toxicity exhibited in aquatic organisms towards pharmaceuticals. The range becomes even wider if other endpoints etc. would be included. Nevertheless, in Table 18 effect concentrations of more sensitive endpoints are presented for fish in comparison to the respective EC_{50} values in order to demonstrate the influence of endpoint selection. Such data was not included into the DB because it is even less available than for the parameter EC_{50}.

Table 18: EC_{50} of some pharmaceutical for fish contained in the DB and some LOEC referring to more sensitive endpoints. All values in µg l^{-1}. (May 09, 2008)

Pharmaceutical	EC_{50} value	Additional remarks	LOEC values	Additional remarks
Carbamazepine	75100	Cytotoxicity toward hepatocyctes (Laville et al., 2004)	1	Reaction in liver, kidney and gills (Triebskorn et al., 2007)
Clofibric acid	14000	Vitality after 48h (LUA Brandenburg, 2002)	5	Reaction in liver, kidney and gills (Triebskorn et al., 2007)
Diclofenac	5600	Cytotoxicity toward hepatocyctes (Laville et al., 2004)	1	Reaction in liver, kidney and gills (Triebskorn et al., 2004; Triebskorn et al., 2007)
			0.5	Various effects after 7-21 d (Hoeger et al., 2005)
Ibuprofen	No data available		1 - 100	Fish alter pattern of reproduction and may reproduce sex-specific responses (Flippin et al., 2006)
Metoprolol	No data available		1	Reaction in liver, kidney and gills (Triebskorn et al., 2007)

The collected K_{OW} values were considered. As they indicate the basic toxicity or the potential to enter a living organism via its membranes, those pharmaceuticals with high K_{OW} values have to be considered as hazardous. Compounds here with high risks are defined as those exceeding a log K_{OW} >2 and additionally being contained in high concentrations in AGU (mean concentrations >100 µg l^{-1}) at the same time. 29 pharmaceuticals were calculated to reach high concentrations in AGU, 5 of which had a log K_{OW} >2. Only one of the five pharmaceuticals above 1000 µg l^{-1} could be included into the group considered hazardous (high AGU concentration and log K_{OW}:> 2): ibuprofen. When looking at all the pharmaceuticals determined for AGU, approximately 50 % of them show log K_{OW} <1. Ryrfeldt (1971) found that biliary excretion of penicillines is higher, the lower their K_{OW} is. This look at the K_{OW} values of pharmaceuticals shows on one hand that high K_{OW} of a few pharmaceuticals can result in good uptake into membranes of living organism, while on the other hand the low K_{OW} values (described in literature for half of all pharmaceuticals to be contained in AGU) have higher potentials to leach through soils and reach groundwater due to their high polarity. Thus supports the assumption of Williams et al. (2006) that distribution of pharmaceuticals in the environment is related to pharmacological data.

As data from this study were weak and insufficient, some additional points of other investigations were taken into account to provide a better overall picture. At first, the efforts of the Stockholm City Council (Stockholms läns landsting, 2008) have to be mentioned including a report of the Swedish Association of the Pharmaceutical Industry (Johansson et al., 2004) providing an environmental classification system for pharmaceuticals. They managed to include more than 300 pharmaceuticals and analysed their risk considering the Swedish volumes consumed annually via PEC/PNEC (Stockholms läns landsting, 2008) as well as their persistence (P), bioaccumulation (B), and toxicity (T) merged in the so-called PBT index. A significant

risk via the PEC/PNEC ratio was only detected for the two hormones 17ß-estradiol and 17α-ethinylestradiol, but 30 substances reached the highest level for the PBT index and pose an environmental hazard. However, it has to be mentioned that for 16 of the 30 pharmaceuticals data is insufficient and the assessment remains uncertain.

Additionally, an ecotoxicological hazard assessment was accomplished by Lienert et al. (2007b) for approx. 40 pharmaceuticals considering their excretion. It was found that metabolic processes in the human body reduced the toxic potential of all but eight investigated drugs. Source separation of urine could remove 50 % of the toxic potential but as already demonstrated does not apply for all pharmaceuticals due to varying excretion routes (3.4.1). Moreover, Lienert et al. (2007b) were able to demonstrate that ibuprofen is dominating a mixture of 30 pharmaceuticals by representing 52 % of the mixture's toxicity. This is an important finding as most investigations focus on single substances and if urine is considered as fertiliser, there will always be a mix of various substances present.

