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Fate of polychlorobiphenyls in *Tenebrio molitor* larvae: consequences for further use as food and feed

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RESEARCH ARTICLE

Abstract

This study explored the ability of *Tenebrio molitor*, one of the most widely bred and traded insect species in Europe, to bioaccumulate Polychlorobiphenyls (PCBs) from their feeding substrate. *T. molitor* larvae were reared for 20 days in a temperature and humidity-controlled incubator and fed with wheat bran artificially contaminated with PCBs at a concentration of 0.67, 4 or 24.4 ppb. The larvae PCB content was then measured based on an analysis by GC-MS and GC-µECD. Whatever the level, a bran contamination by PCBs did not affect the body weight of larvae indicating a high tolerance to PCBs. The bioaccumulation factors (BAF = concentration of PCBs in larvae / concentration of PCBs in wheat bran) obtained with fresh larvae ranged between 0.4 and 1.0 and were significantly higher with the highest contamination level for PCBs 153 and 180. Although there is no bioaccumulation of PCBs in *T. molitor* larvae during rearing, PCBs can be transferred from the diet to the larvae. This study highlights the significant impact of the drying process which induces an increase of the PCB concentration in larvae by a factor of almost 3. This demonstrates the importance of considering the quality of the substrates used for farming insects as food and feed in terms of content in chemical contaminants including persistent organic pollutants like PCBs.

Keywords

PCB, yellow mealworm, bioaccumulation, contaminants, safety

Conflict of interest

The authors declare no conflict of interest.

Funding statement

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1. Introduction

The United Nations estimate that the global human population is expected to reach 9.7 billion in 2050 increasing the consumption of animal products and the growing demand for feed ingredients (United Nations, 2019). Available resources are currently limited and may raise environmental, societal, and economic questions (Tran *et al.*, 2015). The quest for alternative sustainable animal protein sources is therefore expected to become a considerable issue in the feed market. Edible insects could be an interesting solution with a high nutritional value (Van Huis, 2021). Processed animal protein derived from insects are currently authorised for aquafeeds (Commission Regulation (EU) 2017/893) (EU, 2017), poultry and swine feeds in the EU (Commission Regulation (EU) 2021/1372) (EU, 2021). Among the broad variety of insect species, the yellow mealworm (*Tenebrio molitor*) is one of the most widely bred and traded insect species in Europe (Bordiean *et al.*, 2020).

To develop this new sector in future years, it is essential to assess and control the risks associated with the use of insects. Some studies are already available about the microbiological hazards (Vandeweyer *et al.*, 2021). However, there is a lack of knowledge concerning the chemical safety while some chemical contaminants may be found in insect farming environment or in their feeding substrates, can be produced during processing methods or can migrate from packaging material to insect products (Meyer *et al.*, 2021). While some authors have reported that insects may degrade some mycotoxins (Niermans *et al.*, 2019), some antimicrobials (Cai *et al.*, 2018) or even some pesticides (De Almeida *et al.*, 2017), other chemical contaminants like heavy metals can have a negative impact on insect development inducing, for example, an increase in larval mortality (Bulak *et al.*, 2018). Moreover, previous studies have shown that insects like *T. molitor* can bioaccumulate toxic trace elements during rearing, with very variable bioaccumulation factors up to 1.7 for cadmium, 6.2 for mercury and even 34 for lead (Truzzi *et al.*, 2019).

Among chemical contaminants, persistent organic pollutants (POPs) can be transferred from environment or feed to animal products and therefore are of serious concern in animal-derived food products (Engel *et al.*, 2015). In the black soldier fly (*Hermetia illucens*), Van der Fels-Klerx *et al.* (2020) have determined that the bioaccumulation factors of POPs from their substrates (different whole meals and different types of snack products, both with paperboard carton and with plastic packaging materials) ranged between 0.3 and 1.2 for polycyclic aromatic hydrocarbon (PAHs) and between 1.0 and 2.0 for the sum of dioxins and dioxin-like polychlorinated biphenyls (PCBs). However, to our knowledge, there are no studies regarding the bioaccumulation of POPs in *T. molitor.*

Using *T. molitor* larvae as a model and setting up a pilot experimental farming under controlled and safe conditions allowing the implementation of an exposure study with chemical contaminants, the present work explores the ability of insects to bioaccumulate PCBs from their feeding substrate during the larvae growth. For this purpose, the first part of this paper is focused on the set up of an analytical method to monitor PCBs in *T. molitor* larvae and the assessment of the performance of this method in terms of linearity (coefficients of determination, R^2) and sensitivity (limits of detection, LOD). In a second part, the impact of a feed contamination by a mix of 6 non-dioxin like PCBs (nDL-PCBs) on the growth of larvae was studied. The bioaccumulation factors of PCBs in larvae were determined at the end of the rearing and will be discussed in the context of a further use of larvae as food and feed.

2. Materials and Methods

Chemicals

Hexane, dichloromethane, acetone and toluene were organic trace analysis grade solvents (Sigma-Aldrich, Saint-Quentin Fallavier, France). For PCB extraction, Florisil® and activated aluminium oxide (acidic, Brockmann I) were from Sigma-Aldrich. Diatomaceous earth was obtained from Thermo Fisher Scientific (Waltham, MA, USA). The certified reference material AE-00059-H-2X of AccuStandard Europe (Niederbipp, Switzerland) was used for the development of PCB analysis. This mix is composed of the 6 nDL-PCBs (IUPAC numbers 28, 52, 101, 138, 153, and 180) with individual concentrations of 20 ppm. Fluorinated internal standards 3-F-PCB-52 and 5'-F-PCB-126 (Chiron, Trondheim, Norway) were used for the accurate quantification of target compounds.

Larvae

Feeding

Larval feed was dry wheat bran produced by local flour mills in Puy de Dome (France). Larvae were fed with non-contaminated feeds (control group) or with PCB contaminated feeds (exposed groups). For exposed groups, the bran was spiked according to a protocol adapted from Planche *et al.* (2015), based on a contaminant addition to the feed via a volatile solvent. For each PCB exposure condition, the spiking was carried out on the total quantity of wheat bran necessary for all the individual rearing (45g), allowing to ensure the quality of replicates. Briefly, 45 g of dry wheat bran were set in a glass jar (9.5cm high×7cm wide) then immersed in acetone (125 mL) containing the 6 nDL-PCBs. The mixture was evaporated down under a hood and roughly homogenised. Three spiking concentrations, 0.67, 4.0 and 24.4 ppb (ng PCB/g wheat bran), were tested for each PCB congener. This corresponds to levels of Σ(6 nDL-PCBs) that are 0.4, 2.4 and 14.6 times the maximum level (ML) set at 10 ppb by the Regulation (EU) No 277/2012 in feed materials of plant origin. The spiking concentrations are consistent both with the regulation issues and with the scarce literature data dealing with PCB contamination in food of plant origin, like wheat bran, with for example some concentrations of PCBs in bran that can reach 1.84 ppb (Roszko *et al.*, 2014). For the control groups, the bran was either unspiked (bran without solvent or PCB) or spiked with neat solvent (bran with solvent but without PCB).

Rearing

Larvae of *T. molitor* were produced by the startup INVERS (Saint-Ignat, France). Using a balance (Precisa 410 AM-FR, Precisa Instruments Ltd., Switzerland; d: 0.0001), 10g of larvae at L3 growth stage (approximatively 200 individuals) were set on a glass jar containing 45 g of control or contaminated wheat bran. Three glass jars for each modality were prepared and put in an individual incubator (18 L Heratherm™ Compact Microbiological Incubator, Thermo Scientific) customised for sufficient ventilation (300 L/h) (Figure 1). In total, 15 rearing glass jars were distributed in the 5 incubators. These replicates were not randomly distributed over the incubators to avoid a cross contamination between the different exposure conditions. The humidity was maintained at 60% and the temperature regulated at 26°C with a monitoring throughout the rearing (probe: VAISALA HMP110; data acquisition system: AOIP SA32) (Supplementary Figure S1). A water spray was added to each jar containing larvae two times per day. The rearing of larvae was carried out for 20 days in incubators. From the 6th day of the test, the evaluation of larval weight was performed for each trial twice per week. Larvae were separated by using a 1.5 mm mesh sieve. After sieving, the weight of 20 larvae was determined. After each weighting, the sieved content and the larvae were put back in the jar.

Figure 1: Pilot experimental farming of *Tenebrio molitor* larvae.

