Residues of persistent organic pollutants (POPs) in human milk in Hong Kong

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A B S T R A C T

Data on pesticide body load in the south China region are scarce. Here, we report the concentrations of 24 persistent organic pollutants (POPs), in 10 pools of human milk samples, collected at 2–6 weeks postpartum from 238 primiparous women living in Hong Kong and south China, who participated in the 2002–2003 WHO exposure study. Residues were determined by gas chromatography with electron capture detector and confirmed by gas chromatography with mass spectrometry. The mean levels of alpha-HCH (mean 0.6 ng g−1 fat), beta-HCH (940 ng g−1 fat), gamma-HCH (1.8 ng g−1 fat), dieldrin (1.0 ng g−1 fat) and HCB (21.8 ng g−1 fat) were much lower than the 1985 estimates. Mean levels of alpha-HCH, gamma-HCH, dieldrin, cis-heptachlor-epoxide (0.7 ng g−1 fat), sum-chlordane (6.1 ng g−1 fat), trans-nonachlor (12.0 ng g−1 fat), BDE 47 (1.9 ng g−1 fat) and sum-PBDE (3.4 ng g−1 fat) were comparable to the international median levels of the 15 other countries participating in the 2002–03 WHO exposure study. Hong Kong had the highest level of beta-HCH, possibly a residual effect of previous high exposures in the 1970s. Body loads of beta-HCH and chlordane were lower among mothers with younger age while mothers born in mainland China had lower levels of beta-HCH, cis-heptachlor-epoxide, oxy-chlordane and trans-nonachlor. Levels of toxaphene, endrin, endosulfan, bromocyclene and nitrofen were not detected in all or almost all of the milk pools. Continuous monitoring of POPs in human milk, especially beta-HCH, is needed for surveillance and interpretation of time trends, and for linkage to strict enforcement of agricultural regulations.

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1. Introduction

The Stockholm Convention (http://www.pops.int/) was ratified by China, including the Hong Kong Special Administrative Region, in 2004. To achieve the objective of the convention in the protection of human health and the environment from persistent organic pollutants (POP), participating parties should develop action plans to reduce release of these compounds. In Hong Kong, the use of pesticides has been reduced along with the decline of farming activities over several decades. Even so POPs are ubiquitous environmental contaminants and organochlorine pesticides have been reported in ambient air (Louie and Sin, 2003), sediments (Richardson and Zheng, 1999) and fish (Chan et al., 1999) in Hong Kong. Food is the major source of exposure of humans to POPs, therefore the degree of exposure in the population may be estimated from levels of POPs in foods and the consumption of those foods. Such estimates require large amounts of data, particularly in Hong Kong where most of the food is imported, and are subject to a high degree of uncertainty, but by measuring the concentrations of POPs in human tissues, these problems are largely avoided. Assessing the concentration of POPs in humans is also vital for formulating regional strategies and for assessing the long-term usefulness of these strategies in protecting human health. Regular monitoring of POPs in humans can identify
 exposure trends, provide a surveillance system and help identify specific sources of pollutants.

Continuous and systematically collected representative data on pesticide residues in the populations in this region has been lacking. In Hong Kong, only a few POPs, including hexachlorocyclohexanes (HCH), hexachlorobenzene (HCB), lindane and polychlorinated biphenyls (PCB), have been previously documented in the population (Ip, 1983; Ip and Phillips, 1989; Wong et al., 2002). Very few similar studies have been done in mainland China (Kunisue et al., 2004) and the levels in humans of some POPs listed in the Stockholm Convention (including aldrin, chlordane, endrin, heptachlor, mirex and toxaphene) have never been determined in Hong Kong.

