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Biodegradability of some antibiotics, elimination of the genotoxicity and affection of wastewater bacteria in a simple test

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Abstract

Most antibiotics and their metabolites are excreted by humans after administration and therefore reach the municipal sewage with the excretions. Only little is known about their biodegradability in aquatic environments. It was recognised that genotoxic substances may represent a health hazard to humans but also may affect organisms in the environment. Therefore, the biodegradability of some clinically important antibiotic drugs (ciprofloxacin, ofloxacin, metronidazole) and hereby the elimination of their genotoxicity was investigated as the first step of an environmental risk assessment using the Closed Bottle test (CBT) (OECD 301 D) and the SOS chromotest. Additionally, to assess toxicity of the antibiotics tested against aquatic bacteria (i) a growth inhibition test (GIT) with *Pseudomonas putida* was conducted, (ii) a toxicity control was used in the CBT and (iii) the colony forming units (CFUs) were monitored in the test vessels. Worst case concentrations of the antibiotics in hospital effluents were estimated and compared with minimum inhibitory concentrations for susceptible pathogenic bacteria and with the genotoxic potency in the SOS chromotest. Both the concentrations calculated for hospital effluents and the adverse effects in bacteria were in the same order of magnitude. None of the test compounds were biodegraded. The genotoxicity was not eliminated.

Keywords: Ciprofloxacin; Ofloxacin; Metronidazole; Genotoxicity; Biodegradability; Closed bottle test; SOS chromotest

1. Introduction

After administration, a significant part of antibiotic substances is excreted by humans into waste water. Outdated medicaments or reminders are sometimes disposed in drains in households, presumably 20–40% in Germany. Therefore, antibiotic substances enter municipal sewage and sewage treatment plants (STPs). If they are not eliminated during sewage treatment they are emitted into surface water and may reach drinking water. Knowledge of pharmaceuticals in the environment is only little (Halling-Sørensen et al., 1998).

Biodegradation is an important process in STPs for removing substances from sewage water. Since the 1980s pharmaceuticals like clofibrate, various analgesics, cytostatic drugs, antibiotics and others have been reported to be present in surface water and effluent of STPs (Richardson and Bowron, 1985; Kümmerer and Helmers, 1997; Kümmerer et al., 1997a; Stumpf et al., 1996; Hirsch et al., 1999) in part emitted from hospitals (Kümmerer, 1998, 1999; Erbe et al., 1997). More recently, clofibrate was detected in ground and drinking water (Stan et al., 1994).

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Nitroimidazoles are mutagenic in the Ames test and are supposed to be carcinogenic (Simon and Stille, 1993). They inhibit the nucleic acid biosynthesis and are strong bactericides. They are effective against anaerobic bacteria. Toxic effects of metronidazole (Fig. 1) one of the most important nitroimidazoles in low mg/l concentrations against algae and daphnids have been reported recently (Lanzky and Halling-Sørensen, 1997).

Ciprofloxacin (Fig. 1) and ofloxacin are among the most widely used quinolones in hospitals. They inhibit the DNA topoisomerases (gyrases) of bacteria. The gyrases are important enzymes for the nucleic acid synthesis. Ciprofloxacin is effective against aerobic gram-negative and gram-positive bacteria. The effect of ofloxacin against some gram-negative bacteria is slightly lower compared to ciprofloxacin. Ciprofloxacin was not biodegradable in the Closed Bottle test (CBT) (Al-Ahmad et al., 1999). Fluoroquinolone carboxylic acids are photodegradable in aqueous solution (Burhenne et al., 1997a,b). In the case of entering the aquatic environment via sewage water, the photo-degradation route is only of minor importance. Only if the substances are not eliminated in the STP a possible photo-degradation in surface water must be taken into account, if they are not adsorbed to sediments. For other fluoroquinolone carboxylic acids, biodegradability by a terrestrial fungus was only recently reported (Martens et al., 1996).

Genotoxicity is a well-known effect e.g. for metronidazole and fluoroquinolones (Stille and Simon, 1993; Mersch-Sundermann et al., 1994a). Ciprofloxacin was found in concentrations between 0.7 and 124.5 μg/l in effluents from hospitals and was assumed to be the main source of genotoxic effects measured with the umuC test in hospital effluents (Hartmann et al., 1999). Additionally, resistant bacteria may be selected by antibiotic substances in the aeration tanks or anaerobic digestion process of STPs.

