Simultaneous Determination of 13 Synthetic Pyrethroids and Their Metabolite, 3-Phenoxybenzoic Acid, in Tea by Gas Chromatography

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Thirteen synthetic pyrethroid pesticides and their ester cleavage metabolite, 3-phenoxybenzoic acid (PBA), were simultaneously determined. Analytes in tea leaves were extracted with acetone and *n*-hexane. PBA was esterified by the addition of hexafluoroisopropyl alcohol and diisopropylcarbodiimide. The extract was cleaned up using a Florisil column. PBA derivative and pyrethroids other than ethofenprox were determined by ECD-GC, and ethofenprox was determined by GC/MS-(SIM). GC/MS was used to identify the pyrethroids and PBA detected. The recovery of fortified tea leaves, infusion, and leaves after steeping ranged from 80 to 101% at a fortification level of 0.5 or 1.0 μg/g for the 13 pyrethroids. For PBA, recovery was 73–76% at 0.05 μg/g fortification and 80–84% at 0.5 μg/g. Fifty tea samples were analyzed, and several pyrethroids were determined; however, no PBA could be detected. Pyrethroid-containing tea leaves gave 0.5–10.2% of PBA but no pyrethroid in their infusion with hot water.

Keywords: *Pyrethroid; metabolite; tea; gas chromatography*

INTRODUCTION

Pyrethroids comprise an important group of insecticides and are widely used in many countries (Elliott, 1989). However, no systematic analytical method for pyrethroid determination is available. Recently, the authors reported such a method (Nakamura et al., 1993), in which 12 synthetic pyrethroids and natural pyrethrins were simultaneously determined by gas chromatography with electron capture detector (ECD-GC). The structural features of 12 pyrethroids and ethofenprox are listed in Table 1.

Most of the synthetic pyrethroids have an α-cyano-3-phenoxybenzoxy or a 3-phenoxybenzoxy group as the alcohol moiety and produce 3-phenoxybenzoic acid (PBA, shown in Figure 1) as the ester cleavage metabolite (Demoute, 1989). This indicates the possibility of monitoring a wide range of pyrethroids by the detection of only one compound, PBA. To confirm such a possibility, a method for determining 13 pyrethroids and PBA simultaneously was devised and used to analyze 50 tea samples. Tea leaves were used since some pyrethroids could be detected only in them in the previous study. Analyses of infusion from the tea leaves and tea leaves after steeping were also conducted.

PBA has been determined by Fitch et al. (1988) using GC/MS(SIM) after methylation and by Mortimer (1990) using ECD-GC after esterification with hexafluoroisopropyl alcohol. In the present study, the method of Mortimer was used because of its convenience and high sensitivity. The reaction involved is shown in Figure 1. A systematic analysis of PBA and synthetic pyrethroids has been enabled and adopted for food for the first time.

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EXPERIMENTAL PROCEDURES

Tea Leaves and Infusion. Domestic tea leaves were purchased from markets in Osaka, and foreign ones were obtained at Kobe Port by quarantine officers.

Four grams of neat tea leaves was infused with 240 mL of distilled and boiled water for 5 min, followed by filtration through a 420 μm stainless steel filter. The filtrate was cooled in an ice bath (infusion from the tea leaves). The wet tea leaves remaining in the filter were drained by hand-shaking (tea leaves after steeping).

Neat tea leaves were stored in a sealed container with silica gel until extraction for analyses. The infusion from the tea leaves and the tea leaves after steeping were extracted immediately after infusion.

Apparatus. The homogenizer was an Excel autohomogenizer, a product of Nihon Seiki Kaisha Ltd. The rotary evaporator was an RE 111 Rotavapor (Buchi, Shibata Kagaku Kikai Kogyo) equipped with a water bath and a vacuum pump (Model JS-75A, Advantec). The water bath was set at a temperature of 35–40 °C.

Analytical Standards and Working Solutions. Analytical standards of 12 pyrethroids were the same ones as used in the prior study (Nakamura et al., 1993). Ethofenprox was a product of Wako Pure Chemicals Co., and 3-phenoxybenzoic acid was a product of Aldrich Chemical Co.

