Environmental Science & Technology

Article

Cite This: Environ. Sci. Technol. 2020, 54, 364−371

pubs.acs.org/est

Fate of Hexabromocyclododecane (HBCD), A Common Flame Retardant, In Polystyrene-Degrading Mealworms: Elevated HBCD Levels in Egested Polymer but No Bioaccumulation

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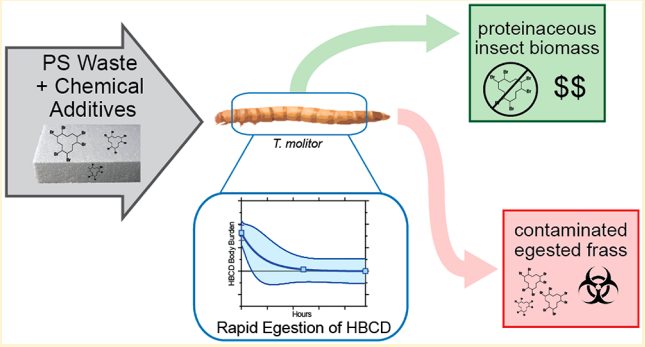
Supporting Information

ABSTRACT: As awareness of the ubiquity and magnitude of plastic pollution has increased, so has interest in the long term fate of plastics. To date, however, the fate of potentially toxic plastic additives has received comparatively little attention. In this study, we investigated the fate of the flame retardant hexabromocyclododecane (HBCD) in polystyrene (PS)-degrading mealworms and in mealworm-fed shrimp. Most of the commercial HBCD consumed by the mealworms was egested in frass within 24 h (1-log removal) with nearly a 3-log removal after 48 h. In mealworms fed PS containing high HBCD levels, only 0.27 ± 0.10%, of the ingested HBCD remained in the mealworm body tissue. This value did not increase over the course of the experiment, indicating little or no bioaccumulation. Additionally, no evidence of higher trophic level bioaccumulation or toxicity was observed when *L. vannamei* (Pacific whiteleg shrimp) were fed mealworm biomass grown with PS containing HBCD. Differences in shrimp survival were attributable to the fraction of mealworm biomass incorporated into the diet, not HBCD. We conclude that the environmental effects of PS ingestion need further evaluation as the generation of smaller, more contaminated particles is possible, and may contribute to toxicity at nanoscale.



INTRODUCTION

Plastic waste is a widespread environmental pollutant and a growing waste management challenge. Polystyrene (PS), one of the five most common thermoplastics, is typically used for packaging and insulation, and is among the least sustainable plastics.[1,2] PS foams are low density and bulky, making them difficult and costly to transport and recycle.[2] Because recycling centers typically do not accept PS wastes, they are generally landfilled or escape into the environment where they persist and can accumulate due to their recalcitrance.[2]

Chemicals added to improve manufacturing properties (e.g., plasticizers or stabilizers) or decrease flammability, for example in insulation in the case of PS, pose an additional sustainability concern.[1,3] Because these additives are not covalently bonded to the polymer, they could potentially partition into fatty tissues and bioaccumulate in food chains or the environment.[3,4] For PS, the most common flame retardant is commercial hexabromocyclododecane (HBCD).[4,5] HBCD is hydrophobic, lipophilic, persistent in the environment, and has been known to bioaccumulate in marine organisms.[4,6−8] It is also an endocrine disruptor and potentially neurotoxic.[4−6,9,10] Due to its toxic nature, HBCD has been the subject of regulatory action in the European Union (to be phased-out and eventually banned)[11] and in the United States HBCD is currently the subject of a risk evaluation by the EPA.[12] Solutions are needed to address HBCD, and other chemical additives, in PS waste.[13]

Biodegradation is of increasing interest for management of PS and other plastic wastes. While researchers have isolated many plastic-degrading microorganisms (e.g., fungi and bacteria), rates of biodegradation are typically low.[14−17] For plastic materials, one rate-limiting factor is access to surface area.[18] Insects that chew natural polymers, such as lignin and wax, convert macro-scale fragments into micron-scale particles, increasing specific surface area accessible to attack by secreted enzymes.[19] Within the insect gut (e.g., *Tenebrio molitor*,[20−23] *Plodia interpunctella*,[24] and *Galleria mellonella*[25]) secreted enzymes and ingested particles are concentrated and co-located, enhancing rates of biodegradation. Of the insects tested to date, mealworms (*T. molitor*) are the most well-studied and can rapidly eat and degrade PS and polyethylene waste, with half-lives for plastic conversion to CO_2 on the order of 15−20 h.[20−23] For chemical fate and effect studies, mealworms are an attractive insect model: they are readily

Received: October 28, 2019
Accepted: December 5, 2019
Published: December 5, 2019

DOI: 10.1021/acs.est.9b06501
Environ. Sci. Technol. 2020, 54, 364−371

cultivated, have a rapid life cycle, and are prey for other animals, enabling assessment of food chain impacts. In addition to their plastic-degrading ability, mealworms are farmed as a valuable protein-rich[26] feed supplement for birds and reptiles as well as aquaculture (e.g., shrimp, prawns, sea bass).[27−32] Further, mealworms are being considered as a more sustainable, lower greenhouse gas emitting source of edible protein for humans.[33−35]

Conversion of plastic wastes into a valuable feed-supplement is appealing, but the fate of plastic additives, such as HBCD, must first be understood. If HBCD accumulates in the tissues of mealworms, then there is a risk of food chain contamination and bioaccumulation. However, if HBCD passes through the mealworm, then there is risk of concentration within the remaining undegraded PS particles in the frass, creating a need for further remediation of these particles, such as frass pyrolysis.[36,37]

In this study, we investigated the fate of HBCD within PS-degrading mealworms using a mass balance and body burden analysis to assess toxicity and bioaccumulation. We also evaluated HBCD bioaccumulation at a secondary trophic level by feeding PS-fed mealworm biomass to a model aquaculture organism, *L. vannamei* (Pacific whiteleg shrimp).

