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ORIGINAL PAPER

Chlorinated paraffin screening in chocolate products and infant formula by planar solid phase extraction

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Abstract

A planar solid phase extraction with visual light detection (pSPE–Vis) was developed to screen chlorinated paraffins (CP) in chocolate products and infant formula. The analysis of selected persistent organic pollutants, such as polychlorinated biphenyls, excluded co-elution with CP. Separation and detection of CP in the target zone were obtained after simple-to-perform sample preparation, including a sulfuric acid treatment. The validation study for CP showed recoveries near 100%, a limit of decision of 7.8 ng g^{-1}, and a limit of detection of 15.7 ng g^{-1} sample. The analysis of 63 samples from the German market revealed CP up to 260 ng g^{-1} in chocolate products and 54 ng g^{-1} in infant formula, which underlines the need for CP monitoring in food. The results are comparable with literature data and were evaluated in an interlaboratory test. The pSPE–Vis method is a cost-effective tool for total CP screening. Developed for initial screening, the method enables the detection of CP-contaminated samples, supporting their prioritization for more detailed follow-up analysis by HRMS.

Keywords Chlorinated paraffins · Planar solid phase extraction · Screening · Chocolate products · Infant formula · Persistent organic pollutants

Introduction

Chlorinated paraffins (CP) are one of the most important industrial chemicals of quantity among the anthropogenic ones, characterized by low production costs, chemical stability, and flame resistance [1–3]. They are used for a wide range of applications, such as plasticizers in polyvinylchloride, flame retardants, and cooling and lubricating agents in metalworking fluids [4, 5]. The complexity of CP results from the manufacturing process, which involves thermally or UV-induced, non-specific, radical chlorination of fractionated petroleum feedstock, resulting in thousands of congeners and isomers [3, 6].

CP are usually categorized into three groups according to their chain lengths: short-chain CP (SCCP, $C_{10–13}$), medium-chain CP (MCCP, $C_{14–17}$), and long-chain CP (LCCP, $C_{18–30}$) [7–9]. SCCP are listed as persistent organic pollutants (POPs) in Annex A of the Stockholm Convention, prohibiting their commercialization because of their persistence, bioaccumulating, and toxic potential [10]. Although not yet regulated, MCCP have raised increasing toxicological concerns and are therefore highly relevant from a food safety perspective [11, 12]. LCCP are actually not considered as substances of high concern. However, available toxicological data suggest that their behavior may be comparable to that of SCCP and MCCP, indicating similar adverse effects and potential health risks [11].

Krätschmer et al. demonstrated that fats and oils, particularly vegetable oils, can be a significant source of CP intake and that infants and toddlers are especially exposed to CP [13]. The investigation of CP in infant formula is very important in controlling CP exposure due to infants' high food intake and low body weight [14–16]. In a similar context, products like chocolate or chocolate spread, which also

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contain fats and oils, may represent an additional source of CP exposure [17], particularly for young children who consume these products regularly. The accumulation of CP in fat-rich matrices is driven by their lipophilic properties and low volatility, making them particularly relevant in the context of high-fat food products.

Current methods for sample preparation of infant formula [14–16] and chocolate spread [17] include the extraction of CP (cold extraction or accelerated solvent extraction) with *n*-hexane or a mixture of dichloromethane (DCM)/*n*-hexane (1:1, *V*/*V*), followed by lipid removal with acidic silica gel or by treatment with sulfuric acid [18]. POPs are removed using a Florisil or sulfuric acidic silica gel column [19].

The instrumental analysis of CP is challenging due to its production-related composition and physical-chemical properties, and the perfect method to reliably quantify CP doesn't exist [20, 21]. Gas chromatography (GC) with electron capture negative ionization (ECNI) (high resolution) mass spectrometry ((HR)MS) [7, 22–24] evaluating CP as a congener group pattern [23] is a widely used method, also for analyzing CP in infant formula or chocolate spread [14, 17]. However, due to volatility, GC analysis is limited to C_{10} to C_{17} congener groups [24]. Liquid chromatography (LC) is suitable for analyzing CP with also longer chain lengths ($C_{\geq 18}$) [25, 26]. Bogdal et al. developed a method based on mathematical data deconvolution, which has been broadly used [26–29]. All of these methods and strategies require expensive equipment and expert operators.

Oellig and Hammel presented a high-performance thin-layer chromatography (HPTLC) based method to screen SCCP and MCCP [30] in vegetable oils and oil-based dietary food supplements, using the concept of planar solid phase extraction with visual light detection (pSPE–Vis). This concept offers the advantages of HPTLC, being cost-effective, reliable, robust, and fast [31]. Applying this method, the CP are focused in a sharp target zone after chromatographic matrix separation. Determination as a sum parameter directly on the thin-layer plate is performed after a UV-induced derivatization product with benzidine derivatives is formed. Several derivatization strategies for CP on pSPE were systematically compared in a previous work, demonstrating that benzidine derivatives such as *o*-tolidine and tetramethylbenzidine provided the highest sensitivity and selectivity while minimizing matrix interferences [30]. Our recent study investigated the derivatization reaction, including an optimized UV-C irradiation, and the method was extended to include LCCP up to C_{23} [32].

In this present study, we aimed to develop the current pSPE methods [30, 32] for cocoa-based sweet food matrices such as chocolate spreads and bars and infant formula as representatives of fat-containing foods. The main objective was to develop and optimize a suitable sample preparation method specifically for this study, building on the basic previously established pSPE concept [30, 32], by integrating diverse sample pre-clean-up procedures and optimizing sample loading to enhance the method's sensitivity. A further aim was to determine the performance parameter (validation) and investigate German market samples. Additionally, results were compared to those of a reference method and investigated which POPs can be excluded or included using this method.

Materials and methods

Chemicals and materials

trans-Chlordane (99.6%, 10 µg mL^{-1} in cyclohexane), chlordecone (86.9%, 10 ng μL^{-1} in isooctane), CP mixture C_{14}–C_{17} 52% Cl (CP_{ref}, 99.9%, 100 µg mL^{-1} in cyclohexane, technical reference CP mixture, CP_{ref}), 4,4'-dichlorodiphenyltrichloroethane (DDT, 99.5%, internal standard (ISTD)), endosulfane (98%), hexabromocyclododecane (HBCD, 93.3%), hexachlorobenzene (HCB, 99%, 10 µg mL^{1} in cyclohexane), *β*-hexachlorocyclohexane (*β*-HCH, 99%, 10 µg mL^{-1} in cyclohexane), 2,2',3,3',4,4',5-heptachlorobiphenyl (PCB 170, 99%), mirex (98.5%), pentabromodiphenylether (PentaBDE, 99%, 10 µg mL^{-1} in cyclohexane), perfluorooctane sulfonic acid (PFOS, 92.9%, 1000 µg mL^{-1}), and toxaphene (99.1%, 500 µg mL^{-1} in cyclohexane) were purchased from Dr. Ehrenstorfer (Augsburg, Germany). Acetone (Rotisolv Pestilyse, ≥ 99.8%), acetonitrile (Rotisolv, HPLC Gradient grade), cyclohexane (Rotisolv UV/IR), dichloroethane (Rotisolv HPLC, ≥ 99.8%), dichloromethane (DCM, Rotisolv HPLC), and diethyl ether (Rotisolv Pestilyse, ≥ 99.8%) were obtained from Carl Roth (Karlsruhe, Germany). Chloroform (for analysis, ≥99.8%) and isopropyl acetate (≥99.6%) were from Merck (Darmstadt, Germany). *n*-Hexane (Chemsolute, for residue analysis, min. 95%), *tert*-butyl methyl ether (*t*BME, Chemsolute, for HPLC, ≥ 99.8%), and 2-propanol (Chemsolute, for HPLC min. 99.8%) were purchased from Th. Geyer (Renningen, Germany). Toluene (for pesticide residue analysis, ≥ 99.7%) was from Honeywell Riedel-de Haën (Seelze, Germany). Calcium chloride ($CaCl_2$, ≥ 98%), hydrochloric acid (HCl, 37% fuming, VLSI grade), sodium bicarbonate ($NaHCO_3$, ≥ 99%), sodium hydroxide (NaOH, ≥ 99%), and sulfuric acid (H_2SO_4, 96%, pure) were obtained from Carl Roth. Magnesium chloride 6-hydrate (pure, pharma grade) was from AppliChem (Darmstadt, Germany), sodium chloride (NaCl, >99.5%) from VWR chemicals (Bruchsal, Germany), and 3,3',5,5'-tetramethylbenzidine (TMB, >98%) from abcr (Karlsruhe, Germany). ChloroFiltr (Enviro Clean, bulk sorbents) was obtained

from UCT (Bristol, USA). Glass cartridges (3 mL) and glass fiber filter circles (9 mm, binder-free, 0.5 µm) were from Macherey-Nagel (Düren, Germany). Graphitized carbon black (GCB, Supelclean ENVI-Carb) and HPTLC silica gel 60 glass plates (20 cm × 10 cm) were from Merck (Darmstadt, Germany). Primary secondary amine (PSA, Bondesil, 40 µm) was from Agilent (Santa Clara, USA).

