Potential biochemical and genetic toxicity of triclosan as an emerging pollutant on earthworms (Eisenia fetida)

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ABSTRACT

Triclosan as an important antimicrobial agent is increasingly detected in the terrestrial environment as sewage sludge and reclaimed water are applied on land, but little is known about its effect on non-target soil organisms. In this study, biochemical responses including changes in the activity of catalase (CAT), superoxide dismutase (SOD), glutathione-S-transferase (GST), and malondialdehyde (MDA) of the earthworm Eisenia fetida were examined in order to assess ecological toxicity of the chemical. The single-cell gel electrophoresis (SCGE) was also used to measure the potential genotoxicity of the chemical. The results showed that the activity of CAT and GST at the highest tested dose could be stimulated after a 2-d exposure, reaching 148% and 123% of that in the control, respectively. However, with prolonged exposure, the activity of CAT and GST at the highest tested dose was inhibited, falling to 47% and 33% of that in the control, respectively. Triclosan induced an increase in the activity of SOD, but no significant changes were observed. The content of MDA was dependent on the dose of triclosan and on the exposure duration. The comet assay demonstrated that triclosan treatments led to a dose-dependent DNA damage of E. fetida after exposures of 7 and 14 d. Our findings can suggest that triclosan has sublethal effects on E. fetida.

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1. Introduction

Pharmaceuticals and personal care products (PPCPs) represent an important type of emerging environmental organic pollutants extensively used in human and veterinary medicine (Kolpin et al., 2002). Although it was designed for specific targets, as introduction into the environment, they may act on non-target species that are consequently disposed of down residential drains later. These include manufactured toothpaste, footwear, shampoo, toilet and hospital hand soap, and medicated cosmetics (Reiss et al., 2009). Triclosan has a moderate water solubility of 12 mg L⁻¹ (Reiss et al., 2002). It is a lipophilic compound (log K ow = 4.8), and relatively persistent in the environment with a half-life of at least 11 d in river water (Bester, 2005). Ying et al. (2007) investigated the biodegradation of triclosan in soils and found that it was degraded in the aerobic soil with a half-life of 18 d, but persisted in the anaerobic soil and sterile aerobic conditions. Triclosan may enter the terrestrial environment through agricultural application of sewage sludge and has been frequently detected in agricultural soils amended by biosolids (Cha and Cupples, 2009). Heidler and Halden (2007) reported that the range of triclosan was wide, from 20 to 55 mg kg⁻¹ in sewage sludge. Therefore, it can pose a toxicological risk to aquatic and terrestrial organisms.

Some studies have assessed the ecotoxicology of triclosan (Orvos et al., 2002; Tatarazako et al., 2004; Ciniglia et al., 2005; Coogan et al., 2007; An et al., 2009). Liu et al. (2009) studied the phytotoxicity of triclosan and found that it inhibited the growth of some plants, with the median effect concentrations (EC₅₀) from 57 mg kg⁻¹ to 108 mg kg⁻¹ in soil. Waller and Kookana (2009) demonstrated that triclosan at doses below 10 mg kg⁻¹ can disturb the nitrogen cycle in soils. Binelli et al. (2009b) observed that triclosan had genotoxic and cytotoxic effect on Zebra mussel hemocytes, connected with both oxidative stress and DNA damage. A study was also carried out on enzyme activities in Mytilus galloprovincialis induced by triclosan. The results indicated that triclosan inhibited the activity of catalase (CAT), involved in the defence against free radicals, and stimulated the activity of glutathione-S-transferase (GST), involved in xenobiotic metabolisms (Canesi

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et al., 2007). Amorim et al. (2010) obtained idiographic data on the effects of triclosan in the terrestrial environment based on a battery of bioassays of soil species. They reported that the EC50 values of triclosan for survival (acute test) and reproduction (chronic test) of *E. andrei* were 866 and 3.8 mg kg⁻¹ soil dry weight, respectively. Reiss et al. (2009) reported that LC50 of triclosan toxic to earthworms (*Eisenia fetida*) after a 14-d exposure was >1026 mg kg⁻¹ soil dry weight, and suggested that the no-observed-effect concentration (NOEC) was at least this level. However, data about the biochemical response and genotoxicity to earthworms is very limited or unavailable. Therefore, additional studies must be performed on triclosan toxicity.

