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ScienceDirect
Food Chemistry 104 (2007) 1315–1319
Food Chemistry
www.elsevier.com/locate/foodchem

Analytical, Nutritional and Clinical Methods

Determination of organochlorine pesticide residues in rice and human and fish fat by simplified two-dimensional gas chromatography

Shubing Chen [a], Lili Shi [b], Zhengjun Shan [b], Qiuhui Hu [a,*]

[a] *Key Laboratory of Food Processing and Quality Control, College of Food Science and Technology, Nanjing Agricultural University, No. 1 Weigang Road, Nanjing, Jiangsu 210095, PR China*

[b] *Institute of Environmental Science, National Environmental Protection, Administration of China, Nanjing 210042, PR China*

Received 11 May 2006; received in revised form 6 July 2006; accepted 16 October 2006

Abstract

The residue levels of four hexachlorocyclohexane (HCH) isomers (α-HCH, β-HCH, γ-HCH and δ-HCH), 4 dichloro-diphenyl-trichloroethane (DDT) congeners (*p*,*p*-DDE, *o*,*p*-DDT, *p*,*p*-DDD, and *p*,*p*-DDT), heptachlor, heptachlor epoxide, aldrin, dieldrin and endrin in rice and its bran from Jiangsu Province, PR China, were investigated by simplified two-dimensional gas chromatography, coupled with micro-electronic capture detector (μECD). Concentrations of organochlorine pesticides (OCPs) for ∑HCH ranged from 0 to 0.039 mg kg^{-1} in the rice and 0 to 0.057 mg kg^{-1} in its bran. For ∑DDT, the concentrations ranged from 0 to 0.053 mg kg^{-1} in the rice and 0 to 0.051 mg kg^{-1} in its bran. The five other OCPs, except HCH and DDT, were not detected. The major HCH isomers and DDT, congeners detected, both in the rice and its bran, were β-HCH and *p*,*p*′-DDE. Compared with the residue levels in the rice, the OCPs levels in fish and human fat were detected at higher residue levels. It is necessary to investigate the OCP residues in foodstuffs of the food chain in order to evaluate the potential health risk to humans.

Keywords: Rice; Fish fat; Human fat; Organochlorine pesticides; Residue

1. Introduction

Organochlorine pesticides (OCPs) have been widely used, globally, since the 1940s because of their strong effects in the control of pests and diseases. In China their consumption was actually more than 50% of total pesticides from the 1960s to 1983 when OCPs were banned for use in agriculture (Hua & Shan, 1996). Hexachlorocyclohexane (HCH) and dichloro-diphenyl-trichloroethane (DDT) were major components of OCPs used in China. The use of HCH and DDT accumulated up to 1983 were approximately 5 and 0.5 million tons, respectively (Cai, 1999).

Although the huge use of OCPs had made great economic profits in Chinese agriculture, it also caused serious environmental pollution in China. Some provinces in China, such as Jiangsu, Zhejiang and Shanghai, were the most severely contaminated areas by OCPs because of their long-term large-scale use. The OCPs in fish, meat, rice, vegetables and mothers' milk were found at high concentrations (Cai, Yang, & Wang, 1983; Zhang, Yang, Fang, & Wei, 1997; Li et al., 2003; Yu, Zhao, Zhang, Zhu, & Liu, 2001; Cao et al., 2000). For example, the HCH and DDT contents, both in soil and the aquatic organisms, in Tai Lake in Jiangsu Province were all at 10^{-6} mass grade in 1980 and the levels of HCH and DDT in the fat tissues of the residents there were at 32 and 29.4 mg kg^{-1}, respectively. The HCH and DDT contents in mother's milk there were 0.62 and 0.54 mg kg^{-1}, respectively.

China has one of the highest rice product consumptions in the world and rice is the staple food. It is of great interest to detect the organochlorine pesticide residues in rice and human and fish fats by simplified two-dimensional gas

* Corresponding author. Tel.: +86 25 84399086; fax: +86 25 84396431.
E-mail address: qiuhuihu@njau.edu.cn (Q. Hu).