We therefore investigated the biodegradability of some typical genotoxic antibiotics: metronidazole, ciprofloxacin and ofloxacin in a screening test, the CBT.

Elimination of genotoxicity caused by the test compounds was monitored using the SOS chromotest. The CBT conditions are low bacterial density and low concentration of organic carbon i.e. test compound. Compounds which are "readily biodegradable" as judged from the CBT are assumed not to accumulate in the aquatic environment and to be readily biodegradable in STPs (Nyholm, 1991). To avoid false negative results in the CBT with antibiotic substances, the effects of the compounds against wastewater bacteria were monitored with different methods, because the test substances have different antibiotic spectra and the test is performed with a mixed bacterial population from the effluent of a municipal STP. It is estimated that only less than 15% of the bacteria present in wastewater cultivated (Hiraishi, 1988). A test using sewage sludge for toxicity assessment (e.g. ISO 17 012) will not indicate total toxicity. As the bacterial density is high in such a test, only inhibition of the most abundant organisms which are growing fast under the tests conditions is indicated. Therefore, we used three different methods for the assessment of the toxicity of the test compounds against the inoculum of the CBT. (i) Growth inhibition test (GIT): *Ps. putida* is used for the large group of Gram-negative environmental bacteria. (ii) Colony forming units (CFUs) were determined, referring to toxicity assessment for all bacteria which can be cultivated on a medium adapted to wastewater bacteria. (iii) A toxicity control in the CBT allows for the monitoring of effects onto bacteria which can use the test compounds as a substrate with oxygen consumption. The inhibitory concentrations obtained by the GIT and published 50%-minimum inhibition concentrations (MIC_{50}) for susceptible pathogenic bacteria were compared with theoretical, i.e. calculated, annual average concentrations of the antibiotic substances in hospital effluents and in municipal sewage to assess the toxicity against bacteria present in aquatic environments.

2. Materials and methods

The selected compounds represent antibiotics with genotoxic activity and the highest expected concentrations in hospital effluents within the group of antibiotics they belong to (Table 1).

2.1. Calculation of predicted environmental concentrations (PECs): concentrations in hospital effluents, municipal sewage, and in surface water

The total annual amounts of antibiotics and sulphonamides used in five hospitals of different size and medical service spectrum were estimated for the years

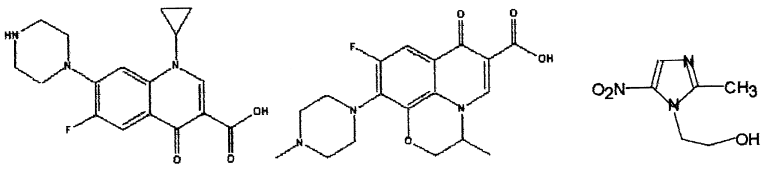

Fig. 1. Formula of the investigated antibiotics: ciprofloxacin, ofloxacin and metronidazole (from left to right).

Table 1
Test compounds and results of the closed bottle test (OECD 301 D)

Test compound	Supplied by	Test concentration (ThOD mg/l)	Test concentration (mg/l)	Biodegradation (%)	
				28 days	40 days
Ciprofloxacin hydrochloride	Bayer AG, Leverkusen	5.95	3.5	0	0
Ofloxacin hydrochloride	Hoechst AG, Frankfurt/M	4.76	2.8	0	0
Metronidazole	Bayer AG, Leverkusen	4.5	5.4	5	5

from 1994 to 1997 (Erbe et al., 1997; Kümmerer, 1998, 1999). With the annual water consumption and the excretion rates of the unchanged drugs, the PECs in the hospital effluents were calculated. Hospital effluent is diluted by a factor of at least 100 by municipal sewage in case of German hospitals, according to our experience (Kümmerer et al., 1997a,b, 1998). Furthermore, a dilution factor of 10 was used to calculate the PEC in surface water from estimated concentrations in municipal sewage (EU, 1995). As no further information was available, both elimination e.g. by adsorption or hydrolysis and biodegradation in STPs were neglected for this purpose. The computational procedures have been described elsewhere in detail (Kümmerer et al., 1997a).