Analysis of PCBs

Sample preparation

At the end of the rearing period, all insects were sacrificed by freezing at -20°C. Larvae were then microwave-dried according to the usual conditions carried out by INVERS company: the power of the microwave was fixed (600 W) and the duration of the drying adapted so that the final relative humidity of the larvae was inferior to 10% (between 5 and 7 min). Prior to extraction, all samples were powdered with a liquid nitrogen grinder. Extraction process was carried out according to Planche *et al.* (2017) with slight modifications. Briefly, 1 g of larvae powder was extracted by accelerated solvent extraction (ASE) using a Dionex ASE 350 extractor (Sunnyvale, CA). Stainless-steel extraction cells (22 mL volume) were used, with 5 g of acidic alumina and 5 g Florisil® placed at the bottom of the cells. Paper filters were placed at the bottom and top of the alumina layer. The cells were then filled with 1 g of ground larvae dispersed in diatomaceous earth and hexane was used as extraction solvent at a temperature of 100 °C and pressure of 1500 psi. ASE extraction included heating (5 min), static time (5 min) and purging (90 s) with two extraction cycles per sample. After filtration through a 1.2 µm glass fiber prefilter and a 0.45 µm nylon filter (Phenomenex, Torrance, CA), the extract was evaporated (Rocket; Genevac Ltd, Ipswich, UK) using toluene as a keeper to minimise analyte losses during the evaporation step, then 4 mL of dichloromethane were added. To clean up extracts, gel permeation chromatography (GPC) (Gilson, Middleton, WI) was carried out on an S-X3 Bio-Beads column (Bio-Rad, Philadelphia, PA) using dichloromethane as eluting solvent at a flow rate of 5 mL/min. The fraction collected between 15 and 37 min was evaporated to dryness (Rocket, Genevac Ltd), and a mix of 100 µL of hexane and internal standard was added (Figure 2).

Figure 2: Simplified diagram of the workflow used to analyse PCBs in *Tenebrio molitor* larvae

PCB detection by GC-ToF/MS

For PCB identification in insect matrix, larvae extracts (obtained according to the conditions detailed in the previous section) were spiked with nDL-PCBs then analyzed with a time-of-flight mass spectrometer (Pegasus 4D, Leco) coupled to a gas chromatograph (6890, Agilent Technologies). 1µL of extract was injected at 280°C in splitless mode into a DB-5MS capillary column (60 m × 0.32 mm × 1 µm; Agilent J&W) with Helium (purity of 99.99995%) as carrier gas at 1 mL/min. Oven temperature was held at 120°C for 1 min, then ramped up to 240°C at a gradient of 20°C/min and to 300°C at a gradient of 2°C/min, and held at 300°C for 15 min. The MS-temperatures were set at 230 °C, 150 °C and 180 °C in the transfer line, the source and the quadrupole, respectively. Electron impact energy was set at 70 eV, and data was collected in full scan in the range of 45 to 600 m/z at a scan range of 10 scans per second.

PCB quantification by GC-µECD

The PCB levels in the larvae experimentally reared were determined by µECD (Agilent) coupled to a gas chromatograph (6890, Agilent Technologies). The GC parameters were the same as for the GC-MS coupling. The micro-ECD system was operated at 300 °C using data acquisition rate of 50 Hz, with nitrogen as make-up gas at a flowrate of 40 ml/min. Calibration curves were preliminary built from dried larvae extracts spiked with the 6 nDL-PCBs (see Table 1) at the following concentrations: 0.2, 0.5, 1.3, 3.0, 8.0, 20.0, 50.0 ng/g. Larvae extracts without PCB added were run in triplicate to check the absence of targeted analytes. Each concentration level was analysed 4 times by GC-µECD. Peak areas were determined, normalized by internal standard, then used for calculations of the standard deviations and the calibration curve equations. The linearity of the calibration curves was assessed for each PCB by calculating the coefficients of determination (R^2). The limit of detection (LOD), using the definition 3s/m (s is the standard deviation of the intercept, and m is the slope of the linear calibration curve), was determined from the calibration curves for each individual PCB studied.

Data treatment

Data were processed with the TIBCO's Statistica software (version 13.0, TIBCO Software Inc.) and the R software (version 3.5.1, The R Foundation for Statistical Computing). The “lm” (linear model) function of R was used on the calibration curve data for the determination of R^2 as well as s and m requested for LOD calculation. Student's t test or one-way analyses of variance (ANOVA) followed by *post hoc* Newman–Keuls test were performed to compare means data. The differences were considered significant when $p < 0.05$.

3. Results

PCB analysis

The recovery rates of the 6 nDL-PCBs measured after spiking in dried powder of *T. molitor* (at 200 ng/g dry matter), extraction, purification and concentration are presented in Table 1. All the recovery rates lay in the classically accepted range of 70–130% according to the EPA Method 8000C (2003), with RSD between 10% and 18%.

Table 1: Recovery rates (%) obtained after spiking, extraction, purification, concentration and analysis of PCBs in dried powder of *Tenebrio molitor* larvae (*n*=3).

Compound name	PCB congener	m/z	Recovery rates ± RSD[1] (%)
2,4,4'-Trichlorobiphenyl	28	256	108 ± 17
2,2',5,5'-Tetrachlorobiphenyl	52	292	110 ± 16
2,2',4,5,5'-Pentachlorobiphenyl	101	326	98 ± 18
2,2',3,4,4',5'-Hexachlorobiphenyl	138	360	107 ± 14
2,2',4,4',5,5'-Hexachlorobiphenyl	153	360	108 ± 16
2,2',3,4,4',5,5'-Heptachlorobiphenyl	180	394	129 ± 10

[1] Relative standard deviation (%)

The LOD measured with GC-µECD for the 6 nDL-PCB congeners in dried powder of *T. molitor* larvae varied from 1.67 ng/g for PCB 153 to 2.98 ng/g for PCB 101, with limits of quantification (LOQ) from 5.56 to 9.93 ng/g respectively (Supplementary Table S1). Coefficients of determination ranged between 0.94 for PCB 101 and 0.98 for PCBs 138, 153 and 180.

Impact of feed contamination with PCBs on larval growth

Table 2 and Figure S2 present the weight of *T. molitor* larvae at days 1, 7, 10, 14, 17 and 20 of rearing depending on their feeding substrate: wheat bran unspiked, spiked with neat solvent without PCB or spiked with nDL-PCBs at 0.67, 4.0 or 24.4 ng/g corresponding to 0.4, 2.4 and 14.6 times the maximum level (ML) for the nDL-PCBs in feed materials of plant origin, respectively (Commission regulation (EU) No 277/2012). One-way ANOVA ($p<0.05$) revealed no significant difference between the different conditions with a mean weight of 54.1 mg per larvae at the beginning of the study and a continuous growth throughout the rearing up to 95.6 mg per larvae on average after 20 days of rearing. Visual observations throughout the rearing period did not reveal differences in terms of larval mortality between the different conditions.

Table 2: Weight of *Tenebrio molitor* larvae (mg) throughout their rearing on wheat bran either unspiked (Control), spiked with neat solvent (Control + acetone) or spiked at 0.67, 4.0 or 24.4 ng PCB/g wheat bran. Data represent mean larvae weight (mg) ± SD which is determined from the 20 larvae measurement for each rearing glass jar (n=3 glass jars for each exposure condition).

Day	Control	Control + acetone	0.67 ppb	4.00 ppb	24.40 ppb	Significance[1]
1	54.5 ± 2.8	50.2 ± 1.8	54.6 ± 1.5	56.5 ± 4.5	54.6 ± 6.8	NS
7	67.1 ± 3.5	67.2 ± 9.2	64.1 ± 3.3	65.3 ± 3.8	73.0 ± 6.4	NS
10	85.6 ± 5.3	76.5 ± 3.3	75.4 ± 5.4	80.7 ± 5.4	82.1 ± 14.4	NS
14	83.4 ± 4.9	81.3 ± 0.8	85.1 ± 3.3	88.9 ± 7.0	86.0 ± 2.5	NS
17	94.1 ± 5.0	92.9 ± 5.8	96.8 ± 3.0	86.7 ± 2.1	93.7 ± 9.4	NS
20	93.1 ± 7.2	92.2 ± 8.6	97.4 ± 5.8	97.2 ± 4.7	98.0 ± 2.4	NS

NS: not significant ($p>0.05$)

[1] p values were calculated for one-way analyses of variance (ANOVA) followed by *post hoc* Newman–Keuls test.

Bioaccumulation factors

Bioaccumulation factors (BAF) were calculated for each congener of PCB in fresh (Table 3) and dried (Table S4) *T. molitor* larvae according to Truzzi *et al.* (2019) and Van der Fels-

Klerx *et al.* (2020), with BAF = concentration of PCB in the organism (fresh or dried larvae) / concentration of PCB in the feed provided (dried wheat bran).

For a spiking concentration of 0.67 ng PCB/g wheat bran, concentrations in dried larvae extracts of the 6 nDL-PCBs were below the detection limits detailed in Supplementary Table S1. For a spiking concentration of 4 ng PCB/g wheat bran, the concentrations of PCB 28, 52 and 101 were between LOD and LOQ (Supplementary Table S2). For all other conditions, PCB congener concentrations are above the LOQ. Regarding these last values, Supplementary Table S4 shows that BAF obtained in dried larvae ranged from 1.0 to 2.8 (raw data enabling BAF calculation given in Table S3). For PCB 138, there is no significant difference in BAF obtained between the two highest spiking levels whereas for PCBs 153 and 180, BAF significantly increased with the spiking level. Moreover, BAF obtained for PCB 52 are significantly lower than for other congeners.