It has been reported that reactive oxygen species (ROS) can be generated in living organisms exposed to environmental contaminants. The increased level of ROS can produce oxidative damage to macromolecules such as proteins, nucleic acids and lipids, finally leading to the damage of different cellular organelles (Sabatini et al., 2009). In living organisms there are protective enzymatic mechanisms and non-enzymatic mechanisms to scavenge ROS and alleviate their deleterious effects. The antioxidant enzymes include superoxide dismutase (SOD), CAT and enzymes involved in the phase II metabolism such as GST. SOD dismutates O₂⁻ to H₂O, and this is decomposed to H₂O by CAT, so that the accumulation of O₂⁻ to H₂O₂ is effectively prevented (Liu et al., 2002). GST involved in xenobiotic metabolisms in organisms is an important detoxification enzyme. The excess active oxygen species and peroxide can be cleared and lipid peroxidation can be prevented by those enzymes working together (Van Assche and Clijsters, 1990). Malondialdehyde (MDA), an indicator of lipid peroxidation contents, has been proven to be a sensitive tool in determining potential genotoxicity of soil pollutants (Van Assche and Clijsters, 1990). All exposures were conducted at 20 ± 1 °C in the dark. Three exposure periods were tested: 2, 7 and 14 d and for each duration or dose, two earthworms, one each for the enzymes and the comet assay, were collected from each replicate at the three durations. There was no mortality observed during the experimental progress.

2. Materials and methods

2.1. Chemicals and reagents

Triclosan (CAS Nos. 3380-34-5, purity > 97%), 1-chloro-2,4-dinitrobenzene and thiobarbituric acid were purchased from the Sigma Company in USA. Nitro blue tetrazolium, l-methionine, ascorbic acid, and trichloroacetic acid were purchased from the Acros Company in USA. All other reagents used in the following tests were purchased from the Jindong Tianzheng Chemical Co. Ltd. (Tianjin, China) and they were of analytical grade with the chemical purity of 97–99%.

2.2. Earthworms and exposure conditions

The earthworm (*E. fetida*) species was selected as a test organism. After having purchased from an earthworm culturing farm in Tianjin, China, the worms were bred with cattle feces for 2 weeks in our laboratory at 20 ± 1 °C and the moisture content was adjusted to 50%. Twenty-four hours prior to use, the earthworms were removed from the cultures, rinsed in distilled water and on clean damp filter paper kept in the dark at 20 ± 1 °C to allow voiding of gut contents (Xue et al., 2009). Fully citellate adults weighing 0.3–0.6 g (live weight) were selected for all experiments.

A surface (5–20 cm) coastal alkali-saline soil was collected from an agricultural field in Tianjin, China. For more than 3 y, sewage sludge or wastewater had not been applied to the agricultural field. The soil had an organic matter content of 22.34 ± 0.51 g kg⁻¹ and a pH of 8.10 ± 0.03. Prior to spiking, the soil was partially air-dried, passed through a 2-mm sieve before use.

Appropriate amounts of triclosan dissolved in acetone were spiked into air-dried soil. Briefly, an initial 50 g of soil was placed in a plastic pot (1000 mL with a diameter of 105 mm), the spike added and blended for 1 min. After acetone evaporated in the fume hood under darkness, the remaining soil (450 g) was added in three equal portions, with blending performed for 3–5 min following each addition, to achieve the following nominal concentrations: 0 (solvent control), 1, 10, 50, 100, and 300 mg kg⁻¹ soil. The moisture content was adjusted to 35% of the final weight. All soil samples were placed in pre-cleaned glass jars and stored in darkness overnight before being used for earthworm toxicity testing. Ten mature worms were added in each soil samples. Each treatment had triplicates. All exposures were conducted at 20 ± 1 °C in the dark. Three exposure periods were tested: 2, 7 and 14 d and for each duration or dose, two earthworms, one each for the enzymes and the comet assay, were collected from each replicate at the three durations. There was no mortality observed during the experimental progress.

