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Persistence of the parabens in soil and their potential toxicity to earthworms

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ABSTRACT

Due to their antimicrobial activity, parabens are commonly used as preservatives in a variety of consumer goods including cosmetics, pharmaceuticals, and personal care products. During the production, usage and disposal of these products, parabens are released into the environment. In this study, the persistence of three widely used parabens; methyl-, propyl-, and butyl paraben in soil and their toxic effects on the soil invertebrate, *Eisenia fetida* was investigated. The results of this study indicate that selected parabens do not negatively affect the survival, growth, and reproduction of *Eisenia fetida* up to 1000 mg Kg^{-1} concentration. Further, these parabens (0–1000 mg Kg^{-1}) exhibited a low persistence in soil with more than 90 % disappearing within three days. In contrast, only 16–54 % degradation of parabens occurred in frozen soil suggesting a microbial role in parabens degradation. This study demonstrates that methyl-, propyl-, and butyl parabens degrade rapidly in the terrestrial environment and therefore, are unlikely to pose a threat to species such as *Eisenia fetida*. To our knowledge, this is the first report on the toxicity of parabens to earthworms.

1. Introduction

Annually, a large number of synthetic chemicals are released to the environment. These chemicals are also referred to as xenobiotics. Only a limited knowledge exists on the anthropogenic stresses caused by xenobiotics to the terrestrial ecosystem. Parabens are one type among the diverse group of xenobiotics released to the environment through personal care products. Parabens are alkyl esters of *p*-hydroxybenzoic acid which has a broad-spectrum antibacterial activity (Soni et al., 2005; Andersen, 2008). They are used as preservatives in many consumer products such as cosmetics, pharmaceuticals, and foods. Methylparaben, propylparaben, and butylparaben are few of the most widely used parabens. More often, different types of parabens are used in combination to obtain a synergistic effect. Methyl- and propylparaben is a popular combination used in many commercial products (Soni et al., 2005). These chemicals are released into the environment during their manufacturing, usage, and disposal. There is, therefore a high chance of these compounds entering the environment as a consequence of the use of products containing parabens.

Entry and accumulation of xenobiotics may lead to alterations in the edaphic environment. Parabens are considered to be readily biodegradable in the environmental matrices (Masden et al., 2001). Regardless of their rapid degradation in various environmental compartments, higher usage and subsequent release of these compounds to the environment has led them to be called “pseudo-persistent contaminants” (Albero et al., 2012). The occurrence and fate of parabens in the aquatic system have been thoroughly investigated (Canosa et al., 2006; Gasperi et al., 2014; Lee et al., 2005; Kasprzyk-Horden et al., 2009). However, the occurrence and effects of parabens in the soil are less studied (Pérez et al., 2012). Once in the environment, chemicals can enter resident organisms and exert harmful effects. Previous work has shown that these compounds are taken up by both animals and humans (Janjua et al., 2008; Kim et al., 2011; Ye et al., 2006; Wu et al., 2017). The toxic effects of parabens, especially their endocrine disrupting (Ramaswamy et al., 2011; Darbre, 2001) and oxidative stress generating (Samarasinghe et al., 2018) potential have been explored previously on humans and several vertebrate species. The occurrence and potential risk of parabens to marine biota have also been reported earlier (Xue and Kannan, 2016)

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and both propyl- and butyl- parabens have been shown to induce harmful effects in aquatic organisms (Dobbins et al., 2009).

To our knowledge, the degradation of methyl-, propyl- and butyl parabens in soils is less understood. Furthermore, the impact of parabens on soil invertebrates has not been reported previously. Soil is the ultimate reservoir for most of the xenobiotics that enter the environment. Chemicals that enter wastewater streams can even end up in the agricultural lands in the form of sewage sludge or biosolids. Earthworms constitute about 80 % of the total biomass in soil (Sinha et al., 2009). They play a crucial role in soil nutrient dynamics and help to improve the biological function of soil through the decomposition of organic matter. The bioavailable fractions of xenobiotics in the soil can enter these organisms either through direct contact or ingestion (Wijayawardena et al., 2017). Earthworms are very important in the terrestrial trophic system and food web (Butt and Grigoropoulou, 2010). They act as a vehicle to carry xenobiotics in the soil up to higher levels in the food chain through predator-prey interactions. *Eisenia fetida* is a useful sentinel soil invertebrate widely used to understand the toxicity of environmental pollutants.