The analytical standards were dissolved in acetone, mixed, and diluted to make the following working solutions: A, mixture of 10 μg of ethophenprox/mL, 10 μg of flucythrinate/mL, and 10 μg of fluvalinate/mL; B, mixture of 20 μg of permethrin/mL, 10 μg of cypermethrin/mL, 10 μg of fenvalerate/mL, and 20 μg of tralomethrin/mL; C, mixture of 10 μg of allethrin/mL, 20 μg of tetramethrin/mL, 20 μg of fenpropathrin/mL, 10 μg of cyhalothrin/mL, 20 μg of cyfluthrin/mL, and 20 μg of deltamethrin/mL; D, 1 μg of PBA/mL; E, 10 μg of PBA/mL.

Reagents. The coagulating reagent (Nakamura et al., 1993) was prepared by dissolving 5 g of ammonium chloride (NH_4Cl) and 10 mL of phosphoric acid (H_3PO_4) in 400 mL of distilled water. Celite 545 was a product of Johns-Manville Sales Corp. Hexafluoroisopropyl alcohol (HFIP) was a product of GL Sciences, and diisopropylcarbodiimide (DIC) was a product of Aldrich. The Florisil column was prepared by packing 10 g of Florisil PR (60–100 mesh, Floridin Co.) as a slurry in a glass column (1.5 cm i.d. × 30 cm) and 10 g of anhydrous sodium sulfate (Na_2SO_4) on it using *n*-hexane.

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Table 1. List of Pyrethroids Studied

common name	abbr[a]	chemical name	production of PBA[b]
allethrin	All	(*RS*)-3-allyl-2-methyl-4-oxocyclopent-2-enyl (1*RS*)-*cis*,*trans*-2,2-dimethyl-3-(2-methylprop-1-enyl)cyclopropanecarboxylate	no
cyfluthrin	Cyf	(*RS*)-α-cyano-4-fluoro-3-phenoxybenzyl (1*RS*,3*RS*), (1*RS*,3*SR*)-3-(2,2-dichlorovinyl)-2,2-dimethyl cyclopropanecarboxylate	no
cyhalothrin	Cyh	(*RS*)-α-cyano-3-phenoxybenzyl (*Z*),(1*RS*,3*RS*)-3-(2-chloro-3,3,3-trifluoropropenyl)-2,2-dimethyl cyclopropanecarboxylate	yes
cypermethrin	Cyp	(*RS*)-α-cyano-3-phenoxybenzyl (1*RS*,3*RS*), (1*RS*,3*SR*)-3-(2,2-dichlorovinyl)-2,2-dimethyl-cyclopropanecarboxylate	yes
deltamethrin	Del	(*S*)-α-cyano-3-phenoxybenzyl (1*R*,3*R*)-3-(2-dibromovinyl)-2,2-dimethylcyclopropanecarboxylate	yes
ethofenprox	Eth	2-(4-ethoxyphenyl)-2-methylpropyl 3-phenoxybenzyl ether	yes
fenpropathrin	Fep	(*RS*)-α-cyano-3-phenoxybenzyl 2,2,3,3-tetramethylcyclopropanecarboxylate	yes
fenvalerate	Fev	(*RS*)-α-cyano-phenoxybenzyl (*RS*)-α-isopropyl-4-chlorophenylacetate	yes
flucythrinate	Flc	(*RS*)-α-cyano-3-phenoxybenzyl (*S*)-2-(4-difluoromethoxyphenyl)-3-methylbutyrate	yes
fluvalinate	Flv	(*RS*)-α-cyano-3-phenoxybenzyl *N*-(2-chloro-α,α,α-trifluoro-*p*-tolyl)-D-valinate	yes
permethrin	Per	3-phenoxybenzyl (1*RS*) *cis*,*trans*-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate	yes
tetramethrin	Tet	cyclohex-1-ene-1,2-dicarboximidomethyl (1*RS*)-*cis*,*trans*-2,2-dimethyl-3-(2-methylprop-1-enyl)cyclopropanecarboxylate	no
tralomethrin	Tra	(*S*)-α-cyano-3-phenoxybenzyl (1*R*,3*S*)-2,2-dimethyl-3-(1,2,2,2-tetrabromoethyl)cyclo-propanecarboxylate	yes

[a] Abbreviation in the present study. [b] Possibility for production of PBA as an ester cleavage metabolite.

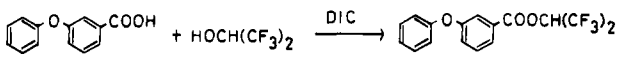

Figure 1. Reaction of 3-phenoxybenzoic acid with hexafluoroisopropyl alcohol.

