Brief Report

Organochlorine pesticide residue in Chinese herbal medicine

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S Supplementary material

Over ten-year routine inspection results on organochlorine pesticide (OCP) residue were summarized, OCPs residues, including BHC isomers (α , β , γ , and δ -BHC), DDT analogs (p,p' -DDD, p,p' -DDE, o,p' -DDT, and p,p' -DDT), and pentachloronitrobenzene (PCNB) and its metabolites (pentachloroaniline and methyl pentachlorophenyl sulfide (MPCPS)), in 1,665 samples for 37 types of Chinese herbal medicine (CHM) using the QuEChERS method coupled with the GC-ECD. Based on the maximal residue levels for OCPs set by Asian pharmacopeias, PCNB contamination in *Ginseng radix* as well as the total DDT and PCNB contamination in *Panax quinquefolii radix* are of concern. OCP residues in different parts of *Panax ginseng* were also compared. The total BHC residue in leaf and fibrous root, as well as the total DDT and PCNB residue in all parts, exceeded MRL of 0.1 mg/kg. Overall, this study provided meaningful results about OCP residue in CHM for pharmaceutical industries and consumers.

Keywords: Chinese herbal medicine, *Ginseng radix*, organochlorine pesticides residue, *Panax quinquefolii radix*.

Introduction

Chinese herbal medicine (CHM) has a long history of world-wide use to cure a variety of diseases. Most CHMs are plant-derived materials and agricultural products; therefore, more attention should be paid to contamination by hazardous agricultural substances, such as pesticides. Organochlorine pesticides (OCPs), a group of chlorinated compounds and the class of persistent organic contaminants, were widely used in agriculture in the past.^{1,2)} Now, the use of most OCPs is banned because they are highly or moderately hazardous.²⁾ The characteristics of OCPs are low biodegradability, a long half-life, and persistence that could be examined in the environment; therefore, the monitoring of OCPs in agricultural products, such as CHM, is necessary to ensure their safety for medication use.²⁾

The aim of the present study is to summarize the results of more than 10 years of routine inspection for OCP residues,

including BHC isomers (α , β , γ , and δ -BHC), DDT analogs (p,p' -DDD, p,p' -DDE, o,p' -DDT, and p,p' -DDT), and pentachloronitrobenzene (PCNB) and its metabolites (pentachloroaniline (PCA) and methyl pentachlorophenyl sulfide (MPCPS)), in 1,665 samples for 37 types of commonly used CHMs. The Quick, Easy, Cheap, Effective, Rugged, and Safe (QuEChERS) method, coupled with gas chromatography (GC) equipped with electron capture detection (ECD), was used to determine the OCP residues in CHMs. The contaminated rate (CR) for 11 types of OCPs in CHM was calculated, and the exceeding rate (ER) was computed based on the diverse maximum residue limits (MRLs) set by different Asian pharmacopeias, such as the Taiwan Herbal Pharmacopeia (THP), the Pharmacopoeia of the People's Republic of China (ChP), the Hong Kong Chinese Materia Medica Standards (HKCMMS), the Japanese Pharmacopoeia (JP), and the Korean Pharmacopoeia (KP). *Panax ginseng*, an ancient medicinal herb, has been used as a dietary supplement in Western countries in the last two decades. Therefore, another objective is to compare OCP residues in different parts, including the leaf, stem, root, and fibrous root, of four-year-old *Panax ginseng*.

Materials and methods

1. CHM collection

A total of 1,665 samples for 37 types of CHMs were imported from various certified pharmaceutical factories in different prov-

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inces of China by diverse suppliers and traders, and voucher specimens were deposited with the quality assurance department of our company. Fresh four-year-old *Panax ginseng* was obtained from the Jinlin Province of China. Four parts, i.e., the leaf, stem, root, and fibrous root, were isolated, rinsed with water, and dried at 50°C in an oven. All collected samples were powdered and sieved by passing through a 40-mesh sifting screen to determine the OCP residues.