2.2. Toxicity assessment

To avoid false negative results in the CBT and to assess the effects of antibiotics on environmental aquatic bacteria, the affection of wastewater bacteria by the test compounds was evaluated with different methods, because the test substances have different antibiotic spectra and the test is performed with a mixed bacterial population from the effluent of a municipal STP. A test using sewage sludge for toxicity assessment (e.g. ISO 17 012) will not indicate total toxicity. As the bacterial density is high in such a test, only inhibition of the most abundant organisms which are growing fast under the tests conditions is indicated. Therefore, we used three different methods for the assessment of the toxicity of the test compounds against the inoculum of the CBT. (i) GIT: *Ps. putida* is used for the large group of Gram-negative environmental bacteria. (ii) CFUs were determined, referring to toxicity assessment for all bacteria which can be cultivated on a medium adapted to wastewater bacteria. (iii) A toxicity control in the CBT allows for the monitoring of effects onto bacteria which can use the test compounds as a substrate with oxygen consumption. The inhibitory concentrations obtained by the GIT and published 50% minimum inhibition concentrations (MIC_{50}) for susceptible pathogenic bacteria were compared with theoretical, i.e. calculated, annual average concentrations of the antibiotic substances in hospital effluents and in municipal sewage to assess possible effects on bacteria present in aquatic environments.

2.2.1. Growth inhibition test

GITs were conducted in duplicates, according to an international method (ISO, 1995). Eight-fold doubling concentrations were used, starting at an initial concentration below the MIC_{50} published for susceptible pathogens (Gerding et al., 1991; Simon and Stille, 1993). The following modifications were made, as described in detail elsewhere (Kümmerer et al., 1996a). The disinfectant benzalkonium chloride was used at a concentration of 32 mg/L as control for 100% inhibition. The incubation temperature was 30°C. The nutrient solution was adapted to wastewater organisms (Brözel and Cloete, 1992). The growth of the bacteria was monitored by measuring the protein content (Lowry et al., 1951).

2.2.2. Colony forming units (CFUs)

CFUs were determined in all test vessels. The nutrient solution was adapted for wastewater bacteria (Brözel and Cloete, 1992; Kümmerer et al., 1996a). Agar plates were inoculated with decadic dilutions of the test aliquots from the CBT in 0.9% sodium chloride solution using a spiral plater (Spiral System Instruments, Lähden, Germany). In preliminary tests, the standard deviation was ±10% (Kümmerer et al., 1996b). The inoculated plates were incubated for two days at 30°C, then the CFUs were counted (BZG 28, WTW, Weilheim, Germany).

2.2.3. Toxicity control in the CBT

In the blank, only mineral salts and phosphate buffer were present. The quality control contained readily biodegradable sodium acetate as organic carbon source corresponding to 5 mg/l theoretical oxygen demand (ThOD), the test compound was added to another series of test vessels. The toxicity control contained sodium acetate and the test compound. Toxicity was assessed by comparing the oxygen consumption measured in the toxicity control with the predicted one which was computed from the oxygen consumption in the quality control and in the test vessel containing only the test compound, respectively. Possible co-metabolism was neglected. Toxicity was stated if the difference between the predicted oxygen consumption and the one measured in the toxicity control exceeded 25% (OECD, 1992).

2.3. Biodegradability – CBT (OECD 301 D)

The CBT is recommended as a first, simple test for the assessment of the biodegradability of organic compounds (Nyholm, 1991; OECD, 1992). The CBT was performed according to test guidelines (OECD, 1992) in the dark at room temperature (20 ± 1°C), as described elsewhere in detail (Kümmerer et al., 1996a). The standard test period of the CBT is 28 days, therefore we reported the results after 28 days of testing. We used some more vessels to prolong the test to 40 days, to favour adaptation of slowly growing bacteria and testing inherent biodegradability (van Ginkel and Stroh, 1992). The judgement of ready biodegradability is based on the results after 28 days. All chemicals used were of at least analytical grade. Each test consisted of four different series: quality control (sodium acetate corresponding to a ThOD of 5 mg/l), blank, test compound (Table 1) and toxicity control (test compound and sodium acetate). Two parallel tests for each series were run. All test vessels were inoculated with an aliquot from the effluent of the municipal STP where the sewage of the Freiburg University Hospital and five other hospitals is treated (Abwasserzweckverband Breisgauer Bucht, Forchheim, population equivalent 600 000). Therefore, some adaptation to the test compounds might already have happened. At the beginning of the test, the CFUs in the test vessels were between 1000 and 10 000 per ml. Each test vessel contained mineral salt solution. Test compounds were obtained from our laboratory stocks as supplied by the manufacturers. The concentrations of the test compounds used are summarised in Table 1. According to our experience a (ThOD) of 4–6 mg/l in the vessels is most suitable for good results (Table 1). Progress of the aerobic biodegradation was monitored by measuring the oxygen concentration in the test vessels with an oxygen electrode (Oxi 196 with EO 196-1.5, WTW Weilheim, Germany) according to international standard methods (ISO, 1990). A test compound is classified as "readily biodegradable" if the biodegradability expressed as percentage of oxygen consumed in the test vessel exceeds 60% of the maximum theoretical consumption within a period of ten days after reaching 10% theoretical oxygen consumption.

