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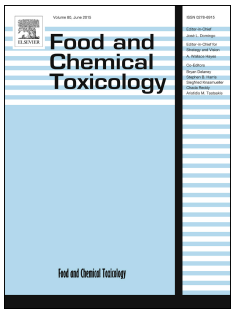

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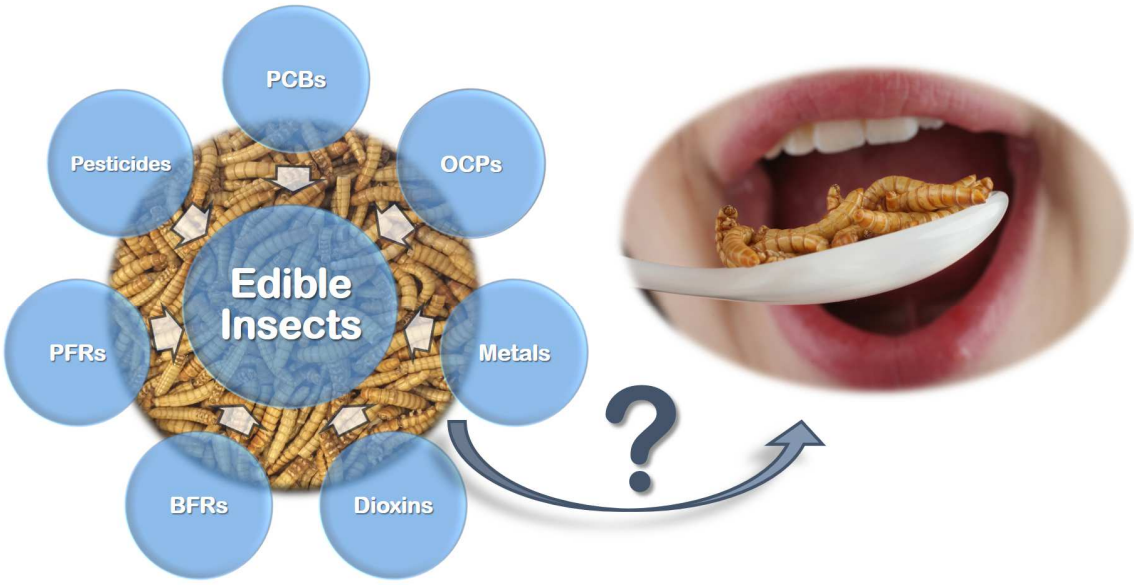

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Evaluation of hazardous chemicals in edible insects and insect-based food intended for human consumption

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Abstract

Due to the rapid increase in world population, the waste of food and resources, and non-sustainable food production practices, the use of alternative food sources is currently strongly promoted. In this perspective, insects may represent a valuable alternative to main animal food sources due to their nutritional value and sustainable production. However, edible insects may be perceived as an unappealing food source and are indeed rarely consumed in developed countries. The food safety of edible insects can thus contribute to the process of acceptance of insects as an alternative food source, changing the perception of developed countries regarding entomophagy. In the present study, the levels of organic contaminants (i.e. flame retardants, PCBs, DDT, dioxin compounds, pesticides) and metals (As, Cd, Co, Cr, Cu, Ni, Pb, Sn, Zn) were investigated in composite samples of several species of edible insects (greater wax moth, migratory locust, mealworm beetle, buffalo worm) and four insect-based food items currently commercialized in Belgium. The organic chemical mass fractions were relatively low (PCBs: 27-2065 pg/g ww; OCPs: 46-368 pg/g ww; BFRs: up to 36 pg/g ww; PFRs 783-23800 pg/g ww; dioxin compounds: up to 0.25 pg WHO-TEQ/g ww) and were generally lower than those measured in common animal products. The untargeted screening analysis revealed the presence of vinyltoluene, tributylphosphate (present in 75 % of the samples), and pirimiphos-methyl (identified in 50 % of the samples). The levels of Cu and Zn in insects were similar to those measured in meat and fish in other studies, whereas As, Co, Cr, Pb, Sn levels were relatively low in all samples (<0.03 mg/kg ww). Our results support the possibility to consume these insect species with no additional hazards in comparison to the more commonly consumed animal products.

Keywords

Edible insects; novel food; food chemical safety; persistent organic compounds; dioxins; metals

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1. Introduction

The world population is continuously increasing, together with its requirement for food. Feeding the growing world population will necessarily require an increase in food production, which will inevitably affect already limited resources, such as land, water, energy, oceans, and fertilizers (FAO, 2013). Especially in developed countries, humans have historically selected a restricted number of plants and animals from which they obtain their energy and nutrient pool, such as livestock and fish (Belluco et al., 2013). With global demand for livestock products expected to more than double between 2000 and 2050 (from 229 to 465 million tons) (FAO, 2013), meeting this demand will require innovative solutions. For this reason, feeding future populations should involve the employment of alternative sources of proteins, such as cultured meat, seaweed, fungi, and insects (FAO, 2013).

In particular, the opportunity for insects to compensate for the rising demand for meat or fish products is enormous, especially considering that hundreds of insect species are worldwide already consumed by humans as food (FAO, 2013; Belluco et al., 2013; Menzel and D'Aluisio 1998; DeFoliart 2002; Paoletti 2005). A number of organizations have already started evaluating the prospect of using insects for food and feed. In a recent document, the Food and Agricultural Organization of the United Nations (FAO, 2013) broadly explored the advantages in consuming insects, as they are considered suitable alternatives to common animal sources of food, such as poultry, pork, beef and fish. Insects have high nutritional value (i.e. contain adequate amounts of calories and essential amino acids, monounsaturated and/or polyunsaturated fatty acids, and micronutrients), high food conversion efficiency compared with conventional livestock, and are responsible for relatively low emissions of greenhouse gases and ammonia.

Although the use of insects as a food source brings important environmental, economic, and food security benefits, consumer acceptance remains one of the largest barriers to the adoption of insects as viable sources of protein (Mlcek et al., 2014). Indeed, entomophagy is mainly associated with feelings of repulsion (Verbeke, 2015) and the general attitude toward insect-based food is

frequently characterized by the rejection of certain food sources for psychological rather than rational reasons (Belluco et al., 2013; DeFoliart 1999; Paoletti and Dreon 2005). European legislation, via Regulation EC 258/97 (repealed from 1 January 2018 by Regulation EU 2015/2283), states that all insect-based products (not only parts of insects or extracts, but also whole insects and their preparations) belong to one of the categories of "Novel Food", but currently there are no specific regulations in Europe on the breeding and marketing of insects intended for human consumption (FASFC, 2014). Nevertheless, some European countries (led by Belgium and the Netherlands) are now investing in production and promotion of insect-based food, which is now available on their respective markets. However, similar to other animal products, insects might accumulate hazardous chemicals, including heavy metals (Handley et al., 2007; Zhuang et al., 2009), dioxins (Devkota and Schmidt, 2000), and flame retardants (Gaylor et al., 2012), but to the best of our knowledge there are no published data on hazardous chemicals in reared insects and in insect-based food. Insect species, stage of harvest, production methods, and especially the chosen substrate (source of nutrients) may all impact the occurrence and accumulation of contaminants in insect-based food (EFSA, 2015). Thus, the possible acceptance and the change in the approach of developed countries with regards to entomophagy would certainly be supported by food chemical safety (Mlcek et al., 2014).