Furthermore, Escher et al. (2005) showed that toxicity of source separated urine with and without pharmaceuticals towards bacteria and algae did not vary significantly. While Muskolus (2008) investigated the overall toxic potential of urine toward soil organisms by looking at earth worms as vector organisms and concluded that application of urine on agricultural land has a positive effect on plants and microbial organisms due to the organic and mineral nutrients it contained. Nevertheless, single constituents such as ammonia are acutely toxic toward worms. Moistening of the skin of earthworms was lethal within a short period of time (Muskolus, 2008). Responsible for this effect are electrical conductivity in combination with pH and ammonium (Nguyen et al., 2008). Therefore, it is important that application is accompanied by an active incorporation into the soil thereby avoiding drainage into worm channels. This direct contact with the worms results in the decrease of populations which recover only slowly in a dry summer or climate.

Overall, it can be concluded that data on acute and chronic effects caused by urine fertilisation as well as changes on soil dwellers are nearly unknown. Additionally, homeopathic effects might be possible. This aspect was never investigated so far and opens a completely new field of research when it comes to soil biota. Hence, further research regarding the ecotoxicological impacts of pharmaceuticals on soil ecosystems is urgently needed.

3.7.2 Modelling approach

Due to the amount of data collected and the additional experiments, the question was raised whether modelling of the interactions with plants of pharmaceutical based on consumption data would be possible (Leinemann, 2008). The first bottleneck was to

determine pharmaceuticals for which information was available: on one hand concerning consumption and concentrations in water and wastewater (mainly available for human pharmaceuticals, see 3.1, 3.2, and 3.3) and on the other hand concerning concentrations in soil and plants (predominantly available for veterinary pharmaceuticals, see 3.5 and 3.6). However, it was difficult to model processes in soil (Leinemann, 2008). Although their sorption capacity and biodegradation potential (3.5.3) were known, it still remained questionable if these processes affect each other (Kay et al., 2005a) or indeed occur independently at the same time (Das et al., 2004). Moreover, specific parameters such as season, temperature, rainfall, and soil type were still not considered. As a result it became clear during the simulation attempts that pharmaceutical concentrations in soil would lay between some ng kg^{-1} DM to several thousand ng kg^{-1} DM (3.6.3) directly after application of AGU.

Furthermore, no existing model was available for the uptake by plants. Only models for pesticides exist so far and authors commonly stated that further improvements are required (Briggs et al., 1987; Trapp and Matthies, 1995; Trapp, 2000; Chiou et al., 2001). Besides, the key question in case of bioaccumulation could not be answered so far: In which plant parts do pharmaceuticals accumulate? From the data collected in the DB no tendency was visible except that they can be found in every plant part. Peterson and Sinha (1977) investigated the distribution of four pharmaceuticals, all antibiotics, between root and aerial plant parts. The outcome was that in the case of aster, marigold, and poppy, pharmaceuticals predominately accumulated in aerial parts whereas in case of caraway, dandelion, and strawberry, mainly roots were the target plant part. In addition, 20 d after exposure was completed, pharmaceuticals could no longer be detected in any aerial plant part of aster, marigold, poppy, dandelion (already after 7 d), and caraway (4 d), while oxytetracycline and tetracycline were still detectable after 30 d in leaves of peach (Peterson and Sinha, 1977).
It became obvious that pharmaceuticals can passively penetrate through the peel into potatoes' tubers due to the osmotic gradient (Boxall et al., 2006b; Dolliver et al., 2007), and are actively excreted by plants in form of guttation drops (Stokes, 1954) on the other side.

Moreover, it should be pointed out that models should also be developed covering additional aspects i.e. the comparison between different concepts and techniques to handle urine as e.g. for hormones with a large impact on aquatic organisms, an application to soil might be better as hormones degrade rapidly in soils (Johnson et al., 2006, Table 15). Also composting might be a promising practice as certain fungi degrade pharmaceuticals (Alder et al., 2001; Cabana et al., 2007).