2. Determination of OCP residues and method validation

Eleven types of OCP standards, i.e., α -BHC, β -BHC, γ -BHC, δ -BHC, p,p' -DDE, o,p' -DDT, p,p' -DDD, p,p' -DDT, PCNB, PCA, and MPCPS, were purchased from Merck (Darmstadt, Germany) with purity higher than 98%. A stock OCP mixed standard solution containing approximately 20 mg/L of individual OCPs was prepared by dissolving around 2 mg of individual OCP powder in 20 mL of acetone, and then isooctane was added to a final volume of 100 mL. A series of working standard solutions containing 10 to 1000 μ g/L was prepared through serial dilution of a stock OCP mixed standard solution using isooctane.

The procedure of sample extraction and clean-up was conducted using the QuEChERS method based on a notice set by the Taiwan authority.³⁾ Briefly, 2.0 g of CHM powder was weighed into 50 mL centrifuge tubes, and 10 mL of precooling deionized water was added, followed by standing for 20 min. Ten mL of acetonitrile containing 1% (v/v) acetic acid, a ceramic homogenizer, 4 g of anhydrous magnesium sulfate, and 2 g of anhydrous sodium acetate was added sequentially. The tube was closed and shaken vigorously for 1 min. After centrifugation at 3000 $\times g$ for 5 min, 6 mL of supernatant was added into a commercial clean-up centrifuge tube containing 900 mg of anhydrous magnesium sulfate, 450 mg of primary-secondary amine, 300 mg of C18, and 50 mg of graphite carbon black (Qu-5CLIII, TWFDA5, Taipei, Taiwan). The tube was closed and shaken vigorously for 1 min. After centrifugation at 3000 $\times g$ for 5 min, 1 mL of supernatant was dried using a pressurized nitrogen blowing concentrator (ChromTech, SC-2800D, Taipei, Taiwan) at 40°C and dissolved in the mixture of acetone and hexane at

the ratio of 1:1, followed by passage through a 0.22 μ m filter.

An Agilent GC 6890 equipped with a 63Ni ECD system and 7683 auto-sampler/injector was used (Santa Clara, CA, USA). 5N5 nitrogen was used as a carrier gas. A fused-silica capillary column, DB-1701 (30 m $\times$ 530 μ m $\times$ 1.0 μ m nominal), was used for separation. The initial column oven temperature was 150°C; it was then ramped up at 2.5°C per min to 270°C and kept for 15 min. The total run time was 63.0 min at a constant flow rate of 1 mL/min. The inlet temperature, detector temperature, and injection volume were 205°C, 280°C, and 1 μ L, respectively.

The linearity for 11 OCPs in the working standard solution of five concentration levels, from 10 to 1000 μ g/L, varied from 0.9990 to 0.9999. The calibration curves were sufficient for the determination of 11 OCPs. For accuracy, free-OCP CHM samples were spiked within a range of 0.3–2.0 mg/kg (three levels and three samples for each level). The calculated recovery rates were between 82.3% and 91.9% (86.7% on average), and the relative standard deviations (RSDs) were below 10% (6.12% on average). For precision, free-OCP CHM samples were spiked with 0.5 mg/kg of 11 OCPs, and the determination was repeated six times. The calculated RSDs were below 10% (4.89% on average). The limits of quantitation were between 3.2 and 9.9 ng/g based on a signal to noise of 10:1.

Results and discussion

The present study investigated 11 types of OCP residues in 1,665 samples for 37 types of CHM using the QuEChERS method coupled with GC-ECD. The typical gas chromatogram for OCPs in a mixed standard solution is illustrated in Fig. 1. The calculated CR and the average detected values for OCPs in CHM samples are shown in Table S1 and Table S2, respectively. To truly reflect the circumstances of OCP contamination, the average detected value was calculated based on the OCP-contaminated CHM samples (Table S2). According to the monitoring data obtained from more than 10 years of routine inspections, the calculated CR for 11 types of OCPs in most CHMs was below 20% (Table S1). CRs over 20% could be found in *Ginseng radix* for α -BHC, δ -BHC, PCNB, and MPCPS; in *Panacis quinquefo-*

Fig. 1. A typical gas chromatogram of a standard solution containing a mixture of 11 types of organochlorine pesticides.