Understanding the fate of parabens in the soil and their impact on earthworms is therefore needed to fully comprehend their environmental risk. Therefore, the aims of this study were; (i) to investigate the degradation of three types of commonly used parabens (methylparaben, propylparaben, butylparaben) in soil to determine their persistence in the terrestrial environment, and (ii) to evaluate the toxicity of these three parabens towards *Eisenia fetida* following acute and chronic exposures.

2. Materials and methods

2.1. Chemicals

All chemicals used in this study were of analytical grade. Methylparaben, propylparaben, and butylparaben were purchased from Sigma-Aldrich (Sigma Chemical Co., Sydney, Australia). Methanol (HPLC grade) was purchased from Thermofisher Scientific, Australia. Ultrapure water (Millipore-type, 18 MΩ/cm) was used in the experiments. Some chemical properties and the structures of the parabens used in this study are listed in Table 1.

2.2. Test soil

Uncontaminated soil for this study was collected from the top 0–15 cm of a field at a bush area in Canoelands (Canoelands, NSW 2157; S 33° 30.087′: E 151° 01.609′). Soils were air-dried, sieved through a 2 mm mesh and stored at room temperature until use. Physicochemical properties of the soil were: 72 % sand, 16 % silt, 11 % clay, 2.3 % total carbon and 5.5 of pH.

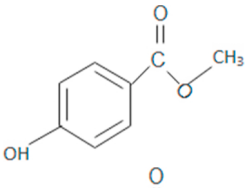
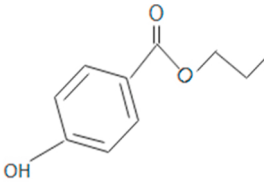
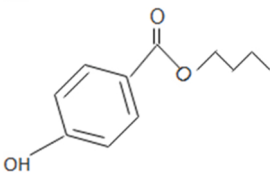
2.3. Earthworms

Healthy adult earthworms (*E. fetida*) used in the study were selected from a earthworm culture maintained in our laboratory at the University of Newcastle. Adult worms (250–300 mg) with a distinct clitellum were selected for the study. Earthworms were depurated for 24 h and washed before exposing either to paraben treated filter papers or soils. The laboratory conditions were: 20 °C ± 2 °C with a controlled light: dark cycle (16: 8 h) and light intensity (600–800 lux). These conditions were maintained throughout the study. Cow manure collected from a known organic source was used as the earthworm food. Cow manure was dried, finely ground, and rewetted before adding to the earthworm containers.

2.4. Acute toxicity to earthworms (Contact Assay)

Acute toxicity test was performed using the OECD standard protocol (OECD 207, 1984) to investigate the direct effects of parabens on the survival of *E. fetida*. The stock solution (1000 mg L^{-1}) of each paraben was prepared by dissolving 10 mg of each paraben in 10 mL of acetone and diluting to 6 working concentrations (0.1, 1, 10, 20, 50 and 100 mg L^{-1}). Five replicates were performed for each concentration. Flat bottom 60 mL glass vials (8cm × 3cm) lined with filter papers that do not overlap on sides were used as the test vessels. Before exposure, 1 mL of the desired concentration of test chemical dissolved in acetone was pipetted on to the filter paper in each vial, and the solvent was evaporated to dryness under a slow stream of compressed filtered air. Then 1 mL of ultrapure water was pipetted to moisten the filter paper. The control vial also received a 1 mL of acetone which was evaporated to dryness before moistening with ultrapure water. The exposure was limited to one worm per vial. The vials were sealed with parafilm®. Small ventilation holes were made in the parafilm® seal to ensure earthworms do not suffocate during the test period. Vials were

Table 1
Chemical properties and structures of the parabens studied.

Paraben	Molecular Formula	Molar Mass (g/ mol)	CAS number	Chemical Structure
Methylparaben	$C_8H_8O_3$	152.15	99-76-3	
Propylparaben	$C_{10}H_{12}O_3$	180.20	94-13-3	
Butylparaben	$C_{11}H_{14}O_3$	194.23	94.26.8	

horizontally placed in a large amber colour basket, and the assay was continued up to 72 h. After 24, 48 and 72 h, worms were assessed for mortality and pathological symptoms (open wounds, secretions, bleeding). The earthworms that fail to respond to a gentle stimulus in the front end were classified as 'dead'.

