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Assessment of dioxins and dioxin-like polychlorinated biphenyls in the palm oil supply chains

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RESEARCH ARTICLE

Abstract

Samples of palm oil and palm kernel oil (PKO) products were collected from two palm oil processing premises which were named as premise A and B. These samples were then analysed for dioxins and dioxin-like polychlorinated biphenyls (PCBs) content. The concentration of dioxins in the palm oil and PKO samples from premise A ranged from 0.128 World Health Organization-toxic equivalency (WHO-TEQ) pg/g fat to 0.189 WHO-TEQ pg/g fat while the concentration in samples collected from premise B ranged from 0.149 WHO-TEQ pg/g fat to 0.540 WHO-TEQ pg/g fat. Meanwhile, dioxin-like PCBs concentration ranged from 0.336 WHO-TEQ pg/g fat to 0.546 WHO-TEQ pg/g fat for premise A and 0.345 WHO-TEQ pg/g fat to 0.346 WHO-TEQ pg/g fat for premise B. The total numbers of samples analysed were 35 and the levels exhibited were below the maximum level of 0.75 WHO-TEQ pg/g fat and 1.25 WHO-TEQ pg/g fat imposed by the European Commission for dioxins and the sum of dioxins + dioxin-like PCBs content in vegetable oils.

Keywords: contamination, dioxins, dioxin-like PCBs, palm oil, palm kernel oil

1. Introduction

Polychlorinated dibenzo-p-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs), collectively known as dioxins and polychlorinated biphenyls (PCBs) are three major persistent organic pollutants (POPs) that are found widely all over the world. Centers for Disease Control (1994, 1998, 2000) has classified these chemical compounds as toxic, stable, do not break down easily and ubiquitously presence in the environmental and food matrices. To date, there are 75 congeners of PCDDs and 135 congeners of PCDFs, and out of these congeners only 7 PCDDs and 10 PCDFs congeners are considered to be toxic. The toxicity and persistence of the congeners depends on the placement of the chlorine atoms in the structure. The most potent and toxic congener within these groups of compounds is 2,3,7,8-tetrachlorodibenzo-p-dioxin (Van den Berg *et al.*, 1998). However, for PCBs there are 209 congeners which comprise non-planar and co-planar configuration. Coplanar PCBs are considered to be most toxic and they are also referred to as dioxin-like PCBs. Dioxin-like PCBs have similar toxicity with dioxins compounds and from 209 congeners of PCBs only 12 of these are classified as dioxin-like PCBs.

Currently, food is one of the major sources of human exposure to dioxins and dioxin-like PCBs due to the lipophilicity nature of the compounds and resistance to metabolic degradation (Van Leeuwen *et al.*, 2000). More than 90% of human exposure to dioxins and dioxin-like PCBs are derived from foodstuffs. Foods, particularly dairy products, meat, oils and fats and fish have been identified as the primary immediate sources of intake of dioxins and dioxin-like PCBs for the general population (Schecter *et al.*, 1997). According to Charnley and Doull (2005), the largest contribution to total dioxins intake for the USA population is meat, followed by dairy products, at 32.3 and 16.4%, respectively, whereas fats and oils contribute 1.5% of the estimated dioxins dietary intakes. Meanwhile for Dutch population the total dietary intake for dioxins and dioxin-like PCBs are mostly contributed by dairy products followed by meat with 27 and 23%, respectively. The contribution of edible oils and fats and fish are 17 and 16%, respectively (Baars *et al.*, 2004). In Asia, Hsu *et al.* (2007) had conducted a total diet study to estimate dioxins and dioxin-like PCBs intake from food in Taiwan. Food samples from 14 food types were collected from 11 locations in Taiwan. The food collected was categorised into 5 groups which were meat and meat products, muscle meat of fish and fishery products, milk and dairy products, fat and oil and lastly eggs. Muscle meat of fish and fishery products contributed the most with 49% estimated monthly intake, followed by meat and meat products with ca. 20% and fat/oil 19%. Dairy and dairy products contributed the least to the estimated monthly intake with a value of 5%. The above studies indicate that the fat/oil is also one of the contributors to the total daily intake of dioxins and dioxin-like PCBs in human diet.