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doi:10.1016/j.foodchem.2006.10.032

chromatography, even though the OCPs have been banned for use for two decades. The result of this investigation can provide new scientific evidence for the estimation of OCP residues in food in Jiangsu Province, China.

2. Materials and methods

2.1. Samples

Ninety-three samples of rice seed were collected from seven different cities (Nanjing, Suzhou, Wuxi, Zhenjiang, Yangzhou, Nantong, Huai'an) in Jiangsu Province, PR China. The rice seed was dried at room temperature. The polished rice was prepared by removing bran which was also kept for analysis. The samples of rice and brans were dried and powdered.

2.2. Fish fats

Live grass carps (two years old) from Wu Xi city, Jiangsu Province were collected in the supermarket. Fish fat was removed from the abdomen of the fish and kept at 4 °C for further use.

2.3. Human fats

The human fats were collected from the abdomen of 11 patients (five males and six females, ages from 15 to 82) living in Nanjing, Suzhou, Wuxi, Zhenjiang, Yangzhou, Nantong and Huai'an in Jiangsu Province, China, where rice was the staple food.

2.4. Standards and reagents

The standard stock solutions of OCPs, including α-HCH, β-HCH, γ-HCH, δ-HCH, *p*,*p*′-DDT, *p*,*p*′-DDD, *p*,*p*′-DDE, *o*,*p*′-DDT, heptachlor, heptachlor epoxide, aldrin, dieldrin and endrin, were purchased from National Research Center for Certified Reference Materials of China.

Ethyl acetate and petroleum ether were of analytical grade. Petroleum ether was redistilled before use.

The standard solutions were prepared from dilution of their stock standard solutions at concentrations of 0.01, 0.05, 0.10, 0.20, 0.25 mg l^{-1} with redistilled petroleum ether.

2.5. Apparatus

The simplified two-dimensional gas chromatography apparatus (Agilent 6890N) equipped with a 7683 series auto-sampler, Deans switch and a ^{63}Ni electron capture detector (μECD) was adopted in this investigation. It can transfer the interference fraction in the first chromatographic column to the second one for a further efficient separation with the heart cutting technique.

Chromatographic columns employed for the analysis were: DB-1701, 30 m and 0.32 mm internal diameter with 1 μm film thickness as pre-elution column and HP-5MS, 30 m and 0.32 mm internal diameter with 1 μm film thickness as the second elution column.

Envi-Florisil cartridges (1 g) for SPE were purchased from Supelco Company, USA.

An ultrasonic water bath (Kedao Co., Ltd, Shanghai, China) was used in the extraction. The generator of this ultrasonic bath has an output of 500 W and a frequency of 59 kHz.

2.6. Sample preparation

The samples or those spiked with the pesticides were placed in 100 ml flasks and redistilled petroleum ether (50 ml) was added into each flask for a 30 min extraction in an ultrasonic water bath at room temperature. 10 ml of filtered extract was blown to dryness by using a gentle nitrogen stream.

