Volatile organic compounds (VOCs) in polyvinyl chloride (PVC) plastic products readily evaporate; as a result, hazardous gases enter the ecosystem, and cause cancer in humans and other animals. Polyethylene vinyl acetate (PEVA) plastic has recently become a popular alternative to PVC since it is chlorine-free. In order to determine whether PEVA is harmful to humans, this research employed the freshwater oligochaete *Lumbriculus variegatus* as a model to compare their oxygen intakes while they were exposed to the original stock solutions of PEVA, PVC or distilled water at a different length of time for one day, four days or eight days. During the exposure periods, the oxygen intakes in both PEVA and PVC groups were much higher than in the distilled water group, indicating that VOCs in both PEVA and PVC were toxins that stressed *L. variegatus*. Furthermore, none of the worms fully recovered during the 24-hr recovery period. Additionally, the *L. variegatus* did not clump together tightly after four or eight days’ exposure to either of the two types of plastic solutions, which meant that both PEVA and PVC negatively affected the social behaviors of these blackworms. The LD50 tests also supported the observations above. For the first time, our results have shown that PEVA plastic has adverse effects on living organisms, and therefore it is not a safe alternative to PVC. Further studies should identify specific compounds causing the adverse effects, and determine whether toxic effect occurs in more complex organisms, especially humans.

Key words: Volatile organic compound, Polyethylene vinyl acetate, Polyvinyl chloride, Environmental toxin, *Lumbriculus variegatus*

INTRODUCTION

Volatile organic compounds (VOCs) are chemicals containing carbon that have high vapor pressures at standard condition. VOCs are used as ingredients in a wide array of household products such as shower curtains, plastic cups, lunch boxes, etc. Their high vapor pressures result from low boiling points, which allow gases to be easily emitted while the products are in storage or in use. Thus, concentrations of many VOCs are up to ten times higher indoors than outdoors (Hester *et al.*, 1995).

VOCs can cause eye, nose, and throat irritation, headaches, loss of coordination, nausea, developmental damage as well as damage to the liver and central nervous, respiratory, and reproductive systems (Lester *et al.*, 2008). Some VOCs like benzene and formaldehyde are known to cause cancer in animals and are suspected to cause cancer in humans (Huff, 2007; Hauptmann *et al.*, 2009). However, at present, not much is known about the health effects of these organic compounds in homes or their ecological effects (Hester *et al.*, 1995).

Polymers derived from vinyl chloride monomer are called polyvinyl chloride (PVC) or “vinyl”. Polyvinyl chloride is used in a plethora of household products from flooring to wall covering.

The addition of a chlorine molecule differentiates PVC from other vinyls. However, during combustion, chlorine produces a carcinogen called dioxin that can cause reproductive and developmental problems, damage the immune system, and interfere with hormones. Low exposure to dioxin is a health concern because the chemical first accumulates in the environment, then in plants, in animals, and finally in humans through food consumption. In fact, food accounts for 95 percent of human exposure to dioxin (Viola, 1969; Struciński *et al.*, 2011). Therefore, the World Health Organization’s International Agency for Research on Cancer (IARC) established vinyl chloride as a known human carcinogen (Aitio, 1999).
In addition to vinyl chloride, PVC has other harmful additives including phenol, cyclohexane, decane, methyl isobutyl ketone, xylene, undecane, ethanol, dipropylene glycol methyl ether, phthalates, organotins, lead, mercury, as well as the carcinogenic compounds toluene and ethylbenzene (Lester et al., 2008). Therefore, a previous study suggested an association between PVC flooring and the development of bronchial obstruction in children (Jaakkola et al., 1999). Also, Di (2-ethyl hexyl) phthalate (DEHP) caused irregularities in male sexual differentiation when given to pregnant rats (Wolf et al., 1999).

Therefore, stores like Target and Wal-Mart have presently replaced PVC shower curtains with chlorine-free vinyls, most commonly polyethylene vinyl acetate (PEVA) plastic (Lester et al., 2008). PEVA is also phthalate-free and biodegradable, which makes it currently a popular alternative to PVC. The absence of chloride has been shown to considerably reduce harmful off-gassing (Nou et al., 2011).