Table 3 shows the BAF of the 6 nDL-PCBs congeners in fresh larvae. BAF in fresh larvae are 2.8 times lower than in dried larvae.

Table 3: Bioaccumulation factors (BAF) of PCB congeners in *Tenebrio molitor* larvae on a fresh weight basis after 20 days of rearing on wheat bran spiked at 0.67, 4.0 or 24.4 ng PCB/g. These values were estimated from data obtained on dried larvae, taking into account the impact of drying on larval weight. Data represent mean BAF ± SD which is determined from 3 analytical replicates of each rearing glass jar (*n*=3 glass jars for each exposure condition).

Congener	**Wheat bran contamination with PCBs**		
	0.67 ppb	**4.0 ppb**	**24.4 ppb**
PCB 28	NA	$0.80 \pm 0.17^{a*}$	0.68 ± 0.03^{b}
PCB 52	NA	$0.39 \pm 0.07^{a*}$	0.36 ± 0.03^{a}
PCB 101	NA	$0.66 \pm 0.10^{a*}$	0.67 ± 0.02^{a}
PCB 138	NA	0.86 ± 0.16^{a}	0.96 ± 0.05^{a}
PCB 153	NA	0.83 ± 0.13^{a}	0.99 ± 0.05^{b}
PCB 180	NA	0.81 ± 0.12^{a}	0.97 ± 0.08^{b}

NA: non-available (PCBs non-detected in larvae extracts)

a–b: different superscript letters within the same row indicate significant differences among values ($p<0.05$). BAF are determined from quantification values in larvae extracts either between LOD and LOQ (*) or greater than LOQ.

4. Discussion

In order to assess the fate of the 6 nDL-PCBs in *T. molitor* larvae during rearing with a contaminated substrate, the first step of this study was to determine their recovery rates after the spiking, extraction, purification, concentration and analysis protocol. Based on a protocol adapted from Planche *et al.* (2015) with a delipidation strengthened by the addition of fat retainers, the PCB recoveries in larvae samples (from 98% to 129%) are of the same order of magnitude as those in raw beef samples (from 87% to 133%) (Planche *et al.*, 2015). This demonstrates that the high fat level of *T. molitor* larvae (56-61% in whole dried *T. molitor* (EFSA, 2021) *vs* 11% in raw ground beef) does not decrease the recovery rates obtained even if PCBs are hydrophobic compounds (Log Kow = 4.09–8.18 according to Hawker and Connell (1988)), thereby confirming the relevance of the protocol used.

Based on this protocol, the impact of a contamination by PCBs on *T. molitor* was assessed using a pilot experimental farming where larvae were fed for 20 days with wheat bran either unspiked, spiked with neat solvent without PCB or spiked with nDL-PCBs at 0.67, 4.0 or 24.4 ng g^{-1}. No significant differences were observed in terms of larval growth between the group fed with unspiked wheat bran and the group fed with wheat bran spiked with neat solvent suggesting that the spiking step had no impact on the further dietary intake of larvae. This confirms that the use of acetone as an inert PCB "vehicle" volatile solvent is relevant contrary, for example, to a mix of chloroform and methanol which has led to a lower *T. molitor* body weight when it was used as a vehicle to spike their feed with Aflatoxin B1 (Bosch *et al.*, 2017).

Moreover, a high PCB concentration in feed has no more impact than a low concentration on the larvae body weight of *T. molitor* contrary to previous results obtained with antimicrobials by Gao *et al.* (2019). These authors showed that the weight of *H. illucens* larvae declined gradually with the increase in sulfonamide concentration from 0 to 10 mg/kg feed (Gao *et al.*, 2019). The present study indicates that a contamination of *T. molitor* by PCBs in an insect farm could not be revealed by monitoring the larvae weight, suggesting that a systematic control of the quality of insect feed substrates used is essential. It would be interesting to determine if these results would be similar with dioxin-like PCBs that have a different mechanism of action than nDL-PCBs on cellular pathways (Elnar *et al.*, 2012).

In order to determine the capacity of the 6 nDL-PCBs to be transferred from feed to *T. molitor* larvae, bioaccumulation factors (BAF) were determined for each PCB congener. If we take into consideration BAF determined from quantification values in larvae extracts greater than LOQ, only BAF results for PCBs 138, 153 and 180 can be discussed for a spiking concentration of 4 ng PCB/g wheat bran (Tables 3 and S4). For PCBs 153 and 180, BAF related to the highest spiking concentration (24.4 ng PCB/g wheat bran) are significantly higher compared to lower spiking concentration (4.0 ng PCB/g wheat bran), suggesting that, for these congeners, BAF increases with the contamination level.

Knowing that a BAF greater than 1 suggests a bioaccumulation of PCBs from wheat bran into *T. molitor* larvae, the BAF obtained with fresh larvae (from 0.4 to 1.0; Table 3) indicate that there is no bioaccumulation of PCBs in larvae. However, PCBs are transferred from the diet to the larvae during rearing and are then concentrated in larvae during the drying process. This concentration effect explains why the BAF calculated on the basis of dried larvae are 2.8 times higher (from 1.0 to 2.8; Supplementary Table S4).

Regarding previous data on nDL-PCBs, Van der Fels-Klerx *et al.* (2020) reported BAF in *H. illucens* dried larvae from 0.8 to 1.2 (based on upper bound values) after rearing on meat or vegetarian substrate containing 3-6% of plastic or paperboard carton packaging material. Their lower values of BAF compared to those obtained in the present study may be explained by the differences in terms of insect species and feeding substrate used. Moreover, the starvation period of 24h set up by Van der Fels-Klerx *et al.* (2020) before harvesting may allow to renew the digestive tract content and thus may decrease the concentration of nDL-PCBs in larvae and therefore the BAF.

PCBs are hydrophobic compounds (Log Kow = 4.09–8.18 according to Hawker and Connell (1988)) which implies that they may be transferred in the lipid fraction of larvae during rearing. Since the drying process does not impact this lipid fraction, it leads to a concentration of PCBs in dried larvae and therefore an increase of BAF. When a PCB contamination is detected in larvae feed substrate, it could therefore be envisaged not to use the whole dried larvae for food and feed but to exploit separately the protein and lipid fractions. In order to limit the risks linked to the presence of chemical contaminants, the lipid fraction (where PCBs are accumulated) could then be used for non-food applications (biofuel production for example) while the protein fraction could possibly be used for food and feed.

5. Conclusion

The pilot experimental farming set up enabled to assess the bioaccumulation of PCBs in *T. molitor* larvae during rearing. A feed substrate contamination with PCBs doesn't seem to have any significant impact on larval development without negative effects on growth. It could be supposed that a much higher PCB level than those assessed in this study could have a significant impact on the development of *T. molitor*, but these concentrations would be unrealistic in insect feed substrate. In addition, it would be interesting to assess the impact on larval development of a longer rearing period on a contaminated substrate. The present study revealed that there is no bioaccumulation of PCBs in *T. molitor* larvae during rearing. However, PCBs can be transferred from the diet to the larvae and are then concentrated in larvae during the drying process. This highlights the significant impact of the drying process which induces a concentration by a factor of almost 3 of PCBs in larvae. Knowing that the nDL-PCB concentration maximum limit is set at 10 µg/kg either for feed materials of plant origin and for feed materials of land animal origin (Commission regulation, EU No 277/2012), the use as larvae feeding substrate of wheat bran containing a residual amount of nDL-PCBs could result, after the rearing period, in dried larvae with an almost 3-fold higher nDL-PCB concentration that could potentially exceed the maximum limit for its use as feed. This highlights the importance of monitoring the quality of the feed substrates used for farming insects for food and feed in order to guarantee a safe content in chemical contaminants including persistent organic pollutants like PCBs. When a PCB contamination is detected in larvae feed substrate, it could therefore be envisaged to use the larvae lipid fraction (where PCBs are accumulated) for non-food applications. Finally, in order to ensure food safety throughout the food chain, it would be interesting to assess the PCB biomagnification factors if *T. molitor* larvae are used as feed, for example for aquaculture since it is expected that most insect production will be targeted towards aquafeed by 2030 (Van Huis, 2022).

