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Article

Improving Product Safety for Edible Insects: Toxicokinetics of Hg in *Tenebrio molitor* and *Hermetia illucens*

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ABSTRACT: Sustainability, circularity, and Zero Waste policies are timely concepts for policy development and strategies in the European Union (EU) and other global regions. Insects can likely become key players in the bioconversion of waste to valuable material and promise one solution to achieve diverse societal goals. Insects further present strategic opportunities as food products; however, it is necessary to understand how insects accumulate and eliminate priority contaminants from different substrates where they can be reared. In the present study, we expanded beyond previous work with mercury (Hg) to examine bioaccumulation kinetics in *Tenebrio molitor* (YMW) and *Hermetia illucens* (BSF). Two-phase bioaccumulation assays, with an uptake (contaminated Hg substrate) and elimination phase (clean substrate), followed by toxicokinetic modeling, showed that both insects have a high capacity to regulate Hg, often reaching an internal steady-state concentration at level responding on the substrate concentration of Hg. Of importance for product safety, both insects quickly eliminated Hg after being transferred to clean substrate. Specifically, BSF eliminated half of the accumulated Hg in approximately 1 day (after 5 days of Hg exposure) and YMW in 4−5 days (after 21 days of Hg exposure). These results provide crucial product safety information for insect producers using possibly contaminated substrates, specifically informing the amount of time for Hg depuration prior to processing and commercialization for food and feed.

KEYWORDS: *edible insects, safety, Toxicokinetics, Hg, uptake, Yellow mealworm, Black soldier fly, depuration, chemical hazard*

1. INTRODUCTION

The growing world population is increasing the demand for food, particularly from animal-derived products.[1,2] Simultaneously, significant changes in food preferences and human consumption patterns are increasing pressure on already limited resources, resulting in intense competition for land and water to produce food and feed.[3] Thus, there is a need to find new, innovative, environmentally friendly, and sustainable solutions to face this problem. The use of insects as a protein source for food and feed is widely accepted as a promising solution for developing a more sustainable food production chain while reducing the impacts of standard animal feed production and consumption.[3] Compared with other conventional animal protein sources, the use of insects leads to lower greenhouse gas and ammonia emissions,[4] requires less land area for production,[5] and presents more efficient feed conversion.[1,6] Furthermore, many insects are also effective bioconverters of organic waste materials to high-quality protein and fat,[1,7−10] which promotes the adoption of circularity principles in food and feed production systems and positions this emerging sector as increasingly important for sustainability, food security, nutrition, and public health.

Simultaneously, food safety is a critical issue that cannot be compromised when considering new food and feed sources. Within the scope of circularity, insects act as waste bioconverters of organic materials from multiple sources, which are often in compliance with limits established by existing regulations concerning the concentrations of pollutants and contaminants. Once these insects consume coproduct streams (e.g., olive pomace, seaweed, coffee roasting, among others), they will be converted to value-added products for feeding purposes.[11] In this sense, research must be conducted to understand bioavailability, define uptake and elimination processes, and characterize the risks of bioaccumulation and biomagnification of contaminants in insects. Microbial substances (e.g., mycotoxins), organic chemicals (e.g., pesticides, veterinary pharmaceuticals, polycyclic aromatic compounds), and metals are some of the contaminants of particular concern.[12] Contaminants potentially present in waste streams could accumulate in insects by various routes of exposure (e.g., uptake via pore water, skin, substrate ingestion). Consequently, the uptake of contaminants and consequent bioaccumulation will depend on the type of exposure experienced.[13−15] The need for a comprehensive understanding of the possible hazardous influences of

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contaminants that can be transferred through the substrate to insects in rearing facilities, in combination with the mechanisms of their accumulation, was highlighted by the European Food Safety Authority (EFSA) as a priority research need.[15]

Several insect studies have reported toxicity and accumulation data for different metals in contaminated substrates,[11,13,16−21] though limited studies have focused on the yellow mealworm (YMW) *Tenebrio molitor* (Linnaeus, 1758),[11,22,23] compared to the black soldier fly (BSF) *Hermetia illucens* (Linnaeus, 1758). Metals are the most studied class of contaminants regarding their bioavailability and bioaccumulation in insects. Though the uptake of Hg in insects was previously examined, few studies have focused on edible insects. This represents a key food and feed safety consideration for edible insects because Hg is considered a priority pollutant by regulatory agencies (European Parliament and Council of European Union, Directive 2000/60/EC), due to its persistence, nondegradable properties, and bioaccumulative and biomagnification potential through food webs.[24] Hg food contamination can elicit adverse health outcomes, with acute and chronic toxicity to animals and humans.[25] These toxic effects are dependent on elemental speciation of Hg, contrasting from the least toxic Hg^0 form (limited bioavailability) to low solubility and highly bioavailable and toxic organomercurial compounds (e.g., methylmercury).[26].