Polyethylene vinyl acetate (PEVA) is a copolymer of polyethylene (PE) and ethylene vinyl acetate (EVA), and is similar to PVC because it is also made from petrochemicals (petroleum and natural gas). A study conducted by SAFC Biosciences in 2006 detected VOCs, such as aromatic compounds, alcohols, aldehydes, alkanes, alkenes, ketones, phenolics, ethers, amines, sulfides, etc., in PE and EVA bags. Although PEVA is chlorine-free, it is full of untested chemicals that may not make it a safe alternative to PVC. Therefore, it is necessary to test the health and environmental effects of those VOCs in PEVA plastic.

A lot of methods are used to measure concentrations of dissolved organic carbon (DOC) of a water sample, among which measuring UV-visible absorbance and then calculating concentration is a convenient and effective way because aromatic molecules of DOC produce vibronic excitations under certain wavelengths of UV (Khan et al., 2014). For example, naphthalene or anthracene has a UV absorbance at 270 nm. Additionally, the absorption of radiation corresponds to functional groups attached to an aromatic ring, and this can include -OH group at 270 nm, -OCH3 at 269 nm, -CN at 271 nm, -CO2 at 268 nm, and -COOH at 273 nm (Williams and Flemming, 1980). Therefore, UV absorbance measurement at 254 nm is usually used as a substitute to estimate organic carbon content in the water (Najm et al., 1994; Eaton, 1995). However, the value of absorbance at 254 nm particularly depends on the concentrations of humic acids in water. When the concentration of humic acids is low, the UV254 may be too low and generate a comparatively high random error (Wang and Hsieh, 2001). Therefore, Khan et al. (2014) developed a correlation with very high coefficient ($R^2 = 0.98$) between UV 272 nm absorbance and concentration of DOC of a sample that had been passed through a filter with 0.45 μm pores (Knap et al., 1996). The DOC includes purgeable (volatile) organic compound (VOC) and non-purgeable organic carbon (NPOC).

*Lumbricus variegatus*, better known as blackworms or California blackworms, are freshwater oligochaetes that live in the sediment and silt of ponds and lakes. A normal behavior for *L. variegatus* is clumping — if several blackworms are together, they will intertwine their bodies into a ball. In nature, numerous *lumbricus* congregate and form large clumped colonies of up to 2,000 individual worms. This allows for increased metabolism, stabilization of body temperatures, effective gas exchange, and collaborative food sharing (Leppanan and Kukkonen, 1998). Moreover, clumped worms burrow their anterior ends in the sediment and extend their posterior up into the overlying water. This orientation not only allows for effective gas exchange via dorsal blood vessels, but also allows for rapid response upon stimuli, to avoid dangers such as predation and acute toxicity (Drewes and Fourtner, 1989; Drewes, 1999; Ding et al., 2001).

*L. variegatus* is an important component of freshwater food webs in Europe and North America because they feed on microorganisms and organic material, and is a major food source for fish. Volatile organic chemicals from deposited plastics end up in sediment of lakes and rivers through wind and rain run-off. These toxins diffuse through *Lumbricus’* skin and bio-accumulates in the blackworm, then in its predators and eventually in humans. As a result the health of *L. variegatus* is a vital indicator of the current and future health of the ecosystem.

This research was conducted to compare effects of VOCs in both PEVA and PVC on the oxygen intake, activity and social behavior of the oligochaete *Lumbricus variegatus* to determine whether PEVA is toxic and whether it is a healthy alternative to PVC, and to provide further information about the health and ecological risk of these plastics.

