ENDOSULFAN AND TOXAPHENE IN BREAST MILK FROM CHINA, KOREA AND JAPAN

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Introduction

Endosulfan has been used as an insecticide around the world[1] and is classified by the World Health Organization as a priority pollutant. In some European countries (Denmark, Finland and Italy), exposure to endosulfan and its metabolites have been determined in breast milk.[2,3] Widespread environmental contamination by endosulfan has been reported in China[4] and Korea.[5] Among legacy POPs, toxaphene has been used in China[6] and Korea[7] but is not registered in Japan. Exposure to toxaphene in seafood from Hong Kong is reported.[8] Historical endosulfan and toxaphene trends showed elevated exposure levels via food intake in Korea and China.[9] However, the recent levels and their temporal trends have never been determined in Asian breast milk.

The present study was conducted to clarify regional differences in recent trends of contamination by endosulfan and toxaphene in human milk from China, Korea and Japan. Our data are compared with other results worldwide to understand the magnitude of contamination and to assess intake of contaminants by infants through breast milk consumption.

Materials and methods

Human milk samples were obtained from the Kyoto University Human Specimen Bank using a standardized protocol.[10] Overall, 70 pooled samples were prepared from 210 human breast milk samples. The samples were collected from volunteers living in China (n = 60 from Beijing, 2007 to 2008), Korea (n = 30 from Seoul in 2007; n = 30 from Busan, 2008 to 2009) and Japan (n = 30 from Sendai, 2009; n = 30 from Takarazuka, 2008; n = 30 from Takayama, 2008). The Ethics Committee of Kyoto University approved the protocol of the present study (E25) and appropriate written informed consent was obtained from all the participants.

Two internal standards, $^{13}C_{12}$-labelled *cis*-chlordane and $^{13}C_{12}$-labelled 2,2',4,4',5,5'-hexachlorobiphenyl (PCB-153), were used for determination of endosulfan and toxaphene. The standards of toxaphenes (Parlar #26, #50, and #62), and endosulfan (α- and β-forms) were purchased from Cambridge Isotope Laboratories (Andover, MA, USA).

The methodology used to analyze organochlorine pesticides in the breast milk samples was based on lipid extraction, gel permeation chromatography (GPC) and silica-gel column cleanup, and gas chromatography–negative chemical ionization– mass spectrometry (GC-ECNI-MS). Briefly, each 15 mL pooled breast milk sample was spiked with an internal standard, namely $^{13}C_{12}$-*cis*-chlordane (2 ng). We extracted the sample with *n*-hexane, after adding potassium oxalate solution, ethanol and ethylether. An aliquot of lipid (300 mg) was dissolved in dichloromethane (DCM):*n*-hexane (1:1), and then subjected to GPC with a Bio-Beads S-X3 column (Bio-Rad Laboratories, CA, USA). The fraction was purified with a silica-gel column (0.2 g Wako gel S-1), by elution with 15 ml DCM:*n*-hexane (12:88, v/v). The fraction was concentrated to 200 µL prior to GC/MS analysis. Endosulfan and toxaphene were measured by GC-ECNI-MS using an Agilent GC/MSD 5973i (Agilent Technologies, CA, USA) coupled with a 6890N gas chromatograph.

The extraction, cleanup, and fractionation steps were evaluated by measurement of the absolute recoveries of the compounds ($^{13}C_{12}$-labelled internal and native surrogate standards) spiked and passed through the entire analytical procedure. Recoveries of endosulfan and toxaphene were between 87% and 94% with the relative standard deviations of <10% (n = 5). When the levels of the target chemicals were less than their LOQs, we allocated half of the LOQ as the value for analysis. The calibration (0.1 to 5 ng/mL of each analyte) was linear and characterized by good correlation coefficients (>0.99) for all compounds studied.

Kruskal-Wallis one-way analysis of variance and the Steel-Dwass test were used to examine differences in the target chemical concentrations among the three countries. Probability values of less than 0.05 were considered to indicate statistical significance.

Results and discussion

Chlordane and heptachlor

In this study, we measured 10 pesticides (19 isomers) in the ECNI mode. The mean concentrations of chlorinated cyclodienes in the breast milk samples are listed in Table 1. The profiles of major contaminants were consistent with the previous results from Japan,[11, 12] and from southern China.[13] The levels of trans-nonachlor, cis-nonachlor, oxychlordane and HCE ranged from 0.7 to 3.9 ng/g lipid in China, from 1.5 to 7.6 ng/g lipid in Korea and from 4.5 to 30 ng/g lipid in Japan. These results indicate that, among the three countries, the levels of cyclodiene pesticides are highest in Japan. No significant difference in concentrations was observed within domestic regions.