MATERIALS AND METHODS

Plastic Test Materials. To determine the fate of HBCD in PS-degrading mealworms, two commercially available expanded polystyrene foam packaging materials were acquired from local vendors. The first PS foam was a commercial insulation material with a high concentration of HBCD, ∼0.25% [w/w] (2385.5 ± 353.0 μg HBCD/g PS) and was utilized as the PS material high in HBCD ("PS-H").[38] The second expanded PS foam was a commercial packing material that contained only trace amounts of HBCD, ∼8.0×10^{-5}% [w/w] (0.83 ± 0.20 μg HBCD/g PS) and was utilized as the PS material low in HBCD ("PS-L"). Table S1 of the Supporting Information (SI) includes additional descriptive characteristics (e.g., molecular weights, density) of the two PS foams used in this study, demonstrating that the concentration of HBCD is the main difference between them. The PS foam blocks were cut into irregular 2−3 cm cubes and cleaned with a stream of air to remove any fine residues prior to being weighed and utilized in experiments following established methods.[20−23]

Mealworm Growth Conditions. Mealworms, larvae of *T. molitor* Linnaeus, (average weight 75−85 mg/worm) were purchased online from Rainbow Mealworms (Compton, CA) and shipped overnight to the laboratories at Stanford University. Prior to arrival, the mealworms were fed bran; after arrival, they were subject to a 48-h depuration (starvation) period before initiating experimental diets.[20] Natural wheat bran used as a control diet[20−23] was purchased from Exotic Nutrition (Newport News, VA).

Five experimental diets were compared, in duplicate: PS-H, PS-H + bran (1:1 [w/w]), PS-L, PS-L + bran (1:1 [w/w]), and a bran only control.[20−23] Each replicate (in 475 mL food grade polypropylene containers) started with 200 randomly selected mealworms along with their respective feeds (Figure S1a). Plastic-fed containers began with 1.80 g of polystyrene cut into 2−3 cm cubes following established methods.[20] Containers that received bran started with 1.80 g of bran, with bran added every 3 days to maintain a 1:1 ratio [w/w] of plastic to bran, including the bran-fed control.[20−23] Containers were stored in incubators maintained at 25 °C and 70% humidity.[22,23]

Every 3 days, mealworm frass (excrement) in the containers was collected and weighed, and mealworm survival was evaluated for the duration of the 32-day experiment. Once a week, the mealworms were cleaned with a stream of air to remove any residual plastic fragments and transferred to a clean container to collect frass for HBCD analysis. After 24 h, the mealworms were returned to their original container, and the frass samples were weighed and stored at −20 °C. In addition, 10 mealworms from each container were sacrificed and dissected (gut track and nongut tissues) as representative weekly biomass samples, which were then freeze-dried for >72 h prior to being stored for future analysis (Figure S1a, SI M2).

To prepare sufficient mealworm biomass for aquaculture feeding experiments, 500 mealworms (from the same order) were raised in larger "bulk-fed" food grade polypropylene containers (volume 780 mL) on either PS-H, PS-L, or bran diets for 32 days following the conditions described above (Figure S1b). At the end of 32 days, the mealworms were sacrificed and the biomass (all the mealworm tissues) was freeze-dried for >72 h prior to being incorporated in aquaculture feed (SI M3).

Depuration tests were conducted to investigate accumulation of HBCD in mealworm tissues; full details are described in the SI (SI M4). Briefly, mealworms were fed either PS-H or PS-L for 72 h and were then subject to depuration for 48 h. Ten mealworms were collected at 0, 24, and 48 h of depuration, in triplicate, for HBCD analysis. The expected intake for each PS diet was calculated (SI M4). Bran was spiked with HBCD to achieve comparable expected intake as PS-H and PS-L, referred to as Bran-H and Bran-L, respectively, to assess the effects of PS on the bioaccumulation or egestion of HBCD.