A recent study observed that Hg and Pb accumulated in the YMW larvae after exposure to different substrates, including olive pomace residues.[11] Despite the observed uptake, the measured Hg values complied with the European Commission maximum limit (ECML) (2002/32/EC), and both substrates and insects could be used for feed (values below 0.1 mg Hg kg^{-1} of substrate, 20% moisture). The accumulation of Hg in the BSF larvae was also evaluated, using media enriched with seaweed as food, and confirmed bioaccumulation of Hg when seaweed content was increased in media.[27] Despite the observed increase in Hg bioaccumulation, the larvae concentrations were again below the ECML set for food and complete feed. Similar conclusions were found by Truzzi et al. (2020) when BSF prepupae were allowed to feed on substrates based on coffee roasting byproducts and microalgae.[28]

Though previous studies have focused on chemical uptake by edible insects, the rate at which an organism eliminates contaminants is key for understanding bioaccumulation. Thus, in the current study, we examined the uptake and elimination of Hg (reported as bioaccumulating in both BSF and YMW) in model edible insects consuming a Hg-contaminated substrate. We then employed toxicokinetic models to assess Hg bioaccumulation dynamics in edible insects. We specifically evaluated, for the first time, whether and how efficiently these model edible insects could eliminate the compound when placed in a clean substrate after exposure to Hg. Two-phase bioaccumulation experiments were conducted with YMW and BSF, initially with a Hg exposure phase and then followed by a depuration phase in a noncontaminated substrate. Thus, this information is crucial to increase the knowledge about using contaminated substrates in insect production, opening the possibility to increase this sector's impact on waste management. However, in order to do that, insect producers need this information to improve their production and management practices for protecting product safety before processing insects for commercialization.

2. MATERIALS AND METHODS

2.1. Test Organisms and Substrate Spiking with Hg. *2.1.1. Tenebrio molitor.* Yellow mealworm larvae were provided by Thunder Foods (Santarém, Portugal) from an established YMW colony that has been running since 2021, in which all developmental stages of *T. molitor* are reared. Newly hatched larvae were reared using semolina wheat bran as feed substrate and raw potato slices as a water source, provided twice a week. The larvae used in the current experiments were, on average, 17 weeks old, or at the 12−13° instar of development,[29] and had an approximate mean weight of 15−20 mg.

After they arrived at the applEE (applied Ecology and Ecotoxicology laboratory, CESAM, University of Aveiro (Portugal)), an acclimatization period of 1 week was established. Two days before initiating the bioaccumulation experiments, YMW were acclimated to the climate-controlled conditions maintained at a fixed temperature of 25 ± 2 °C with a 16:8 h (light/dark) photoperiod.

Throughout the experiments, YMW larvae were exposed to semolina wheat contaminated with 0.1 and 1 mg Hg kg^{-1} substrate, using Mercury(II) chloride ($HgCl_2$, CAS no. 7487-94-7) purchased from Merck Millipore (Darmstadt, Germany, 99.5% purity). Briefly, a stock solution was prepared, and the corresponding amount of working solution was added to 400 g of semolina wheat for each Hg concentration. In summary, 400 mL of ultrapure water containing Hg working solution was added to each Hg treatment level to obtain a proper spiking homogenization. Simultaneously, noncontaminated semolina wheat was submitted to the same process, adding the same amount of water (without Hg). After mixing accordingly, containers containing semolina wheat were lyophilized. This step was crucial to avoid fungal growth during the studies, considering the duration of the experiment (21 d) and the experimental temperature (25 ± 2 °C).

2.1.2. Hermetia illucens. The company Ingredient Odyssey SA - EntoGreen, also located in Santarém, Portugal, provided the BSF larvae used in this study from an established colony that has been running since 2015. Eggs were collected at the company and incubated for 2 days when the larvae started hatching. Larvae were then reared on a standard diet (Gainesville or GV diet, composed of 50% wheat bran, 30% alfalfa meal, 20% corn meal)[30] for 5 days. Due to their short life cycle, the larvae were shipped to the applEE (applied Ecology and Ecotoxicology laboratory, CESAM, University of Aveiro (Portugal)), where they were acclimated for 4 days at a fixed temperature (25 ± 2 °C) with a 16:8h (light/dark) photoperiod, prior to initiating the experiments. Subsequently, the larvae (3−4 mg) were exposed to a contaminated substrate made of the GV diet, specifically using 200 mL of ultrapure water per 100 g, at the same Hg concentrations used in the mealworm experiments (0.1 and 1 mg Hg kg^{-1} of substrate), and the same stock solution used in the YMW studies. Preliminary assays demonstrated that fungal growth was an obstacle to the bioaccumulation experimentation when BSF was exposed to a moistened GV diet. However, due to a higher need for water for BSF growth, the Gainesville substrate was autoclaved for 20 min before introducing contamination media to avoid fungal growth during experiments.

2.2. Bioaccumulation Studies. Both YMW and BSF Hg two-phase bioaccumulation assays were conducted based on previous experimental approaches using the YMW exposed to silver nanoparticles via soil or food,[32] the springtail *Folsomia candida* exposed to Hg contaminated food,[31] and the isopod *Porcellionides pruinosus* exposed to nano and non-nano $Cu(OH)_2$ forms via soil,[33] with adaptations related to the time of exposure of both insect species used in the present study.