Florisil was activated at 130 °C for 12 h prior to use. Acetone, *n*-hexane, diethyl ether, and ethyl acetate were of special grade for pesticide analysis. Sodium chloride (NaCl), potassium carbonate (K_2CO_3), tin acetate [$Pb(CH_3COO)_2$], NH_4Cl, H_3PO_4, and Na_2SO_4 were of analytical grade. Filter paper was ashless No. 5A and No. 5C (Advantec Toyo Co., Ltd.).

Extraction and Cleanup. *(a) Neat Tea Leaves.* Ten grams of neat tea leaves was homogenized with 100 mL of acetone twice, followed by filtration using the No. 5A filter paper. The filtrate was evaporated to less than 50 mL. To the residue was added acetone to make up to 50 mL, and 50 mL of coagulating reagent and 5 g of Celite 545 were added. After 30 min, the mixture was filtered using the No. 5C filter paper, and the residue was washed with 50 mL of coagulating reagent and acetone mixture (1:1). To the filtrate was added 100 mL of 10% (w/v) NaCl solution, and the mixture was extracted with 100 mL of *n*-hexane three times. The organic layer was combined and dehydrated by addition of anhydrous Na_2SO_4 and evaporated to dryness. The residue was dissolved in 5 mL of *n*-hexane followed by addition of 10 μL of hexafluoroisopropyl alcohol and 15 μL of diisopropylcarbodiimide to produce hexafluoroisopropyl 3-phenoxybenzoate (PBA-HFIP). HFIP and DIC were added directly, not in any solvent. Because of their toxicity, they were handled using disposable gloves and a ventilation system. After 1 min, 5 mL of 5% (w/v) K_2CO_3 was added and the organic layer was transferred to a Florisil column. Impurities from tea leaves were washed away by 50 mL of *n*-hexane, and the esterified PBA was eluted by 50 mL of 2% (v/v) diethyl ether in *n*-hexane. Then pyrethroids were eluted by 50 mL of 30% (v/v) ethyl acetate in *n*-hexane. Each eluate was evaporated and reconstituted in 5 mL of *n*-hexane for GC analysis.

(b) Infusion from Tea Leaves. Infusion from 4 g of neat tea leaves was treated with 100 mL of acetone, 2 mL of saturated tin acetate solution, and 5 g of Celite 545 followed by filtration using the No. 5C filter paper. The residue was washed with 50 mL of acetone and distilled water mixture (1:1), and to the filtrate were added 20 g of NaCl and 2 mL of H_3PO_4. Then the analytes of interest were extracted three times with 100 mL of *n*-hexane. The combined organic layer was treated as in the case of the neat tea leaves, except that the final volume of the test solution was 2 mL.

(c) Tea Leaves after Steeping. Tea leaves after steeping of 4 g of neat tea leaves were treated as neat tea leaves. The final volume of the test solution was 2 mL.

Detection and Quantification. All analytes of interest were detected by gas chromatography. Quantification of them was accomplished using standard curves prepared by diluting the working solutions with *n*-hexane (from 0.1 or 0.2 to 1 or 2 μg/mL for pyrethroids and from 0.01 to 1 μg/mL for PBA). The working solutions for PBA were esterified with HFIP and DIC as the treatment for the sample extract prior to GC analyses.

(a) Detection of Pyrethroids Other than Ethofenprox. The gas chromatograph with an electron capture detector (ECD-GC) was a Yanaco G-2800 equipped with a methyl silicone coated fused-silica capillary column CBP-1 (0.2 mm i.d. × 25 m, 0.25 μm film thickness, Shimadzu Corp.). The oven temperature was 250 °C. Injection volume was 2 μL. For more detailed conditions, see the previous paper (Nakamura et al., 1993). Some pyrethroids gave double or quadruple peaks on the chromatogram originating from the isomers. The sum of peak areas of the isomers was used for calculation of the concentration of these pyrethroids.