HBCD Quantification. Full details of the HBCD analysis are described in the SI (SI M2, Figure S3). Briefly, analytical methods to quantify total HBCD were developed following modified EPA methods using extraction via ultrasonication (sample extraction: 3350B;[39] sample cleanup: 3630C[40]) on lyophilized biomass or PS samples. Quantification of total HBCD was performed using an Agilent gas chromatograph[41−43] (model 6890) equipped with a micro electron capture detector (GC-μECD) following modified EPA methods 8082[44] and 8081.[45] Surrogate standards (a mixture of PCBs, BZ# 14, 65, 166; 20 μL of 400 μg/L) were used to assess recovery efficiencies (Figure S6, accepted range: 40−100%) and internal standards (a mixture of PCBs, BZ# 30, 204; 10 μL of 400 μg/L) were utilized for quantification.[8,43] Standards of the three main stereoisomers were tested independently and as a mixture. Retention times and response factors of the three stereoisomers were statistically indistinguishable (Figure S8), justifying the use of one HBCD signal to quantify total HBCD.[42,43] Method detection limit (MDL) for mealworm biomass samples was 0.20 ng HBCD/g dry weight and for shrimp biomass the MDL was 1.00 ng HBCD/g dry weight. Our limit of detection (LOD) and limit of quantitation (LOQ) were determined to be 40 pg and 1000 pg, respectively, based on established definitions.[46,47] To increase the robustness of our analysis, we do not utilize any values below the MDL for statistical quantification, but we plot all values above our LOD.

Aquaculture Experimental Conditions. Postlarvae of *L. vannamei* were used as a model aquaculture organism. *L.*

DOI: 10.1021/acs.est.9b06501
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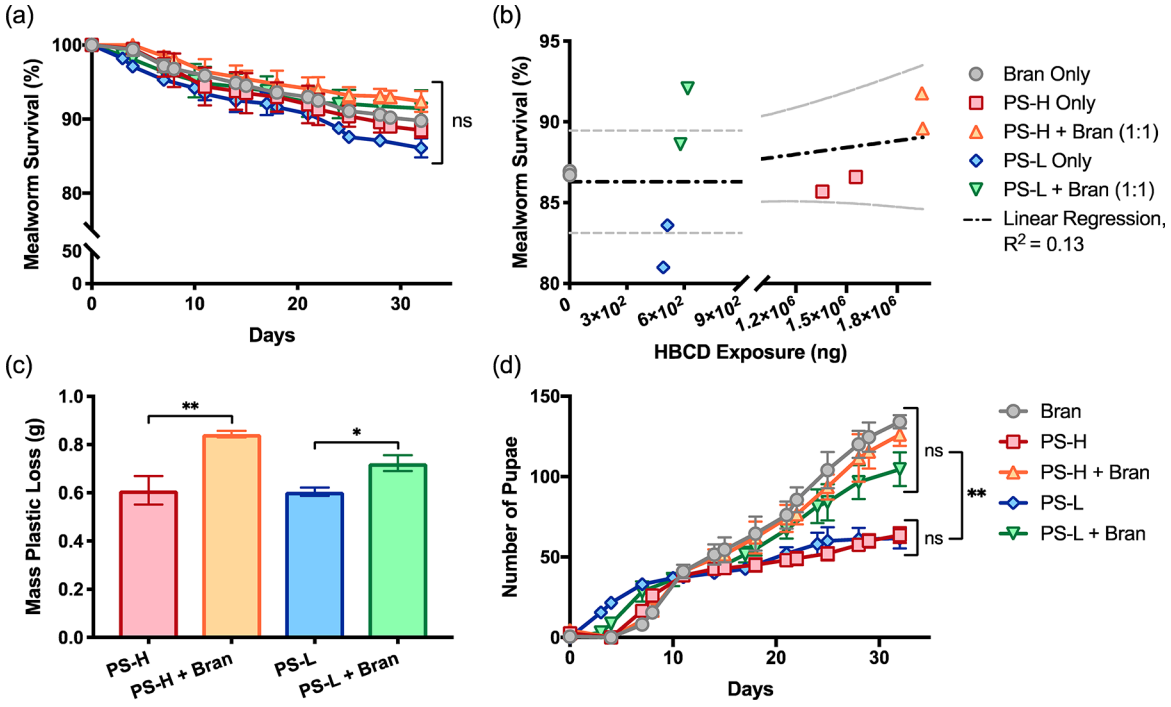

Figure 1. Survival rate and HBCD consumption by *T. molitor*. (a) Survival rate of mealworms over 32-day experiment, significance analyzed using Kaplan−Meier survival analysis. (b) Scatter plot and linear regression (black line) with 95% confidence interval (gray lines), of final mealworm survival versus HBCD exposure, by diet. HBCD exposure was calculated for each experimental container as total plastic consumed over the course of the experiment [g] × the concentration of HBCD in the plastic [ng/g]. (c) Plastic mass loss [g] at the end of the experiment, used in the HBCD exposure calculation above, by diet. (d) Total number of larvae that pupated over the course of the experiment by diet. All values represent mean ± SD, $n = 2$. Significance (one-way ANOVA, Bonferroni multiple test correction, for all tests not already described) $p \leq 0.05$ indicated by *, $p \leq 0.005$ indicated by **, no statistical significance indicated by ns.

vannamei were reared for two experimental tests. In the first, postlarvae were placed in individual containers to assess toxicity of experimental feeds through daily survival monitoring. The second test evaluated total HBCB bioaccumulation. Postlarvae were grown in larger containers with the same experimental diets to enable collection of sufficient biomass for HBCD analysis (Figure S2). The experimental diets included bran-fed mealworm biomass (MWB), PS-L fed MWB, PS-H fed MWB; each type of MWB was integrated into the shrimp diets at 3 different rates: 10%, 50%, and 100% w/w (for a total of 9 experimental diets). Control shrimp were fed commercially available feed (FRiPPAK RW+500, INVE Aquaculture, Deception Bay, Queensland, AUS). Detailed experimental conditions used to assess bioaccumulation studies in aquaculture are available in the SI (SI M3, Figure S2).