Standard solutions

n-Hexane was used to prepare all stock solutions and dilutions. A stock solution of DDT with a concentration of 400 ng μL^{-1} was prepared and diluted to 40 ng μL^{-1} for the working solution. For method optimization, the CP_{ref} was diluted to a working solution with a concentration of 5.0 ng μL^{-1}. The spiking solutions for the validation study were prepared by diluting the CP_{ref} to 0, 0.175, 0.35, 0.7, 1.4, 2.8, 4.2, and 7.0 ng μL^{-1}, including the ISTD DDT at 6.0 ng μL^{-1}. For the calibration, the CP_{ref} was diluted to 0.2, 0.3, 0.5, 1.0, 2.5, 4.0, 6.0, and 8.0 ng μL^{-1}, including the ISTD DDT at 6.0 ng μL^{-1}.

For the analysis of POP, stock solutions of 5.0 ng μL^{-1} were prepared for DDT, endosulfane, HBCD, mirex, and PCB 170. The solutions of HCB, PFOS, and toxaphene were diluted to 5.0 ng μL^{-1}. The solutions of *trans*-chlordane, chlordecone, *β*-HCH, and PentaBDE, with concentrations of 10 ng μL^{-1}, were directly used for pSPE–Vis.

Sample preparation

Samples

A total of 25 chocolate spread and chocolate product samples and 38 infant formula samples from the German market, collected between 2019 and 2024, were investigated. Among the chocolate products, six cake glaze samples, two milk chocolate bar samples, and 17 chocolate and hazelnut spread samples were analyzed. Detailed information is given in Table S1. In accordance with Regulation (EU) No 609/2013, 15 samples of the infant formulas were infant formula (from birth), and 23 samples were follow-on formula (6 + months). They primarily comprised cow milk, with two samples made from goat milk. One sample was hypoallergenic (Table S2).

Chocolate spread and solid chocolate products

For sample extraction, 3.5 g chocolate spread or shredded chocolate product were weighed into a 50 mL PTFE tube with a screw cap, and 15 µL ISTD solution (600 ng DDT) and 5 mL cyclohexane were added. The sample was completely dissolved by vigorous shaking by hand. 20 mL sulfuric acid were added, and the solution was shaken for 5 min on a horizontal shaker (KS 125 basic, IKA Labortechnik, Staufen im Breisgau, Germany) at 500 rpm. The solution was then centrifuged (Multifuge X1R, Thermo Scientific, Waltham, USA) at 4000 rpm and room temperature (RT) until phase separation (5–10 min), and the organic phase was transferred with a glass pipette into a 20 mL glass centrifugal tube with a screw cap. The sulfuric acid phase was extracted a second time with another 5 mL cyclohexane as described above. 5 mL 1 M NaOH solution was added to the combined organic phases, shaken for 5 min at 1500 rpm on a horizontal shaker, and centrifuged for 5 min. The organic phase was transferred with a glass pipette into a 15 mL glass centrifuge tube with a screw cap, and the solvent was removed by a gentle air stream. Then, 20 mg GCB (prewashed with *t*BME, 5 mL per 100 mg in a glass cartridge with a binder-free glass fiber filter) and 2 mL *t*BME were added. After shaking for 3 min on a horizontal shaker at 1500 rpm, the solution was separated from the GCB using a 3 mL glass cartridge equipped with a binder-free glass fiber filter. The cartridge was rinsed with *t*BME, the solvent evaporated, and the residue resolved in 200 µL *n*-hexane. The final sample extract contained 17.5 mg sample per µL extract and was used for pSPE–Vis analysis according to Sect. "Planar solid phase extraction with visual light detection (pSPE–Vis)".

Maximum sample weighing To determine the maximum sample quantity, 3.0, 3.5, 4.0, and 4.5 g of chocolate spread were weighted into 50 mL PTFE tubes. ISTD solution (600 ng DDT), 5 mL cyclohexane, and 20 mL sulfuric acid were added, and the samples were extracted as described above, including the following NaOH washing step and GCB clean-up. Samples with the final sample concentration of 15.0, 17.5, 20.0, and 22.5 mg μL^{-1} were analyzed by pSPE–Vis according to Sect. "Planar solid phase extraction with visual light detection (pSPE–Vis)".

Infant formula

For sample digestion, 3.5 g powder was weighed in a 50 mL PTFE tube with a screw cap, 20 mL sulfuric acid were added, and the mixture was shaken for 20 min on a horizontal shaker at 500 rpm. 15 µL ISTD solution (600 ng DDT) and 5 mL cyclohexane were added, and the sample was shaken for 5 min at 500 rpm on the horizontal shaker. After centrifugation at 4000 rpm and RT until phase separation (5–10 min), the organic phase was transferred into a 20 mL glass centrifuge tube with a screw cap. The extraction of the sulfuric acid was repeated with 5 mL cyclohexane, and the combined organic phase was further treated as described above for chocolate products (Sect. Samples").

Maximum sample weighing To determine the maximum sample quantity, the sample was prepared with different weights (3.0–4.5 g) analog to the chocolate products (chocolate spread). The sample preparation method described above for infant formula was used for extraction and clean-up. Samples with the final sample concentration of 15.0, 17.5, 20.0, and 22.5 mg μL^{-1} were analyzed by pSPE–Vis according to Sect. "Planar solid phase extraction with visual light detection (pSPE–Vis)".

Planar solid phase extraction with visual light detection (pSPE–Vis)

The analysis was performed based on Oellig's and Geyer's methods [30, 32] with slight modifications. Standards and samples were applied on 20 cm × 10 cm KG 60 HPTLC plates prewashed with DCM using the Automatic TLC Sampler 4 (ATS4, CAMAG, Muttenz, Switzerland). The application started at 8 mm along the x- and y-directions with a band length of 6 mm, a bandwidth of 3 mm, and a track spacing of 8.7 mm, resulting in 22 tracks. *n*-Hexane was used as the rinsing solvent for all applications (filling speed 24 µL min^{-1}, predosage volume 200 nL, retraction volume 200 nL, dosage speed 1200 nL s^{-1}, rinsing vacuum time 4 s, and filling vacuum time 0 s). Between each application step, the syringe was rinsed twice. The application volume was 50 µL for all calibration solutions and 100 µL for all sample and validation extracts.

