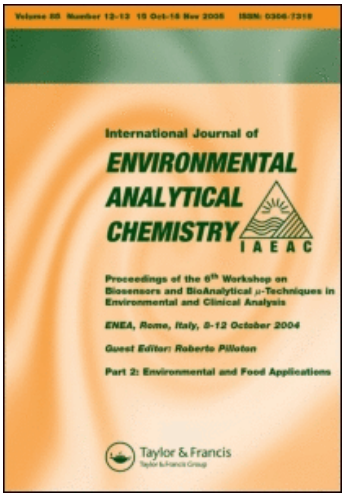

International Journal of Environmental Analytical Chemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713640455

Degradation of the antiviral drug oseltamivir carboxylate in surface water samples

Cesare Accinelli[a]; Anna Barra Caracciolo[b]; Paola Grenni[b]
[a] Department of Agro-Environmental Science and Technology, University of Bologna, Bologna, Italy [b] Water Research Institute, National Research Council, Rome, Italy

To cite this Article Accinelli, Cesare , Caracciolo, Anna Barra and Grenni, Paola(2007) 'Degradation of the antiviral drug oseltamivir carboxylate in surface water samples', International Journal of Environmental Analytical Chemistry, 87: 8, 579 — 587
To link to this Article: DOI: 10.1080/03067310601151894
URL: http://dx.doi.org/10.1080/03067310601151894

Intern. J. Environ. Anal. Chem.
Vol. 87, No. 8, 15 July 2007, 579–587

Taylor & Francis
Taylor & Francis Group

Degradation of the antiviral drug oseltamivir carboxylate in surface water samples

CESARE ACCINELLI*†, ANNA BARRA CARACCIOLO‡ and PAOLA GRENNI‡

†Department of Agro-Environmental Science and Technology, University of Bologna, Viale Fanin 44, 40127 Bologna, Italy
‡Water Research Institute, National Research Council, Via Reno 1, 00198 Rome, Italy

(*Received 20 October 2006; in final form 1 December 2006*)

Numerous studies have documented that a wide number of pharmaceuticals used in human and veterinary medicine have the potential to enter the aquatic ecosystem. The antiviral prodrug oseltamivir phosphate has received recent attention with regard to its possible use against the highly pathogenic H5N1 virus. This preliminary laboratory study investigated the persistence of the active antiviral drug, oseltamivir carboxylate (OSC), in water samples taken from an irrigation canal. After an initial rapid decrease, OSC concentrations slowly decreased during the remaining incubation period. Approximately 65% of the initial OSC amount remained in water at the end of the 36-day incubation period. A small amount of OSC was lost both from sterilized water and from sterilized water/sediment samples, suggesting a significant role for microbial degradation. Stimulating microbial processes by the addition of sediments resulted in reduced OSC persistence. Presence of OSC ($1.5\,\mu g\,mL^{-1}$) did not significantly affect the metabolic potential of the water microbial population, estimated by glyphosate and metolachlor mineralization. In contrast, OSC caused an initial transient decrease in the size of the indigenous microbial population of water samples.

Keywords: Tamiflu; Pharmaceuticals; Bird flu; Environmental fate; Surface water

1. Introduction

Nowadays, a wide number of pharmaceuticals are routinely used to prevent and control human and animal diseases. After administration, a variety of pharmaceuticals are discharged as parent compounds or metabolites into wastewater treatment plants via excretion with urine and faeces. Research has shown that many pharmaceuticals are not completely removed during wastewater treatments, and as a result, pharmaceuticals have been found in a wide range of environmental samples including surface and groundwater [1]. The occurrence of pharmaceuticals in the aquatic environment has become a subject of scientific and public concern. Since pharmaceuticals are specifically designed to perform some sort of biological effects, the presence of these compounds in

*Corresponding author. Fax: +39-2096241. Email: accinel@agrsci.unibo.it

International Journal of Environmental and Analytical Chemistry
ISSN 0306-7319 print/ISSN 1029-0397 online © 2007 Taylor & Francis
DOI: 10.1080/03067310601151894

the environment is viewed as potentially harmful to wildlife and human health [2, 3]. Pharmaceuticals are considered by regulatory agencies as emerging contaminants that may require regulatory action [4]. Although pharmaceuticals are used in large quantities, their environmental fate has not been adequately addressed.

