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Occurrence of Selected Organic Contaminants in Edible Insects and Assessment of Their Chemical Safety

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Background: Feeding the continuously growing world population is challenging, and edible insects offer a sustainable alternative to conventional sources of animal proteins. As with any food source, the potential presence of hazardous organic chemicals, such as persistent organic pollutants (POPs), plasticizers and flame retardants (FRs), must be investigated to guarantee consumer chemical safety.

Objectives: Here, we have investigated the contamination levels of several classes of organic compounds in edible insects. To evaluate their chemical safety, a dietary exposure risk assessment was then performed by combining the measured chemical contamination with the most recent food consumption data from local surveys.

Methods: Insect samples, belonging to six orders (Orthoptera, Coleoptera, Lepidoptera, Hemiptera, Odonata, Hymenoptera) were purchased from five European and three Asian countries. POPs and halogenated FRs were analyzed by gas chromatography–mass spectrometry (GC/MS) and organophosphorus FRs and plasticizers were quantified by liquid chromatography–MS/MS, according to validated protocols.

Results: The overall levels of chemical contamination varied greatly among the insect orders and country of purchase, but they were generally low and comparable with other commonly consumed animal products.

Discussion: Here we show that, besides the activities during rearing, the industrial post-harvesting handling and addition of ingredients are supplementary factors influencing the chemical load of the final insect food-product. The total estimated dietary intakes of the considered classes of compounds through insect consumption are comparable with those generally assessed in common food of animal origin worldwide and, when compared with existing reference dose values, suggest that the risk of adverse health effects from exposure to the targeted organic compounds via insect consumption is unlikely. https://doi.org/10.1289/EHP5782

Introduction

Due to the exponential growth of the world's population, which is expected to reach 9 billion people by 2050, current agricultural practices and water consumption are predicted to increase pressure on the environment and biodiversity (van Huis 2013a). It is well-known that many current food production systems are not sustainable from a global perspective, due to the growing demand for animal proteins and the decreasing availability of agricultural land and freshwater supplies (van Huis 2015). To anticipate this challenge, there is an urgent need to invest in innovative solutions for food production, such as new protein sources (van Huis 2015). In this perspective, the opportunity for edible insects as an alternative to the rising demand for meat and fish products is remarkable, especially considering that many insect species are already consumed by humans in a large part of the world (a practice known as entomophagy) (van Huis 2013a, 2015; Jansson and Berggren 2015). Insects have a high nutritional value, generate low emissions of greenhouse gases and ammonia, and are significantly more efficient than other livestock in terms of feed conversion (Dobermann et al. 2017; van Huis 2013b). In addition to nutritional and environmental merits, feeding insects to humans or livestock offers economic benefits through farming and trading in both developing and developed countries (Dobermann et al. 2017). However, in Western societies, entomophagy is still met with instinctive resistance and negative reactions. Consumer acceptance remains thus the largest barrier toward global insect consumption, even when the advantages over conventional protein sources are acknowledged (Dobermann et al. 2017; Jansson and Berggren 2015; Mlcek et al. 2014). The production and marketing of edible insects in Europe is regulated by the Novel Foods legislation (EU Reg. No. 2015/2283) (EC 2015), which states that insect producers must conform with the same general rules that apply to operators in other food/feed sectors, making them responsible for the safety of the marketed products (IPIFF 2019). As is observed in other animals, there is in fact a potential for insects to accumulate hazardous chemicals, including persistent organic pollutants (POPs), flame retardants, and metals during the rearing process (Poma et al. 2017a).

In this study, the contamination status of selected classes of hazardous organic compounds was assessed in different insect species commercialized and purchased exclusively for human consumption. Differences were explored between samples purchased in Europe, where entomophagy is still in its infancy, and in Asia, where the practice is already well established. To evaluate the chemical safety of the analyzed edible insects, a dietary exposure risk assessment was performed by combining measured chemical contamination levels with the most recent consumption data from local surveys.

Methods

Information about the characteristics of the targeted groups of compounds are provided in the "Characteristics of targeted organic compounds" section in the Supplemental Material. The data set generated during or analyzed during the current study is available as Supplemental Excel File.

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Sample Purchasing and Preparation

Selected species of edible insects were purchased between September 2017 and September 2018 from five European and three Asian countries at various shops, e-shops, and supermarkets (Table 1). All purchased composite samples ($n = 35$) were authorized for human consumption and belonged to six different orders (Orthoptera, 49%; Coleoptera, 34%; Lepidoptera, 9%; Hemiptera, 3%; Odonata, 3%; Hymenoptera, 3%). Only Orthoptera and Coleoptera were available for purchase in Europe, whereas all orders were available in Asia. Thirty samples were purchased in their natural state (i.e., without addition of any ingredients), whereas the other five were purchased seasoned (i.e., with added flavors and dressings). Due to their small individual size, multiple insects from the same species and from the same retailer/country were pooled for analysis into composite samples (henceforth simply called samples), freeze-dried, homogenized, and stored in aluminum foil at −20°C until analysis. The lipid content of each sample was determined using a gravimetric method. About 1 g of dry sample was weighed and solid-liquid extracted with 5 mL of *n*-hexane:acetone (3:1, vol/vol). After extraction, a volume of 1 mL of extract was transferred to a small precleaned metal tray and dried at 110°C for 1 h. The weight difference of the tray, corrected by the loss of water of the sample during lyophilization, was further used for lipid content calculation on wet weight (ww) basis (Table 1).

Chemicals, Materials, and Reagents

Chemicals. The list of targeted compounds consisted of 31 persistent organic pollutants (POPs), including 20 polychlorinated biphenyls (PCBs) and 11 organochlorine compounds (OCPs); 11 halogenated flame retardants (HFRs), including 9 polybrominated diphenyl ethers (PBDEs) and 2 dechlorane plus (DPs); 18 plasticizers, including 7 legacy plasticizers (LPs), and 11 alternative plasticizers (APs); 17 phosphorous flame retardants (PFRs), including 12 legacy PFR, and 5 emerging PFRs (ePFRs); 8 LP biotransformation products (LPs-BT), 11 AP biotransformation products (APs-BT), and 12 PFR biotransformation products (PFRs-BT) (see Table S1).

Standards of PFR parent compounds, PCBs, PBDEs, and DPs were purchased from Wellington Laboratories, and PFRs-BT and PFR deuterated internal standards (ISs) were custom synthesized by V. Belov (Max Planck Institute, Göttingen, Germany). Bromodiphenyl ether (BDE)-77, OCPs, ePFRs, and plasticizers were purchased from AccuStandard, and recovery standard (RS)

Table 1. Sample details.

