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Spatial Distribution and Accumulation of Organohalogen Compounds in Human Breast Milk from the Philippines

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Abstract—Organohalogen compounds that enter the human body from the external environment—food, water, and air—are the sources for our biologic composition. The present study determined the concentrations of organohalogen compounds such as dichlorodiphenyltrichloroethane and its metabolites (DDTs), polychlorinated biphenyls (PCBs), chlordane compounds (CHLs), hexachlorocyclohexane isomers (HCHs), hexachlorobenzene (HCB), tris (4-chlorophenyl) methane (TCPMe), polybrominated diphenyl ethers (PBDEs) and hexabromocyclododecanes (HBCDs) in human breast milk samples from the Philippines. The detection of these compounds in all the samples analyzed indicates that widespread contamination in the Philippines. DDTs and PCBs were the predominantly identified compounds. PBDEs in the Philippines are higher than those in Japan and many other Asian countries. In the case of HBCDs, this is the first comprehensive monitoring research on human breast milk in Asian developing countries. No statistical difference was observed for the organohalogen compounds between dumping and reference sites. Primipara mothers had significantly higher organochlorines (DDTs, CHLs and HCHs) than multipara mothers, but this phenomenon was not observed for PBDEs and HBCDs, probably because of their continuous exposure and ongoing usage at present. A few individuals accumulated CHLs close to or even higher than the TDI (Tolerable Daily Intake) guidelines proposed by Health Canada.

Keywords: human breast milk, the Philippines, organohalogen compounds, Asia, accumulation kinetics, health risk assessment

INTRODUCTION

Breastfeeding provides a significant source of exposure to persistent organic pollutants (POPs) in early human life, the effects of which are unknown, and is the subject of a growing body of research. Apart from POPs, several other halogenated chemicals also do exist in the environment as a result of their use in many industrial processes. Because of their environmental stability, persistence and high production volume, PBDEs and HBCDs are among the most abundant brominated flame retardants (BFRs) detected in the environment and in wildlife and human tissues (Alaee *et al.*, 2003). Recently, concern on these emerging classes of chemicals has increased because of their occurrence in the environment and biota.

The Philippines is one of the archipelagic countries in the Southeast Asian region, with a population of 85 million. The data on human exposure to organohalogen compounds in the Philippines is rather scarce and comprehensive studies have not been made and so detailed studies on the present scenario are needed. In the present study on the concentrations of persistent organohalogens such as PCBs, OC pesticides, PBDEs and HBCDs in human breast milk samples from the Philippines were carried out.

MATERIALS AND METHODS

Samples

Human breast milk samples (n = 33) were collected from primipara (n = 6) and multipara mothers (n = 27) living at two different locations; Payatas a dumping site and Malate a reference site in the Philippines during 2004. These milk samples were shipped to the laboratory in Japan under frozen conditions and stored at –20°C in the Environmental Specimen Bank (*es*-Bank) of Ehime University (Tanabe, 2006) until analysis. We randomly selected the breast milk donors. Almost all the donors are housewives in the age range of 17 to 41 and their biological characteristics are relatively similar for both locations. Eleven samples are from the reference site and twenty two samples are from the mothers living in the periphery of the dumping site.

Chemical analysis

Analysis of PBDEs was performed following the procedure by Ueno *et al.* (2004), with slight modification. Analysis of HBCDs was performed on the basis of an analytical method reported by Tomy *et al.* (2004) and all the HBCD congeners were quantified by LC-MS-MS analysis. Organochlorine compounds (OCs) were analyzed following the methods described earlier by Minh *et al.* (2004). OCs including DDTs, PCBs, CHLs, HCHs, HCB and TCPMe were determined by gas chromatography with an electron capture detector (GC-ECD) and Mass Spectrometer (GC-MS). Procedural blanks were analyzed simultaneously with samples to check for interference or contamination from solvents and glassware. The concentration of OCs, PBDEs and HBCDs were expressed in ng/g lipid weight basis unless otherwise specified.

RESULTS AND DISCUSSION

Organohalogen compounds were detected in all the breast milk samples (Table 1), indicating their widespread contaminations in the Philippines. From our results we suggest that the residents in both the sites in the Philippines have

Table 1. Mean and range concentrations (ng/g lipid wt.) of organohalogen compounds in human breast milk from the Philippines.

