Chemosphere 77 (2009) 922–927

Contents lists available at ScienceDirect

Chemosphere

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

Toxicity assessment of sequential leachates of tire powder using a battery of toxicity tests and toxicity identification evaluations

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ARTICLE INFO

Article history:
Received 16 April 2009
Received in revised form 19 August 2009
Accepted 23 August 2009
Available online 15 September 2009

Keywords:
Tire wear
Sequential leaching
Daphnia magna
Ceriodaphnia dubia
Pseudokirchneriella subcapitata
Danio rerio

ABSTRACT

Approximately 460 000 ton of rubber are dispersed annually along the European roads due to tire wear. Tire rubber is known to leach compounds that are toxic to aquatic organisms. However, the potential effects of tire wear material on aquatic organisms at environmental relevant concentrations, and over time have so far not been extensively studied. In this study, rubber from three different tires was abraded and the powder leached in deionised water. The rubber powder was leached six times sequentially. All leachates were tested for toxicity using standardized toxicity tests including green algae (*Pseudokirchneriella subcapitata*, 72 h growth inhibition), crustaceans (*Daphnia magna*, 24 and 48 h immobility and *Ceriodaphnia dubia*, 48 h survival and 9 d reproduction and survival), and zebra fish eggs (*Danio rerio*, 48 h lethality). The reproduction of *C. dubia* was the most sensitive endpoint tested, with an EC50 of 0.013 g L^{-1} up to the third leaching of the most toxic tire, which is similar to a predicted concentration in road runoffs. The toxicity of all tires was reduced by the sequential leachings and after the sixth leaching the EC50s were >0.1 g L^{-1} for all endpoints. Toxicity identification evaluations indicated that the toxicity was caused by zinc and organic compounds.

1. Introduction

Due to increased regulations and more efficient techniques for pollution control in factories, most of today's pollution in western countries occurs during the use phase of products (Rydén et al., 2003). This causes a diffuse dispersion of pollutants, which to a large extent impedes the implementation of the European Water Framework Directive (WFD, 2000/60/EC), aiming to achieve good ecological status in all water bodies by 2015. About 10–20% of a tire's weight is being worn off during its use phase due to tire wear (Ahlbom and Duus, 1994). Based on the annual tire sale on the European market (ETRMA, 2007), this implies that approximately 460 000 ton of rubber is being dispersed along the European roads annually. Tire rubber is known to leach compounds that are toxic to aquatic organisms (Day et al., 1993; Evans, 1997; Gualtieri et al., 2005). In a previous study we determined 48-h EC50s for *Daphnia magna* exposed to tire wear leachates that were as low as 0.1 g L^{-1} (Wik and Dave, 2005). This EC50 is approximately a factor of 10 higher than the predicted environmental concentration that was calculated based on reports on a tire component found in environmental samples, which emphasizes the need for further risk assessment of tire wear (Wik and Dave, 2006). In one previous study tire pieces were found to leach toxic compounds still after 52 d of daily renewals of water (Goudey and Barton, 1992). To our knowledge, there are no reports on the long term leachability of toxic compounds from tire wear material. Therefore, the main objective of this study was to examine the toxicity of sequential leachates of tire wear material using a battery of test organisms. Furthermore, there are no reports on what types of toxic compounds that are present in leachates from sequential leachings of tire wear. Therefore, in this study we performed toxicity identification evaluations to characterise the toxic compounds in leachates from sequential leachings.

2. Materials and methods

2.1. Preparation of leachates and experimental setup

Three different tires (labelled "A", "B", and "C") were tested, and information on the tires is given in Table 1.

For each tire type there were two replicated tires. All tires had been used; tires "A" were worn only to some extent, whereas tires "B" and "C" were all heavily worn. Before testing, the tires were thoroughly washed using a brush and water, and finally rinsed with distilled water. Twelve grams of rubber was then abraded from each tire using a rasp. The same rasp and the same rasping technique were used for all tires, and the rasp was rinsed with

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doi:10.1016/j.chemosphere.2009.08.034

Table 1
Brand, type and manufacturing details on the tested tires.

Label in this study	Manufacturer	Tire	Country of manufacture	Date of manufacture (week/year)
A	Kumho	Marshal KR11	Korea	51/2003
B	Hankook	Zowac HPW 401	Korea	32/1999
C	Good Year	Eagle touring	France	2/1992

distilled water between tires. Tire powder from each tire was then placed at different quantities in four 2.5 L brown glass bottles with screw caps (10, 1, 0.1, and 0.01 g rubber in the four bottles, respectively), and 1 L milliQ water was added to each bottle. Each concentration series also included one bottle containing only milliQ water that was treated as the other concentrations during the whole test procedure and served as blanks in the toxicity tests. The six sequential leachings lasted for 5, 9, 20, 7, 5, and 11 d, respectively. During each leaching period, the bottles were shaken vigorously for 20 s, twice a day. Before toxicity tests were started, the leaching water was filtered through filter paper (Munktell®, Quality 3, Ø = 185 mm) to remove the rubber from the leachate. The leachate was collected in a 1 L Erlenmeyer flask, and the filter paper was allowed to dry until the next sequential leaching, when the rubber was rinsed from the filter paper using milliQ water and again transferred to the same glass bottle as was used before, and the volume was again adjusted to 1 L using milliQ water.