		Species									
ID	Order	Scientific name	Common name	Life stage	Purchasing continent	Purchasing country	Origin country	Purchasing status	Purchasing year	Packaging type	Lipid (% on ww)
EU-AT-01	Orthoptera	*Acheta domesticus*	Cricket	Adult	Europe	Austria	Austria	Natural	Jul 2018	Plastic	6.4
EU-BE-01	Orthoptera	*Acheta domesticus*	Cricket	Adult	Europe	Belgium	Netherlands	Natural	Jul 2018	Plastic-PET	5.4
EU-FR-01	Orthoptera	*Acheta domesticus*	Cricket	Adult	Europe	France	Thailand	Seasoned	Jul 2018	Plastic	4.2
EU-NL-01	Orthoptera	*Acheta domesticus*	Cricket	Adult	Europe	Netherlands	Netherlands	Natural	Jul 2018	Plastic	7.9
EU-UK-01	Orthoptera	*Acheta domesticus*	Cricket	Adult	Europe	UK	Netherlands	Natural	Jul 2018	Plastic	8.5
EU-AT-02	Orthoptera	*Locusta migratoria*	Grasshopper	Adult	Europe	Austria	Austria	Natural	Jul 2018	Plastic	6.1
EU-BE-02	Orthoptera	*Locusta migratoria*	Grasshopper	Adult	Europe	Belgium	Netherlands	Natural	Jul 2018	Plastic-PET	4.4
EU-FR-02	Orthoptera	*Locusta migratoria*	Grasshopper	Adult	Europe	France	Thailand	Natural	Jul 2018	Plastic	2.4
EU-NL-02	Orthoptera	*Locusta migratoria*	Grasshopper	Adult	Europe	Netherlands	Netherlands	Natural	Jul 2018	Plastic	8.1
EU-UK-02	Orthoptera	*Locusta migratoria*	Grasshopper	Adult	Europe	UK	Netherlands	Natural	Jul 2018	Plastic	6.8
EU-AT-03	Coleoptera	*Tenebrio molitor*	Mealworm	Larva	Europe	Austria	Austria	Natural	Jul 2018	Plastic	9.7
EU-BE-03	Coleoptera	*Tenebrio molitor*	Mealworm	Larva	Europe	Belgium	Netherlands	Natural	Jul 2018	Plastic-PET	10.4
EU-FR-03	Coleoptera	*Tenebrio molitor*	Mealworm	Larva	Europe	France	Thailand	Natural	Jul 2018	Plastic	15.3
EU-NL-03	Coleoptera	*Tenebrio molitor*	Mealworm	Larva	Europe	Netherlands	Netherlands	Natural	Jul 2018	Plastic	9.9
EU-UK-03	Coleoptera	*Tenebrio molitor*	Mealworm	Larva	Europe	UK	Netherlands	Natural	Jul 2018	Plastic	8.6
AS-PRC-01	Coleoptera	*Tenebrio molitor*	Mealworm	Larva	Asia	P.R. China	P.R. China	Natural	Sept 2017	Plastic	10.6
AS-PRC-02	Lepidoptera	*Bombyx mori*	Silkworm	Larva	Asia	P.R. China	P.R. China	Natural	Sept 2017	Plastic	10.1
AS-PRC-03	Hemiptera	*Cryptotympana atrata*	Cicada	Larva	Asia	P.R. China	P.R. China	Natural	Sept 2017	Plastic	3.1
AS-PRC-04	Orthoptera	*Acheta domesticus*	Cricket	Adult	Asia	P.R. China	P.R. China	Natural	Sept 2017	Plastic	3.4
AS-PRC-05	Orthoptera	*Locusta migratoria*	Grasshopper	Adult	Asia	P.R. China	P.R. China	Natural	Sept 2017	Plastic	3.8
AS-PRC-06	Odonata	*Pantala flavescens Fabricius*	Dragonfly	Larva	Asia	P.R. China	P.R. China	Natural	Sept 2017	Plastic	7.6
AS-JPN-01	Lepidoptera	*Bombyx mori*	Silkworm	Larva	Asia	Japan	Japan	Seasoned	Jul 2018	Glass	9.2
AS-JPN-02	Orthoptera	*Locusta migratoria*	Grasshopper	Adult	Asia	Japan	Japan	Seasoned	Jul 2018	Glass	2.3
AS-JPN-03	Hymenoptera	*Vespula flaviceps*	Bee	Larva	Asia	Japan	Japan	Seasoned	Jul 2018	Tin can	5.8
AS-SK-01	Coleoptera	*Protaetia brevitarsis seulensis*	Grub	Larva	Asia	R. Korea	R. Korea	Natural	Sept 2018	Plastic	6.8
AS-SK-02	Coleoptera	*Protaetia brevitarsis seulensis*	Grub	Larva	Asia	R. Korea	R. Korea	Natural	Sept 2018	Plastic	5.4
AS-SK-03	Orthoptera	*Oxya japonica Thunberg*	Grasshopper	Adult	Asia	R. Korea	P.R. China	Natural	Sept 2018	Plastic-PE	1.0
AS-SK-04	Orthoptera	*Oxya japonica Thunberg*	Grasshopper	Adult	Asia	R. Korea	R. Korea	Natural	Sept 2018	Plastic	1.8
AS-SK-05	Orthoptera	*Nemobius sylvestris*	Cricket	Adult	Asia	R. Korea	P.R. China	Natural	Sept 2018	Plastic	4.0
AS-SK-06	Orthoptera	*Nemobius sylvestris*	Cricket	Adult	Asia	R. Korea	R. Korea	Natural	Sept 2018	Plastic	4.2
AS-SK-07	Coleoptera	*Tenebrio molitor*	Mealworm	Larva	Asia	R. Korea	R. Korea	Natural	Sept 2018	Plastic	9.6
AS-SK-08	Coleoptera	*Tenebrio molitor*	Mealworm	Larva	Asia	R. Korea	R. Korea	Natural	Sept 2018	Plastic	11.8
AS-SK-09	Coleoptera	*Tenebrio molitor*	Mealworm	Larva	Asia	R. Korea	P.R. China	Natural	Sept 2018	Plastic-PE	4.7
AS-SK-10	Coleoptera	*Tenebrio molitor*	Mealworm	Larva	Asia	R. Korea	P.R. China	Natural	Sept 2018	Plastic	7.4
AS-SK-11	Lepidoptera	*Bombyx mori*	Silkworm	Larva	Asia	R. Korea	R. Korea	Seasoned	Jul 2018	Tin can	8.6

Note: Analyzed samples ($n = 35$). AS, Asia; AT, Austria; BE, Belgium; EU, Europe; FR, France; ID, identification code; JPN, Japan; NL, Netherlands; PE, polyethylene; PET, polyethylene terephthalate; PRC, People's Republic of China; SK, Republic (R) of Korea; UK, United Kingdom; ww, wet weight.

triamyl phosphate (TAP) was obtained from TCI Europe. RS chlorobiphenyl CB-207 and IS CB-143, ^{13}C-hexachlorobenzene (^{13}C-HCB), and ε-hexachlorocyclohexanes (ε-HCH) were purchased from Dr. Ehrenstorfer Laboratories.

Materials. Polypropylene (PP) tubes (15 mL) were obtained from Greiner Bio-One. Sodium chloride (NaCl) was purchased from Sigma-Aldrich. C18 sorbent powder, primary-secondary amine (PSA) and Florisil® ENVI cartridges (500 mg, 3 mL) were purchased from Supelco. Centrifugal filters (nylon membrane, 0.2 μm) were obtained from VWR International. Empty PP cartridges (25 mL) were purchased from Agilent Technologies, and silica gel from Merck.

Reagents. *n*-Hexane (hex) was purchased from Acros Organics; ethyl acetate (EtAc), dichloromethane (DCM), iso-octane, acetone, acetonitrile (ACN), toluene, and concentrated sulfuric acid (H_2SO_4, 98%) were purchased from Merck. Methanol (MeOH), analytical grade formic acid (FA, 99–100%) and ammonium acetate (NH_4Ac) were purchased from Sigma-Aldrich. All solvents were analytical reagent grade or equivalent analytical purity. liquid chromatography (LC)-grade ultrapure water (UPW) was obtained from a PURELAB Flex system ($\rho = 18.2$ MΩ/cm; Elga Veolia).

Analysis of Persistent Organic Pollutants and Halogenated Flame Retardants

Analysis of POPs and HFRs was performed according to the protocol reported by (Poma et al. 2017a). Briefly, 1.0 g of dry sample was weighed in a prewashed 15-mL PP tube, spiked with an IS mixture (including CB-143: 200 pg/μL and ^{13}C-HCB, ε-HCH, BDE-77: 25 pg/μL) and added to 5 mL of hex:DCM (1:1, vol/vol). The tube was vortexed 1 min, centrifuged at 3,000 rpm for 5 min, and the supernatant was transferred to a precleaned glass tube. This procedure, called solid-liquid extraction, was repeated once more with 5 mL of the same clean solvent. The extract was concentrated under a gentle nitrogen stream to a volume of 2 mL and cleaned up by passage onto 6 g of acidified silica (AS) self-packed in a 25-mL empty cartridge (AS 44% H_2SO_4, wt/wt, activated with 15 mL hex) and eluted with 20 mL of hex and 15 mL of DCM. The final extract was concentrated under a gentle nitrogen to near dryness, reconstituted in 100 μL isooctane and RS (CB-207, 50 pg/μL) and further analyzed with an Agilent 6890 gas chromatographic (GC) system coupled to an Agilent 5973 mass spectrometry (MS) system operated in electron capture negative ionization (ECNI) mode. The GC system was equipped with a DB-5 capillary column (30 m × 0.25 mm × 0.25 μm), with electronic pressure control and a programmable-temperature vaporizer (PTV) inlet. Injection and acquisition details have been reported previously by Poma et al. (2017a). Briefly, the injection temperature was set at 92°C, held 0.03 min, ramped at 700°C/min to 300°C, held 30 min. Injection (1 mL) was performed under a pressure of 0.69 bar until 1.25 min and purge flow to split vent of 50 mL/min after 1.25 min. The GC temperature ramp started at 92°C and was held 1.25 min, ramped at 10°C/min to 300°C, held 1 min, ramped at 40°C/min to 310°C, and held 9.5 min. Helium was used as a carrier gas with a flow rate of 1.0 mL/min until 25 min, then increased to 1.5 mL/min. The ion source and quadrupole temperatures were set at 170°C and 150°C, respectively. The MS was operated in selected ion monitoring (SIM) for the quantification of targeted POPs and HFRs.

Analysis of Phosphorous Flame Retardants and Plasticizers

Parent compounds. Analysis of targeted PFRs and plasticizers was carried out based on the analytical protocol described by Christia et al. (2019), slightly modified to allow their determination and quantification in insect matrices. A dry sample (150 mg) was spiked with an IS mixture [including tris(2-chloroethyl) phosphate-d12 (TCEP-d12), tris(1,3-dichloro-2-propyl) phosphate-d15 (TDCIPP-d15), triphenyl phosphate-d15 (TPhP-d15), tris(2-butoxyethyl) phosphate-d6 (TBOEP-d6): 2 ng/μL and di-benzyl phthalate-d4 (DBzP-d4), bis(2-ethylhexyl) phthalate (DEHP)-d4, di-N-butyl phthalate-d4 (DNBP-d4): 10 ng/μL], added to 100 mg NaCl and solid-liquid extracted with 5 mL of ACN:toluene (9:1, vol/vol). The extract was then concentrated under a gentle nitrogen stream to a volume of 2 mL and subjected to a first cleanup step by dispersive solid phase extraction (d-SPE), performed by adding 100 mg of C18 and 50 mg of PSA sorbent powder to the sample. After being vortexed for 1 min and centrifuged at 3,000 rpm for 5 min, the supernatant was transferred to a clean glass tube, evaporated to dryness under a gentle nitrogen stream, exchanged to 1 mL hex and further cleaned up by being eluted onto a Florisil® cartridge (activated with 4 mL acetone, 6 mL EtAc, and 6 mL hex). Fractionation was achieved with 12 mL of a hex:DCM mixture [4:1, vol/vol; Fraction 1 (F1), discarded] and 10 mL of EtAc (F2, containing the targeted compounds). After F2 was evaporated to 4 mL under a gentle nitrogen stream, the Florisil® cartridge was further eluted with 8 mL acetone, and the eluate was collected in the same tube with F2. F2 was lastly evaporated under a gentle nitrogen stream and redissolved in 100 μL MeOH and RS (TAP, 1 ng/μL). A small aliquot (15 μL) of the final volume was added to 135 μL EtAc for the quantitative analysis of DEHP and bis (2-ethylhexyl) terephthalate (DEHT) with an Agilent GC coupled to an Agilent 5973 MS operated in electron ionization (EI) mode. The remaining volume was filtered on 0.2-μm centrifugal filters (10,000 rpm, 3 min), and analyzed with an Agilent 1200 Infinity LC system coupled to an Agilent 6410 Triple Quadrupole MS (Agilent Technologies). Details of the instrumental analysis have been reported previously by Christia et al. (2019).