2.5. Earthworm reproduction assay

The chronic toxicity of parabens towards earthworms was assessed according to the standard OECD procedure (2004). In this test, earthworms were exposed to 0, 0.1, 1, 10, 100, 1000 mg Kg^{-1} concentrations of each paraben (methyl-, propyl-, butyl-) in soil for a period of 28 days and their cocoon formation and juvenile production were assessed. Individual parabens were spiked in soil using the following procedure: stock solutions were prepared in acetone. A 20 mL solution of acetone containing an appropriate dilution of parabens was added to 100 g soil, thoroughly mixed and solvent was evaporated in fume hood. This 100 g paraben treated soil was mixed with 1400 g of soil and thoroughly shaken for 5 h in an end-over-end shaker to make the soil homogeneous and then divided into three 500 g replicate samples (Mayilswami et al., 2016). For the negative control, soil received only solvent acetone and the same procedure was followed. Soil treated with 1000 mg kg^{-1} Zn ($ZnCl_2$) served as a positive control. Polypropylene containers with a wide mouth were filled with 500 g of the spiked soil with each test concentration and made up to 60 % of water holding capacity (WHC) by adding the deionized water.

The tests were performed with three replicates (soil containers) for each treatment and control, and each test container/ replicate obtained ten earthworms. Five grams of cow manure was added on the surface of the container before starting the experiment and after that once a week, until 28 days. Soil moisture was replenished as needed. The weights of the earthworms were recorded before and after the exposure. Worms were removed from the test containers, washed, and depurated for 24 h before measuring the weight. Earthworm weights before and after the experiments were used to assess the weight change during the parabens exposure.

Mortality of earthworms was assessed after 28 days. The number of cocoons were counted in each container. After counting, cocoons were returned to the respective container and the test continued for another 28 days. Food (5 g of cow dung) was added before starting the cocoon incubation period. After cocoon incubation period, the number of hatched juveniles were counted.

2.6. Instrumentation and method validation

Parabens analysis was performed with Agilent 1100 series High-Performance Liquid Chromatography (Agilent, Australia) equipped with a UV–vis detector set at the wavelength of 255 nm. Analysis was performed on an Agilent C_{18} column (4.6 × 150 mm; 3.5 μm) using methanol and water (70:30 v/v) as the mobile phase, in an isocratic mode, at a flow rate of 1 mL min^{-1} (injection volume 10 μL) (Mincea et al., 2009; Ebrahimpour et al., 2012). The calibration curves for all three parabens exhibited a good linear relationship over the concentration range of 100–500 μg L^{-1} with the correlation coefficient (r) of 0.9989. The limit of detection was 20 μg L^{-1} for methylparaben and 50 μg L^{-1} for both propyl- and butylparabens. The relative standard deviations were below 5.4 % (n = 3) for all the parabens.

2.7. Degradation of parabens in soil

A time-course biodegradation assay for parabens was conducted by spiking the soil samples with individual paraben at 10 mg kg^{-1} level. Also, parabens concentrations in the soils exposed to earthworms were measured at start (day 0) and completion (day 28) of the earthworm chronic assay.

2.7.1. Biodegradation assay

The biodegradation of methyl-, propyl-, and butyl parabens were studied for the selected concentration of 10 mg Kg^{-1}. Soils were spiked with 10 mg Kg^{-1} of individual parabens (as previously explained) and incubated under laboratory conditions (20 ± 2 °C; soil in field capacity moisture level; 16:8 h dark: light cycle). Initial paraben concentration was determined by immediate extraction of parabens from soil sampled at 0 time (same day as spiked; day 1). Then, soils spiked with individual parabens were sampled at 3, 5, 7, 14, and 28 days to establish the degradation pattern of each paraben. To evaluate the abiotic degradation of parabens in the soil, a separate set of soils were spiked and stored in the freezer at −20 °C (to minimize the biological activity) until the end of the experiment (28 days). Soils incubated under these frozen conditions were assumed to have nil or minimum biological activity (control) while the soil incubated under the normal conditions are referred to as biotic in this context.