2.2.1. Tenebrio molitor Experiments. Bioaccumulation studies using YMW were performed in plastic 6-well plates filled with 3 g of semolina wheat (noncontaminated, or 0.1 or 1 mg Hg kg^{-1} of substrate), maintained at a constant temperature of 25 ± 2 °C and 16:8 h light/dark. Organisms with sizes between 15 and 25 mg were exposed individually. This amount of feed was calculated based on the quantity provided to the larvae at Thunder Foods to guarantee that the insects had enough feed to consume throughout the entire experiment. This experiment consisted of an uptake phase followed by

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an elimination period, and each were 21 days in duration. In the uptake phase, organisms were exposed to Hg-contaminated semolina wheat (0.1 and 1 mg Hg kg^{-1} of substrate), and five organisms (replicates) were sampled on days 1, 3, 7, 14, and 21. At the beginning of the elimination phase (on day 21), organisms were moved to a noncontaminated substrate, and then organisms were sampled after 22, 24, 28, 35, and 42 days. The same procedure used in the uptake phase was conducted for the elimination phase. As a control, *T. molitor* larvae were maintained in noncontaminated semolina for 42 days. Three sampling times were performed (on days 0, 21, and 42) in the same experimental conditions. Five individual replicates/organisms were collected at each sampling time. For all conditions and at each sampling time, a depuration period was performed where organisms were left in individually empty plastic 6-well plates for 24 h to clear gut content.[32] Subsequently, organisms were weighed and frozen at −20 °C for Hg measurements at each sampling time for all treatments.

2.2.2. Hermetia illucens Experiments. Experiments with BSF larvae were conducted in plastic vessels with 4 cm diameter and 6 cm height, containing 7 g of moistened GV diet (noncontaminated, or 0.1 mg Hg kg^{-1} or 1 mg Hg kg^{-1} of substrate) at a constant temperature of 25 ± 2 °C. Organisms with 3−4 mg (third developmental instar)[34] were exposed individually. The same number of sampling times during the uptake (contaminated substrate) and the elimination phases (clean substrate) used in *T. molitor* experiments were conducted on different sampling days. In the uptake phase, organisms were sampled every day until day 5 at which time organisms were transferred to a clean substrate and then sampled every day until day 10 during the elimination period. A control condition was also designed, with BSF larvae maintained in a noncontaminated GV diet for the entire experimental period (10 days) with three sampling times (on days 0, 5, 10) under the same conditions. Five individual replicates/organisms were collected at each sampling time point, and organisms were left for 12 h to empty their gut in plastic containers (for organisms to depurate) at each sampling time for all treatment levels. After that, BSF larvae were weighed and frozen at −20 °C for Hg measurements.

2.2.3. Mercury Analysis. To determine the total mercury content in the insects and the different substrates, a Milestone DMA-80 Evo direct Mercury Analyzer was used, following US-EPA Method 7473.[35] Samples were dried inside the DMA-80 for 60 s at 300 °C, then the temperature was increased to 650 °C for 180 s, allowing for sample decomposition. Hg vapors were transported to the catalyst tube, removing the impurities and converting all forms of Hg to elemental Hg. The vapor was then transported to the gold amalgamator. After completing its flash heat cycle at 850 °C, Hg was released to an atomic absorption spectrophotometer, which measured absorption intensity at 253.65 nm. The Hg peak heights were then integrated, and the results were presented in mg Hg kg^{-1} sample.

Analytical quality and accuracy of the procedure were certified using the reference material PACS-3 (Marine Sediment Certified Reference Material for total and extractable metal content −2.98 ± 0.36 mg Hg/kg) and TORT-3 (Lobster hepatopancreas reference material for trace metals, National Research Council of Canada -0.292 ± 0.022 mg Hg/kg). Calibration blanks were run initially and between samples from different sampling times to check for and avoid possible contamination.

The mean Hg recovery from the reference material in the analytical procedure was 102.4% (±8.2%) for insect tissue analyses and 101.2% (±6.3%) for substrate analyses.

2.2.4. Toxicokinetics and Data Analysis. Since both YMW and BSF individuals can substantially change their biomass throughout the experiments, potentially introducing bias to the estimation of uptake and elimination kinetics, a growth dilution factor was included in the toxicokinetics models in the form of growth rate (k_{growth}), as proposed in refs 36 and 37. The parameter k_{growth} was estimated for each specific insect and experimental condition by fitting an exponential growth model (eq 1) to the biomass of organisms, for which the biomass values (mg, dry weight (DW)) obtained at each sampling time (n = 5) were used for this calculation:

$$B_t = B_0 e^{(K_{growth} t)} \quad (1)$$

where B_t is the biomass (mg) at time t (days), B_0 is the larvae's initial biomass (mg), and K_{growth} is the growth rate constant (day^{-1}).

Regarding the toxicokinetic evaluation of the data, uptake and elimination kinetics of Hg by both YMW and BSF were modeled using a one-compartment model, which considers the animal as a homogeneous compartment with single uptake and elimination rates, considering the K_{growth} in the model in SPSS (version 28).