In response to the recommendations expressed in a recent scientific opinion adopted by EFSA (2015) on the “risk profile related to production and consumption of insects as food and feed”, the present study intended to assess, for the first time, the chemical content of four species of edible insects (greater wax moth, migratory locust, mealworm beetle, and buffalo worm) and four insect-based food items currently commercialized in Belgium. In particular, our study aimed (i) to provide a comprehensive overview of the residual levels of different chemical compounds, including flame retardants (BFRs, DPs, and PFRs), organochlorine compounds (PCBs, OCPs: DDT, HCH, HCB), dioxins and dioxin-like PCBs, pesticides, and metals (As, Cd, Co, Cr, Cu, Ni, Pb, Sn, Zn), in insects

and insect-based food intended for human consumption, and (ii) to compare these levels to those measured in common animal products (such as fish, meat, and eggs).

2. Materials and Methods

2.1 Sample preparation

Several species of edible insects (including larvae of greater wax moth - *Galleria mellonella*, adults of migratory locust - *Locusta migratoria*, larvae of mealworm beetle - *Tenebrio molitor*, and larvae of buffalo worm - *Alphitobius diaperinus*) and different types of insect-based food (including locust- and buffalo worm-based "bugballs", cricket croquettes, and buffalo worm-based "bugburger") were purchased in November 2015 – February 2016 from three different European companies at various shops, e-shops, and supermarkets from Antwerp (Belgium) (Table 1). All the samples were certified and authorized for human consumption.

For each species of edible insects, individuals were pooled and homogenized with a blender; the same was done for insect-based food samples. Then, the samples where freeze-dried and stored at -20 °C until chemical analysis. The lipid content of each sample was determined using a gravimetric method, as described by Xu et al. (2015).

2.2 Chemicals and materials

BDE-103 and BDE-128 (internal standards (IS)) were obtained from AccuStandard Inc. (New Haven, CT, USA). ^{13}C-syn-DP and ^{13}C-anti-DP (IS) were purchased from Cambridge Isotope Laboratories (CIL) (Andover, MA, US). Triamyl phosphate (TAP) (IS) was purchased from TCI Europe (Zwijndrecht, Belgium). Labeled internal standards, TPHP-d15, TDCIPP-d15, TBOEP-d6, and TCEP-d12 (IS) were custom synthesized. CB-143 (IS) and recovery standard (RS) CB-207 were purchased from Dr. Ehrenstorfer Laboratories (Augsburg, Germany). IS for mono-ortho PCBs (MO-PCBs, including PCB-105, 114, 118, 123, 156, 157, 167, and 189) (MBP-MKX) was purchased from Wellington Laboratories (Guelph, ON, Canada). The 2,3,7,8-substituted PCDD/F

congeners and coplanar PCBs (PCB-77, 81, 126, and 169) were quantitated using IS EDF-4144 from CIL. RS EDF-4145 was used for recoveries of PCDD/Fs and co-PCBs: $^{13}C_6$1,2,3,4-TCDD for tetra- and penta-chlorinated compounds; $^{13}C_{12}$1,2,3,4,7,8,9-HpCDF for hexa-, hepta-, and octa-chlorinated species and $^{13}C_{12}$CB-80 for co-PCBs; RS EC-1414 containing $^{13}C_{12}$ PCB-80 for MO-PCBs (description of the calibration curve available at L'Homme et al., 2015). All RSs were purchased from CIL.

Polypropylene-PP tubes (15 mL) were obtained from Greiner Bio-one (Belgium), while PP syringe, $MgSO_4$, and NaCl were purchased by Sigma-Aldrich (St. Louis, MO, USA). Disposable PTFE columns for EconoPrep™ automated clean-up system were obtained from Fluid Management Systems (FMS Inc., Waltham, MA, USA). Empty polypropylene cartridges (25 mL) were purchased from Grace (Lokeren, Belgium), while C18 sorbent, Z-SEP sorbent, primary-secondary amine (PSA), Florisil® cartridges (500 mg, 3 mL) were purchased from Supelco (Bellefonte, PA, USA); sodium sulfate and diatomaceous earth were purchased from VWR International (Radnor, PA, USA). Silica gel, concentrated sulfuric acid (H_2SO_4, 98%), and formic acid (FA, for analysis, 98-100 %) were purchased from Merck (Darmstadt, Germany). All solvents were chromatography grade: *n*-hexane (*n*-Hex) was purchased from Acros Organics (Belgium); ethyl acetate (ETAC), dichloromethane (DCM), iso-octane, and acetonitrile (ACN) were purchased from Merck, nonane was purchased from Fluka (Steinheim, Germany), ethanol was purchased from Sigma Aldrich (St. Louis, MO, USA). Hexane and toluene for dioxins and dioxin-like compound analysis were Picograde® reagents (LGC Promochem, Wesel, Germany). All solvent batches were tested for the investigated analytes contamination before use. All plastic-ware used for metal analysis was new and cleaned by soaking in 10% (*v/v*) HNO_3 for ≥ 24 h followed by thorough rinsing with deionized water (18.2 MΩ·cm, Milli-Q, Millipore). All chemicals were analytical reagent grade or equivalent analytical purity.

The target analytes are listed in Table S1 in the Supplementary Material.

2.2.1 PCBs, OCPs, BFRs

For the analysis of BFRs, PCBs, and OCPs, 1 g of dry sample was weighed in a pre-washed 15 mL polypropylene (PP) tube, and each sample was spiked with the IS mixture (including BDE-103, BDE-128, ^{13}C-syn-DP, ^{13}C-anti-DP, and CB-143). After spiking, 10 mL of *n*-Hex:DCM (1:1 *v/v*) was added to the samples. The tube was capped, vortexed for 1 min, and place overnight at 4 °C. The following day, the sample was first vortexed 1 min, and then centrifuged at 2178 g for 5 min. The supernatant was transferred to pre-cleaned glass tubes and concentrated under a gentle nitrogen stream to a volume of 2 mL. The solution was loaded onto 25 mL cartridges containing 8 g of acidified silica (AS, 44%, *w/w*, prewashed with 15 mL *n*-Hex) and eluted with 20 mL of *n*-Hex and 15 mL of DCM. The final extract was concentrated to near dryness, reconstituted in 50 µL of iso-octane and 50 µL of the recovery standard (RS) (CB-207 in iso-octane:toluene; 9:1, *v/v*) and transferred to amber injection vials for GC-ECNI/MS and GC-EI/MS analysis.

2.2.2 PFRs

For the PFR analysis, 0.5 g of dry sample was added into a 25 mL PP syringe with a frit and the cap at the bottom. Each sample was spiked with IS mixture (including TAP, TPHP-d15, TDCIPP-d15, TBOEP-d6, and TCEP-d12) and added with 5 mL of ACN. The syringe was then closed with the piston, vortexed 1 min, and placed overnight at 4 °C. The following day, the sample was first vortexed 1 min, and then the solvent was transferred in a pre-cleaned glass tube. The extract was concentrated to 2 mL and added with 160 mg Z-SEP sorbent. After performing the d-SPE, the extract was centrifuged and the supernatant was first transferred to a pre-cleaned glass tube, concentrated to near dryness and finally reconstituted with 0.5 mL of *n*-Hex. The solution was loaded on a Florisil® cartridge (pre-conditioned with 6 mL of ETAC and 6 mL of *n*-Hex). The fractionation was achieved with 12 mL of *n*-Hex:DCM (1:1 *v/v*) (F1) and 10 mL ETAC (F2). F1 was discarded, while F2, containing the target compounds, was concentrated to near dryness, and reconstituted with 50 µL of RS and 50 µL of iso-octane:ETAC (8:2 *v/v*). The sample was then

transferred to injection vial and stored under -20 °C for at least 30 min before analysis to precipitate residual lipids (if present).