**MATERIALS AND METHODS**

**Preparation of PEVA and PVC solutions**

To prepare the solution containing volatile organic compounds from PEVA plastics, twenty squares measuring 2 inches by 2 inches were cut from a 100% PEVA plastic shower liner. 200 mL of distilled water was poured into a water bath, and then the pieces of plastic were even-
VOCs of PEVA plastic are toxic

ly spread out in the water. The cover of the water bath was sealed with tape to prevent gases from escaping. The water bath was heated to 66 degrees Celsius, and kept at this temperature for two hours, and then was turned off and left overnight to allow the VOCs condense. The same procedure was repeated to obtain the solution containing VOCs from a 100% PVC plastic shower liner.

Measuring concentrations of DOC in six solutions

Six solutions, including one distilled water, one tap water, the PEVA solution, the PVC solution, one lake water (from North Carolina, USA), and one pond water (from North Carolina, USA), were separately filtered with sterile and endotoxin-free polyethersulfone filters with 0.45 μm pores (Whatman™), and then read UV absorbance at 272 nm with NanoDrop ND-1000 V3.3.0 (Thermo Scientific). The settings were as follows: UV-Vis, 1.0 Max absorbance, Normalize ON, and HiAbs ON. Next, the concentrations were calculated with the following equation:

\[
\text{DOC (mg/L)} = 518.93 \times \text{Absorbance (272 nm)} + 1.065
\]

(Khan et al., 2014)

Obtaining and maintaining L. variegatus

L. variegatus from the same source was purchased from Carolina Biological Supply Company. Each of these worms was at a young age — just two weeks old — and measured 1 to 2 inches long. It was not necessary to account for gender in the experiment because each worm has both male and female sex organs, and reproduces through asexual fragmentation. Also, it was not necessary to feed the worms because the blackworms are advantageous at long-term exposure without feeding (Sardo and Soares, 2010) and this experiment did not require the worms to be maintained for an extended period. The experimental condition for these worms was at room temperature (25°C) in PEVA, PVC or distilled water.

Determining what concentrations of solutions are used

A LD50 test was performed prior to the experiment to determine the toxicity of both PEVA and PVC plastics as well as the appropriate concentrations to use in the actual experiment. When establishing a LD50 test, a serial dilution is used to drop the concentration of the substance to levels of interest. In this experiment, the concentrations 100%, 10%, 1%, 0.01% and 0.001% of the stock solution were desired. Thus, the five desired concentrations were diluted and poured into five dishes, and then ten blackworms were put in each dish.

After 24-hr, a blackworm died in the 100% PEVA solution and another blackworm died in the 100% PVC solution. The blackworms in the 100% solutions did not clump and tended to be less active than the ones in the diluted solutions. Although the mortality rates of Lumbriculus during the LD50 were very low, the VOCs in the 100% solutions did prove to have adverse effect, so the 100% stock solutions from both PEVA and PVC were chosen to be used in the experiment.

Measuring oxygen intakes

Since the goal of this experiment was to test consequence when Lumbriculus variegatus was exposed to volatile organic compounds in both PVC and PEVA, three exposures in different lengths were tested: one day, four days, and eight days. Each length of exposure had three petri dishes: PEVA (that is, in PEVA solution), PVC (in PVC solution), and the control (in distilled water). Using a graduated cylinder, each petri dish of one exposure was filled with 15 mL of solution of PEVA, PVC or distilled water, and then 10 blackworms were transferred into it.

The blackworms in each petri dish were transferred to an appropriate container using a funnel 24-hr before the due time of an exposure. Then the opening of the container was sealed with an oxygen probe. The amount of oxygen in the container was measured for 24-hr with a Vernier oxygen gas sensor. After measuring the oxygen, the blackworms in each container were transferred back into their original petri dish, and then the solution was sucked out with a pipette and replaced with 15 mL of distilled water, and then transferred back into the container again. The oxygen during blackworms’ recovery was monitored for 24-hr to see whether the effects of the exposure to PEVA or PVC were reversible.

The oxygen intakes were calculated after the amounts of remaining oxygen (in ppm) in the container were measured with the Vernier oxygen gas sensor. That is, an oxygen intake at a specific time was determined to be equal to 210,000 ppm (the oxygen concentration in the empty container) minus the amount of remaining oxygen at that time. The ppm was then converted to mg by multiplying the container’s total volume (317 mL).