Statistical Analysis. Statistical analyses were performed in Prism (GraphPad Software, version 8.1.1). To assess differences in plastic consumption and pupation rates, one-way ANOVAs were performed, followed by pairwise comparisons using Student's *t* test with Bonferroni multiple test correction to assess differences between diets. Differences in survival were assayed using Kaplan−Meier survival analysis. All *p*-values are adjusted *p*-values, and all error values are average ± standard deviation.

RESULTS AND DISCUSSION

Effects of HBCD on Mealworm Survival and Plastic Consumption. Mealworm survival rate was unaffected by the amount of total HBCD consumed in PS over the course of the experiment (Figure 1a, b). There was no significant difference in final survival curves among the experimental diets nor the control diet based on Kaplan−Meier survival analysis (Figure 1a). However, a pairwise analysis of the final survival by diet found that mealworms fed PS-L + bran had a significantly higher survival than mealworms fed PS-L alone ($p = 0.02$). No other significant differences in survival based on the inclusion of bran in the diet were found (PS-H vs PS-H + bran, PS-H vs bran, PS-L vs bran). Previous studies have reported differences in survival based on inclusion of bran.[21] Further research is needed to determine why different types of PS may result in differential survival outcomes among mealworms when cofed with bran. Importantly, a linear regression analysis shows that total HBCD exposure from each diet was not a significant determinant of final survival (Figure 1b, $R^2 = 0.13$).

Mealworms consumed a similar amount of either type of PS, showing that higher concentration of HBCD in the PS did not affect mealworm consumption patterns (Figure 1c). Mealworms fed plastic plus bran consumed significantly more plastic than those fed PS alone, a trend established in previous studies[20,21] (Figure 1c). The increase in PS consumption in bran-fed mealworms was not as large as previously reported,[20,23] perhaps due to feedstock differences. Overall, mealworm plastic consumption rates in all experimental diets are comparable to those of previous studies.[20−23]

Mealworm pupation is a hormonally controlled process[48,49] that is known to be impacted by endocrine disrupting chemicals.[50,51] Therefore, if HBCD (an endocrine disrupting compound[5,52]) were bioaccumulating in mealworm tissues, we would hypothesize observable differences in the rates of pupation across experimental diets on the basis of HCBD exposure. However, our results indicate that the pupation rate was not affected by HBCD exposure (Figure 1d). The major

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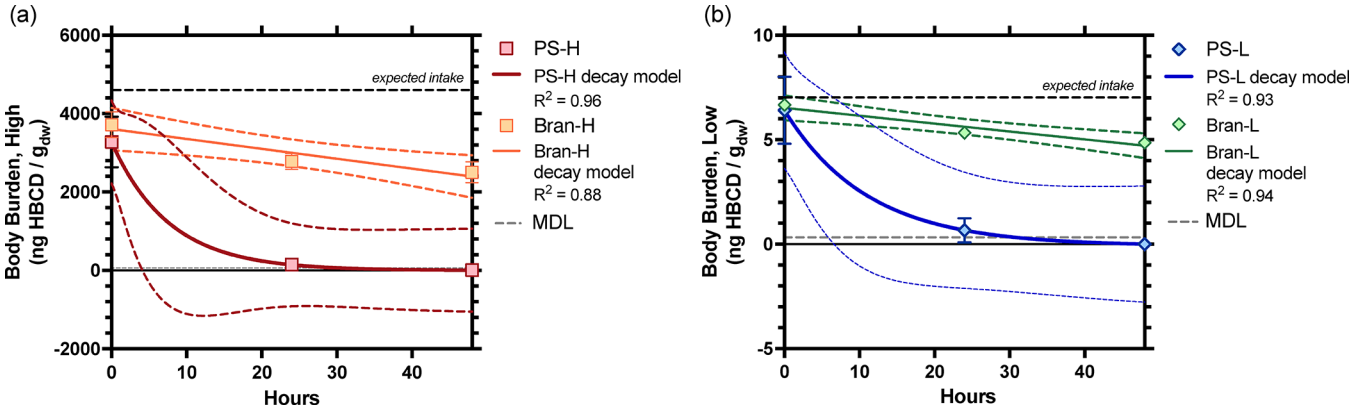

Figure 2. Reduction in *T. molitor* whole-body burden of HBCD with starvation. Body burden [ng HBCD/g dry weight] over time (points) plotted with one-phase exponential decay model (solid lines) and 95% confidence interval (dashed lines) (a) for PS-H and for control bran spiked with HBCD at a comparable concentration (b) for PS-L and for control bran spiked with HBCD at a comparable concentration. From the models, the half-lives for HBCD in the mealworm gut are 5.3 and 7.6 h for PS-H and PS-L, respectively. The half-lives for HBCD spiked directly into control bran are 14 and 16 h for Bran-H and Bran-L. The rate constant (k) is 0.13 h^{-1} for PS-H, 0.09 h^{-1} for PS-L, 0.05 h^{-1} for Bran-H, and 0.04 h^{-1} for Bran-L. All values represent mean ± SD, n = 3.

difference in the rate of pupation appeared to be driven by the inclusion of bran in the diet, rather than HBCD. Both PS diets that included bran (PS-H + bran, PS-L + bran), had a comparable pupation rate as the control group (Figure 1d). This finding was further explored with a multiple regression model to assess the effects of bran, plastic, and HBCD consumption on pupation rates (Figure S4, model R^2 = 0.95). Bran consumption was found to be a significant predictor of pupation rate ($p < 0.0001$) while HBCD consumption was not ($p > 0.05$). This suggests that differing rates of pupation are driven by the added nutritional benefits from bran in the diet rather than any effect from HBCD.