2.2.3 Dioxins and dioxin-like PCBs

The insect samples (with the exception of EI-5, due to the small amount of sample available), freeze-dried and milled in their entirety as commercially available, one procedural blank, and one freeze-dried egg yolk quality control (QCE) were analyzed for the quantification of PCDDs, PCDFs, co-PCBs, and MO-PCBs at the University of Liège. As no average levels and no regulation were available for edible insects, the amount considered for each sample ranged from 15 to 25 g ww, based on the estimated average serving (Table S3). The freeze dried samples were first mixed with diatomaceous earth, then spiked with labelled IS solution and extracted with accelerated solvent extraction (ASE^{TM} 350, Dionex, Thermo Fisher Scientific) using toluene/ethanol (9:1, *v/v*) at 150 °C for 5 min for 2 cycles. Extracts were filtrated over Na_2SO_4 and the filtrate solvent was evaporated to dryness for fat determination. The extracted fat, ranging from 0.3 to 3 g depending on the sample, was cleaned-up with the EconoPrep^{TM} automated system equipped with disposable PTFE columns. Due to commercial column fat capacity, for fat amount ranging from 1 to 3 g (EI-1, -2, -3, -4, -8 and QCE), a 2-step automated approach was used: i) fat degradation with high capacity acidic silica column (HC-ACD, 22 g of 44% acidic silica), and ii) after hexane evaporation up to approximately 1 mL (SuperVap 6 positions from FMS, 40°C, N_2 stream 10 psi, sensor mode), further clean-up and fractionation with classic ABN silica column (4 g acid, 2 g basic, 1.5 neutral), carbon-Celite column and mini-basic alumina column (3 g). For the blank and for samples with fat content up to 1 g (EI-6, -7, -9), preliminary fat degradation was not necessary and single clean-up and fractionation step was directly carried out using mid-capacity multilayer ABN silica column (MID-ABN) connected to carbon-Celite column, followed by a mini-alumina column. The first procedure lasted 42 minutes (excluding evaporation time) and consumed 310 mL of solvents (260 mL of hexane and 50 mL of toluene). The second procedure lasted 28 min and required 150 mL of

solvent (100 mL of hexane and 50 mL of toluene). No DCM was used for sample clean-up because of the new column composition and a new plumbing diagram of the EconoPrep^{TM}, where the silica column is followed by carbon-Celite column connected to an alumina column. Two fractions were collected from the automated system, both with toluene in backflush: fraction A (FA) containing MO-PCBs, and fraction B (FB) containing co-PCBs and PCDD/Fs. Fraction solvents were evaporated to approximately 500 µL in dedicated tubes using a sensor-equipped SuperVap 6 positions from FMS (55 °C, N_2 stream 12 psi, sensor mode) and then transferred in GC vials containing nonane (90 µL for FA and 10 µL FB) as a keeper. The final evaporation for complete solvent exchange to nonane was done with SuperVap 24 from FMS (35 °C, N_2 stream 1 psi until complete solvent exchange to nonane). RS was added to each fraction prior the instrumental analysis.

2.2.4 Pesticide suspect-screening

For the suspect-screening analysis of pesticides, 0.5 g of dry sample was extracted and purified by the modified QuEChERS method described below. Each homogenized sample was weighed into a 15 mL PP Falcon tube and 7 mL of ACN was added. After 1 min of vortexing, 1 g of $MgSO_4$ and 0.25 g of NaCl were added and immediately vortexed for 1 min. This ratio of salts was shown to be the most effective in selectivity and in capacity to separate aqueous and organic phases, maintaining low co-extraction of interferences (González-Curbelo et al., 2015). The sample was centrifuged at 4500 g for 5 min, then 2 mL of the supernatant was transferred into a pre-cleaned glass tube and concentrated to 1 mL under a gentle nitrogen stream. The clean-up phase was then performed by adding 50 mg of PSA (to remove polar interferences, including fatty acids, other organic acids, and pigments) and 100 mg of C18 (for the removal of lipids and non-polar co-extractives) to the extract and the mixture was vortexed for 1 min. After a second centrifugation at 4500 g for 5 min, the supernatant was transferred into a pre-cleaned glass tube, concentrated to dryness under gentle

nitrogen stream, reconstituted in 150 µL ACN:MilliQ (1:1 *v/v*), and filtered through a 0.22 µm nylon membrane to an autosampler vial for analysis by LC-Q-TOFMS.

2.2.5 Metals

In a pre-weighted vial, 2 mL of concentrated HNO_3 (67-69% Trace Metal Grade, Fisher Chemical) was added to 0.1 g of dry sample. Metals were extracted at room temperature overnight followed by heating on a hot block (110 °C) for 1 h. After 30 min of heating, 200 µL of H_2O_2 (30% AnalaR Normapur, VWR Chemicals) were added to samples. Once samples cooled down, vials were re-weighted and diluted 5-fold (*v/v*) using deionized water (18.2 MΩ·cm, Milli-Q, Millipore). A further 10-fold dilution (*v/v*) was performed before analysis.

2.3 Instrumental analysis

2.3.1 PCBs, OCPs, BFRs

GC-ECNI/MS: The analysis was performed with an Agilent 6890 GC (Palo Alto, CA, USA) coupled to an Agilent 5973 MS operated in electron capture negative ionization (ECNI) mode and equipped with a DB-5 capillary column (30 m × 0.25 mm × 0.25 µm). The GC system was equipped with electronic pressure control and a programmable-temperature vaporizer (PTV) inlet. 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 µL) was performed under a pressure of 10.06 psi until 1.25 min and purge flow to split vent of 50 mL/min after 1.25 min. The GC temperature ramp started from 92 °C, held 1.25 min, ramped at 10 °C/min to 300 °C, held 1 min, ramped at 40 °C/min to 310 °C, 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 mass spectrometer was operated in selected ion monitoring (SIM) for the quantification of BDE-28, 47, 100, 99, 154, 153, 183, BTBPE, syn-DP, anti-DP, CB-101, 99, 118, 153, 138, 187, 183, 180, 170, oxychlordane (OxC), trans-nonachlor (TN), HCB, pp'-DDE, pp'-DDT, α-HCH, β-

HCH, γ-HCH. BDE-103 and BDE-128 were used as IS for all PBDE congeners and BTBPE; ^{13}C-syn-DP, and ^{13}C-anti-DP, were used as IS for syn-DP, and anti-DP, respectively; CB-143 was used as IS for the targeted PCBs and OCPs.

GC-EI/MS: The analysis was performed with an Agilent 6890 GC coupled to an Agilent 5973 MS operated in electron impact ionization (EI) mode and equipped with an HT-8 column (25 m × 0.22 mm × 0.25 µm). The GC system was equipped with electronic pressure control and a PTV inlet. The injection temperature was set at 100 °C, held 0.03 min, ramped at 700 °C/min to 300 °C, held 40 min. Injection (1 µL) was performed under a pressure of 14.34 psi until 1.5 min and purge flow to split vent of 50 mL/min after 1.5 min. The GC temperature ramp started from 90 °C, held 1.5 min, ramped at 15 °C/min to 180 °C, ramped at 5 °C/min to 280 °C, ramped at 40 °C/min to 310 °C, held 11.75 min. Helium was used as a carrier gas with a flow rate of 1.0 mL/min. The mass spectrometer was operated in selected ion monitoring (SIM) for the quantification of CB-28, 52, 149, HCB. CB-143 was used as IS for the targeted PCBs and OCPs.