2.4. Genotoxicty assay

2.4.1. Materials

All media and reagents described by Quillardet and Hofnung (1985) and Mersch-Sundermann et al. (1993) for the SOS chromotest were obtained from Merck Darmstadt, Germany. Due to the experiences with topoisomerases-II-inhibitors we used the SOS chromotest only without S9-mix.

2.4.2. Tester strain

The tester strain *Escherichia coli* PQ37 was kindly provided by Maurice Hofnung and Philippe Quillardet, Institut Pasteur, Paris, France. The genetic markers of the tester strain including the sfiA::lacZ operon fusion have been described elsewhere (Quillardet and Hofnung, 1985). Cultures of *E.coli* PQ37 were grown from frozen stocks (−80°C) in LA-medium overnight (16 h) at 37°C with agitation (100 rpm).

2.4.3. Test principle

The SOS chromotest for the identification of DNA damage produced by chemicals is a bacterial mutagenicity assay which employs a specific mutant, *E. coli* PQ37 (Quillardet and Hofnung, 1985). In *E.coli* PQ37, a β-galactosidase gene (*lacZ*) is placed under control of the *sfiA* gene operon, which belongs to the SOS-repair system (*sfiA*::*lacZ* fusion). Accordingly, upon induction of DNA damage not only the *sfiA* gene, but also the *lacZ* gene is expressed. Consequently, the gene product of *lacZ*, β-galactosidase, can be measured by a colorimetric assay which provides an indirect measurement of DNA damage capable of inducing the SOS repair system (genotoxicity assay). Additionally, the measurement of the constitutive production of alkaline phosphatase (PHOC) in *E.coli* PQ37 can be used as an indicator of non-specific toxicity, i.e. bacteriotoxicity.

2.4.4. Test protocol

To carry out the SOS chromotest, we followed the procedure described by Quillardet and Hofnung (1985) in a modified version recommended by Mersch-Sundermann et al., 1991; Mersch-Sundermann et al., 1993; Mersch-Sundermann, 1993. 100 μl of a 16 h overnight culture of *E. coli* PQ37 was subcultivated in 10 ml of fresh LA-medium for about 2 h at 37°C to a bacterial density of 200×10^6 bacteria/ml. 0.25 ml of the subculture was poured into 9.75 ml fresh LA-medium. 300 μl of these suspensions was added to tubes containing 10 μl of the polycyclic musks and positive controls, respectively. After 2 h of incubation two 150 μl aliquots were withdrawn from each tube for β-galactosidase and alkaline phosphatase assays. For the β-galactosidase assay, 900 μl portions of β-galactosidase buffer (pH 7.7) were added to one of the tube series and incubated for 10 min at 37°C for temperature equilibration. Following, the enzyme assay was started by adding 200 μl of 4-nitrophenyl-β-D-galactopyranoside (4 mg/ml ONPG) solution. After incubation at 37°C for 20 min with agitation the substrate conversion was stopped by adding 660 μl of 1 M sodium carbonate. The β-galactosidase activity was measured as the o-nitrophenol concentration by photometric measurement at 420 nm. For the alkaline phosphatase assay, 900 μl portions of alkaline-phosphatase buffer (pH 8.0) were added to the other tube series and incubated for 10 min at 37°C. The

ap-activity was measured as the turnover of the substrate 4-nitrophenyl phosphate (200 μl of 4 mg/ml PNPP). 4-nitroquinoline-N-oxide (4-NQO) was used as a positive control. All compounds were tested three times ($n = 3$) on different days (for details see Mersch-Sundermann, 1993).

