

Title

Development of an Analytical Method for Pentachlorothiophenol (PCTP) in Food and Risk Assessment

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Body

Pentachlorothiophenol (PCTP) is a persistent organic pollutant released from tire vulcanization, chemical intermediate production, and the environmental degradation of hexachlorobenzene (HCB) and pentachloronitrobenzene (PCNB). Owing to its high resistance to degradation and bioaccumulative potential, PCTP can persist in soil and water and enter the food chain through contaminated agricultural, livestock, and fishery products. However, its reactive thiol (-SH) group readily degrades during conventional clean-up, and no analytical method for food had previously been reported. This study developed a gas chromatography-tandem mass spectrometry (GC-MS/MS) method incorporating thiol methylation with trimethylsilyl diazomethane (TMS-DM) to stabilize PCTP for reliable quantification, and the method was validated across six representative food matrices, meeting international performance criteria. The validated method was applied to nationwide food monitoring, and a dietary risk assessment for the Korean population showed that PCTP exposure remained well below the health-based reference level, with the highest estimated risk at only 4.126% (95th percentile, 1-2-year age group), confirming a low health risk from current dietary PCTP exposure.

Development of an Analytical Method for Pentachlorothiophenol (PCTP) in Food and Risk Assessment

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Research Background

Pentachlorothiophenol (PCTP) is a persistent and bioaccumulative pollutant used industrially in tyre vulcanization and as a chemical intermediate, and it can also form as a degradation product of hexachlorobenzene and pentachloronitrobenzene, both of which have been widely used as pesticides and industrial chemicals. Owing to its high environmental persistence, PCTP released from these sources can remain in soil and water for extended periods, bioaccumulate through the food chain, and potentially contaminate agricultural, livestock, and fishery products. However, PCTP contamination in food remains poorly characterized, as its reactive thiol group readily degrades during conventional clean-up procedures and adsorbs onto laboratory equipment, resulting in reduced analytical sensitivity. To overcome these limitations, this study employed a thiol methylation approach using trimethylsilyldiazo-methane (TMS-DM) to stabilize the thiol group prior to instrumental analysis. An optimized GC-MS/MS method was developed and validated for the determination of PCTP in Korean food samples, and the resulting occurrence data were used to conduct a dietary exposure and risk assessment for the Korean population.

Objectives

Pentachlorothiophenol (PCTP) has garnered increasing attention due to its high production volume, environmental persistence, and bioaccumulative nature (Howard & Muir, 2010). However, the detection of PCTP poses significant analytical challenges due to the reactivity of its thiol group (-SH), which is prone to degradation during oxidative clean-up procedures and adsorption onto laboratory equipment, leading to reduced sensitivity. Therefore, this study aimed to (1) develop and optimize a GC-MS/MS method incorporating TMS-DM-based thiol methylation for the determination of PCTP in food, (2) apply the validated method to characterize PCTP occurrence in Korean agricultural, livestock, and fishery products, and (3) conduct a dietary exposure and risk assessment to evaluate the potential health risk of PCTP to the Korean population through food consumption.

Materials and Methods

Chemicals and Materials

PCTP standard (Tokyo Chemical Industry) and PCTA-D3 (HPC Standards) were used as the target analyte and internal standard, respectively. PCTP working concentrations were prepared at concentrations of 50–500 µg/kg, while PCTA-D3 was used at 100 µg/kg. Analytical-grade solvents, including dichloromethane, n-hexane, benzene, and anhydrous sodium sulfate were used for extraction. Sulfuric acid (95–97%) and silica SPE cartridges were used for fat removal and clean-up. TMS-DM (Thermo Scientific) and acetic acid were used for thiol methylation and reaction termination, respectively.

Sample Preparation

A total of 870 samples from agricultural, livestock, and fishery products were purchased from Korean markets. Six representative food matrices (apple, canola oil, egg, pork, clam, and mackerel) were selected for method validation. Samples were homogenized and extracted according to their fat content. Briefly, 2g of each sample was spiked with the internal standard and extracted with hexane using ultrasonic-assisted extraction. High-fat samples were additionally treated with sulfuric acid for fat removal. The extracts were dehydrated with anhydrous sodium sulfate, concentrated, and purified using silica-based solid-phase extraction. The purified eluates were concentrated and finally subjected to thiol methylation prior to GC-MS/MS analysis.

Methylation Optimization

The methylation conditions were optimized using canola oil as a representative high-fat matrix by evaluating the effects of TMS-DM volume (100–400 μL), reaction time (0.5–45 min), and temperature (15–45 $^{\circ}\text{C}$) on PCTP recovery. The optimal conditions were determined to be 300 μL of TMS-DM, 35 $^{\circ}\text{C}$, and 30 min. Under these conditions, 100 μL of methanol and 300 μL of TMS-DM were added to the purified eluate, followed by vortexing and methylation at 35 $^{\circ}\text{C}$ for 30 min. The reaction was quenched with acetic acid, and the resulting methylated product was concentrated to 100 μL under nitrogen prior to GC-MS/MS analysis. The optimized derivatization step was intended to stabilize the reactive thiol group and improve the reliability of PCTP determination.