The twofold development was performed in the Automated Developing Chamber 2 (ADC2, CAMAG) equipped with a twin-trough chamber (20 cm × 10 cm). Before both developments, the plate activity was set to 33% relative humidity with a saturated $MgCl_2$ solution (ADC2 humidity control for 5 min). The first development was conducted with a mixture of cyclohexane/toluene (95:5, *V*/*V*) up to 80 mm, followed by a drying step (3 min). The second development was performed with DCM/*n*-hexane (75:25, *V*/*V*) up to 50 mm, followed by 3 min drying. After drying, plate images were captured with the TLC Visualizer (CAMAG) under UV 254 nm, UV 366 nm, and white light illumination (for method optimization and matrix evaluation). Before derivatization, the plate activity was adjusted in the ADC2 using a saturated $MgCl_2$ solution for 10 min, and the plate was derivatized immediately after. The TLC Chromatogram Immersion Device III (CAMAG, immersion speed 3, immersion time 1) was used to dip the plate into a TMB solution (0.4% in acetone). Thereafter, the plate was evenly dried in a stream of cold air for 4 min. To start the derivatization reaction, the plate was exposed to UV-C radiation in an irradiation chamber, according to Geyer et al. [32]. After 5 min irradiation, a plate image was captured under white light illumination with the TLC Visualizer and scans of the plate were recorded with the TLC Scanner 4 (CAMAG) in the absorption mode at 645 nm using the tungsten lamp (scanning speed of 20 mm s^{-1} and data resolution of 25 µm per step). The control of the HPTLC instruments and the data evaluation were performed with the software winCATS, version 1.4.6.2002 (CAMAG). Data evaluation was done using the ratio of the peak areas of CP and DDT.

Validation study

For the validation study, 3.5 g chocolate spread or infant formula sample, which had previously been tested with our method and found to contain CP levels below the limit of decision, were weighed for each CP level and spiked with 100 µL of the prepared standard solutions of CP_{ref} for the validation study (spiking solutions), including the ISTD (Sect. "Standard solutions"). Four spiking series were carried out, each at eight CP amounts: 0, 5, 10, 20, 40, 80, 120, and 200 ng g^{-1} (ISTD in all samples 600 ng g^{-1}). The first and the last two series were prepared, each with a different matrix and all on different days. The extracts were prepared analogously to the sample preparation described in Sect. "Sample preparation", but without the addition of the ISTD. For infant formula, the samples were spiked after sulfuric acid treatment analogous to the addition of the ISTD described in Sect. "Sample preparation". pSPE–Vis was performed according to Sect. "Planar solid phase extraction with visual light detection (pSPE–Vis)", including the application of 50 µL of each corresponding spiking solution mentioned above.

To calculate the performance characteristics (linearity, limit of decision, limit of detection (LOD), and recovery rate), a weighted least-square model [33–35] was fitted to the experimental data set, whereby the weights for the CP contents were calculated according to Steliopoulos and Stickel [36]. The uncertainty of the measurements was determined as part of the validation process (detailed information: SI Description 1: Validation).

Analysis of spiked samples with planar solid phase extraction with visual light detection (pSPE–Vis) and gas chromatography with electron capture negative ion high-resolution mass spectrometry (GC–ECNI/HRMS)

A chocolate spread sample was spiked at two different CP_{ref} levels (50 and 150 ng g^{-1}) in our laboratory. The unspiked and spiked samples were then prepared in duplicate according to Sect. "Samples" and measured according to Sect. "Planar solid phase extraction with

visual light detection (pSPE–Vis)". Quantification was done using the applied calibration solutions mentioned in Sect. "Standard solutions", and the data evaluation was based on the ratios of the peak areas of CP and DDT (ISTD). Additionally, the CVUA Freiburg prepared and analyzed the samples in triplicate, using a Q Exactive GC Orbitrap mass spectrometer coupled to a TRACE 1310 GC (Thermo Scientific, Waltham, USA) equipped with a HP-5MS UI capillary column connected to uncoated precolumn (Agilent Technologies, Santa Clara, USA), as described by Krätschmer et al. [37] with the following adjustments in sample preparation: An aliquot of each sample, containing approximately 1.5 g of fat after extraction, was mixed with anhydrous silica to receive a dry mixture and recovery standards were added. Extraction was performed three times with DCM/*n*-hexane (1:1, *V*/*V*) using an Ultra-Turrax disperser (IKA, Germany).

Analysis of samples from the German market

The samples were prepared in duplicate according to Sect. "Sample preparation" for chocolate spread, solid chocolate products, and infant formula. pSPE–Vis was performed according to Sect. "Planar solid phase extraction with visual light detection (pSPE–Vis)", and the quantification was done using the applied calibration solutions mentioned in Sect. "Standard solutions". Samples with measured values above the highest calibration standard were diluted and measured again. The data evaluation was based on the ratios of the peak areas of CP and DDT (ISTD). The confidence intervals about the measured sample results were derived from the validation study.

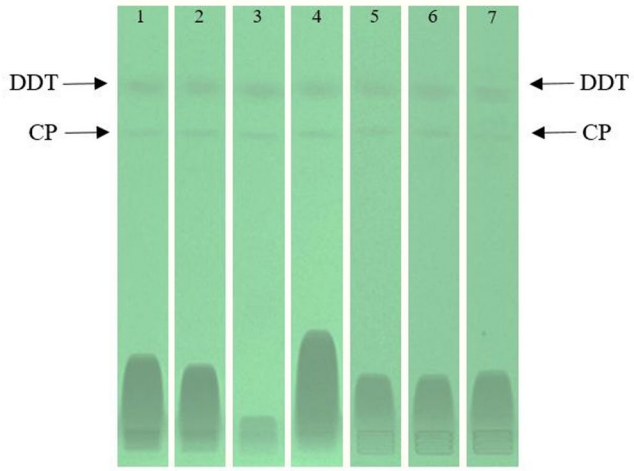

Fig. 1 Plate image of a chocolate spread sample (1.25 g per zone) spiked with CP_{ref} (125 ng per zone) and DDT (300 ng per zone) under white light illumination after pSPE and derivatization with tetramethylbenzidine and different sample treatments; (1) after sulfuric acid treatment and (2–7) additional treatments or liquid-liquid extraction with (2) 10% calcium chloride, (3) 1 M sodium hydroxide, (4) water, (5) 1 M hydrochloric acid, (6) 10% sodium bicarbonate, and (7) sulfuric acid. CP and DDT zones are marked with arrows. CP_{ref}: technical reference chlorinated paraffin mixture C_{14}–C_{17} 52% Cl, DDT: 4,4'-dichlorodiphenyltrichloroethane

Results and discussion

Sample preparation

Chocolate spread and chocolate products

The method development was conducted with a CP-free chocolate spread (previously determined by the pSPE–Vis screening), which was spiked with 250 ng CP_{ref} and 600 ng ISTD (to evaluate matrix separation/clean-up and analyte recovery). In the first studies, the sample weighing and the added volume of *n*-hexane and sulfuric acid described in the work of Oellig and Hammel [30] were scaled up to enhance sensitivity. An aliquot of 2.5 g sample was mixed with 5 mL *n*-hexane as a starting point. For chocolate spread, direct weighing and dissolving were possible; other solid chocolate products had to be shredded before dissolving. The mixture was treated with 20 mL sulfuric acid, shaken for 3 min, and centrifuged until phase separation. After separating the organic phase, a second extraction step followed with another 5 mL *n*-hexane to enhance recovery. pSPE–Vis of the combined and concentrated extracts (200 µL) showed CP and DDT to be well separated, but with a relatively high matrix load at lower hR_F values (Fig. 1 track 1).

Due to this remaining high matrix load on the tracks, additional investigations were conducted to optimize sample preparation and the clean-up process, aiming to increase the application volume and improve the method's sensitivity. Alternatively to the conc. sulfuric acid treatment, decomposing matrix with semi-conc. sulfuric acid and alkaline saponification were investigated using the same sample amount. For the saponification, 7.5 mL aqueous potassium hydroxide solution (w = 3.3 g 100 g^{-1}) was applied at 60 °C for 30 min, followed by adding 2.5 mL ethanol and 5 mL *n*-hexane and shaking for 30 min. Both approaches were ineffective regarding matrix reduction (Fig. S1) and were not pursued further. Additionally, it was tested whether extraction and separation of the *n*-hexane phase before adding the sulfuric acid leads to a reduction of matrix load, but this was also not the case (Fig. S1).