	Reference site		Dumping site		All donars
	Primipara	Multipara	Primipara	Multipara	
Age of mothers	22 (18–25)	31 (23–39)	22 (17–28)	31 (24–41)	29 (17–41)
Lipid content (%)	2.8 (1.8–3.8)	2.2 (1.4–3.4)	2.2 (1.1–3.9)	2.1 (1.0–3.9)	2.2 (1.0–3.9)
Number of samples	2	9	4	18	33
Compounds					
PBDEs					
mono to hepta	6.3 (4.5–8.1)	2.6 (1.4–3.7)	5.3 (3.9–6.30)	10 (1.6–52)	7.2 (1.4–52)
Sum PBDEs (mono-deca)	7.8 (7.0–8.5)	2.6 (1.5–3.8)	5.6 (4.2–6.9)	10 (1.7–52)	7.5 (1.5–52)
HBCDs	0.31 (0.22–0.40)	1.0 (0.15–3.2)	0.98 (0.40–1.6)	0.81 (0.13–2.0)	0.86 (0.13–3.2)
PCBs	75 (40–110)	50 (10–80)	120 (55–260)	65 (20–280)	70 (10–280)
DDTs					
p,p′-DDE	585 (220–950)	63 (10–155)	620 (40–2000)	56 (10–190)	158 (10–2000)
p,p′-DDD	4.4 (4.1–4.6)	0.57 (0.35–1.7)	4.1 (1.1–11)	0.62 (0.064–2.9)	1.2 (0.064–11)
p,p′-DDT	24 (12–35)	5.1 (3.3–8.1)	13 (7.6–26)	5.0 (1.6–14)	7.1 (1.6–26)
Sum DDTs	615 (260–970)	70 (15–170)	655 (60–2100)	60 (10–210)	170 (10–2100)
HCHs					
α-HCH	0.25 (0.16–0.33)	0.28 (0.070–0.66)	0.55 (0.25–0.78)	0.24 (0.036–0.86)	0.29 (0.036–0.86)
β-HCH	11 (5.2–17)	4.0 (2.2–6.7)	11 (6.7–17)	3.2 (0.95–7.0)	4.9 (0.95–17)
γ-HCH	0.99 (0.68–1.3)	0.18 (0.064–0.37)	1.2 (0.30–2.2)	0.15 (0.049–0.36)	0.34 (0.049–2.2)
Sum HCHs	12 (5.9–18)	4.4 (2.4–7.3)	13 (7.7–20)	3.6 (1.1–8.2)	5.5 (1.1–20)
CHLs					
Oxy-CHL	8.5 (3.5–14)	2.6 (1.1–4.6)	7.4 (3.1–14)	1.7 (0.35–4.1)	3.0 (0.35–14)
Cis-CHL	1.3 (1.1–1.5)	1.3 (<0.05–4.2)	4.4 (0.69–12)	0.59 (<0.05–1.5)	1.4 (<0.05–12)
Trans-nona	16 (6.1–2.5)	5.1 (1.8–8.3)	15 (5.6–27)	3.8 (1.2–9.5)	6.2 (1.2–2.7)
Cis-nona	2.6 (<0.05–2.6)	1.2 (0.41–22)	6 (<0.05–10)	0.75 (0.15–1.5)	1.3 (<0.05–10)
Sum CHLs	27 (13–40)	10 (3.7–15)	30 (17–55)	6.7 (1.8–15)	12 (1.8–55)
HCB	3.4 (1.8–4.9)	1.7 (0.76–3.3)	4.4 (1.0–6.9)	2.2 (1.0–5.0)	2.5 (0.76–6.9)
TCPMe	3.1 (0.94–5.2)	0.95 (ND–2.7)	7.6 (0.61–28)	1.3 (ND–8.7)	2.2 (ND–28)

ND - Not Detected.

been widely exposed to these contaminants. The residue levels were in the order of DDTs > PCBs > CHLs > PBDEs > HCHs > HCB > TCPMe > HBCDs.