POPs are transferred to the fetus and expressed in human milk during lactation and concentrations in human milk from primiparae mothers represent life-long exposure. POPs levels decrease with subsequent pregnancies and prolonged lactation. The WHO Regional Office for Europe (WHO/EURO) initiated a series of international studies to monitor the concentrations of polychlorinated dibenzo-para-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and PCBs in human breast milk of primiparous women (Yrjanheikki, 1989; WHO/EO, 1996; Malisch and van Leeuwen, 2002a). In 2002, 13 pools of milk samples from Hong Kong mothers, comprising 316 individual samples from “first-time” mothers giving birth in Hong Kong were analyzed in the 2002–2003 WHO/EURO study on PCDDs, PCDFs and PCBs in human breast milk (Hedley et al., 2006). This study was co-sponsored by the WHO Global Environment Monitoring System/food Contamination Monitoring and Assessment Programme (GEMS/Food). In order to monitor the human exposure to other POPs, WHO completed additional analyses on the concentration of various POPs in milk samples from 16 participating countries and regions, including 10 pooled samples from Hong Kong (Malisch et al., 2008). This report presents the concentrations of POPs determined in this survey and their comparison with levels from previous local studies and contemporary world-wide levels.

2. Materials and methods

Milk samples were collected at 2–6 weeks postpartum from 316 primiparae women who gave birth to a singleton during the period December 2001 to September 2002 in Hong Kong (Hedley et al., 2006). Mothers were interviewed face-to-face to collect dietary and residential information. The questionnaire design, milk sampling method and pooling strategy were adapted from the protocol for the 2002–2003 WHO co-ordinated dioxin exposure study (Malisch and van Leeuwen, 2002a). In 2002, 13 pools of milk samples from Hong Kong mothers, comprising 316 individual samples from “first-time” mothers giving birth in Hong Kong were analyzed in the 2002–2003 WHO/EURO study on PCDDs, PCDFs and PCBs in human breast milk (Hedley et al., 2006). This study was co-sponsored by the WHO Global Environment Monitoring System/food Contamination Monitoring and Assessment Programme (GEMS/Food). In order to monitor the human exposure to other POPs, WHO completed additional analyses on the concentration of various POPs in milk samples from 16 participating countries and regions, including 10 pooled samples from Hong Kong (Malisch et al., 2008). This report presents the concentrations of POPs determined in this survey and their comparison with levels from previous local studies and contemporary world-wide levels.

Ten pools of human milk, each comprising samples from 12 to 42 mothers, a total of 238, each represented relatively homogeneous characteristics in terms of the mothers’ residential background (Hong Kong, Mainland China, China Immigrant and Overseas), dietary habits (consumption of dairy products and seafood in Pools 2–7) and smoking history (Pool 1) (Table 1). All participants gave written consent before taking part in the study. The study was approved by the Ethics Committees of the University of Hong Kong, the Chinese University of Hong Kong and the Department of Health, Hong Kong SAR Government.

Mass concentrations of POPs were determined by gas chromatography (GC) with electron capture detector (ECD) and confirmed by GC with mass spectrometry (GC/MS) in the State Institute for Chemical and Veterinary Analysis of Freiburg, Germany. This laboratory has successfully participated in 35 proficiency tests in 1994–2004. The quality control procedures followed the Guidelines for Residues Monitoring in the European Union – Quality Control Procedures for Pesticide Residues Analysis – second edition 1999/2000, Document No. SANCO/3103/2000, updated by third edition 2003, Document No. SANCO/10476/2003 of 05/February/2004. The following relevant criteria were performed for each batch, amongst others: Reagent blanks were analyzed by performance of a complete analysis using the solvents and reagents only, in the absence of any sample. Different quality control samples of certified reference material were analyzed. Uncontaminated fat samples were analyzed as QC/IA samples after spiking of all the analytes that were reported. The recovery rates of the internal standards in the samples as well as the analytes in the QC/IA samples were in the range of 70–120% which met the requirements of the Guidelines. Calibration was based on a multilevel (3 or 4 levels) calibration curve including bracketing calibration to control the drift of the relative response.

POPs were extracted from freeze-dried human milk samples by means of a continuous hot extraction device (Twisselmann extractor) with ethanol/toluene (70/30) for 8 h. The crude fat extract was purified with butyl methyl ether (Malisch and van Leeuwen, 2002b). Up to 0.5 g of the fat extract was dissolved in cyclohexane/ethyl acetate (1:1 v/v) and the internal standards 2,4,5-trichlorobiphenyl and mirex were added.

