



Emerging investigator series: Degradation of phthalate esters in floor dust at elevated relative humidity

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3 **Emerging investigator series: Degradation of phthalate esters in floor**
4 **dust at elevated relative humidity**
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Abstract

Phthalate esters are present at elevated concentrations in floor dust, and resuspension of dust represents a major source for human exposure to chemicals. Biodegradation of phthalates occurs in aquatic systems and soils but has not been demonstrated in house dust. The goal of this study was to quantify indoor phthalate ester degradation through both biotic and abiotic mechanisms. Worn carpet squares were embedded with dust and incubated for one to six weeks at equilibrium relative humidity (ERH) levels of 50, 80, 85, 90, 95, and 100%, and nine phthalates were measured. Removal was observed for DEHP, BBzP, DiNP, DiDP, and DMP ($p < 0.05$) when incubated under elevated relative humidity conditions. Abiotic and biotic losses were examined separately using dust spiked with deuterated di(2-ethylhexyl) phthalate (d-DEHP) that was embedded in carpet and incubated at 100% ERH. Abiotic processes resulted in a 10.1% ($\pm 1.1\%$, standard error) to 69.6% ($\pm 4.8\%$) decrease in total d-DEHP after one week ($p = 0.03$) and a 27.2% ($\pm 1.4\%$) to 52.0% ($\pm 2.1\%$) decrease after three weeks ($p = 0.008$). Biodegradation resulted in a decrease in total d-DEHP after one week, ranging from 5.9% ($\pm 8.9\%$) to 8.5% ($\pm 1.7\%$) ($p = 0.07$) and a 1.7% ($\pm 3.9\%$) to 10.3% ($\pm 4.5\%$) decrease after three weeks ($p = 0.044$). Metatranscriptomic-based analysis indicates that fungi found in carpet dust express genes capable of degrading phthalate esters via various biochemical processes (including β -oxidation and hydrolysis). Overall, these results support the hypothesis that phthalate losses in floor dust are due to a combination of abiotic and microbial degradation at $\geq 80\%$ ERH.

Environmental Significance

Phthalate esters are only one example of human-made esters that are used as additives in commercial products and are known constituents of house dust. Other examples include

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3 organophosphate-, adipate-, and sebacate esters. A number of these are of public health concern.
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5 In this study, we observed degradation of phthalates in house dust at elevated relative humidity
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7 (RH) ($\geq 80\%$), with both biotic and abiotic mechanisms contributing to the observed degradation.
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10 Similar degradation processes may impact other human-made esters that are found in house dust.
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12 This work contributes to improved understanding of microbial activity and the fate of human-
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14 made esters in our homes, especially under conditions with available moisture.
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19 **Introduction**

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21 Phthalate esters (phthalates) are commonly found in the indoor environment. These
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23 plasticizers originate from building materials, PVC plastics, and personal care products ¹ and are
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25 readily released into the environment since they are not covalently bonded to the polymers.
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27 Exposure sources include diet, unintentional house dust ingestion, indoor air inhalation, and
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29 dermal absorption ². Exposure from non-dietary pathways alone can exceed tolerable daily intake
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31 levels for children ³. A survey conducted by the National Health and Nutrition Examination
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33 Survey (NHANES) in the United States found that all participants had phthalate metabolites
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35 present in their urine ⁴. Phthalates are a significant concern to human health because certain
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37 phthalates cause endocrine disruption by mimicking estrogenic compounds ⁵⁻⁸. Specific concerns
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39 include development and reproductive effects ¹ such as altered semen quality ⁹ and altered
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41 response to allergens ¹⁰.
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46 Phthalate degradation can be efficient in systems such as wastewater ^{11, 12} and soils ¹³.
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48 Degradation in the environment can occur both biologically ¹²⁻¹⁷ and chemically ^{18, 19}, though
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50 biological degradation is the predominant route in surface waters, soils, and sediments ¹⁷.
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53 Biological degradation reactions include hydrolysis and transesterification ^{14, 15}. One pathway
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3 includes β -oxidation with either de-esterification/dealkylation or trans-
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5 esterification/demethylation^{20, 21}. Chemical reactions include hydrolysis, oxidation by hydroxyl
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7 radicals, and direct photolysis¹⁷. Hydrolysis typically produces a monoester and alcohol,
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9 followed by a second hydrolysis step to form phthalic acid and another alcohol. Under
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11 appropriate conditions, phthalic acid can dehydrate to form phthalic anhydride. Our previous
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13 work showed that microbial growth and activity in house dust can occur at elevated relative
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15 humidity²², and that a diversity of microbial functions occur²³. The availability of moisture in
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17 the air above about 80% relative humidity produces microbial growth and activity in dust^{22, 23}.
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19 However, there have been no investigations of the contributions of chemical and biological
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21 processes to degradation in house dust, where phthalates can contribute up to 1 mg/g to the
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23 weight of the dust²⁴ but moisture is often limited.
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29 The goal of this project was to determine percent loss and degradation rates for common
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31 phthalates in the home environment under elevated relative humidity conditions ($\geq 80\%$) through
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33 both abiotic and biotic mechanisms. We hypothesize that elevated relative humidity conditions
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35 ($\geq 80\%$) will lead to phthalate degradation in house dust through both chemical and microbial
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37 processes, and we will determine the relative contribution of each. This study demonstrates that
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39 elevated relative humidity results in both chemical and microbial degradation of phthalates in
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41 house dust embedded in carpet. Further study is needed to determine removal under lower
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43 moisture conditions.
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49 **Methods**