Contamination status of BFRs

Twelve PBDE congeners from di- to deca-BDE were identified. The concentrations of the sum of the 12 congeners (∑PBDEs) found in human breast milk samples varied widely, from 1.5 to 52 ng/g lipid wt., with an overall mean of 7.5 ng/g lipid wt. (Table 1). Interestingly, mean concentrations of PBDEs found in our study may become a major environmental concern in the Philippines as they were higher than those in Japan an industrially developed country and many other Asian developing countries. However, the levels were one or two orders of magnitude lower than in Canada (Gill *et al.*, 2004) and USA (Restrepo *et al.*, 2007).

Among PBDEs, BDE-47, -99, -100, -153 and -183 were the predominant congeners (Fig. 1), similar to other studies (Schecter *et al.*, 2003; Gill *et al.*, 2004). The observed difference in the human milk samples from the two different sites seemed to indicate a spatial variability that might be due to the corresponding background residue levels in the environment. Interestingly, despite its lower bioavailability as a result of its high molecular weight (de Wit, 2002), a few donors had BDE-183 and -209 at high levels (Fig. 1). Food is the major source for BDE-47 (Sjödin *et al.*, 2003), whereas airborne intake/indoor air and house dust seems to be more pronounced for BDE-183 and -209 (Sjödin *et al.*, 2003; Wu *et al.*, 2007). Major seafood items of Asian people, such as mussel, tuna and other fish accumulated higher proportion of BDE-47 (Ueno *et al.*, 2004; Ramu *et al.*, 2007), which could be one of the reasons for the high proportion of BDE-47 in the present study. However, study on the accumulation of PBDEs in the air and/or house dust has not been made in the Asian countries. Collectively, due to lack of information on PBDE commercial mixtures used in the Philippines and food habits of donors, it is difficult to identify the major source and pathways of nursing mothers.

In the case of HBCDs, this is the first comprehensive monitoring research on human breast milk in the Asian developing countries and the concentrations of the sum of the three congeners (∑HBCDs) found in breast milk samples varied widely, from 0.13 to 3.2 ng/g lipid wt., with an overall mean of 0.86 ng/g lipid wt. (Table 1). Kakimoto *et al.* (2008) reported that the over all mean concentration of ∑HBCDs in human breast milk samples collected between 2000 and 2006 in Japan was in the range 1.0–4.0 ng/g lipid wt., and in our study the mean concentrations of ∑HBCDs levels were fewer than in Japan. However, the levels of α-HBCD were (0.66 ng/g lipid wt.) within the range observed in Texas and lower than in Ontario (Ryan *et al.*, 2006). There are few reports regarding stereoisomer-specific concentrations of HBCD in human breast milk in the world. In this study, the stable isomer, α-HBCD was predominant followed by γ- and β-isomers in both locations. The inconsistency in the presence of γ- and β-HBCD in human breast milk samples in our study may be due to individual

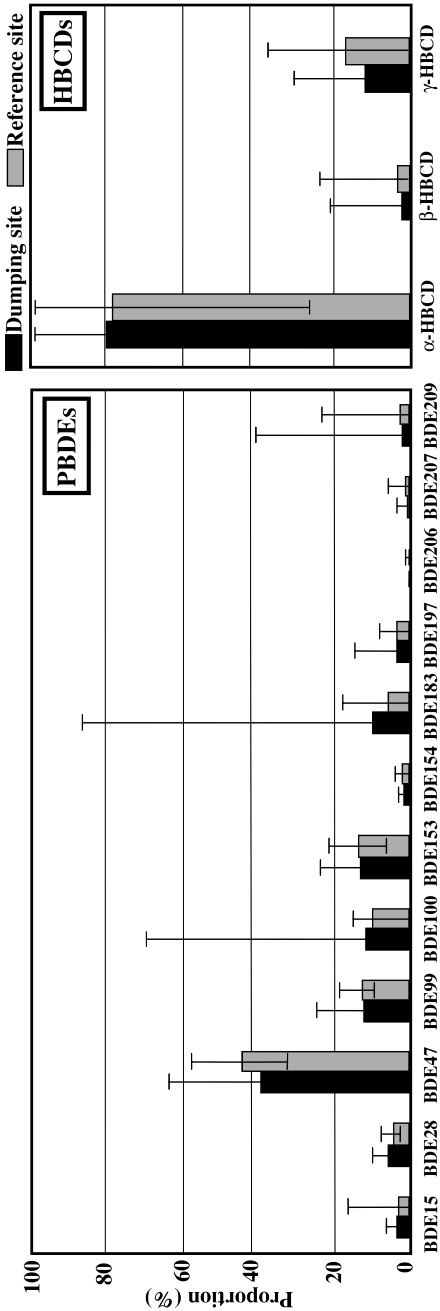

Fig. 1. Profiles of congener/isomer patterns of PBDEs and HBCDs in human breast milk samples collected in 2004 from the Philippines.