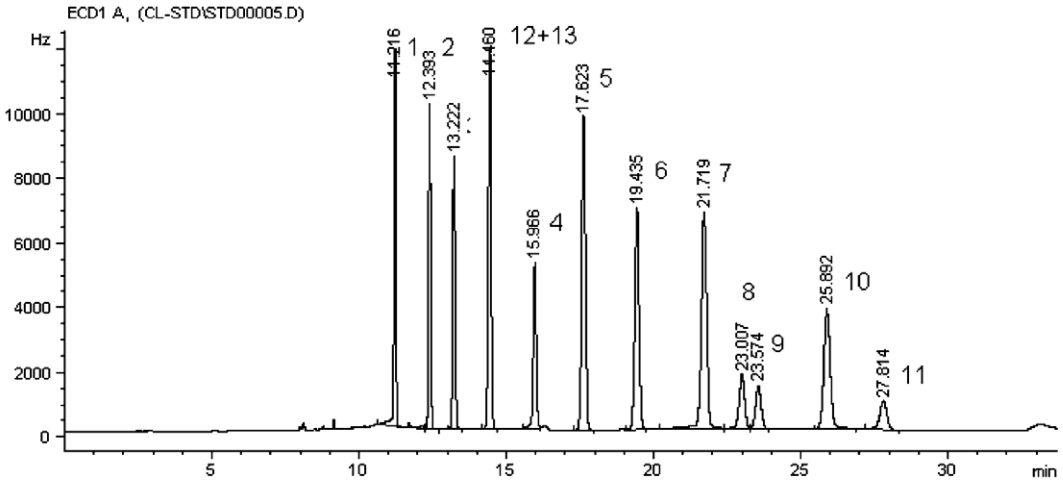

Fig. 1. Single-column gas chromatogram of 13 organochlorine pesticides. Peaks: 1. α-HCH; 2. γ-HCH; 3. Heptachlor; 4. δ-HCH; 5. Heptachlor epoxide; 6. *p*,*p*′-DDE; 7. Dieldrin; 8. *o*,*p*′-DDT; 9. Endrin; 10. *p*,*p*′-DDD; 11. *p*,*p*′-DDT; 12. β-HCH; 13. Aldrin.

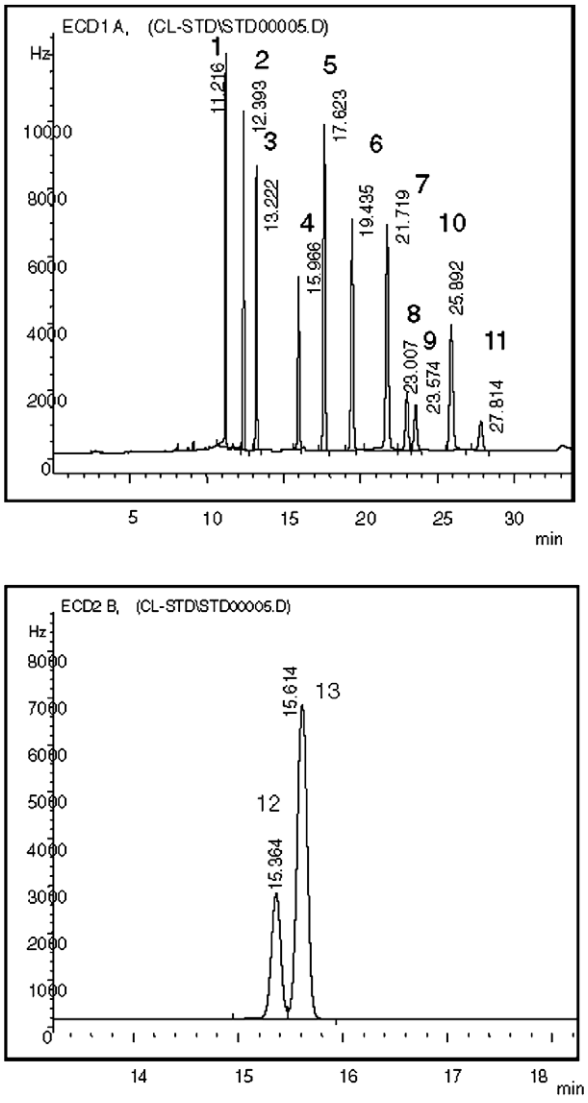

Fig. 2. Two-dimensional chromatogram of 13 organochlorine pesticides. Peaks: 1. α-HCH; 2. γ-HCH; 3. Heptachlor; 4. δ-HCH; 5. Heptachlor epoxide; 6. *p*,*p*′-DDE; 7. Dieldrin; 8. *o*,*p*′-DDT; 9. Endrin; 10. *p*,*p*′-DDD; 11. *p*,*p*′-DDT; 12. β-HCH; 13. Aldrin.

Two millilitres of redistilled petroleum ether was added to dissolve the dry extract. The florisil cartridges for SPE were loaded with the 2 ml of extract after being activated and eluted with 2 ml of ethyl acetate and petroleum ether (9:1). Finally, the eluate was blown to dryness with a gentle nitrogen stream and dissolved with 1 ml of redistilled petroleum ether for analysis.