For the uptake phase, the following equation was used (eq 2):

$$Q_{(t)} = C_0 + \frac{K_1}{K_2 + K_{growth}} C_{exp}(1 - e^{-(K_2 + K_{growth})t}) \quad (2)$$

where $Q_{(t)}$ is the concentration of Hg in the animal at t days (μg Hg/$g_{organism}$); C_0 is the basal internal Hg concentration (μg Hg $g_{organism}^{-1}$) calculated from the mean measured Hg body concentration at t = 0; K_1 is the uptake rate constant ($g_{substrate}$ $g_{organism}^{-1}$ day^{-1}); K_2 is the elimination rate constant (day^{-1}); C_{exp} is the measured Hg concentration in the exposure medium (mg Hg $kg_{substrate}^{-1}$); K_{growth} is the growth rate estimated using an exponential growth model (day^{-1}), and t is the time (days).

For the elimination phase (eq 3), the same approach was taken, and considered the growth rate constant in the model (model 1):

$$Q_{(t)} = C_0 + \frac{K_1}{K_2 + K_{growth}} C_{exp}(e^{-(K_2 + K_{growth})(t - t_c)} - e^{-(K_2 + K_{growth})t}) \quad (3)$$

where t_c is the last day of the uptake phase when the animals were transferred to clean substrate. Other parameters are described in eq 1 description.

The toxicokinetic parameters were estimated by nonlinear regression in SPSS (version 28). Potential differences between K_1 and K_2 values in the different Hg treatments were tested using a Generalized Likelihood Ratio Test.[32]

The Kinetic Bioaccumulation Factor ($BAF_{kinetic}$) ($kg_{substrate}$ per $kg_{organism}$) was calculated as the ratio of the uptake (K_1) and the elimination rate constants (K_2) (eq 4):

$$BAF_{kinetic} = \frac{K_1}{K_2} \quad (4)$$

The half-life values of Hg (DT_{50}) in YMW and BSF larvae were calculated (eq 5) as

$$DT_{50} = \frac{\ln(2)}{K_2} \quad (5)$$

The depuration times of both YMW (24 h) and BSF (12 h) were added to the obtained DT_{50}s of both species and Hg concentrations.

The Biota-Substrate Accumulation Factor (BSAF) (kg substrate per kg organism) was calculated by dividing the insects' internal Hg content at the last day of exposure (21 d for YMW and 5 d for BSF) (C_{org}) by the Hg concentrations in the substrate (C_{subst}) (eq 6):

$$BSAF = \frac{C_{org}}{C_{subst}} \quad (6)$$

3. RESULTS

3.1. Mercury Analysis in Substrates. The basal levels of Hg ranged from 0.006 ± 0.001 mg Hg kg^{-1} (mean ± SD, n = 5) in the GV diet for BSF to 0.007 ± 0.001 mg Hg kg^{-1} (n = 5) in semolina wheat provided to YMW.

Since differences between nominal and measured values of Hg were lower than 16% in all treatments, nominal concentrations were used throughout the manuscript, and measured concentrations were used in the data analysis and interpretation. All measurements are presented using dry weight (DW) values.

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3.2. - Bioaccumulation Studies: Growth Rates and Toxicokinetics of Hg in Insects. *3.2.1. Tenebrio molitor Experiments.* At the beginning of the experiments, the background Hg concentration in *T. molitor* larvae was 0.051 ± 0.019 mg Hg kg^{-1}, dry body weight (Table 1). Longer exposures to Hg in substrate led to higher Hg content in insects, especially when exposed to semolina at 1 mg Hg kg^{-1} (Figure 1). On day 21, the last day of exposure to the contaminated substrate, tissue levels in mealworm larvae exposed to 0.1 and 1 mg Hg kg^{-1} of Hg were 0.1319 ± 0.0575 mg Hg kg^{-1} and 1.0590 ± 0.2867 mg Hg kg^{-1}, respectively (Table 1). For the 1 mg Hg kg^{-1} treatment level, larvae reached their maximum Hg internal concentration on day 21, the last day of the uptake phase. YMW exposed to 1 mg Hg kg^{-1} reached their maximum Hg content on day 14. After moving the organisms to a clean substrate, Hg body concentration in mealworms declined over time in both Hg treatment levels (Figure 1). After 21 days in the clean substrate, YMW eliminated Hg from their bodies, reaching the same Hg levels as control individuals (Table 1).

The k_{growth}, estimated by fitting an exponential growth model to the biomass of all organisms sampled throughout the experiments, was 0.020 ± 0.002 and 0.027 ± 0.002 day^{-1} for 0.1 and 1 mg Hg kg^{-1} treatment levels, respectively (Figure SD1; Table 2). These values were included in the toxicokinetic models (eqs 2 and 3) to improve the estimates for uptake and elimination kinetics, displayed in Figure 1 and Table 2, where we can confirm a similarity between both K_1 and K_2 in the exposure to both Hg concentrations. Therefore, exposure of mealworms to 0.1 and 1 mg Hg kg^{-1} led to nonsignificantly different K_1 ($X^2_{(1)}$= 0.067; $p > 0.05$) and K_2 ($X^2_{(1)}$ = 0; $p > 0.05$) values between these two exposures. Similar uptake and elimination constants were also observed in the kinetic BAF, with values of 0.529 $g_{substrate}$ $g_{organism}^{-1}$ and 0.898 $g_{substrate}$ $g_{organism}^{-1}$ following exposure to 0.1 and 1 mg Hg kg^{-1}, respectively (Table 2). Similar BSAF values were also obtained when organisms were exposed to 0.1 and 1 mg Hg kg^{-1} (1.133 $g_{substrate}$ $g_{organism}^{-1}$ and 0.919 $g_{substrate}$ $g_{organism}^{-1}$, respectively). The half-life for Hg elimination (DT_{50}) (Table 2) was also quite similar between both Hg concentrations in the substrate, ranging from 4.6 to 4.8 days.