The parabens in soil were extracted using ultrasound-assisted extraction (UAE). First, 10 mL of methanol was added to each tube and placed in an ultrasonic bath for 30 min (400 W) followed by shaking in the end-over-end rotator for 4 h and centrifugation for 20 min at 4000 rpm. The extraction was repeated with an additional 10 mL methanol, and the extracts were pooled and filtered using 0.22 μm PTFE syringe filter. Then the parabens were determined by HPLC. Each exposure concentration, including the controls, had three replicates.

2.7.2. Paraben concentration in spiked soil before and after earthworm chronic assay

Parabens were extracted from soils of all treatment groups before and after the exposure period (28 days) of earthworm chronic reproductive assay and analyzed by HPLC. Even though the paraben concentrations were measured in the soil after spiking, for simplicity, the concentrations referred in this context are nominal concentrations.

2.8. Statistical analysis

One-way analysis of variance (ANOVA) was performed using SPSS statistics software version 24 (IBM Corp, USA) to analyze survival and reproductive response. Results were expressed as means ± SE ($p \leq 0.05$). Where differences are found, a comparison between the control group and specific treatment concentrations were determined by Dunnett's test ($p < 0.05$).

3. Results

3.1. Acute toxicity tests – Filter paper contact test

No mortality of earthworms was observed during the filter paper contact test. All earthworms survived following 24, 48, and 72 h exposure to every concentration of all three parabens (methyl-, propyl-, and butyl paraben). All groups of paraben-exposed worms on filter paper did not exhibit any secretions or detectable pathological changes at any concentration.

3.2. Chronic exposure

None of the studied parabens were lethal to earthworms in soil at the selected concentrations (0.1–1000 mg Kg^{-1}). There were no significant differences in the weight of earthworms between the controls and paraben treatments after 28 days exposure to all three types of parabens (data not shown). Therefore, the earthworm survival and growth are not considered to be negatively affected by the paraben treatment. Reproduction of earthworms was not significantly affected due to the exposure of parabens (0.1–1000 mg Kg^{-1}) to earthworms (Fig. 1). The positive control used in this experiment (1000 mg Zn Kg^{-1}), showed a significant reproductive inhibition compared to the untreated group, which validated the experimental conditions.

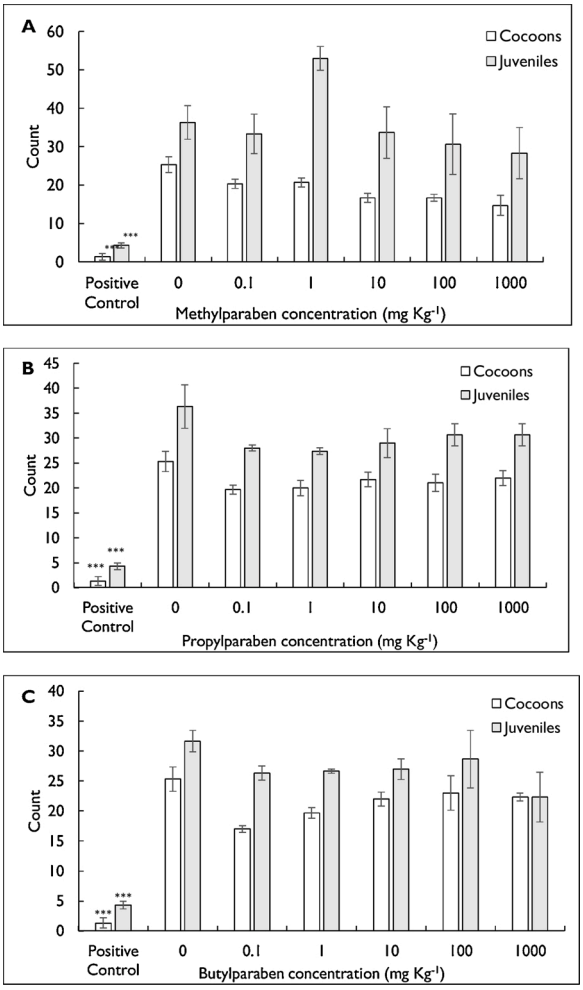


Fig. 1. Reproductive effects of *E. fetida* exposed to different concentrations of the parabens studied. (Mean ± SE; n = 3 independent samples). ***$p < 0.001$.