variability in metabolizing capacity and/or other unknown factors such as the frequency of exposure to HBCD. In the technical mixture, γ-HBCD was the most dominant isomer (>70%) at room temperature, followed by α-HBCD and β-HBCD (Tomy *et al.*, 2004). However, the composition of HBCD isomers varies according to the type of environmental matrix. Among HBCDs, α-HBCD was reported to have a relatively longer environmental and biological half-life than those of β- and γ-HBCD.

No statistically significant difference was observed for BFRs such as PBDEs and HBCDs isomers between dumping and reference sites. However, PBDEs and HBCDs were found to be somewhat higher in dumping site than the reference site, indicating additional source of these compounds in dumping site. Massive amounts of waste electric products (television, computer etc.) used in developed countries were exported as trash to Asian developing countries (Hileman, 2002) and such imported products and materials may also be the sources of BFRs in the Philippines. This fact, coupled with the relatively high amount of PBDEs in breast milk from the Philippines may indicate that some of the developing countries may become important global sources for BFRs contamination in future (Ueno *et al.*, 2006). The variations of PBDE and HBCD congener profiles in human breast milk between individuals may support the fact that human were exposed to multiple sources and pathways, and also possibly to different commercial mixtures.

Contamination status of OCs

Among OCs, DDTs and PCBs were the predominantly identified compounds in all the breast milk samples. Concentrations of other compounds were 1–3 orders of magnitude lesser than those of DDTs and PCBs (Table 1). The concentrations of OCs varied widely, the distribution pattern were in the order of DDTs > PCBs > CHLs > HCHs > HCB > TCPMe. No statistical difference was observed for OCs between dumping and reference sites. The accumulation pattern of OCs in human breast milk was different from that found in mussels from the Philippines coastal environment (Ramu *et al.*, 2007), which showed higher levels of PCBs and CHLs, probably because of the difference in exposure routes. However, DDTs was consistently the prevalent OC in both samples, indicating that DDTs is the major environmental contaminant in the Philippine environment.

In comparison with worldwide OC levels (Tsydenova *et al.*, 2007), the human breast milk from the Philippines contained higher levels of PCBs and CHLs than those reported in other Asian developing countries like China, Indonesia and Cambodia, indicating elevated sources of these compounds in this country. In the case of PCBs, apart from industrial usage-based on the inventory, there were 2,089,000 kg of PCB oils in the country contained in electric transformers, capacitors and circuit breakers (Prudente *et al.*, 2007) may also be a potential source. For chlordane, technical mixture of CHLs is still in use for public health purposes, for example as termiticides, in urban and industrial areas

(Prudente *et al.*, 2007) were the possible reasons for the elevated levels in the human milk of the Philippines. Among chlordane compounds, the most abundant were *trans*-nonachlor, oxychlordane and *cis*-nonachlor. This is because CHLs are rapidly metabolized in organisms into oxychlordane, γ-chlordane or into impurities such as *trans*-nonachlor or *cis*-nonachlor, and these breakdown products persist in various biological tissues (Solomon and Weiss, 2002). TCPMe accumulation in the Philippine human milk samples is reported for the first time in this work. Significant positive correlation of TCPMe levels were found with DDTs and other OCs (data not shown), indicating that the bioaccumulative nature of TCPMe is similar to other OCs, such as DDTs and PCBs.