2.4.5. Calculation and evaluation of measurements

In order to obtain consistent and comparable results we used the following calculation and evaluation procedure: The β-galactosidase (βg) and alkaline phosphatase (ap) activities were calculated as enzyme units $U_{(\beta g)}$ and $U_{(ap)}$ with $U = A_{420} \times 1000/t$ (A_{420} = optical density at 420 nm; t = substrate conversion time in minutes). The induction factor IF was calculated as the ratio R_X/R_0 ($R_X = U_{(\beta g)}/U_{(ap)}$ determined for the test chemical concentration $k_1, \ldots, k_n$; $R_0 = U_{(\beta g)}/U_{(ap)}$ with $k = 0$, negative control). A compound was classified as "not genotoxic" if the induction factor remained <1.5, as "marginal" if the induction factor ranged between 1.5 and 2.0 and as "genotoxic" if (i) the induction factor was in excess of 2.0 and (ii) a continuous increase of the βg-activity with increasing compound concentration was detectable.

3. Results

The biodegradability of some clinically important antibiotic substances in the CBT as well as their effects against wastewater bacteria were investigated. As a first step, theoretical concentrations of the investigated substances in hospital effluents and surface water were calculated and compared (i) with MIC_{50} published for susceptible pathogens and (ii) the results of the microbiological monitoring of the CBT.

3.1. PEC of hospital effluents, municipal sewage and surface water

The PECs of antibiotics in hospital effluents were in the range of the published MIC_{50} and inhibition concentrations measured in the GIT for all tested antibiotics (Table 2). In municipal sewage, the PEC of ciprofloxacin and ofloxacin was one order of magnitude below the MIC_{50} of pathogens and the EC_0 of *Ps. putida*, Table 2). The PECs of metronidazole in all wastewater types and surface water were far below the inhibition thresholds. As expected when concentrations in surface water are assumed to be 10% of the sewage concentration, none of the tested antibiotic substances reached the MIC_{50} of susceptible pathogenic bacteria or EC_0 of *Ps. putida*.

3.2. Affection of wastewater bacteria

Effect concentrations (EC_x) measured in the GIT with the Gram-negative bacterium *Ps. putida* varied with the test compound (Table 2) but were in the same range as the MIC_{50} for susceptible pathogenic bacteria. The most toxic compounds were the quinolones ciprofloxacin and ofloxacin, with 50% growth inhibition at a concentration of 80 μg/l in the case of ciprofloxacin.

Table 2
Inhibition concentration of test compounds against different bacteria and concentrations expected in the aquatic environment

	Unit	Ciprofloxacin	Ofloxacin	Metronidazole
Effects onto bacteria				
Genotoxicity (SOS chromotest)	μg/l	0.2–0.4	1–2	Marginal
MIC_{50} (susceptible pathogens)[a]	μg/l	2–8000	7.5	60
EC_0 (GIT, *Ps. putida*)[b] ($n = 2$)	μg/l	10	<10	64,000
EC_{50} (GIT, *Ps. putida*)[b] ($n = 2$)	μg/l	80	10	>64,000
EC_{100} (GIT, *Ps. putida*)[b] ($n = 2$)	μg/l	320	40	>64,000
CFU (CBT)[b]		[c]	[d]	[d]
Toxicity control (CBT)		[d]	[c]	[d]
Input into the aquatic environment				
Excretion rate (unchanged drug)	%	40	70	40
Theoretical concentration in hospital wastewater[e]	μg/l	2–30	0.5–50	70–110
Theoretical concentration in municipal wastewater[f]	μg/l	0.6	0.5	0.1
Theoretical concentration in surface water[f]	μg/l	0.06	0.05	0.01

[a] Lowest concentration, at which 50% of the susceptible pathogenic bacteria used were inhibited (Simon and Stille, 1993; Gerding et al., 1991); no data available for bacteria in STPs and the aquatic environment.
[b] Average of two replicates, difference of values less than 10%.
[c] Weak but significant effect, i.e., difference to between test vessel and blank more than one logarithmic unit.
[d] No effect.
[e] Five German hospitals of different size and medical service spectrum.
[f] Annual average for Germany, if no elimination occurs in sewage treatment.

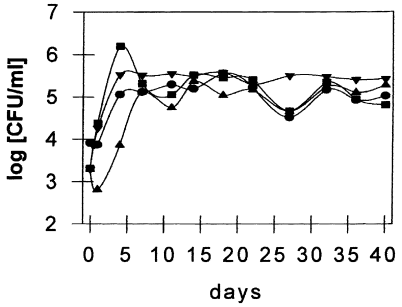

Fig. 2. CFUs in the CBT of ofloxacin: ● test compound; ■ quality control; ▲ toxicity control; ▼ toxicity control theoretical.