The continuing spread of the highly pathogenic H5N1 virus has forced several countries to set up strategies and contingency measures to protect citizens and to contain the disease [5]. These strategies include various precautionary measures and strengthened surveillance on wild birds and poultry farms, vaccination, and prophylactic assumption of antiviral drugs. Although vaccination is the primary strategy for the prevention of influenza, there are a number of likely scenarios for which antiviral agents would have considerable importance [6]. Among approved antiviral drugs, oseltamivir phosphate (commercially known as Tamiflu) is currently indicated for treatment of clinically confirmed cases and for post-exposure prophylaxis to control recent H5N1 avian influenza outbreaks [7, 8]. Oseltamivir phosphate (OSP) is an orally available prodrug of the active metabolite oseltamivir carboxylate (OSC). OSP is absorbed from the gastrointestinal tract and converted by hepatic esterases to the active metabolite (OSC). OSC is a selective inhibitor of influenza virus neuramidase enzymes, interrupting an established infection in respiratory cells [9]. Pharmacological studies indicated that OSC is not further metabolized and is entirely ($>90\%$) eliminated by renal excretion [7]. In recent months, governments of several countries have begun stockpiling OSP in order to protect 20–25% of the population against a potential bird flu pandemic [8]. In cases of urban areas with a large number of patients under OSP medication, and considering that the adult dosage is 75 mg twice daily for 1–6 weeks, potential contamination of water reservoirs should be considered. To date, no information concerning the potential of OSC to contaminate surface water is available. Considering the importance of this antiviral drug, research on the environmental fate of this drug is consequently needed.

This preliminary study was performed to investigate the degradation of the active antiviral drug OSC in surface water. Effects of OSC on bacteria abundance and metabolic potential of the water microbial community were also estimated.

2. Experimental

2.1 *Water sampling and sample incubation*

Water samples were collected from the irrigation canal Canale Emiliano Romagnolo (CER). CER is a 133-km-long irrigation canal that receives water from the Po river and runs from Salvadonica di Bondeno (Ferrara) downs to Donegaglia di Bellaria (Rimini) before empting in the Uso river. Sampling operations were conducted on April 2006 in the proximity of Medicina (44°27′59″ N, 11°42′25″ E). Samples were collected manually by immersing 2-L sterile glass bottles approximately 10 cm below the water surface. Collected samples were transported to the laboratory within 2 h from sampling and were kept at 4°C in the dark. Prior to use, water samples were left at 20°C overnight. A portion of the collected water was sterilized by autoclaving for 1 h at 121°C and 103 kPa. Some of the physico-chemical properties of the collected water are given in table 1.

Table 1. Physico-chemical properties of water taken from the irrigation canal CER.

pH	DO[a] ($mg\,L^{-1}$)	DOC[b] ($mg\,L^{-1}$)	HCO_3^- ($mg\,L^{-1}$)	F^- ($mg\,L^{-1}$)	Cl^- ($mg\,L^{-1}$)	NO_2^- ($mg\,L^{-1}$)	NO_3^- ($mg\,L^{-1}$)	PO_4^{3-} ($mg\,L^{-1}$)	SO_4^{2-} ($mg\,L^{-1}$)
8.3	13.1	4.7	158.0	0.17	28.5	0.07	13.2	< 0.02	83.8

[a]DO: dissolved oxygen. [b]DOC: dissolved organic carbon.

Water samples (80 mL) were transferred into 250-mL sterile Erlenmeyer flasks under aseptic conditions. Samples were treated with oseltamivir carboxylate (OSC) dissolved in 50 mM NaH_2PO_4 to give a final concentration of $1.5\,\mu g\,mL^{-1}$. Flasks were sealed, and samples were incubated at 20°C on an orbital shaker (125 rpm) in the dark. At selected time intervals, duplicate 1-mL aliquots were taken for analytical or microbiological analysis. All operations were conducted under sterile conditions. Control samples consisting of untreated water and samples containing 5% (w/v) of sterilized or non-sterilized sediments taken from the same irrigation canal were included. The sand, silt, clay, and organic carbon content of sediments were 7.3, 38.9, 53.8, and 2.8%, respectively. Sediments were air-dried and passed through a 5-mm sieve. The size of the sediment microbial population was estimated using the dilution plate count onto 0.1 strength tryptone soy agar as described in Accinelli *et al*. [10]. The whole experiment was conducted in triplicate.