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51 We collected carpet and dust from a home and incubated these samples at various
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53 relative humidity levels while measuring phthalate concentrations before and after. Additionally,
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3 we conducted a spike experiment to separate chemical and microbial degradation contributions.
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5 In this experiment, some carpet samples were spiked with a deuterated phthalate (di(2-
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7 ethylhexyl-3,4,5,6-d4) phthalate) and compared to sterile controls.
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10 **Carpet and dust collection.** Initial analysis was conducted on the same carpet used in a previous
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12 study and with methods described previously^{22, 23}. Briefly, worn, medium-pile, nylon carpet
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14 samples were collected from a single-family home in Massachusetts, USA in September 2014
15
16 (Site A). This carpet was used exclusively for the analysis described here, except for an
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18 additional analysis in the spiked experiment. For this additional analysis, designed to distinguish
19
20 microbial from abiotic degradation, carpet and dust were collected from three homes in Ohio,
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22 USA over the course of 2017-2018 (Sites B, C, and D). Sections were removed, wrapped in
23
24 aluminum foil, and sealed in polyethylene bags. Dust from the same home was collected from
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26 the occupants' vacuum cleaner bags for inoculation in the matched carpet. Dust was sieved to
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28 300 μm , mixed, and stored at room temperature. Dust and carpet were never frozen prior to use
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31 to avoid disruption of microbial communities.
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35 Carpets sections were cut into 10 cm x 10 cm squares and embedded with 0.5 g of dust
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37 collected from the same home as the carpet. Dust was not embedded in the 1 cm section near the
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39 edge of the carpet to avoid edge effects. Samples were placed in 3.8 L glass temperature and
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41 relative humidity-controlled chambers covered with parafilm.
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45 For the first "natural" experiment, we analyzed samples incubated in the previous study (Site
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47 A), including one week at 50, 80, 85, 90, 95, and 100% equilibrium relative humidity (ERH) to
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49 determine how change in relative humidity might influence degradation over one week. We
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51 extended the time period to one to six weeks at 85 and 100% ERH to determine degradation rates
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53 at these two ERH levels. The samples at 100% ERH were excluded after week 3 when extensive
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3 visible mold growth was present on the sample. For this study, we consider values $\geq 80\%$ ERH to
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5 be elevated.
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8 For the second “spiked” experiment, we spiked dust samples from Sites A-D (250 mg each)
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10 with 10 μg di(2-ethylhexyl) phthalate-3,4,5,6-d4 (d-DEHP) in 100 μL methanol (AccuStandard,
11
12 New Haven, CT, USA) and incubated them at 100% ERH for one and three weeks. These spiked
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14 samples were monitored over time. Three different sample types were prepared as follows: 1) a
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16 set of carpet squares and dust samples for each site were sterilized by autoclaving for 60 min and
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18 drying for 24 hours prior to spiking; 2) a set of unsterilized dust samples were spiked then
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20 embedded into carpet just as in the “natural” experiment; 3) a final set of unsterilized dust
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22 samples were spiked and embedded in carpet then frozen at $-80\text{ }^{\circ}\text{C}$ and never incubated. This
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24 final set of frozen samples was used as controls to confirm the spiked concentration. All samples
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26 were prepared in triplicate, and the 100% ERH samples set for one week had six replicates as
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28 noted previously²². After incubation, dust was collected from the
29
30 carpet using a 19 mm x 90 mm cellulose Whatman thimble inserted into a Eureka Mighty Mite
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32 vacuum cleaner with an adapter. Dust was stored at $-20\text{ }^{\circ}\text{C}$ prior to analysis after incubation.
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40 **RNA analysis to determine microbial function.** The purpose of the metatranscriptomic
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42 analysis was to determine microbial function in the “natural,” non-spiked samples. RNA analysis
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44 was conducted on non-spiked samples incubated at 50%, 85%, and 100% ERH, as described
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46 previously²³. Briefly, after incubation, collected dust was extracted in a sterile, laminar flow
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48 hood using the PowerMicrobiome™ RNA Isolation Kit (MoBio, Carlsbad, CA, USA) modified
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50 by increasing the amount of β -mercaptoethanol to 10x the recommended concentration. RNA
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52 was reverse-transcribed into cDNA using a polyA selection protocol to select for eukaryotic
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3 DNA and sequenced on an Illumina HiSeq 4000 at the Yale University Center for Genomic
4 Analysis. The 75 base pair paired-end reads are archived in the European Nucleotide Archive
5 and can be accessed at PRJEB25059. Read filtering and *de novo* metatranscriptome assembly
6 were carried out using the Trinity pipeline²⁵ with details published previously²³.
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12 Sequencing of the fungal metatranscriptome allowed us to look at several potential pathways
13 for phthalate degradation. We compiled a list of genes known to have the ability to degrade
14 phthalates, as well as some ligninolytic enzymes that have been hypothesized to have phthalate
15 degradation potential^{26,27}. Among these, di(2-ethylhexyl)phthalate esterase and fungal cutinase
16 are known to be able to degrade di(2-ethylhexyl)phthalate (DEHP)²⁶. Potential products may
17 include mono(2-ethylhexyl)phthalate (MEHP) and phthalic acid²⁸⁻³⁰. Long-chain phthalates,
18 including DEHP, have been shown to also degrade via β -oxidation (removing one ethyl group
19 from a side chain at a time) to DEP and then either de-esterified/dealkylated or trans-
20 esterified/demethylated to phthalic acid^{20,21}. Additionally, we examined whether any annotated
21 sequences had a “phthalate metabolic process” or “phthalate catabolic process” gene ontology
22 (GO) term annotation. As no contigs were found to have either of these GO terms, we also
23 compiled a list of parent GO terms related to the specific enzymes known to degrade phthalates
24 as a proxy for phthalate degradation potential.
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44 **Phthalate detection.** An aliquot of 100 mg of collected dust was weighed into a 40 mL glass
45 vial that had been baked at 450 °C for 8 hours with a Teflon cap. A total of 3 mL of a 3:1
46 hexane:isopropanol solution was added, and the sample was sonicated for 30 minutes. The
47 solution was transferred to a phthalate-free polypropylene tube (cat. no. 339650, Nalgene Nunc
48 International, Rochester, NY, USA) and centrifuged at 10,000 g for 10 minutes. The supernatant
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3 was transferred to a new baked glass vial. For the one-week spiked samples only, the pellet and 3
4 mL of methanol was added to the original glass vial and the mixture was sonicated for 30
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6 minutes. After centrifugation as described above, the two fractions were injected separately. The
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8 methanol extraction was discontinued after the initial round of sample processing because no
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10 additional compounds were detected in the methanol fraction on the gas chromatograph/mass
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12 spectrometer. Solutions were injected into an Agilent gas chromatograph with Agilent HP5MS,
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14 30 mx 0.250 mm column connected to an Agilent mass spectrometer using a 1 μ L splitless
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16 injection. The oven program consisted of 120 °C for 1 min then 20 °C/min to 300 °C for 5 min.
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18 Results were analyzed and quantified with ChemStation software (Agilent). A dilution series of
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20 standards was used to create a standard curve. We detected dimethyl phthalate (DMP), diethyl
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22 phthalate (DEP), diisobutyl phthalate (DIBP), di(n-butyl) phthalate (DnBP), di(2-
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24 ethylhexyl)phthalate (DEHP), butyl benzyl phthalate (BBzP), di-n-octyl phthalate (DnOP),
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26 diisononyl phthalate (DINP), and diisodecyl phthalate (DiDP). We used dimethyl isophthalate
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28 and dibenzyl phthalate as recovery standards and benzyl benzoate as an injection standard.
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30 Benzyl benzoate served as an internal standard for quantitation and was used as a response
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32 reference for the standard curve (SI Figure 1) and remained constant in all samples. Dimethyl
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34 isophthalate and dibenzyl phthalate served as recovery standards. A sample blank without dust
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36 was processed using the same protocol as the spiked samples. Instrument blanks were run in
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38 triplicate before each set of samples and standards.
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47 Samples were also injected on an Agilent LC MS with a C18 column using the following
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49 program: ramp from 5% to 95% solvent B (acetonitrile and 0.1% formic acid) in solvent A
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51 (water and 0.1% formic acid) over 15 minutes with a flow rate of 0.5 mL/min. Standard curves
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3 from a dilution series were generated to measure mono(2-ethylhexyl)phthalate (MEHP) and
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5 phthalic acid in a subset of samples.
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8 After initial sample processing, subsequent samples of carpet and dust from three additional
9
10 different homes were tested for phthalate degradation in the spiked experiment. These samples
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12 were processed in the same manner as the initial analysis with the following exceptions.
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14 Solutions were injected into a Thermoquest Trace GC 2000 gas chromatograph with Agilent
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16 DB5MS, 30 mx 0.250 mm column connected to a Finnigan PolarisQ mass spectrometer. The
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18 oven program consisted of 80 °C for 1 min then 20 °C/min to 300 °C for 3 min. The injection
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20 was a 1 µL splitless injection. Results were analyzed and quantified with Xcalibur data system
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22 (ThermoFisher). No additional LC MS analysis was performed.
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28 **Statistics.** Statistical analyses were performed using Stata Statistical Software, release 15
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30 (StataCorp LLC., College Station, TX, USA) to evaluate phthalate degradation indicated by a
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32 decrease in concentrations within our experiments. Simple linear regression was used to evaluate
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34 the degradation of each phthalate detected in the natural experiment over time at 100% and 85%
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36 ERH. Data from the nine phthalates incubated at 100% ERH were tested first for a statistically
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38 significant decrease in phthalate concentration over the three weeks tested. Only the phthalates
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40 with $p < 0.05$ were evaluated for degradation at 85% ERH over the six weeks tested. We also
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42 tested BBzP and DEHP for degradation at 85% ERH due to their steep slope values at 100%
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44 ERH and because it is possible that the degradation is not linear. Degradation of the spike D-
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46 DEHP was measured as the percent loss of the phthalate from the total concentration delivered
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48 over a three-week period, but it was determined that it did not follow a linear trend, so linear
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50 regression was not used. Instead, we performed a two-sample t-test to compare the mean percent
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3 loss between week zero and both week one and week three of the incubation. Phthalate
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5 degradation for the spike experiment was assessed using the mean percent loss of all four sites.
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8 9 10 **Results**