2.3.2 PFRs

PFRs were analyzed using an Agilent 6890 GC coupled to an Agilent 5973 MS operated in EI mode. The GC system was equipped with an HT-8 column (25 m × 0.22 mm × 0.25 µm). The GC system was equipped with electronic pressure control and a PTV inlet. The injection temperature was set at 80 °C, held 0.03 min, ramped at 700 °C/min to 300 °C, held 40 min. Injection (1 mL) was performed under a pressure of 13.65 psi until 1.25 min and purge flow to split vent of 50 mL/min after 1.25 min. The GC temperature ramp started from 80 °C, held 1.25 min, ramped at 15 °C/min to 200 °C, held 3 min, ramped at 5 °C/min to 270 °C, ramped at 20 °C/min to 310 °C, held 12 min. Helium was used as a carrier gas with a flow rate of 1.0 mL/min until 28 min, then increased to 1.5 mL/min. The mass spectrometer was run in SIM mode and TEHP, TCEP, TCIPP (2isomers), EHDPHP, TPHP, TDCIPP, TNBP and TBOEP were analyzed. TAP was used as IS for TEHP,

TNBP; TCEP-d12 was used for TCEP and TCIPP (2 isomers); TBOEP-d6 was used for TBOEP; TPHP-d15 was used for TPHP and EHDPHP; TDCIPP-d15 was used for TDCIPP.

2.3.3 Dioxins and dioxin-like PCBs

The detection and quantification of target compounds were performed by GC-HRMS Double Focusing Magnetic Sector (DFS, Thermo Fisher Scientific, Bremen) connected with heated transfer lines to two chromatographic ovens (Trace 1300 series from Thermo Scientific), one equipped with an Agilent DB-5ms ultra inert (60 m × 0.25 mm × 0.25 µm) column, and the other one containing HT-8 (25 m × 0.22 mm ID × 0.25 µm film thickness) chromatographic column (SGE, Villebon, France). Both chromatographic ovens were equipped with DualData XL device for fast in-series acquisition.

Measurements of the compounds in FA were carried out using helium as the carrier gas at constant flow rate of 0.8 mL/min. A volume of 1 µL from the final extract in nonane (100 µL) was injected into a split/splitless (SSL) injector held at 290 °C in splitless mode. The oven temperature was maintained at 140 °C for 2 min, ramped at 15.0 °C/min to 220 °C held for 7.5 min, ramped at 6.0 °C/min to 250 °C, ramped at 2.0 °C/min to 265 °C, and finally ramped at 28 °C/min to 320 °C. The HRMS instrument was operated in selected ion monitoring (SIM) mode with a static resolving power of 10,000. Two ions were monitored for both native and labelled compounds for isotope ratio check. For FB, the transfer line was heated at 275 °C, helium was used as the carrier gas at constant flow rate of 1.5 mL/min. A volume of 1 µL out of the final extract in nonane (15 µL) was injected into (SSL) injector held at 290 °C in splitless mode. The oven temperature was maintained at 130 °C for 1.50 min, ramped at 20 °C/min to 250 °C, ramped at 2.5 °C/min to 285 °C and ramped at 10 °C/min to 320 °C for 4 min. The HRMS instrument was operated in selected ion monitoring (SIM) mode with a mass resolution of at least 10,000 at a 10 % valley. The specificity was insured by monitoring the signal of two ions and comparing their ion ratio to the theoretical chlorine isotope ratio.

2.3.4 Pesticide suspect-screening

The separation was performed on a Kinetex XB-C18 (150 x 2.1 mm; 1.7 µm particle size) RP-column (Phenomenex, Utrecht, the Netherlands), connected to an Agilent 1290 Infinity UPLC binary pump (Santa Clara, USA). Mobile phase A was 0.04 % *v/v* of formic acid in water (pH = 2.86), mobile phase B was composed of 95 % ACN and 5 % mobile phase A. A gradient was made at a flow rate of 0.3 mL/min starting with 5 % B for 2 min, increasing to 90 % of B at 15 min and 100 % of B at 18 min. The column was rinsed for 6 min and re-equilibrated at starting conditions for 4 min. Column and mobile phases were heated up to 30 °C. Injection volume was 3 µl and 1 µL for positive and negative ionization mode, respectively. Eluting analytes were detected with an Agilent 6530 Q-TOF-MS with Agilent-Jet-Stream-Electrospray Ionization (AJS-ESI). For both ionization modes, the drying gas was 250 °C at a flow rate of 10 L/min, the sheath gas was 350 °C at a flow rate of 12 L/min. The pressure on the nebulizer was 45 psig. The voltages of the capillary and the nozzle were 3500 V and 500 V, respectively. The fragmentor voltage was 120 V. For the acquisition of full HRMS spectra, the MS scan rate was 59-1100 m/z with a cycle of 250 ms. Fragmentation spectra were acquired by auto-MS/MS at a scan rate of 125 ms. Precursor ions were selected with a narrow bandwidth (m/z=1.3) and fragmented with a collision energy of 20 V. For each cycle, maximum 3 precursors were selected, the threshold was 1000 counts and active exclusion for 4 s was used to increase the coverage of analytes fragmented. A static exclusion list was used to prevent fragmentation of background ions. All data were stored in centroid mode for further data-analysis.

The data were analyzed using Mass-Hunter Qualitative analysis (version B.06.00; Agilent Technologies). The method was based on an untargeted screening approach: the molecular formulas representing different contaminants were searched by the Find by Formula algorithm (Agilent Technologies). Parameters were set as followed: match tolerance 10 ppm, expected variation 2 mDa ± 8 ppm, no match for a score below 80. The algorithm was instructed to look for proton, sodium

and ammonium adducts in the positive ionization mode ($M+H/M+Na/M+NH_4$). In negative mode, the data were screened for anionic molecules and formic acid adducts ($M-H/M+HCOO^-$). The databases used were the Maurer Pfleger Weber (MPW, version 2007) and the Agilent ForensicsTox_AM PCDL (version 4 Agilent, 2011), both commercially available.

2.3.5 Metals

Levels of As, Cd, Co, Cr, Cu, Ni, Pb, Sn, Zn in digested insect samples were assessed by inductively coupled plasma-mass spectrometry (ICP−MS, Agilent Technologies Series 7700x). ICP multi-element standard solution IV and VI (Merck) were used to prepare calibration standards. Final working standards were prepared in 2 % HNO_3. An ICP-MS autosampler (ASX-500 Series, Agilent Technologies) was used for automated sampling. During analysis, a nebulizer pump rate of 0.1 rps was used to introduce the sample in the spray chamber through a MicroMist nebulizer. After introduction of the sample in the spray chamber, a stabilization time of 40 s was used before actual measurement. The general plasma mode was used for analysis with the following parameters: 1550W RF Power, 10 mm sampling depth and 1.01 L/min carrier gas flow (Ar), monitored signals ^{52}Cr (230 ms), ^{59}Co (230 ms), ^{60}Ni (230 ms), ^{63}Cu (220 ms), ^{66}Zn (190 ms), ^{75}As (230 ms), ^{111}Cd (220 ms), ^{118}Sn (220 ms), ^{208}Pb (220 ms). Concentrations of calibration standards were verified by comparison with National Institute of Standards and Technology (NIST) spectrophotometric standards (SRM 1640a).

