RESIDUES AND TRACE ELEMENTS

Survey of Organochlorine Pesticide Residues in Milk in Hong Kong (1993–1995)

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A survey was conducted from 1993 through 1995 to monitor organochlorine pesticides and their metabolite residues in milk available in local Hong Kong markets. Of 252 samples analyzed, including pasteurized milk, fresh milk, and raw milk, 42 contained organochloride pesticide residues at levels exceeding the Extraneous Maximum Residue Limits of the Codex Committee on Pesticide Residues. DDE and HCH isomer levels were substantially higher than those found in a 1984–1987 survey, probably because the source of cow's milk has shifted from local dairy industries to mainland China over the past decade. Although organochlorine pesticides such as DDT and HCH have been banned in China since 1983, residues of such compounds may still persist in the environment and cause contamination through the food chain.

Organochlorine pesticide compounds display various types and degrees of toxicity (1, 2). Researchers have linked exposure to them with increased risk of cancer in humans (3). A controversial hypothesis, based on both biochemical and epidemiological findings, suggests that chlorinated pesticides could trigger breast cancer by adversely affecting the metabolism of estrogen (4).

Organochlorine pesticides, which are lipophilic and lack chemical activity, accumulate in animal and human fatty tissue and are excreted in milk. Thus, the concentrations of organochlorines in breast milk are a reliable indicator of the body load of organochlorine in human females, and hence of population exposure to such compounds. In a 1985 Hong Kong survey, high concentrations of DDT, DDE, and HCH isomers were detected in human breast milk (5). However, the results obtained might not be representative because of the lack of demographic data on the mothers who participated in the study. Moreover, detailed information, including data on whether participants lived all their lives in a specified region or came from other areas, whether they were city or rural people, or what their normal diet consisted of, was not available. However, because the main route of human exposure to many organochlorine pesticides is through food of animal origin where milk is an important product, milk has been used as an indicator of organochlorine pesticide exposure in recent surveys (6–8).

From 1984 through 1987, 2 surveys of organochlorine pesticide levels in foods revealed that milk and dairy products were important sources of organochlorine pesticide intake in Hong Kong (9). From 1993 through 1995, a comprehensive survey was conducted to review the organochlorine pesticide levels in milk intended for human consumption and collected from frontier control points and local markets. A total of 252 milk samples were analyzed for the presence of α, β, γ, and δ-HCH, hexachlorobenzene (HCB), dieldrin, heptachlor epoxide, and DDT and its metabolites.

Experimental

Apparatus

(a) *Gas chromatograph.*—Hewlett Packard (Palo Alto, CA) HP-5890 equipped with electron capture detector with the column DB 1701 (30 m × 0.53 mm id × 1.0 μm film thickness). Operating conditions: carrier gas and make-up gas flow set at 10 and 25 mL nitrogen/min, respectively; column temperature 195°C; injector temperature 210°C; detector temperature 300°C, injection volume 1 μL.

(b) *Gel permeation chromatograph.*—ABC Laboratories (Columbia, MO) AS-2000 gel permeation chromatograh with a column of Bio-bead SX-3 (45 cm × 25 mm id). Operating conditions: mobile phase, dichloromethane–cyclohexane, 1 + 1; flow rate, 5 mL/min; dump time, 24 min; collect time, 25 min; sample loop volume, 5 mL.

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Reagents

(a) *Solvents.*—Acetone, petroleum ether (40°–60°C), dichloromethane, cyclohexane, isooctane. All solvents were distilled in glass apparatus.

(b) *Adsorbent.*—Florisil, PR grade, 60–100 mesh; activated at 130°C overnight before use.

(c) *Pesticide reference standards.*—DDT, DDE, TDE, α-, β-, γ-, δ-hexachlorocyclohexane (HCH), heptachlor epoxide, dieldrin, and hexachlorobenzene (HCB) were obtained from Riedel-de Haen, Inc. (Seelze, Germany). Working standard solutions were prepared by dissolving the appropriate amount of individual organochlorine pesticide with distilled isooctane.

Sampling

About 80 milk samples, including pasteurized milk, UHT milk, fresh milk and raw milk, were collected each year. Samples were collected evenly according to the types of milk and the supplier. Collection was not resticted to local sources; UHT milk imported from other countries and raw milk from southern Guangdong were also sampled.