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Figure 1

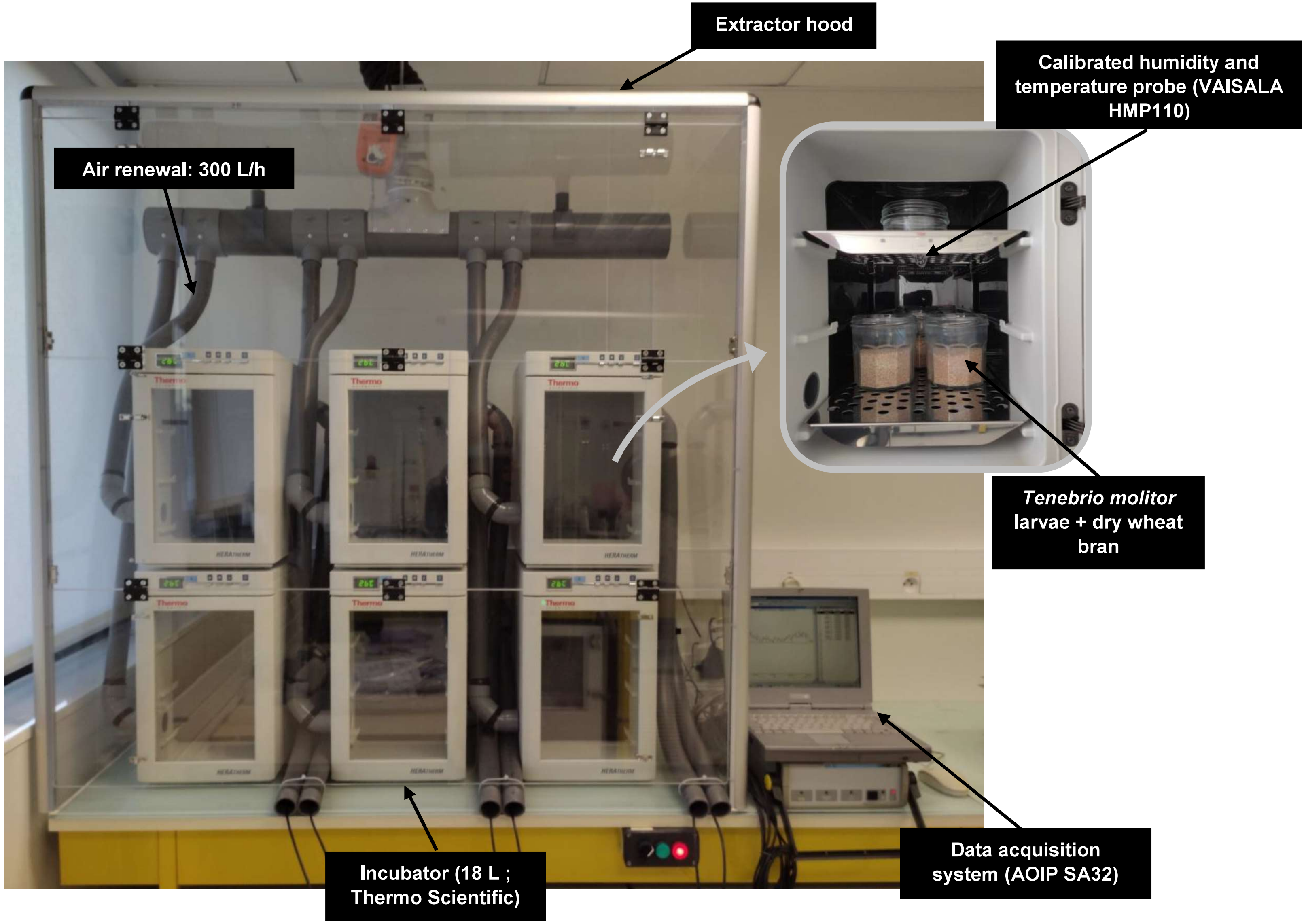
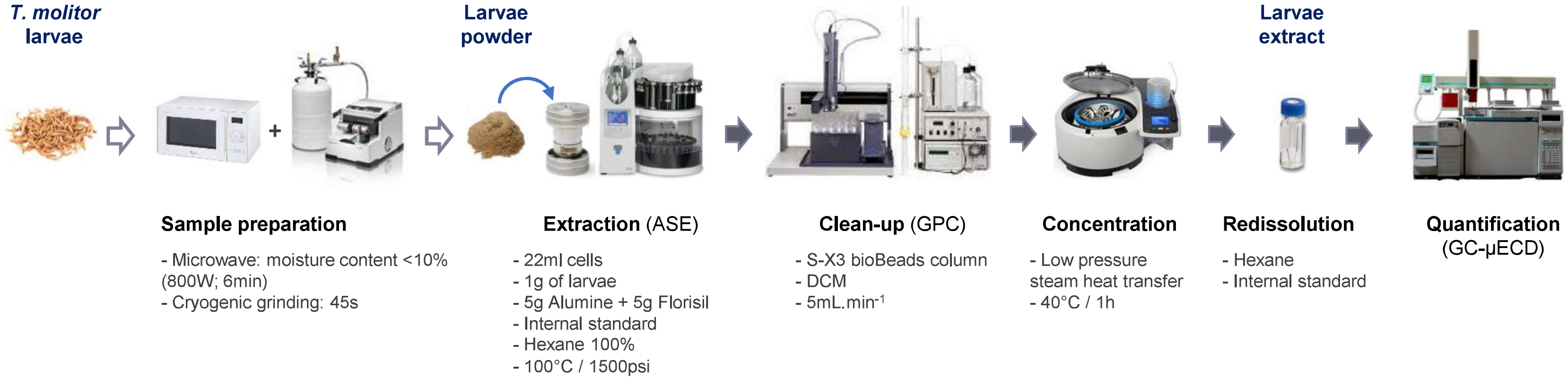

Extractor hood
Calibrated humidity and temperature probe (VAISALA HMP110)
Air renewal: 300 L/h
Tenebrio molitor larvae + dry wheat bran
Incubator (18 L ; Thermo Scientific)
Data acquisition system (AOIP SA32)

Figure 2


T. molitor larvae
Larvae powder
Larvae extract
+
Sample preparation
- Microwave: moisture content <10% (800W; 6min)
- Cryogenic grinding: 45s
Extraction (ASE)
- 22ml cells
- 1g of larvae
- 5g Alumine + 5g Florisil
- Internal standard
- Hexane 100%
- 100°C / 1500psi
Clean-up (GPC)
- S-X3 bioBeads column
- DCM
- 5mL.min-1
Concentration
- Low pressure steam heat transfer
- 40°C / 1h
Redissolution
- Hexane
- Internal standard
Quantification (GC-µECD)

Supplementary material

Fate of polychlorobiphenyls in ~~the insect~~ *Tenebrio molitor* larvae: consequences for further use as food and feed

J. Ratel[1], F. Mercier[1], J. Rivas[1], H. Wang[1], M. Angénieux[1], B. Calmont[2], S. Crépieux[3], C. Planche[1]*, E. Engel[1]

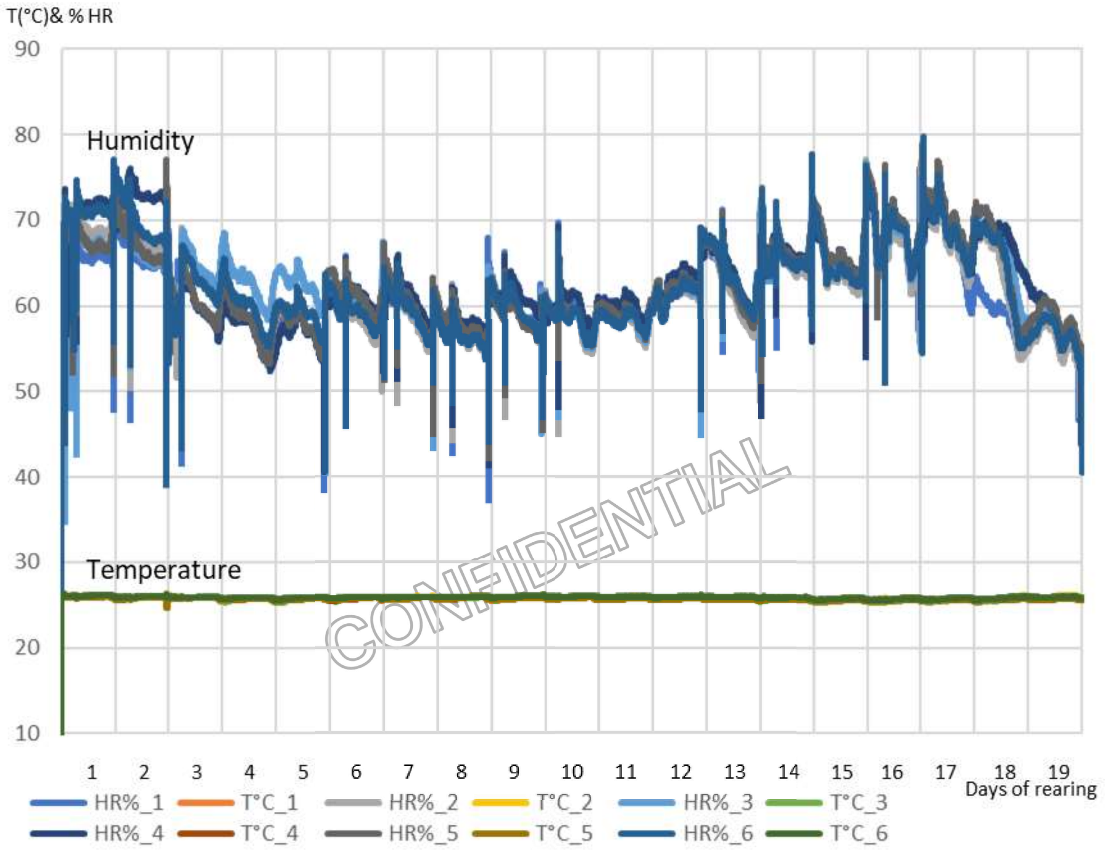


Figure S1: Temperature (°C) and relative humidity (%) readings of each incubator during the larvae rearing.