In Malaysia, the primary oil used for cooking is palm oil. Almost every Malaysian use palm oil as a frying medium because it is cheaper compared to other vegetable oils. Other than frying, palm oil products are also widely used in food industry such as in the production of margarine, shortening, ghee and vanaspati. Malaysian palm oil is exported to over 90 countries all over the world and the major importers are China, Pakistan and the European Union. Palm oil also contributed about 26.7% to the world oil market and 37% of that comes from Malaysia (Oil World, 2011). Since palm oil is the major frying oil in the country and Malaysia is also one of the largest producers of palm oil in the world, continuous monitoring of this vegetable oil and its products for dioxins and dioxin-like PCBs contamination is pertinent. This is important to ensure that palm oil and its products are free from contamination and thus will increase the consumers' confidence towards the safety of palm oil products.

The palm fruit consist of an outer layer known as mesocarp which surrounds the kernels. The mesocarp and kernel are extracted to obtain crude palm oil (CPO) and crude palm kernel oil (CPKO), respectively. The CPO is processed into various products which include refined, bleached, deodorised (RBD) palm olein and palm stearin; neutralised bleached deodorised (NBD) palm olein and palm stearin. The kernel of the palm fruits is processed into a variety of different products, namely (1) CPKO; (2) RBD palm kernel oil (PKO); (3) NBD palm kernel olein; and (4) NBD palm kernel stearin. All of these products have to undergo various processing before they are used to manufacture food products. This study aim to assess the level of dioxins and dioxin-like PCBs in the palm oil supply chains. Data from this study could also be used to alleviate consumers' anxiety on the presence of dioxins and dioxin-like PCBs in palm oil products.

2. Materials and methods

Sample collection

The palm oil products were collected at the mill and refinery. The collection of samples was carried out at two different palm oil premises and for the purpose of this study they were named as premise A and premise B. Figure 1 shows the flow of palm oil processing. Samples collected at the mill were CPO from three different stages which were from production line, storage tank and transportation tanker. As for the refinery, the samples collected consisted of (1) CPO for feed oil; (2) bleached palm oil (BPO); (3) RBD palm oil; (4) RBD olein; (5) RBD stearin; (6) neutralised palm oil; (7) neutralised BPO; (8) NBD olein; (9) NBD stearin; (10) CPKO; (11) bleached palm kernel oil (BPKO); (12) RBD PKO; (13) neutralised palm kernel oil; (14) neutralised bleached PKO; (15) NBD PKO; (16) NBD palm kernel olein; and (17) NBD palm kernel stearin. The palm oil products from refinery were also collected in the three stages as in the mill. The collected palm oil products were then stored in amber glass bottles.

Chemical, reagents and apparatus

Pesticide grade hexane, dichloromethane, acetone and toluene were purchased from Fisher Scientific (Loughborough, UK). Sulphuric acid was obtained from Labscan Asia (Bangkok, Thailand). PCB free classical disposable basic silica ABN column, PCB free disposable basic alumina column and PCB free disposable carbon/celite column were purchased from Fluid Management System Inc. (Watertown, MA, USA). Beaker and test tube were purchased from Schott Duran (Mainz, Germany) while pear shape flasks were obtained from Favorit (Kuala Lumpur, Malaysia). Sodium sulfate anhydrous granular was obtained from Fisher Scientific (Loughborough, UK). Analytical standard solutions of ^{13}C labelled PCDDs/PCDFs and dioxin-like PCBs congeners were obtained from Cambridge Isotope Laboratories (Andover, MA, USA).

Extraction and clean-up of palm oil samples

10 g of palm oil products sample was weighed. The sample was mixed with 140 ml n-hexane and stirred to dissolve. The mixture was spiked with clean-up standard EDF 8999 (contained 15 ^{13}C-labeled tetra to octa PCDD/PCDF congeners) and clean-up standard EC 4937 (contained 12 ^{13}C-labeled tetra to hepta CB congeners). The mixture was added with 10 ml concentrated Sulphuric acid drop by drop until two layers of the solution was formed. The clear supernatant was transferred into pear shaped flask and concentrated using a rotary evaporator. The distilled water was then added into the concentrated sample. The organic phase (samples) was drained through column packed