In summary, these results demonstrate that HBCD in commercial PS was not toxic to mealworms and did not affect mealworm survival and pupation behavior. Taken together, these results suggest that total HBCD does not bioaccumulate within mealworm tissue. This finding was further explored through analysis of mealworm tissue.

Exponential Removal of HBCD from Mealworm Biomass. Depuration tests post feeding of PS were conducted to investigate accumulation of HBCD in mealworm tissues over time periods that were comparable to the retention time of PS in the mealworm gut (15−20 h).[20] This analysis revealed that HBCD was rapidly excreted from the mealworm body (whole body tissues, including the gut track) when subject to depuration (i.e., starvation) (Figure 2). The HBCD concentration from both types of PS (PS-H and PS-L) decreased significantly over 48 h, in good agreement with a one-phase exponential decay model (Figure 2, R^2 = 0.96 for PS-H, 0.93 for PS-L). From the fitted decay models, the half-lives for HBCD in the mealworm gut 5.3 and 7.6 h for PS-H and PS-L, respecitvely. The decay rate constant (k) for PS-H is 0.13 h^{-1} and for PS-L is 0.09 h^{-1}. This suggests that HBCD from both types of plastic are subject to similar processes within the gut and are removed at comparable rates. After 24 h of starvation, there was a 1-log reduction in the HBCD body burden (ng HBCD/g dry weight of mealworm tissue) from both types of plastic (Figure 2). After 48 h of starvation, tissue from mealworms fed either type of PS experienced a nearly 3-log reduction in HBCD body burden (Figure 2). There was not a significant difference in the log-reduction of HBCD between the PS-H and PS-L diets at either time point (Figure S9).

To assess the impact of the presence of polystyrene on the egestion of HBCD, mealworms were fed bran directly spiked with HBCD at a comparable expected intake of HBCD as PS-H and PS-L referred to as Bran-H and Bran-L, respectively (SI M4). The concentration of HBCD in mealworm tissues were measured over the same depuration period as mealworms fed PS (at 0, 24, and 48 h) and were then fit to a one-phase decay model (Figure 2, R^2 = 0.88 for Bran-H, 0.94 for Bran-L). The decay rate constants (k = 0.05 h^{-1} for Bran-H and 0.04 h^{-1} for Bran-L) and half-lives (13.7 h for Bran-H and 16.0 h for Bran-L) indicate a significantly slower egestion of HBCD than that of mealworms fed PS. The log-reduction of HBCD from both Bran-H and Bran-L diets was significantly lower than the reduction of HBCD from either PS diet (Figure S9). These findings suggest that the presence of PS within the gut may play a significant role in concentrating the HBCD in the frass, leading to its egestion.

These findings suggest that the majority of the HBCD is transient and that when mealworms are subject to depuration periods longer than the gut retention time, HBCD passes through the gut and is egested when PS is present. The rates of HBCD egestion are not significantly different between PS-H and PS-L, suggesting that HBCD behaves similarly in either PS diet. Previous studies of mealworm PS-degradation have demonstrated that a fraction of the PS is egested in the frass as partially degraded polymer.[20,22,23] Given the lipophilic nature of HBCD[5] and the rapid egestion of HBCD in mealworm tissues, this could suggest that the HBCD is predominantly partitioned to and thus concentrating within the egested PS polymer residues. The increased half-life of HBCD delivered without PS (spiked into bran) further supports this hypothesis. Taken together, our findings suggest that the presence of depolymerizing PS plays an important role in concentrating HBCD and removing it from the mealworm; however, further work is needed to confirm the role of PS in the egestion of HBCD.

Fate of HBCD in Mealworm Biomass over Time. To further assess whether any of the ingested HBCD bioaccumulates in the mealworm tissues, a mass balance was conducted to monitor the fate of HBCD in various mealworm tissues over time. The amount of HBCD that entered the mealworm (based on consumption of PS), the amount of HBCD that left

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Environ. Sci. Technol. 2020, 54, 364−371