(b) Detection of Ethofenprox. A gas chromatograph with a mass spectrometer (GC/MS) was used in selected ion monitoring (SIM) mode. The gas chromatograph with a mass spectrometer was a JEOL JMS DX-302 equipped with a capillary column CBP-5 (0.2 mm i.d. × 25 m, 0.25 μm film thickness, Shimadzu). The oven temperature was held at 60 °C for the initial 2 min and then ramped at 32 °C/min to 250 °C. Injection port temperature was 250 °C. The carrier gas was helium at 1 mL/min. The monitored ions were *m/z* 163 and 135 in electron impact mode. The injection volume was 2 μL. For more detailed conditions, see the previous paper (Nakamura et al., 1993).

(c) Detection of PBA-HFIP. ECD-GC was used. All conditions were the same as for the analysis of pyrethroids except that the oven temperature was 160 °C.

(d) Confirmation of Pyrethroids and PBA-HFIP. In case some peaks of interest were detected in ECD-GC or GC/MS-(SIM) analysis, the mass spectra of them were compared with those of the standards for confirmation. The sample extract was evaporated to dryness followed by addition of 100 μL of *n*-hexane. Two microliters of that was injected to GC/MS. The conditions were almost the same as for detection of ethofenprox except that the scan range was *m/z* 50−700 and the scan cycle was 1 s. The retention time (RT) and mass spectral character were as follows: ethofenprox, RT 28 min 3 s, highest ion *m/z* 376 ($[M]^+$), strongest ion *m/z* 163; PBA-HFIP, RT 7 min 58 s, highest ion *m/z* 364 ($[M]^+$), strongest ion *m/z* 364, second strongest ion *m/z* 197 ($[M - OCH(CF_3)_2]^+$); fenpropathrin, RT 15 min 34 s. The relative retention times of the other pyrethroids to that of fenpropathrin are shown in the previous paper; their mass spectra are also in that paper.

Recovery. Recoveries were determined in triplicate for tea samples that were checked for the absence of any pyrethroid or PBA. The fortification levels were 0.5 μg/g for allethrin,

Table 2. Recoveries (Percent)[a] of Pyrethroids and PBA in Neat Tea Leaves, the Infusion from Tea Leaves, and the Tea Leaves after Steeping

	compd added, μg/g														
	All, 0.5	Cyf, 1.0	Cyh, 0.5	Cyp, 0.5	Del, 1.0	Eth, 0.5	Fep, 1.0	Fev, 0.5	Flc, 0.5	Flv, 0.5	Per, 1.0	Tet, 1.0	Tra, 1.0	PBA 0.05	PBA 0.5
neat tea leaves	98	98	90	84	86	96	84	95	92	94	88	92	96	76	84
infusion from tea leaves[b]	99	94	86	89	84	93	86	93	85	91	80	90	88	73	80
leaves after steeping[b]	101	93	88	86	85	91	85	91	88	92	81	91	92	75	81

[a] Average of three trials. [b] Fortification levels were calculated on the basis of the weight of neat tea leaves before steeping.

cyhalothrin, cypermethrin, ethofenprox, fenvalerate, flucythrinate, fluvalinate, and PBA and 1 μg/g for cyfluthrin, deltamethrin, fenpropathrin, permethrin, tetramethrin, and tralomethrin. The lower fortification level (0.05 μg/g) was tried for PBA. Working solutions of A–E were added to each test portion 2 h before analysis. Pyrethroids were divided into three groups because some of their peaks overlapped on the ECD gas chromatogram when injected together.

RESULTS AND DISCUSSION

Extraction and Cleanup. Colored materials from tea leaves were precipitated by addition of coagulating reagent, adsorbed by Celite, and eliminated by filtration with filter paper.

PBA is more soluble in water than pyrethroids, and its extraction by *n*-hexane is difficult. The first, second, and third extractions were 61, 16, and 5%, respectively. Three extractions are thus necessary.

An emulsion made the extraction of PBA from tea infusion very difficult, possibly due to tannin in tea. Tin acetate was used to solve this problem (Onoda and Imamura, 1980). An emulsion was sometimes produced even with tin acetate, and thus 100 mL of acetone was applied to the infusion. When the second or third extraction was hindered by an emulsion, 1–5 mL of acetone was added with good result.

Reaction of PBA. One minute was sufficient to complete the reaction of PBA with HFIP at room temperature. A longer reaction time (up to 3 h) and higher temperature (50 °C) did not increase recovery of the ester. Organic solvents other than *n*-hexane prevented the reaction. Acetone, methanol, and ethyl acetate inhibited production of the ester. Complete evaporation of these solvents from the residue samples prior to the derivatization was thus necessary for satisfactory results. Dehydration with anhydrous Na_2SO_4 was required since DIC is deactivated by even a small amount of water.