3.7.3 Social considerations

Aside from all these considerations based on natural sciences (such as pharmaceutical concentrations in urine, soil, and plants, their behaviour in soil and plant, as well as their ecotoxicological potential, just to state a few) a major factor should be kept in mind: the social impact. Last but not least, the success in application of urine on food crops depends upon people's acceptance. As long as farmers and consumers mention pharmaceuticals as a major concern the use of urine as fertiliser (Muskolus, 2008), it will not be accepted by the public.
Moreover, many people do not even know about effects pharmaceuticals can cause in the environment (Keil, 2006). Hence, aside from urgently needed further research, education of and information for the public are extremely important.

Additionally, some precautionary principles should be respected in order to protect the environment and reach a better acceptance. First of all, not every type of urine should be used for every crop. Pharmaceutical concentrations in urine rise with each person under medication contributing to a collection system. Urine collected within one family and applied in the own garden is considered as relatively safe (Clemens et al., 2008), urine collected from homes for the elderly or from hospitals is not appropriate for fertilisation of food crops. Moreover, in settlements where people are provided with urine-diverting (UD) toilets and a direct use as fertiliser is planned, inhabitants have to be informed about the aspect of pharmaceutical residues. Pharmaceuticals have to be an integral part of the introduction and education activities upon the occasion of UD toilets installation. Educating the users that people under medication should not use the common toilet (whereby the urine goes directly without further processing to agriculture) but a separate system, would achieve an improvement.

Furthermore, we have to be aware that "end-of-the-pipe" solutions usually are not the best ones. In the long run, we have to go to the sources and address the main actors of the health care system: physicians, pharmacists, patients. As Götz and Deffner (2008) stated, people have to become aware of the problem of pharmaceuticals in the environment. Only then will a behavioural change of handling pharmaceuticals become possible. A good moment to start such a change might be the introduction of a UD toilet.

4 Conclusion

Overall, the database contained information for 330 substances out of 700 articles which were detected in the environment. Concentrations for different types of media could be determined by means of theoretical calculations. Evaluation of the data showed that concentrations decrease along the pathway of pharmaceuticals through the environment; from raw wastewater, via effluent of WWTP and surface waters to groundwater.

As only few investigations measured concentrations of pharmaceuticals in yellowwater, it became necessary to calculate them. Predicted concentrations were in the range of 0.1 to 10^3 µg l^{-1} urine and were determined for 124 substances. The theoretical model showed very good correlations with the analytical data obtained for the public waterless urinal in Hamburg (R^2: 0.98) and for the waterless urinals and source separating toilets in offices and flats in Berlin (R^2: 0.90). The calculated values were slightly overestimated implying that the model includes a safety margin and generally overestimates the expected concentration. Furthermore, the comparison showed the importance of a large user group of ≥100 people for measuring average values close to those predicted by the calculation for mean German concentrations. This result was also verified by using additional datasets for pharmaceuticals' concentrations in yellowwater from other studies although the correlation was worse (R^2: 0.54). As reasons for weak correlation, smaller groups of donors in most cases and differences in pharmaceutical consumption between the countries are assumed. More investigation of pharmaceuticals in urine collected in large communities with regard to consumption of pharmaceuticals within these communities is needed.

Comparing pharmaceutical concentrations in urine and raw municipal wastewater showed that pharmaceuticals and their metabolites detected in municipal wastewaters originated to a major degree from urine although some substances show reasonable excretion via faeces. Pharmacokinetic data are a key aspect in understanding and estimating the release of pharmaceutical residues to the environment. Although, theoretical assumptions concerning average pharmaceutical concentrations in urine fit to some extent the levels detected in raw sewage, only a statistically weakly provable relation between concentrations of pharmaceuticals in raw domestic wastewater and urine could be shown in this study. This is likely due to the environmental effects occurring during the passage from human excretion to influents of wastewater treatment plants. Overall it can be concluded that urine separation and separate handling of this wastewater stream represents a promising approach to lower the pharmaceutical load of raw domestic wastewater, to disburden wastewater treatment plants, and to protect the aquatic environment safely from pharmaceuticals.