Ciprofloxacin had also a weak but significant toxicity in the CFU monitoring but no affection of the bacteria in the toxicity control of the CBT by CFU monitoring. A weak but significant affection was detected in the toxicity control of the CBT with ofloxacin which disappeared after a few days (Fig. 2). For both quinolones, an inhibition was observed within the first 8–11 days in the toxicity control of the CBT (Fig. 3).

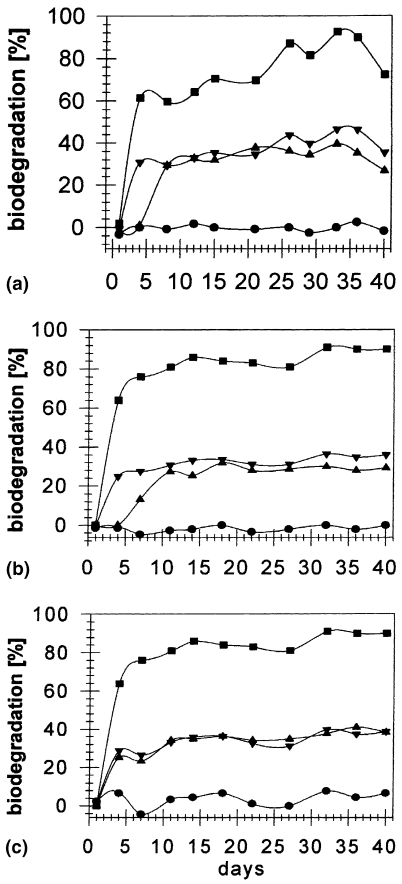

Fig. 3. Biodegradation of the test compounds: ● test compound; ■ quality control; ▲ toxicity control; ▼ toxicity control theoretical; from top to bottom: (a) ciprofloxacin; (b) ofloxacin and (c) metronidazole.

3.3. Biodegradability in the CBT

The activity of the inoculum in the quality control (sodium acetate, see Fig. 3) was adequate and the tests were valid according to the established guidelines. None of the test compounds were biodegraded in this test system to a higher extent (Table 2). Therefore, none of the antibiotics can be classified as "readily biodegradable". In the toxicity controls of the tests with the fluoroquinolones ciprofloxacin and ofloxacin, an inhibition of the biodegradation was observed within the first days of the test. The inhibition disappeared after 8 and 11 days, respectively. The ciprofloxacin concentration in the test vessels was analysed by HPLC. Within the analytical error no elimination of ciprofloxacin was found (data not shown). A prolongation of the test period did not influence the results.

3.4. Elimination of genotoxic activity

The quinolones, ciprofloxacin and ofloxacin are of high genotoxic activity. Their genotoxicity was not eliminated during the test period of 40 days (Fig. 4). For ciprofloxacin only the day 40 sample is shown with IF(max.) = 22.9. For ofloxacin IF(max.) was 19.7. Metronidazole was only marginal genotoxic with IF(max.) = 1.8. Its genotoxicity was also not eliminated during the test period of 40 days. IF(max.) for the SOS chromotest control (4-NQO) was between 40.9 and 53.

4. Discussion

The substances under investigation represent two clinically important types of antibiotic drugs (chinolones

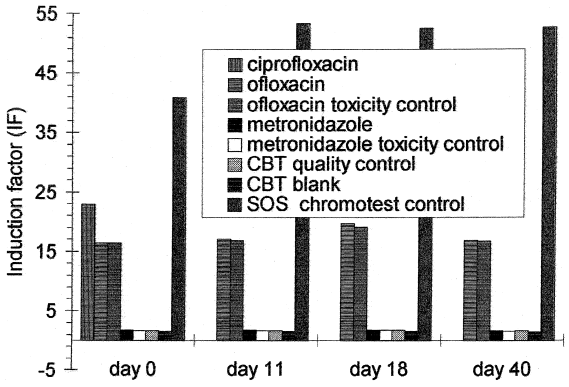

Fig. 4. Elimination of genotoxicity in the CBTs.