3.3. Degradation of parabens in soil

3.3.1. Biodegradation study

Soils spiked with 10 mg Kg^{-1} of individual parabens and incubated under the biotic conditions were analyzed for three parabens (methyl-, propyl-, and butyl-) at different time intervals (0, 3, 7, 14, and 28 days). Paraben concentration of soils under abiotic conditions (frozen) were determined only at the beginning and end of the experiment (0 and 28 day). Paraben degradation patterns of soils under both biotic and frozen conditions are shown in Fig. 2. Under biotic conditions, all three parabens showed an initial rapid rate of degradation (> 90 % within ~ 3 days). The degradation of methylparaben under biotic conditions was 96.6 % and 99.2 % at day 3 and 7, respectively. At the 14 day time point, no traces of methylparaben was detected in soil. The propylparaben concentration in biotic soil was 90.3 %, 98.3 %, and 98.6 % on 3rd, 7th and 14th days, respectively. The degradation pattern of butylparaben in soil was 92.5 %, 97.2 %, and 98.0 % on 3rd, 7th and 14th days. No detectable levels of propyl- and butylparabens were observed at the end of the 28 days.

Under frozen conditions (soil incubated at -20 °C), > 50 % of the parabens were detected after 28 days. The remaining paraben levels in these soils were 79 %, 54 % and 84 % for methyl, propyl and butyl paraben, respectively.

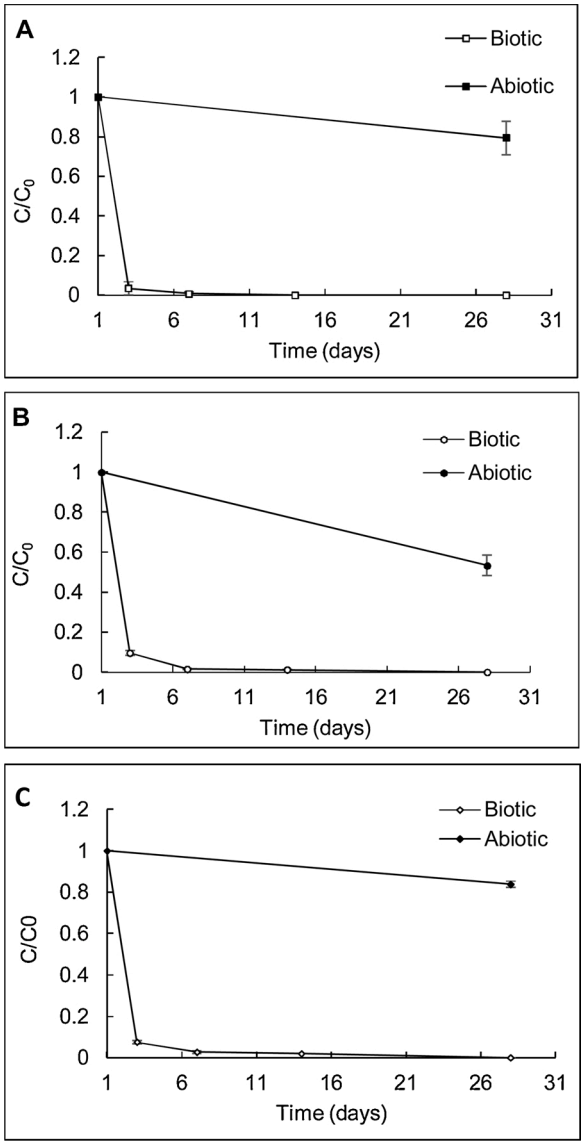


Fig. 2. Paraben degradation in abiotic and biotic soils along the incubation time in days: (A) Mehtylparaben; (B) Propylparaben; (C) Butylparaben. C = Concentration at time t; C_0= Initial Concentration; (Mean ± SE, n = 3 independent samples).