Extraction and Cleanup

Samples were analyzed essentially as described by Tessari and Savage (10) except that gel permeation chromatography (GPC) was used in the cleanup procedure instead of solvent partitioning. A 50 g homogenized sample was weighed into a 380 mL centrifuge bottle; 250 mL acetone–petroleum ether (1 + 2) at 40°–60°C was added to the bottle, and the mixture was homogenized for 4 min and then centrifuged at 2000 rpm for 10 min. The organic layer was transferred to a separatory funnel containing 200 mL 2% sodium sulfate solution; 25 mL acetone–petroleum ether (1 + 2) was added to the centrifuge bottle to rinse the sample, and the organic layer was collected in the same separatory funnel. The separatory funnel was shaken for 30 s, and the lower aqueous layer was discarded. Another 250 mL 2% sodium sulfate solution was added to wash the organic layer. The organic layer was then filtered through anhydrous sodium sulfate and concentrated just to dryness with a rotary evaporator at 40°C under reduced pressure. The residue was redissolved and the volume was adjusted to 10 mL in dichloromethane–cyclohexane (1 + 1). The extract was cleaned up with a GPC using a 5 mL sample loop. The fraction collected was automatically concentrated and reconstituted in 1 mL petroleum ether. A florisil column was prepared by slowly introducing 5 g activated florisil followed by 2 g anhydrous sodium sulfate into a 1 cm id column. The column was first washed with 10 mL petroleum ether and the extract was allowed to drain through the column. The organochlorine pesticide residues retained in the column were eluted with 25 mL 15% diethylether in petroleum ether. The eluent was evaporated to dryness under a gentle stream of nitrogen. The final residue was reconstituted in 1 mL isooctane. A reagent blank using water as the sample was also analyzed. Residues were quantitated by the external standard calibration method. For the recovery study, milk samples that contained no organochlorine residues were spiked at concentrations ranging from 0.02 to 0.7 mg/kg organochlorine pesticide standards, and mixed before analysis.

Results and Discussion

To compare the findings from the present work with those of a previous survey conducted by the University of Hong Kong (9), the sampling and analytical methodology of the earlier survey were adopted in the present study. However, because most of the local dairy farms had been moved to mainland China, raw milk samples were collected at frontier control points and had increased to about 40 % of the total samples collected in the study. In addition, GPC was proposed in the cleanup procedure to increase the throughput. To validate the modification in the analytical procedures, recovery studies were thus needed. Average recoveries of organochlorine pesticides and limits of quantitation attained by the present method are summarized in Table 1. Concentrations of organochlorine pesticide found in the samples are shown in Table 2. All data are expressed as mg/kg fat, which is considered the most appropriate basis for reporting concentration of lipophilic contaminants in fatty food.

Table 1. Limit of quantitation and average recovery of organochlorine pesticides in milk

Organochloride pesticide	Limit of quantitation[a]	Avg. recovery, %	Relative standard deviation, %[b]
HCB	0.004	104.1	11.5
o,p'-DDE	0.005	93.6	5.0
p,p'-DDE	0.005	94.9	6.5
o,p'-TDE	0.005	88.5	5.3
p,p'-TDE	0.005	93.9	3.5
o,p'-DDT	0.005	89.6	4.8
p,p'-DDT	0.005	88.6	4.5
Dieldrin	0.010	97.9	12.6
α-HCH	0.002	90.6	6.2
β-HCH	0.002	89.4	4.1
γ-HCH	0.002	86.6	2.5
δ-HCH	0.002	86.8	7.4
Heptachlor epoxide	0.006	89.5	6.5

[a] Expressed as mg/kg fat.
[b] $n = 10$.

Table 2. Organochlorine pesticide residues in positive milk samples collected from 1993 through 1995[a]

Pesticides	Frequency 1993	Frequency 1994	Frequency 1995	Concentration range[b] (mean)[c] 1993	Concentration range[b] (mean)[c] 1994	Concentration range[b] (mean)[c] 1995
HCB	19	61	52	0.004–0.18 (0.03)	0.004–0.60 (0.10)	0.004–0.74 (0.09)
HCH[d]	76	41	42	0.008–0.88 (0.11)	0.008–0.73 (0.11)	0.008–0.79 (0.10)
DDE[e]	89	63	43	0.01–0.69 (0.14)	0.01–6.20 (0.25)	0.01–0.32 (0.10)
TDE[e]	11	4	5	0.03–0.52 (0.17)	0.01–0.59 (0.16)	0.03–0.81 (0.08)
DDT[e]	4	5	—	0.01–0.07 (0.04)	0.01–0.08 (0.05)	0
Dieldrin	34	62	18	0.01–0.23 (0.09)	0.01–0.34 (0.07)	0.01–0.44 (0.09)
Heptachlor epoxide	7	3	1	0.006–0.03 (0.02)	0.05–0.49 (0.20)	0.09

[a] Number of samples analyzed = 98 (1993), 84 (1994), 70 (1995).
[b] Expressed as mg/kg fat, not corrected for recovery. Mean fat content = 3.5%.
[c] Average values for positive samples.
[d] Including α-, β-, γ-, and δ-isomers.
[e] Including *o,p'* and *p,p'*-isomers.

During the past 3 years, HCB residues were found in 52% of samples, with a mean level of 0.09 mg/kg. The highest concentration, 0.74 mg/kg, was found in a raw milk sample. The mean value of total HCH, detected in 63% of samples, was 0.11 mg/kg. The highest concentration was 0.88 mg/kg in a raw milk sample. Dieldrin residues were found in 45% of samples, with a mean level at 0.08 mg/kg. Moreover, heptachlor epoxide residues were found in 11 samples, with mean levels of 0.08 mg/kg. For DDT and metabolites, 73% of samples contained DDE, with a mean level of 0.16 mg/kg, whereas only 8 and 5% of samples contained TDE and DDT, with mean levels of 0.14 and 0.05 mg/kg, respectively. The highest DDE concentration was 6.2 mg/kg in a raw milk sample.