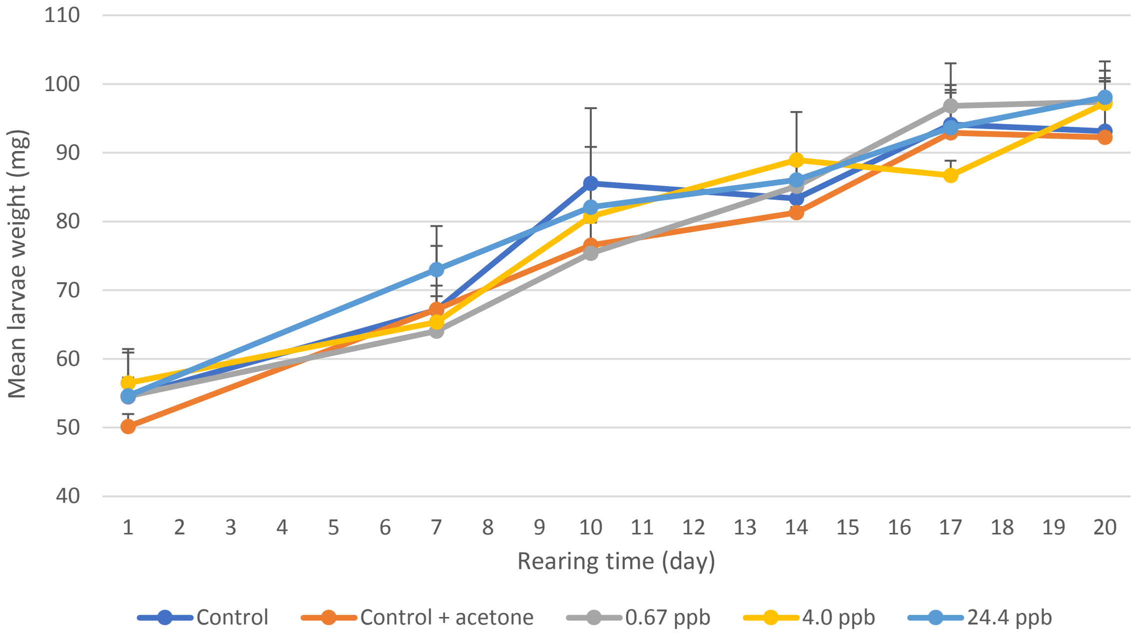


Figure S2: Weight of *Tenebrio molitor* larvae (mg) throughout their rearing on wheat bran either unspiked (Control), spiked with neat solvent (Control + acetone) or spiked at 0.67, 4.0 or 24.4 ng PCB/g wheat bran. Data represent mean larvae weight (mg) ± SD which is determined from the 20 larvae measurement for each rearing glass jar (*n*=3 glass jars for each exposure condition).

Table S1: Performance of GC-µECD for quantification of the 6 nDL-PCB congeners in dried powder of *Tenebrio molitor* larvae (linearity range: 0.2-50.0 ng/g).

PCB congener	Coefficient of determination (R^2)	Limit of detection (LOD) in ng/g	Limit of quantification (LOQ) in ng/g
28	0.95	2.80	9.32
52	0.96	2.40	8.00
101	0.94	2.98	9.93
138	0.98	1.76	5.88
153	0.98	1.67	5.56
180	0.98	1.68	5.61

Table S2: Concentrations (ng/g) of PCB congeners in *Tenebrio molitor* larvae on a dried weight basis after 20 days of rearing on wheat bran spiked at 0.67, 4.0 or 24.4 ng PCB/g. For each exposure condition, three analytical replicates of each rearing glass jar were carried out (*n*=3 glass jars for each exposure condition).

Congener	**Wheat bran contamination with PCBs**		
	0.67 ppb	**4.0 ppb**	**24.4 ppb**
PCB 28	NA	5.26*	44.03
	NA	6.73*	51.78
	NA	9.98	48.67
	NA	8.98*	48.94
	NA	9.67	44.26
	NA	10.71	45.12
	NA	9.94	47.06
	NA	10.89	47.77
	NA	8.79*	46.21
PCB 52	NA	3.28*	20.82
	NA	3.28*	25.54
	NA	5.38*	25.38
	NA	4.25*	26.93
	NA	4.43*	25.05
	NA	5.09*	24.18
	NA	4.28*	25.57
	NA	5.12*	25.87
	NA	4.31*	25.44
PCB 101	NA	5.60*	44.90
	NA	5.77*	47.35
	NA	7.81*	46.75
	NA	6.75*	48.12
	NA	7.56*	44.12
	NA	8.85*	44.60
	NA	7.84*	47.83
	NA	8.88*	48.04
	NA	7.20*	46.44
PCB 138	<LOD	7.17	67.28
	<LOD	7.21	62.43
	<LOD	9.32	63.24
	<LOD	8.26	69.15
	<LOD	10.05	64.03
	<LOD	11.55	63.56
	<LOD	10.58	70.78
	<LOD	12.40	69.11
	<LOD	10.00	68.47
PCB 153	1.96*	7.65	68.68
	<LOD	7.70	64.16
	<LOD	8.84	65.71
	<LOD	7.43	70.40

	<LOD	9.56	65.13
	<LOD	10.69	65.32
	1.77*	10.71	73.80
	<LOD	11.61	73.50
	<LOD	9.60	71.97
PCB 180	1.86*	7.89	71.41
	<LOD	8.23	59.18
	<LOD	7.58	61.84
	<LOD	7.46	65.84
	<LOD	8.91	63.52
	<LOD	9.92	63.93
	1.71*	9.80	75.07
	<LOD	11.33	73.20
	<LOD	10.70	73.05

NA: non-available (non-detected in larvae extracts)

LOD: Limit of detection

[*] Quantification values between LOD and LOQ (see Table S1)

Table S3: Raw data enabling the calculation of bioaccumulation factors (BAF) of PCB congeners in *Tenebrio molitor* larvae on a dry weight basis after 20 days of rearing on wheat bran spiked at 0.67, 4.0 or 24.4 ng PCB/g.