Accumulation kinetics

In this study, concentrations of OCs such as DDTs, CHLs and HCHs significantly decreased with increasing number of children (data not shown), indicating that parity plays an important role in these compounds in lactating women. The relationship however, is less pronounced for other compounds like PCBs, HCB and TCPMe (data not shown), probably because of the difference in exposure routes. In lactating women, several factors such as parity, age of mother, food intake preferences, period of breast-feeding and several other external factors have been reported as related to the concentrations of lipophilic contaminants in human breast milk (Harris *et al.*, 2001). In order to understand whether OCs and BFRs are built up during a woman's life, correlation between concentrations of these compounds and age of mothers were examined in primipara samples. No significant correlation was observed between OC levels and age of mothers in this study (data not shown), which may be due to small number of primipara samples ($n = 6$). However, the oldest mother accumulated OCs at highest level, which may support the fact that OCs accumulate with age resulting in higher concentration in older women. In contrast, PBDEs and HBCDs did not correlate with maternal characteristics, either with number of children or age of mothers, as also seen in other studies (Schecter *et al.*, 2003). This may be due to the fact that recent exposures to these two chemicals are still low at present and/or might be accumulation level of these chemicals are higher than the excretion levels in those mothers milk.

Health risk assessment

Daily intakes of OCs and PBDEs by infants' were calculated based on the assumption that the average breast milk consumption of a 5 kg infant is 700 g/day (Oostdam *et al.*, 1999). The infants' tolerable daily intakes (TDI) for OCs in the present study were generally lower than the guideline standards proposed by Health Canada. However, the elevated levels of CHLs in some donors resulted in TDI close to or even higher than the guidelines (Oostdam *et al.*, 1999). This fact may raise greater concern for infant health because they are highly susceptible to effects from environmental contaminants. Unlike OCs, no guideline standard for PBDEs has been proposed for human. The infants' daily intake of total PBDEs via milk in the Philippines are still below the experimental body burden associated with various development effects in neonatal mice and rats (Eriksson *et al.*, 2001; Viberg *et al.*, 2003; Kuriyama *et al.*, 2005), implying minimal risk caused by PBDEs at present. Therefore, it is not clear whether current concentrations of PBDEs in human tissues may be expected to adversely affect human health and we need to have more toxicological studies to assess the infants' health risk.

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REFERENCES

Alaee, M., P. Arias, A. Sjödin and Å. Bergman (2003): An overview of commercially used brominated flame retardants, their applications, their use patterns in different countries/regions and possible modes of release. *Environ. Int.*, **29**, 683–689.

de Wit, C. A. (2002): An overview of brominated flame retardants in the environment. *Chemosphere*, **46**, 583–624.

Eriksson, P., H. Viberg and A. Fredrikson (2001): Brominated flame retardant: A novel class of developmental neurotoxicants in our environment? *Environ. Health Perspect.*, **109**, 903–908.

Gill, U., I. Chu, J. J. Ryan and M. Feedley (2004): Polybrominated diphenyl ethers: Human tissue levels and toxicology. *Arch. Environ. Contam. Toxicol.*, **183**, 55–97.

Harris, C. A., M. W. Woolridge and A. W. M. Hay (2001): Factors affecting the transfer of organochlorine pesticide residues to breast milk. *Chemosphere*, **43**, 243–256.

Hileman, B. (2002): Electronic waste. *Chem. Eng. News*, **80**(26), 15–18.

Kakimoto, K., K. Akutsu, Y. Konishi and Y. Tanaka (2008): Time trend of hexabromocyclododecane in the breast milk of Japanese women. *Chemosphere*, **71**, 1110–1114.

Kuriyama, S. N., E. Chris, G. K. Talsness and I. Chahoud (2005): Developmental exposure to low dose PBDE 99: 1-effects on male fertility and neurobehavior in rat offspring. *Environ. Health Perspect.*, **113**, 149–154.

Minh, N. H., M. Someya, T. B. Minh, T. Kunisue, H. Iwata, M. Watanabe, S. Tanabe, P. H. Viet and B. C. Tuyen (2004): Persistent organochlorine residues in human breast milk from Hanoi and Hochiminh city, Vietnam: contamination, accumulation kinetics and risk assessment for infants. *Environ. Pollut.*, **129**, 431–441.

Oostdam, J. V., A. Gilman, E. Dewailly, P. Usher, B. Wheatley and H. Kuhnlein (1999): Human health implications of environmental contaminants in Arctic Canada: a review. *Sci. Total Environ.*, **230**, 1–82.