and nitroimidazoles) that possess different chemical structures, different activity spectra and various modes of antimicrobial action. None of the compounds investigated were "readily biodegradable". Our findings are in agreement with the low biodegradation rates reported for ciprofloxacin and other antibiotics in a CBT (Al-Ahmad et al., 1999). Richardson and Bowron (1985) reported results of primary biodegradation in tests with high bacterial density between not biodegradable (e.g. erythromycin) and 48% (ampicillin, a β-lactam compound). Abiotic elimination processes like adsorption, hydrolysis or partial biodegradation of active moieties may take place but were not studied. In an aquatic model ecosystem, elimination of ciprofloxacin took place by adsorption onto the sediment, but the mineralising process was slow (Bayer, 1991). Low elimination was also reported for other antibiotics in soil thus underlining our results. Virginiamycin, an antibiotic food additive for livestock administered orally as a growth promoter, was found to biodegrade in various soils only with a long half-life of 87–173 days (Weerasinghe and Towner, 1997). Cyclosporine A was shown to be degraded very slowly after some months in moist samples of garden soils, despite the fact that several micro-organisms capable of degradation have been isolated from soils (Hübener et al., 1992). Sarafloxacin, a fluoroquinolone registered for use against poultry diseases, was mineralised by less than 1% in various soils within 80 days, probably because of its strong binding to soil (Marengo et al., 1997). Therefore the results of our tests and published results of the biodegradability of antibiotics show that further investigations into the biodegradability of antibiotics in the environment are necessary. In our tests there was no biodegradation or other elimination process like adsorption of the quinolones in the test vessels as was confirmed for ciprofloxacin by HPLC analysis. The amount of oxygen used was exactly the ThOD of sodium acetate amount present in the toxicity controls. Therefore, inhibitory effects of the quinolones cannot be the reason for the observed low biodegradability.

Ciprofloxacin and ofloxacin are broad spectrum compounds and affect both Gram-negative and Gram-positive bacterial species. Therefore, low inhibition concentration against *Ps. putida* in the GIT is not surprising. The concentration of ciprofloxacin and ofloxacin was in the range of the EC_0 (GIT) and one could suppose that there should be inhibition of the bacteria by the test substances. In the toxicity controls there was indeed an inhibitory effect within the first days (Figs. 2 and 3), probably on bacteria able to degrade sodium acetate, because HPLC analysis showed that there was no elimination of ciprofloxacin. After a short adaptation period the inoculum biodegraded readily biodegradable sodium acetate well in the presence of the quinolones. As with the toxicity control, mainly the fast growing groups of bacteria which can use the substrate under oxygen consumption are monitored. Effects on other groups are not reported with this setting.

The inhibitory effect of ofloxacin was also observed in the test vessel by CFU counting (Fig. 2) but not for ciprofloxacin (data not shown), which refers to their somewhat different antibiotic spectrum. There was only a weak inhibitory effect in the CFU monitoring and none in the toxicity control. But in all test vessels containing a quinolone the CFUs were higher than in the blank after the initial test period. This indicates that despite the broad spectrum of pathogenic bacterial species affected by ciprofloxacin only a minority of the aquatic species monitored by CFU counting were inhibited.

The CBT is a screening test using low bacteria density. The number of bacteria present which are able to biodegrade a chemical is lower than in tests with higher bacterial density and diversity. Therefore, only the most numerable species will be present in higher individual numbers and the results of CFU monitoring and the toxicity control are referring only to the most abundant bacteria, because the other species are only present in small numbers, if present at all. Therefore, in further studies, methods like chemotaxonomy and tests with higher inoculum density have to be used and the whole population dynamics in the test vessels should be studied which has already been applied successfully in biodegradability testing of disinfectants (Kümmerer and Al-Ahmad, 1999).

For metronidazole the EC_0 against *Ps. putida* was much higher than the test concentration. The test concentration was higher than the MIC_{50} published for pathogens. But it is effective against anaerobic bacteria. Therefore, inhibition by the test compound can be excluded. This is in agreement with the result of the toxicity control and CFU monitoring. Because of its non-biodegradability for metronidazole its affection of anaerobic bacteria in sludge digestion and sediments has to be assessed.

The concentration calculated for ciprofloxacin in hospital effluents was in the range reported by Hartmann et al. (1999) for 24 h sampling. Therefore, we assume that the concentrations calculated for the two other compounds are also realistic. The concentrations calculated for the antibiotics in hospital effluents and municipal sewage are annual average concentrations. Peak concentrations during the day time can reach much higher concentrations than the calculated annual average concentrations. This is in agreement with results obtained for cytotoxic compounds cisplatin and carboplatin and the disinfectant benzalkonium chloride within 2 h sampling over 24 h (Kümmerer et al., 1997a,b; Kümmerer, 1998). Dilution of the hospital effluents by municipal sewage will lower antibiotic concentration only moderately, because municipal wastewater also

contains antibiotic substances and disinfectants from households and to a minor extent from livestock.