Observations of activity, mortality, and social behavior

Observations of activity, mortality and clumping were recorded after each exposure and each recovery period. Activity was measured as follows: Blackworms in a control group were gently probed with a pipette and their reaction was recorded as normal. Activity rating ranged from 1 to 3, where 1 = inactive (the worm did...
not react with one touch), 2 = normal activity level (the worm reacted like the control group with one touch), and 3 = very active (one touch triggered others to react, too).

Interpreting data
After the oxygen intakes were calculated, the trends of the oxygen intakes in each case and their lines of best fit were graphed with Microsoft Excel. The slope of the best fit line was the oxygen change rate per hour (Δmg/hr), which reflected the health effects when the worms were exposed to VOCs. The similar analysis was done on the data from each of the recovery periods.

RESULTS

The UV absorbance of the six solutions (one distilled water, one tap water, the PEV A solution, the PVC solution, one lake water, and one pond water) at 272 nm were as follows: 0.005, 0.009, 0.012, 0.021, 0.023, and 0.015, and their estimated concentrations (mg/L) were as follows: 3.66, 5.74, 7.29, 11.96, 13, and 8.85.

For the one day exposure, the oxygen intakes of both PEV A (average 9,250 mg) and PVC (23,529 mg) groups were much higher than the distilled water group (3,041 mg) (see Fig. 1). Furthermore, the high oxygen intakes of both PEV A (average 11,729 mg) and PVC (17,891 mg) groups did not fully recover after a 24-hr healing period in distilled water (3,205 mg) (see Fig. 2). The same was observed from oxygen intake data in four day exposure, eight day exposure and their 24-hr recovery periods (see Table 1).

Generally, the PEV A group had more fluctuations than the control and PVC groups (see Figs. 2 and 3). The change in oxygen intake (Δmg/hr) per hour is the slope of the best fit line. During the exposures (see Figs. 1, 3, 5), the slope of the control (distilled water) first decreased from one day exposure 29.06 to four day exposure -19.74 then remained constant from four day to eight day exposure. The Δmg/hr of PVC first decreased from 27.91 to 2.87 then increased from 2.87 to 47.59. Meanwhile, the Δmg/hr of PEV A increased from 21.38 to 96.94 then decreased from 96.94 to 9.99 (see Table 2).

When comparing the Δmg/hr of exposure to recovery, PEVA’s rate of oxygen intake first increased in one day exposure and subsequent recovery (from 21.38 to 106.90), then decreased in the four day set (96.94 to 1.36) and the eight day set (9.99 to 2.69). PVC’s Δmg/hr (27.91 to 19.47, 2.87 to -38.26, 47.59 to 25.23) decreased from exposure to recovery. The control (29.06 to -12.72, -19.74 to 14.05, -19.92 to 4.57) decreased then increased (see Table 2).

Additionally, the blackworms did not clump together tightly after four or eight day exposure to either of the two plastic solutions (Table 3). The number of worms clumped together mainly decreased with greater time of exposure. Recovery periods generally had greater worms clumping together than the exposure period, but the worms in the PEV A and PVC solutions did not fully recover (see Table 3).

The control groups during all periods of exposure and recovery had normal activity. The activity level of PEV A and PVC was initially higher than normal and decreased to below normal by the eight day of exposure (see Table 4).

During all the exposure and recovery periods, all of

![Fig. 1. One day exposure. The equation for the trend line is above each curve. The trend line does not show up if it completely overlaps with its corresponding curve. The same rules apply thereafter.](image)

![Fig. 2. One day recovery. The dash line crossing the PEVA curve is its trend line.](image)
the blackworms were respiratory (that is, their tails curled and were out of the water) and none of the blackworms died under observation.