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12 All targeted phthalates were detected in the dust from Site A. DEHP had the highest initial
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14 concentration at 0.029 $\mu\text{g}/\text{mg}$ dust, while other concentrations ranged from 0.0021 – 0.0057
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16 $\mu\text{g}/\text{mg}$ dust (DIBP – BBzP) (**Figures 1 and 2**). Over the time range and ERH levels measured,
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18 there was no evidence for degradation of DEP, DIBP, DnBP, or DnOP. There was indication of
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20 biotic/abiotic degradation for higher molecular weight phthalates DINP, and DiDP ($p < 0.05$) and,
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22 to a lesser extent, BBzP ($p = 0.055$) and lower molecular weight phthalate DMP ($P < 0.05$).
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26 Although the decrease in DEHP in the natural experiment was not statistically significant, there
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28 was a mean 13.5% decrease in DEHP concentrations per week. All phthalates except DEP
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30 showed a negative rate of change suggesting there is an overall downward trend in phthalate
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32 concentration over time (**Table 1**). Degradation rates are also listed in **Table 1** and range from -
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34 0.11 to -1.1 $\mu\text{g}/\text{g-dust}/\text{week}$ for statistically significant compounds. The degradation rate for
35
36 DEHP was -3.9 $\mu\text{g}/\text{g-dust}/\text{week}$ but was not statistically significant, possibly because the data
37
38 was not linear. Thus, this was tested using a t-test for the spike experiment.
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42 We also tested for losses at 85% ERH for phthalates that showed a statistically significant
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44 reduction at 100% ERH, in addition to BBzP and DEHP which had two of the top three largest
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46 degradation rates. BBzP and DINP ($p < 0.05$) concentrations were significantly reduced under
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48 these conditions. All phthalates except DMP exhibited a negative rate of change demonstrating
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50 an overall downward trend in phthalate concentrations over time (**Table 1**).
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3 This analysis, shown in **Figures 1 and 2**, was not designed to determine the relative
4 contributions of biotic and abiotic degradation mechanisms. Rather, the measurements quantified
5 total losses of the phthalate in the house dust via any process.
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12 **Microbial and abiotic degradation.** To determine the relative contribution of biotic and abiotic
13 losses, we spiked d-DEHP into both sterilized and non-sterilized dust and incubated it in carpet
14 for one and three weeks. The total concentration of d-DEHP decreased in incubated samples
15 undergoing both microbial growth and in sterile controls (**Figure 3A**). In this figure, the line
16 labeled “chemical” indicates degradation from sterilized samples. The line labeled “microbial”
17 indicates the difference in degradation between non-sterile and sterile samples (i.e., degradation
18 due to microbial growth only, not including the chemical component).
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28 Chemical degradation rates were always higher than microbial degradation rates. For the
29 incubated sample from Site A, after one week about 8.5% d-DEHP was lost to microbial
30 degradation and an additional 10% was lost to chemical degradation. After three weeks, there
31 was no additional microbial driven loss, but chemical driven loss continued, totaling about 27%.
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33 We repeated this experiment with carpet and dust from three additional homes (**Figure 3B-D**).
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35 After one week, chemical driven loss was 22.5%, 32.6% and 69.6% for samples from Sites B-D,
36 while microbial driven loss was substantially smaller (5.9%, 7.3% and 0% for Sites B-D,
37 respectively). At three weeks, chemical driven loss continued for samples from Sites B and C,
38 but appeared to reverse for the sample from Site D. This apparent reversal may be a sampling
39 artifact. No further microbial driven loss was observed for Site B and Site C samples, while
40 microbial driven loss increased to 10% for the Site D sample. A two-sample t-test of the mean
41 percent losses for all four sites combined indicated there was a statistically significant difference
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3 in the percent loss at the start of the experiment and at week one of the incubation for abiotic
4 (p=0.03) but not microbial (p=0.07) degradation. At week three, both abiotic (p=0.008) and
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6 microbial (p=0.044) percent losses for all the sites were statistically significant. Although DEHP
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8 did not show degradation in our natural experiment based on linear regression, we demonstrated
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10 that d-DEHP is degraded in our spike experiment.
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15 The first step in the degradation of DEHP is typically the production of its monoester,
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17 mono(2-ethylhexyl)phthalate (MEHP). Measurement of MEHP in Site A from both sets of
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19 experiments (non-spiked and spiked) provide additional insights regarding the loss of DEHP
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21 from settled dust. In the sterile control after one week, MEHP's concentration increased slightly
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23 compared with its initial concentration; the increase in MEHP's concentration was even larger in
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25 the non-sterile "growth" sample. d-MEHP was observed in the initial sample, and after one week
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27 its concentration in the sterile sample was no different, while its concentration in the "growth"
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29 sample was slightly higher (**Figure 4A**). Phthalic acid, a degradation product of MEHP, was also
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31 measured in these samples and was above the detection limit in only the sample that experienced
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33 growth. d-Phthalic acid had a substantially higher concentration in the "growth" sample than in
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35 either the sterile control or the initial sample (**Figure 4B**). d-MEHP and d-phthalic acid in the
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37 "initial" samples are hypothesized to come from the d-DEHP solution used for spiking. MEHP
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39 was measured in samples incubated at different ERH levels for one week, and an increase
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41 relative to its initial concentration was seen only at 100% ERH (**Figure 4C**). MEHP was also
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43 measured in samples at 100% ERH for up to three weeks and 85% ERH for up to six weeks. At
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45 100% ERH, the concentration increased initially, and then appeared to decrease from weeks two
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47 to three (perhaps due to degradation of MEHP to phthalic acid). At 85% ERH, the concentration
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3 gradually increased over the six weeks, but at the end of six weeks MEHP's concentration was
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5 still less than half the level measured after only two weeks at 100% ERH (**Figure 4D**).
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10 **Metatranscriptomics and microbial function.** To determine whether the metatranscriptomic
11 data provides evidence of phthalate degradation we identified 16 enzymes with phthalate
12 degradation potential and 15 Gene Ontology (GO) terms associated with phthalate
13 degradation. No direct evidence of phthalate degradation potential was found using the GO terms; however,
14 indirect evidence points to the metabolic processes necessary for phthalate degradation,
15 including contigs annotated with GO:0046274, or lignin catabolic process (**Table 2**). Similarly,
16 the enzymatic evidence points to the potential of these cells for phthalate degradation (**Table 3**).
17 Phthalate degradation enzymes fall into three main classes: hydrolases (EC:3.-.-.),
18 oxidoreductases (EC:1.-.-.), and lyases (EC:4.-.-.). Of those enzymes with a known ability to
19 degrade phthalates, only E10 3,4-protocatechuate deoxygenase and fungal cutinase were found
20 in the metatranscriptome. This evidence is suggestive but cannot confirm whether DEHP is
21 degraded in this manner in our samples.
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38 The metatranscriptomics data suggests some differential expression of genes with phthalate
39 degradation potential at the three relative humidity levels tested (**SI Figure 2**). Although most
40 contigs with a cutinase annotation had no statistically significant changes in gene expression
41 ($p_{\text{adj}} < 0.05$), for one out of the three contigs, gene expression increased at 85% compared to 50%
42 ERH. None of the contigs with an E10 3,4-protocatechuate deoxygenase annotation were found
43 to be differentially expressed between any of the conditions. For those contigs with a laccase
44 annotation, 7 out of 26 contigs were up-regulated at 100% compared to 50% ERH, 10 out of 26
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3 contigs were up-regulated at 100% compared to 85% ERH, and none were differentially
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5 expressed between 85% and 50% ERH.
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8 Previous studies have shown that phthalates with side chains longer than DEP can be
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10 shortened via β -oxidation and then converted to phthalic acid via either trans-esterification or de-
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12 esterification^{20,21}. Although evidence of this pathway can be seen in the GO terms and enzymes
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14 (**Tables 1 and 2**), we cannot say definitively whether they are acting on phthalate esters. While
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16 most contigs with a β -oxidation annotation are not differentially expressed, nearly twice as many
17
18 contigs are up-regulated at 100% versus 50% ERH and nearly four times as many contigs are up-
19
20 regulated at 100% versus 85% ERH (**SI Figure 3**). We also see evidence of possible microbial
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22 degradation via hydrolysis (**Table 3**), and we cannot definitively say which pathway(s) is/are
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24 used.
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30 31 **Discussion**