Detection and Quantification. Ethofenprox was separately determined by GC/MS(SIM) from the other pyrethroids, having neither a halogen nor a cyclopropane ring and thus being undetectable by ECD. Carotene from tea leaves showed a retention time virtually the same as that of ethofenprox and hindered determination when CBP-1 was used as the capillary column. CBP-5 gave better resolution of carotene and ethofenprox and thus was used in place of CBP-1.

Recovery Test. Recoveries of pyrethroids and PBA after fortification of neat tea leaves, infusion from the tea leaves, and tea leaves after steeping are shown in Table 2. The recoveries for pyrethroids exceeded 80% in all cases; the recovery for PBA at low fortification was lower, possibly due to the water solubility of PBA. CV of the recovery for each pyrethroid and PBA was within 10% (data not shown). Typical gas chromatograms for PBA-HFIP and ethofenprox are shown in Figures 2 and 3, respectively. Chromatograms of the pyrethroids other than ethofenprox appear in our previous paper. Detection limits were 0.005–0.1 μg/g for the pyrethroids and 0.005 μg/g for PBA.

Figure 2. Typical chromatograms of PBA-HFIP by ECD-GC: (upper) PBA-HFIP derived from 0.5 μg/mL of PBA (the arrow indicates the peak of PBA-HFIP); (lower) extract of tea leaves (the broken line indicates the retention time of PBA-HFIP).

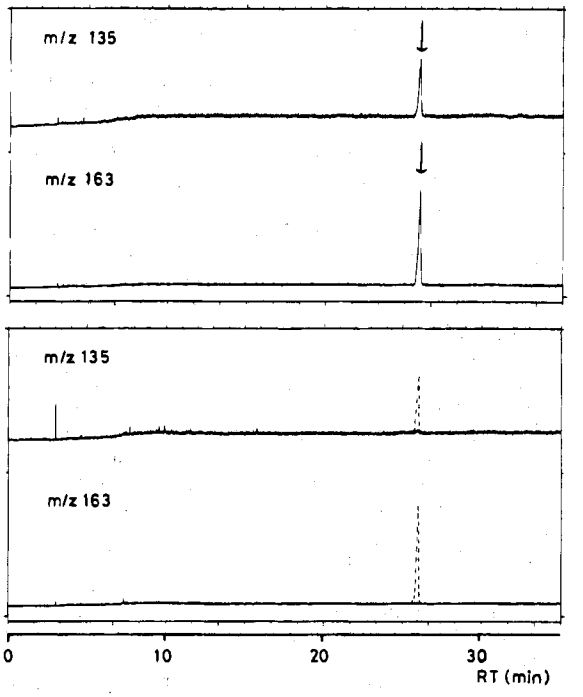

Figure 3. Typical chromatograms of ethofenprox by GC/MS(SIM): (upper) ethofenprox 0.5 μg/mL (the arrows indicate the peaks of ethofenprox); (lower) extract of tea leaves (the broken lines indicate the retention time of ethofenprox).

Detection of Pyrethroids and PBA from Samples. Fifty tea samples of domestic or foreign origin were analyzed for 13 pyrethroids and PBA. The results are shown in Table 3. Ethofenprox, fenpropathrin, and

Table 3. Number of Tea Samples in Which Pyrethroids Were Detected[a]

country	class	no.[b]	pyrethroid detected[c] Eth	Fep	Fev	Flc	Flv	Per
Japan	green tea	28	3 (0.09–0.27)	13 (0.02–0.60)	1 (0.25)	0	21 (0.02–0.61)	0
	roasted tea	3	0	0	0	0	0	0
	Genmaicha[d]	4	0	0	0	0	0	0
	powdered tea	2	0	1 (1.25)	0	0	1 (0.17)	0
Formosa	green tea	2	0	0	0	0	0	1 (1.36)
	oolong tea	2	0	0	1 (1.64)	1 (0.45)	0	1 (1.21)
	po-chan tea	1	0	1 (0.05)	0	0	0	0
	black tea	1	0	0	0	0	0	0
	jasmin tea	1	0	0	0	0	0	1 (0.21)
China	oolong tea	3	0	0	0	0	0	0
	pual tea	2	0	0	0	0	0	0
Srilanka	black tea	1	0	0	0	0	0	0
total		50	3	15	2	1	22	3

[a] All, Cyf, Cyh, Cyp, Del, Tet, Tra, and PBA were not detected in any sample. [b] The total number of samples analyzed. [c] Range of pesticide levels (μg/g) is shown in parentheses. [d] A kind of green tea with roasted rice.