2.3. Preparation of earthworm extracts

All procedures were carried out at 4 °C. Earthworms were placed into a perchedil mortar and pestled under ice-cold conditions in 50 mM Tris–HCl buffer (1:9, w/v, pH 7.5) containing 250 mM sucrose, and 1 mM EDTA. The homogenate was centrifuged at 9000 rpm for 30 min to yield the postmitochondrial fraction. The supernatant was used for the analysis of enzymes and protein determination.

2.4. Biochemical assays

The protein concentration in the supernatant was determined by the dye-binding method according to Bradford (1976) using bovine serum albumin as standard. The activity of SOD was determined by measuring its ability to inhibit the photochemical reduction of nitro blue tetrazolium as described by Dhindsa et al. (1980). One unit (U) of the SOD activity was defined as the amount of the enzyme that caused 50% inhibition of the photoreduction rate of nitro blue tetrazolium, and the result was expressed as U mg⁻¹ of fresh mass (FM). The activity of CAT and GST was assayed immediately after centrifugation of the homogenates according to the method of Saint-Denis et al. (1998). One unit of the CAT activity was defined as the enzyme quantity required to consume half of H₂O₂ in 100 s at 25 °C. The activity of GST was measured using 1-chloro-2,4-dinitrobenzene as its substrate. A unit of the GST activity is defined as the amount of the enzyme catalyzing the formation of 1 μmol of the product per min under the conditions of the specific assay. The specific activity is defined as the units of the enzyme activity per mg of protein. The content of MDA was estimated by formation of thiobarbituric acid reactive substances according to the method described by Livingstone et al. (1990). All measurements were made using a dual beam temperature-controlled ultraviolet absorption.

2.5. Comet assay

After exposure to the pollutant at various levels, earthworm coelomocytes were obtained using the non-invasive extrusion
method described by Eyambe et al. (1991) with a slight modification. Briefly, individual earthworms were rinsed in chilled extraction medium (5% ethanol, 95% saline, 2.5 mg mL\(^{-1}\) EDTA, and 10 mg mL\(^{-1}\) guaiacol glyceryl ether, pH 7.3) for 3 min. Coelomocytes were spontaneously secreted in the medium, and then the extraction medium were centrifuged (4\(^\circ\)C, 9000 rpm, 10 min) and the supernatant was removed. Coelomocytes were washed three times in phosphate-buffered saline and the final cell density was adjusted to about 1 \(\times\) 10\(^{5}\)–1 \(\times\) 10\(^{6}\) cells mL\(^{-1}\) with phosphate-buffered saline. Viability of the cells obtained by the trypan blue exclusion method was in the range of 90–98% for all groups.

The alkaline comet assay was performed as described originally by Singh et al. (1988) with a slight modification. All steps were conducted under dim yellow light and performed at 4\(^\circ\)C to prevent additional DNA damage. Fifty micro-liter of the cell suspension was mixed with 50 \(\mu\)L of 1.0% (w/v in phosphate-buffered saline) low melting agar at 37 \(^\circ\)C and pipette onto normal microscope slides precoated with a layer of 100 \(\mu\)L of 0.6% (w/v in phosphate-buffered saline) normal melting agar. After solidification at 4\(^\circ\)C for 15 min, cover slips were removed and another layer of 85 \(\mu\)L low melting agar was added, slides were left at 4\(^\circ\)C again for 15 min to solidify and the cover slips were removed. Then slides were immersed into a lysis solution for 2 h (4\(^\circ\)C, 2.5 M NaCl, 10 mM Tris, 100 mM Na\(_2\)EDTA, 1% Na-sarcosinate, supplemented with 10% dimethyl sulfoxide and 1% Triton X-100 just before use, pH 10). Slides were then placed in an electrophoresis tank containing 300 mM NaOH with 1 mM Na\(_2\)EDTA (pH > 13) for 30 min at 4 \(^\circ\)C prior to electrophoresis in the same buffer for 20 min at 150 mA, 15 V (1 V cm\(^{-1}\)). The slides were then neutralized (0.4 M Tris, pH 7.5) thrice at 5 min intervals and fixed with absolute ethanol for 10 min, dried at room temperature and kept under refrigeration until analyses.