Further on, clean-up steps after the initial sulfuric acid treatment (2.5 g sample with 20 mL) and the extraction of CP with *n*-hexane were investigated to reduce the matrix on the planar layer. A loss of CP and DDT during processing was evaluated by preparing standards without an additional

purification step. Liquid-liquid extraction (LLE) or other treatments of the organic phase were evaluated. LLE was done with 10% $CaCl_2$, 1 M NaOH, water, 1 M HCl, 10% $NaHCO_3$ (5 mL each). A second amount of sulfuric acid was also tested for further matrix decomposition and separation. The LLE with 1 M NaOH showed an effective matrix reduction at the application area, while the other procedures showed no reduction or, in the case of water, a slight increase in matrix load (Fig. 1). Lower or higher concentrated NaOH solutions did not further reduce the remaining matrix. However, for some chocolate product samples, co-elution of the matrix with the CP remained, which required further optimization of the method.

Analyzing lower amounts of CP, an additional zone of further matrix became visible shortly below the CP target zone (Fig. S2). Dispersive solid phase extraction (dSPE) with different adsorption materials was investigated after the sulfuric acid treatment and the LLE with NaOH to reduce the additional matrix that was hindering reliable integration. After solvent exchange to *t*BME, different amounts (20–80 mg) of PSA, silica gel, ChloroFiltr, and GCB were studied. PSA, silica gel, and ChloroFiltr did not impact the matrix load, independent of the amount and solvent used (*t*BME, acetonitrile, and acetone). Conversely, GCB could reduce the matrix below the CP target zone, enabling better integration (Fig. S2). Increasing the quantity of GCB above 20 mg did not further reduce the matrix load. Analyzing standards without dSPE with GCB ensured no losses of CP or DDT.

During extraction with *n*-hexane, a slimy layer between the sulfuric acid and the organic phases was often formed, making it hard to transfer the organic phase quantitatively. Investigating alternative solvents (DCM, DCM/*n*-hexane (1:1 *V*/*V*), and cyclohexane) delivered cyclohexane instead of *n*-hexane, showing no slimy layer and no loss of CP and DDT.

Finally, the maximum possible sample quantity was determined to provide the highest sensitivity. Matrix load along the entire development distance increased above a sample quantity of 2.0 g per zone, as shown in Fig. S3. An increasing bright background under UV 366 nm (left) and increasing blue areas below the CP target zone after derivatization (under white light, right) occurred. Therefore, the maximum application amount was set at 1.75 g per zone, equivalent to a 3.5 g sample weight (Fig. S3).

In conclusion, the sample preparation involved dissolving the sample in 5 mL cyclohexane, followed by sulfuric acid treatment and subsequent extraction with 5 mL cyclohexane. An additional LLE with 5 mL 1 M NaOH solution and a dSPE clean-up with 20 mg GCB was necessary to ensure the best matrix reduction.

Infant formula

With the preliminary results gained from the sample preparation of chocolate products, using sulfuric acid treatment, LLE of the organic phase with 1 M NaOH, and dSPE clean-up with GCB for matrix reduction, infant formula was studied. All samples for evaluating the sample preparation (2.0 g) were CP-free and spiked with 250 ng CP_{ref} and 600 ng DDT to evaluate analyte recovery. First, the dissolving of the infant formula powder in water was investigated. In initial tests, the powder was dissolved in as little as possible volume of water (1:5, *w*/*w*) and then extracted with 5 mL *n*-hexane. This led to a slimy organic phase, and separating the *n*-hexane was impossible. As adding sulfuric acid to the water-dissolved powder was not possible due to the heat released in this process, the extraction of the total lipids, according to Folch et al. [38], was investigated with a mixture of chloroform/methanol/water (10:5:1, *V*/*V*), followed by solvent evaporation, dissolving in *n*-hexane, and the subsequent already applied preparation with sulfuric acid, NaOH, and GCB. For evaluating the extraction, the gravimetric recovery of the fat was determined after evaporating the extraction solvents, showing suitable recovery of fat with 94–105% (n = 3) based on the nutritional information of the powder package. To avoid chloroform, extraction with DCM was evaluated, and likewise, good gravimetric results (recoveries between 92 and 104%, n = 3) for the fat content were obtained. Further, the volume of extraction solvent was evaluated with 10, 20, and 30 mL for a 2.0 g sample. In doing so, a correlation between the amount of used extraction solvent and the matrix load was found (Fig. S4). The formation of a slimy layer during subsequent preparation steps and a generally high matrix load on the planar layer were observed, even when using only 10 mL extraction solvent. Additionally, due to a processing loss of ~ 50% DDT, the fat extraction method based on Folch et al. [38] was not further pursued. As an alternative, hydrochloric acid digestion for human milk following Noti et al. [39] was adapted to our samples (treatment of 2.0 g sample with 10 mL conc. hydrochloric acid for 30 min at 80 °C) with subsequent extraction of the fat with a mixture of *n*-hexane/2-propanol (3:1, *V*/*V*) according to extraction of infant formula by Cesa et al. [40], as well as with *t*BME, DCM, and cyclohexane. Extraction with *n*-hexane/2-propanol showed a little slimy layer but yielded good gravimetric results in fat content after three-fold extraction with 10 mL for 15 min (recoveries between 95 and 107%, n = 6) and relatively low entire matrix load (Fig. S5). *t*BME extracts showed gravimetric recoveries up to 130% (n = 4), which indicated extraction besides fat and resulted in a high matrix load after pSPE (Fig. S5). DCM extraction was not investigated intensively due to a huge slimy layer. The extraction

with cyclohexane showed no slimy layer, a good gravimetric yield of fat (recoveries between 94 and 99%, $n = 4$), and the least matrix load.

Based on these promising results, the original sulfuric acid treatment of the dry powder was investigated instead of hydrochloric acid digestion, including heat processing and cyclohexane as an extraction solvent, to simplify and uniform the process. The sulfuric acid treatment was performed with 2.5 g spiked sample and 20 mL sulfuric acid for 5–30 min at 80 °C followed by twofold cyclohexane extraction and matrix reduction by LLE of the organic phase with 1 M NaOH and dSPE clean-up with GCB. DDT degraded partly after 5 min and completely after 10 min in the heat, which is why the heat treatment was not investigated further. Repeating the procedure for 10 min at RT showed no DDT degradation. Adding cyclohexane as the first step before adding sulfuric acid, as applied in the preparation of chocolate products, was not possible, as it resulted in a higher matrix load on the plate. The entire dissolving of the powder particles of the infant formula in sulfuric acid was achieved after 20 min for various samples, ensuring complete release of CP from the sample. To evaluate CP and DDT stability during the sulfuric acid treatment, this treatment, including the following sample preparation steps (extraction and clean-up), was additionally carried out with CP_{ref} (250 ng) and DDT (600 ng) in pure solvent, and results were compared to an approach omitting the 20-min sulfuric acid treatment. CP recoveries were between 92 and 96%, but there was a loss of ~20% DDT ($n = 3$). Based on this finding, DDT was added after the sulfuric acid treatment with the extraction solvent cyclohexane.

Experiments with increased sample weighing were done as described for chocolate products to finally investigate the maximum amount of applied sample. As for chocolate products, 3.5 g infant formula sample and a final sample amount of 1.75 g per zone was the optimal balance between the highest sample amount and the maximal possible matrix load.

The final sample preparation consists of adding 20 mL sulfuric acid to the sample, treatment for 20 min at RT, and twofold extraction of the mixture with 5 mL cyclohexane. An additional LLE with 5 mL 1 M NaOH solution and a dSPE clean-up using 20 mg GCB was followed to achieve the best matrix reduction.

Optimization of pSPE

An intensive matrix zone was present after TMB derivatization shortly below the CP target zone, making integration impossible (Fig. S6). To effectively separate the matrix from the target zone and avoid matrix interferences, the solvent composition of the mobile phase for the second development was investigated. Different mixtures of DCM/*n*-hexane (9:1 to 7:3, *V*/*V*) and dichloroethane, diethyl ether, isopropyl acetate, *t*BME, and toluene instead of DCM were evaluated. Using solvents other than DCM in the mixture also resulted in well-focused target zones but did not lead to improved matrix separation. Finally, complete separation of this matrix component and still well-focused target zones were achieved by increasing the *n*-hexane content in the mixture with DCM from 10 to 25% (Fig. S6).