The clean-up parts of the analytical method follow the principles of the European standardized methods, Fatty food-Determination of pesticides and PCBs, EN 1528 part 1–4, 1996-10 (confirmed 2001). To remove the fat, gel permeation chromatography was performed on a chromatography column (length 580 mm, 25 mm i.d., filling level 330 mm) using Bio-Beads S-X3 with cyclohexane/ethyl acetate (1:1 v/v) as eluting solvent. The eluate was concentrated with iso-octane added and evaporated to about 1 ml. Chromatography on a small column of partially deactivated silica gel was determined as the final clean-up step using toluene as eluent. The silica gel (70–230 mesh) was heated overnight at 130 °C and allowed to cool in a desiccator. After adding 1.5% of water, it was shaken for 30 min and then stored in a tightly sealed container. The chromatographic tube was packed with 1 g of deactivated silica gel.

Routine determination was performed with GC/ECD using a GC (Fisons Mega 2) with two custom-made columns of different polarity parallel (fused silica no. 1: 30 m PS-088 [97.5% dimethyl-2.5% diphenyl siloxane copolymer], 0.32 mm i.d., 0.32 μm film thickness, fused silica no. 2: 30 m OV-1701-OH, 0.32 mm i.d., 0.25 μm film thickness). Results were confirmed by GC–MS (GC: HP 6890/MS: HP 5973; 30 m HP-5MS, 0.25 mm i.d., 0.25 μm film thickness + 2.5 m pre-column; detection mode: MSD-El). PBDEs were determined by GC/MS-El, SIM mode. Identification and quantification of the analytes was based on choice of the masses of one target-ion and three qualifier-ions.
Table 1
Concentrations (ng g\(^{-1}\) fat) of pesticides in 10 pooled milk samples from mothers delivered in Hong Kong.

| Pool number | Pool characteristics | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | Mean
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<tbody>
<tr>
<td></td>
<td>Hong Kong-eversmokers</td>
<td>17.0</td>
<td>18.2</td>
<td>22</td>
<td>20.0</td>
<td>27</td>
<td>18.5</td>
<td>29.6</td>
<td>24.2</td>
<td>18.1</td>
<td>17.9</td>
<td>21.8</td>
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<td></td>
<td>Hong Kong-high dairy</td>
<td>0.7</td>
<td>0.7</td>
<td>2</td>
<td>1.4</td>
<td>2</td>
<td>nd</td>
<td>nd</td>
<td>&lt;0.5</td>
<td>1.4</td>
<td>1.8</td>
<td>1.0</td>
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<tr>
<td></td>
<td>Hong Kong-high seafood</td>
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<td>Hong Kong-low dairy and seafood</td>
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<td>0.8</td>
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<td>1</td>
<td>0.9</td>
<td>1</td>
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<td>0.9</td>
<td>1.00</td>
<td>0.7</td>
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<td>Mainland China-high dairy/seafood</td>
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<td></td>
<td>China immigrants-2–6 years in Hong Kong</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
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<td>nd</td>
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<tr>
<td></td>
<td>China immigrants-7 years + in Hong Kong</td>
<td>&lt;0.5</td>
<td>&lt;0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
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<td>0.5</td>
<td>0.5</td>
<td>0.6</td>
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<tr>
<td></td>
<td>Overseas-stay for 1–10 years</td>
<td>4.9</td>
<td>6.2</td>
<td>9</td>
<td>8.2</td>
<td>9</td>
<td>2.7</td>
<td>2.4</td>
<td>3.5</td>
<td>6.7</td>
<td>9.4</td>
<td>6.1</td>
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<tr>
<td></td>
<td>Mean</td>
<td>2.7</td>
<td>2.4</td>
<td>3.5</td>
<td>6.7</td>
<td>9.4</td>
<td>9.4</td>
<td>6.1</td>
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\(a\) nd – the level is below the Limit of Detection (LOD). If the level was found to be between LOD and Limit of Quantification (LOQ), it will be stated as <LOQ (1 ng g\(^{-1}\) fat for Parlar compounds; 0.5 pg g\(^{-1}\) fat for others, except for Pools 3 and 5 where 0.5 ng g\(^{-1}\) fat for PBDE and 1 ng g\(^{-1}\) fat for others).