Fig. 2. The calculated contaminated rate (CR) for 11 types of organochlorine pesticides (OCs), *i.e.*, BHC isomers (α -BHC, γ -BHC, β -BHC, and δ -BHC), DDT analogs (p,p' -DDE, o,p' -DDT, p,p' -DDD, and p,p' -DDT), and PCNB and its metabolites (PCA and MPCPS), in Chinese herbal medicines. The CR was calculated as the number of contaminated samples divided by the number of its corresponding total samples multiplied by 100%. Abbreviations (alphabetically): ATRMR, *Atractylodis macrocephalae* rhizome; GINR, *Ginseng radix*; PANQR, *Panacis quinquefolii* radix; POLR, *Polygalae radix*; SENF, *Sennae folium*.

lii radix for BHC isomers, DDT analogs, PCNB, and MPCPS; in *Sennae folium* for γ -BHC; in *Polygalae radix* for p,p' -DDE; and in *Atractylodis macrocephalae* rhizome for PCNB (Fig. 2).

As indicated in Table 1, we summarized the diverse MRLs for OCP residues in CHM set by different Asian pharmacopeias. The Taiwan authority established the MRLs only for total BHC, total DDT, and total PCNB in 16 types of CHM (Table 1) and incorporated them into the THP. In addition to these regulated CHMs, the MRLs for these OCPs in another 21 types of com-

monly used CHMs were set by law as 0.9, 1.0, and 1.0 mg/kg, respectively. Only four types of CHMs, *i.e.*, *Ginseng radix*, *Panacis quinquefolii* radix, *Astragali radix*, and *Glycyrrhizae radix et rhizome*, were included in the ChP and the MRLs only set for PCNB, heptachlor, and chlordane (Table 1). The JP only established MRLs of 0.2 mg/kg for the total BHC and total DDT in 14 types of CHMs (Table 1). By contrast, the HKCMMS and the KP formulated the MRLs for 20 and 11 types of OCP residues, respectively, as the general rule in listed CHMs derived from plants (Table 1). For medication use safety, the MRLs set by the HKCMMS provided stricter requirements for OCP residues in CHMs.

The calculated ERs in most types of CHMs, except *Ginseng radix* and *Panacis quinquefolii* radix, were below 10% under the diverse MRLs for total BHC, total DDT, total PCNB, and PCNB alone set by different Asian pharmacopeias (Fig. 3). Previous studies from China surveyed BHC isomers, DDT analogs, and PCNB residues in *Ginseng radix*, indicating that the calculated CRs were over 80% for BHC isomers and PCNB, and the calculated ERs were over 80% and 50% under MRLs of 0.1 mg/kg for total BHC and PCNB, respectively.⁴⁻⁶ However, the sample sizes in these surveys were relatively small. In 22 types of OCP residues in 186 batches of *Ginseng radix* samples, more attention should be paid to PCNB contamination, with a calculated CR of 52%.⁷ Our findings in 132 batches of *Ginseng radix* samples revealed that the calculated CRs were 21%, 27%, 61%, and 31% for α -BHC, δ -BHC, PCNB, and MPCPS, respectively (Fig. 2), and the calculated ER was 26% for PCNB under an MRL of 0.1 mg/kg (Fig. 3). Concisely, PCNB contamination in *Ginseng radix* is a

Table 1. The maximum residue limits (MRLs) for organochlorine pesticide (OCP) residues in Chinese herbal medicines (CHMs) set by different Asian pharmacopeias