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33 Microbial activity in the indoor environment may alter chemicals, and we need to understand
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35 the conditions under which this may occur. Degradation of phthalate esters could be either
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37 beneficial (due to phthalate removal) or harmful (because exposure to the degradation products
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39 may also have negative health effects³¹⁻³³). We provided preliminary evidence that
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41 biodegradation of phthalate esters in the indoor environment occurs and may contribute to
42
43 removal of these potentially harmful compounds under elevated relative humidity.
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47 **Degradation in sampled dust/carpet.** In this proof-of-concept study, we employed extreme
48
49 relative humidity conditions. Future studies are required to determine whether microbial
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51 degradation occurs under more typical building conditions; and, if it does, to what extent. Our
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53 data at 85% ERH does point to the potential for this to also occur over longer periods of time
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3 (such as many weeks, several months, or possibly years) at a more realistic humidity level. In the
4 present investigation, DMP, BBzP, DINP all showed a decrease in concentration over time. In
5 other systems, such degradation has been stimulated with the addition of appropriate
6 microorganisms¹¹. However, we did not attempt to separate biotic and abiotic processes for any
7 of these compounds except for DEHP.

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9
10 At this time, we are unable to provide an explanation for why microbial degradation of DEHP
11 appears to cease after one week in samples from a home in Massachusetts (**Figure 3A**) and two
12 homes in Ohio (**Figures 3B and 3C**). This is a complex and dynamic system with many
13 microbes and chemicals present and changing in concentration. One potential hypothesis is that
14 another degradation product, such as MEHP, 2-ethyl hexanol or the product of another pathway,
15 has built up to inhibit the microbe(s) responsible for degradation. MEHP is known to inhibit
16 cellular processes in other organisms^{34,35}, and an increase in MEHP concentration was observed
17 at week two in the Massachusetts home (**Figure 4D**). 2-Ethyl hexanol is reported to inhibit
18 phthalate degradation under methanogenic conditions³⁶. It is also possible that a more preferred
19 energy source became available in the samples from Sites A - C. However, further investigation
20 of this observation is beyond the scope of the present study and will need to be examined in
21 future work.

22
23 At 100% ERH, the increasing concentration of MEHP at week one and week two
24 measurements was followed by a decreasing concentration at week three (**Figure 4D**).
25 Presumably, the initial increase is due to the degradation of DEHP. However, MEHP is also
26 susceptible to hydrolysis and other degradation reactions, producing phthalic acid as one of its
27 products (**Figure 4B**). Thus, MEHP is both produced and consumed as a consequence of
28 degradation processes. Taken together, Figures 4A and 4B suggest that these reactions are driven