2.2 *Chemical analysis*

OSC concentrations of incubated water samples were determined by HPLC after derivatization with 20 mM naphthalene-2,3-dialdehyde (Sigma-Aldrich Italia s.r.l., Milan, Italy) and 20 mM potassium cyanide (Ultra Scientific Italia s.r.l., Bologna, Italy) as described in Eisenberg and Cundy [11]. Aliquots of the derivatized mixtures were analysed by a chromatography system equipped with a 250×0.46 mm Prodigy ODS-2 column (Phenomenex Inc., Torrance, CA), and an RF-10AXL spectrofluorometric detector (Shimadzu Italia s.r.l., Milan, Italy). Isocratic elution was carried out at 40°C, and the eluent flow was set at $1.0\,mL\,min^{-1}$ with 50 mM sodium acetate in acetonitrile/water (27 : 73, v/v). Detection of OSC was achieved by setting the detector at excitation and emission wavelengths of 420 and 472 nm, respectively. OSC was quantified on the basis of external standards. OSC was obtained from analytical grade OSP (≥99% purity; Sequoia Research Product, Pangbourne, UK) by chemical hydrolysis at elevated pH. Samples from flasks containing water and sediments were extracted with ethanol, centrifuged at 5000 *g* for 10 min, redissolved in 50 mM monosodium phosphate and analysed as described above. Recoveries of OSC from water and water/sediment samples were 97.1 and 87.7%, respectively.

2.3 *Size of the bacteria population and relative abundance of viable bacteria*

The size of the bacteria population and relative abundance of viable bacteria of water samples were estimated by direct count. At different time intervals, 1-mL aliquots of water were fixed with 1 mL of phosphate-buffer saline (PBS) containing formaldehyde

(2% w/v), Tween 20 (0.5 v/v) and sodium pyrophosphate (0.1 M). After sonication, samples were treated with the fluorescent agent 4′6-diamino-2-phenylindole (1 μg mL^{-1}), filtered onto a 0.22 μm black polycarbonate filter, and cells enumerated using an epifluorescence microscope (DM LB30, Leica GmBH, Heideberg, Germany), as described in Barra Caracciolo *et al.* [12]. Cell viability was estimated following the method proposed by Haglund *et al.* (2003), using two different fluorescence dyes, SYBR Green II, and propidium iodide, as viability and membrane-compromised cell marker, respectively. Aliquots (1 mL) of water samples were incubated in the presence of SYBR Green II (1/10,000 dilution; Sigma-Aldrich, Germany) and propidium iodide (20 mM). After incubation, samples were filtered through a black polycarbonate filter (0.22 μm pore size) and viable bacteria enumerated by a direct count using the above-mentioned epifluorescence microscope.

