ARTICLE IN PRESS

Chemosphere xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

Contrasting effects of two antimicrobial agents (triclosan and triclocarban) on biomineralisation of an organophosphate pesticide in soils

R.S. Kookana [a,b,*], A. Ali [a,c], L. Smith [a], M. Arshad [a,c]

[a] CSIRO Land and Water, PMB 2, Glen Osmond 5064, Australia
[b] University of Adelaide, PMB 2, Glen Osmond 5064, Australia
[c] Institute of Soil and Environmental Sciences, University of Agriculture, Faisalabad, Pakistan

HIGHLIGHTS

- Cadusafos was mainly biomineralised and was little affected by soil type.
- Mineralisation of cadusafos decreased with increasing concentration of triclosan.
- Glucose mineralisation in soil was little affected by the presence of antimicrobial compounds.
- The effect of triclosan was greatest in an alkaline soil, most likely due to its ionisation state.
- Triclocarban had little effect on the cadusafos mineralisation, even at 450 mg kg^{-1} concentration in soil.

ARTICLE INFO

Article history:
Received 30 September 2013
Received in revised form 20 December 2013
Accepted 20 December 2013
Available online xxxx

Keywords:
Cadusafos
Biocides
Antimicrobials
Mineralisation
Biodegradation
Soil microbial respiration

ABSTRACT

We examined the impact of triclosan (TCS) and triclocarban (TCC) antimicrobial compounds on the biomineralisation of glucose and cadusafos pesticide in three Australian soils. Mineralisations of radiolabelled (^{14}C) compounds were measured over a period of up to 77 d in sterile and non-sterile soils treated with different concentrations of TCS and TCC (0–450 mg kg^{-1}). The rates of mineralisation of cadusafos were found to decrease with increasing concentration of TCS in all soils, but varied with soil type. Soils treated with TCS at the highest concentration (270 mg kg^{-1}) reduced cadusafos mineralisation by up to 58%. However, glucose mineralisation was not significantly affected by the presence of TCS. While TCS, significantly reduced the mineralisation of cadusafos (by 17%; $p < 0.05$) even at the lowest studied concentration (30 mg kg^{-1}), no significant effect of TCC was observed on cadusafos or glucose mineralisation even at the highest concentration used (450 mg kg^{-1}).

1. Introduction

Triclosan [5-chloro-2 (2,4-dichlorophenoxy)phenol] and triclocarban [3,4,4'-trichlorocarbanilide] are broad-spectrum antimicrobial compounds used in many contemporary consumer and professional health care products, such as hand soaps, shower gels, deodorant soaps, toothpastes, mouthwashes, deodorants, and surgical scrubs. (Jones et al., 2000; Schweizer, 2001). Their concentrations in personal care products are typically in the range of 0.1–0.3% (w/w) (Sabaliunas et al., 2003). Due to their broad spectrum biocidal activity and extensive usage, TCC and TCS are 'high production volume' chemicals in the USA (USEPA, 2002).

Triclosan is a persistent and bioaccumulative compound known for its high toxicity to algae, daphnids, fish, and other aquatic organisms (Orvos et al., 2002; Singer et al., 2002; Neumegen et al., 2005). Data from wastewater treatment plants (WWTPs) from several countries have shown ubiquitous presence of triclosan (TCS) in sewage effluents and biosolids (Ying and Kookana, 2007; Cha and Cupples, 2009). TCS and TCC have been detected in streams, seawater, sediments and soils (Kolpin et al., 2002; Singer et al., 2002; Halden and Paull, 2005; Ying and Kookana, 2007; Coogan et al., 2007; Cha and Cupples, 2009).

Wastewaters containing such biocides and other organic contaminants (e.g. pesticides, pharmaceuticals, steroid hormones, alkylphenols) are discharged into aquatic ecosystems. Similarly biosolids are applied on land potentially releasing such contaminants in the terrestrial environment. Due to their hydrophobic nature (Table 1), TCS and TCC accumulate in the sludge during the

* Corresponding author at: CSIRO Land and Water, PMB 2, Glen Osmond 5064, Australia. Tel.: +61 8 83038450.
E-mail address: Rai.Kookana@csiro.au (R.S. Kookana).

0045-6535/$ - see front matter © 2014 Published by Elsevier Ltd.
http://dx.doi.org/10.1016/j.chemosphere.2013.12.090

treatment process and consequently, dried sludge (biosolids) can have concentrations of TCS and TCC up to 30 and 55 mg kg^{-1} respectively (Halden and Paull, 2005; Heidler and Halden, 2007). Two Australian studies (Ying and Kookana, 2007; Langdon et al., 2011) have shown that TCS was present in all of the tested biosolids at concentrations ranging from <0.03 to 16.8 mg kg^{-1} on dry weight basis, with a mean concentrations ranging from 3.77 to 5.58 mg kg^{-1}. These values are similar to those reported previously in the literature (McAvoy et al., 2002; Cha and Cupples, 2009). Nearly 60% of the total amount of biosolids produced in USA (5.6 million dry tons) is applied on agricultural land (NRC, 2002). According to Heidler and Halden (2007) annual inputs of TCS to the terrestrial environments including agricultural soils in USA were in the order of 30–130 t yr^{-1}. Based on residue analysis on 110 archived biosolids in USA, McClellan and Halden (2009) reported that TCC and TCS were the most abundant compounds with mean concentrations of 36 ± 8 and 12.6 ± 3.8 mg kg^{-1}, respectively, accounting for nearly two thirds of the total mass of the 72 analytes tested. Concentrations as high as 55 mg kg^{-1} (Heidler and Halden, 2007) and 61 mg kg^{-1} (Xia et al., 2010) in sewage sludge have been reported.