2.4 QA/QC

QA/QC of the analytical method for BFRs, PCBs, and OCPs was carried out using the Cod Liver 1402-CLA (EURL-PT-BFR_1402-CL). All measured values were within the certified range (± 20 %). The mean recoveries of the spiked IS standards ranged from 60 to 120 %. The QA/QC method for the PFRs was performed using the fish oil used in the first worldwide interlaboratory study (ILS) on PFRs (Brandsma et al., 2013). The measured values were within the range of the

consensus concentrations (±15 %), while the mean recoveries of the spiked IS standards ranged from 50 to 70%. A procedural blank was analyzed every ten samples to check for laboratory contamination. If analytes were detected in the procedural blanks, the blank concentrations were subtracted from the values found in the samples.

The limit of quantification (LOQ) was ten times the signal to noise ratio of each peak for compounds not detected in the procedural blanks or it was calculated as average blank values plus three times the standard deviation of the blanks for compounds present in the blanks. LOQs ranged from 10 to 3000 pg/g ww for the organic compounds, between 0.0004 to 232 pg/g ww for dioxin compounds, and from 0.03 to 0.30 mg/kg for metals. Individual LOQs for each compound are listed in the Table S1.

Egg yolk was chosen for quality control (QCE) for the dioxin compound analysis, since no specific quality control sample for edible insects was available and because it followed the same sample preparation of edible insects. We assumed that the fat extraction had the same efficiency for eggs and insects because the same extraction method proved to be effective for a great variety of food and feed matrices and it is suggested at the EURL level. Recovery tests to check for chitin adsorption were carried out on lobster samples, ground in their entirety, shell included, undergoing the complete analytical procedure. QCE contamination levels, measured in the same series of samples as edible insects were in accordance with the control chart drawn in our routine laboratory, demonstrating the validity of the analytical procedure without full method validation, not performable due to the lack of Regulation and MRLs on edible insects. A single procedural blank was considered even if two sample clean-up approaches were used, as that average blank levels in the two cases were not statistically different. Contamination levels for PCDD/Fs, co- and MO-PCBs in the blank (0.61 pg WHO-TEQ/g) were in accordance with the blank acceptability criteria set in our accredited laboratory for other regulated food and feed matrices (10% of the MRL in pg TEQ/g lw or ww).

The quality of identification during the suspect-screening analysis was evaluated according to Schymanski et al. (2014), as reported in Fig S1. Specifically, the identification of contaminants of which the molecular formula is known without the presence of any MS/MS spectrum is considered of low quality and received a score of 4. When MS/MS patterns are available to suggest a chemical class, but unequivocal confirmation is not possible, the compound received a score of 3. When the MS/MS spectrum was of good quality and the fragments matched libraries and could be explained, the identification quality was classified as level 2. When standards were available, they were injected to confirm the identification of the compounds. The comparison was based on retention time, MS profile and MS/MS fragments. Compounds which had the same retention time ± 0.1 min and have the same MS pattern (5 ppm, isotope pattern 3 %; MS/MS: match with all major peaks) were confirmed as level 1. The samples were injected in triplicate, 1/10 dilutions were injected in duplicate, making a total of 5 replicates for each sample. To reduce the number of false positives, the extracted features were grouped and qualitatively evaluated using Mass Profiler Professional (version 12.5, Agilent Technologies). For each sample, the detected features were only retained if they were present in 100 % of the samples of which the signal was higher in the original samples than in the diluted samples and the blank. The reduced list was further manually evaluated on peak shape, MS pattern and MS/MS pattern to confirm the identification. We validated the approach by spiking a mixture of five compounds (carbaryl, ethiofencarb, diuron, diazinone, chlorpyrifos-methyl) to an aliquot of sample EI-2. All spiked compounds have been found correctly in the extracted dataset. The proposed method is an effective way to clear false positive contaminants without the increment of false negatives.

For the metal analysis, duplicates of all samples, blanks (n=3), and certified reference material (CRM, National Institute of Standards & Technology, mussel tissue, SRM 2976, n=3) were analyzed. Duplicates were generally within 20 % and no significant differences between CRM measured concentrations and certified values were observed (based on the comparison between the absolute difference between measured and certified values and combined (expanded) uncertainties).

3. Results and discussion

3.1 Levels of chemicals in edible insects and insect-based food

3.1.1 Chlorinated compounds

The individual and total levels, based on lower bound levels (LB), of PCBs, OCPs, BFRs, and PFRs in the edible insects and insect-based food are given in Table 2. An overview of the total contaminant levels presented as lower, medium, and upper bound (LB, MB, and UB) levels (pg/g ww) is given in the Supplementary Material, Table S4. Levels of total PCBs largely varied among samples, ranging from 26.5 pg/g ww (EI-3) to 2065 pg/g ww (EI-2), with an average mass fraction of 743 (±745, SD) pg/g ww. Overall similar PCB levels were measured between samples of edible insect (EI-1 to EI-5) and insect-based food (EI-6 to EI-9), and between larvae (EI-1, EI-3, EI-4) and adult organisms (EI-2, EI-5). PCB levels in samples EI-2 appeared to be higher than those measured in EI-5, although samples contained the same insect species (*Locusta migratoria*). The two samples were purchased from different suppliers, suggesting that the organisms may have been exposed to different environmental and rearing conditions, including the use of a different substrate. The PCB levels determined in edible insects and in insect-based food were compared with the current European Regulation (1881/2006) setting the maximum UB levels for the sum of six PCB indicators (PCB-28, 52, 101, 138, 153, 180). The average values of the sum of PCBs considered in the present study (including the six PCB indicators), 0.98 ± 0.66 ng/g ww and 19.7 ± 19.5 ng/g lw based on UB levels, were below the regulated maximum residual levels set for beef, poultry, milk, and eggs (40 ng/g lw) and fish (75 ng/g ww).

Among the various organochlorine compounds (OCPs) examined, HCB, DDT and HCHs were detected in almost all the analyzed samples, contributing to total OCPs levels ranging between 46.3 (EI-4) and 368 (EI-1) pg/g ww, whereas OxC and TN were always <LOQ (Table 2). HCB was measured in all the edible insects and in one processed food (EI-6) (29.6 – 70.1 pg/g ww). The metabolite p,p'-DDE was detected in seven samples (34.9 – 176 pg/g ww), the parental compound

p,p'-DDT in six samples (24.5 – 91.1 pg/g ww), and p,p'-DDD was <LOQ in all samples. For HCH, the γ-isomer (lindane) was found in all the edible insects and in two insect-based food samples (12.2 – 34.3 pg/g ww), whereas α-HCH was measured only in EI-1 (18.2 pg/g ww), and β-HCH was never detected in any of the analyzed samples. Due to the high lipophilicity of these compounds, widely produced and used as insecticides, and their strong resistance to degradation, it is likely that the detected OCPs were accumulated by the animals during the rearing process, possibly from the rearing soil and the substrate used for feeding. This is supported by previous studies indicating that OCP residues can be absorbed by multiclass plants and accumulate in terrestrial animals through food chains (Tang et al., 2016; Pan et al. 2014). A similar chemical transfer was suggested by Belluco et al. (2013), stating that the chemical hazards in insects depend on, in most cases, habitat and plant feed contamination.

3.1.2 Flame retardants

The individual and total levels of BFRs and PFRs in the edible insects and insect-based food are given in Table 2. PBDE levels were generally <LOD and never exceeded 35.5 pg/g ww. BDE-47 was detected in four out of nine samples (EI-1, EI-2, EI-5, and EI-7) and BDE-99 was detected only in sample EI-5. Also, no BTBPE and DPs were detected in the edible insects and in the insect-based food. PFR levels in the samples were generally two/three orders of magnitude higher than the levels of PBDEs (total mass fractions ranging from 783 to 23786 pg/g ww). A total of six PFRs were detected: TCIPP was the most frequently detected contaminant, found in seven samples (783 – 1996 pg/g ww), followed by EHDPHP (1159 – 15344 pg/g ww), and TPHP (718 – 8442 pg/g ww). In addition to possible bioaccumulation of flame retardants from the substrate and the rearing soil, the presence of PFRs in the analyzed samples could be also explained by their use during the food treatment processes and packaging (Campone et al., 2010).