\(b\) High dairy means 17 highest dairy product intake and dairy product intake >2.5 \(\times\) seafood intake. High seafood means 17 highest seafood intake and seafood intake >2.5 \(\times\) dairy product intake. Low dairy and seafood means <2 kg month\(^{-1}\) dairy product intake and <2 kg month\(^{-1}\) seafood intake. High dairy/seafood means >2 kg month\(^{-1}\) dairy product intake OR >2 kg month\(^{-1}\) seafood intake.

\(c\) Mean was weighted by the number of mothers in each pool. (Undetected levels “nd” were assumed to be zero and those between LOD and LOQ were assumed to be half of the LOQ.)
The contents of the following 24 POPs were determined: HCB, dieldrin, endrin, endrin ketone, cis-heptachlorepoxide, alphachlordane, gamma-chlordane, oxy-chlordane, trans-nonachlor, toxaphene congeners (Parlar 26, Parlar 50 and Parlar 62), alpha-HCH, beta-HCH, gamma-HCH, sum endosulfan, bromocyclene, nitrofen and PBDE (polybrominated diphenylether) congeners (BDE 28, BDE 47, BDE 100, BDE 99, BDE 154 and BDE 153). Data on DDT and its metabolites has been reported elsewhere (Hui et al., 2008). The concentrations of chemicals are reported as nano-grams per gram of milk fat (ng g⁻¹). The limit of quantification (LOQ) was 1 ng g⁻¹/C₀. The limit of detection (LOD) was 0.1 ng g⁻¹/C₀ for all POPs except for toxaphene congeners 0.5 ng g⁻¹/C₀. The mean level for each pesticide residue was calculated by weighing the number of mothers in each pool, with the assumption of zero for undetected values and half LOQ for levels determined between LOD and LOQ. The level designated undetected (nd) if it was below LOD. Levels between LOD and LOQ were stated as <LOQ.

Data analysis was performed by using the Statistical Package for Social Sciences (SPSS for Windows, version 10.1; SPSS Inc., Chicago, United States). Correlation between mothers’ characteristics and POP concentrations was assessed by Spearman rank correlation coefficients.

3. Results

3.1. Levels of POPs in human milk

Nine out of twenty-four compounds analyzed in this study (i.e. endrin, endrin ketone, alpha-chlordane, gamma-chlordane, Parlar 62, sum endosulfan, bromocyclene, nitrofen and BDE 154) were not detected in all 10 pooled milk samples (Table 1).

Eight other compounds were detected in all ten pools including HCB (range: 17.0–29.6 ng g⁻¹/C₀; mean: 21.8 ng g⁻¹/C₀), cis-heptachlorepoxide (<0.5–1.0 ng g⁻¹/C₀; 0.7 ng g⁻¹/C₀), oxy-chlordane (2.4–9.4 ng g⁻¹/C₀; 6.1 ng g⁻¹/C₀), trans-nonachlor (4.1–18 ng g⁻¹/C₀; 12.0 ng g⁻¹/C₀), alpha-HCH (<0.5–1.0 ng g⁻¹/C₀; 0.6 ng g⁻¹/C₀), beta-HCH (288–1380 ng g⁻¹/C₀; 940 ng g⁻¹/C₀), BDE 47 (0.9–4.6 ng g⁻¹/C₀; 1.5 ng g⁻¹/C₀) and BDE 153 (0.6–1.4 ng g⁻¹/C₀; 1.0 ng g⁻¹/C₀).