Prudente, M., G. Malarvannan and S. Tanabe (2007): Persistent toxic substances in the Philippine environment. *Persistent Organic Pollutants in Asia: Sources, Distributions, Transport and Fate. Developments in Environmental Science (7)* 559–585.

Ramu, K., N. Kajiwara, A. Sudaryanto, T. Isobe, S. Takahashi, A. Subramanian, D. Ueno, J. G. Zheng, P. K. S. Lam, H. Takada, P. M. Zakaria, P. H. Viet, M. Prudente, T. S. Tana and S. Tanabe (2007): Asian mussel watch program: Contamination status of polybrominated diphenyl ethers and organochlorines in coastal waters of Asian countries. *Environ. Sci. Technol.*, **41**, 4580–4586.

Restrepo, B. J., R. Addink, C. Wong, K. Arcaro and K. Kannan (2007): Polybrominated diphenyl ethers and organochlorine pesticides in human breast milk from Massachusetts, USA. *J. Environ. Monit.*, **9**, 1205–1212.

Ryan, J. J., B. C. Wainman, A. Schecter, J. Moisey, I. Kosarac and W. F. Sun (2006): Trends of the brominated flame retardants, PBDEs and HBCD, in human milks from North America. *Organohalogen Compd.*, **68**, 778–781.

Schecter, A., M. Pavuk, O. Päpke, J. J. Ryan, L. Birnbaum and R. Rosen (2003): Polybrominated diphenyl ethers (PBDEs) in U.S. mothers' milk. *Environ. Health Perspect.*, **111**, 1723–1729.

Sjödin, A., G. Donald, D. G. Patterson and Å. Bergman (2003): A review on human exposure to brominated flame retardants-particularly polybrominated diphenyl ethers. *Environ. Int.*, **29**, 829–839.

Solomon, G. M. and P. M. Weiss (2002): Chemical contaminants in breast milk: time trends and regional variability. *Environ. Health Perspect.*, **110**, 339–347.

Tanabe, S. (2006): Environmental specimen bank in Ehime University (*es*-BANK), Japan for global monitoring. *J. Environ. Monit.*, **8**, 782–790.

Tomy, G. T., W. Budakowski, T. Halldorson, D. M. Whittle, M. J. Keir, C. Marvin, G. MacInnis and M. Alaee (2004): Biomagnification of alpha- and gamma-hexabromocyclododecane isomers in a Lake Ontario food web. *Environ. Sci. Technol.*, **38**, 2298–2303.

Tsydenova, O., A. Sudaryanto, N. Kajiwara, T. Kunisue, B. V. Batoev and S. Tanabe (2007): Organohalogen compounds in human breast milk from Republic of Buryatia, Russia. *Environ. Pollut.*, **146**, 225–232.

Ueno, D., N. Kajiwara, H. Tanaka, A. Subramanian, G. Fillmann, P. K. S. Lam, G. J. Zheng, M. Muchtar, H. Razak, M. Prudente, K. H. Chung and S. Tanabe (2004): Global pollution monitoring of polybrominated diphenyl ethers using Skipjack Tuna as bioindicator. *Environ. Sci. Technol.*, **38**, 2312–2316.

Ueno, D., M. Alaee, C. Marvin, D. C. G. Muir, G. Macinnis, E. Reiner, P. Crozier, I. V. Furdui, A. Subramanian, G. Fillmann, P. K. S. Lam, G. J. Zheng, M. Muchtar, H. Razak, M. Prudente, K. H. Chung and S. Tanabe (2006): Distribution and transportability of hexabromocyclododecane (HBCD) in the Asia-Pacific region using skipjack tuna as a bioindicator. *Environ. Pollut.*, **144**, 238–247.

Viberg, H., A. Fredrikson and P. Eriksson (2003): Neonatal exposure to polybrominated diphenyl ether (BDE 153) disrupts spontaneous behavior, impairs learning and memory, and decreases hippocampal cholinergic receptors in adult mice. *Toxicol. Appl. Pharmacol.*, **192**, 95–106.

Wu, N., T. Herrmann, O. Paepke, J. Tickner, R. Hale, E. Harvey, M. La Guardia, M. D. McClean and T .F. Webster (2007): Human exposure to PBDEs: Associations of PBDE body burdens with food consumption and house dust concentrations. *Environ. Sci. Technol.*, **41**, 1584–1589.

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