3.2.2. Hermetia illucens Experiments. Mercury background levels in *H. illucens* were 0.015 ± 0,004 mg Hg kg^{-1} DW, and thus were consistent with the other two control sampling times (days 5 and 10) (Table 1). For both Hg treatment levels in the substrate, larvae reached maximum Hg internal body content levels on day 2 for 0.1 mg Hg kg^{-1} and day 3 for 1 mg Hg kg^{-1}. Internal Hg body content then decreased until the last day of the uptake phase, where organisms reached 0.138 ± 0.044 mg Hg kg^{-1} and 0.967 ± 0.247 mg Hg kg^{-1} for the substrate's lower and higher Hg contamination, respectively (Table 1). Here again, all measurements were performed using DW values.

Regarding the elimination of Hg by BSF, levels of Hg in the organisms decreased over time (Figure 1), reaching their lowest levels on day 10 of the depuration phase for both Hg treatments (Table 1). At that point, internal Hg body content was similar to control values, indicating that BSF had eliminated Hg taken up from substrates.

Prior to performing toxicokinetic modeling, growth of the insects in both Hg concentrations was evaluated, obtaining a K_{growth} of 0.319 ± 0.016 day^{-1} and 0.306 ± 0.015 day^{-1} for 0.1 and 1 mg Hg kg^{-1} treatment levels (Figure 1; Table 2).

Table 1. Measured Total Hg Concentrations (mg Hg kg^{-1}) in *Tenebrio molitor* and *Hermetia illucens* Larvae Exposed to Contaminated Substrates with Two Different Hg Concentrations (0.1 and 1 mg Hg kg^{-1}) and Negative Control (Substrate without Hg), in Time[a]

		Uptake phase						Elimination phase					
Species	Treatment	Day 0	Day 1	Day 3	Day 7	Day 14	Day 21	Day 22	Day 24	Day 28	Day 35		Day 42
Tenebrio molitor	Control 0 mg kg^{-1}	0.0515 (0.0192)	-	-	-	-	0.0733 (0.0235)	-	-	-	-		0.0186 (0.0086)
	0.1 mg kg^{-1}	0.0515 (0.0192)	0.0497 (0.0249)	0.0351 (0.0181)	0.0702 (0.0280)	0.1344 (0.0206)	0.1319 (0.0575)	0.0772 (0.0266)	0.0439 (0.0167)	0.0250 (0.1563)	0.0169 (0.0031)		0.0359 (0.0176)
	1 mg kg^{-1}	0.0515 (0.0192)	0.0719 (0.0875)	0.5913 (0.3337)	0.8520 (0.2887)	0.8282 (0.5848)	1.0590 (0.2867)	0.5773 (0.2581)	0.5425 (0.5821)	0.1108 (0.5157)	0.0338 (0.0163)		0.0215 (0.0098)
		Uptake phase						Elimination phase					
Species	Treatment	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9		Day 10
Hermetia illucens	Control 0 mg kg^{-1}	0.0146 (0.0041)	-	-	-	-	0.0163 (0.0061)	-	-	-	-		0.0066 (0.0006)
	0.1 mg kg^{-1}	0.0146 (0.0041)	0.0867 (0.0199)	0.2060 (0.1752)	0.1204 (0.0302)	0.1316 (0.0186)	0.1385 (0.0438)	0.0571 (0.0360)	0.0472 (0.0110)	0.0200 (0.0039)	0.0252 (0.0198		0.0183 (0.0035)
	1 mg kg^{-1}	0.0146 (0.0041)	0.9602 (0.1309)	1.5349 (0.4406)	1.6294 (0.2199)	1.544 (0.2992)	0.9669 (0.2469)	0.8301 (0.3872)	0.1906 (0.3238)	0.1796 (0.0787)	0.1050 (0.0620)		0.0681 (0.0375)

[a]Data are shown as average ± SD with five replicates with one organism sampled per day and Hg concentration.