Biotransformation products. Analysis of targeted biotransformation products of PFRs and plasticizers was carried out based on the analytical protocols described by Tang et al. (2019) and Yin et al. (2019), with minor modifications. A dry sample (100 mg) was spiked with an IS mixture (including a mix of PFRs-BT: 0.1 ng/μL, LPs-BT: 0.5 ng/μL, APs-BT: 0.1 ng/μL) and solid-liquid extracted with 2 mL MeOH. The clean extract was then transferred to a precleaned glass tube, evaporated to near dryness under a gentle nitrogen stream, redissolved in 150 μL UPW:MeOH (1:1, vol/vol), filtered on 0.2-μm centrifugal filters (10,000 rpm, 3 min), and analyzed with an Agilent 1290 Infinity LC system coupled to an Agilent 6460 Triple Quadrupole mass MS (Agilent Technologies) with electrospray ionization (ESI) source. The chromatographic details and MS acquisition parameters for the quantification of targeted PFRs-BT, LPs-BT, and APs-BT are described in Table S2.

Quality Assurance and Quality Control

Due to the lack of a certified insect matrix for the targeted organic compounds, the reliability of the applied analytical method for the quantification of POPs and HFRs was ensured by determining the recoveries of the spiked ISs and by means of three replicate analyses of the National Institute of Standards and Technology standard reference material (SRM) 1945 (Organics in Whale Blubber; percentage accuracy = 100 × measured content/certified value; performance criteria: ±25%). Outside this defined range, due to the general low variability of the data, a correction factor (determined by 100 divided by the average percentage accuracy per each compound) was applied during data treatment (see Table S1). All values were within the accuracy certified range, except for *p*,*p*′-dichlorodiphenyltrichloroethane (*p*,*p*′-DDT) (see Table S1). The mean recoveries of the spiked ISs ranged from 73% to 97%.

As the analytical methods used for the quantification of PFRs and plasticizers (both parent compounds and biodegradation products) were based on previously published papers but applied with few modifications, a specific validation for insect matrices (using grasshopper, cricket, and mealworm as model organisms) was performed to guarantee the consistency of the generated data. The reliability of the analysis was thus ensured by performing fortification experiments on a clean solvent blank (in triplicate) and subsequently calculating the recoveries (percentage recovery = 100 × measured content/fortification level; performance criteria: ±25%) and repeatability [expressed as relative standard deviation (RSD) <15%] of the measurements (EC 2002). Also in this case, outside the defined range, a correction factor was applied during data treatment (see Table S1).

Parent PFRs and plasticizers. For the determination of recovery and repeatability of the analytical method, fortification experiments at a single level relevant for the analysis [PFRs and ePFRs: 75 ng; LPs and APs: 500 ng; cyclohexane dicarboxylic acid diisononyl ester (DINCH), di-isodecyl phthalate (DIDP), and di-isononyl phthalate (DINP): 1,300 ng] were conducted in triplicate and the average recoveries were calculated (see Table S3). Thirty-eight percent of the compounds showed values outside the performance criteria (see Table S1). The mean recoveries of the spiked ISs ranged from 92% to 118%.

Biotransformation products of legacy PFRs and plasticizers. For the determination of recoveries and repeatability of the analytical method, fortification experiments at two levels relevant for the analysis (Low—PFRs-BT: 1 ng, LPs-BT: 5 ng, APs-BT: 4 ng; High—PFRs-BT: 8 ng, LPs-BT and APs-BT: 40 ng) were conducted in triplicate and the average recoveries were calculated (see Table S4). For PFRs-BT, recoveries were generally satisfactory (for about 70% of measurements), but consistently outside the range of acceptability for bis(1-chloro-2-propyl) phosphate (BCIPP), ethylhexyl phenyl phosphate (EHPHP), and 3-HO-TBOEP. This is likely ascribable to an enhanced instrumental sensitivity caused by a matrix effect. Because such an effect did not occur during the validation of this method for fish tissue samples (Tang et al. 2019), it may possibly be caused by the presence of interfering factors other than lipids (such as chitin) in the insect matrices. For LPs-BT and APs-BT, 70% of the values were within the performance criteria. The mean recoveries of the spiked IS ranged from 91% to 104% for PFRs-BT and from 103% to 120% for plasticizer BTs.

For GC-ECNI/MS, GC-EI/MS, and LC-MS/MS analysis, average procedural blank levels were subtracted from the sample results, and a value equal to 3 × the standard deviation (SD) of the blank measurement was used as the limit of quantification (LOQ). For compounds undetected in the blanks, LOQs were based on a signal/noise ratio of 10. LOQs per each compound (nanograms per gram wet weight) are listed in Table S1.

Data Handling and Statistical Analyses

Due to their highly lipophilic nature, POP and HFR concentrations were expressed in nanograms per gram lipid weight (lw). Concentrations of PFRs and plasticizers were expressed in nanograms per gram wet weight because they have been shown to have a lower tendency to accumulate in fatty tissues than POPs (Greaves et al. 2016; Greaves and Letcher 2017) and have been observed to quickly metabolize in humans (Meeker et al. 2013; Yin et al. 2019) and biota (Tang et al. 2019). During data processing, concentrations below LOQs were treated as LOQ × df, where df is the detection frequency of the compound above LOQs in the samples (James et al. 2002). All statistical elaborations were performed in the R environment (version 3.6.0; R Development Core Team). To reduce sparsity in the data set, the individual contaminants were grouped and summed according to their chemical properties (see Table S1). Statistical comparisons of all contaminant groups were performed for orders purchased in both Europe and Asia (i.e., Coleoptera and Orthoptera) using a Kruskal-Wallis nonparametric test, followed by a Dunn post hoc test using the multtest and dunn.test package (Dinno 2017; Pollard et al. 2005). p-Values were corrected for multiple testing using Bonferroni correction (significant raw p-values and corrected q-values are reported in Tables S5 and S6). Next to the univariate analyses, a multivariate principal component analysis (PCA) was performed on the chemical classes to investigate potential trends related to other sample grouping categories. A PCA plot projects the maximum variance of a multidimensional space in principal components, reducing the complexity of the data. Being unsupervised, this exploratory technique can reveal correlations previously unaccounted for (Jollife and Cadima 2016). Hence, it has a complementary value to univariate tests, in which all variables are considered independent. Briefly, all variables were z-transformed to remove any bias toward specific contaminant groups concerning contamination range. Biplots, in which the contribution of the variables toward the principal components are plotted on top of the projection, were generated using different colors and figure shapes according to the factors in Table 1.

Dietary Exposure Assessment

To perform the dietary exposure assessment, the most recent national surveys from the purchasing countries were screened to obtain food consumption data (ANSES 2017; Elmadfa 2012; MHLW 2017; MOHW 2017; van Rossum et al. 2016; Whitton et al. 2011; WIV-ISP 2016; Wu et al. 2018). Because data regarding the consumption of edible insects were not available, the dietary intake of the adult population to the targeted compounds was estimated based on consumption data for common food of animal origin (i.e., meat and meat products, fish and seafood, eggs) (see Table S7). The estimated dietary intake (EDI) of organic compounds [in nanograms per day and nanograms per kilogram body weight (BW) per day] was calculated for each targeted class by multiplying the median concentration of that class (in nanograms per gram wet weight) by the average consumption rate of a particular food group (in grams per day) by the adult population and dividing by the average body weight of the adult population for each country. The total EDI per individual/class of compounds was then obtained by summing the respective intakes from each considered food group. The potential risk of noncarcinogenic effects [hazard quotient (HQ)] per individual/class of compounds was calculated by dividing the total EDI (in milligrams per kilogram BW per day) by the relative oral reference dose factor (RfD, in milligrams per kilogram BW per day) (Table 2). HQ values ≥1 indicate a potential exposure risk for the population (Li et al. 2018; Poma et al. 2018; Qian et al. 2017; U.S. EPA 2017). The potential carcinogenic risk (CR) was calculated by multiplying the EDI (in milligrams per kilogram BW per day) by the relative oral cancer slope factor (SFO; in milligrams per kilogram BW per day) (Table 2). A public screening criterion set to 1 in 1 million is used as a threshold for the CR ($CR > 10^{-4}$ is considered unacceptable, $10^{-6} < CR < 10^{-4}$ is an area of concern, and $CR < 10^{-6}$ is acceptable) (Li et al. 2018; Qian et al. 2017; U.S. EPA 2017).

Results

A PCA plot (Figure 1) revealed a higher occurrence of alkylated- and chlorinated-PFRs in European samples in comparison with Asian products. The distinction between edible insects purchased in

Table 2. Dietary risk assessment.