GC-MS/MS Analysis

PCTP analysis was performed using a Trace 1310 GC coupled with a TSQ 9000 triple quadrupole MS (Thermo Scientific, USA) and equipped with a TriPlus RSH autosampler. Chromatographic separation was achieved on a DB-5MS Ultra-Inert column (30 m $\times$ 0.25 mm $\times$ 0.25 μm) using helium as the carrier gas at a constant flow rate of 1 mL/min. Samples were injected in splitless mode, and the mass spectrometer was operated in electron ionization mode at 70 eV with selected reaction monitoring. PCTP and PCTA-D3 were monitored using specified precursor and product ion transitions for quantitative and confirmatory analysis.

Method Validation

The analytical method was validated according to AOAC guidelines using six representative food matrices (apple, egg, clam, canola oil, pork, and mackerel). Validation parameters included specificity, linearity, LOD, LOQ, recovery, and precision. Matrix-matched calibration curves were prepared at 50–500 $\mu\text{g}/\text{kg}$, and recovery and precision were assessed at three fortification levels (5, 20, and 50 $\mu\text{g}/\text{kg}$) using repeatability and reproducibility tests.

Monitoring and Risk Assessment

Dietary risk assessment was conducted using individual food consumption and body weight data extracted from the Korea National Health and Nutrition Examination Survey (KNHANES, 2019–2021), and monitoring targets were selected among frequently consumed, high-fat foods and items prone to POPs accumulation. Food consumption data were stratified by age group, and moisture-corrected mean and 95th-percentile intake values were calculated with survey-year weighting. Daily exposure was estimated by combining food-specific contamination levels with individual consumption and body weight. Non-detected values were statistically estimated using the regression on order statistics (ROS) method because of the low detection frequency of PCTP.

Results and Discussions

The developed method, incorporating the optimized thiol methylation procedure, showed satisfactory performance across the six representative food matrices. Matrix-matched calibration curves showed good linearity ($R^2 > 0.99$), with LODs and LOQs ranging from 1.67 to 1.93 ng/g and 5.06 to 5.86 ng/g, respectively. Recoveries ranged from 72.8% to 107.6%, and repeatability (RSDr) and reproducibility (RSDR) met the acceptable criteria established by AOAC. Measurement uncertainty was also evaluated, with reproducibility identified as the major contributing factor. The validated method was subsequently applied to 870 food samples from agricultural, livestock, and fishery products. PCTP concentrations ranged from not detected (ND) to 13.63 ng/g wet weight. Across the food categories, the highest concentration was observed in dairy products (ND–4.97 ng/g), followed by grains and their products (ND–4.90 ng/g), vegetables (ND–4.05 ng/g), and tea (ND–3.94 ng/g). In animal-derived foods, PCTP

was detected in eggs (ND–3.10 ng/g) and mussels (ND–4.36 ng/g). The observed variation in PCTP concentrations among food types may reflect differences in environmental conditions and production practices, as well as the persistence of related organochlorine compounds. Given the limited occurrence data for PCTP in food, these findings provide a reference for future monitoring of PCTP contamination.

Because no PCTP-specific toxicity reference value has been established, a provisional health-based guidance value of 0.001 mg/kg bw/day was derived through read-across from the oral reference dose of hexachlorobenzene (HCB, 0.0008 mg/kg bw/day), considering PCTP as a major urinary metabolite of HCB. Dietary exposure was estimated by combining the food monitoring data with individual food consumption data from the 2019–2021 KNHANES, and non-detected values were substituted using the ROS method. The estimated risk was highest in the 1–2-year age group, with mean and 95th-percentile risk levels of 2.249% and 4.126%, respectively, both far below 5% of the provisional health-based guidance value. Agricultural products contributed the most to overall exposure, followed by livestock and fishery products. These results indicate that current dietary exposure to PCTP among the Korean population poses a low health risk, although continued monitoring is warranted given its environmental persistence and bioaccumulative potential.

Conclusions

This study developed and validated a GC-MS/MS method incorporating an optimized TMS-DM-mediated thiol methylation for the determination of PCTP in food. The method met AOAC validation criteria and was successfully applied to nationwide food monitoring. Dietary risk assessment based on Korean food consumption data showed that PCTP exposure through food consumption was low, with the highest estimated risk of 4.126% of the provisional health-based guidance value at the 95th percentile in the 1–2-year age group. These findings demonstrate the applicability of the developed method for PCTP monitoring in food and provide information for evaluating dietary exposure and risk. continued monitoring of PCTP in food may be warranted given its environmental persistence and bioaccumulative potential.

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