2.4 *Metabolic potential of indigenous microorganisms*

The effects of OSC on the metabolic potential of microorganisms were assessed by measuring mineralization of the pesticide glyphosate and metolachlor in water samples of the CER irrigation canal. Samples of non-sterilized and sterilized water containing OSC (1.5 μg L^{-1}) were prepared as described above. Glyphosate and metolachlor were applied as water solutions using a mixture of unlabelled and ^{14}C-labelled compound in order to obtain a final concentration of 1 μg a.i. L^{-1}. Unlabelled glyphosate (purity > 99%) and ^{14}C-glyphosate (*N*-phosphonomethyl-2-14C-glycine; radiopurity > 99%, specific activity 5.4 mCi $mmol^{-1}$) were purchased from Sigma-Aldrich Italia (Milan, Italy). Unlabelled metolachlor (purity > 96%) and ^{14}C-metolachlor (2-chloro-*N*-(2-ethyl-6-methyl-[U-14C]phenyl)-*N*-(2-methoxy-1-methyl-ethyl)acetamide; radiopurity > 99%, specific activity 13 mCi $mmol^{-1}$) were donated by Syngenta Crop Protection AG (Basel, CH). Treated water samples were incubated at 20°C on an orbital shaker (125 rpm) in the dark. Metolachlor and glyphosate mineralization was monitored by trapping the evolved $^{14}CO_2$ in vials containing 4 mL of a 1 M NaOH solution. The NaOH solution was replaced at sampling, facilitating flask aeration. Aliquots (1 mL) of NaOH solution were mixed with 4 mL of HiSafe 3 liquid scintillation cocktail (PerkinElmer, Boston, MA) and radioactivity quantified using a Wallac 1490 liquid scintillation counter (Wallac Oy, Turku, Finland). Samples were kept in the dark for 12 h prior to analysis. Experiment was conducted in triplicate, and untreated samples (control) were included. Experiment was repeated with samples consisting of water/sediment mixture prepared as described above. Metabolic potential was expressed as the percentage of added glyphosate and metolachlor mineralized.

3. Results and discussion

3.1 *Degradation of OSC in water*

Degradation of OSC in water and water/sediment samples over the course of the 36-day incubation period is shown in figure 1. Degradation of OSC in water did not adequately fit the first-order model ($r^2 \leq 0.80$). After a rapid decrease, OSC concentrations slowly decreased during the remaining incubation period. Approximately 65% of the applied

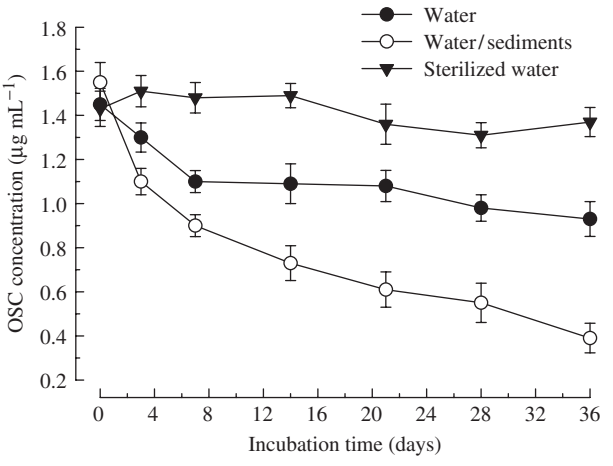

Figure 1. Degradation of oseltamivir carboxylate (OSC) in water and water/sediment samples. Bars represent standard deviations of the means.

amount dissipated in water samples within 36 days. These findings suggested that degradation of OSC in water is a complex process, not simply described by the linear model. OSC was less persistent in samples containing sediments (5% w/v). In contrast to water samples, the linear model gave a strong fit ($r^2 \geq 0.96$) to the degradation of OSC in water/sediment mixtures. The estimated half-life of OSC in the water/sediment microcosm was 21 days. Chemical analysis revealed that approximately 5% of the applied OSC was degraded within 36 days in sterilized water samples (figure 1). Similar values were observed in the sterilized water/sediment mixture (data not shown). This information provides supporting evidence that OSC degradation was mainly driven by microbial processes. Considering the size of the culturable bacteria population (7.9 ± 0.28 log CFUs g^{-1} air-dried sediments), the effect of sediments is consequently compatible with the increasing microbial abundance and metabolic potential of the microcosm. Enhanced biodegradation of xenobiotics in the presence of sediments has been reported for a number of compounds, including pesticides and antimicrobials [13–15]. According to Walker *et al.* [13], this effect could result from a greater number of microorganisms on the surface of sediment particles, an increased activity of microorganisms in the presence of sediments due to greater availability of nutrient, or an ability of sediments to concentrate chemical through sorption. Degradation of xenobiotics in the aquatic ecosystem depends on a variety of factors, including compound properties and environmental factors [16]. Under some circumstances, abiotic processes (i.e. hydrolysis and photolysis) can have an important role in the dissipation of pharmaceutical in water [17]. The investigation described here was conducted in the dark, and consequently it cannot be excluded that abiotic processes would have a greater importance in the dissipation of OSC under normal light conditions.