Dieldrin with concentrations ranging from <0.5–2 ng g⁻¹/C₀ was detected in eight pools but not in Pools 6 and 7 comprising mothers from mainland China, while gamma-HCH was found in all but one pool (range <0.5–8.3 ng g⁻¹/C₀). Parlar 26, Parlar 50, BDE 28, BDE 100 and BDE 99 were detected in some pools at levels under or very close to the LOQ.

3.2. Comparison with previous local studies

Most of the insecticide and herbicide residues in human milk were determined for the first time in this region and so their trends in body loads cannot be deduced. The concentrations of the pesticides, HCB, dieldrin and beta-HCH were documented in the two previous studies using human milk samples collected in 1979 (Ip, 1983) and 1985; (Ip and Phillips, 1989). An apparent decreasing trend in these three pesticide residues in human milk in Hong Kong was observed in the 1980s and a further decrease was found in the present study (Table 2). Lower levels of alpha-HCH and gamma-HCH were also found compared with the 1985 level (Ip and Phillips, 1989).

3.3. Factors associated with body load

We analyzed social and demographic factors associated with the measured POPs concentrations. Levels of beta-HCH, cis-heptachlorepoxide, sum-chlordane and trans-nonachlor, were lower in milk pools from mothers who had mainly lived in mainland China (Fig. 1A–D) but no geographic specific characteristics were demonstrated for HCB and PBDE (Fig. 1E and F). A strong positive correlation between mother’s age and concentrations of beta-HCH and sum-chlordane (Fig. 2A and B) suggested that body load for these pesticides was age dependent, but there was no age relationship for other pesticides.

No specific trend was observed between pesticide levels in human milk and other characteristics of mothers, including smoking habits, body mass index and dietary habits for seafood, meat and dairy products (data not shown).

4. Discussion

4.1. Decreasing trend in levels of POPs listed in the Stockholm Convention

This is the first report on the human body load of a wide range of POPs including most of the organochlorine pesticides listed in the Stockholm Convention in a representative population sample from women living in Hong Kong and mainland China. These data serve as a baseline for further monitoring of exposure to POPs and can be used to formulate action plans based on the Stockholm Convention for this region. The results indicate there has been a decreasing trend in the concentrations of the body levels of HCH isomers, dieldrin and HCB in Hong Kong since their first determination in the 1970s and similar decreasing trends have been documented for DDT (Hui et al., 2008). The decrease recorded in the 1980s is probably attributable to the decline in local farming and the reduced use of pesticides. Since mainland China is the major food source for Hong Kong and was one of the biggest users of HCH containing pesticides (Li, 1999), the bans and restrictions on pesticide use in mainland China that started in 1983, would have resulted in a further decrease in pesticide body load in this population. The higher beta-HCH level in Hong Kong mothers compared to mothers from mainland China is probably due to their older age (Dirtu et al., 2006; Thomas et al., 2006) and a habitual higher seafood and other animal food intake (Woo et al., 1999).

4.2. Comparison with other regions

Being part of the 2002–2003 WHO POPs exposure study, a valid comparison with contemporary levels on the body load of a wide range of POPs in other participating countries can be made. The
mean levels of alpha-HCH, gamma-HCH, HCB and dieldrin in our sample are comparable to the median levels (respectively 0.5 ng g\(^{-1}\) fat, 1.4 ng g\(^{-1}\) fat, 16.5 ng g\(^{-1}\) fat and 3.7 ng g\(^{-1}\) fat) of the 27 international samples (two from Hong Kong [Pools 3 and 5] and 25 from 15 other countries including Brazil, Bulgaria, Czech Republic, Egypt, Fiji, Germany, Ireland, Italy, Luxemburg, Norway, Philippines, Russia, Spain, Ukraine and USA) in which POPs levels were determined in the 2002–2003 WHO exposure study (Personal Communications, R. Malisch). The Hong Kong levels of beta-HCH were about forty times higher than the WHO median level (25.3 ng g\(^{-1}\) fat), even though its content in human milk has decreased to less than 10% of its 1980 estimated level. The concentrations of lindane in fish (Chan et al., 1999) and vegetables (Personal Communications from local Government Laboratory) purchased in Hong Kong were low comparing with the Acceptable Daily Intake, therefore the high beta-HCH content found in Hong Kong milk samples probably reflects a residual effect from the very high exposure in the 1970s (Ip, 1983).