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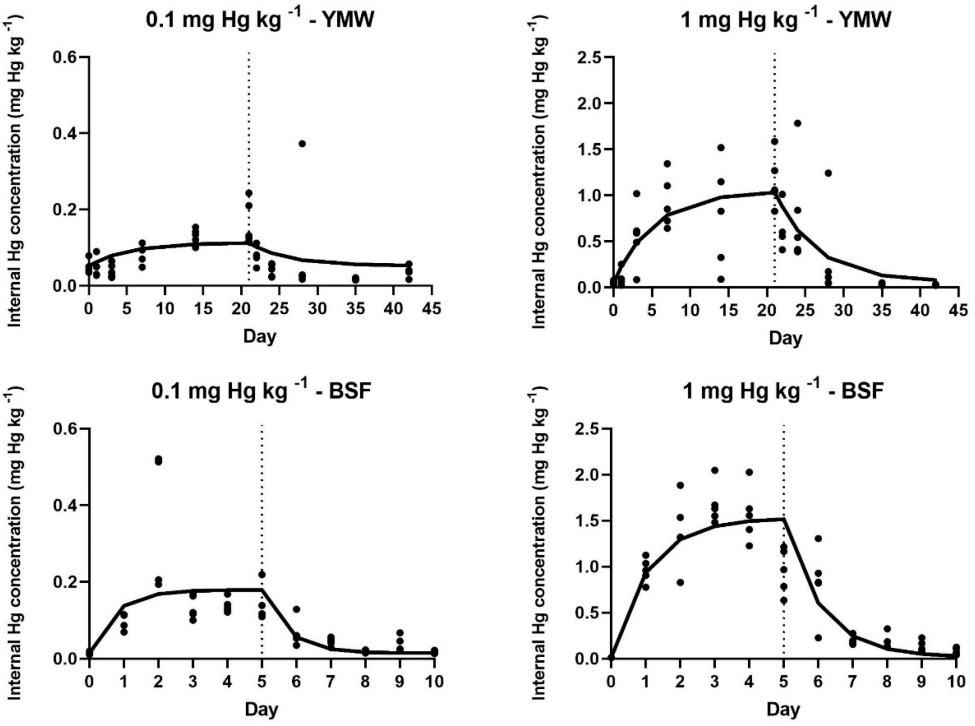

Figure 1. Uptake and elimination kinetics of Hg in *Tenebrio molitor* (YMW) and *Hermetia illucens* (BSF) (top and bottom graphs, respectively) exposed to 0.1 and 1 mg Hg kg^{-1} of Hg in the substrate (left and right-side graphs, respectively) for 21 days plus 21 days in a clean substrate for YMW and 5 plus 5 days for BSF. Points show measured values (n = 5). Solid lines show the fit of a one-compartment model with growth dilution (eqs 2 and 3). The dashed vertical line defines the last day of exposure to the contaminated substrate, ending the uptake and elimination phases of the experiment.

Table 2. Uptake and Elimination Kinetic Parameters for Hg in *Tenebrio molitor* and *Hermetia illucens* Exposed to 0.1 and 1 mg Hg kg^{-1} Substrate[a]

	Treatment	K_{growth} (day^{-1})	K_1 ($g_{substrate}$ $g_{organism}^{-1}$ day^{-1})	K_2 (day^{-1})	DT_{50} (days)	$BAF_{kinetic}$ ($g_{substrate}$ $g_{organism}^{-1}$)	BSAF ($g_{substrate}$ $g_{organism}^{-1}$)
Tenebrio molitor	0.1 mg kg^{-1}	0.020 (0.002)	0.096 (0.057)	0.161 (0.113)	4.815	0.529	1.133
	1 mg kg^{-1}	0.027 (0.002)	0.172 (0.037)	0.164 (0.043)	4.628	0.898	0.919
Hermetia illucens	0.1 mg kg^{-1}	0.319 (0.016)	2.056 (0.771)	1.071 (0.554)	0.975	1.438	1.247
	1 mg kg^{-1}	0.306 (0.015)	1.402 (0.176)	0.631 (0.128)	1.60	2.222	0.954

[a] K_1 (uptake rate constant) and K_2 (elimination rate constant) were estimated using the one-compartment model, assuming a growth dilution. K_{growth} (growth rate constant) was derived using an exponential growth curve (eq 1) for each insect species, Hg exposure concentrations, and DT_{50} (half-life for Hg elimination + depuration period), $BAF_{kinetic}$ (Kinetic Bioaccumulation Factor), and BSAF (The Biota-Substrate Accumulation Factor) were derived according to eqs 4, 5, and 6, respectively. Values in brackets represent the standard error for each parameter.

Toxicokinetic analysis demonstrated constant uptake rates of 2.056 ± 0.771 ($g_{substrate}$ $g_{organism}^{-1}$ day^{-1}) and 1.402 ± 1.76 ($g_{substrate}$ $g_{organism}^{-1}$ day^{-1}) when exposed to 0.1 and 1 mg Hg kg^{-1} in the substrate, respectively (Table 2). Elimination constant rates were 1.071 ± 0.554 per day and 0.631 ± 0.128 per day when exposed to substrate with 0.1 and 1 mg Hg kg^{-1}, respectively (Table 2). Toxicokinetic evaluation confirmed that organisms exposed to 0.1 mg Hg kg^{-1} reached steady state on day 4, but this did not happen to organisms exposed to 1 mg Hg kg^{-1}, and calculated BAFs were 1.438 $g_{substrate}$ $g_{organism}^{-1}$ and 2.222 $g_{substrate}$ $g_{organism}^{-1}$ for the lowest and highest concentration of Hg in the substrate, respectively (Table 2). BSAFs were 1.247 $g_{substrate}$ $g_{organism}^{-1}$ and 0.954 $g_{substrate}$ $g_{organism}^{-1}$ for the same exposures, respectively. Exposure of BSF to 0.1 and 1 mg Hg kg^{-1} led to nonsignificantly different K_1 ($X^2_{(1)}$ = 0.197; p > 0.05) and K_2 ($X^2_{(1)}$ = 0.175; p > 0.05) values between these two treatment levels. Due to the high elimination rates observed for BSF, very short half-lives for Hg elimination were observed, specifically at only 0.975 days for 0.1 mg Hg kg^{-1} and 1.6 days for the 1 mg Hg kg^{-1} treatment level (Table 2).