Slides stained with 40 \(\mu\)L ethidium bromide (20 \(\mu\)g mL\(^{-1}\)) and viewed using a fluorescence microscope (Zeiss, Axio Imager Z1) equipped with a CCD camera. For each parallel set of slides, 50 randomly select and non-overlapping cells were captured at 400\(^\times\) magnification and a total of 100 cells were scored for each sample. The captured images were analyzed using CASP (Kočka et al., 2003).

2.6. Statistical analysis

Each treatment was analyzed with three replicates. All the values are presented as mean ± standard deviation (SD). Parametric tests were preceded by tests for normal distribution and for homogeneity of variances. Comet assay data were found to be non-normally distributed. The log 10-transformed the ratio between migration length and diameter of the comet head (LDR) values were used to normalize the variance of the comet test results. Differences in enzyme activities (CAT, GST, SOD and MDA) were made by analysis of variance (ANOVA). In addition, post hoc comparison (LSD test) was carried out to check for differences between the exposed and control groups. All statistics were performed using the SPSS software (SPSS 13.0).

3. Results

3.1. Biochemical assays

The possible biochemical effects of triclosan on the activity of various enzymes in E. fetida were evaluated and the relevant results were listed in Table 1. As shown in Table 1, the activity of CAT at the highest tested dose was significantly (ANOVA, \(F = 9.60, p < 0.05\)) enhanced as the concentration of triclosan increased after a 2-d exposure, reaching about 148% of that in the control. After a 7-d exposure, the activity returned to the level in the control, and there were no significant (ANOVA, \(F = 1.77, p > 0.05\)) changes observed at all doses. Finally, after a 14-d exposure, the activity of CAT was inhibited and decreased. This decrease was significant (\(p < 0.05\)), up to 39% and 47% with respect to the controls at the dose of 50 mg kg\(^{-1}\) and 300 mg kg\(^{-1}\), respectively. The statistical analysis confirmed this result (Table 2).

After a 2-d exposure, triclosan induced an increase in the activity of SOD, but there were no significant effects observed at all doses of triclosan. After a 7- and 14-d exposure, there were no regular changes in the activity of SOD at the tested doses (Tables 1 and 2).

The activity of GST in the earthworms is also shown in Table 1. After a 2-d exposure, triclosan also stimulated the activity of GST, which is involved in xenobiotic metabolisms. However, after a 7-d exposure, there were no significant (ANOVA, \(F = 0.732, p > 0.05\)) changes observed in the activity of GST between the controls and triclosan treatments. On the contrary, at the longer exposure time (after 14 d), triclosan inhibited significantly (ANOVA, \(F = 6.46, p < 0.05\)) the activity of GST, and the activity of GST at 100 mg kg\(^{-1}\) and 300 mg kg\(^{-1}\) fell by 24% and 33% of that in the control, respectively. This inhibition of the GST activity may be attributed to the toxic stress.

The content of MDA increased obviously by triclosan at the concentration between 50 mg kg\(^{-1}\) and 300 mg kg\(^{-1}\) after a 7-d exposure and at the dose of 100 mg kg\(^{-1}\) and 300 mg kg\(^{-1}\) after a 14-d exposure (Table 1). The univariate analyses (ANOVA) revealed that there were significant influences of duration (\(p < 0.001\)) and concentrations (\(p < 0.001\)) on the content of MDA (Table 2).