In Germany the daily water consumption per capita dropped from 150 to 130 l due to water saving. Within the last few years the usage of antibiotics increased every year whereas the usage of other pharmaceuticals was constant or dropped slightly. If these trends will continue, the concentrations of antibiotics in wastewater will rise and an increased impact of antibiotics on bacteria in STPs has to be expected. In some areas in Germany (e.g. Berlin, Frankfurt) the dilution factor of STP effluent by surface water as recommended by the EU (EU, 1995) is by far less than 10 in summer time. Therefore, the concentrations expected in surface water could be higher than the ones presented in this paper. But other factors like binding to sediments or sludge and other solids or photolysis have to be regarded also.

Because of the fact that 56% of all rodent carcinogens are Salmonella mutagens (Ashby and Morrod, 1991), bacterial mutagenicity assays play an important role in the screening and identification of carcinogenic compounds. Moreover, mutagenic rodent carcinogens tend to cause cancers in male and female rats and mice at multiple sites while non-mutagenic carcinogens cause cancers mostly at single sites in male or female rats or mice (Ashby and Tennant, 1988). Therefore, bacterial mutagenicity tests are widely used instruments to identify potential carcinogens. Former Studies dealing with (i) the structure activity of chemical compounds in the SOS chromotest (Mersch-Sundermann et al., 1996; Rosenkranz et al., 1999), (ii) the comparison of the widely used Salmonella assay (Amestest) and the SOS chromotest (Mersch-Sundermann et al., 1994b), and (iii) the use of the SOS chromotest and other microbial assays for the detection of mutagens in complex aqueous mixtures (Helma et al., 1996) have demonstrated that the SOS chromotest is a rapid, sensitive and discriminative screening procedure for mutagens in water and other environmental samples. Therefore, in the present study we used the SOS chromotest for the detection of genotoxicity caused by residues of antibiotics in water and to estimate their biodegradation.

Hartmann et al. (1999) found positive effects in the umuC test in samples of hospital wastewater containing ciprofloxacin in concentrations of 0.7 μg/l (lowest observed effect level). As shown in former studies (Mersch-Sundermann et al., 1994b) topoisomerases-II inhibitors like ciprofloxacin and ofloxacin are very strong genotoxicants in *E.scherichia coli* PQ37 (SOS chromotest) and mutagens in *Salmonella typhimurium* TA102 (Amestest) in the absence of an exogenous metabolising system. DNA damaging effects could be shown in concentration of approximately 0.2–0.4 μg/l for ciprofloxacin and 1–2 μg/l for ofloxacin (lowest observed effect level). These concentrations causing DNA damages in bacteria are approximately 10–150-fold lower than the concentrations expected in hospital wastewater and very similar to those expected in municipal wastewater (Table 2). Therefore, genetic damages of susceptible bacteria which are responsible for biodegradation in water and sediments could be concluded. On the other hand, the results of the GITs and CFU measurements led to the conclusion that the DNA damages probably caused by ciprofloxacin and ofloxacin in the concentrations calculated are not sufficient to cause significant changes in the composition and interaction of the biotope. Additionally, due to the fact that ciprofloxacin and ofloxacin inhibit bacterial topoisomerases in 1000-fold lower concentrations than the correspondingly enzymes in eukrayontic cells, a genetic risk for plants, animals and humans by residues of topoisomerases-II inhibitors is unlikely.

5. Conclusions

The antibiotics tested were not biodegraded in the CBT. Consequently, their genotoxicity was not eliminated. The results indicate that the various antibiotics were active against different groups of bacteria present in wastewater. Inhibition monitoring by the toxicity control of the CBT only is not suitable to assess the toxicity of the different wastewater bacteria species by antibiotic substances because of the complexity of the inoculum and the different antibiotic spectra of the test compounds. But even if the antibiotics are broad spectrum compounds, they are not inhibiting or killing all of the bacteria present. But additional microbiological monitoring is recommended to assess the effects of test substances onto the inoculum.

The work presented suggests further that biodegradation of antibiotics in STPs might not be a reliable expectation for the removal of antibiotic substances and needs more detailed investigations. In tests with a higher degree of simulating an STP or an STP, higher biodegradability and non-biotic elimination processes like adsorption, hydrolysis or partial degradation of active moieties may take place in a higher extension. Further investigations into their behaviour in STPs are necessary.

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