Additionally, it became obvious that information available for soils and agricultural land is limited. Only 11 pharmaceuticals have been detected in soils so far and mainly substances used in veterinary medicine. Nearly exclusively antibiotics were detected in soils with concentrations in the range of 100 µg kg^{-1} DM. When the relation between concentration and depth was analysed, the result was that no pharmaceuticals were detected at levels below 90 cm soil depth, in most studies even not below 40 cm. By means of calculations, it can be concluded that concentrations in soil would range from 10 to several thousand ng kg^{-1} DM directly after application of urine. For the two main processes occurring (sorption, biodegradation), a larger base of data was available. K_{OC} is a very appropriate parameter regarding sorption and mobility of pharmaceuticals in soil. A large K_{OC} database for many pharmaceuticals was available. Mobility in sand is very high regardless of the specific pharmaceutical. Evaluation of data upon biodegradation is very difficult as kinetics were mostly unknown. Again, predominantly data for pharmaceuticals implemented in animal husbandry was available. Further research is urgently needed. Pot experiments proved the findings from literature. Ibuprofen and 17α-ethinylestradiol were not detected in soil anymore, while approx. 49 % of carbamazepine originally applied by urine was determined in soil samples taken after 3 months.

The collected literature data showed that pharmaceuticals can cause phytotoxic reactions of plants and that bioaccumulation of pharmaceuticals occurs. Plants take up pharmaceuticals, which reside in the roots as well as in the aerial plant parts. Concentrations reported to be measured in plant parts were in the range of ng kg^{-1}. They were also found in edible plant parts such as carrot roots and cereals. However, discrepancies between different plant species and pharmaceuticals were observed. Experiments carried out in this study in the range of µg kg^{-1} showed that relevant concentrations of carbamazepine, ibuprofen, and ethinylestradiol (as calculated for AGU) did not affect production of aerial parts of rye grass during the whole growth period. Only carbamazepine could be quantified in the rye grass when it was applied in its artificial concentration (its ten-fold concentration in AGU). Mean concentrations were 225 µg kg^{-1} DM in roots and 4950 µg kg^{-1} DM in aerial plants parts. This leads to the assumption that only pharmaceuticals which are persistent in soil might be transferred to plants in higher concentrations. 30 % of CZ was taken up into aerial plant parts and 0.2 % into roots while IBU was not detected (LOD: 20 µg kg^{-1} DM in roots), which correlates with their behaviour in soil determined in this research and known from literature.

No evaluation of potential toxic effects of pharmaceuticals ingested by humans with crops is possible at the moment with respect to the findings of this research. However, there are concerns and as long as the concerns are not allayed, it is recommended not to use urine of people under medication for fertilisation of food crops. Might it be a solution that people under medication use a separate toilet?

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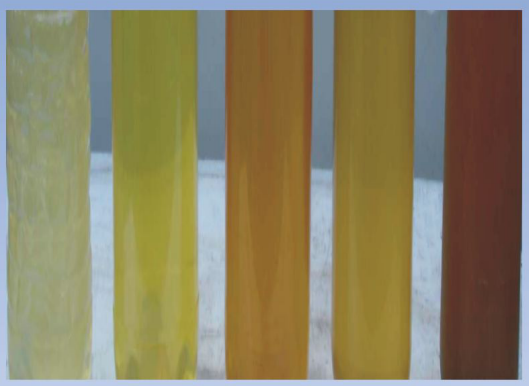
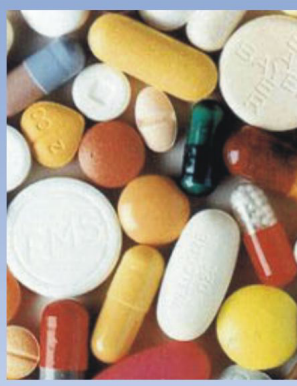
Aim of this study was to determine the potential risks of pharmaceuticals contained in urine when used as fertiliser by means of a database and by greenhouse experiments. Pharmaceutical concentrations in average German urine could be identified by consumption and pharmacokinetic data and were related to concentrations found in wastewater as well as separately colleted urine. Certain pharmaceuticals persist in soil and are taken up by plants, a potential concern with respect to urine fertilisation.





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