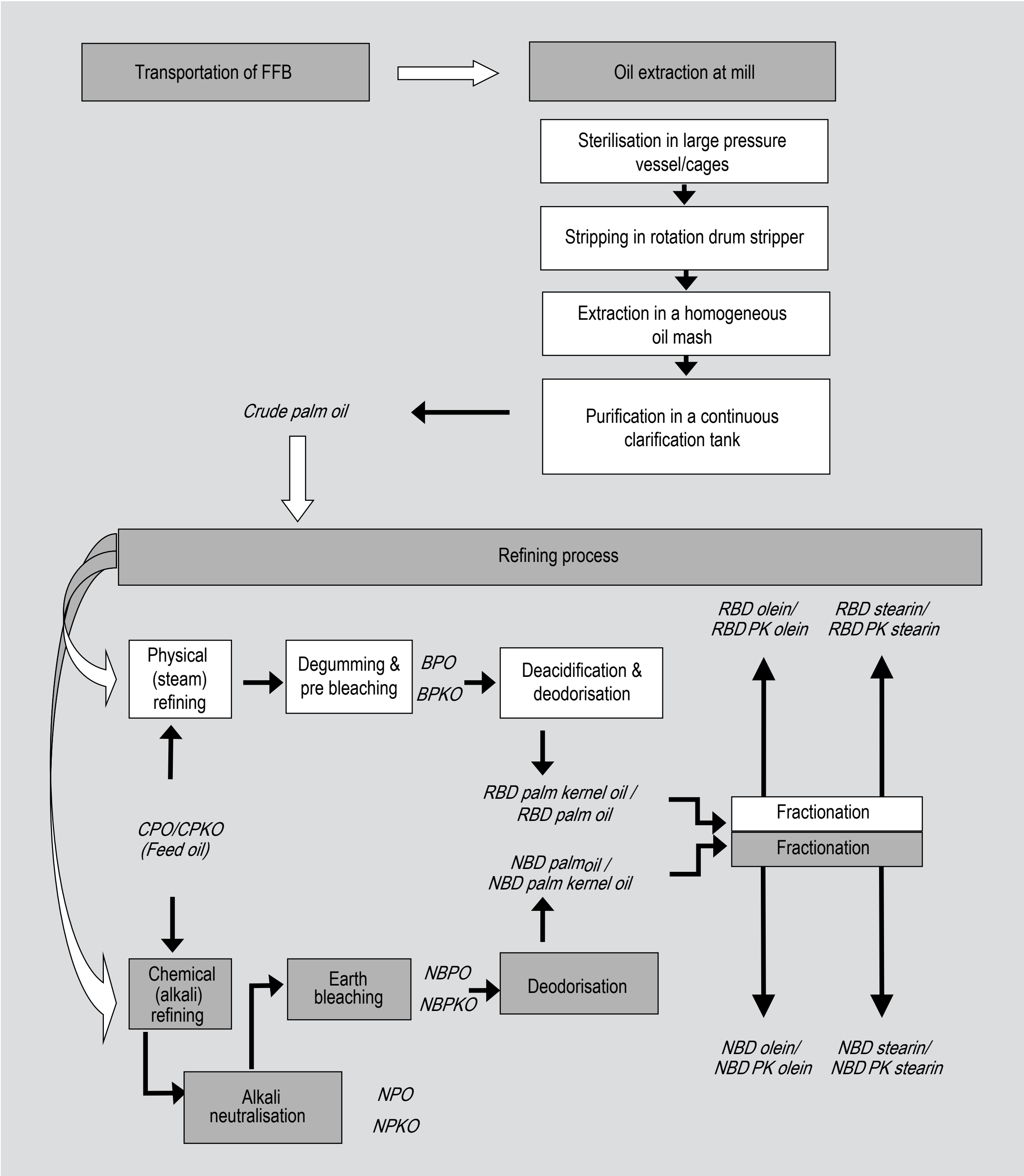

Figure 1. Palm oil processing flow chart. BPKO = bleached palm kernel oil; BPO = bleached palm oil; CPO = crude palm oil; FFB = fresh fruit bunches; NBD olein = neutralised bleached deodorised olein; NBD palm kernel oil = neutralised bleached deodorised palm kernel oil; NBD palm oil = neutralised bleached deodorised palm oil; NBD PK olein = neutralised bleached deodorised palm kernel olein; NBD PK stearin = neutralised bleached deodorised palm kernel stearin; NBD stearin = neutralised bleached deodorised stearin; NBPKO = neutralised bleach palm kernel oil; NBPO = neutralised bleached palm oil; NPKO = neutralised palm kernel oil; NPO = neutralised palm oil; RBD olein = refined bleached deodorised olein; RBD palm kernel oil = refined bleached deodorised palm kernel oil; RBD palm oil = refined bleached deodorised palm oil; RBD PK olein = refined bleached deodorised palm kernel olein; RBD PK stearin = refined bleached deodorised palm kernel stearin; RBD stearin = refined bleached deodorised stearin

with the mixture of Ottawa sand and anhydrous sodium sulfate and reconstituted with hexane to approximately 18 ml. The sample was then subjected to purification using multi column Power-Prep^{TM} clean up system supplied by Fluid Management System Inc.. Two eluates were collected from Power-Prep^{TM} clean-up process: dioxins fraction and dioxin-like PCBs fraction, respectively. The eluates were concentrated using rotary evaporator. The concentrated extracts were transferred into 1.5 ml tapered vial, spiked with internal standard EDF 5999 (contained 2 ^{13}C-labeled tetra and hexa chlorinated dioxins congeners) and internal standard EC 4978 (contained 3 ^{13}C-labeled tri to hepta CB congeners) before further concentrated under a nitrogen stream to approximately 10-15 µl. The final extracts were then transferred into an auto sampler vials for gas chromatography/high resolution mass spectrometry (GC/HRMS) analysis. The