3.1.3 Dioxins and dioxin-like PCBs

The individual and total levels of dioxins and dioxin-like PCBs in the edible insects and insect-based food are given in Table S5. The total levels of dioxin compounds ranged from 0.0001 to 0.25 pg WHO-TEQ/g ww (based on LB approach), two times higher in the insects than in the insect-based food, likely because of the "dilution effect" due to the presence of other ingredients than insects in these samples (Table S2). The total mass fractions were also expressed in total pg WHO-TEQ/g ww and lw, based on UB levels, to compare our data with the current European Regulation setting the maximum levels for dioxin compounds in foodstuffs (Commission Regulation 1881/2006 and its amendments). From this comparison, the total levels of dioxin compounds measured in the analyzed edible insects (ranging from 0.05 to 0.28 pg WHO-TEQ/g ww, and from 1.40 to 5.98 pg WHO-TEQ/g lw) were mostly below the regulated maximum residual levels set for beef (4.0 pg WHO (2005) TEQ/g lw), poultry (3.0 pg WHO (2005) TEQ/g lw), fish (6.5 pg WHO (2005) TEQ/g ww), milk (5.5 pg WHO (2005) TEQ/g lw), and eggs (5.0 pg WHO (2005) TEQ/g lw). Our results prove that these food samples do not present any higher risk of human exposure to dioxins and dioxin-like PCBs than regular food of animal origin.

3.1.4 Pesticide suspect-screening

Based on the "Find by Formula" algorithm alone, many compounds were claimed to be detected, and a list with the number of detected features for positive and negative ionization mode is available in Table S6. After filtering, the number of possible contaminants was significantly reduced and all possible positive hits are listed for each sample separately in SM. The list of the contaminants identified in the samples and the level of confidence obtained during the identification process are shown in Table 3. Due to the small amount of sample available, sample EI-5 was not considered for the suspect-screening.

Most hits had a low level of identification, only matching the molecular formula. Other compounds lacked a high quality MS/MS spectrum and their identification could not be confirmed. A possible reason for the lack of high quality spectra is the presence of high abundant co-eluting compounds,

which get fragmented first in the auto MS/MS acquisition method used in our study. Contaminants are usually present in a lower abundance, which increases the risk of not getting fragmented. Of the possible hits, in-house standards of aldicarb, azoxystrobin, carbaryl, carbofuran, metacrifos, mevinphos, pirimiphos-methyl were available, and the retention time, m/z values, and MS/MS patterns were compared to the signals in the samples to confirm the putative identification. Only azoxystrobine and pirimiphos-methyl had a positive match and their presence was confirmed, providing level 1 of identification. The other five compounds did not match properly and were therefore discarded from the detected list.

Since the screening procedure is a qualitative approach, it was only possible to state the presence of the detected compounds, according to the level of identification, and no quantification was performed. Certain contaminants were more frequently detected in samples, such as vinyltoluene (always detected), tributylphosphate, and pentafluoropropionic acid (present in 75 % of the samples). The first two compounds might derive from their industrial applications in the areas intended for the insect rearing or for the insect-based food preparation. Tributylphosphate finds also its use as herbicide and fungicide concentrates. In addition, different pesticides were detected in multiple samples, most of them used as insecticides (e.g. methoprene, empenthrine, pirimiphos-methyl). In particular, pirimiphos-methyl was the only compound identified at the highest level of confidence in 50 % of the samples, mostly in the insect-based food (likely due to the high vegetable composition rate of samples EI-6, EI-7, and EI-8, up to 75%). Pirimiphos-methyl is indeed an organophosphorus compound used for controlling a wide range of pests and mites in stored grain, animal houses, domestic and industrial premises, and on vegetables (flowers, sugar cane, maize, sorghum, rice, citrus and other fruit, olives, vines, and cereals) (FAO, 2004). However, it was not possible to identify a clear contamination pattern between the insects and the insect-based food.

3.1.5 Metals

In this study, we chose to investigate three essential metals (Co, Cu, Zn,), and six metals with no dietary requirements (As, Cd, Cr, Ni, Pb, Sn). In all samples, Cu and Zn were consistently the most abundant metals, with mass fractions ranging from 0.85 to 9.12 mg/kg ww and from 6.44 to 58.60 mg/kg ww, respectively (Table 4). Lower levels were measured for Co (<0.05 mg/kg ww), Cd (<0.06 mg/kg ww), Cr (<0.24 mg/kg ww), and Ni (<0.28 mg/kg ww)), while As, Pb, and Sn were consistently <LOQ. Similar levels of Cr and Ni were measured between samples of edible insects and insect-based food, whereas considerably higher levels of Cu and Zn were found in edible insects, which were about five times as high as those measured in insect-based food samples. This is likely due to the lower content of insect in these samples (15.8 %), and the presence of additional ingredients with a lower metal content.

The values of Cu and Zn measured in this work are comparable to those found in three species of edible insects (adult cricket, larvae of mealworm, and adult locust) by Zielińska et al. (2015), confirming that edible insects are likely to provide these essential micronutrients. The levels of As, Cd, and Pb measured in this study were lower than those found by Hyun et al. (2012) in edible grasshoppers from Korea (mean levels of 0.12, 0.02, and 0.73 mg/kg ww, respectively), considered as safe for human consumption. However, published data on the presence and levels of metals in comparable edible insects are rather scarce, which makes it difficult to further compare our results with other studies. When compared to maximum levels of metals in foodstuffs set by the European Commission (Commission Regulation 1881/2006), Cd, Pb, and Sn levels in insect samples were always lower than maximum values for all types of foods, including meat of bovine animals, sheep, pig, and poultry, muscle meat of fish, cephalopods, crustaceans, and bivalves. However, only four metals (Cd, Hg, Pb, Sn) are currently regulated by the EU. Overall, our results indicate that the risk of exposure to metals from consumption of edible insects and insect-based food is relatively low and in compliance with European Union regulations.

3.2 Implications for human consumption

Recent studies suggested that the global population will reach 9 billion people by the half of this century and that the world will need a 70 to 100 % higher food production by 2050 (FAO, 2013; Godfray et al., 2010). Currently, one of the major challenges for the food industry is the rapidly increasing demand for meat, fish, and dairy products over the past 50 years (Godfray et al., 2010). Insects can be a valuable alternative to traditional food of animal origin, such as beef, poultry, pork, fish, and eggs (FAO, 2013; Shockley and Dossey, 2014; EFSA, 2015). Out of the recorded number of edible insect species consumed all over the world, and based on regional and national estimations, 250 species were identified to be used as food in Africa, more than 200 species in China and South-East Asia, and more than 400 species were estimated as food in South America, whereas only 12 species of edible insects have now the biggest potential to be farmed on commercial basis in Europe (FAO, 2013; EFSA, 2015). However, edible insects and insect-based food may contain hazardous chemicals (EFSA, 2015) similarly to other animal products. To investigate the possibility for humans to consume certain insect species with no additional hazards in comparison to common animal products, the levels of chemicals present in our samples (including 5 of the 12 insect species potentially commercialized in EU) were compared with those found in other studies reporting contamination levels in meat, fish and seafood, and eggs at levels considered safe for human consumption.