2.7. GC analysis

One microlitre of each sample was injected in a splitless mode. Oven temperature increased from 150 °C (held for 1 min) to 270 °C (held for 28 min) at the rate of 25 °C min^{-1}. The temperatures of injector and detector were 250 °C and 300 °C, respectively. High purity nitrogen was used as the carrier gas at the flow rate of 1 ml min^{-1} in column DB-1701 and 2 ml min^{-1} in column HP-5MS and the make-up gas was at a flow rate of 60 ml min^{-1}. The peaks of β-HCH and aldrin could not be separated by a single column (Fig. 1). After applying the technology of two-dimensional gas chromatography, the two substances were well separated (Fig. 2). According to the result, the Deans switch was turned on at 14.20 min and turned off at 14.80 min.

The levels of OCPs were quantitatively determined by the external standard method using peak area. The correlation coefficients (r) of calibration curves of OCPs were all higher than 0.996. Matrix spike recovery experiments were also undertaken. The recoveries of OCPs were in the range 81.4–90.4%. The quantitative limit of the method was calculated as three times the signal noise value. It ranges from 0.6 μg kg^{-1} for aldrin and 8 μg kg^{-1} for *p*,*p*′-DDT. The relative standard deviation varied from 0.8% to 9.8%.

3. Results and discussion

3.1. OCPs levels in the rice and its bran

The concentrations of organochlorine pesticides (OCPs) in the rice and its bran are shown in Tables 1 and 2, respectively. Concentrations of organochlorine pesticides (OCPs) in the rice were in the range 0–0.039 mg kg^{-1} (mean 0.030 mg kg^{-1}) for ∑HCH, and 0–0.053 mg kg^{-1} (mean 0.029 mg kg^{-1}) for ∑DDT, and in its bran ranged from 0 to 0.057 mg kg^{-1} (mean 0.031 mg kg^{-1}) for ∑HCH, and from 0 to 0.051 mg kg^{-1} (mean 0.034 mg kg^{-1}) for ∑DDT, respectively. The detection rates of total HCH and DDT in the rice were 22.8% and 35.9% compared with 26.0% and 30.0% in rice bran from Jiangsu Province, China. The other five OCPs, except HCH and DDT, were not detected. Rice bran in China is usually used for animal and fish feeds. OCPs residues in rice bran can be bioaccumulated in animal products and then in humans eventually.

3.2. OCPs levels in human and fish fats

Table 3 shows the investigation results of OCP levels in human and fish fats. The total HCH and DDT in fish fats averaged 0.028 mg kg^{-1} and 0.666 mg kg^{-1}, respectively. However, the total HCH and DDT in human fats reached,

Table 1
Organochlorine pesticide residues in rice from Jiangsu Province, China

Pesticides	Sample amount	Concentration range (mg kg^{-1})	Average concentration (mg kg^{-1})	Detected rate (%)
∑HCH	92	0–0.039	0.030	22.8
∑DDT	92	0–0.053	0.029	35.9
Heptachlor	92	0	0	0
Heptachlor epoxide	92	0	0	0
Aldrin	92	0	0	0
Dieldrin	92	0	0	0
Endrin	92	0	0	0

Table 2
Organochlorine pesticide residues in rice bran from Jiangsu Province, China

Pesticides	Sample amount	Concentration range (mg kg^{-1})	Average concentration (mg kg^{-1})	Detected rate (%)
∑HCH	50	0–0.057	0.031	26.0
∑DDT	50	0–0.051	0.034	30.0
Heptachlor	50	0	0	0
Heptachlor epoxide	50	0	0	0
Aldrin	50	0	0	0
Dieldrin	50	0	0	0
Endrin	50	0	0	0

Table 3
The concentrations of organochlorine pesticides in human and fish fats from Jiangsu Province, China

Pesticides	Mean concentration of OCPs (mg kg^{-1})	
	Human fat	Fish fat
HCH	0.898	0.028
DDT	3.80	0.666

on average, 0.898 mg kg^{-1} and 3.80 mg kg^{-1}, much higher than the residues in fish fats.