3.3.2. Paraben concentrations in spiked soil before and after earthworm chronic toxicity assay

No parabens were detected in the uncontaminated soil. The measured paraben concentration in spiked soils were within ± 20 % of nominal concentration, at the beginning of the earthworm chronic toxicity test (Table 2). At the end of the earthworm exposure period (after 28 days), no parabens were detected in soils spiked with 0.1, 1 and 10 mg Kg^{-1}. The measured concentrations were very low at 100 and 1000 mg Kg^{-1} at the end of the 28 days. The methylparaben levels in the soils with initial doses of 100 and 1000 mg Kg^{-1} were 0.36 and 4.1 mg Kg^{-1}, respectively. Only ≤ 1–2 % of 100 and 1000 mg Kg^{-1} of propylparaben and butylparaben were detected in the soil after 28 days.

4. Discussion

Xenobiotics may invoke acute as well as chronic deleterious effects on beneficial soil biota. Parabens are one group of xenobiotics with weak intrinsic estrogenic activity that attracted more attention due to their usage in many consumer products thereby leading to continuous release

Table 2
Measured concentrations of (A) Methylparaben; (B) Propylparaben and (C) Butylparaben in the soil before during the earthworm chronic assay.

Nominal Concentration (mg/Kg)	Measured Concentration (mg/Kg) Day 1-	Measured Concentration (mg/Kg) Day 28-	% degradation in 28 days
(A.) Methylparaben			
0 (Control soil)	0 ± 0	0 ± 0	–
0.1	0.096 ± 0.002	0 ± 0	100 %
1	0.88 ± 0.02	0 ± 0	100 %
10	11.89 ± 0.7	0 ± 0	100 %
100	118.7 ± 2.2	0.4 ± 0.04	99.9 %
1000	1199.4 ± 75.1	4.1 ± 0.4	99.7 %
(B.) Propylparaben			
0 (Control soil)	0 ± 0	0 ± 0	–
0.1	0 ± 0	0 ± 0	–
1	0.96 ± 0.01	0 ± 0	100 %
10	11.52 ± 0.6	0 ± 0	100 %
100	112.2 ± 1.4	0.4 ± 0.04	99.6 %
1000	1218.2 ± 85. 7	15.7 ± 0.3	98.7%
(C.) Butylparaben			
0 (Control soil)	0 ± 0	0 ± 0	–
0.1	0.12 ± 0.01	0 ± 0	100 %
1	1.1 ± 0.09	0 ± 0	100 %
10	12.6 ± 0.19	0 ± 0	100 %
100	108.0 ± 2.1	0.7 ± 0.005	99.4 %
1000	1228.0 ± 20.1	13.0 ± 0.02	98.9 %

into the environment (Okubo et al., 2001). Although considerable literature exists on the toxicity of parabens on aquatic organisms, studies on terrestrial organisms are scarce. Therefore, the present study was conducted to generate new information on the persistence of parabens in soil and their effects on the earthworms.

The environmental concentrations of these compounds in the soil are not well understood. Parabens have been identified in the soil at the concentrations of 127, 5 and 23 ng g^{-1} d.w. for methyl-, propyl-, and butyl paraben, respectively (Viglino et al., 2011). A few studies conducted in Spain support these findings (Núñez et al., 2008; Pérez et al., 2012). Wang and Kannan (2016) reported that the environmental release of parabens and their metabolites was 4.85–6.16 and 1270–2050 mg/ day/1000 people, respectively, as estimated from two wastewater treatments plants in the Albany area of New York State (USA). A recent study revealed the occurrence of parabens and metabolites in animal tissues at various levels of the food chain: fish, birds, and bears (Xue and Kannan, 2016). In their study, methylparaben was found to be the most abundant with up to 690 ng g^{-1}, wet weight in fish tissues and 8 – 657 ng g^{-1}, wet weight in birds. The major metabolite found at relatively high concentrations in the tissues analyzed was 4-hydroxybenzoate (up to 68, 600 ng g^{-1}, wet weight). The concentrations used in the acute assay were not sufficient to derive the LC_{50} value; a further unrealistic concentration of 1000 mg Kg^{-1} was also used in the chronic test to determine a safe limit of parabens.