4. DISCUSSION

The United Nations projects that a 50% increase in food will be needed globally, and a 200% increase will be required in low and middle-income countries by 2050.[38] Herein, edible insects promise to help address this pressing need that lies at the intersections of sustainability and circularity and hunger and poverty. We designed the present study to understand whether and to what extent Hg is accumulated and also eliminated in two of the most used insects reared for food or feed with high commercial value. Here we focused on Hg, given previous reports of Hg accumulation in insects,[11] and we specifically examined Hg bioaccumulation dynamics in edible insects for the first time, which has implications for improving product safety practices.

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The use of toxicokinetic tools extends beyond descriptive analysis toward more mechanistic, time-efficient, and potentially predictive research that contributes to defining strategies to improve food/feed safety in insect farming. Evaluating chemical hazards in edible insects is necessary using toxicokinetic evaluation because it can develop a predictive understanding of how rapidly insects take up, eliminate, and thus bioaccumulate a chemical. It is possible to estimate if insects can regulate (by looking at the steady state of the chemical in the organism) and how long it takes to reach the maximum equilibrium of an internal concentration, considering the exposure scenario. At the same time, identifying and ideally confirming if an organism eliminates a chemical when contaminant exposure ceases is critical knowledge to calculate elimination rates and thus inform the time it takes for contaminants to reach acceptable food and feed safety levels. Thus, elimination kinetic information can directly define minimum temporal thresholds for depuration needed to manage the rearing of edible insects within a food safety context.

Though previous work by Morgado et al. (2022) and Khodaparast et al. (2021) evaluated the bioaccumulative capacity of different chemicals (e.g., copper and silver nanoparticles, respectively) in YMW, to the best of our knowledge, this is the first time that the uptake and elimination of Hg over time was defined using two valuable insect species for industrial purposes.[32,33] We observed both insect species to have a high capacity to regulate internal tissue levels of Hg. This accumulation depended on the concentration of Hg in the substrate and exposure time. Such observations are generally consistent with the findings of Biancarosa et al. (2018) and Truzzi et al. (2020, 2019).[11,27,28] Truzzi et al. (2019) highlighted that Hg in larvae tissue levels was clearly influenced by Hg content in the feeding substrate (which included olive pomace).[11] In that study, despite feeding substrates containing low Hg content (well below the 0.1 mg Hg kg^{-1} ECML (2002/32/EC)), significant differences in Hg content in insects were observed among all feeding substrates tested during a four-month-long (from the first instar to the last instar) experiment. This led to a statistically significant linear correlation between Hg content in feeding substrates (with the increased % of olive pomace in the substrate) and larvae, with the BAF for Hg ranging from 1.5 to 6.2 in YMW, higher values than the ones observed here in this study (both $BAF_{kinetic}$ and BSAF). These differences highlight that the contaminant concentration in the substrate and the time of exposure will influence the bioaccumulation of the compound by the organism. In all cases, Hg content in larvae was below the current ECML for Hg in feed.[11]

Accumulation of Hg in insects used for food and feed has also been studied by Biancarosa et al. (2018), in which larval accumulation of Hg was reported during 8 days when BSF was fed on seaweed-enriched media.[27] Our findings in the present study are consistent with these previous observations, where an increased Hg concentration was observed in organisms when more seaweed was added to the feeding media. A BAF closer to 1 was observed by Biancarosa et al. (2018), which is similar to our current results, even though the studies contained different substrates and exposure times. Though accumulation of Hg by insect larval was below the current ECML in complete feed, other metals (cadmium and total arsenic) were above corresponding regulatory limits when seaweed inclusion exceeded 20% in the media. Clearly, future research is needed to understand the toxicokinetic dynamics of Cd, As, and other contaminants in edible insects.