OCPs	MRL (mg/kg)						
	THP	ChP	HKCMMS	JP	KP		
Total BHC ^{a)}	0.9 ^{e)}	0.9 ^{g)}	0.2 ^{h)}	—	α -+ β -+ δ -0.3 γ -0.6	0.2 ^{h)}	0.2
Total DDT ^{b)}	1.0 ^{e)}	0.2 ^{g)}	0.2 ^{h)}	—	1.0	0.2 ^{h)}	0.1
PCNB	1.0 ^{e,f)}	1.0 ^{f,g)}	—	0.1 ^{i,j)}	1.0 ^{f)}	—	—
Heptachlor ^{c)}	—	—	0.05 ⁱ⁾	0.05	—	—	—
Chlordane ^{d)}	—	—	0.1 ⁱ⁾	0.05	—	—	—
Aldrin	—	—	—	0.05 ^{k)}	—	—	0.01
Dieldrin	—	—	—	—	—	—	0.01
Endrin	—	—	—	0.05	—	—	0.01
Hexachlorobenzene	—	—	—	0.1	—	—	—

^{a)} Total DDT is the sum of p,p' -DDD, p,p' -DDE, o,p' -DDT, and p,p' -DDT. ^{b)} Total BHC is the sum of α , β , γ , and δ -BHC. ^{c)} Heptachlor is the sum of heptachlor and heptachlor epoxide. ^{d)} Chlordane is the sum of *cis*-chlordane, *trans*-chlordane, and oxychlordane. ^{e)} This MRL applies to *Astragali radix*, *Hedysari radix*, *Glycyrrhizae radix et rhizoma*, *Ginseng radix*, and *Panacis quinquefolii* radix. ^{f)} This MRL is the sum of pentachloronitrobenzene (PCNB), pentachloroaniline (PCA), and methyl pentachlorophenyl sulfide (MPCPS). ^{g)} This MRL applies to *Sennae folium*. ^{h)} This MRL applies to *Moutan radialis cortex*, *Polygalae radix*, *Asari radix et rhizoma*, *Eriobotryae folium*, *Cinnamomi cortex*, *Cinnamomi ramulus*, *Jujubae fructus*, *Corni Sarcocarpium*, *Citri reticulatae pericarpium*, and *Perillae folium*. ⁱ⁾ This MRL is applies to *Ginseng radix* and *Panacis quinquefolii* radix. ^{j)} This MRL is applies to *Astragali radix* and *Glycyrrhizae radix et rhizome*. ^{k)} This MRL is the sum of Aldrin and Dieldrin. ^{l)} This MRL applies to *Asiasari radix*, *Astragali radix*, *Cinnamomi cortex*, *Citri reticulatae pericarpium*, *Corni fructus*, *Ginseng radix*, *Glycyrrhizae radix et rhizoma*, *Zizyphi fructus*, *Eriobotryae folium*, *Moutan cortex*, *Perillae herba*, *Polygalae radix*, *Ginseng radix rubra*, and *Sennae folium*. Abbreviations: THP, the Taiwan Herbal Pharmacopeia; ChP, the Pharmacopoeia of the People's Republic of China; HKCMMS, the Hong Kong Chinese Materia Medica Standards; JP, the Japan Pharmacopeia; KP, the Korean Pharmacopeia.

Fig. 3. The calculated exceeding rate (ER) for organochlorine pesticides (OCPs) in *Ginseng radix* and *Panacis quinquefolii radix* under the diverse maximum residue levels (MRLs) set by Asian pharmacopeias. The ER was calculated as the number of samples that exceeded the MRLs divided by the number of its corresponding total samples multiplied by 100%. Abbreviations (alphabetically): ChP, the Pharmacopeia of the People's Republic of China; HKCMMS, the Hong Kong Chinese Materia Medica Standards; JP, the Japanese Pharmacopeia; KP, the Korean Pharmacopeia; THP, the Taiwan Herbal Pharmacopeia.

matter of concern.