The comparison between the mean lower bound levels of PCBs, OCPs, PBDEs, PFRs, dioxins and dioxin-like PCBs measured in edible insects, insect-based food and meat, fish, and eggs is shown in the Supplementary Material, Table S7. To improve the readability of the table and to allow a more direct comparison with other data, the values of PCBs, OCPs, PBDEs, and dioxins were expressed both based on wet weight and lipid weight (lw). The levels of PCBs in edible insects were found similar or lower than those measured in fish and seafood (200-22000 pg/g ww; 3800-16400 pg/g lw), meat (298-481 pg/g ww; 7000-32000 pg/g lw), and eggs (60-1330 pg/g ww; 5200-15700 pg/g lw), considering different animals and their country of origin (Fattore et al., 2008; Tornkvist et al., 2011; Arnich et al., 2009; Baars et al., 2004; Nakata et al., 2002). Similarly, low levels of OCPs

were measured in edible insects when compared with those present in fish and seafood, meat, and eggs from Sweden (Darnerud et al., 2006) and China (Nakata et al., 2002). The levels of dioxin compounds measured in the analyzed edible insects were lower than those found in fish samples from Finland (Kiviranta et al., 2004) and Sweden (Torknvist et al., 2011; Darnerud et al., 2006), similar to those measured by Bocio and Domingo (2004) in fish samples from Spain, and by Darnerud et al. (2006) and Baars et al. (2004) in eggs from Sweden and the Netherlands, and slightly higher than those observed by Kiviranta et al. (2004), Stachel et al (2006), and Torknvist et al. (2011) in meat samples from Finland, Germany, and Sweden. Concerning flame retardants, the levels of PBDEs measured in edible insects were similar to those found in fish, meat, and eggs from a recent Belgian study (Xu et al., 2015), but lower than those detected in fish and seafood, meat, and eggs from Belgium, Sweden, Finland, and China, (Voorspoels et al., 2007; Darnerud et al., 2006; Kiviranta et al., 2004; Chen et al., 2013; Nakata et al., 2002). On the contrary, the concentrations of PFRs in insects were found slightly higher than those measured in fish, meat and eggs from Xu et al. (2015), maybe due to their use during food treatment processes and packaging (Campone et al., 2010).

The comparison between the mean levels of metals measured in edible insects, insect-based food, meat, fish, and eggs is shown in Table S8. Most of the studies focused on the levels of Zn and Cu in meat, fish, and seafood. The general concentrations of Zn ranged between 5.43 and 47.5 mg/kg ww, while the concentrations of Cu ranged from 0.01 to 1.3 mg/kg ww. Similar or higher levels of Cu and Zn were measured in this study (Table 5), confirming that edible insects are a good dietary source of these elements and a valid alternative source to meat. Regarding the concentrations of other metals (Co, Cr, Ni), similar values were found in insects, fish and eggs (Guérin et al., 2011; Esposito et al., 2016).

Considering the above information, the possibility for these insect species to be consumed by humans with no additional hazards in comparison with commonly consumed animal products was

demonstrated. It was also confirmed that these edible insects can be a valuable and chemically safe alternative to protein-based foodstuff.

3.3 Evaluation of possible contamination sources

According to van der Spiegel et al. (2013), there are mainly two sources for the presence of hazardous substances in insects, such as the production of natural toxins by the insects themselves (at a certain stage of their development) and the intake of contaminants via substrate and soil. Only the evaluation of the second source does fit with the purpose of this study. Based on the current legislation, animals in the EU must be fed only with safe feed (Commission Regulation (EC) No 68/20136, Regulation (EC) No 178/2002, and Regulation (EC) No 767/2009), and the Regulation (EC) No 1069/20097, considering insects as "farmed animals", does not allow the use of certain substrates (e.g. manure, catering waste or former foodstuff containing meat and fish) for their feeding. Thus, the main substrates currently applied in the European insect production include commercial animal feed (mostly chicken feed), former foodstuffs not containing meat and fish (i.e. plant discarded food and co-products from primary production of food of non-animal origin), and clean, fresh-cut, or potted versions of the plant material on which they feed in nature (EFSA, 2015). Unfortunately, no information was available on the kind of substrate and soil used for rearing the analyzed insects and thus it was not possible to evaluate an eventual transfer of contaminants from the substrate to the insects. For this reason, considering the potential key-role played by the substrate and rearing conditions in the chemical contamination of the insects, further studies focused on the comparison between the levels of contaminants in the insects and in the substrate or rearing soil would be of great interest.

3.4 Limitations of the study

The limited number of samples analyzed in this study was mainly due to the scarce availability of edible insects and insect-based food in Belgian grocery stores, which do not (yet) find convenient to

have in stock and sell these items due to the general skepticism of Europeans towards entomophagy. Moreover, for insect-based food, it was not possible to distinguish the contamination of the insects from that of other ingredients, which might have strongly influenced the final concentrations of the food items. However, from a food safety perspective, also the insect-based foods reflect the current EU market assortment of insect products and, as such, were included in this study

4. Conclusions

In this work, the presence and levels of hazardous chemicals in four species of edible insects and in insect-based food currently commercialized in Europe were assessed, and the extent of their accumulation in insects in comparison with accumulation in food-producing animals was also evaluated. Our results suggest the possibility for several chemicals to accumulate in the farmed insect, but the levels of contamination were relatively low and the chemical concentrations were similar or lower than those measured in commonly eaten animal products, such as meat, fish and eggs. In addition, our study suggests that these species of edible insects have the potential to provide specific micronutrients, such as Cu and Zn. Overall, our results support the possibility for humans to consume these insect species with no additional hazards in comparison with commonly eaten animal products, and indicate that the analyzed insect food could be considered a valuable alternative to common sources of proteins.

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Table 1. Identification codes, species, insect content (%) in the food items, dry fraction, and lipid content of the considered pooled samples. The specific composition of samples EI-6 – EI-9 is showed in the Supplementary Material, Table S2.

id	sample	order	species	% insect	dry fraction	lipid %*
EI-1	Greater wax moth	Lepidoptera	*Galleria mellonella*	100	0.38	23.7
EI-2	Locusta 1	Orthoptera	*Locusta migratoria*	100	0.27	11.7
EI-3	Mealworm	Coleoptera	*Tenebrio molitor*	100	0.38	6.9
EI-4	Buffalo worm	Coleoptera	*Alphitobius diaperinus*	100	0.35	5.5
EI-5	Locusta 2	Orthoptera	*Locusta migratoria*	100	0.27	7.7
EI-6	Bug-balls red	Orthoptera	*Locusta migratoria*	7.4	0.39	2.0
EI-7	Bug-balls green	Coleoptera	*Alphitobius diaperinus*	15.8	0.36	5.0
EI-8	Cricket croquette	Orthoptera	*Acheta domesticus*	8.9	0.32	5.0
EI-9	Buggie burger	Coleoptera	*Alphitobius diaperinus*	10	0.28	2.7

*determined on ww basis

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Table 2. Individual and total mass fractions (based on lower bound levels) of PCBs, OCPs, BFRs, and PFRs in the considered edible insects and insect-based food (pg/g ww). LOQs for each compound are listed in Table S1.