The present study highlights the need for kinetic-based bioaccumulation studies of Hg and other chemical contaminants in edible insects. Here we observed that at the end of the uptake phase (21 days of YMW and 5 days for BSF), both insects presented a Hg concentration above the 0.1 mg Hg kg^{-1} ECML (15−20% more), leading to kinetic BAFs of 0.529 g_{food}/g_{tissue} and 1.438 g_{food}/g_{tissue} as well as BSAFs of 1.133 g_{food}/g_{tissue} and 1.247 g_{food}/g_{tissue} for YMW and BSF, respectively. Differences in the obtained kinetic BAF and BSAF highlight the importance of this toxicokinetic evaluation, which considers the time of exposure, the growth of the organisms and also the uptake and elimination of the compound, rather than looking at the concentration of the compound inside the organism at a specific time and the exposure concentration. These results identify a potential issue when considering the product safety of insects as a protein source for other animals and humans. Such uptake of Hg by YMW and BSF was expected based on previous studies. However, data from the current bioaccumulation experiments demonstrated that both insects could quickly eliminate Hg from their tissues back to background levels. When the DT_{50} values, a key parameter in this study, are considered, then BSF eliminates half of the accumulated Hg in only a day. Therefore, even though BSF accumulated Hg to levels during the uptake phase that would not allow these organisms to be used as feed or food for other animals, a one-day period was sufficient for these edible insects to decrease tissue levels of Hg to below the legal limit for product safety and animal consumption. Similar rapid elimination of Hg was observed by YMW, which took 4.6−4.8 days to eliminate half of the Hg accumulated. Though this time for elimination was higher than BSF, the life cycle of the YMW is different such that their time to be ready for harvesting in rearing facilities is also longer. This life cycle difference is thus related to a similar proportion of exposure time and the time to eliminate half of the accumulated Hg.

Our results suggest that instead of redefining levels in the current legislation for insects to feed, a short period of elimination/depuration in clean standard substrate could be proposed for insects after exposure to the feeding substrate, mainly when reared in other wastes that are not so well characterized (olive pomace, seaweed, roasting coffee, among others). To advance the translation of bioaccumulation science to food safety practices for edible insects, information about the elimination capacity of the different contaminants will be needed in future studies. If relatively rapid, first-order elimination kinetics are observed for other contaminants, then the use of waste streams could be revised, augmenting the circularity of this waste management technology with insects. This contribution could be significant since insects can only be currently used for processing preconsumer vegetable waste in the EU and other locations worldwide. If an understanding of bioaccumulation kinetics is advanced, then product safety concerns related to the reuse and bioconversion of waste by insects could be managed, and insects would provide a sustainable biotechnology solution for use in more circular practices. It should also be highlighted that both insects can regulate higher concentrations of Hg. Even when exposed to 10-fold higher levels of Hg in the substrate than the ECML, such observations for both insects with Hg were similar, despite the expected increase of Hg tissue residues.

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Both YMW and BSF present a highly efficient opportunity to transform waste biomass into protein- and fat-rich insect biomass.[39,40] In the present study, we observe BSF grow faster than YMW, based on the tested substrates, and BSF larvae take up more Hg from these substrates due to their higher metabolic activity. However, these larvae could also quickly eliminate Hg from their tissues. YMW presented lower uptake and constant elimination rates, with higher DT_{50} related to the elimination of Hg from their bodies. These studies are critical since, for example, the marketing of dried mealworms was recently authorized in May 2021 and further received authorization to be placed on the market as a novel food due to its acceptable food safety.[41] With the pressure to use insects as bioconverters and protein sources, developing a predictive understanding of bioaccumulation and improved management practices will be important for other contaminants to ensure acceptable product safety.

In summary, the present study demonstrated that the commercially important insect species YMW and BSF could eliminate tissue levels of Hg acquired during uptake from a Hg-contaminated substrate. Our toxicokinetic evaluation afforded derivation of uptake and elimination rates, which allowed the calculation of kinetic-based BAFs for Hg and a half-life for eliminating Hg in insects. We observed that BSF take up more Hg from the substrate than YMW, which led to a higher BAF for this species. However, elimination by BSF also took place faster. After exposing these model insects to substrate with Hg at the maximum allowed levels by EU for feed, we identified that both insects could accumulate Hg levels that were unsuitable for feed to be consumed by other animals, such as fish, cattle, or pigs. However, due to the fast elimination of Hg from their bodies, especially in the case of BSF, a short depuration period of a few days was sufficient to achieve tissue levels considered acceptable for these insects' consumption. This observation is timely because if the insects are used as waste converters, some concerns exist for contaminants that may be present. Due to life-cyle differences, the elimination period necessary to achieve acceptable product safety levels of Hg was observed to be species-dependent. It is essential to highlight that this study was designed to understand Hg (as model chemical) uptake and elimination kinetics at a laboratory scale. Future work should focus on improving and scaling up this assessment strategy, with more replicates, more organisms, and additional samplings times, that would be used to validate our findings for broader implementation within the practice. Future studies with other contaminants and organisms are needed, and these additional efforts would benefit from using the toxicokinetic approach presented here to understand uptake and elimination dynamics in insects intended to be used for food and feed.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsfoodscitech.3c00051.

Body weight (mg, DW) of *Tenebrio molitor* and *Hermetia illucens* recorded during a 21-day and 5-day exposure, respectively, to Hg-contaminated substrate (PDF)

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Notes

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■ ABREVIATIONS

EU European Union
Hg mercury
YMW *Tenebrio molitor*
BSF *Hermetia illucens*
EFSA European Food Safety Authority
ECML European Commission maximum limit
k_{growth} Growth rate
DW Dry weight
K_1 Uptake rate constant
K_2 Elimination rate constant
$BAF_{kinetic}$ Kinetic Bioaccumulation Factor
DT_{50} Half-life Hg in organism
BSAF Biota-Substrate Accumulation Factor

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