Regarding OCP pollution in *Panacis quinquefolii radix*, several previous surveys indicated that calculated CRs over 50% could be observed in BHC isomers,^{5,6,8,9} DDT analogs,^{8,9} PCNB,^{5,6,8,9} MPCPS,⁹ PCA,⁹ dieldrin,⁹ heptachlor,⁹ heptachlor epoxide,⁹ and hexachlorobenzene.⁸ In addition, calculated ERs over 50% could be found in total BHC,^{5,6,9} total DDT,⁹ and PCNB^{5,8,9} under MRLs of 0.9, 1.0, and 1.0 mg/kg, respectively, set by THP. Consistently, the present findings found that the calculated CRs were 20% for BHC isomers, 35% for DDT analogs, 52% for PCNB, and 40% for MPCPS in 25 batches of

Panacis quinquefolii radix samples (Fig. 2). In addition, the calculated ERs were 44% and 56% under MRLs of 0.2 and 0.1 mg/kg, respectively, for total DDT as well as 36% with an MRL of 0.1 mg/kg for PCNB alone (Fig. 3). In brief, the DDT analogs and PCNB contamination in *Panacis quinquefolii radix* are of concern. The medicinal part of *Ginseng radix* and *Panacis quinquefolii radix* are the root belonging to perennials that easily absorbed and accumulated OCPs from the soil. Bioaccumulation, one of the features of OCP, is the accumulation over time in the food chain; it plays a critical role in determining the extent of environmental pollution.^{10,11)}

Fig. 4. Gas chromatogram and quantitative analysis of 11 types of organochlorine pesticides (OCPs) in different parts of *Panax ginseng*: (A) ginseng leaf, (B) ginseng stem, (C) ginseng root, and (D) ginseng fibrous root. Data was expressed as the means $\pm$ S.D. from three independent experiments.

The massive sample size in this study was sufficient to be representative of OCP-contaminated CHMs. In addition, the calculated ERs based on the diverse MRLs set by different Asian pharmacopeias could demonstrate the circumstance of OCP contamination in CHMs. However, only 11 types of OCPs were found in CHMs during our past routine examination due to regulations of the Taiwan authority.

Eleven types of OCP residues in the different parts of four-year-old *Panax ginseng*, including the leaf, stem, root, and fibrous root, were also compared. According to the Chinese National Standard, the MRLs for OCPs in different parts of *Panax ginseng* were set as 0.1 mg/kg for total BHC, total DDT, and PCNB; 0.02 mg/kg for heptachlor and the sum of aldrin and dieldrin; and 0.2 mg/kg for cypermethrin.¹²⁾ The typical gas chromatogram and quantitative analysis for OCPs in different parts of *Panax ginseng* are illustrated in Fig. 4. The results revealed that the total BHC residue in the leaf and fibrous root as well as the total DDT and PCNB residue in all parts of *Panax ginseng* exceeded the MRL of 0.1 mg/kg (Fig. 4). Notably, PCNB residues in the leaf and stem were extremely high, with detected values of 4.06 and 8.16 mg/kg, respectively (Fig. 4). Furthermore, PCA and MPCPS residue in the leaf and stem should be noticed due to the detected value at around 2 mg/kg (Fig. 4). To the best of our knowledge, this is the first report to compare the OCP residues in different parts of *Panax ginseng*, although the sample size is small.

In summary, the present study concluded that more attention should be paid to the PCNB contamination in *Ginseng radix* as well as DDT analogs and PCNB contamination in *Panacis quinquefolii radix*. In addition, OCP contamination in the leaf and stem of *Panax ginseng* is a matter of concern. We hope that these findings will raise awareness of OCP contamination in certain CHMs that could impact the safety of medications, affecting farmers, manufacturers, and consumers.

Declaration of Interest Statement

All authors listed in this study are from our company, and the results obtained from the present study were part of the quality control process in our company. Therefore, we declare that there is no conflict of interest.

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Electronic supplementary materials

The online version of this article contains supplementary materials (Supplemental Tables S1 and S2), which are available at <https://www.jstage.jst.go.jp/browse/jpestics/>.

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