**DISCUSSION**

**Oxygen consumption is an effective parameter of physical stress**

Measure of oxygen consumption is an indirect but common technique to measure energy expenditure (Levine, 2005) because most of energy in the body of an organism is produced aerobically, and monitoring energy expenditure is a standard method to measure the effects of stressors in aquatic organisms (Giesy, 1988). Therefore, it is reasonable to measure oxygen intakes of the worms to indicate the stress that they endure. Actually, the rate of respiration/intake of oxygen of earth-dwelling worms has been shown to increase by introducing stress such as light stimuli (Billstein et al., 2002). Moreover, direct measurement of exercise respiratory gas exchange is a valuable and standard testing of cardiopulmonary exercise stress in patients (Crespo et al., 2009).

As Giesy (1988) stated, the methods to measure the effects of stressors in aquatic organisms can be placed into six categories: 1) Hormones; 2) Energetics; 3) Enzyme activities; 4) Osmoregulatory electrolytes; 5) RNA/DNA and protein content; and 6) other substrates. Obviously, measuring oxygen consumption belongs to the second category, and measuring heat shock protein or immediate-early gene products falls into the fifth category. It is no doubt that all of them, including oxygen consumption, induction of heat shock proteins, induction of immediate-early gene products, hormones, and osmoregulatory electrolytes, are effective parameters to measure stress.

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Table 1. Average Oxygen Intake (mg)

<table>
<thead>
<tr>
<th>Exposure</th>
<th>Avg. Oxygen Intake (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Length of Exposure</td>
<td>Control</td>
</tr>
<tr>
<td>One Day</td>
<td>3,041</td>
</tr>
<tr>
<td>Four Days</td>
<td>2,719</td>
</tr>
<tr>
<td>Eight Days</td>
<td>2,420</td>
</tr>
</tbody>
</table>

Table 2. Change in Oxygen Intake (Δmg/hr)

<table>
<thead>
<tr>
<th>Exposure (Δmg/hr)</th>
<th>Length of Exposure</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
</tr>
<tr>
<td>One Day</td>
<td>29.06</td>
</tr>
<tr>
<td>Four Days</td>
<td>-19.74</td>
</tr>
<tr>
<td>Eight Days</td>
<td>-19.92</td>
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</table>

Table 3. The Amount of Clumping Worms

<table>
<thead>
<tr>
<th>Exposure (# Clumping)</th>
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<tbody>
<tr>
<td>Length of Exposure</td>
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<tr>
<td>One Day</td>
</tr>
<tr>
<td>Four Days</td>
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<tr>
<td>Eight Days</td>
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Table 4. Activity Level

<table>
<thead>
<tr>
<th>Exposure (Activity Level)</th>
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<tbody>
<tr>
<td>Length of Exposure</td>
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<tr>
<td>One Day</td>
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<tr>
<td>Four Days</td>
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<td>Eight Days</td>
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Note: 1 = inactive, 2 = normal activity, and 3 = very active

VOCs of PEVA plastic are toxic
Both solutions stressed the worms and irreversibly increased their oxygen intakes

Immediate responses of the blackworms to VOCs were observed at the LD50 test. That is, a blackworm died in both the original PEVA stock solution and the original PVC stock solution. Furthermore, the blackworms in the stock solutions did not clump and tended to be less active than the ones in the diluted solutions. Therefore the LD50 test demonstrated that the greater the concentrations, the more toxic were the effects of the VOCs, as is consistent with toxicity studies while treated blackworms with caffeine, nicotine, etc. (Bohrer, 2006), and with extracts from various hosta varieties (Rivera and Myers, 2012).

And, one day, four day and eight day exposures were compared to see the effect of VOCs on the oxygen intake of *L. variegatus*. PVC had the highest oxygen intake in all of the graphs (total average 15,605 mg), however, PEVA (9,209 mg) still had very high intake compared to the control (2,726 mg) (see Figs. 1-6). Therefore, these results clearly indicated that PEVA, like PVC, contained toxins that stressed *L. variegatus* and thus increased their oxygen intakes.

The 24-hr recovery period after the exposure represented detoxification. When comparing exposure to recovery, there was not much difference in the average oxygen intake. The recovery data showed that complete recovery did not occur within the given time period (as shown from the avg. oxygen intake and clumping). These results...
led to the conclusion that the adverse effects of exposure are irreversible in a 24-hr period.