The number of detections of organochlorines, except HCB and dieldrin, declined within the survey period. However, compared with the findings of the 1984–1987 survey conducted by the University of Hong Kong (9), a great increase in the levels of DDT and its metabolites was noted in the recent study (Table 3). Similarly, a substantial increase in the levels of HCH isomers in milk was observed in the recent study as compared with those in the 1985 survey (9) (Table 4). Some samples contained organochlorine pesticides at levels exceeding the Codex Extraneous Maximum Residue Limits (EMRLs; 11) (Table 5). A plausible reason for the elevated levels of organochlorine pesticides may be the change of sources of cow's milk. In the past decade, due to the increase in operating costs and the introduction of more stringent environmental protection regulations, essentially all local dairy farms were closed. Local dairy industries now rely mainly on mainland China, particularly Southern Guangdong, for the supply of raw milk. At present, about 1.7 to 1.8 million kg of raw milk is imported from Shenzhen every month. Although organochlorine pesticides such as DDT and HCH have been banned in China since 1983 (12), residues of such compounds may still persist in the environment. In fact, HCB and DDT and its metabolites were actually detected at levels ranging from 0.01 to 0.12 μg/L in river and sewage systems in Guangzhou, Shenzhen, and Zhaoqing, cities in southern Guangdong (13). Through the food chain, organochlorine pesticides would then accumulate and be concentrated in cow's milk. Nonetheless, the elevated levels found in this study indicate that further attention should be paid to control and monitor the sources of dairy products,

Table 4. Levels of HCH isomers in milk sampled in Hong Kong (1/1/85–7/30/85)

Sample	No. of positives · samples	HCH level range[a] (average value)
Milk: fresh, raw, UHT	27	0.01–0.09 (0.02)

[a] Expressed as mg/kg fat.

Table 3. Levels of DDT and metabolites in milk samples collected in Hong Kong (1984–1987)

Sample	No. of samples analyzed	Pesticide level range[a] (mean)[b] DDT	DDE	TDE
Milk: raw, fresh, UHT	96	ND[c]–0.04 (0.01)	ND–0.12 (0.01)	ND

[a] Expressed as mg/kg fat.
[b] Average values for positive samples.
[c] ND, not detected.

Table 5. Summary of samples containing organochlorine pesticide at levels exceeding Codex EMRL values

Pesticide	EMRL value[a]	No. of samples exceeding EMRL values (No. of positive samples)		
		1993	1994	1995
HCB	0.5	0 (19)	1 (61)	1 (52)
Dieldrin	0.15	7 (34)	3 (62)	1 (18)
HCH[b]	0.25	19 (76)	6 (41)	3 (42)
DDT and metabolites	1.25	0 (86)	1 (62)	0 (42)
Heptachlor epoxide	0.15	0 (7)	0 (3)	0 (1)

[a] EMRL (Extraneous Maximum Residue Limits), expressed as mg/kg fat.
[b] For γ-isomer.

particularly raw milk, that are imported into Hong Kong.

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References

(1) Briggs, A.A. (1992) *Basic Guide to Pesticides: Their Characteristics and Hazards*, Hemisphere Publishing Corporation, Washington, DC, pp. 212–213

(2) Matsumura, F. (1976) *Toxicology of Insecticides*, Plenum Press, New York, NY, pp. 53–64

(3) Hileman, B. (1994) *Chem. Eng. News* **72**, 19–23

(4) Patlak, M. (1996) *Environ. Sci. Technol.* **30**, 210A–211A

(5) Ip, H.M.N. (1989) *Arch. Environ. Contam. Toxicol.* **18**, 490–494

(6) Mukherjee, I., & Gopal, M. (1993) *J. AOAC Int.* **76**, 283–286

(7) Vaz, R. (1995) *Food Addit. Contam.* **12**, 543–558

(8) Waliszewski, S.M., Pardio, V.T., Waliszewski, S.K.N., Chantiri, J.N., Infanzon, R.M., & Rivera, J. (1996) *Food Addit. Contam.* **13**, 231–235

(9) Ip, H.M.N. (1990) *Arch. Environ. Contam. Toxicol.* **19**, 291–296

(10) Tessari, J.D., & Savage, E.P. (1980) *J. Assoc. Off. Anal. Chem.* **63**, 736–741

(11) *Guide to Codex Recommendations Concerning Pesticide Residues* (1993) Codex Alimentarius Commission, FAO and WHO, Rome, Italy

(12) Wolfe, D.A., Champ, M.A., Cross, F.A., Kester, D.R., Park, P.F., & Swanson, R.L. (1984) *Mar. Pollut. Bull.* **15**,