			IS[b]	PCB 28			PCB 52			PCB 101			PCB 138			PCB 153			PCB 180		
Wheat bran PCB conc. (ppb)	Replicate[a]	Sample (g)	Area	Area	Conc. (ppb)[c]	BAF[d]	Area	Conc. (ppb)	BAF	Area	Conc. (ppb)	BAF	Area	Conc. (ppb)	BAF	Area	Conc. (ppb)	BAF	Area	Conc. (ppb)	BAF
0,67	1-R1	1,0019	5,56E+07	NA	NA	NA	NA	NA	NA	NA	NA	NA	9,94E+07	1,69	2,53	1,46E+08	1,96	2,94	1,21E+08	1,86	2,79
0,67	1-R2	0,9993	6,60E+07	NA	NA	NA	NA	NA	NA	NA	NA	NA	8,49E+07	1,17	1,75	1,37E+08	1,52	2,29	1,20E+08	1,54	2,31
0,67	1-R3	0,9991	7,14E+07	NA	NA	NA	NA	NA	NA	NA	NA	NA	1,13E+08	1,48	2,22	1,29E+08	1,31	1,97	1,11E+08	1,31	1,97
0,67	2-R1	1,0002	6,44E+07	NA	NA	NA	NA	NA	NA	NA	NA	NA	9,85E+07	1,42	2,13	1,16E+08	1,30	1,95	9,84E+07	1,29	1,93
0,67	2-R2	1,0003	5,79E+07	NA	NA	NA	NA	NA	NA	NA	NA	NA	8,16E+07	1,30	1,94	1,10E+08	1,39	2,08	1,05E+08	1,54	2,32
0,67	2-R3	1,0011	7,27E+07	NA	NA	NA	NA	NA	NA	NA	NA	NA	1,08E+08	1,37	2,06	1,43E+08	1,44	2,15	1,16E+08	1,35	2,02
0,67	3-R1	0,9982	6,14E+07	NA	NA	NA	NA	NA	NA	NA	NA	NA	1,05E+08	1,60	2,41	1,47E+08	1,77	2,66	1,23E+08	1,71	2,56
0,67	3-R2	0,9994	7,31E+07	NA	NA	NA	NA	NA	NA	NA	NA	NA	1,28E+08	1,66	2,49	1,37E+08	1,36	2,04	1,18E+08	1,37	2,05
0,67	3-R3	0,9984	6,51E+07	NA	NA	NA	NA	NA	NA	NA	NA	NA	1,11E+08	1,61	2,42	1,36E+08	1,53	2,30	1,14E+08	1,49	2,23
4	1-R1	1,0012	5,49E+07	3,95E+08	5,26	1,32	1,55E+08	3,28	0,82	3,32E+08	5,60	1,40	3,88E+08	7,17	1,79	5,38E+08	7,65	1,91	4,95E+08	7,89	1,97
4	1-R2	1,0011	4,59E+07	4,16E+08	6,73	1,68	1,30E+08	3,28	0,82	2,85E+08	5,77	1,44	3,26E+08	7,21	1,80	4,52E+08	7,70	1,93	4,31E+08	8,23	2,06
4	1-R3	1,0006	4,28E+07	5,65E+08	9,98	2,49	1,94E+08	5,38	1,34	3,58E+08	7,81	1,95	3,91E+08	9,32	2,33	4,83E+08	8,84	2,21	3,71E+08	7,58	1,89
4	2-R1	1,0006	4,65E+07	5,54E+08	8,98	2,25	1,68E+08	4,25	1,06	3,36E+08	6,75	1,69	3,76E+08	8,26	2,06	4,42E+08	7,43	1,86	3,96E+08	7,46	1,86
4	2-R2	1,0009	4,43E+07	5,67E+08	9,67	2,42	1,66E+08	4,43	1,11	3,58E+08	7,56	1,89	4,35E+08	10,05	2,51	5,40E+08	9,56	2,39	4,50E+08	8,91	2,23
4	2-R3	1,0004	3,92E+07	5,54E+08	10,71	2,68	1,68E+08	5,09	1,27	3,70E+08	8,85	2,21	4,41E+08	11,55	2,89	5,34E+08	10,69	2,67	4,43E+08	9,92	2,48
4	3-R1	1,0009	4,01E+07	5,28E+08	9,94	2,49	1,46E+08	4,28	1,07	3,36E+08	7,84	1,96	4,14E+08	10,58	2,65	5,47E+08	10,71	2,68	4,48E+08	9,80	2,45
4	3-R2	1,0006	4,11E+07	5,90E+08	10,89	2,72	1,77E+08	5,12	1,28	3,89E+08	8,88	2,22	4,96E+08	12,40	3,10	6,07E+08	11,61	2,90	5,30E+08	11,33	2,83
4	3-R3	1,0006	4,32E+07	5,05E+08	8,79	2,20	1,58E+08	4,31	1,08	3,33E+08	7,20	1,80	4,22E+08	10,00	2,50	5,29E+08	9,60	2,40	5,27E+08	10,70	2,67
24,4	1-R1	1,0002	4,35E+07	2,47E+09	44,03	1,80	7,43E+08	20,82	0,85	2,05E+09	44,90	1,84	2,82E+09	67,28	2,75	3,77E+09	68,68	2,81	3,52E+09	71,41	2,92
24,4	1-R2	1,001	4,37E+07	2,91E+09	51,78	2,12	9,13E+08	25,54	1,04	2,17E+09	47,35	1,94	2,63E+09	62,43	2,55	3,54E+09	64,16	2,62	2,93E+09	59,18	2,42
24,4	1-R3	1,0006	3,80E+07	2,38E+09	48,67	1,99	7,89E+08	25,38	1,04	1,86E+09	46,75	1,91	2,31E+09	63,24	2,59	3,15E+09	65,71	2,69	2,66E+09	61,84	2,53
24,4	2-R1	1,0009	4,20E+07	2,65E+09	48,94	2,00	9,26E+08	26,93	1,10	2,12E+09	48,12	1,97	2,80E+09	69,15	2,83	3,73E+09	70,40	2,88	3,14E+09	65,84	2,69
24,4	2-R2	1,001	4,50E+07	2,56E+09	44,26	1,81	9,23E+08	25,05	1,02	2,08E+09	44,12	1,80	2,78E+09	64,03	2,62	3,70E+09	65,13	2,66	3,24E+09	63,52	2,60
24,4	2-R3	1,0001	4,56E+07	2,65E+09	45,12	1,85	9,02E+08	24,18	0,99	2,13E+09	44,60	1,82	2,79E+09	63,56	2,60	3,75E+09	65,32	2,67	3,30E+09	63,93	2,62
24,4	3-R1	1,0007	4,25E+07	2,58E+09	47,06	1,93	8,91E+08	25,57	1,05	2,13E+09	47,83	1,96	2,90E+09	70,78	2,90	3,96E+09	73,80	3,02	3,62E+09	75,07	3,07
24,4	3-R2	0,9997	4,51E+07	2,77E+09	47,77	1,95	9,54E+08	25,87	1,06	2,27E+09	48,04	1,97	3,00E+09	69,11	2,83	4,18E+09	73,50	3,01	3,74E+09	73,20	2,99
24,4	3-R3	1,0011	4,16E+07	2,47E+09	46,21	1,89	8,67E+08	25,44	1,04	2,03E+09	46,44	1,90	2,75E+09	68,47	2,80	3,78E+09	71,97	2,94	3,44E+09	73,05	2,99

NA: non-available (PCBs non-detected in larvae extracts)

[a] For each exposure condition, three analyses (R1 to R3) of approximatively 1 g of dried larvae sampled in each of the 3 glass jars (1 to 3) were carried out.

[b] Internal standard (5'-F-PCB-126) used for the accurate quantification of the nDL-PCBs

[c] PCB concentration in larvae was calculated according to the formula: Concentration = [((Area PCB$_x$ / Area IS) – b) /a] / (Sample weight × CF)
where CF is the concentration factor due to the extraction procedure (CF=10), and a and b are the slope and y-intercept, respectively, of the calibration curves:

	PCB 28	PCB 52	PCB 101	PCB 138	PCB 153	PCB 180
R²	0,95	0,96	0,94	0,98	0,98	0,98
a	0,13	0,08	0,10	0,10	0,13	0,11
b	0,46	0,16	0,19	0,16	0,16	0,07

[d] BAF was calculated according to the formula: BAF = (Larvae PCB$_x$ concentration) / (Wheat bran PCB$_x$ concentration)

Table S4: Bioaccumulation factors (BAF) of PCB congeners in *Tenebrio molitor* larvae on a dry weight basis after 20 days of rearing on wheat bran spiked at 0.67, 4.0 or 24.4 ng PCB/g. Data represent mean BAF ± SD which is determined from 3 analytical replicates of each rearing glass jar (*n*=3 glass jars for each exposure condition).

Congener	**Wheat bran contamination with PCBs**		
	0.67 ppb	**4.0 ppb**	**24.4 ppb**
PCB 28	NA	2.25 ± 0.47^{a**}	1.93 ± 0.10^{a}
PCB 52	NA	1.10 ± 0.19^{a**}	1.02 ± 0.07^{a}
PCB 101	NA	1.84 ± 0.29^{a**}	1.90 ± 0.06^{a}
PCB 138	NA	2.40 ± 0.45^{a}	2.72 ± 0. 13^{a}
PCB 153	NA	2.33 ± 0.38^{a}	2.81 ± 0.16^{b}
PCB 180	NA	2.27 ± 0.35^{a}	2.76 ± 0.24^{b}

NA: non-available (PCBs non-detected in larvae extracts)

a–b: different superscript letters within the same row indicate significant differences among values (p<0.05). BAF are determined from quantification values in larvae extracts between LOD and LOQ (*) or greater than LOQ.