Levels of toxaphene, endrin and its metabolites were undetectable in most of the 10 Hong Kong milk pools indicating that these pesticides are not used in our region. Two organochlorine pesticides determined for the first time in Hong Kong, cis-heptachlor epoxide and oxy-chlordane, were detected in all of the Hong Kong milk pools but their levels were lower than the median of the samples from other participating countries in the 2002–2003 WHO study. No trend in body load can be inferred from these data, but the findings suggest that the Hong Kong population is not currently exposed to high levels of these four POPs.

4.3. POPs not listed in Stockholm Convention

In contrast to the POPs which peaked in the 1970s and now show an apparent downward trend, the body load of PBDE showed an increasing trend until the mid-1990s causing concern in several countries, including the USA (Petreas et al., 2003), Sweden (Meironyte and Noren, 1999) and Germany (Schroter-Kermani et al., 2000). Food, especially fatty fish from contaminated areas, is a major source of exposure (Sjodin et al., 2003). The Pearl River Delta is adjacent to areas associated with heavy electronic industry and unregulated disposal of e-wastes in mainland China (Puckett et al., 2002; Schmidt, 2002), creating risks of increasing exposure to PBDE (Martin et al., 2004). Although both body loads of PBDE in
humans and concentrations of PBDE in sediments Hong Kong and Pearl River Delta (Wang et al., 2007) were relatively low compared with other countries, close monitoring of the levels and time trends of PBDE in both the environment and human milk is warranted. On the other hand, levels of nitrogen, endosulfan and bromocyclene were undetected in all the Hong Kong human milk samples as well as in the pools from the other countries participating in the 2002–2003 WHO exposure study, indicating a general low global exposure level.

4.4. Factors associated with POPs body load

Similar to a recent surveillance study undertaken in Belgium, maternal age and geographic factor were the strongest within-country determinants of human milk levels of some POPs among first time mothers (Colles et al., 2008). A positive association between pesticide residues and age has been well established (Harris et al., 1999; Sim et al., 1998) and observations in our study showed a similar pattern for some but not all pesticide compounds. Duration of stay in Hong Kong and mainland China was also associated with the concentrations of pesticides such as oxy-chlordane, trans-nonachlor and beta-HCH. Since food intake is the major source of exposure to POPs, we believe such geographic variation is partly attributable to greater consumption of seafood and animal products in Hong Kong than in the mainland (Woo et al., 1999). However, the present study was limited by a relatively small number of samples. Further, well-controlled analyses would be needed to elucidate life-style factors associated with pesticide body load in humans.

5. Conclusion

Although Hong Kong had the highest level of beta-HCH and DDT (Hui et al., 2008) in human milk in the 2002–2003 WHO exposure study, this may be a legacy of previous high exposures of communities in this region rather than resulting from recent local exposure. Given the recent evidence on the increased risk of diabetes by body loads of various POPs detected in unexposed populations (Lee et al., 2006), the public health concerns about the exposure to POPs are not limited to cancers (Wolf and Schecter, 1991) and disruptions of reproductive (Damgaard et al., 2006) and immune systems (Karmaus et al., 2001), but also related to common degenerative diseases which are the leading causes of death in both developed and developing countries. Continuous monitoring of concentrations of POPs, especially DDT and beta-HCH in human milk is needed to reliably determine time trends and to evaluate environmental protection measures. Food is the major source of exposure to POPs in humans, therefore close monitoring of the POPs in food is needed to avoid any dietary exposure from new source of contaminants. Despite the presence of such contaminants in human milk, it is important to emphasize that public health recommendations are still to strongly support breastfeeding on the basis of its significant gains on the development and growth of infants and children.

Acknowledgement

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