### Table 1

<table>
<thead>
<tr>
<th>Biochemical measurement</th>
<th>Duration of exposure (d)</th>
<th>Dose of TCS (mg kg(^{-1}) soil)</th>
<th>0</th>
<th>1</th>
<th>10</th>
<th>50</th>
<th>100</th>
<th>300</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAT activity (U mg(^{-1}) protein)</td>
<td>2</td>
<td>5.50 ± 0.62</td>
<td>4.86 ± 0.40</td>
<td>5.35 ± 0.79</td>
<td>6.99 ± 0.25</td>
<td>7.68 ± 0.67 (^*)</td>
<td>8.13 ± 0.79 (^*)</td>
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</tr>
<tr>
<td></td>
<td>7</td>
<td>5.18 ± 0.55</td>
<td>5.22 ± 1.00</td>
<td>4.30 ± 0.29</td>
<td>5.20 ± 0.22</td>
<td>5.98 ± 0.67</td>
<td>5.49 ± 0.31</td>
<td></td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>6.71 ± 0.38</td>
<td>6.59 ± 0.45</td>
<td>5.87 ± 0.39</td>
<td>4.09 ± 0.62 (^*)</td>
<td>4.41 ± 0.29 (^*)</td>
<td>3.65 ± 0.54 (^*)</td>
<td></td>
</tr>
<tr>
<td>GST activity (U mg(^{-1}) protein)</td>
<td>2</td>
<td>54.07 ± 7.42</td>
<td>50.89 ± 4.22</td>
<td>60.62 ± 5.91</td>
<td>58.51 ± 9.50</td>
<td>62.24 ± 7.18</td>
<td>66.32 ± 2.97</td>
<td></td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>51.35 ± 3.83</td>
<td>52.60 ± 6.58</td>
<td>49.50 ± 5.81</td>
<td>46.23 ± 6.36</td>
<td>44.98 ± 6.51</td>
<td>54.73 ± 6.91</td>
<td></td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>57.58 ± 6.34</td>
<td>58.54 ± 6.29</td>
<td>59.04 ± 5.00</td>
<td>62.53 ± 5.44</td>
<td>43.82 ± 1.13 (^*)</td>
<td>38.47 ± 1.72 (^*)</td>
<td></td>
</tr>
<tr>
<td>SOD activity (U mg(^{-1}) protein)</td>
<td>2</td>
<td>1.05 ± 0.13</td>
<td>0.92 ± 0.11</td>
<td>1.20 ± 0.12</td>
<td>1.47 ± 0.30 (^*)</td>
<td>1.36 ± 0.27</td>
<td>1.41 ± 0.23</td>
<td></td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>1.03 ± 0.07</td>
<td>0.84 ± 0.13</td>
<td>0.94 ± 0.21</td>
<td>1.24 ± 0.12</td>
<td>1.19 ± 0.16</td>
<td>1.27 ± 0.33</td>
<td></td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>1.13 ± 0.10</td>
<td>1.10 ± 0.17</td>
<td>1.06 ± 0.15</td>
<td>1.21 ± 0.06</td>
<td>0.99 ± 0.20</td>
<td>0.85 ± 0.17</td>
<td></td>
</tr>
<tr>
<td>MDA content (nmol g(^{-1}))</td>
<td>2</td>
<td>0.44 ± 0.03</td>
<td>0.36 ± 0.04</td>
<td>0.44 ± 0.10</td>
<td>0.41 ± 0.06</td>
<td>0.55 ± 0.04</td>
<td>0.47 ± 0.11</td>
<td></td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>0.46 ± 0.06</td>
<td>0.52 ± 0.08</td>
<td>0.50 ± 0.05</td>
<td>0.62 ± 0.06</td>
<td>0.81 ± 0.13 (^*)</td>
<td>0.86 ± 0.12 (^*)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>0.35 ± 0.03</td>
<td>0.26 ± 0.07</td>
<td>0.35 ± 0.09</td>
<td>0.44 ± 0.01</td>
<td>0.53 ± 0.04 (^*)</td>
<td>0.63 ± 0.05 (^*)</td>
<td></td>
</tr>
</tbody>
</table>
Moreover, using ANOVA to detect the interaction of doses and duration, it was significant only on activity of CAT and GST (Table 2).

3.2. Comet assay

The DNA damage was measured as LDR (Bolognesi et al., 2004), connected with the percentage of tail DNA and the following DNA damage classes proposed by Mitchelmore et al. (1998): minimal <10%, low damage 10–25%, mid damage 25–50%, high damage 50–75%, and extreme damage >75%.