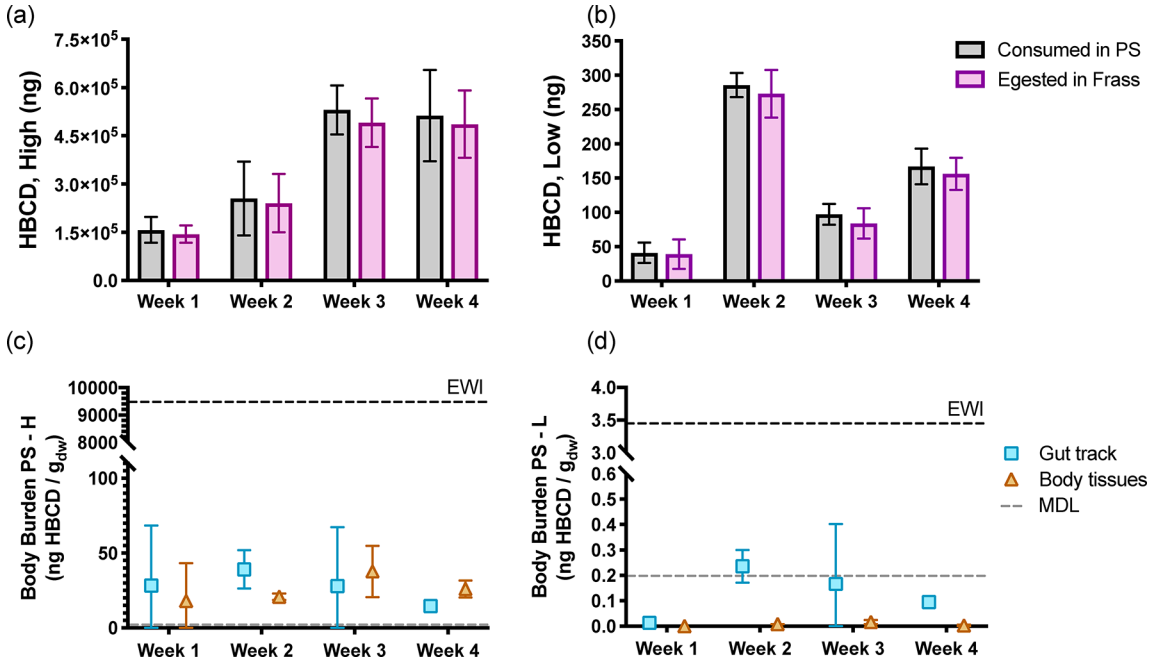

Figure 3. Fate tracking of HBCD in *T. molitor* fed PS-H (left) or PS-L (right). (a,b) Amount of HBCD consumed in PS (gray bars) coupled with the amount of HBCD egested in frass (purple bars) from each container weekly (a: PS-H, b: PS-L). Amount of HBCD consumed was calculated as total plastic consumed per week [g] × the concentration of HBCD in the plastic [ng/g]). Amount of HBCD egested was calculated as the concentration of HBCD in the weekly representative clean frass sample [ng/g] × the total frass collected for the week [g]. (c, d) Body burden of HBCD [ng/g dry weight] in mealworm tissues (gut or nongut tissues) weekly, plotted with the method detection limit (MDL) (c: PS-H, d: PS-L). Expected weekly intake (EWI) of HBCD was calculated as the average amount of HBCD consumed for each diet [ng/week], normalized to 10 mealworms, and is plotted for comparison. All values represent mean ± SD, n = 2.

the mealworm system (measured in the frass), and the amount of HBCD remaining in mealworm (gut and nongut tissues) was determined on a weekly basis. This approach enabled the tracking of HBCD within the mealworms over the course of the 32-day experiment to assess bioaccumulation (Figure S7). The results indicate that the majority of the HBCD consumed by mealworms, fed either type of PS, is egested in their frass (Figure 3a,b). On average, of the amount of HBCD consumed from PS on a weekly basis, 93.20 ± 1.45% was egested in PS-H fed-mealworms and 92.56 ± 4.17% was egested from PS-L fed mealworms. This trend was also observed in PS diets cofed with bran, where the total HBCD consumption was higher than those fed PS alone (Figure S5a,b). This further supports evidence that HBCD is rapidly excreted from mealworms (Figure 2). Moreover, while total consumption of HBCD varied over the course of the experiment (as PS consumption by the mealworms varied), the HBCD recovered in frass remained consistently high.

The weekly HBCD body burden analysis, based on 10 randomly selected representative mealworms pooled together from each container, showed that little HBCD remained in the mealworm tissues (Figure 3c,d). On the basis of total PS weekly consumption and number of mealworms per container, the expected weekly intake of HBCD (EWI, normalized to 10 mealworms) was calculated and compared to the amount of HBCD in mealworm tissues. For mealworms fed PS-H, on average only 0.29 ± 0.11% of the EWI was found in the mealworm gut (Figure 3c). The representative mealworms were first subjected to a 24-h depuration, which from analysis in Figure 2 enables ∼90% removal of transient HBCD, explaining why trace amounts of HBCD may still have been detected within the gut track. For the same mealworms fed PS-H, on average only 0.27 ± 0.10% of the EWI, or 25.6 ± 8.7 ng HBCD g^{-1} dry weight, was found in the nongut tissues of the mealworms; this amount remained consistently low throughout the experiment (Figure 3c). For mealworms fed PS-L, the HBCD concentration of the majority of body tissue samples were below the method detection limit (MDL, 0.2 ng HBCD/g dry weight) and thus were not used for quantification (plotted in Figure 3d). The HBCD detected in the gut tissues was elevated compared to the HBCD in the nongut tissues, which again, was likely due to the limited (∼90%) removal of transient HBCD during the 24-h depuration period. For mealworms fed either type of PS, the levels of HBCD in the nongut tissue remained stable over the course of the experiment, suggesting that HBCD was not bioaccumulating, as bioaccumulation would likely have resulted in an increase in HBCD concentration in nongut tissues over time.