As discussed below, OSC determined a transient decrease in bacteria abundance. This is partly consistent with the observed patterns of OSC concentration in water samples, suggesting that other factors are involved in the degradation of OSC in surface water. Results discussed here indicate that OSC is moderately persistent in the water of

the CER irrigation canal. This appears to be mainly due to the reduced intensity of microbial degradation processes as further evidenced by results of the glyphosate and metolachlor mineralization experiment. The potential of a wide number of pharmaceuticals to slowly dissipate in surface water has been documented [18].

3.2 *Effects of OSC on bacterial abundance and metabolic activity of water microorganisms*

The size of the bacterial population of untreated water samples (control) remained approximately constant during the whole 36-day incubation period, except that a transient decrease was observed at the end of the second week of sample incubation. Addition of the antiviral drug OSC ($1.5\,\mu g\,mL^{-1}$) led to a significant decrease in the number of bacteria during the first half of the incubation period. In the remaining period, the number of bacteria remained significantly higher in samples treated with OSC than in the control. In both the treated and control water, the ratio of viable to dead bacteria decreased significantly over the incubation time. The highest decrease was observed in samples containing the antiviral drug (table 2).

Even though pharmaceuticals are specifically designed to perform some sort of biological effect, the direct and indirect effects of pharmaceuticals on non-target organisms have received little attention [10]. OSC is a selective inhibitor of influenza virus neuraminidase, an enzyme involved in the release of new virus particles from infected cells [11]. Based on its specific mode of action and in contrast to antimicrobials, which are active against bacteria, no direct toxic effect of OSC on water microorganisms would be expected. However, this does not exclude the possibility that OSC may have indirect effects on non-target microorganisms. This phenomenon has been evidenced for a wide number of compounds, including pharmaceuticals [1, 19, 20].

3.3 *Metabolic potential of water microorganisms*

General microbial activity was estimated from the mineralization of radiolabelled glyphosate and metolachlor. These two herbicides were chosen as models of chemicals

Table 2. Size of the bacteria population and relative abundance of viable bacteria of untreated water (control) and water samples treated with oseltamivir carboxylate (OSC) over the incubation period.[a]

	Size of bacteria population (log cell number mL^{-1})		Viable bacteria (% of total bacteria)	
Incubation time (days)	Control	OSC	Control	OSC
0	6.24* ± 5.64	6.12 ± 5.35	77.04 ± 15.01	66.31 ± 4.89
3	5.95 ± 4.28	5.22 ± 5.05	62.86 ± 6.30	54.45 ± 2.15
7	5.83 ± 4.92	5.37 ± 4.51	72.13 ± 3.92	53.81 ± 1.13
14	6.53 ± 5.46	5.78 ± 4.81	61.04 ± 7.88	50.46 ± 8.64
21	6.27 ± 4.93	6.92 ± 6.22	72.04 ± 15.00	63.31 ± 4.89
28	6.07 ± 5.28	6.42 ± 5.45	41.10 ± 18.85	20.73 ± 5.35
36	6.01 ± 4.97	6.55 ± 5.87	66.64 ± 9.37	51.77 ± 18.37

[a]Values are means of three replicates ± standard deviations.

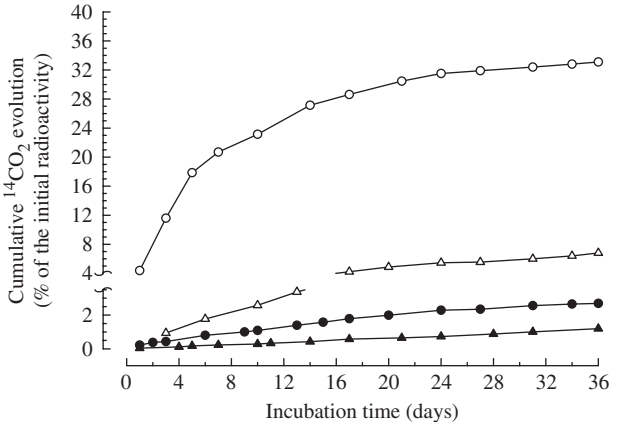

Figure 2. Glyphosate (circles) and metolachlor (triangles) mineralization in water (empty symbols) and water/sediment (full symbols) samples.