Analysis of selected persistent organic pollutants by planar solid phase extraction with visual light detection

To evaluate potential co-elution of selected POPs with the CP target zone and the ISTD DDT, POPs from the Stockholm Convention with different chemical constitutions were analyzed by pSPE–Vis according to Sect. "Planar solid phase extraction with visual light detection (pSPE–Vis)", and results are shown in Fig. 2. A co-elution with the CP target zone was detected for *β*-HCH, HBCD, *trans*-chlordane, and toxaphene. These compounds have been subject to strict regulatory restrictions for decades and are nowadays not part of food contaminant monitoring in the EU. However, their determination could potentially be ruled out by coupling pSPE with HRMS [41], which enables compound-specific verification. In the context of a conservative screening approach, such unspecific signals are acceptable, as they ensure that no potentially hazardous halogenated compounds are overlooked. Mirex and PCB 170 showed an hR_F distinctly above the CP target zone and DDT. Endosulfane delivered an hR_F below the target zone. PFOS was visible in the starting zone. Chlordecone, PentaBDE, and HCB were not detected with TMB, even at amounts of 350 ng per g sample, and therefore pose no problem for the CP detection/analysis.

Generally, PCBs pose a particular problem when analyzing CP with GC– or LC–low-resolution MS methods, as they have the same nominal mass [24, 42], and HRMS is necessary to differentiate between CP and PCB. In our pSPE–Vis method, PCBs do not interfere with CP detection/quantification due to their higher hR_F values, omitting the need for HRMS in CP analysis.

Method validation study

The suitability of the applied screening method for CP was assessed by the performance parameters: linearity, recovery rate, limit of decision, and LOD. A validation study was done with the investigated matrices chocolate spread and infant formula according to Sect. Validation study. As the processing for both sample types is almost identical, the validation

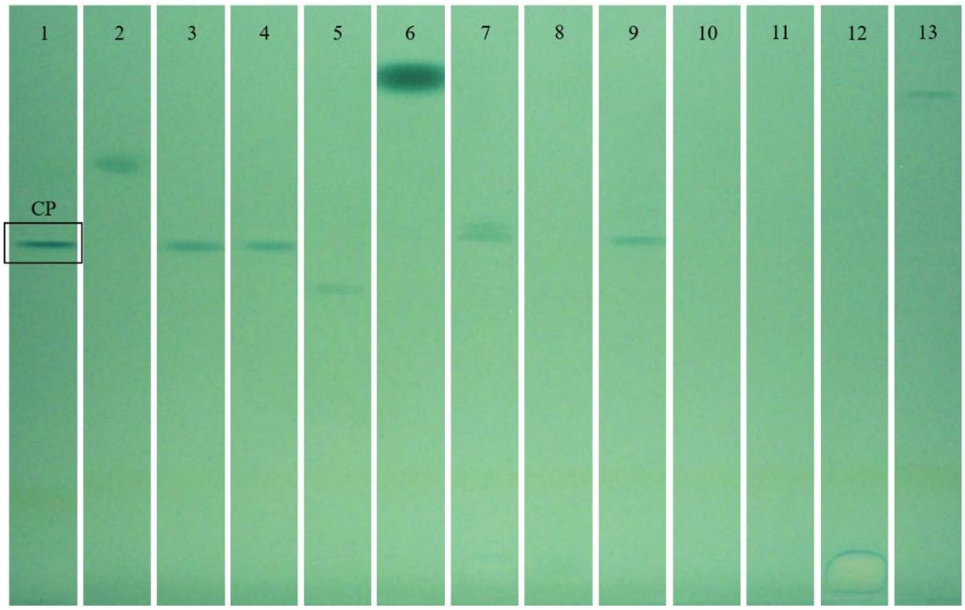

Fig. 2 Plate image of persistent organic pollutants under white light illumination after pSPE and derivatization with tetramethylbenzidine. (1) reference chlorinated paraffin (CP, 125 ng per zone), (2) DDT (300 ng per zone), (3) *β*-HCH (125 ng per zone), (4) HBCD (125 ng per zone), (5) endosulfane (125 ng per zone), (6) mirex (40 ng per zone), (7) *trans*-chlordane (125 ng per zone), (8) chlordecone (200 ng per zone), (9) toxaphene (125 ng per zone), (10) PentaBDE (200 ng per zone), (11) HCB (200 ng per zone), (12) PFOS (200 ng per zone), and (13) PCB 170 (300 ng per zone). Abbreviations according to Sect. "Standard solutions".

was carried out for both samples together. The linearity in the range of 0 to 350 ng CP per zone was good, with R^2_1 = 0.998 and R^2_2 = 0.932 for chocolate spread and R^2_1 = 0.988 and R^2_2 = 0.995 for infant formula. No significant difference was observed between repeatability and within-laboratory reproducibility (Fig. S7). Since the variance of measured values was strongly related to the spiked level, and no significant difference was observed between repeatability and within-laboratory reproducibility, a weighted least-squares regression model was applied to the data (Fig. S8), following the IUPAC recommendations [43]. Estimating weights and calculating performance characteristics were accomplished as described by Steliopoulos and Stickel [36]. The performance parameters of the method were determined based on the calibration function. For quantities > 25 ng CP g^{-1} sample, the recovery was close to 100% (102–105%). For quantities < 25 ng CP g^{-1} sample, the recovery increased up to 120% (Figs. S9 and S10). The limit of decision was 7.8 ng CP g^{-1} sample, and the LOD 15.7 ng CP g^{-1} sample.

A comparison of the performance parameters of commonly used GC–MS- or LC–MS-based methods with the parameters of the pSPE–Vis is challenging, as they were often determined as ng CP μL^{-1} injection solutions. Tomy et al. published a method detection limit (MDL) of 23 ng CP g^{-1} sample for GC–ECNI-HRMS analysis of environmental samples, which is in the same order of magnitude as in our method [44] published a method detection limit (MDL) of 23 ng CP g^{-1} sample for GC–ECNI-HRMS analysis of environmental samples, which is in the same order of magnitude as in our method. An MDL for the analysis of CP in human blood by UPLC–QTOF-MS was determined to < 1 ng CP g^{-1} sample [45], and the MDL of a UPLC–Orbitrap-MS method ranges from 0.79 to 2.5 ng CP g^{-1} soil or chicken sample [46].

Reference measurements

To evaluate our measurement's method performance and quality, reference measurements were performed using GC–ECNI/HRMS. These measurements served as an independent benchmark to evaluate our results in comparison with the values of an established method and to ensure the reliability and robustness of our approach. This kind of quality assurance step is important to confirm the applicability of our method for chocolate spread, chocolate products, and infant formula.

A visual impression of the quantification strategy of the pSPE approach, including the plate image and the corresponding 3D scan of the samples and calibration standards, is shown in Fig. 3. The comparison between our method and the reference measurements showed a general matching, which underlines the reliability of our analytical approach (Table 1). By pSPE–Vis, the CP content of the native sample was estimated at 7.2 ng g^{-1}, slightly below the limit of decision of the method, while GC–ECNI/HRMS delivered 3.0 ng g^{-1}. These amounts determined for the native sample indicate that the discrepancy between the two methods is more apparent at low amounts. For the spiked samples, the results obtained by pSPE–Vis mainly agreed with those by GC–ECNI/HRMS. Generally, the deviations are within the expected uncertainties and variations in the field of the challenging CP analysis.