recoveries for the samples analysed were in the range of 71 to 85% for dioxins while dioxin-like PCBs were in the range of 50 to 89%. The recoveries for all palm products analysed either for dioxins or dioxin-like PCBs were satisfactory based on USEPA method 1613B, 8290, and 1668A (USEPA, 1994, 1999, 2007).

Instrumentation

The chromatographic separations for dioxins and dioxin-like PCBs congeners were done using GC/HRMS system. Separations for dioxins congener were carried out on Trace GC Ultra gas chromatography coupled to double focusing sector high resolution mass spectrometry (Thermo Fisher Scientific, Bremen, Germany). The MS system was operated at 10,000 resolution with perfluorotributylamine as reference standard for mass calibration. Helium was used as carrier gas at a constant flow rate of 1.5 ml/min. The GC oven temperature was programmed at 130 °C for a hold of 1 min; increased to 215 °C at a rate 42 °C/min; increased to 280 °C at a rate of 4 °C/min; increased to 320 °C at a rate 6 °C/min and hold at the final temperature for 7 min. The chromatography column used was Rtx®-Dioxin2 (60 m, 0.25 mm i.d. capillary column) obtained from Restek (Bellefonte, PA, USA). Meanwhile for dioxin-like PCBs, the chromatographic separation was done using Agilent 6890 Gas Chromatography coupled to MAT 95-XL high resolution mass spectrometry (Thermo Fisher Scientific). The system was operated at 10,000 resolution with perfluorophenanthrene as reference standard for mass calibration. Helium was used as carrier gas at a constant flow rate of 0.8 ml/min. The GC oven temperature was programmed at 130 °C for a hold of 1 min; increased to 215 °C at a rate 42 °C/min; increased to 280 °C at a rate of 4 °C/min; increased to 320 °C at a rate 6 °C/min and hold at the final temperature for 7 min. The chromatography column used was HT8-8% phenyl polycarborane-siloxane (60 m, 0.25 mm i.d capillary column) obtained from SGE International (Ringwood, Australia).