Chemical group	EDI (mg/kg BW per day)		RfDs[a]	HQ		SFO[a]	CR	
Individual compound	EU	Asia	(mg/kg BW per day)	EU	Asia	(mg/kg BW per day)	EU	Asia
HFRs	1.30×10^{-7}	2.30×10^{-7}	—	—	—	—	—	—
BDE-99	6.70×10^{-9}	1.20×10^{-8}	1.00×10^{-4}	6.70×10^{-5}	1.20×10^{-4}	—	—	—
BDE-153	4.50×10^{-10}	7.70×10^{-10}	2.00×10^{-4}	2.20×10^{-6}	3.90×10^{-6}	—	—	—
OCPs	4.40×10^{-7}	7.60×10^{-7}	—	—	—	—	—	—
DDT	7.10×10^{-8}	1.20×10^{-7}	5.00×10^{-4}	1.40×10^{-4}	2.40×10^{-4}	3.40×10^{-1}	2.40×10^{-8}	4.10×10^{-8}
HCB	5.50×10^{-8}	9.50×10^{-8}	8.00×10^{-4}	6.90×10^{-5}	1.20×10^{-4}	1.60×10^{0}	8.80×10^{-8}	1.50×10^{-7}
α-HCH	3.40×10^{-9}	5.80×10^{-9}	8.00×10^{-3}	4.20×10^{-7}	7.20×10^{-7}	6.30×10^{0}	2.10×10^{-8}	3.70×10^{-8}
β-HCH	6.70×10^{-9}	1.20×10^{-8}	—	—	—	1.80×10^{0}	1.20×10^{-8}	2.10×10^{-8}
γ-HCH	6.70×10^{-9}	1.20×10^{-8}	3.00×10^{-4}	2.20×10^{-5}	3.90×10^{-5}	1.10×10^{0}	7.40×10^{-9}	1.30×10^{-8}
PCBs	1.60×10^{-7}	2.80×10^{-7}	2.00×10^{-5}	8.20×10^{-3}	1.40×10^{-2}	4.00×10^{-1}	6.50×10^{-8}	1.10×10^{-7}
PFRs	1.30×10^{-5}	2.20×10^{-5}	—	—	—	—	—	—
TCEP	1.90×10^{-7}	3.20×10^{-7}	7.00×10^{-3}	2.70×10^{-5}	4.60×10^{-5}	2.00×10^{-2}	3.70×10^{-9}	6.40×10^{-9}
TCIPP	6.40×10^{-7}	1.10×10^{-6}	1.00×10^{-2}	6.40×10^{-5}	1.10×10^{-4}	—	—	—
TPHP	2.60×10^{-6}	4.40×10^{-6}	2.00×10^{-2}	1.30×10^{-4}	2.20×10^{-4}	—	—	—
EHDPHP[b]	1.90×10^{-7}	3.20×10^{-7}	1.50×10^{-2}	1.20×10^{-5}	2.10×10^{-5}	—	—	—
TEHP	5.60×10^{-8}	9.70×10^{-8}	1.00×10^{-1}	5.60×10^{-7}	9.70×10^{-7}	3.20×10^{-3}	1.80×10^{-10}	3.10×10^{-10}
Plasticizers	1.20×10^{-3}	2.10×10^{-3}	—	—	—	—	—	—
DEHP	9.80×10^{-4}	1.70×10^{-3}	2.00×10^{-2}	4.90×10^{-2}	8.40×10^{-2}	1.40×10^{-2}	1.40×10^{-5}	2.40×10^{-5}

Note: Assessment based on median measured concentrations of individual/classes of compounds present in edible insects ($n=35$). EDI is reported in mg/kg BW per day to allow a direct comparison with the established reference dose (RfD) and oral cancer slope (SFO) factors (U.S. EPA 2017). —, no data; BDE, bromodiphenyl ether; CR, potential carcinogenic risk; DDT, dichlorodiphenyltrichloroethane; DEHP, bis(2-ethylhexyl) phthalate; EDI, estimated dietary intake; EHDPHP, 2-ethylhexyl diphenyl phosphate; EU, Europe; HCB, hexachlorobenzene; HFRs, halogenated flame retardants; HQ, hazard quotient; OCPs, organochlorinated pesticides; PCBs, polychlorinated biphenyls; PFRs, phosphorus flame retardants; TCEP, tris(2-chloroethyl) phosphate; TCIPP, tris(chloro-2-propyl) phosphate; TEHP, tris(2-ethylhexyl) phosphate; TPHP, triphenyl phosphate; α, β, γ-HCHs, hexachlorocyclohexanes.
[a]Data of RfD and SFO are from U.S. EPA (2017).
[b]Data of Rfd for EHDPHP are from Poma et al. (2018).

the two continents was mostly related to the higher presence of OCPs, HFRs, LPs, and ePFRs in Asia than in Europe (Figure 1). Coloring by country revealed contamination patterns according to three geographical areas (i.e., Europe—EU, China—PRC, and Japan/Republic of Korea—JPN/SK), of which the most contaminated samples were purchased in JPN/SK (see Figure S1A). In addition, when coloring by insect order, the PCA revealed a positive correlation between both Hymenoptera and Lepidoptera and the presence of PCBs, aromatic-PFRs, and APs (Figure 1; Figure S1B).

The levels and patterns of chemical contamination varied greatly among the considered insect orders (Figures 2–5). Measured medians of the main POP categories and HFRs were 1.5 ng/g lw for polychlorinated biphenyls (PCBs), 3.9 ng/g lw for organochlorine compounds (OCPs), and 1.2 ng/g lw for HFRs (Figure 2; Table S8 and Excel Table S1). The most abundant OCPs were dichlorodiphenyldichloroethylene (*p,p′*-DDE, median 0.63 ng/g lw) and hexachlorobenzene (HCB, median 0.49 ng/g lw), whereas the most prevalent PCBs and HFRs were CB-153 (median 0.39 ng/g lw), CB-138 (median 0.2 ng/g lw), and DPs (median *anti*-DP 0.37 ng/g lw, *syn*-DP 0.13 ng/g lw), respectively. Median concentrations of POPs and HFRs in EU, PRC, and JPN/SK were 7.6, 8.8, and 38 ng/g lw, respectively, mostly represented by PCBs in Europe and by OCPs in Asia (see Figure S2). Among insect orders, Hymenoptera showed the highest contamination with HFRs and relatively high levels of PCBs, whereas Lepidoptera were mostly contaminated with PCBs and OCPs (Figure 2).

When comparing insect orders purchased in both continents (i.e., Coleoptera and Orthoptera), a general higher contamination of HFRs and OCPs in Asia versus Europe was found, reaching significance for Asian Orthoptera ($q=0.007$) (Figure 2B). An opposite trend was found for PCBs, but no significant differences between continent/country of purchase or between orders were apparent.

Measured medians of the main PFR subscript-categories were below 2.0 ng/g ww and up to 6.8 ng/g ww for the total PFRs, whereas higher values were measured for individual plasticizers (up to 2,441 ng/g ww), with medians for LPs about 10-fold higher than those of APs (Figures 3–5; Table S9 and Excel Table S1). The general-purpose legacy plasticizer bis(2-ethylhexyl) phthalate (DEHP, median 522 ng/g ww) was the predominant compound in all insect samples, whereas the aromatic-PFR triphenyl phosphate (TPHP, median 1.4 ng/g ww) and isodecyl diphenyl phosphate (iDPP, median 0.09 ng/g ww) were the most abundant legacy and emerging PFRs, respectively. The median contamination with PFRs and plasticizers in insect samples was similar between the EU, PRC, and JPN/SK, always dominated by aromatic-PFRs (see Figure S2) and DEHP. Among the analyzed insect orders, Odonata, Lepidoptera, and Hymenoptera were the most contaminated with PFRs (Figure 3A), whereas Hemiptera, Lepidoptera, and Coleoptera had the highest contamination with plasticizers (Figure 4A). Comparing Coleoptera and Orthoptera purchased in both continents, European samples were generally more contaminated with PFRs, but were less affected by the presence of ePFRs (Figure 3B) and plasticizers. In addition, Orthoptera showed higher medians of PFR-BTs than Coleoptera, but relatively lower levels of parent compounds.

This was also observed for plasticizers, where Asian Orthoptera had significantly higher ($q=0.03$) median concentrations of BTs than Coleoptera, mostly due to the presence of LPs-BT (Figure 4D and E) [especially mono-*n*-butyl phthalate—MnBP and mono (2-ethylhexyl) phthalate—MEHP]. However, although the parent compound of MEHP (DEHP) was detected in all samples, the parent compound for MnBP (i.e., DnBP) was always below the LOQ.

The total estimated dietary intake (EDI) of the considered classes of compounds for the average adult population ranged from 0.1 to 1 ng/kg BW per day for POPs and HFRs in both continents, whereas it was 13 and 22 ng/kg BW per day for PFRs and 1,201 and 2,069 ng/kg BW per day for plasticizers, in Europe and Asia, respectively (see Table S10). The calculated HQ ranged between 4.2×10^{-7} and 8.4×10^{-2}, whereas the calculated potential CR was $<10^{-6}$ for all individual/classes of compounds, except for DEHP in both Europe and Asia (values up to 2.4×10^{-5}).