Overall, the results confirm that our method provides comparable results to the reference measurements, which

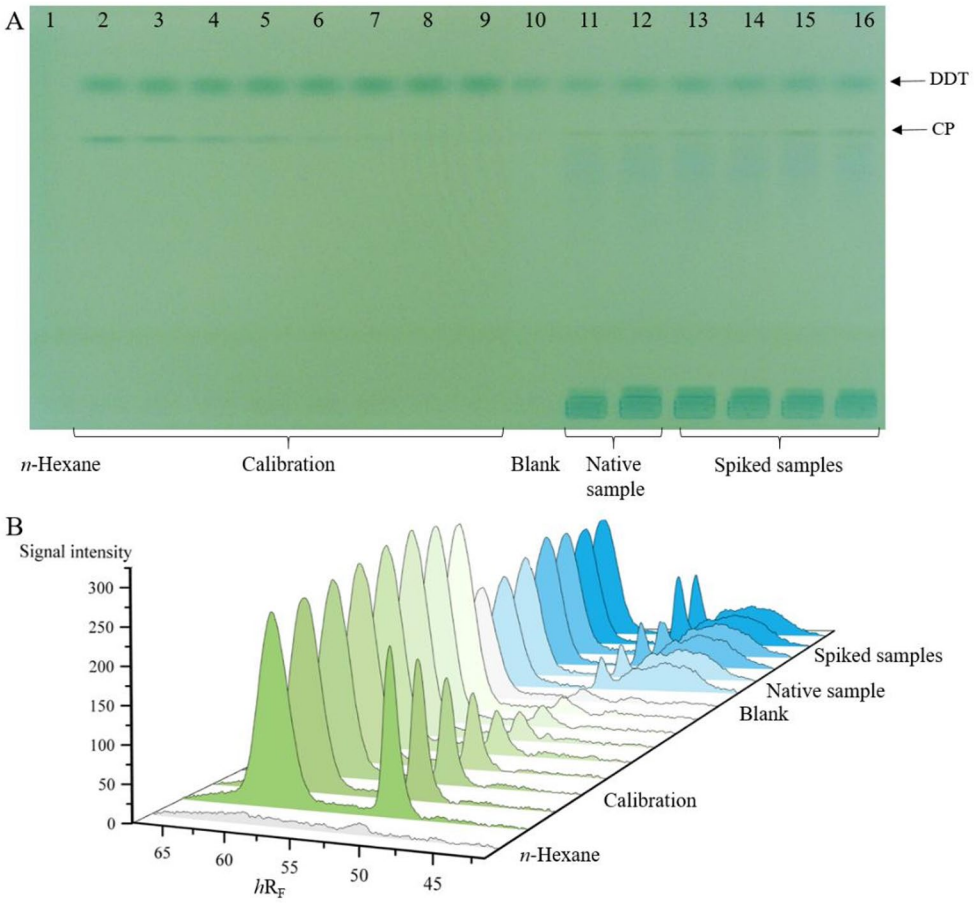

Fig. 3 **A** Plate image after derivatization with tetramethylbenzidine under white light illumination and (**B**) corresponding 3D scan at 645 nm of *n*-hexane (track 1), calibration standards (tracks 2–9), reagent blank (track 10), native sample (tracks 11 and 12), and spiked samples (each level two samples, tracks 13–16)

Table 1 CP contents [ng g^{-1} sample] ± half-width of the 95% confidence interval *u* of native and spiked chocolate spread samples analyzed with GC–ECNI/HRMS ($n=3$) and pSPE–Vis ($n=2$)

	GC–ECNI/HRMS CP ± *u* [ng g^{-1}]	pSPE–Vis CP ± *u* [ng g]
Native sample	< limit of decision	< limit of decision
Spiking level 1	28.1 ± 6.3	30.8 ± 7.5
Spiking level 2	70 ± 29	101 ± 13

Table 2 Chocolate spread and chocolate cake glaze samples (each sample $n=2$) with detected CP content [ng g^{-1} sample] ± half-width of the 95% confidence interval *u*

Sample no.	Product	CP ± *u* [ng g^{-1}]
6	Cocoa-containing vegetable fat glaze	170 ± 20
7	Chocolate spread	25.2 ± 7.3
8	Cocoa-containing vegetable fat glaze	12.3 ± 7.0
10	Cocoa-containing vegetable fat glaze	71.2 ± 10.0
11	Chocolate spread	250 ± 27
15	Chocolate spread	158 ± 19
18	Chocolate spread	102 ± 13
19	Chocolate spread	258 ± 27
23	Chocolate spread	151 ± 18
25	Chocolate spread	201 ± 23

Only samples with contents above the limit of detection are listed

underpins its suitability for reliable quantification in this challenging analytical context.

Screening of CP in samples from the German market

Chocolate spread and chocolate products

In ten of the 25 samples, CP could be detected above the LOD. The content of one sample was between the limit of decision and LOD; the other nine samples ranged from 25 to 260 ng g^{-1} (Table 2). A comparison of the CP content with the fat composition of the analyzed samples indicated that all samples with detectable CP levels contained palm oil. Although no direct quantitative correlation was observed, this recurring presence suggests that palm oil might be a relevant factor in CP contamination. In contrast, rapeseed oil was only found in samples without detectable CP, potentially indicating a lower risk of contamination for this fat source. For example, high CP contents were found in samples containing only palm oil (e.g., sample 6, 170 ng g^{-1}) as well as in those with mixed oil compositions, including hazelnut and sunflower oil (e.g., samples 11 and 19, 250 and 258 ng g^{-1}, respectively). However, since palm oil was also present in several samples without CP contamination, no

Table 3 Infant formula samples (each sample $n=2$) with detected CP content [ng g^{-1} sample] ± half-width of the 95% confidence interval u

Sample no.	Definition according to Regulation (EU) No 609/2013	CP ± u [ng g^{-1}]
15	Follow-on formula	10.5 ± 6.9
16	Follow-on formula	18.6 ± 7.1
17	Follow-on formula	48.3 ± 8.4
19	Infant formula	33.0 ± 7.6
34	Follow-on formula	54.1 ± 8.8
36	Infant formula	12.3 ± 7.0

Only samples with contents above the limit of detection are listed

direct correlation can be established between the presence of CP and palm oil. CP are known to accumulate in fat-rich food matrices due to their lipophilic nature and low volatility. Within each product group (chocolate products and infant formula) investigated, however, the variation in total fat content was minimal. Therefore, no conclusion can be drawn regarding a potential correlation between fat content and CP concentration. All chocolate spread samples were packaged in glass, while cake glazes were sold in plastic containers. Therefore, no conclusions can be drawn regarding potential CP migration from packaging materials. These findings suggest that the occurrence of CP may be influenced not solely by the fat composition but also by external factors such as raw material quality, sourcing regions, or specific production and processing conditions.

Sprengel et al. [17] investigated hazelnut spread from the German market and determined contents between 7.5 and 270 g CP g^{-1} sample, with SCCP levels between 7.5 and 50 ng g^{-1} sample and MCCP between 17 and 270 ng g^{-1} sample. This showed that, in general, the contents of our study agree well with the data in the literature. However, it has to be noted that our method does not differentiate between the prohibited SCCP and the currently still unrestricted MCCP, and further investigations should be conducted with positive samples to enable a legal classification of the samples. Although M- and LCCP are currently not regulated, both have been associated with toxicological effects comparable to SCCP [11]. This supports the relevance of a total CP signal for food safety monitoring, even in the absence of congener-specific data. A coupling of pSPE to HRMS, currently under development, will enable congener-specific differentiation and thus allow more precise risk assessment of SCCP, MCCP, and LCCP.

Infant formula

CP was detected in six of the 38 samples above the LOD, with the content in two samples being between the limit of decision and the LOD. The content of the remaining four samples varied from 18 to 54 ng g^{-1} (Table 3), whereby only one sample represented infant formula according to Regulation (EU) No. 609/2013, and the other samples were representatives of the group of follow-on formula. Similar to the findings in chocolate-based products, no correlation was observed between CP content and fat composition (see Table S2 and discussion above). However, all six positive samples contained palm oil, which was the only fat component consistently present in CP-contaminated infant formulas. These findings align with the observations in chocolate-based products and may suggest a general relevance of palm oil for CP entry. Moreover, all positive samples also contained sunflower and rapeseed oil, although these oils were equally common in negative samples and, therefore, not indicative. In three cases, fish oil or other specialized fats such as coconut oil or Mortierella oil were present, but no consistent relationship with CP levels could be established. The results are consistent with the observations gained for the chocolate spread and chocolate products. This supports the assumption that the CP content is independent of these parameters and is probably influenced by external (production) factors unrelated to the sample matrix. All infant formula powders were packaged in multilayer composite materials, presumably including an internal barrier layer based on aluminum foil combined with plastic sealing layers such as polypropylene. Since no alternative packaging types were present in the dataset, no conclusions can be drawn regarding the potential influence of packaging on CP contamination.