which are degraded by a wide number of microorganisms [21, 22]. Representative mineralization values of glyphosate and metolachlor, expressed as $^{14}CO_2$ evolution, in water and water/sediment samples are shown in figure 2. As expected, mineralization of glyphosate and metolachlor proceeded without a lag phase, thus confirming that these two chemicals are degraded by a variety of microorganisms and that microbial adaptation is not strictly necessary. During the 36-day incubation period, cumulative $^{14}CO_2$ evolution in water samples accounted for 2.9 and 0.9% of the total applied ^{14}C as glyphosate and metolachlor, respectively (table 3). Even though mineralization of glyphosate can vary among environmental samples, these values are considerably lower than those reported for soil ecosystems [22–24]. Addition of sediments resulted in an intense increase in glyphosate and metolachlor mineralization. At the end of the incubation period, mineralization of both glyphosate and metholachlor in samples containing 5% sediments was approximately 10 times higher than that observed in water samples. These findings confirmed the important role of microorganisms in glyphosate and metolachlor mineralization. Moreover, the lack of herbicide mineralization in sterilized water suggested that chemical degradation is not a major pathway of degradation of these two chemicals (table 3). Results from this mineralization experiment reinforced the concept that a major factor limiting a more rapid degradation of OSC in surface water of the CER irrigation canal is represented by the low metabolic potential of this ecosystem. Information concerning degradation and other environmental aspects of these two chemicals in surface water is scarce [21, 25]. Based on the results from a recent monitoring investigation conducted in the USA, Kolpin *et al*. [26] speculated that glyphosate would be much more persistent in surface waters than in soil.

The presence of a low concentration ($1.5\,\mu g\,mL^{-1}$) of the antiviral drug OSC did not reduce the potential of water from the irrigation canal to mineralize the two studied herbicides (table 3). In contrast to other pharmaceuticals, no data concerning environmental aspects of the antiviral drug OSC or other neuraminidase antivirals have been published. Considering that degradation of glyphosate and metolachlor is to some extent related to the size of indigenous bacteria, these findings suggest that the

Table 3. Effect of oseltamivir carboxylate (OSC) on $^{14}CO_2$ evolution from radiolabelled glyphosate and metolachlor within the 36-day incubation period.

	Cumulative $^{14}CO_2$ evolution			
	^{14}C-Glyphosate		^{14}C-Metolachlor	
	(% of initial radioactivity)			
	Control	OSC	Control	OSC
Non-sterilized water	2.85[a] ± 0.15	2.91 ± 0.22	0.85 ± 0.07	0.81 ± 0.09
Sterilized water	0.82 ± 0.06	0.88 ± 0.06	0.11 ± 0.02	0.09 ± 0.02
Water/sediment	38.22 ± 3.98	36.35 ± 4.11	9.91 ± 1.21	8.75 ± 1.39

[a]Values are means of three replicates ± standard deviations.

moderate persistence of OSC in water was mainly caused by the low metabolic potential of the water microbial community rather than indirect effects of OSC on microorganisms.

4. Conclusions

This preliminary study showed the important role played by microbial processes in the dissipation of the antiviral drug OSC in surface water. Even though a low amount of OSC was lost from surface water samples, the potential of OSC degradation was significantly greater in water samples containing sediments, which resulted in an increase in the size of the microbial community. Considering that the present experiment was conducted under laboratory conditions with a single OSC concentration value, additional dissipation studies would be necessary for a better understanding of dissipation of OSC in other environmental scenarios and to elucidate potential effects of this antiviral drug on the structure of the microbial community of surface water.

Acknowledgements

We greatly appreciate the scientific and technical support by Dr R. Piccaglia and Dr S. Grandi of the University of Bologna. Special thanks are given to Dr Maria Ludovica Saccà and to Dr Francesca Falconi for microbiological analysis.