Similar trends were observed in the PS plus bran cofed diets (Figure S5). Previous studies have established that mealworms cofed bran gain more weight over the course of the experiment than those fed PS alone.[20,23] Despite the increased growth and metabolic activity of mealworms fed these diets, mealworm tissues still only contained trace levels of HBCD (0.24 ± 0.04% and 0.15 ± 0.12% of EWI for PH-H + bran remains in the gut and nongut tissues, respectively). As observed in the PS-L group, the HBCD concentration in the tissues of mealworms fed PS-L plus bran were below the MDL, suggesting little or no bioaccumulation. This also suggests that bioaccumulation of HBCD was not affected by an increase in metabolic activities.

Overall, the rapid excretion of HBCD from the gut and the consistently low body burden analysis in all diets over the duration of the experiment suggest that HBCD is not bioaccumulating in mealworm tissues. The mass balance

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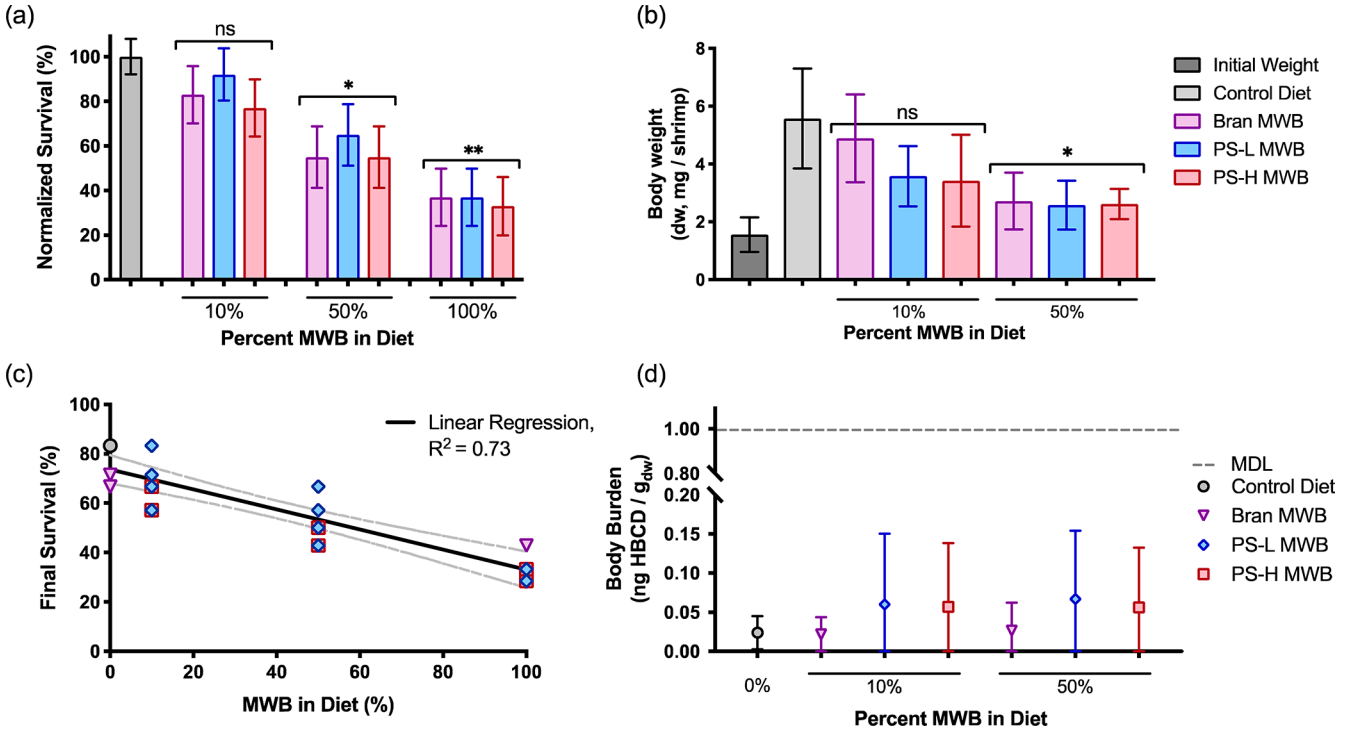

Figure 4. Bioaccumulation effects from plastic-fed mealworm biomass on *L. vannamei*. (a, c) Survival of *L. vannamei* from toxicity experimental setup. (a) Final survival proportions from Kaplan−Meier survival analysis by diet normalized to control diet and (c) scatter plot and linear regression of final survival versus percent of mealworm biomass (MWB) included in the diet. (b, d) Analysis of *L. vannamei* from bulk experimental set up. (b) Final body weight (dry weight, dw) based on diet. (d) Body burden of HBCD [ng/g dry weight] in shrimp biomass at the end of the bulk experiment. All samples are below the method detection limit (MDL, plotted in dashed line), thus raw data are shown but not used for quantification. All values represent mean ± SD, $n = 3-5$ depending on diet. Significance versus control (one-way ANOVA, Bonferroni multiple test correction) $p \leq 0.05$ indicated by *, $p \leq 0.005$ indicated by **, and no statistical significance indicated by ns.

instead demonstrates that total HBCD is concentrating in the frass egested by the mealworms. Further research is needed to better understand the mechanism driving rapid egestion of HBCD and to assess the environmental impacts of HBCD within the smaller, more concentrated particles in the frass.