2.2. Toxicity tests

Sub samples of the filtered tire leachates from leachings 1, 2, 3, and 6 were instantaneously frozen (−18 °C) and sent on the same occasion to the Norwegian Institute for Water Research in Oslo for tests with algae (*Pseudokirchneriella subcapitata*) and zebra fish eggs (*Danio rerio*). Toxicity tests with daphnids were conducted directly after the test solutions had been prepared at the Department of Plant and Environmental Sciences at the University of Gothenburg (*Ceriodaphnia dubia* after leachings 1, 2, 3, and 6, and *D. magna* after all six leachings). Stock solutions of $CaCl_2{\cdot}2H_2O$, $MgSO_4{\cdot}7H_2O$, $NaHCO_3$, and KCl were added to the leachates to provide 50% synthetic water according to ISO (1996), before the *D. magna* and *C. dubia* tests were started. This 50% ISO water has a hardness of 125 mg L^{-1} (as $CaCO_3$) and a pH of ~7.5.

2.2.1. Tests with D. magna

Acute toxicity tests with *D. magna* were performed according to ISO (1996) but with 50% strength of standard dilution water (as explained above). Ten milliliter of each sample was pipetted to one well on a NUNC® 6 well plate and ten neonates (<24 h) of *D. magna* were added to each well. The temperature during testing was 20 ± 2 °C, and the photoperiod was 16 h light:8 h darkness. Immobility was recorded after 24- and 48-h exposures. These tests with *D. magna* also served as baseline tests for the toxicity identification evaluation (TIE) manipulations (see below). Tests were considered valid if the control immobility was <10% at the end of the test. A reference toxicity test with $K_2Cr_2O_7$ was conducted in parallel to the experimental tests.

2.2.2. Tests with C. dubia

For chronic tests with *C. dubia* we used the method developed by Mount and Norberg (1984) as modified and described by Unger and Ek (1994). Ten milliliter of each tire leachate was pipetted to six wells on a NUNC® 6 well plate. One neonate (3–24 h) was added to each well, so that 12 daphnids were exposed for each test concentration of each tire replicate. During testing, the plates were kept in a climate chamber holding a temperature of 25 °C and a 16 h:8 h light: dark cycle. The samples were stored dark and cooled (+4 °C) during the reproduction tests, and samples were renewed and food added (50 μL yeast-trout chow-Cerophyll + ~10^6 cells of the green algae *P. subcapitata*) on a daily basis (except for on day 8 when the samples were not renewed). Survival and number of live neonates per female were determined daily. Median effective concentrations (EC50s) were calculated for survival after 48 h and 9 d exposures and for reproduction (young female^{-1}) after 9 d exposure. Tests were considered valid if the control survival was >80% at the end of the test, and if the number of offspring born per live mother in the control was >15.

2.2.3. Tests with P. subcapitata

Stock solutions of nutrients were added to the leachates in accordance with ISO (2004). The samples were inoculated with 5×10^3cells mL^{-1} of *P. subcapitata* from an exponentially growing culture, maintained in the same growth medium. The samples were split in three replicates of 2 mL in glass vials. The vials were covered with a plastic film and incubated on a reciprocating shaking table under continuous illumination from cool-white fluorescent tubes providing an irradiance of 70 μM m^{-2} s^{-1}. The temperature was 21 ± 1 °C. After 72 h incubation, the cell density in the cultures was determined from measurement of chlorophyll fluorescence on a Millipore Cytofluor 2300 plate scanner. Fluorescence was measured at 685 nm with excitation wavelength 480 nm. Five micromolar 3(3,4-dichloro-phenyl)-1,1-diphenyl urea (DCMU) was added prior to the measurement to enhance the fluorescence signal as described by Kallqvist et al. (2008). The control cultures were also counted on a Coulter Multisizer M3 and a factor for conversion of fluorescence to cell density was obtained from the measurements in the controls. The specific growth rate (μ) of the algae was calculated as the logarithmic increase of cell density during 72 h according to:

$$\mu = \frac{\ln N_L - \ln N_0}{t_L - t_0}$$

where t_0 is the time of test start, t_L the end of the time interval, N_0 the initial cell density, and N_L is the cell density at t_L.

For each concentration series, the growth rate was calculated relative to the control values and plotted against the concentration. The growth rates in control cultures were between 1.6 and 1.9 d^{-1}, which is normal for exponential growth of *P. subcapitata* under the conditions applied.

2.2.4. Tests with zebra fish (D. rerio) eggs

All 1 and 10 g L^{-1} tire leachates were tested for acute toxicity with zebra fish eggs (*D. rerio*) according to ISO (2007). If no significant effect was observed at 1 g L^{-1}, no further testing of lower concentrations was performed. Approximately 15 newly laid eggs (1–3 h old) were added to about 30 mL of sample. One fertilised egg and 2 mL of sample was transferred to one well on a 24 well polystyrene micro plate. A minimum of 10 eggs were exposed for each sample. For each micro plate four wells were used as control using dilution water as defined in the ISO standard (2007). The eggs were inspected after 48 h and numbers of dead were determined according to the definitions in the standard.