In the exposures (see Figs. 1, 3, 5), the slopes of the control (distilled water) first decreased then remained constant, which indicated that the blackworms adjusted to the new environment and then continued to take in the same amount of oxygen. The Δmg/hr of PVC first decreased then increased. Similarly, Billstein et al. (2002) showed that when earth-dwelling worms were exposed to stress such as light stimulus, their oxygen intake level increased. Thus, the longer blackworms were exposed to PVC, the more stressed they became. However, Δmg/hr of PEVA first increased then decreased significantly (96.94 mg to 9.99 mg), perhaps because the harmful compounds of PEVA, which affected the ability of L. variegatus to aspirate oxygen, were different from the toxins of PVC.

Both solutions adversely affected the worms' behavior

As exposure length increased, the number of worms clumping together decreased. Activity level of worms in both PEVA and PVC solutions was initially higher than normal and then decreased to below normal by the eighth day of exposure, which meant that the stress excited the blackworms' body systems, and then weakened their ability to respond. Therefore, there was no doubt that increases in concentration and exposure length to either PEVA or PVC solution negatively affected blackworms' social behavior. A similar phenomenon was observed by other researchers: Bohrer (2006) noticed that the blackworms were not or less clumping while living in water treated with caffeine or nicotine.

Experimental conditions were well controlled

Not all of the blackworms at each exposure length demonstrated the same behavior (see Table 3, Table 4). This can be explained by variability or differences among the worms. Both extrinsic and intrinsic factors may affect results of experiments. However, extrinsic factors, such as temperature and pressure, were controlled via conducting the experiment in standard room conditions. Intrinsic factors are within individual organisms, such as age, metabolism, and genetic difference. In the experiment, L. variegatus picked for each group were selected to be all roughly the same length and coloring, thus age and body size was controlled. Still, genetic difference between blackworms could have affected results of exposure to VOCs. However, the genetic difference among the worms should be small since all of them were fresh young, were from the same source at the same time, and were almost the same size.

Toxic organic compounds are potential health risk to human and other animals

UV absorption of organic solutes is directly proportion-al to their concentration in aromatic compounds (Traina et al., 1990; Chin et al., 1994), and thus measuring UV-Vis absorbance is a convenient and effective way to estimate concentrations of DOC in water (Najm et al., 1994; Eaton, 1995; Khan et al., 2014).

The DOC of PVC solution is lower than that of the lake water (11.96 mg/L vs. 13 mg/L), and higher than that of the pond water (11.96 vs. 8.85). The DOC of PEVA solution is close to that of the pond water (7.29 vs. 8.85), and higher than the tap water (7.29 vs. 5.74). These data indicate that toxic organic compounds are possibly accumulated in the environment such as lakes, ponds etc., representing a potential health risk to human and other animals. Further steps will include determining what components in the two plastics are toxic to the blackworms, and how much they have accumulated in the ecosystem.

As a side note, the concept Dissolved Organic Matter (DOM) in the reference Khan et al. (2014) is the same as the concept Dissolved Organic Carbon (DOC) in this article because of two reasons: Firstly, samples in the two studies were passed through filters with 0.45 μm pores and then were measured UV-Vis absorbance. Secondly, the practical definition of DOC is the "dissolved" fraction of organic carbon below 0.45 μm (Knap et al., 1996), which includes VOC and NPOC.

In summary, this research clearly showed that PEVA has toxic effects on the oxygen intake, social behavior and activity of Lumbriculus variegatus, even though it is chlorine-free. And, this research proves that there is a need for further study on the toxicity of VOCs in PEVA plastic, especially to see whether it causes cancer in humans. In the near future, further studies should determine what compounds caused these adverse effects, how much they have accumulated in the ecosystem, and whether the toxic effect occurs in more complex organisms, especially humans.

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Conflict of interest---- The authors declare that there is no conflict of interest.

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