Endosulfan

Endosulfan was detected as the α-form in the range of 0.85–1.4 ng/g lipid and as the β-form in the range of 0.05–0.11 ng/g lipid in all breast milk samples from China, Korea and Japan. The levels from Korea were significantly higher ($p < 0.05$) than those from China. The present levels in these three countries appear to be lower than those from European countries.[2, 3] A recent dietary exposure study reported that endosulfan is present at similar ratios of α- and β-forms in the diet,[9] while technical endosulfan consists of 70% α-form and 30% β-form. Higher ratios (>10) of α-form to β-form in human milk might be explained by their different physicochemical properties. The α-endosulfan has a higher Henry's Law constant and, as a result, air samples are dominated by the α-form, which is easily transported in the atmosphere.[1] In contrast, β-endosulfan has markedly higher aqueous solubility than the α-form and it will therefore partition aqueous phases more readily.[1] Furthermore, β-endosulfan is possibly converted to the α-form or endosulfan sulfate in the human body.[1] These findings support the hypothesis that the source of α-endosulfan in breast milk from Asian countries is attributable to inhalation of endosulfan from the atmosphere rather than dietary intake. Endosulfan has been used widely to control a number of insects on crops and fruits in China[4] and Korea,[5] although exposure to endosulfan via seafood products in southern China in 2005 is reported.[8] A recent survey of endosulfan levels in the diet showed an exponentially increasing trend in China and Korea.[9] No such historical trends of endosulfan levels have been observed in Japan. As its agricultural registration expired in 2010, exposure from local usage would be expected to decrease in future. However, endosulfan is one of the most abundant pesticides in the Arctic air and has a propensity to undergo atmospheric long-range transport.[5]

Toxaphenes

The mean concentration of toxaphene in breast milk from Japan was 2.5 ng/g lipid, which was significantly higher than those from Korea (0.73 ng/g lipid) and China (0.36 ng/g lipid). These levels were comparable to recent reports from eastern Asia,[12, 13] but appears to be much lower than those in mothers from Germany,[14] Russia[15] and southern Canada.[16] Technical toxaphene was used until 1999 in Korea[7] and until 1982 in China,[6] but has never been registered in Japan. The source of toxaphene in breast milk from Japan might be through dietary intake of imported foods, or long-distant transported samples from the Arctic air, since the Arctic environment contains higher levels of toxaphene than the temperate regional environment.[17] Due to differences in the persistency of congeners, a much smaller number of toxaphene congeners are found in biota and only two (Parlar #26 and #50) are present in humans.[14] Recently, the trend for increasing levels of toxaphenes in the diet in China and Korea has been reported,[9] therefore future monitoring of toxaphenes in human samples is required.

Health-risk assessment for infants

The calculation of estimated daily intake (EDI) values by infants was based on the assumption that the average milk consumption of a 5 kg infant is 750 mL/day (or g/day). The EDI values were compared with acceptable daily intake (ADI)/ provisional tolerable daily intake established by FAO/WHO[18] and Health Canada.[17]

The mean EDIs of CHLs, HCE, toxaphene and endosulfan for breastfed infants are compared with the guidelines. The EDI of HCE, toxaphene, and endosulfan for all samples from the three countries did not exceed the guideline values, although EDI of CHLs in Japanese infants accounted for 43% of ADIs. Owing to the limitations of the experimental design (using pooled samples), we could possibly overlook certain individual samples that may exceed their ADIs. Even if the EDI of these pesticides exceeded the guideline values, it is unclear if the current concentrations may cause adverse effects.

Conclusion

The present study indicates that the levels of α- and β-endosulfans are relatively higher in breast milk from Korea, whereas the other chlorinated cyclodiene such as toxaphenes and chlordanes are still contaminants in samples from Japan. Therefore, endosulfan and toxaphene need further monitoring in the future.