Supplementary material

Fate of polychlorobiphenyls in *Tenebrio molitor* larvae: consequences for further use as food and feed

J. Ratel[1], F. Mercier[1], J. Rivas[1], H. Wang[1], M. Angénieux[1], B. Calmont[2], S. Crépieux[3], C. Planche[1]*, E. Engel[1]

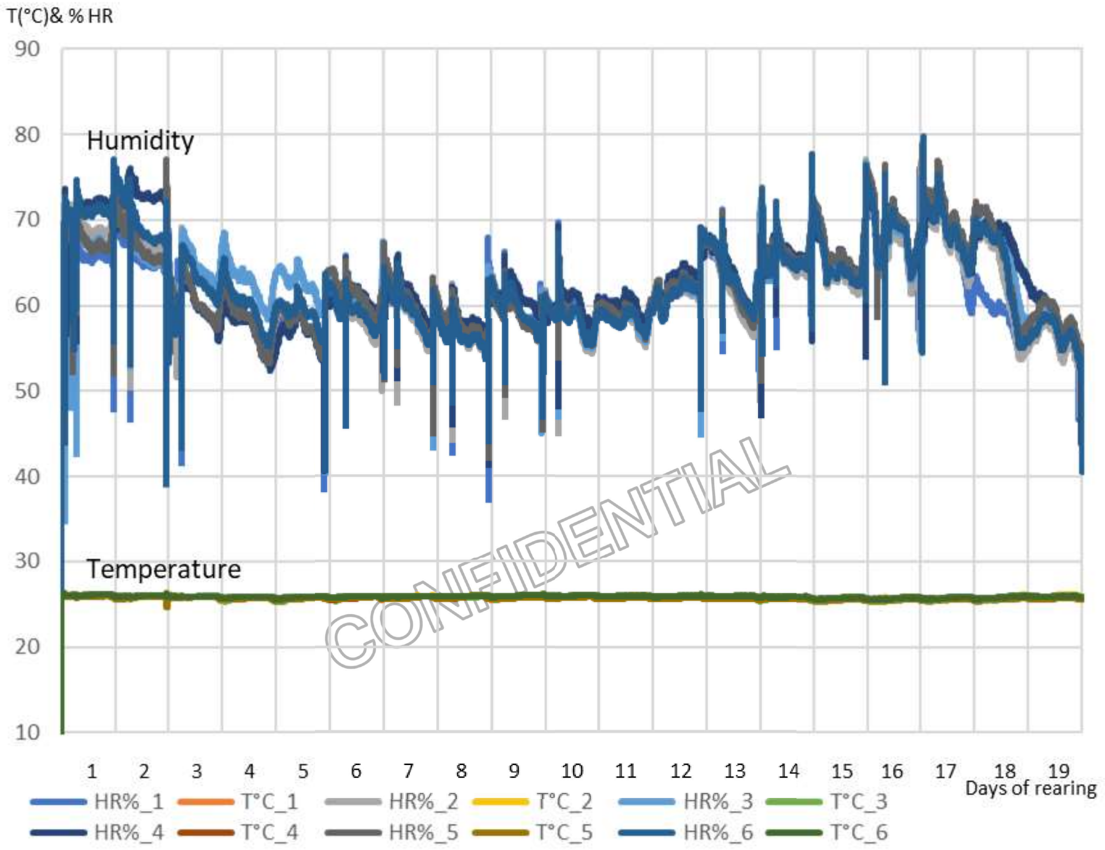


Figure S1: Temperature (°C) and relative humidity (%) readings of each incubator during the larvae rearing.

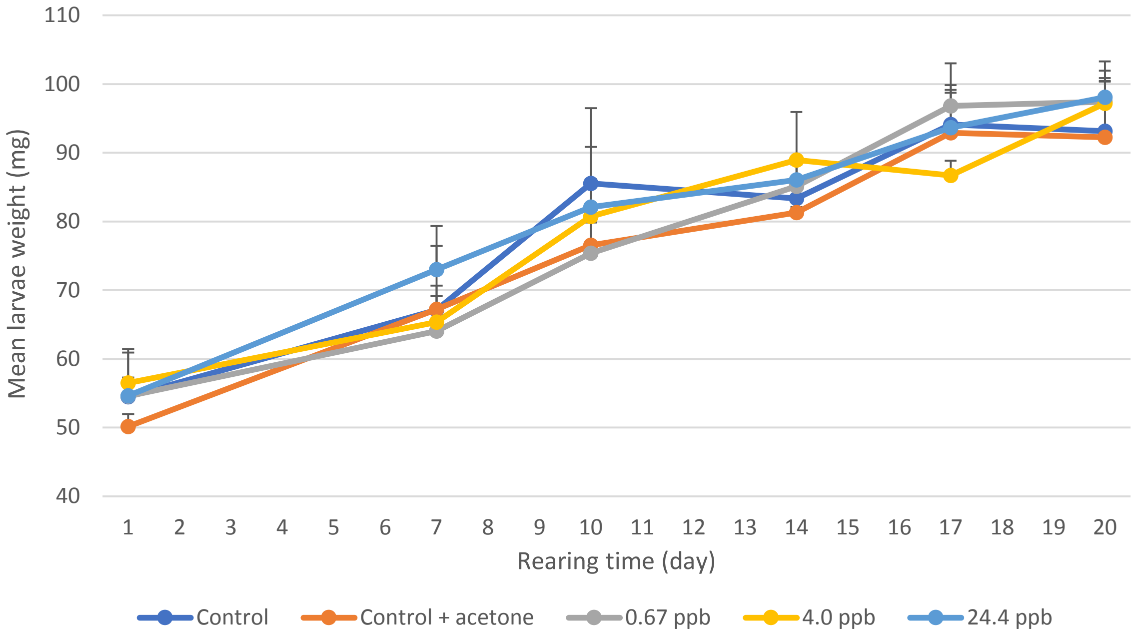


Figure S2: Weight of *Tenebrio molitor* larvae (mg) throughout their rearing on wheat bran either unspiked (Control), spiked with neat solvent (Control + acetone) or spiked at 0.67, 4.0 or 24.4 ng PCB/g wheat bran. Data represent mean larvae weight (mg) ± SD which is determined from the 20 larvae measurement for each rearing glass jar (*n*=3 glass jars for each exposure condition).

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101	0.94	2.98	9.93
138	0.98	1.76	5.88
153	0.98	1.67	5.56
180	0.98	1.68	5.61

Table S2: Concentrations (ng/g) of PCB congeners in *Tenebrio molitor* larvae on a dried weight basis after 20 days of rearing on wheat bran spiked at 0.67, 4.0 or 24.4 ng PCB/g. For each exposure condition, three analytical replicates of each rearing glass jar were carried out (*n*=3 glass jars for each exposure condition).

Congener	Wheat bran contamination with PCBs		
	0.67 ppb	4.0 ppb	24.4 ppb
PCB 28	NA	5.26*	44.03
	NA	6.73*	51.78
	NA	9.98	48.67
	NA	8.98*	48.94
	NA	9.67	44.26
	NA	10.71	45.12
	NA	9.94	47.06
	NA	10.89	47.77
	NA	8.79*	46.21
PCB 52	NA	3.28*	20.82
	NA	3.28*	25.54
	NA	5.38*	25.38
	NA	4.25*	26.93
	NA	4.43*	25.05
	NA	5.09*	24.18
	NA	4.28*	25.57
	NA	5.12*	25.87
	NA	4.31*	25.44
PCB 101	NA	5.60*	44.90
	NA	5.77*	47.35
	NA	7.81*	46.75
	NA	6.75*	48.12
	NA	7.56*	44.12
	NA	8.85*	44.60
	NA	7.84*	47.83
	NA	8.88*	48.04
	NA	7.20*	46.44
PCB 138	<LOD	7.17	67.28
	<LOD	7.21	62.43
	<LOD	9.32	63.24
	<LOD	8.26	69.15
	<LOD	10.05	64.03
	<LOD	11.55	63.56
	<LOD	10.58	70.78
	<LOD	12.40	69.11
	<LOD	10.00	68.47
PCB 153	1.96*	7.65	68.68
	<LOD	7.70	64.16
	<LOD	8.84	65.71
	<LOD	7.43	70.40

	<LOD	9.56	65.13
	<LOD	10.69	65.32
	1.77*	10.71	73.80
	<LOD	11.61	73.50
	<LOD	9.60	71.97
PCB 180	1.86*	7.89	71.41
	<LOD	8.23	59.18
	<LOD	7.58	61.84
	<LOD	7.46	65.84
	<LOD	8.91	63.52
	<LOD	9.92	63.93
	1.71*	9.80	75.07
	<LOD	11.33	73.20
	<LOD	10.70	73.05

NA: non-available (non-detected in larvae extracts)

LOD: Limit of detection

[*] Quantification values between LOD and LOQ (see Table S1)

Table S3: Raw data enabling the calculation of bioaccumulation factors (BAF) of PCB congeners in *Tenebrio molitor* larvae on a dry weight basis after 20 days of rearing on wheat bran spiked at 0.67, 4.0 or 24.4 ng PCB/g.