Krätschmer et al. determined levels of up to 210 ng g^{-1} CP (combined value for SCCP and MCCP analyzed by GC–HRMS) relative to the fat content for infant formula from the German market [14]. Based on an average fat content of 23.2%, this corresponds to approx. 46 ng CP g^{-1} powdered sample. This is nearly equivalent to the CP content in the sample with the highest contamination in our study. The research group led by Han et al. also confirmed CP contents of this magnitude in infant formula from the Chinese market [16]. In contrast to our results and those of Krätschmer et al. [14] and Han et al. [16], another study examining infant formula from the Chinese market with LC–HRMS found distinctly higher contents ranging from 9 to 146 ng SCCP g^{-1}, 13–775 ng MCCP g^{-1}, and 1–48 ng LCCP g^{-1} sample [15].

Our findings illustrate the value of pSPE–Vis as a cost-effective, high-throughput method to identify potentially contaminated products, which can then be subjected to HRMS-based congener-specific analysis if regulatory clarification or detailed profiling is required.

Conclusion

The study demonstrates a simple and reliable method by pSPE for screening the total CP in complex food matrices such as chocolate products and infant formula. By

systematically optimizing the sample preparation, including the extraction and purification, and adjusting pSPE steps, the interfering matrix was effectively removed. Co-elution of CP with most of the investigated POPs was omitted except for *β*-HCH, HBCD, *trans*-chlordane, and toxaphene, which depicts a minor problem in the EU. The pSPE–Vis method showed good performance parameters in terms of linearity, recovery (near 100% for CP quantities > 25 ng g^{-1} sample, limit of decision (7.8 ng CP g^{-1} sample), and limit of detection (LOD, 15.7 ng CP g^{1} sample), making it suitable for regulatory purposes. A reference measurement with GC–ECNI/HRMS yielded comparable results for spiked chocolate product samples, confirming the method's reliability. Although the method does not differentiate between CP homologous groups, it is intentionally designed as a cost-effective, high-throughput screening tool that allows clearly uncontaminated samples to be ruled out early, while positive results can be confirmed by HRMS if detailed congener information is required. Thus, the developed pSPE–Vis approach provides a practical basis for routine and scalable monitoring of total CP contamination in food, triggering further analytical or regulatory action where necessary.

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Declarations

Conflict of interest The authors declare that they have no conflict of interest or competing interests.

Ethical approval and consent to participate Not applicable.

Human Rights Not applicable.

References

1. Chen C, Chen A, Zhan F, Wania F, Zhang S, Li L, Liu J (2022) Global historical production, use, in-use stocks, and emissions of short-, medium-, and long-chain chlorinated Araffins. *Environ. Sci. Technol.* 56:7895–7904. https://doi.org/10.1021/acs.est.2c00264
2. van Mourik LM, Gaus C, Leonards PEG, de Boer J (2016) Chlorinated paraffins in the environment: A review on their production, fate, levels and trends between 2010 and 2015. *Chemosphere* 155:415–428. https://doi.org/10.1016/j.chemosphere.2016.04.037
3. Vetter W, Sprengel J, Krätschmer K (2022) Chlorinated paraffins - A historical consideration including remarks on their complexity. *Chemosphere* 287:132032. https://doi.org/10.1016/j.chemosphere.2021.132032
4. Glüge J, Wang Z, Bogdal C, Scheringer M, Hungerbühler K (2016) Global production, use, and emission volumes of short-chain chlorinated paraffins - A minimum scenario. *Sci. Total Environ.* 573:1132–1146. https://doi.org/10.1016/j.scitotenv.2016.08.105
5. Kobetičová K, Černý R (2018) Ecotoxicity assessment of short- and medium-chain chlorinated paraffins used in polyvinyl-chloride products for construction industry. *Sci.Total Environ.* 640–641:523–528. https://doi.org/10.1016/j.scitotenv.2018.05.300
6. Nikiforov VA (2010) Synthesis of polychloroalkanes. In: de Boer J (ed) Chlorinated Paraffins. The handbook of environmental chemistry, vol 10. Springer, Berlin, Heidelberg, pp 41–82. DOI = 10.1007/698_2009_40.
7. Muir D, Stern GA, Tomy GT (2000) Chlorinated Paraffins. In O. Hutzinger, & P. Paasivirta (Eds.), Volume 3 Anthropogenic Compounds Part K, The Handbook of Environmental Chemistry, vol 3K (pp. 203–236). Berlin, Heidelberg: Springer. DOI = 10.1007/3-540-48915-0_8
8. Pakalin S, Aschberger K, Sosgrove O, Paya Perez A, Vegro S (2008) Updated European Union Risk Assessment Report of Alkanes, C10-13, Chloro. Publications Office of the European Union. Luxembourg, JRC45867
9. European Commission, Joint Research Centre. Institute for Health and Consumer Protection. (2011) ALKANES, C14-17, CHLORO.Addendum to the final report (2007) of the risk assessment - Environment part. Publications Office. https://doi.org/10.2788/69599
10. Persistent Organic Pollutants Review Committee (2016) Report of the persistent organic pollutants review committee on the work of its twelfth meeting: risk management evaluation on short-chain chlorinated paraffins. Rome, Italy. UNEP/POPS/POPRC.12/11
11. EFSA Panel on Contaminants in the Food Chain (2020) Risk assessment of chlorinated paraffins in feed and food. EFSA Support Publ 18. https://doi.org/10.2903/sp.efsa.2020.EN-1815
12. Persistent Organic Pollutants Review Committee (2024) Report of the Persistent Organic Pollutants Review Committee on the Work of its Twentieth Meeting: Consideration of recommendations to the Conference of the Parties: Chlorinated paraffins with carbon chain lengths in the range C14–17 and chlorination levels