References

[1] K. Kümmerer, A. Al-Ahmad, V. Mersch-Sundermann. *Chemosphere*, **40**, 701 (2000).
[2] P. Kay, P.A. Blackwell, A.B.A. Boxall. *Environ. Toxicol. Chem.*, **23**, 1136 (2004).
[3] M.W. Lam, C.J. Young, R.A. Brain, D.J. Johnson, M.A. Hanson, C.J. Wilson, S.M. Richards, K.R. Solomon, S.A. Mabury. *Environ. Toxicol. Chem.*, **23**, 1431 (2004).
[4] B. Gross, J. Montgomery-Brown, A. Naumann, M. Reinhard. *Environ. Toxicol. Chem.*, **23**, 2074 (2004).

[5] N.M. Ferguson, D.A.T. Cummings, S. Cauchemez, C. Fraser, S. Riley, A. Meeyai, S. Iamsirithaworn, D.S. Burke. *Nature*, **437**, 209 (2004).
[6] A. Moscona. *New Engl. J. Med.*, **29**, 1363 (2005).
[7] P. Ward, I. Small, J. Smith, P. Suter, R. Dutkowski. *J. Antimicrob. Chemother.*, **55**, 5 (2005).
[8] J.S. Oxford, R. Lambkin. *Int. J. Antimicrob. Agents*, **27**, 271 (2006).
[9] E.J. Eisenberg, A. Bidgood, K.C. Cundy. *Antimicrob. Ag. Chemother.*, **41**, 1949 (1997).
[10] C. Accinelli, M. Hashim, R. Epifani, R.J. Schneider, A. Vicari. *Chemosphere*, **63**, 1539 (2006).
[11] E.J. Eisenberg, K.C. Cundy. *J. Chromatogr.*, **716**, 267 (1998).
[12] A. Barra Caracciolo, P. Grenni, C. Cupo, S. Rossetti. *FEMS Microbiol. Lett.*, **253**, 55 (2005).
[13] W.W. Walker, C.R. Cripe, P.H. Pritchard, A.W. Bourquin. *Chemosphere*, **13**, 1283 (1984).
[14] P.H. Pitchard, C.R. Cripe, W.W. Walker, J.C. Spain, A.W. Bourquin. *Chemosphere*, **16**, 1509 (1987).
[15] Y.H. Kim, K. Pak, V. Pothuluri, C.E. Cerniglia. *FEMS Microbiol. Lett.*, **234**, 169 (2004).
[16] J. Lu, L. Wu, J. Newman, B. Faber, J. Gan. *J. Agric. Food Chem.*, **54**, 2658 (2006).
[17] B. Liu, L.L. McConnell, A. Torrents. *Chemosphere*, **44**, 1315 (2001).
[18] R. Alexy, T. Kümpel, K. Kümmerer. *Chemosphere*, **57**, 505 (2004).
[19] B. Engelen, K. Meinken, F. von Wintzingerode, H. Heuer, H.P. Malkomes, H. Backhaus. *Appl. Environ. Microbiol.*, **64**, 2814 (1998).
[20] M.D. Busse, A.W. Ratcliff, C.J. Shestak, R.F. Powers. *Soil Biol. Biochem.*, **33**, 1777 (2001).
[21] C. Accinelli, G. Dinelli, A. Vicari, P. Catizone. *Soil Biol. Soils*, **33**, 495 (2001).
[22] C. Accinelli, W.C. Koskinen, J.D. Seebinger, A. Vicari, M.J. Sadowsky. *J. Agric. Food Chem.*, **53**, 4110 (2005).
[23] Z.M. Getenga, F.O. Kengara. *Bull. Environ. Contam. Toxicol.*, **72**, 266 (2004).
[24] R. Strange-Hansen, P.E. Holm, O.S. Jacobsen, C.S. Jacobsen. *Pest Manag. Sci.*, **60**, 570 (2004).
[25] M.T. Tsui, L.M. Chu. *Chemosphere*, **52**, 1189 (2003).
[26] D.W. Kolpin, E.M. Thurman, E.A. Lee, M.M. Meyer, E.T. Furlong, S.T. Glassmeyer. *Sci. Total Environ.*, **354**, 191 (2006).