A relativly high residue level of OCPs in human was reported in Shanghai, Beijing and Changsha, China. The β-HCH and *p*,*p*′-DDE concentrations in mother's milk were 1.73 and 1.16 mg kg^{-1}, respectively, in residents of Shanghai (Li et al., 2003), 1.18 and 2.04 mg kg^{-1} in residents of Beijing (Yu et al., 2001), and 2.20 and 2.43 mg kg^{-1} in residents of Changsha (Cao et al., 2000).

The investigations of OCP levels in different organisms in the food chain showed wide occurrence in the environment. OCP levels in rice have decreased sharply after being banned from use for 20 years, but could still be intensified by the food chain and bio-accumulated to a final high residue level in humans because of their low biodegradability and high persistence.

3.3. Compositional analysis of OCPs

Compositional differences of HCH isomers or DDT congeners in the environment could indicate different contamination sources (Iwata, Tanabe, Ueda, & Tatsukawa, 1995). The typical HCH generally contains 55–80% of α-HCH, 5–14% of β-HCH, 8–15% of γ-HCH, 2–16% of δ-HCH (Lee, Tanabe, & Koh, 2001). The physicochemical properties of these HCH isomers are different. β-HCH has the lowest water solubility, vapour pressure, and is the most stable and relatively resistant to microbial degradation (Ramesh, Tanabe, Murase, Subramanina, & Tatsukawa, 1991). Also it should be noted that α-HCH can be converted to β-HCH in the environment (Wu, Xu, Schramm, & Kettrup, 1997). Many studies have reported that β-HCH was dominant in environmental samples after long-term migration and transformation (Wu, Zhang, & Zhou, 1999). In this study, a high percentage of β isomers was recorded and this is shown in Table 4. The average compositions of HCH isomers measured in the rice were α: 19.7%, β: 80.3%, γ: 0%, δ: 0%, and, in its bran, these were α: 15.3%, β: 84.7%, γ: 0%, δ: 0%.

Table 4
Compositions of organochlorine pesticide in rice from Jiangsu Province, China

Pesticides	Composition of OCPs in rice (%)	Composition of OCPs in rice bran (%)
α-HCH	19.7	15.3
β-HCH	80.3	84.7
γ-HCH	0	0
δ-HCH	0	0
p,*p*′-DDT	1.5	0
p,*p*′-DDD	0	0
p,*p*′-DDE	98.5	100
o,*p*′-DDT	0	0

Technical DDT generally contains 75% *p*,*p*′-DDT, 15% *o*,*p*′-DDT, 5% *p*,*p*′-DDE, and less than 5% others. DDT can be biodegraded to DDE under aerobic condition and to DDD under anaerobic conditions (Kalantzi et al., 2001). Comparing the concentrations of *p*,*p*′-DDT and its metabolites can distinguish whether the DDT input is recent or not (Phuong, Son, Sauvain, & Tarradellas, 2001). A ratio of (DDE + DDD)/∑DDT of more than 0.5 can be concluded to result from a long-term biodegradation from DDT to DDE or DDD (Hong, Chen, Xu, Wang, & Zhang, 1999). In most of the present samples, DDE occupied the predominant percentage, as shown in Table 4. The mean ratio is considerably higher than 0.5, which indicated that the degradation occurred significantly.

In conclusion, the OCPs residues in rice and bran have decreased sharply since being banned for two decades in Jiangsu Province, China. β-HCH and *p*,*p*′-DDE were the major detected isomers of HCH and DDT in rice. However, the OCPs levels in fish and human fat were found to be higher. It can be concluded that trace OCPs residues in the environment can be bio-accumulated to a final high level in humans by the food chain. This study indicates that a large-scale investigation of OCP residues in different organisms in the food chain should be launched in order to adequately evaluate the potential health risk to humans.

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