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3 at least partially by microbes, since in both instances the amount of product measured after one
4 week at 100% ERH is substantially larger for the “growth” condition compared to the sterile
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6 condition.
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10 **Degradation pathways and trends.** Our data provides suggestive evidence of the microbial
11 pathways that may be responsible for phthalate degradation in house dust. Degradation of
12 phthalates via β -oxidation is consistent with our data from the first “natural” experiment, which
13 demonstrated preferential degradation of phthalates with longer side chains (**Figures 1 and 2**).
14 However, we cannot conclusively link the degradation pathway with the degradation measured
15 in this study based on our data. The presence of fungal cutinase and other ligninolytic enzymes
16 in our metatranscriptome offer another potential avenue of phthalate degradation. Taken
17 together, these results show that microbes growing on dust at various relative humidity levels are
18 expressing genes with phthalate degradation potential, but do not definitively point to a specific
19 enzyme or pathway as being responsible for the microbial degradation of phthalates that we saw
20 in our experiments.
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35 Previous studies have generally found that low molecular weight phthalates are more prone to
36 abiotic and biotic degradation than high molecular weight phthalates^{17, 20, 28, 37-39}. While the
37 opposite trend was seen here, none of these prior studies were conducted using house dust
38 embedded in carpet and there are other potential reasons that could be investigated further to
39 explain this difference in our samples. Water, soil, and other outdoor systems may not be
40 representative of what occurs in a home. Factors besides molecular weight can also affect
41 degradation. These include temperature, pH, metal content, dissolved oxygen, certain organics,
42 available nutrients, and microbial composition^{37, 38, 40}. In general, phthalates must be dissolved
43 to be bioavailable, and sorption of phthalates to humic material can influence dissolution¹⁷.
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3 Bioavailability of esters is also dependent on hydrophobicity, charge, size, and even steric effects
4 associated with certain side chains⁴⁰. The trend observed in our study was seen in one home
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6 (Site A), which has a certain microbial community and contains dust with a certain chemical
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8 composition. Acclimation of the organisms at Site A to certain compounds might further
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10 influence degradation rates^{20,40}, and high concentrations of DEHP (as occurs in our system) can
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12 reduce degradation rates for lower molecular weight phthalates, including DnBP, DEP, and DMP
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20. In summary, multiple factors influence phthalate degradation rates. Trends in such rates might vary with the nature of a home, a possibility that should be explored in future work.

Relative humidity level may alter microbial degradation of phthalates. Our previous work demonstrated that fungi in house dust at 100% ERH increased gene expression compared to either 50% or 85% ERH²³. In particular, fungal cells at 50% ERH mainly expressed genes related to primary metabolism, while genes for more secondary metabolic processes were expressed as ERH increased²³. Genes with phthalate degradation potential are associated with both primary and secondary metabolism. For instance, lignin catabolism is a secondary metabolic process⁴¹ with enzymes capable of phthalate degradation. In this study, some ligninolytic genes demonstrate this pattern of increasing expression with increasing ERH: fungal cutinase expression is up-regulated at 85% compared to 50% ERH and laccase degradation is up-regulated at 100% compared to both 50% and 85% ERH. Fatty acid β -oxidation is a primary metabolic process⁴¹ with phthalate degradation potential; overall, those contigs with a β -oxidation annotation are up-regulated at 100% compared to both 50% and 85% ERH. This mirrors the overall increase in gene expression with humidity seen in our previous work and may be because cells need to conserve resources due to the lack of moisture at 50% ERH²³. As β -

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3 oxidation is a primary metabolic process, it is possible that even at lower relative humidity levels
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5 phthalates could be slowly degraded in this way.
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8 **Exposure implications.** As noted at the beginning of this section, the health consequences of
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10 these degradation processes may be mixed. Removal of DEHP itself is beneficial for reducing
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12 exposure to this compound. However, degradation products such as 2-ethyl hexanol, MEHP,
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14 phthalic acid and phthalic anhydride are potentially more irritating or harmful than the parent
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16 compound³¹. For instance, 2-ethyl hexanol has an offensive odor, while phthalic anhydride is
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18 known to cause allergic rhinitis and asthma⁴²⁻⁴⁵. Future work is needed to investigate the extent
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20 to which these processes occur under more realistic, long-term building conditions and measure
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22 the quasi steady-state levels of intermediate products such as MEHP. Our study was short term
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24 (up to six weeks) but at elevated relative humidity levels, and it is unknown how changing either
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26 of these parameters will affect concentrations. Dust in the indoor environment typically has a
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28 residence time of months to years depending on its location within the structure.
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33 Extended periods with extremely high relative humidity should be avoided in homes.
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35 Although phthalate esters in dust may degrade faster under such conditions, the production of
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37 harmful degradation products coupled with mold growth are anticipated to contribute to adverse
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39 health effects^{46,47}.
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43 **Limitations.** 1) This proof-of-concept study was conducted with dust and carpet that came
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45 primarily from one Massachusetts home, with some exploration of d-DEHP degradation in
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47 samples from three additional Ohio homes (**Figure 3B-D**). 2) The portion of the study that
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49 explored biotic and abiotic degradation of deuterated DEHP from Sites A-D occurred under
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51 extreme conditions of 100% relative humidity over one to three weeks and needs to be validated
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53 under more typical building conditions and more realistic timeframes. With a dataset limited to
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3 only two measurements post spike, linear interpolations are required between weeks one and
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5 three. 3) This study was not designed to determine the specific microbial organism(s) responsible
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7 for this degradation; this should also be evaluated in future work. 4) The gene expression
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9 analysis was only able to generate suggestive (rather than definitive) information on potential
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11 phthalate degradation pathways that will also need to be explored in future studies. Future
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13 degradation experiments could use radiolabeled compounds to determine if the radioactive label
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15 becomes incorporated into the biomass. If we had sequenced to a greater depth, we might have
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17 found evidence of more genes that were expressed at very low levels, including more phthalate
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19 degradation genes. We were also limited in the metatranscriptomics analysis by a lack of
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21 definitive work on which enzymes are responsible for degrading phthalates.
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28 **Conclusions**

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30 This work demonstrates that phthalate degradation in house dust is possible through both
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32 biotic and abiotic mechanisms. At elevated relative humidity, both processes contribute to the
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34 overall degradation rate. The removal of higher molecular weight phthalates from indoor dust
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36 can be viewed as a positive outcome, but degradation results in the production of alcohols,
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38 monoesters, and phthalic acid, which can be dehydrated to form phthalic anhydride. Some of
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40 these products may be of greater health concern than their precursors. This study was conducted
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42 at elevated relative humidity levels to demonstrate proof-of-concept, and the work needs to be
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44 repeated at lower relative humidity levels and for more extended periods of time.
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51 **Conflicts of Interest**

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53 The authors declare no conflicts of interest.
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Figure Legends

Figure 1. Measured values found in the site A dust for DMP, DEP, DIBP, and DnBP at different ERH levels for one week, at 85% ERH for up to six weeks, or at 100% ERH for up to three weeks. Samples were analyzed in triplicate. Points represent mean values and error bars represent standard error. Excepting DMP, there were indications of degradation for lower molecular weight phthalates under 100% ERH conditions ($p < 0.05$).

Figure 2. Measured values found in the site A dust for BBzP, DEHP, DnOP, DINP, and DiDP at different ERH levels for one week, at 85% ERH for up to six weeks, or at 100% ERH for up to three weeks. Note the larger ordinate range for DEHP. Samples were analyzed in triplicate, except the point 100% ERH for one week which was analyzed with six replicates. Points represent mean values and error bars represent standard error. Under 100% ERH conditions, there were indications of degradation for higher molecular weight phthalates DINP and DiDP ($p < 0.05$) and, to a lesser extent, BBzP ($p = 0.055$). Under 85% ERH conditions, BBzP and DINP concentrations were significantly reduced ($p < 0.05$).