Lack of HBCD Bioaccumulation in Secondary Trophic Level. Mealworm biomass represents a valuable supplemental protein source in many agricultural and aquaculture feeds.[27−32] Therefore, we investigated the toxicity of HBCD-exposed mealworm biomass (MWB) on the growth and survival of a model aquaculture organism, Pacific whiteleg shrimp (*L. vannamei*). Further, we investigated whether HBCD from MWB was bioaccumulating through a body burden analysis of the shrimp biomass. Mealworm biomass was incorporated into experimental shrimp diets both at a realistic levels (10%)[27,30,53] and at higher level to assess the upper bound for HBCD bioaccumulation and toxicity.

Our results from the toxicity experiments showed an increase in mortality with increasing incorporation of MWB (Figure 4a). The increase in mortality was observed for MWB from mealworms fed PS-H as well as mealworms fed PS-L and bran-fed mealworms (Figure 4a). This suggests that changes in shrimp survival are driven by the total amount of mealworm biomass included in the diet, and not by HBCD. The amount of MWB included in the shrimp-feed, regardless of the diet of the mealworms, explained >70% of the difference in survival (Figure 4c, $R^2 = 0.73$), with a greater inclusion of MWB leading to an increase in mortality. This suggests that MWB of any type (bran, PS-H, or PS-L) affects mortality at higher inclusion rates, likely due to lack of nutrition, rather than presence of HBCD. Shrimp mortality in this study was higher than has been previously observed when supplementing *L. vannamei* diet with MWB.[29] Unlike previous work, we studied postlarvae, which are more sensitive than adult shrimp,[54] likely explaining the higher mortality rate. Increases in weight in the *L. vannamei* over the course of the experiment also appear to be driven by percent of MWB incorporated in the diet rather than HBCD exposure (Figure 4b).

To further confirm that total HBCD was not bioaccumulating in the shrimp, body burden analysis was conducted on shrimp fed the same experimental diets, excluding the high mortality 100% MWB diets, for 2 weeks (Figure S2b). The HBCD concentration in all shrimp biomass including those fed PS-L and PS-H MWB were below the MDL and not significantly different than zero. Moreover, the concentration of HBCD in the shrimp biomass was unaffected by the experimental diet (bran-fed, PS-H fed, or PS-L fed MWB). Taken together, these results suggest no significant bioaccumulation of HBCD in the shrimp fed mealworm biomass. While the starting concentration of HBCD in the shrimp feed was likely relatively low (given the 24 h depuration before collection of MWB), this case-study demonstrates that that inclusion of PS-fed mealworm biomass under realistic aquaculture growth conditions does not lead to the bioaccumulation of total HBCD.

IMPLICATIONS

This is the first study to assess the fate of chemical additives in a plastic biodegradation system. This is important because chemical additives, such as HBCD, can have significant

DOI: 10.1021/acs.est.9b06501
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environmental and health impacts. We demonstrated that total HBCD does not bioaccumulate in PS-degrading mealworms, but is instead egested in the frass where it likely concentrated in the partially degraded PS. This study did not investigate the fate of individual stereoisomers of HBCD, and such an analysis would not change the conclusions of this study. Nevertheless, future research should assess whether the distribution of isomers egested in the frass are different from those in the initial PS.[55]

This study serves as a proof-of-concept for deriving valuable biomass from plastic waste. Through the egestion of HBCD, the proteinaceous mealworm biomass is preserved as a potential resource, which in this study we demonstrated could be used as an aquaculture feed supplement without leading to bioaccumulation at a higher trophic level. However, only one chemical additive for one type of plastic was investigated. Other common chemical additives (e.g., plasticizers, stabilizers, pigments, other types of flame retardants)[1,3] have variable chemical properties (e.g., hydrophobicity), which will result in a different fate within plastic-degrading mealworms. This study lays the foundation for additional investigations into the fate of plastic additives within plastic degrading systems. Future work should focus on whether these findings are generalizable to other plastic additives and in other systems that degrade plastic.

This research shows that in working to address the issue of plastic pollution, it is important to avoid creating a new concern as residual plastic particles become smaller and smaller, increasingly concentrating hydrophobic contaminants in the particles. Future research should focus on the environmental impacts of such particles. Further, this research demonstrates the need for biodegradable plastic replacement materials and green chemistry to ensure that future materials and additives are nonbioaccumulative and nontoxic.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.est.9b06501.

Additional methods (M1-M4), table (S1), and figures (S1−S9) with full details on HBCD chemical analysis and quality assurance measures, mealworm and aquaculture growth conditions, and sample preparation (PDF)

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This work was supported by the Stanford Woods Institute for Environment (award 1197667-10-WTAZB). A.M.B. was supported by the National Science Foundation Graduate Research Fellowship Program (DGE-1656518). S.H.E. was supported by the Stanford Interdisciplinary Graduate Fellowship. The authors thank Dr. Daniel McCurry, University of Southern California, for initial method development help. We thank Mr. Jack Chieuh, Stanford University, for administrative help.

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