Compound	EI-1	EI-2	EI-3	EI-4	EI-5	EI-6	EI-7	EI-8	EI-9
PCBs									
CB-28	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
CB-52	<LOQ	498	<LOQ	<LOQ	<LOQ	256	294	525	<LOQ
CB-101	294	502	<LOQ	<LOQ	43.5	415	245	542	86.0
CB-99	116	155	<LOQ	<LOQ	<LOQ	122	72.0	157	<LOQ
CB-118	93.3	167	<LOQ	<LOQ	32.0	116	66.4	152	24.0
CB-149	<LOQ	203	<LOQ	<LOQ	<LOQ	78.4	<LOQ	138	<LOQ
CB-153	53.6	210	16.1	18.6	48.3	70.0	58.8	97.5	24.9
CB-138	58.8	163	10.5	15.2	41.8	76.0	46.3	100	20.7
CB-187	<LOQ	45.2	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
CB-183	<LOQ	22.5	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
CB-180	<LOQ	70.2	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
CB-170	<LOQ	29.8	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
total	*616*	*2065*	*26.5*	*33.8*	*166*	*1133*	*783*	*1712*	*156*
OCPs									
OxC	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
TN	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
HCB	48.0	46.2	32.9	29.6	70.1	39.0	<LOQ	<LOQ	<LOQ
p,p'-DDE	176	144	97.9	<LOQ	106	34.9	<LOQ	35.0	36.2
p,p'-DDD	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
p,p'-DDT	91.0	54.3	<LOQ	<LOQ	31.3	45.6	34.1	24.5	<LOQ
α-HCH	18.2	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
β-HCH	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
γ-HCH	34.3	15.7	24.7	16.7	26.8	<LOQ	12.2	<LOQ	20.7
total	*368*	*260*	*156*	*46.3*	*235*	*119*	*46.3*	*59.5*	*56.9*
PBDEs									
BDE-28	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
BDE-47	35.5	13.9	<LOQ	<LOQ	13.4	<LOQ	16.2	<LOQ	<LOQ
BDE-100	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
BDE-99	<LOQ	<LOQ	<LOQ	<LOQ	13.5	<LOQ	<LOQ	<LOQ	<LOQ
BDE-154	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
BDE-153	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
BDE-183	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
total	*35.5*	*13.9*	*<LOQ*	*<LOQ*	*26.9*	*<LOQ*	*16.2*	*<LOQ*	*<LOQ*
HFRs									
BTBPE	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
syn-DP	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
anti-DP	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
total	*<LOQ*	*<LOQ*	*<LOQ*	*<LOQ*	*<LOQ*	*<LOQ*	*<LOQ*	*<LOQ*	*<LOQ*
PFRs									
TEHP	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
TNBP	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	2532	<LOQ	<LOQ
TCEP	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	818	<LOQ	<LOQ
TBOEP	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
TPHP	718	<LOQ	8407	8442	4005	<LOQ	<LOQ	<LOQ	<LOQ
EHDPHP	<LOQ	<LOQ	1159	15344	2289	2414	4594	4248	<LOQ
TDCIPP	<LOQ	680	<LOQ	<LOQ	<LOQ	<LOQ	901	<LOQ	<LOQ
TCIPP	1993	862	1996	<LOQ	1951	1662	<LOQ	843	783
total	*2711*	*1542*	*11562*	*23786*	*8245*	*4075*	*8845*	*5090*	*783*

Table 3. List of contaminants identified in the samples. Values are levels of confidence obtained during the identification process, according to Schymanski et al. (2014).

Compound	Class	EI-1	EI-2	EI-3	EI-4	EI-6	EI-7	EI-8	EI-9
hydroxylated geranylgeranyl-methylhydroquinone	unknown	-	-	3	-	-	-	-	-
4-(imidazo[1,2-a]pyridin-2-yl)phenol	unknown	-	-	3	-	-	-	3	3
4-fluorophenylacetic acid	chemical intermediate	2	-	-	-	-	-	-	-
affinine class	insecticide	-	-	-	-	-	-	-	3
ammoidin	psoralene	-	-	-	-	-	-	2	2
azoxystrobine	fungicide	-	-	-	-	-	1	-	-
butylhydroxybenzoic acid	storage additive	-	-	-	3	-	-	-	-
class of chlorbufam	herbicide	-	-	-	-	-	3	-	3
curcumine	flavour	-	-	-	-	-	-	2	-
cycloheximide	fungicide	-	-	-	3	-	-	-	-
diethylphtalate	plasticizer	-	-	2	-	-	-	2	-
difenzoquate	herbicide	3	-	-	-	-	-	-	4
dimantine	antihelmintic	3	-	-	-	-	-	-	-
empenthrine	insectide	-	4	-	-	-	2	-	2
ethacrylic acid-ethylester	polymer	-	2	-	2	-	-	-	-
ethinylcyclohexanol	chemical intermediate	-	-	-	-	-	-	-	3
flavonoid	colouring	-	-	-	-	-	-	-	3
herbicide class morfamquate	herbicide	-	-	3	-	-	-	-	-
methoprene	insecticide	3	-	2	-	-	-	-	-
methoxy-2-methyl-1-[2-(3,4,5-trimethoxyphenyl)ethyl]-1,2,3,4-tetrahydro-7-isoquinolinol	unknown	-	-	-	-	-	3	-	-
methoxybenzaldehyde	fragrant	-	-	-	-	-	3	-	-
methoxyfedrine	stimulant	-	3	-	-	-	-	-	-
n-[1-Amino-3-oxo-1-propen-2-yl]formamide	unknown	-	-	-	-	-	-	-	3
netilmicine	antibiotic	3	3	-	-	-	-	3	-
oxydi-2,1-ethanediyl dibenzoate	plasticizer	-	3	-	-	-	-	-	-
pentafluoropropionic acid	organic chemistry	-	2	2	2	2	2	2	-
piperine	flavour	-	-	-	-	-	-	-	2
pirimiphos-methyl	insecticide	-	1	-	-	1	1	1	-
prostaglandines	endogenous	3	3	3	3	-	-	-	-
streptovaricine G	antibiotic	-	-	-	-	-	3	-	-
terfluranol	anti-neoplastic	-	-	-	-	-	-	-	3
tributylphosphate	flame retardant	2	2	2	2	2	-	2	-
trimethylbenzene	organic chemistry	-	-	-	-	-	3	3	3
vinyltoluene	polymer	3	3	3	3	3	3	3	3
total number of identified compounds		*8*	*10*	*9*	*7*	*4*	*10*	*10*	*13*

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Table 4. Measured levels of metals in edible insects and insect-based food (mg/kg ww).

Sample	Cr	Co	Ni	Cu	Zn	As	Cd	Sn	Pb
Edible insects									
EI-1	0.17	<0.03	0.04	3.47	25.80	<0.03	0.04	<0.03	<0.03
EI-2	0.12	<0.03	0.20	9.12	37.10	<0.03	0.03	<0.03	<0.03
EI-3	0.18	0.05	0.28	5.81	58.60	<0.03	0.06	<0.03	<0.03
EI-4	0.15	<0.03	<0.03	7.72	54.10	<0.03	<0.03	<0.03	<0.03
EI-5	0.11	<0.03	0.20	5.31	38.30	<0.03	<0.03	<0.03	<0.03
Insect-based food									
EI-6	0.12	<0.03	<0.03	1.62	6.44	<0.03	<0.03	<0.03	<0.03
EI-7	0.12	<0.03	0.18	1.67	11.40	<0.03	<0.03	<0.03	<0.03
EI-8	0.24	<0.03	0.18	0.85	8.47	<0.03	<0.03	<0.03	<0.03
EI-9	0.12	<0.03	0.23	1.51	7.33	<0.03	<0.03	<0.03	<0.03

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Highlights

- Chemical levels in insects were lower than those in common animal products
- Non-target screening identified few compounds present in most insect food samples
- Edible insects have the potential to provide specific micronutrients (Cu and Zn)
- Food safety can lead to acceptance of insects as an alternative food sources

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