at or exceeding 45 per cent chlorine by weight. UNEP/POPS/POPRC.20/10. Rome, Italy
13. Krätschmer K, Schächtele A, Vetter W (2021) Short- and medium-chain chlorinated paraffin exposure in South germany: A total diet, meal and market basket study. *Environ. Pollut.* 272:116019. https://doi.org/10.1016/j.envpol.2020.116019
14. Krätschmer K, Schächtele A, Vetter W (2021) Chlorinated paraffins in baby food from the German market. *Food Control* 123:107689. https://doi.org/10.1016/j.foodcont.2020.107689
15. Luo Y, Li J, Gao W, Gao L, Ke R, Yang C, Wang Y, Gao Y, Wang Y, Jiang G (2022) Exposure to short-, medium-, and long-chain chlorinated paraffins for infant via cow infant formula, goat infant formula and baby food. *Food Chem. Toxicol.* 165:113178. https://doi.org/10.1016/j.fct.2022.113178
16. Han X, Chen H, Deng M, Du B, Zeng L (2021) Chlorinated paraffins in infant foods from the Chinese market and estimated dietary intake by infants. *J. Hazard. Mater.* 411:125073. https://doi.org/10.1016/j.jhazmat.2021.125073
17. Sprengel J, Rixen S, Tietz T, Zellmer S, Schumacher DM, Lüth A, Kappenstein O, Vetter W (2023) Chlorinated paraffins in nut-nougat and chocolate spreads from the German market. *Food Control* 145, 109385. DOI = 10.1016/j.foodcont.2022.109385
18. Sprengel J, Wieselmann S, Kröpfl A, Vetter W (2019) High amounts of chlorinated paraffins in oil-based vitamin E dietary supplements on the German market. *Environ. Int.* 128:438–445. https://doi.org/10.1016/j.envint.2019.04.065
19. Muir DC, Grift NP, Lockhart WL, Wilkinson P, Billeck BN, Brunskill GJ (1995) Spatial trends and historical profiles of organochlorine pesticides in arctic lake sediments. *Sci. Total Environ.* 160–161, 447–457. DOI = 10.1016/0048-9697(95)04378-E
20. Krätschmer K, Schächtele A (2019) Interlaboratory studies on chlorinated paraffins: evaluation of different methods for food matrices. *Chemosphere* 234:252–259. https://doi.org/10.1016/j.chemosphere.2019.06.022
21. Yuan B, Muir D, MacLeod M (2019) Methods for trace analysis of short-, medium-, and long-chain chlorinated paraffins: critical review and recommendations. *Anal. Chim. Acta* 1074:16–32. https://doi.org/10.1016/j.aca.2019.02.051
22. van Mourik LM, Leonards PEG, Gaus C, de Boer J (2015) Recent developments in capabilities for analysing chlorinated paraffins in environmental matrices: A review. *Chemosphere* 136:259–272. https://doi.org/10.1016/j.chemosphere.2015.05.045
23. Reth M, Zencak Z, Oehme M (2005) First study of congener group patterns and concentrations of short- and medium-chain chlorinated paraffins in fish from the North and Baltic sea. *Chemosphere* 58:847–854. https://doi.org/10.1016/j.chemosphere.2004.09.036
24. Reth M, Oehme M (2004) Limitations of low resolution mass spectrometry in the electron capture negative ionization mode for the analysis of short- and medium-chain chlorinated paraffins. *Anal. Bioanal. Chem.* 378, 1741–1747. DOI = 10.1007/s00216-004-2546-9
25. Amoura C, Larvor F, Marchand P, Le Bizec B, Cariou R, Bichon E (2024) Quantification of chlorinated paraffins by chromatography coupled to high-resolution mass spectrometry – Part B: influence of liquid chromatography separation. *Chemosphere* 352:141401. https://doi.org/10.1016/j.chemosphere.2024.141401
26. Mézière M, Cariou R, Larvor F, Bichon E, Guitton Y, Marchand P, Dervilly G, Bizec L (2020) B. Optimized characterization of short-, medium, and long-chain chlorinated paraffins in liquid chromatography-high resolution mass spectrometry. *J. Chrom. A* 1619, 460927. DOI = 10.1016/j.chroma.2020.460927
27. Bogdal C, Alsberg T, Diefenbacher PS, MacLeod M, Berger U (2015) Fast quantification of chlorinated paraffins in environmental samples by direct injection high-resolution mass spectrometry with pattern Deconvolution. *Anal. Chem.* 87:2852–2860. https://doi.org/10.1021/ac504444d
28. Yuan B, Alsberg T, Bogdal C, MacLeod M, Berger U, Gao W, Wang Y, de Wit CA (2016) Deconvolution of soft ionization mass spectra of chlorinated paraffins to resolve congener groups. *Anal. Chem.* 88:8980–8988. https://doi.org/10.1021/acs.analchem.6b01172
29. Cui J, Hua R, Wu Y, Wang H, Wang D, Ren G, An J, Quan S, Yu Z (2025) Identification of hydroxylated chlorinated paraffins in human serum and their potential metabolic pathways. *Environ. Sci. Technol.* 59:5487–5495. https://doi.org/10.1021/acs.est.5c00091
30. Oellig C, Hammel Y-A (2019) Screening for chlorinated paraffins in vegetable oils and oil-based dietary supplements by planar solid phase extraction. *J. Chrom. A* 1606:460380. https://doi.org/10.1016/j.chroma.2019.460380
31. Oellig C, Schwack W (2011) Planar solid phase extraction – a new clean-up concept in multi-residue analysis of pesticides by liquid chromatography-mass spectrometry. *J. Chrom. A* 1218:6540–6547. https://doi.org/10.1016/j.chroma.2011.06.108
32. Geyer S, Götz S, Oellig C (2025) Planar solid phase extraction for chlorinated paraffin analysis – irradiation chamber for standardized derivatization on planar thin-layers. *J. Chrom. A* 1741:465618. https://doi.org/10.1016/j.chroma.2024.465618
33. Sayago A, Asuero AG (2004) Fitting straight lines with replicated observations by linear regression: part II. Testing for homogeneity of variances. *Cri. Rev. Anal. Chem.* 34:133–146 DOI = 10.1080/10408340490888599
34. Zorn ME, Gibbons RD, Sonzogni WC (1997) Weighted least-squares approach to calculating limits of detection and quantification by modeling variability as a function of concentration. *Anal. Chem.* 69:3069–3075. https://doi.org/10.1021/ac970082i
35. Oppenheimer L, Capizzi TP, Weppelman RM, Mehta H (1983) Determining the lowest limit of reliable assay measurement. *Anal. Chem.* 55:638–643. https://doi.org/10.1021/ac00255a013
36. Steliopoulos P, Stickel E (2007) Estimation of performance characteristics of a confirmation method for thyreostats in plasma by means of a weighted least-squares approach. *Anal. Chim. Acta* 592:181–186. https://doi.org/10.1016/j.aca.2007.04.026
37. Krätschmer K, Schächtele A, Malisch R, Vetter W (2019) Chlorinated paraffins (CPs) in salmon sold in Southern germany: Concentrations, homologue patterns and relation to other persistent organic pollutants. *Chemosphere* 227:630–637. https://doi.org/10.1016/j.chemosphere.2019.04.016
38. Folch J, Lees M, Stanley GS (1957) A simple method for the isolation and purification of total lipids from animal tissues. J Biol Chem 226:497–509. https://doi.org/10.1016/S0021-9258(18)64849-5
39. Noti A, Grob K, Biedermann M, Deiss U, Brüschweiler BJ (2003) Exposure of babies to C15-C45 mineral paraffins from human milk and breast salves. *Regul. Toxicol. Pharmacol.* 38:317–325 DOI = 10.1016/S0273-2300(03)00098 – 9
40. Cesa S, Casadei MA, Cerreto F, Paolicelli P (2012) Influence of fat extraction methods on the peroxide value in infant formulas. *Food. Res. Int.* 48:584–591. https://doi.org/10.1016/j.foodres.2012.06.002
41. Morlock G, Schwack W (2010) Coupling of planar chromatography to mass spectrometry. TrAC *Trends Anal. Chem.* 29, 1157–1171. DOI = 10.1016/j.trac.2010.07.010
42. Krätschmer K, Cojocariu C, Schächtele A, Malisch R, Vetter W (2018) Chlorinated paraffin analysis by gas chromatography Orbitrap high-resolution mass spectrometry: Method performance, investigation of possible interferences and analysis of fish samples. *J. Chrom. A* 1539, 53–61. DOI = 10.1016/j.chroma.2018.01.034

43. Danzer K, Currie LA (1998) Guidelines for calibration in analytical chemistry. Part I. Fundamentals and single component calibration (IUPAC Recommendations 1998). *Pure A. Chem.* 70, 993–1014. DOI = 10.1351/pac199870040993
44. Tomy GT, Stern GA, Muir DC, Fisk AT, Cymbalisty CD, Westmore JB (1997) Quantifying C10-C13 polychloroalkanes in environmental samples by high-resolution gas chromatography electron capture negative ion high-resolution mass spectrometry. *Anal. Chem.* 69, 2762–2771. DOI = 10.1021/ac961244y
45. Li T, Wan Y, Gao S, Wang B, Hu J (2017) High-Throughput Determination and Characterization of Short-, Medium-, and Long-Chain Chlorinated Paraffins in Human Blood. *Environ. Sci. Technol.* 51, 3346–3354. DOI = 10.1021/acs.est.6b05149
46. Huang X, Ding C, Su Q, Wang Y, Cui Z, Yin Q, Wang X (2021) A simplified method for determination of short-, medium-, and long-chain chlorinated paraffins using tetramethyl ammonium chloride as mobile phase modifier. *J. Chrom. A* 1642, 462002. DOI = 10.1016/j.chroma.2021.462002