Although short-term toxicity tests provide valuable data for deriving threshold values for the chemical risk assessments, reproduction is considered to be a more sensitive indicator than mortality or growth parameters. Previous studies in the literature have reported the potential of parabens to exert harmful effects on the reproduction of different organisms. Dobbins et al. (2009) demonstrated the low-level estrogenic potential of parabens using *Daphnia magna* (invertebrate) and *Pimephales promelas* (fish) as model organisms with adverse effects on growth and reproduction as endpoints. The involvement of propyl- and butyl paraben on inhibition of spermatogenesis in rats was also evident without any significant change in the body weight (Oishi, 2002). Beside other reported effects on the reproduction of different species by parabens, there are no previous reports on the reproductive toxicity of these compounds on earthworms. In this study, we did not observe any adverse effects on the reproduction of the earthworms up to the maximum exposure of 1000 mg Kg^{-1} concentration. In. contrast, the positive control (1000 mg Zn Kg^{-1}) exhibited significant toxicity to the earthworms as expected thereby confirming the validity of the test. The observed lack of effect on reproduction could be explained by the rapid degradation of these compounds in the soil within a short period of time. Therefore, as a consequence of their high biodegradability, all tested concentrations of methyl- propyl-, and butyl- parabens did not seem to affect the life cycle of earthworms in the soil. Further, there was no significant effect on the survival and growth of earthworms upon acute and chronic exposure to methyl-, propyl- and butyl parabens. Apparently, as shown in the degradation experiment in this study, >90 % of parabens degrade in soil within 3 days. Measuring the concentration of parabens at the end of the study also showed that even very high concentrations of these compounds (1000 mg Kg^{-1}) are not retained in soil for any more than 28 days. We did not assess the metabolites, if any, formed during parabens degradation in this study. However, lack of chronic toxicity of parabens to earthworms shows that even putative metabolites are not toxic to earthworms. These results demonstrate that even though parabens are continuously released into the environment, they are not likely to pose a risk to earthworms. However, the metabolomics analysis would have provided a better understanding of the sub-lethal molecular responses towards parabens in earthworms. Hence, a further assessment on the metabolomic profile post exposure to parabens is suggested for future studies.

The degradation study demonstrated that a comparatively high amount of parabens persisted in soils maintained at frozen conditions for 28 days. Retention of propylparaben (>50 %) was less than the other two (methyl- and butylparaben), which was >79 % in this soil. Contrary to this, all three parabens showed a faster degradation in the biotic soil, where >97 % disappeared within 3 days for methylparaben and within 7 days for propyl- and butylparabens. This demonstrates the influence of biotic factors in parabens degradation in soil. In agreement with our findings, González-Maríño et al. (2011) showed that these three parabens rapidly degrade under biotic conditions. They studied the degradability of parabens in activated sewage sludge and found that methylparaben degrades faster than the other two and > 99 % of all three parabens degrade in less than 5 days. Hurtado et al. (2017) also showed that another structurally similar paraben compound, ethylparaben degrades faster with more than 90 % degradation within the first few days in non-sterilized soil, with a reported half-life of 3.7 days. Most of the previous studies have first sterilised (autoclaved) the soil and then spiked with the chemical before incubation. In our study, we spiked the soil with 10 mg Kg^{-1} concentration of each paraben and incubated under frozen conditions to minimize any microbial activity as well as changes in soil properties. However, in contrast to these findings, more than 50 % of the parent molecules were present in the soil under abiotic conditions in our study. Another study by Camino-Sánchez et al. (2016) reported that these three parabens have half-life of less than 7 days in non-sterile soil. Using PBT (Persistence, Bioaccumulation, and Toxicity) profiler and other hazard screening tools, Bazin et al. (2010) have predicted the persistence of all three types of parabens as 15 days, which is in agreement with our findings.

To the best of our knowledge, this is the first study on the risk assessment of parabens to earthworms in the soil. This study suggests parabens are unlikely to pose a risk to the earthworms at environmental concentrations.

5. Conclusion

This study demonstrates that methyl-, propyl-, and butyl parabens are unlikely to cause measurable acute or chronic toxic effects to *E.fetida*. The degradation of these parabens is minimal in soils under frozen conditions. However, methyl-, propyl-, and butyl parabens degrade rapidly under biotic conditions.

CRediT authorship contribution statement

Samarasinghe Vidane Arachchige Chamila Samarasinghe: Investigation, Methodology, Data curation, Validation, Writing - original draft. **Kannan Krishnan:** Writing - review & editing. **Robert John Aitken:** Investigation, Writing - review & editing. **Ravi Naidu:** Investigation, Writing - review & editing. **Mallavarapu Megharaj:** Conceptualization, Supervision, Investigation, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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