E. fetida exposed to different concentrations of triclosan had a significant increase in DNA damage after a 7- and 14-d exposure (Fig. 1). The very high DNA damage (LDR = 1.76 ± 0.36) was already observed at the lowest tested dose (1 mg kg⁻¹) after a 7-d exposure. Noticeably, the LDR value exposed to triclosan at the highest dose was about 32% higher than that at the lowest tested dose. There was a similar dose-dependent increase in DNA damage also observed after a 14-d exposure. The damaging degree was related to the dose/effect relationship. The percentage of coelomocytes that fell into the high and extreme DNA damage increased from 1.7% at 1 mg kg⁻¹ of triclosan to 22% at 300 mg kg⁻¹ after a 7-d exposure, and from about 1.0% at 1 mg kg⁻¹ to 24% at 300 mg kg⁻¹ after a 14-d exposure. In the control, the majority of coelomocytes percentage fell into the minimal and low damaging degree (Table 3).

Although the LDR values increased slightly after a 2-d exposure as the concentration of triclosan increased. There were no significant (p > 0.05) differences observed between the treated groups and the control. The majority of coelomocytes percentage fell into the minimal and low damaging degree (Table 3).

4. Discussion

Toxic effects of triclosan on E. fetida at the biochemical levels and DNA damage in coelomocytes of E. fetida were observed in this study. The data presented in this work shows that activities of the enzymes varied according to the tested doses and exposure duration, although not all differences were significant. There was a real toxic effect due to triclosan exposure, even though the tested concentrations were much lower than the LC₅₀ value (866 mg kg⁻¹ soil dry weight) (Amorim et al., 2010).

CAT is an antioxidant enzyme that indirectly takes part in the contaminant metabolism by targeting reactive oxygen species, hydrogen peroxide (H₂O₂) (Brown et al., 2004). In this work the activity of CAT in earthworms treated by triclosan was higher than that in the control after a 2-d exposure and later returned to the levels in the control. While at the longer exposure (after 14 d), the activity of CAT became lower than that in the control. Our results on the activity of CAT support well our work done on herbicides (Wang and Zhou, 2006a,b). The study by Canesi et al. (2007) indicated that triclosan inhibited the catalase activity of CAT in hemocytes of M. galloprovincialis. The results of this work could suggest that the earthworm has the capacity to tolerate the oxidative stress and activate the antioxidant system at the first stage of stress at all doses of triclosan. A decrease in the activity of CAT in earthworms could be explained by ROS (O₂⁻). When the generated superfluous ROS exceeds the capacity of scavenging ROS by SOD and CAT, they become inhibitors of CAT (Kono and Fridovich, 1982; Geret et al., 2002). Our data also showed that 300 mg triclosan kg⁻¹ soil treatment significantly decreased the activity of CAT after a 14-d exposure. The decrease might be caused by damage of the antioxidant defense system. This demonstrated that the defensive effect of antioxidant enzymes would be lost with an
increase in toxic stress by triclosan, which is consistent with previous reports by Canesi et al. (2007) and Schreck et al. (2008).

GST is involved in xenobiotic metabolisms in living organisms, evaluated as the activity of GST utilizing 1-chloro-2,4-dinitrobenzene as a substrate (Canesi et al., 2007). Because it is an important detoxification enzyme and its activity has been used as a potential biomarker of earthworms exposed to heavy metals (Lukkari et al., 2004) and pesticides (Booth et al., 2001). According to the data obtained from this work, after a 2-d exposure, triclosan could stimulate the activity of GST in earthworms, which catalyze the conjugation of glutathione with both endogenous and exogenous substrates. Thus, it might be concluded from the fact of enhancing the activity of GST in earthworms after a 2-d exposure that triclosan might be used as a substrate for phase II enzymes in earthworms. This hypothesis is confirmed by data obtained by Moss et al. (2000) and Canesi et al. (2007), who found that triclosan is a substrate for phase II enzymes in bivalve tissues and mammalian cells.