Discussion

The PCA highlighted that the pollutant levels in certain insect orders (i.e., Hymenoptera and Lepidoptera) were strongly influenced by

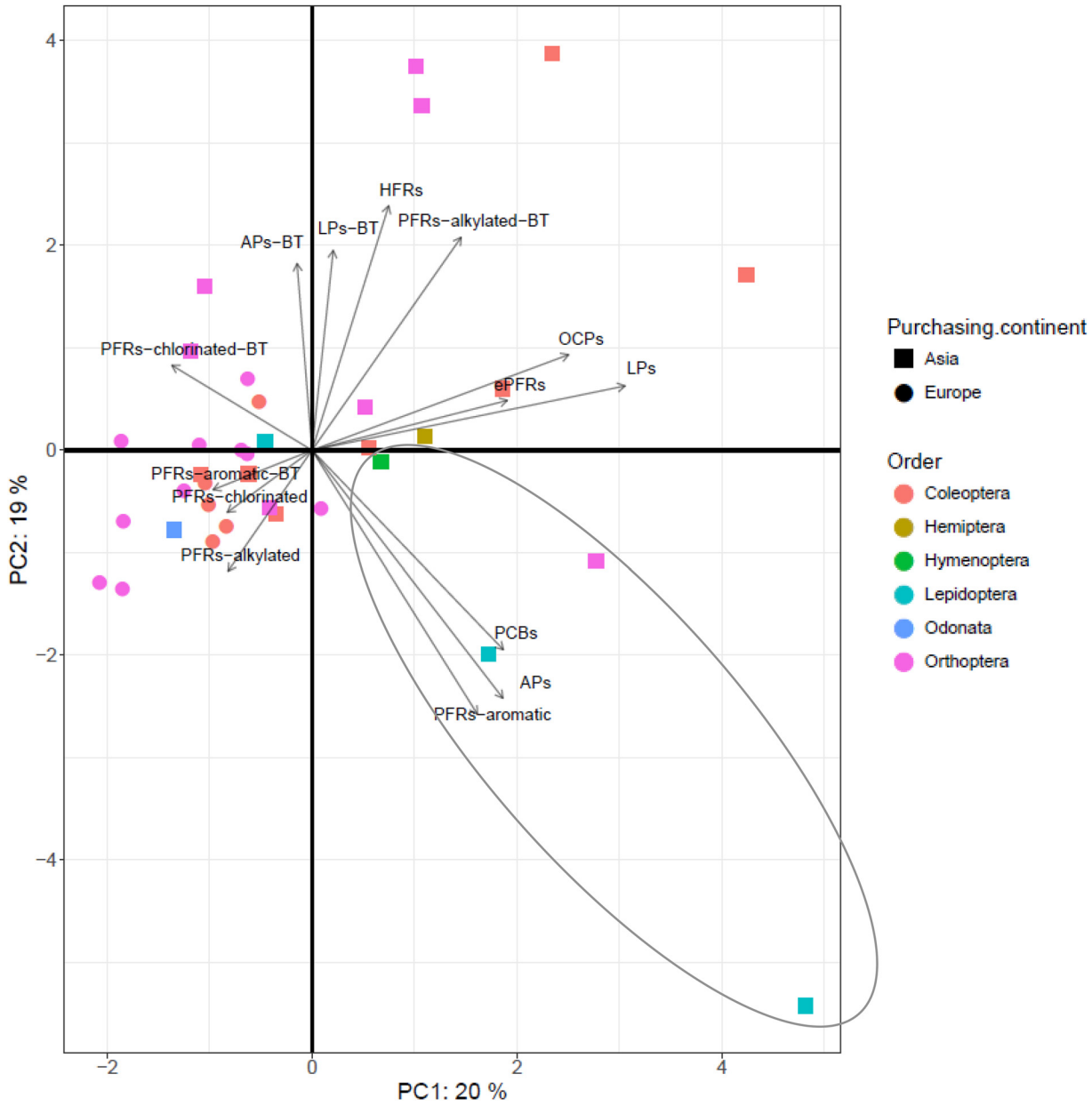

Figure 1. Biplot of the principal component analysis (PCA) of the summed variables. The first principal component explains 20% of the variation and is oriented according to continent of purchase. The biplot represents the samples (squares and circles) in the projection of the multivariate space, which is a summary of the contamination categories. The contribution of each contaminant is shown as an arrow, hence samples oriented toward specific arrow directions tend to have a higher concentration of that specific contaminant (Jollife and Cadima 2016). Note: APs, alternative plasticizers; BT, biotransformation products; ePFRs, emerging phosphorus flame retardants; HFRs, halogenated flame retardants; LPs, legacy plasticizers; OCPs, organochlorinated pesticides; PCBs, polychlorinated biphenyls; PFRs, phosphorus flame retardants.

seasoning, which suggests a relationship between sample contamination and industrial processing, rather than order-related toxicokinetics (Poma et al. 2018). This hypothesis was additionally supported by the poor general correlation between the parent PFRs and plasticizers and their specific BTs. These findings indicate that the contamination likely occurred during industrial manipulation after harvesting, rather than being related to bioaccumulation and/or biotransformation during rearing. This was clearly the case for APs, aromatic-PFRs, and alkylated-PFRs, where the divergent load from their BTs suggested that contamination occurred during insect seasoning and industrial processing after harvesting in JPN/SK and in EU, respectively. Conversely, better correlations were observed between chlorinated-PFRs and LPs and their respective BTs, suggesting that the contamination occurred during rearing, but that the compounds were not completely biotransformed (Figure 1). Besides the identification of the likely causal source of contamination, the general divergent loads of parent PFRs and plasticizers from their BTs could be indicative of different biotransformation capacities among insect orders.

Measured median concentrations of POPs and HFRs from the present study (Figure 2; Table S8) were comparable with the results from our previous investigation on chemical compounds in a limited number of edible insects and insect-based food for human consumption purchased in Belgium (Poma et al. 2017a). In addition, the relative median concentrations of the DP *anti*- and *syn*-isomers were in a 3:1 ratio, which matches the technical formulation (Sverko et al. 2011). Because insects are located mostly

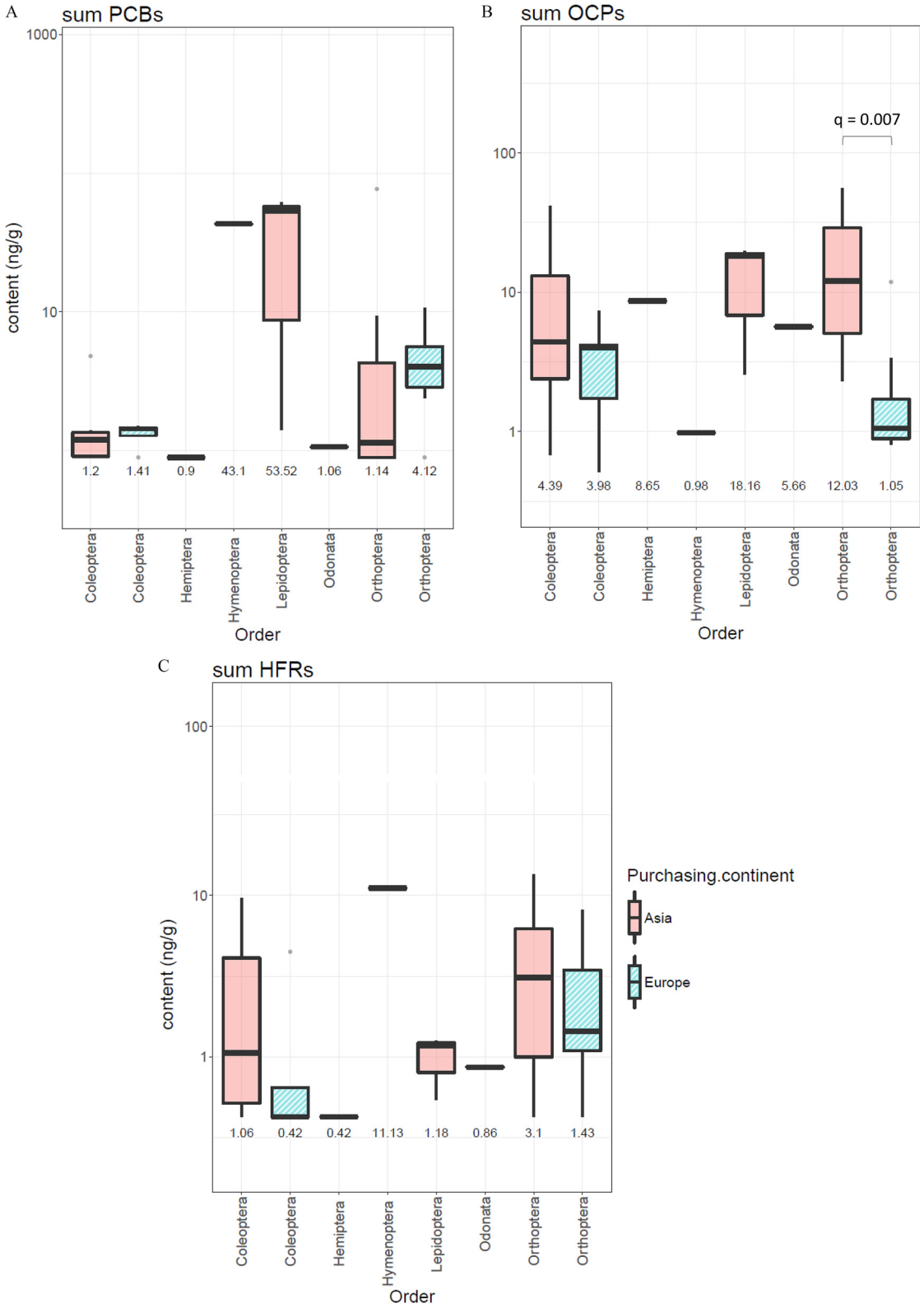

Figure 2. Box plots of lipophilic compounds (A) polychlorinated biphenyls (PCBs), (B) organochlorinated pesticides (OCPs), and (C) halogenated flame retardants (HFRs) according to insect order and purchasing continents (*y*-axis content in ng/g lw). Due to the limited availability of order purchasing in Europe, only Orthoptera and Coleoptera were comparable between continents. Box plots showing the median (horizontal line in box), interquartile range (IQR; shaded box), and the samples within 1.5 times the IQR (vertical lines). Samples outside 1.5 times the IQR are shown as dots. Sample size was sufficiently large for Kruskal-Wallis nonparametric testing with Dunn's post hoc testing for Asian Coleoptera ($n = 7$), European Coleoptera ($n = 5$), Asian Orthoptera ($n = 7$), and European Orthoptera ($n = 10$) (see Tables S5 and S6). The box plots belonging to Hemiptera ($n = 1$), Hymenoptera ($n = 1$), Lepidoptera ($n = 3$), and Odonata ($n = 1$) were added to include all samples in the representation.