Table 4. Pyrethroids and PBA Detected in Neat Tea Leaves, the Infusion from Tea Leaves, and the Tea Leaves after Steeping

sample	pesticide	neat leaves concn (μg/g)	infusion pesticide[a] (%)	infusion PBA[b] (%)	tea leaves after steeping pesticide[a] (%)	tea leaves after steeping PBA[b] (%)
A	Flv	0.55	ND[c]	3.4	95.6	ND
B	Fep	0.48	ND	10.2	89.7	ND
C	Per	1.36	ND	0.5	85.4	ND
D	Fev	1.64	ND		103.8	
	Flc	0.45	ND	8.1	90.1	tr
	Per	1.21	ND		80.0	

[a] The ratio of pesticide detected after steeping of tea leaves. [b] The ratio of pesticides converted into PBA after steeping of tea leaves. [c] ND, not detected.

fluvalinate were frequently detected in Japanese tea leaves, while permethrin was detected only in Formosan samples. Pyrethroid detection in all cases was confirmed by GC/MS. Although more permethrin, fenvalerate, and fenpropathrin were detected in some samples, no PBA could be found. The possibility of monitoring pyrethroids by PBA determination is thus ruled out for tea leaves, demonstrating the stability of pyrethroids in dry tea leaves or rapid metabolism of PBA into lower molecules.

Effects of Infusion. Pyrethroid-containing tea leaves were infused by hot water; infusion from the tea leaves and tea leaves after steeping were analyzed for pyrethroids and PBA. Table 4 shows their ratios. It is of interest that the infusion contained no pyrethroid but 0.5–10.2% PBA. Tea leaves after steeping continued to retain their pyrethroids but no PBA. Pesticides in tea leaves are known to be partitioned between infusion to residues, the extent depending on water solubility (Zongmao and Haibin, 1988; Zimmerli and Blaser, 1982; Wan et al., 1991). Pyrethroids are less water soluble and remain in the leaves, though the degradation product PBA has been shown to be completely infused. The other moiety of pyrethroids, the acid part, may thus possibly be infused in tea for drinking. The acid moiety of pyrethroids often contains halogens, thus posing more hazards to human health than PBA. This matter is presently being studied.

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LITERATURE CITED

Demoute, J. P. A brief review of the environmental fate and metabolism of pyrethroids. *Pestic. Sci.* **1989**, *27*, 375–385.

Elliott, M. The pyrethroids: early discovery, recent advances and the future. *Pestic. Sci.* **1989**, *27*, 337–351.

Fitch, W. L.; Sjolander, A. C.; Miller, W. W. Determination of fluvalinate metabolite residues in cottonseed, apples, tomatoes, and soil. *J. Agric. Food Chem.* **1988**, *36*, 764–766.

Mortimer, R. D. Rapid method for esterification of trace levels of carboxylic acids for analysis by gas chromatography-electron-capture detection. *J. Chromatogr.* **1990**, *516*, 427–432.

Nakamura, Y.; Tonogai, Y.; Tsumura, Y.; Ito, Y. Analysis of pyrethroid residues in vegetables, fruits, grains, beans and green tea leaves. Applications to pyrethroid residue monitoring studies. *J. Assoc. Off. Anal. Chem.* **1993**, *76*, 1348–1361.

Onoda, Y.; Imamura, M. Utility of basic lead acetate treatment for analysis of pesticides residues in tea infusion. *J. Pestic. Sci.* **1980**, *5*, 101–105.

Wan, H.; Xia, H.; Chen, Z. Extraction of pesticide residues in tea by water during the infusion process. *Food Addit. Contam.* **1991**, *8*, 497–500.

Zimmerli, B.; Blaser, O. Preliminary study of the transfer of pesticides from tea leaves to the infusion. *Mitt. Geb. Lebensmittelunters. Hyg.* **1982**, *73*, 174–185.

Zongmao, C.; Haibin, W. Factors affecting residues of pesticides in tea. *Pestic. Sci.* **1988**, *23*, 109–118.

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