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References

1. Weber J, Halsall CJ, Muir D, Teixeira C, Small J, Solomon K, Hermanson M, Hung H, Bidleman T. (2010); *Sci Total Environ* 408: 2966-2984.
2. Cerrillo I, Granada A, López-Espinosa MJ, Olmos B, Jiménez M, Caño A, Olea N, Fátima Olea-Serrano M. (2005); *Environ Res* 98: 233-239.
3. Shen H, Main KM, Andersson AM, Damgaard IN, Virtanen HE, Skakkebaek NE, Toppari J, Schramm KW, (2008); *Hum Reprod* 23: 201-210.
4. Li J, Zhang G, Guo L, Xu W, Li X, Lee CSL, Ding A, Wang T. (2007); *Atmospheric Environment* 41: 3889-3903.
5. Yeo HG, Choi M, Sunwoo Y. (2004); *Atmospheric Environment* 38: 4779-4788.
6. Wong MH, Leung AO, Chan JK, Choi MP. (2005); *Chemosphere* 60: 740-752.
7. de Geus HJ, Besselink H, Brouwer A, Klungsøyr J, McHugh B, Nixon E, Rimkus GG, Wester PG, de Boer J. (1999); *Environ Health Perspect* 107 Suppl 1: 115-144.
8. Guo JY, Zeng EY, Wu FC, Meng XZ, Mai BX, Luo XJ. (2007); *Environ Toxicol Chem* 26: 1109-1115.
9. Desalegn B, Takasuga T, Harada KH, Hitomi T, Fujii Y, Yang HR, Wang P, Senevirathna S, Koizumi A. (2011); *Chemosphere* in press.
10. Koizumi A, Harada KH, Inoue K, Hitomi T, Yang HR, Moon CS, Wang P, Hung NN, Watanabe T, Shimbo S, Ikeda M. (2009); *Environ Health Prev Med* 14: 307-318.
11. Haraguchi K, Koizumi A, Inoue K, Harada KH, Hitomi T, Minata M, Tanabe M, Kato Y, Nishimura E, Yamamoto Y, Watanabe T, Takenaka K, Uehara S, Yang HR, Kim MY, Moon CS, Kim HS, Wang P, Liu A, Hung NN. (2009); *Environ Int* 35: 1072-1079.
12. Nakai K, Nakamura T, Murata K, Satoh H. (2009); *Nippon Eiseigaku Zasshi* 64: 749-758.
13. Hedley AJ, Hui LL, Kypke K, Malisch R, van Leeuwen FX, Moy G, Wong TW, Nelson EA. (2010); *Chemosphere* 79: 259-265.
14. Skopp S, Oehme M, Fürst P. (2002); *J Environ Monit* 4: 389-394.
15. Polder A, Becher G, Savinova TN, Skaare JU. (1998); *Chemosphere* 37: 1795-1806.
16. Newsome WH, Ryan JJ. (1999); *Chemosphere* 39: 519-526.
17. van Oostdam J, Gilman A, Dewailly E, Usher P, Wheatley B, Kuhnlein H, Neve S, Walker J, Tracy B, Feeley M, Jerome V, Kwavnick B. (1999); *Sci Total Environ* 230: 1-82.
18. FAO/WHO (2009) http://www.who.int/ipcs/publications/jmpr/pesticide_inventory_edition10.pdf

Table 1. Mean concentrations (ng/g lipid) of chlorinated cyclodienes in pooled breast milk samples from China, Korea and Japan (n = 70).

	China			Korea			Japan		
	n=20		N > LOQ*	n=20		N > LOQ*	n=30		N > LOQ*
α-endosulfan	0.95	n.s.**	20	1.3	n.s.	20	1.1	n.s.	30
β-endosulfan	0.06	B***	19	0.09	A	20	0.09	AB	30
ΣEndosulfan	1.0	B	20	1.4	A	20	1.2	AB	30
Toxaphene Parlar26	0.17	C	11	0.32	B	19	0.99	A	30
Toxaphene Parlar50	0.19	C	8	0.41	B	19	1.6	A	30
ΣToxaphene	0.36	C	11	0.73	B	19	2.5	A	30
trans-nonachlor	3.9	C	20	7.6	B	20	30	A	30
cis-nonachlor	0.7	C	20	1.5	B	20	4.8	A	30
oxychlordane	2.2	C	20	4.9	B	20	11	A	30
Σ Chlordane	6.8	C	20	14.0	B	20	18.8	A	30
cis-heptachlor epoxide	0.96	B	20	4.7	A	20	4.5	A	30

* Concentrations below the detection limit was treated as half the LOQ for the arithmetic mean and statistics.
**n.s.: not significant.
*** Significant difference ($p < 0.05$) according to the Steel-Dwass test. Means followed by different letters differed significantly from other countries ($p < 0.05$);