			IS[b]	PCB 28			PCB 52			PCB 101			PCB 138			PCB 153			PCB 180		
Wheat bran PCB conc. (ppb)	Replicate[a]	Sample (g)	Area	Area	Conc. (ppb)[c]	BAF[d]	Area	Conc. (ppb)	BAF	Area	Conc. (ppb)	BAF	Area	Conc. (ppb)	BAF	Area	Conc. (ppb)	BAF	Area	Conc. (ppb)	BAF
0,67	1-R1	1,0019	5,56E+07	NA	NA	NA	NA	NA	NA	NA	NA	NA	9,94E+07	1,69	2,53	1,46E+08	1,96	2,94	1,21E+08	1,86	2,79
0,67	1-R2	0,9993	6,60E+07	NA	NA	NA	NA	NA	NA	NA	NA	NA	8,49E+07	1,17	1,75	1,37E+08	1,52	2,29	1,20E+08	1,54	2,31
0,67	1-R3	0,9991	7,14E+07	NA	NA	NA	NA	NA	NA	NA	NA	NA	1,13E+08	1,48	2,22	1,29E+08	1,31	1,97	1,11E+08	1,31	1,97
0,67	2-R1	1,0002	6,44E+07	NA	NA	NA	NA	NA	NA	NA	NA	NA	9,85E+07	1,42	2,13	1,16E+08	1,30	1,95	9,84E+07	1,29	1,93
0,67	2-R2	1,0003	5,79E+07	NA	NA	NA	NA	NA	NA	NA	NA	NA	8,16E+07	1,30	1,94	1,10E+08	1,39	2,08	1,05E+08	1,54	2,32
0,67	2-R3	1,0011	7,27E+07	NA	NA	NA	NA	NA	NA	NA	NA	NA	1,08E+08	1,37	2,06	1,43E+08	1,44	2,15	1,16E+08	1,35	2,02
0,67	3-R1	0,9982	6,14E+07	NA	NA	NA	NA	NA	NA	NA	NA	NA	1,05E+08	1,60	2,41	1,47E+08	1,77	2,66	1,23E+08	1,71	2,56
0,67	3-R2	0,9994	7,31E+07	NA	NA	NA	NA	NA	NA	NA	NA	NA	1,28E+08	1,66	2,49	1,37E+08	1,36	2,04	1,18E+08	1,37	2,05
0,67	3-R3	0,9984	6,51E+07	NA	NA	NA	NA	NA	NA	NA	NA	NA	1,11E+08	1,61	2,42	1,36E+08	1,53	2,30	1,14E+08	1,49	2,23
4	1-R1	1,0012	5,49E+07	3,95E+08	5,26	1,32	1,55E+08	3,28	0,82	3,32E+08	5,60	1,40	3,88E+08	7,17	1,79	5,38E+08	7,65	1,91	4,95E+08	7,89	1,97
4	1-R2	1,0011	4,59E+07	4,16E+08	6,73	1,68	1,30E+08	3,28	0,82	2,85E+08	5,77	1,44	3,26E+08	7,21	1,80	4,52E+08	7,70	1,93	4,31E+08	8,23	2,06
4	1-R3	1,0006	4,28E+07	5,65E+08	9,98	2,49	1,94E+08	5,38	1,34	3,58E+08	7,81	1,95	3,91E+08	9,32	2,33	4,83E+08	8,84	2,21	3,71E+08	7,58	1,89
4	2-R1	1,0006	4,65E+07	5,54E+08	8,98	2,25	1,68E+08	4,25	1,06	3,36E+08	6,75	1,69	3,76E+08	8,26	2,06	4,42E+08	7,43	1,86	3,96E+08	7,46	1,86
4	2-R2	1,0009	4,43E+07	5,67E+08	9,67	2,42	1,66E+08	4,43	1,11	3,58E+08	7,56	1,89	4,35E+08	10,05	2,51	5,40E+08	9,56	2,39	4,50E+08	8,91	2,23
4	2-R3	1,0004	3,92E+07	5,54E+08	10,71	2,68	1,68E+08	5,09	1,27	3,70E+08	8,85	2,21	4,41E+08	11,55	2,89	5,34E+08	10,69	2,67	4,43E+08	9,92	2,48
4	3-R1	1,0009	4,01E+07	5,28E+08	9,94	2,49	1,46E+08	4,28	1,07	3,36E+08	7,84	1,96	4,14E+08	10,58	2,65	5,47E+08	10,71	2,68	4,48E+08	9,80	2,45
4	3-R2	1,0006	4,11E+07	5,90E+08	10,89	2,72	1,77E+08	5,12	1,28	3,89E+08	8,88	2,22	4,96E+08	12,40	3,10	6,07E+08	11,61	2,90	5,30E+08	11,33	2,83
4	3-R3	1,0006	4,32E+07	5,05E+08	8,79	2,20	1,58E+08	4,31	1,08	3,33E+08	7,20	1,80	4,22E+08	10,00	2,50	5,29E+08	9,60	2,40	5,27E+08	10,70	2,67
24,4	1-R1	1,0002	4,35E+07	2,47E+09	44,03	1,80	7,43E+08	20,82	0,85	2,05E+09	44,90	1,84	2,82E+09	67,28	2,75	3,77E+09	68,68	2,81	3,52E+09	71,41	2,92
24,4	1-R2	1,001	4,37E+07	2,91E+09	51,78	2,12	9,13E+08	25,54	1,04	2,17E+09	47,35	1,94	2,63E+09	62,43	2,55	3,54E+09	64,16	2,62	2,93E+09	59,18	2,42
24,4	1-R3	1,0006	3,80E+07	2,38E+09	48,67	1,99	7,89E+08	25,38	1,04	1,86E+09	46,75	1,91	2,31E+09	63,24	2,59	3,15E+09	65,71	2,69	2,66E+09	61,84	2,53
24,4	2-R1	1,0009	4,20E+07	2,65E+09	48,94	2,00	9,26E+08	26,93	1,10	2,12E+09	48,12	1,97	2,80E+09	69,15	2,83	3,73E+09	70,40	2,88	3,14E+09	65,84	2,69
24,4	2-R2	1,001	4,50E+07	2,56E+09	44,26	1,81	9,23E+08	25,05	1,02	2,08E+09	44,12	1,80	2,78E+09	64,03	2,62	3,70E+09	65,13	2,66	3,24E+09	63,52	2,60
24,4	2-R3	1,0001	4,56E+07	2,65E+09	45,12	1,85	9,02E+08	24,18	0,99	2,13E+09	44,60	1,82	2,79E+09	63,56	2,60	3,75E+09	65,32	2,67	3,30E+09	63,93	2,62
24,4	3-R1	1,0007	4,25E+07	2,58E+09	47,06	1,93	8,91E+08	25,57	1,05	2,13E+09	47,83	1,96	2,90E+09	70,78	2,90	3,96E+09	73,80	3,02	3,62E+09	75,07	3,07
24,4	3-R2	0,9997	4,51E+07	2,77E+09	47,77	1,95	9,54E+08	25,87	1,06	2,27E+09	48,04	1,97	3,00E+09	69,11	2,83	4,18E+09	73,50	3,01	3,74E+09	73,20	2,99
24,4	3-R3	1,0011	4,16E+07	2,47E+09	46,21	1,89	8,67E+08	25,44	1,04	2,03E+09	46,44	1,90	2,75E+09	68,47	2,80	3,78E+09	71,97	2,94	3,44E+09	73,05	2,99

NA: non-available (PCBs non-detected in larvae extracts)

[a] For each exposure condition, three analyses (R1 to R3) of approximatively 1 g of dried larvae sampled in each of the 3 glass jars (1 to 3) were carried out.

[b] Internal standard (5'-F-PCB-126) used for the accurate quantification of the nDL-PCBs

[c] PCB concentration in larvae was calculated according to the formula: Concentration = [((Area PCB_x / Area IS) – b) /a] / (Sample weight × CF)
where CF is the concentration factor due to the extraction procedure (CF=10), and a and b are the slope and y-intercept, respectively, of the calibration curves:

	PCB 28	PCB 52	PCB 101	PCB 138	PCB 153	PCB 180
R²	0,95	0,96	0,94	0,98	0,98	0,98
a	0,13	0,08	0,10	0,10	0,13	0,11
b	0,46	0,16	0,19	0,16	0,16	0,07

[d] BAF was calculated according to the formula: BAF = (Larvae PCB_x concentration) / (Wheat bran PCB_x concentration)

Table S4: Bioaccumulation factors (BAF) of PCB congeners in *Tenebrio molitor* larvae on a dry weight basis after 20 days of rearing on wheat bran spiked at 0.67, 4.0 or 24.4 ng PCB/g. Data represent mean BAF ± SD which is determined from 3 analytical replicates of each rearing glass jar (*n*=3 glass jars for each exposure condition).

Congener	**Wheat bran contamination with PCBs**		
	0.67 ppb	**4.0 ppb**	**24.4 ppb**
PCB 28	NA	$2.25 \pm 0.47^{a**}$	1.93 ± 0.10^{a}
PCB 52	NA	$1.10 \pm 0.19^{a**}$	1.02 ± 0.07^{a}
PCB 101	NA	$1.84 \pm 0.29^{a**}$	1.90 ± 0.06^{a}
PCB 138	NA	2.40 ± 0.45^{a}	$2.72 \pm 0. 13^{a}$
PCB 153	NA	2.33 ± 0.38^{a}	2.81 ± 0.16^{b}
PCB 180	NA	2.27 ± 0.35^{a}	2.76 ± 0.24^{b}

NA: non-available (PCBs non-detected in larvae extracts)

a–b: different superscript letters within the same row indicate significant differences among values ($p<0.05$). BAF are determined from quantification values in larvae extracts between LOD and LOQ (*) or greater than LOQ.