Figure 3. Biotic and abiotic degradation of deuterated DEHP spiked into carpet from Sites A-D and measured at one and three weeks. The dashed blue line labeled “chemical” indicates degradation from sterilized samples. The solid orange line labeled “microbial” indicates the difference in degradation between non-sterile and sterile samples (i.e., degradation due to microbial growth only, not including the chemical component). The lines indicating microbial and chemical degradation between the measured points at week one and week three are linear

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3 interpolations of the data. Samples were analyzed in triplicate. Points represent mean values and
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5 error bars represent standard error. Abiotic processes resulted in a significant decrease in total d-
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7 DEHP after one week ($p=0.03$) and after three weeks ($p=0.008$). Biodegradation resulted in a
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9 decrease in total d-DEHP after one week ($p=0.07$) and after three weeks ($p=0.044$).
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15 Figure 4. Phthalate degradation products. A. MEHP and D-MEHP in dust of spike experiment
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17 after one week. “Initial” is the amount spiked in for D-MEHP or the initial amount measured of
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19 MEHP naturally in the dust. “Sterile” represents the autoclaved dust that was spiked with D-
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21 DEHP, and “Growth” represents the non-sterilized dust spiked with D-DEHP. All samples
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23 (including initial) were spiked with D-DEHP, which may have contained some D-MEHP. B.
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25 Phthalic acid under the same conditions as in A. * represents values that were not detectable. C.
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27 Values of MEHP found in the “OD” (original dust) and after incubation at the listed ERH level
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29 for one week. D. MEHP in the dust at either 85% ERH or 100% ERH for zero to three weeks.
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32 Samples were analyzed in triplicate, with the exception of points in C and D at 100% ERH for
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34 one week, which were analyzed with six replicates. Points represent mean values and error bars
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36 represent standard error.
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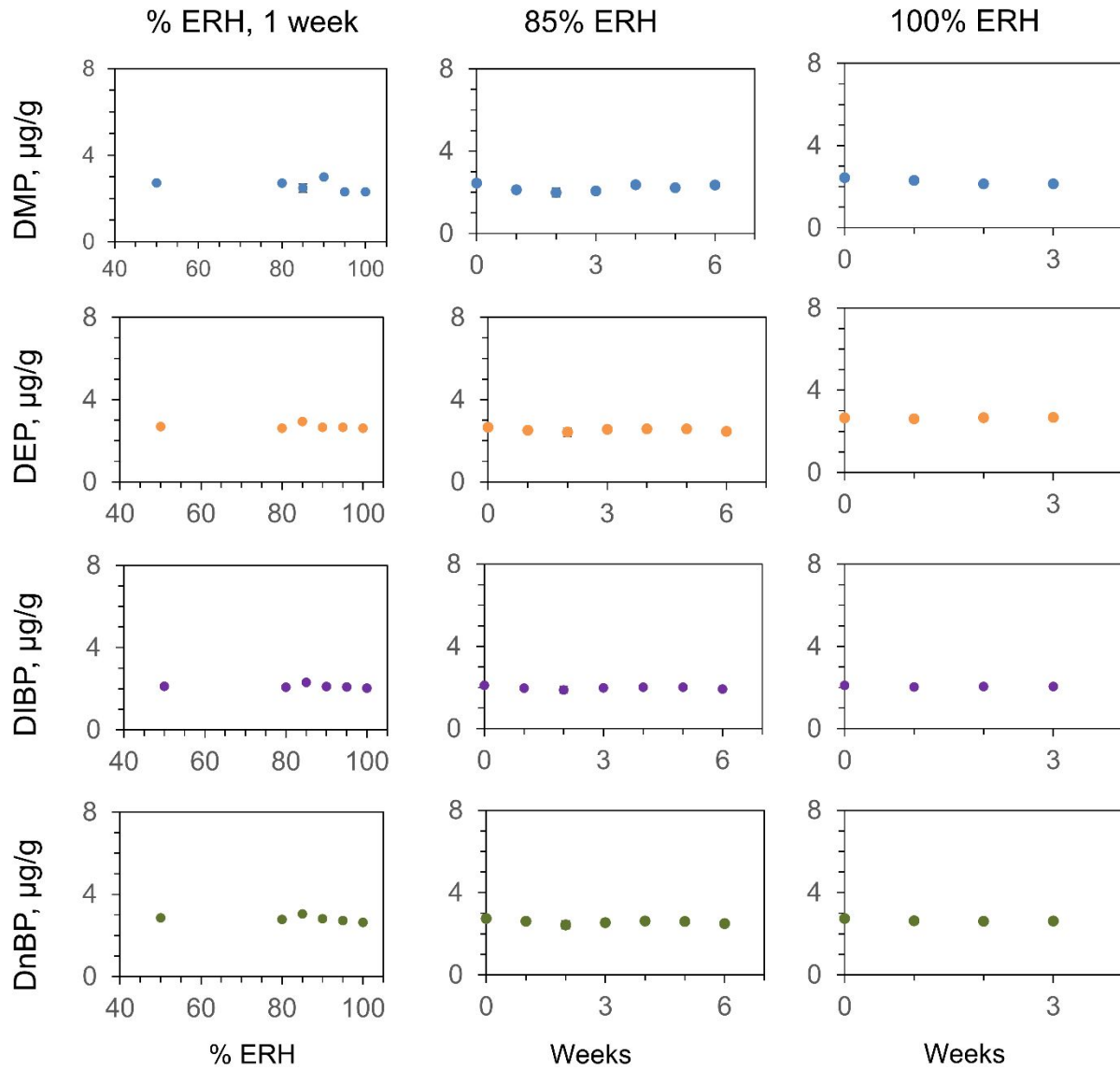