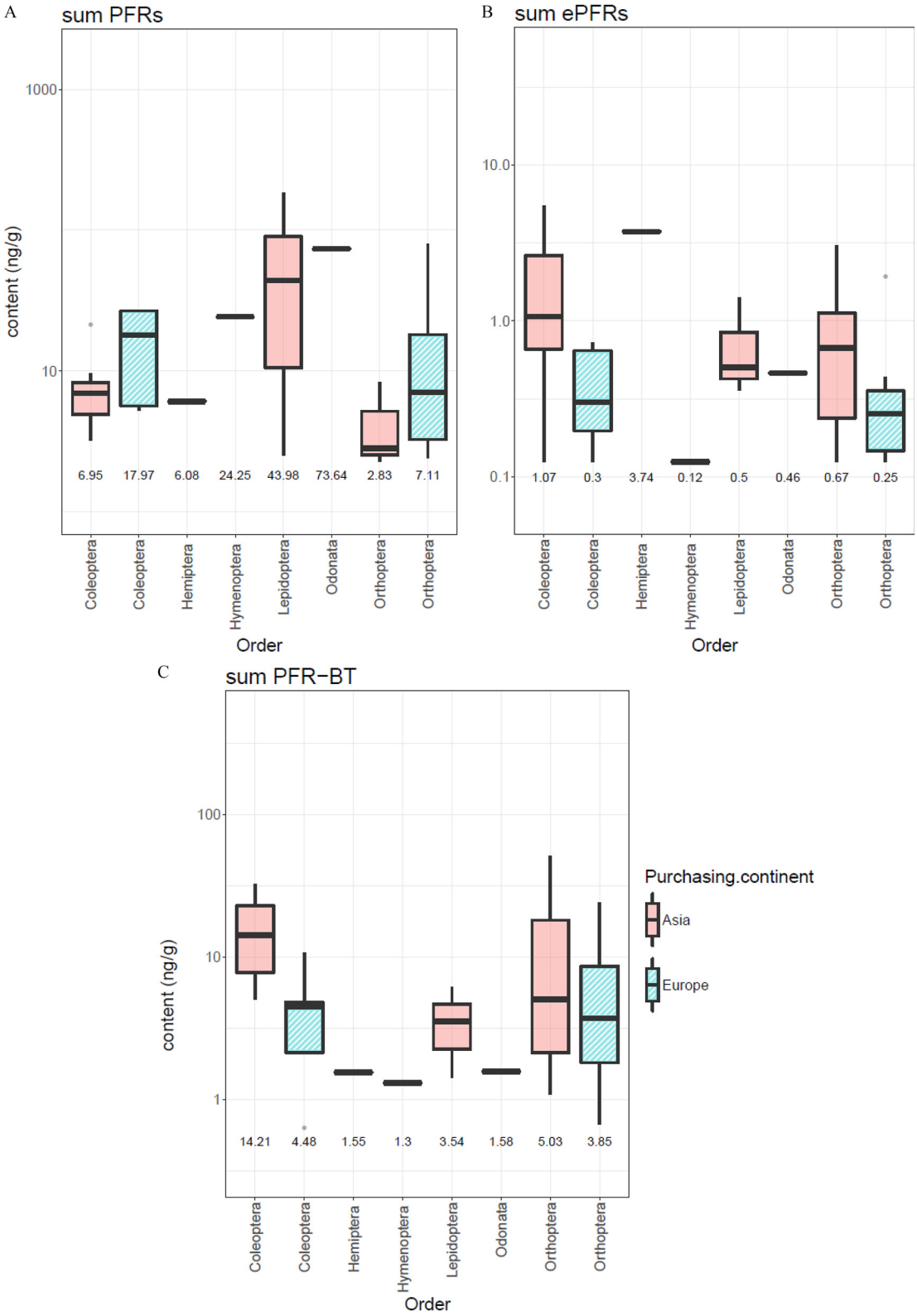

Figure 3. Box plots of the total sum of (A) phosphorus flame retardants (PFRs), (B) emerging phosphorus flame retardants (ePFRs), and (C) their biotransformation products (BT) (*y*-axis content in ng/g ww). Box plots showing the median (horizontal line in box), interquartile range (IQR; shaded box), and the samples within 1.5 times the IQR (vertical lines). Samples outside 1.5 times the IQR are shown as dots. Sample size was sufficiently large for Kruskal-Wallis nonparametric testing with Dunn's post hoc testing for Asian Coleoptera ($n = 7$), European Coleoptera ($n = 5$), Asian Orthoptera ($n = 7$), and European Orthoptera ($n = 10$) (see Tables S5 and S6). There were no statistically significant differences observed. The box plots belonging to Hemiptera ($n = 1$), Hymenoptera ($n = 1$), Lepidoptera ($n = 3$), and Odonata ($n = 1$) were added to include all samples in the representation.

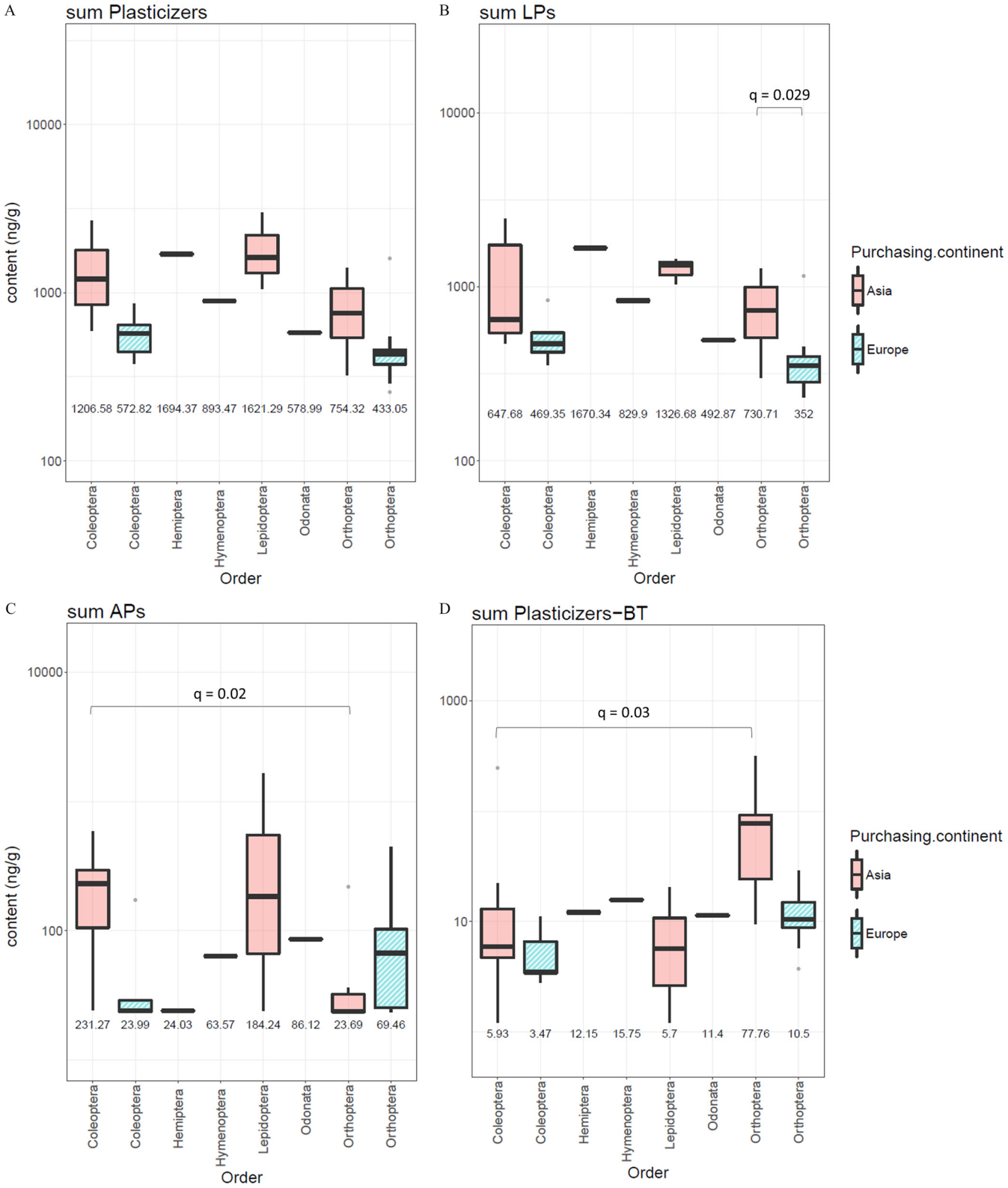

Figure 4. Box plots of total (A) plasticizers, (B) legacy, and (C) alternative plasticizers and (D–F) their corresponding biotransformation products (BT) (*y*-axis content in ng/g ww). Box plots showing the median (horizontal line in box), interquartile range (IQR; shaded box), and the samples within 1.5 times the IQR (vertical lines). Samples outside 1.5 times the IQR are shown as dots. Sample size was sufficiently large for Kruskal-Wallis nonparametric testing with Dunn's post hoc testing for Asian Coleoptera ($n = 7$), European Coleoptera ($n = 5$), Asian Orthoptera ($n = 7$), and European Orthoptera ($n = 10$) (see Tables S5 and S6). The box plots belonging to Hemiptera ($n = 1$), Hymenoptera ($n = 1$), Lepidoptera ($n = 3$), and Odonata ($n = 1$) were added to include all samples in the representation.