Relatively high concentrations of TCS present in biosolids may lead to exposure of organisms in terrestrial ecosystems when biosolids are used as soil conditioners (Cha and Cupples, 2009). Due to their strong antimicrobial properties, the accumulation of TCS and TCC in soil and sediments could affect the soil microbial activity/processes and biodegradation of xenobiotics (Waller and Kookana, 2009; Svenningsen et al., 2011). Svenningsen et al. (2011) reported that presence of 4 mg kg^{-1} of triclosan resulted in a decrease in cultivable microbial populations by 22-fold while enriching triclosan-resistant *Pseudomonas strains*.

In biosolids or in sediments, trace organic contaminants coexist with biocides such as TCC and TCS. There is little information in literature on the impact of TCS and TCC on biodegradation of co-contaminants. We are aware of only two studies published recently (Shareef et al., 2009; Svenningsen et al., 2011) in which effect of triclosan on persistence of other xenobiotics was studied. These studies reported contradictory findings on the effect of triclosan. Shareef et al. (2009) did not see any inhibitory effect on biodegradation of a synthetic and a natural hormone (17 α ethinylestradiol and 17 β estradiol, respectively) even at very high TCS concentrations (up to 1000 mg kg^{-1}). However, Svenningsen et al. (2011) noted that the persistence of a linear alkylbenezene sulfonate (LAS) and ibuprofen increased with increasing concentration of TCS (0.16–100 mg kg^{-1}). In this study TCC was not included as a test compound.

The objective of this study was to assess the impact of two widely used antimicrobial compounds (TCC and TCS) on the biomineralisation of two model compounds, namely a readily assimilable substrate (^{14}C glucose as an indicator of overall microbial activity in soil) and an organophosphate pesticide (^{14}C cadusafos) in three Australian soils with contrasting properties. The studies compared directly the degradation of glucose and cadusafos with and without the addition of biocides on both sterilised and non-sterile soils. A comparative assessment of impact of two compounds on biomineralisation of the test compounds was made at several concentrations in soil. Deliberately high concentrations (up to an order of magnitude higher than reported in biosolids) of biocides were used to see at what concentration the antimicrobial effect, if any, may become significant.

Table 1
General properties of chemicals used in this study.

Property	Triclosan	Triclocarban	Cadusafos
CAS number	3380-34-35	101-20-2	95465-99-9
Molecular formula	$C_{12}H_7Cl_3O_2$	$C_{13}H_9Cl_3N_2O$	$C_{10}H_{23}O_2PS_2$
Water solubility (mg L^{-1} at 25 °C)	4.6	0.65	245 (at 20 °C)
pK_a	7.9	12.7	Not applicable
K_{ow}	50119	79433	70800
K_{oc} (for soil)	9200	54,800 (activated sludge)	227
Persistence in soil (DT_{50}) in days	18	Not available	38

2. Materials and methods

2.1. Chemicals

Cadusafos [$C_{10}H_{23}O_2PS_2$;S,S-di-sec-butyl O-ethyl phosphorodithioate] is an organophosphate insecticide/nematicide, extensively used worldwide against soil insects and nematodes in crops such as banana, citrus, maize, potato, tobacco, and tomato (Lamberti et al., 1998; Giannakou et al., 2005; Karpouzas and Singh, 2006). The use of cadusafos as a non-fumigant nematicide has increased in recent years because of the phasing out of other chemicals, e.g. methyl bromide (Dungan et al., 2003). Cadusafos was chosen as a test compound because of ready biodegradation by a taxonomically wide range of microorganisms (Karpouzas and Singh, 2006).

[S-isobuthyl-^{14}C] Cadusafos, (>95.0% purity), activity 9.250 MBq mL^{-1} (250 μCi) was supplied by Institute of Isotopes Co., Ltd., Budapest. D-[U-^{14}C] Glucose, (98% purity), activity 7.400 MBq mL^{-1} (200 μCi), was procured from Amersham Pharmacia Biotech UK Ltd. Scintillation liquid, OptiPhase 'HiSafe 3' was supplied by PerkinElmer Life and Analytical Sciences B.V., The Netherlands. Analytical grade samples on the two biocides triclosan (97% pure) and triclocarban (99% pure) were purchased from Sigma–Aldrich. Some relevant physico-chemical properties of the test compounds have been presented in Table 1.

2.2. Soils

Three agricultural soils were collected from different regions of South Australia. They were selected for a range of properties that are similar to other arid soils in Australia and overseas with low organic matter content and where biosolids are applied or wastewater/effluent is being used. The soils were air dried, (40 °C), ground and sieved to 2 mm prior to storage in air tight plastic containers. Selected physico-chemical characteristics of these soils are given in Table 2. The soils had a good representation of the variation in soil organic matter content (9–19 g kg^{-1}) and pH_w (1:5 soil to water ratio) values ranging from 5.8 (Freeling) to 9.0 (Avon) that are commonly found.

2.3. Mineralisation of ^{14}C-cadusafos and ^{14}C-glucose

A one kg sample of each of the prepared soils was weighed into 2 L plastic containers. Sterile deionised water was then added to obtain 45% maximum water holding capacity (MWHC) listed in Table 2. Soils were incubated in the dark at 28 ± 2 °C for 3 weeks to re-establish microbial populations prior to use in the incubation study, as recommended by OECD (2002). Soils were monitored on a regular basis to maintain the initial water content. After the 3 week equilibration period, an aliquot of 10 g air dried soil was weighed into plastic vials. The control samples were spiked with 50 μg of ^{14}C Cadusafos, without the addition of biocides. This application rate is equivalent to 5 mg kg^{-1} soil and equals approximately 4 kg of active ingredient per ha (assuming a soil incorporation depth of 10 cm and a bulk density 1.25 g cm^{-3}). This rate was