Figure 1

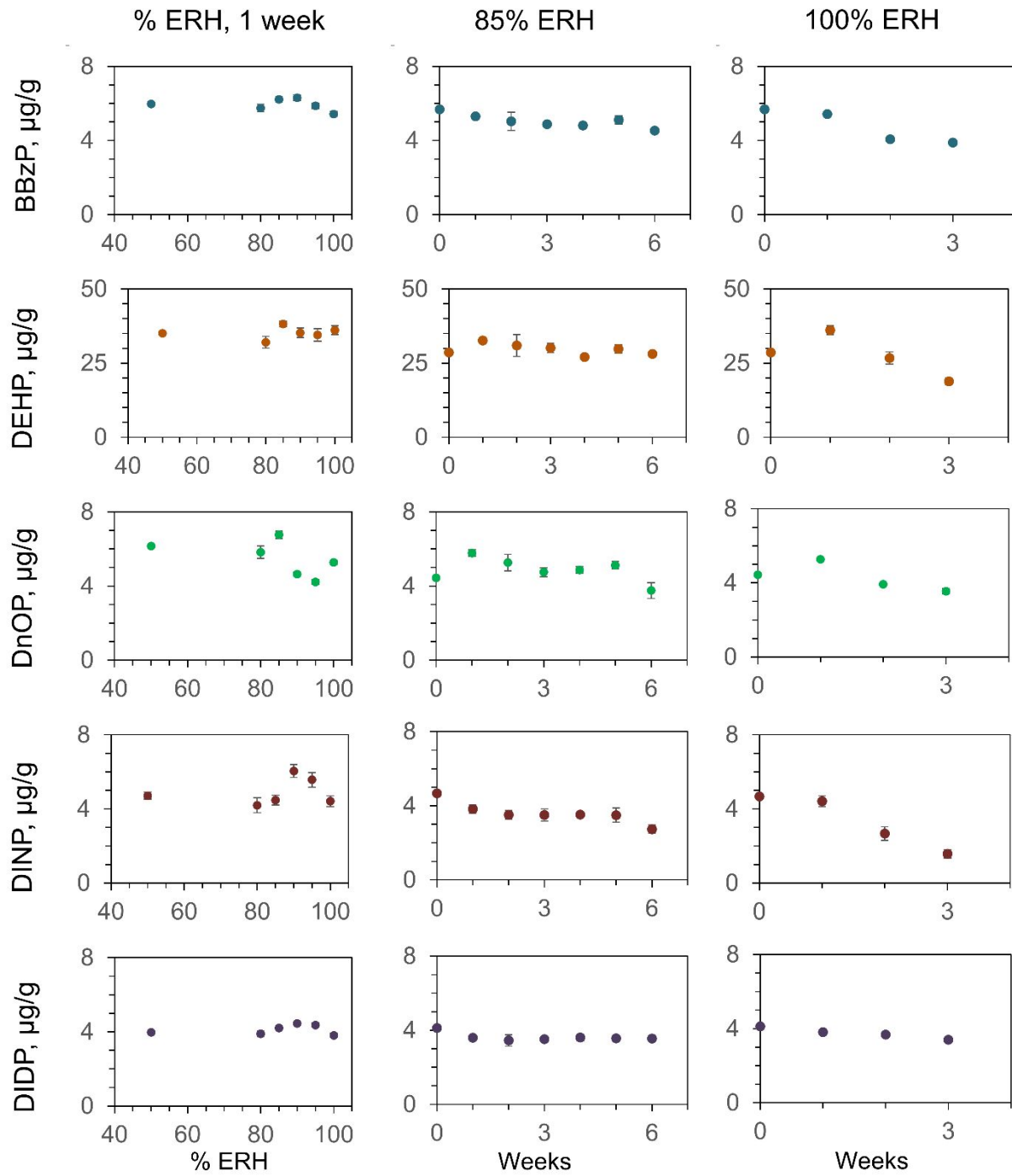


Figure 2

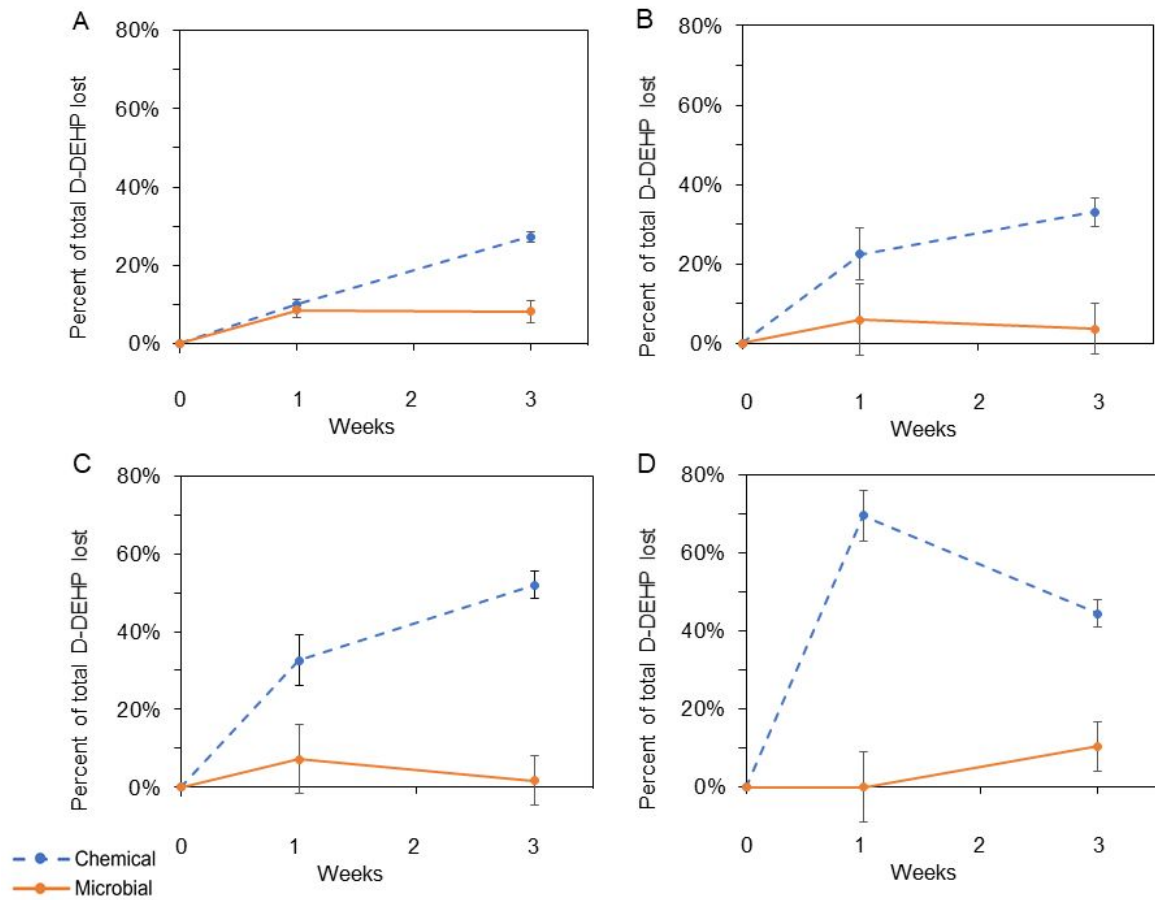


Figure 3

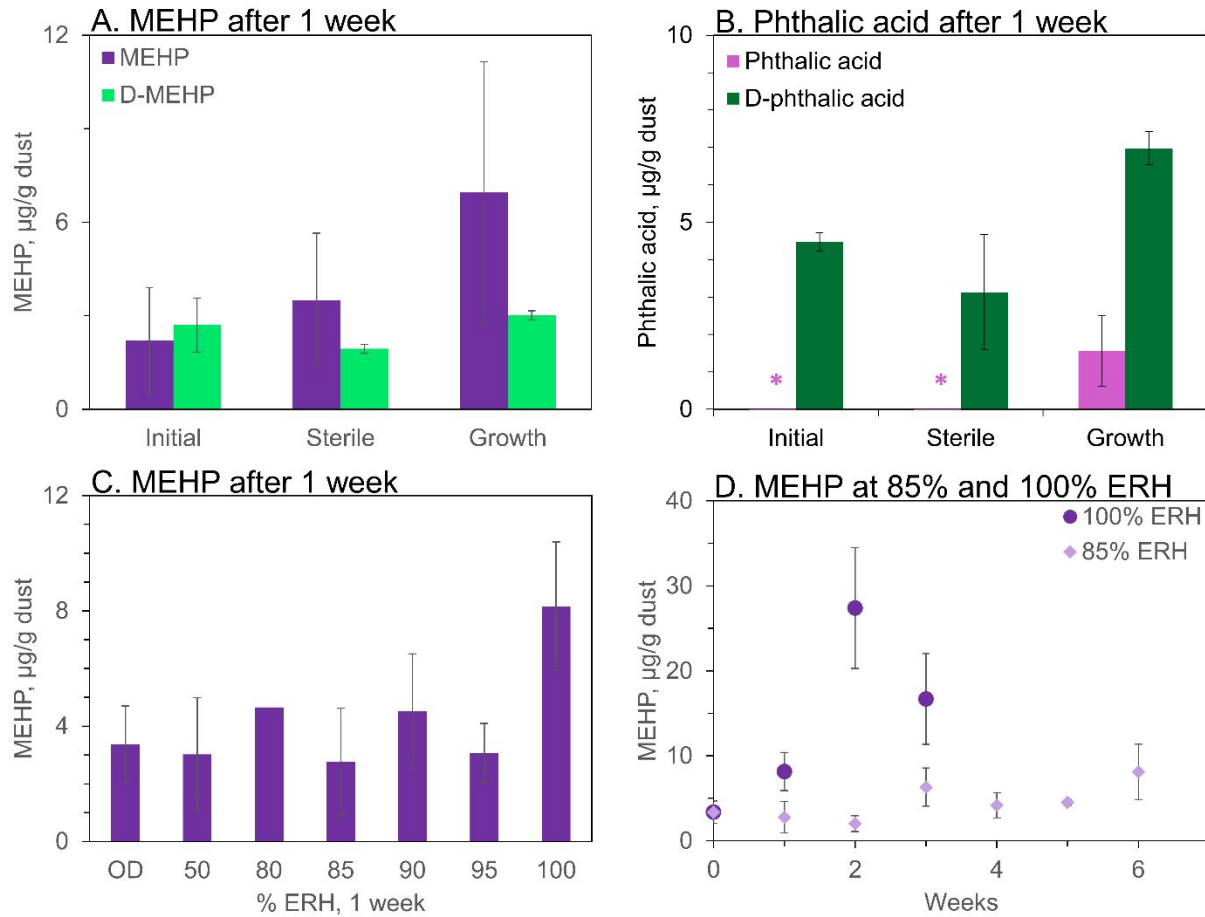


Figure 4

Table 1 Degradation rates for all phthalates at 100% ERH and select phthalates at 85% ERH. Only phthalates that showed potential for degradation at 100% ERH were evaluated at 85% ERH. The p-values <0.05 are in bold. BBzP and DEHP were also evaluated at 85% ERH due to their high slope values.

Phthalate	ERH	p-value	Slope ($\mu\text{g/g dust/week}$)	% Slope (% decrease/week)	Figure
DMP	100%	0.048	-0.11	-4.4%	1
DEP	100%	0.46	0.011	0.43%	1
DIBP	100%	0.39	-0.014	-0.68%	1
DnBP	100%	0.19	-0.039	-1.4%	1
BBzP	100%	0.055	-0.67	-12%	2
DEHP	100%	0.30	-3.9	-14%	2
DnOP	100%	0.31	-0.40	-9.0%	2
DINP	100%	0.03	-1.1	-24%	2
DiDP	100%	0.01	-0.23	-5.6%	2
DMP	85%	0.77	0.011	0.45%	1
BBzP	85%	0.02	-0.15	-2.6%	2
DEHP	85%	0.30	-0.40	-1.4%	2
DINP	85%	0.01	-0.23	-5.0%	2
DiDP	85%	0.19	-0.058	-1.4%	2

Table 2 Gene ontology (GO) terms with phthalate degradation associations.

Gene Ontology (GO) Term	GO Number	Found in metatranscriptome?
phthalate catabolic process	GO:0046239	No
phthalate metabolic process	GO:0018963	No
carboxylic acid metabolic process (parent of phthalate metabolic process)	GO:0019752	Yes
benzyene-containing compound metabolic process (parent of phthalate metabolic process)	GO:0042537	Yes
peroxidase activity	GO:0004601	Yes
fatty acid β -oxidation	GO:0006635	Yes
lipase activity	GO:0016298	Yes
oxidoreductase activity, acting on the CH-CH group of donors, NAD or NADP as acceptor	GO:0016628	Yes
oxidoreductase activity, acting on paired donors, with incorporation or reduction of molecular oxygen, NAD(P)H as one donor, and incorporation of two atoms of oxygen into one donor	GO:0016708	Yes