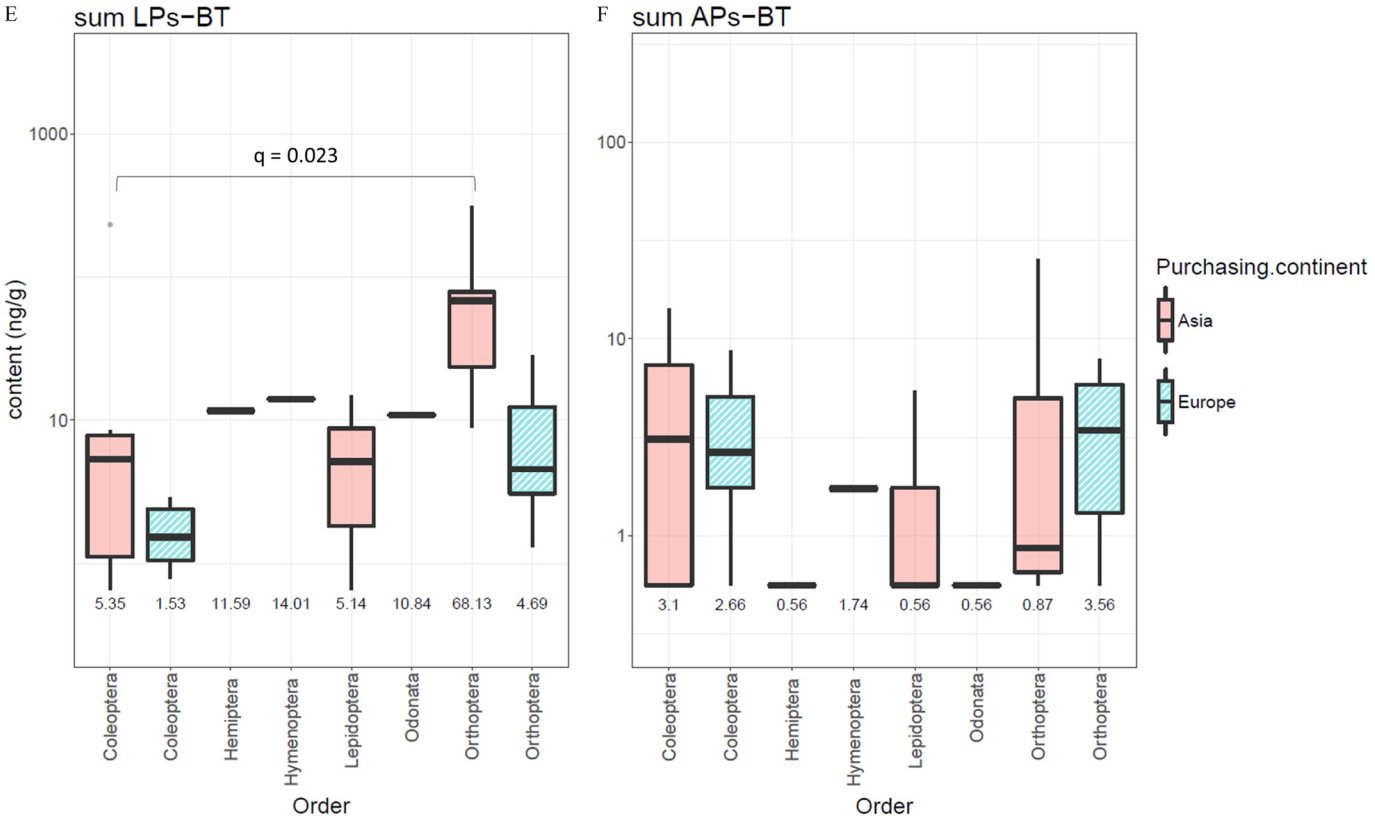

Figure 4. (*Continued.*)

at the bottom of trophic food webs, these results are in accordance with previous studies suggesting that the DP isomer ratio shifts during bioaccumulative propagation (Sverko et al. 2011). Even though DPs are worldwide spread contaminants, their levels were about 3-fold lower in samples from Europe than in Asia, which concurs with the region's production volumes (Wang et al. 2010).

The differences in POP and HFR contamination patterns in EU, PRC, and JPN/SK (see Figure S2), mostly due to the prevalence of PCBs in Europe and OCPs in Asia, emphasize the difference in contamination both between the two Asian regions and between continents. All samples showed contamination values below the legally tolerated maximum residue levels (MRL) in common food of animal origin of the country of purchasing (see Table S11). However, when applying the strictest tolerance thresholds, one sample (JPN-02) exceeded the PCB value of 40 ng/g lw and three samples (JPN-02, SK-05, and SK-09) had HCB values higher than the 10-ng/g lw stipulated by EU regulation (EC 2006).

These compounds are highly lipophilic, bioaccumulative, and resistant to biodegradation (Mackay et al. 2006), suggesting that they bioaccumulated in the insects from the rearing substrate, with noticeable differences among the orders. However, it is worth pointing out that most samples included in the abovementioned orders were purchased seasoned, which additionally supports an industrial source of contamination.

Our measured median concentrations of PFRs (Figure 3) were comparable with the levels detected in edible insects and insect-based food for human consumption from our previous study (Poma et al. 2017a). In the same way, the median contamination with PFRs and plasticizers in insect samples was similar between the EU, PRC, and JPN/SK, always dominated by aromatic-PFRs (see Figure S2) and DEHP, and was comparable with median levels measured in foodstuffs of animal origin from worldwide studies (Cariou et al. 2016; Cheng et al. 2016; Ding et al. 2018; He et al. 2015; Poma et al. 2017b, 2018; Van Holderbeke et al. 2014; Wang and Kannan 2018; Yang et al. 2018). For these two groups of compounds, the contamination in the insects could have occurred during production [e.g., from the rearing substrate, from the polyvinyl chloride (PVC) gloves used by workers during insect rearing and handling, etc.], industrial processing, storage (e.g., in PVC tanks), transportation, and/or as a result of direct transfer from food-packaging material (Guo and Kannan 2012). The strong prevalence of TPHP in the seasoned samples, with a median value nearly 14-fold higher than the natural ones, strongly suggests a post-harvesting, industrial origin of PFR contamination. This hypothesis is supported by the outcomes of a recent investigation on the occurrence of legacy PFRs in several foodstuffs, which pointed to TPHP as the predominant compound in industrially processed food groups (Poma et al. 2018). In addition, DEHP was the most predominant compound in all insect samples, likely attributed to its presence in food containers and food packaging (Guo and Kannan 2012), with levels below the legally tolerated specific migration limits (SMLs) according to local regulations (see Table S11). However, when applying the strictest tolerance threshold, four samples (PRC-03, SK-01, SK-02, and SK-09) exceeded the value of 1,500 ng/g ww allowed for DEHP by EU regulation (EC 2011).

Differences in PFRs and plasticizers contamination (both parent compounds and their BTs) (Figures 3–5) among insects emphasize the likely key role of insect orders (or even species) in the bioaccumulation of these two classes of compounds, suggesting a higher biotransformation capacity of Orthoptera than Coleoptera. In addition, the different pattern of contamination with LPs-BT (e.g., MnBP and MEHP) in these two orders (Figure 4E) might suggest two scenarios of interpretation, where *a*) DEHP contamination mostly occurred after harvesting, whereas DnBP was accumulated