SOD occurs in various cell compartments and catalyzes the dismutation of superoxide into oxygen and hydrogen peroxide (Salin, 1987). It is a primary remover of O2· radicals and can play an important role in defending against accumulation of toxic activated oxygen species (AOS). The enhanced SOD activity resulted in increased H2O2. Thus, the inducement of SOD coincided with an increase in the activity of enzymes removing H2O2 (Bowler et al., 1994). Oruc et al. (2004) found the increase in the activity of SOD in fish exposed to 2,4-D and azinphosmethyl. In our study, we also observed the inducement of the SOD activity after a 2-d exposure, although there were no significant (p > 0.05) differences. So the increasing CAT activity might be induced by H2O2 produced from sources of SOD. This phenomenon has already been observed in our previous work (Li et al., 2008).

The oxidative stress induced by triclosan can be demonstrated by MDA formation, after the 7-d and 14-d exposures. The increased level of MDA could be detected in the two time intervals to indicate the presence of AOS. This was in agreement with our previous work (Li et al., 2008) and other reports (Xue et al., 2009). Shalata and Tal (1998) suggested that one of the most damaging effects of ROS and their products in cells is the peroxidation of membrane lipids, which can be indicated by MDA detection. Our study has shown that MDA levels in earthworms were strongly enhanced after the 7-d and 14-d exposures, and the effect of dose of triclosan and duration of exposure on the content of MDA was also significant (Table 2). Thus, it can be concluded that the increased MDA content in E. fetida responded to oxidative stress caused by triclosan exposure.

Data obtained in this study showed that antioxidative enzymes were not affected significantly (p > 0.05) when exposed to the low dose of triclosan. However, the interaction between the dose of triclosan and duration of exposure was significant (p < 0.05) on the activity of CAT and GST, and appeared at different durations: The activity of CAT increased after the 2-d exposure and declined after the 14-d exposure, whereas the activity of GST decreased after the 14-d exposure.

Assessment of the ecological and genetic impact of PPCPs such as triclosan on living organisms in soil environments is of great importance. In this research, the comet assay was applied to evaluate the genotoxic potential of triclosan in E. fetida. The results obtained from the comet assay indicated that triclosan can genetically impact E. fetida. After the 2-d exposure there were no regular changes in LDR detected with increasing doses of triclosan, whereas after the 7-d and 14-d exposures, triclosan induced a significant (p < 0.001) increase of LDR (Fig. 1) at increased doses of triclosan and duration of exposure. As indicated in Table 3, after the 2-d exposure, the majority of the coelomocyte percentage fell into the minimal and low damage classes, and no extreme damage class was found at this duration. While after the 7-d and 14-d exposures, the extreme damage class increased with an increase in the tested doses. Moreover, many of the comet images from the treated groups, especially at the highest dose of triclosan, were of small heads and large fan-like tails. This may be associated with necrotic or apoptotic DNA fragmentation (Gichner et al., 2006). Our results are supported by Binelli et al. (2009b), who suggested that the genotoxic effects of triclosan increased in a dose-dependent manner and could significantly affect hemocyte functionality due to severe DNA injuries when Zebra mussel were exposed to triclosan at very low doses.

The correlation analysis showed that there were no significant (p > 0.05) correlations between genotoxicity and oxidative stress after the exposure of 2, 7 and 14 d, indicating that the inducement of DNA damage might be attributed to the apoptosis of coelomocytes. Martin et al. (1994) found that apoptosis is responsible for the activation of endogenous nucleases that cleave nuclear DNA into oligonucleosomal-sized fragments. Our results in this work were consistent with the previous studies (Binelli et al., 2009a,b). They suggested that the genotoxic effects of triclosan on hemocytes were closely related to apoptosis because a positive correlation (p < 0.001) between LDRs and apoptosis frequency was found. In the same work, Binelli et al. (2009b) also found that oxidative stress and the consequent production of ROS also can lead to DNA damage.

5. Conclusion

Our results suggested that environmental exposure of triclosan was potentially harmful to E. fetida as the biochemical responses and genotoxicity were observed at low or moderate tested doses. Triclosan toxicity caused oxidative stress and DNA damage of E. fetida and the consequent production of oxidative stress can lead to DNA damage. The comet assay was very sensitive, with clear effects observed even at the lowest concentration (1.0 mg triclosan kg \(^{-1}\) soil) (p < 0.01).

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