oxidoreductase activity, acting on diphenols and related substances as donors, oxygen as acceptor (synonym: laccase activity)	GO:0016682	Yes
hydroquinone:oxygen oxidoreductase activity	GO:0052716	Yes
carboxylase activity	GO:0016831	Yes
lignin catabolic process	GO:0046274	Yes
dioxygenase activity	GO:0051213	Yes
carboxylic ester hydrolase activity	GO:0052689	Yes

Table 3 Enzymes with known and potential phthalate degradation capabilities. Enzymes in bold were found in our metatranscriptome.

Enzyme	EC number	Pathway	Phthalate degradation?	Found in metatranscriptome?
E1 DAP esterase	3.1.1.-	phthalate biodegradation	known	No
E2 MAP esterase	3.1.1.-	phthalate biodegradation	known	No
bis(2-ethylhexyl) phthalate esterase	3.1.1.60	phthalate biodegradation	known	No
E3 PA 4,5-dioxygenase	1.14.12.7	PA and TA biodegradation	known	No
E4 PA 3,4-dioxygenase	1.14.12.-	PA and TA biodegradation	known	No
E5 cis-4,5-dihydroxy-4,5-dihydrophthalate dehydrogenase	1.3.1.-	PA and TA biodegradation	known	No
E6 cis-3,4-dihydroxy-3,4-dihydrophthalate dehydrogenase	1.3.1.-	PA and TA biodegradation	known	No
E7 4,5-dihydroxyphthalate decarboxylase	4.1.1.55	PA and TA biodegradation	known	No
E8 3,4-dihydroxyphthalate decarboxylase	4.1.1.69	PA and TA biodegradation	known	No
E9 4,5-protocatechuate dioxygenase	1.13.11.8	PA and TA biodegradation	known	No
E10 3,4-protocatechuate deoxygenase	1.13.11.3	PA and TA biodegradation	known	Yes
fungal cutinase	3.1.1.74	ligninolytic enzymes	known	Yes
yeast esterase	3.1.1.-	ligninolytic enzymes	known	Yes
lignin peroxidase	1.11.1.14	ligninolytic enzymes	possible	No
magnanese-dependent peroxidase	1.11.1.13	ligninolytic enzymes	possible	No
laccase	1.10.3.2	ligninolytic enzymes	possible	Yes

Phthalate losses in floor dust are due to abiotic chemical degradation as well as microbial degradation of phthalates under elevated relative humidity conditions.