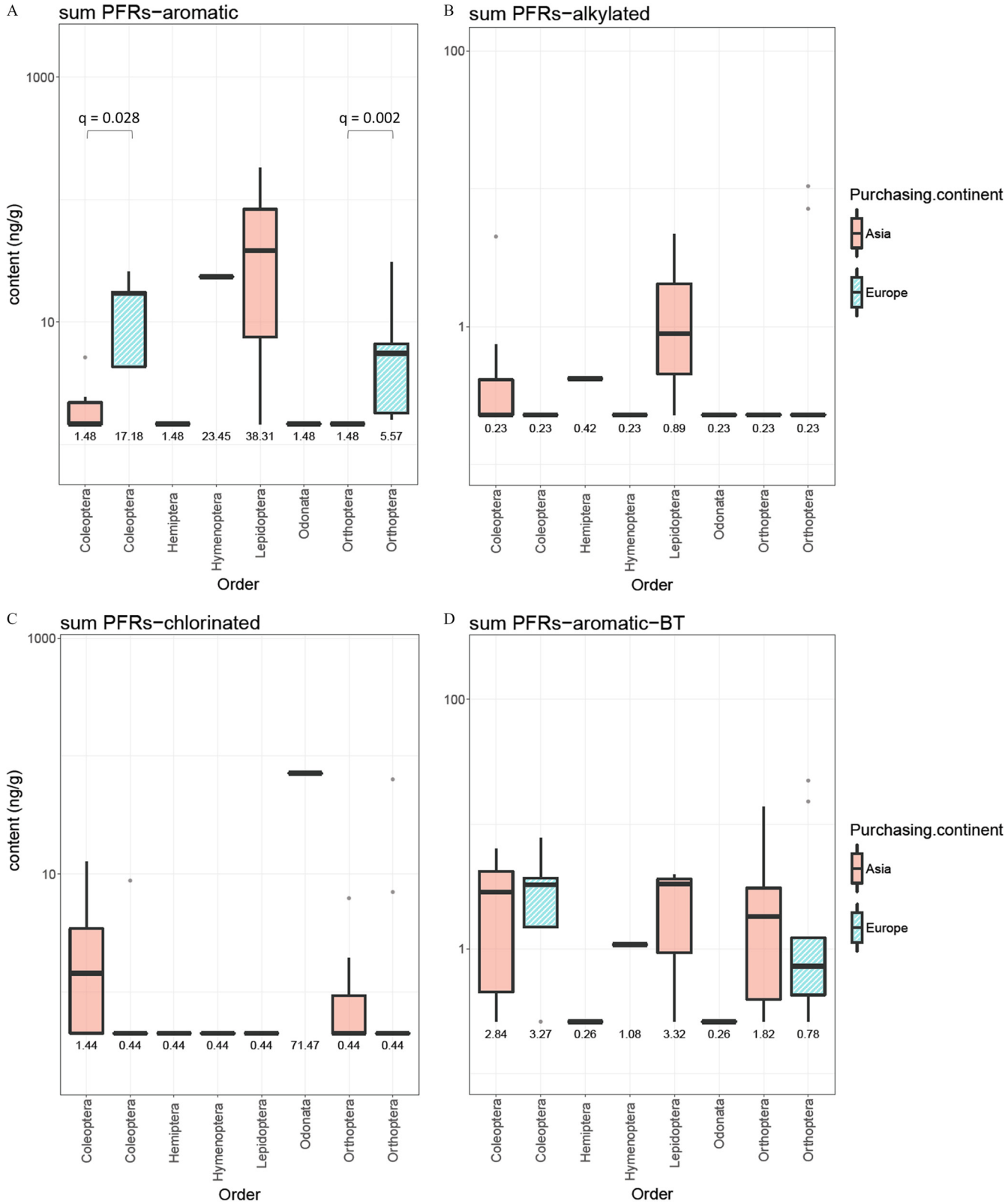

Figure 5. Box plots of (A) aromatic-, (B) alkylated-, and (C) chlorinated-phosphorus flame retardants (PFRs) and (D–F) their corresponding biotransformation products (BT) (*y*-axis content in ng/g ww). Box plots showing the median (horizontal line in box), interquartile range (IQR; shaded box), and the samples within 1.5 times the IQR (vertical lines). Samples outside 1.5 times the IQR are shown as dots. Sample size was sufficiently large for Kruskal-Wallis nonparametric testing with Dunn's post hoc testing for Asian Coleoptera ($n = 7$), European Coleoptera ($n = 5$), Asian Orthoptera ($n = 7$), and European Orthoptera ($n = 10$) (see Tables S5 and S6). The box plots belonging to Hemiptera ($n = 1$), Hymenoptera ($n = 1$), Lepidoptera ($n = 3$), and Odonata ($n = 1$) were added to include all samples in the representation.

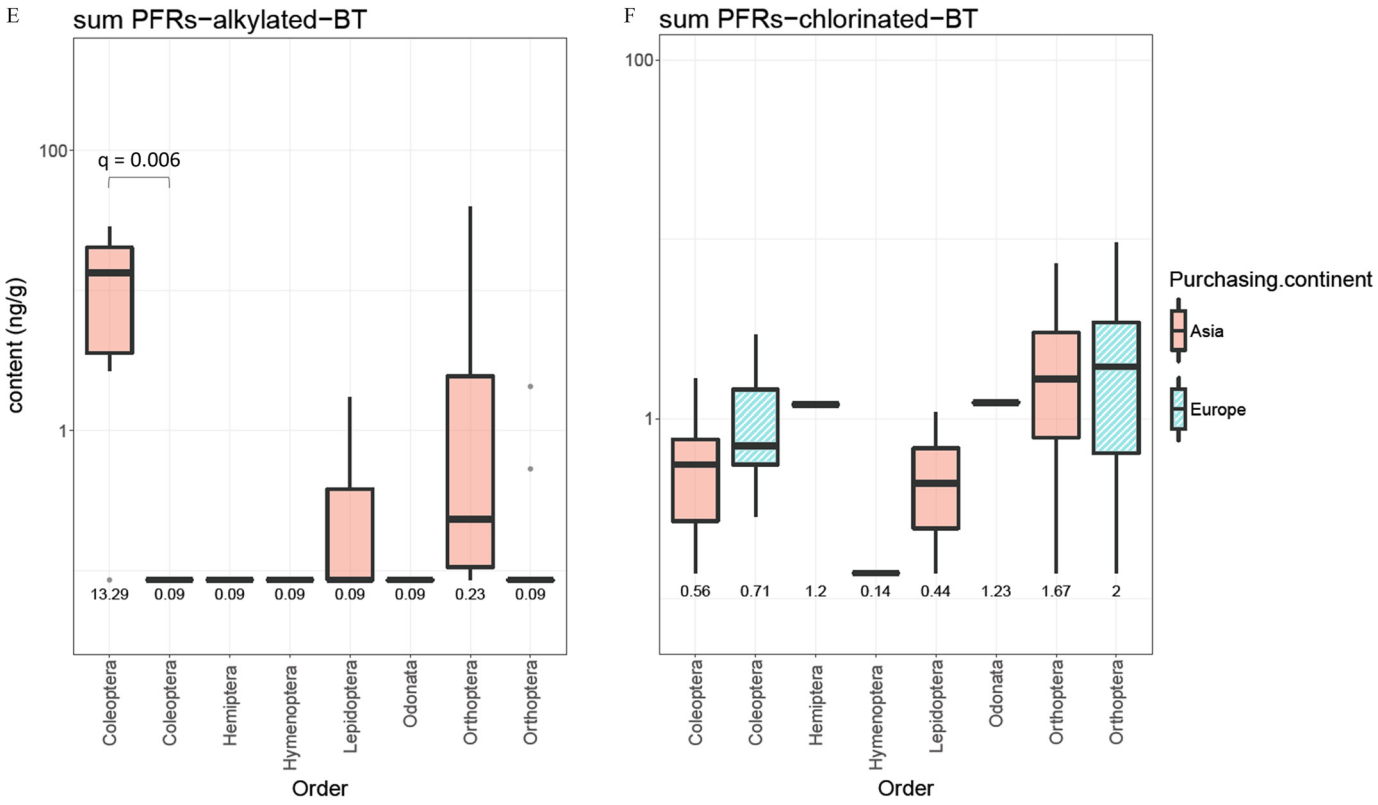

Figure 5. (*Continued.*)

by the insects during rearing and completely biotransformed during their lifetime; or *b*) Orthoptera have a different (stronger) biotransformation capacity than Coleoptera, as observed for PFRs. However, too little is known about the biotransformation potential of these contaminants in insects to speculate on which scenario might be the most plausible.

Evaluation of Chemical Safety of Edible Insects

A reliable way to evaluate the chemical safety of edible insects is through a dietary exposure risk assessment, which combines food chemical contamination findings with consumption data, to generate an EDI (IPCS 2008). Because data regarding the consumption of edible insects were not available in any of the considered countries, the EDI of the targeted compounds for the adult population via insect consumption was calculated based on an assumptive scenario in which common food of animal origin (i.e., meat, fish, and eggs) was completely replaced by edible insects (Table 2; Table S10). Given that this is currently an unlikely scenario, the calculated estimated dietary intake represents an implicit overestimation of the health risk for the adult population.

The EDI-estimation resulted similar for Europe and Asia and comparable with levels generally estimated for the same groups of compounds in common food of animal origin worldwide (Ding et al. 2018; Giovanoulis et al. 2018; Poma et al. 2017b, 2018; Qian et al. 2017; Wang et al. 2018, 2019; Yang et al. 2018). However, when making this comparison, it is important to consider that the estimated dietary intake can vary strongly among countries, likely due to differences in dietary habits of the population, number, and typology of food items included in the food categories as well as individual targeted compounds considered within a group of chemicals, etc. The calculated HQs were always within the acceptable level (HQ <1) (U.S. EPA 2017) (Table 2). The calculated CRs were also within the acceptable level of 10^{-6} for all individual/classes of compounds, except for DEHP in both Europe and Asia (classified as within an area of concern) (Li et al. 2018; U.S. EPA 2017) (Table 2). However, the exposure of the population to DEHP is expected to decrease due to its replacement with alternative plasticizers in both food processing equipment and packaging materials (Giovanoulis et al. 2018), complying with a recent EU restriction because of its reproductive toxicity and endocrine disrupting properties (EC 2018). Therefore, even when voluntarily overestimated, the results of the performed dietary risk assessment for the described toxicants show that the adult population from Europe and Asia has a low exposure to the targeted compounds following ingestion of the analyzed edible insects.

In this study, the chemical contamination with selected hazardous organic compounds was assessed in several species of edible insects purchased in Europe and Asia, followed by an evaluation of their chemical safety. Our results revealed an overall low level of chemical contamination with POPs, plasticizers, and PFRs, comparable with other commonly consumed animal proteins and not exceeding the legal limits in food of animal origin according to local regulations. Our results emphasize that, besides the insect species, the insect production environment and the eventual industrial manipulation and addition of ingredients after harvesting are additional factors influencing the chemical safety of the final insect food-product. The results from the performed risk assessment showed that adverse health effects for the adult population of Europe and Asia through exposure to these compounds due to insect consumption are unlikely, but these findings should be read considering the limitations deriving from analyzing a selected group of organic chemical compounds in a rather small data set and should not be further generalized. Future studies are thus necessary to broaden our insights regarding

chemical contamination in edible insects by *a*) expanding the investigation to other classes of compounds (e.g., emerging pesticides); *b*) investigating the mechanisms of accumulation and transfer of organic compounds to the insects during and after harvesting; and *c*) extrapolating the impact of multiple classes of chemical compounds on the final chemical safety of insect-food products.

Acknowledgments

G.P. conceived of the study and designed the experiments. S.S.Y. and B.T. performed the experiments. Y.F. provided the Japanese samples and helped with data interpretation. M.C. performed statistical tests and elaborations. A.C. supervised the research. All authors contributed to data analysis and the drafting and editing of the manuscript.

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