3. Results and discussion

Level of dioxins and dioxin-like PCBs concentration in palm oil products

Table S1 and S2 show World Health Organization-toxic equivalency (WHO-TEQ) concentration of dioxins, dioxin-like PCBs and sum of dioxins and dioxin-like PCBs in palm oil and PKO products collected at mill and refinery of premise A and premise B, respectively. The highest concentration of dioxins from palm oil samples analysed was observed from CPO used as feed oil (0.189 WHO-TEQ pg/g fat) while RBD palm stearin from transportation tanker and RBD palm olein from production line contained the lowest concentration of dioxins with a level of 0.131 WHO-TEQ pg/g fat. As for PKO supply chain, the highest concentration of dioxins was detected in RBD PKO from storage tank (0.132 WHO-TEQ pg/g fat) while the lowest concentration was detected in RBD PKO from production line with a level of 0.128 WHO-TEQ pg/g fat. For refinery B, the highest concentration of dioxins from palm oil supply chain products was observed in the sample of NBD palm olein from the storage tank with 0.323 WHO-TEQ pg/g fat while the lowest was from sample of neutralised palm oil from the production line with a level of dioxins at 0.151 WHO-TEQ pg/g fat. The highest concentration of dioxins in PKO products was recorded for NBD palm kernel olein from storage tank with 0.540 WHO-TEQ pg/g fat, while the lowest concentration was exhibited by neutralised PKO samples with level of 0.149 WHO-TEQ pg/g fat.

Dioxin-like PCBs concentration in palm oil and PKO products for both premises A and B were relatively similar for most of the samples. Table S1 and S2 depicted the concentration of dioxin-like PCBs in the supply chain samples collected from premise A and premise B. For refinery A, dioxin-like PCBs concentration in palm oil products ranged from 0.337 WHO-TEQ pg/g fat to 0.546 WHO-TEQ pg/g fat while for PKO supply chain products the concentration was in the range of 0.336 WHO-TEQ pg/g fat to 0.365 WHO-TEQ pg/g fat. The highest concentration of dioxin-like PCBs in the palm oil and PKO products of refinery A was observed from samples of RBD palm olein (Storage tank) and CPKO of feed oil, respectively. The lowest concentration was observed from sample of CPO for feed oil and RBD PKO from transportation tanker. As for refinery B, the concentration of dioxin-like PCBs for palm oil products ranged from 0.345 WHO-TEQ pg/g fat to 0.346 WHO-TEQ pg/g fat while similar ranged of dioxin-like PCBs concentration were observed for PKO products. Dioxin-like PCBs congeners in the palm products of refinery B were mostly detected below the quantification limit and therefore the limit of quantification values were used to calculate the total concentration (WHO-TEQ) of the samples analysed. This explains the narrow range of dioxin-like PCBs concentration in palm oil and PKO supply chains products. The total concentration of dioxins and dioxin-like PCBs in WHO-TEQ is an important value which will be used for regulatory purposes. TEQ values were calculated by multiplying concentration of each dioxins and dioxin-like PCBS congeners (obtained from GC/HRMS analysis) with WHO-TEF values (Van den Berg *et al.*, 2006) which was then sum up to get total WHO-TEQ concentration for dioxins and dioxin-like PCBs. For monitoring the contaminants in the food and feed samples, the concentration of dioxins and the sum of dioxins + dioxin-like PCBs will be used as a regulatory measure to monitor the level of these contaminants. The commission of European Communities (EC 1881/2006; EC, 2006) has set the maximum level of dioxins and sum of dioxins + dioxin-like PCBs in vegetable oil and its by-products at 0.75 WHO-TEQ pg/g fat for dioxins and 1.25 WHO-TEQ pg/g fat for the sum of dioxins + dioxin-like PCBs. The level of dioxins and sum of dioxins + dioxin-like PCBs in all palm products samples collected from refinery A and refinery B were below the maximum level.

Comparison with other vegetable oils indicates that the level dioxins analysed in this study were in the same range of concentration. Abad *et al.* (2002) reported concentration ranged from 0.155 WHO-TEQ pg/g fat to 0.378 WHO-TEQ pg/g fat for dioxin and 0.145 WHO-TEQ pg/g fat to 0.440 for dioxin-like PCBs in olive oil samples collected from Catalonia, Spain. Meanwhile, Papadopoulus *et al.* (2004) reported the value between 0.23 WHO-TEQ pg/g fat to 0.36 WHO-TEQ pg/g fat and 0.01 WHO-TEQ pg/g fat to 0.09 WHO-TEQ pg/g fat for dioxins and dioxin-like PCBs in the samples of olive oil collected from Greek market. Recent study by Hsu *et al.* (2007) shows an increasing concentration of dioxins and dioxin-like level in vegetable oil analysed. The concentration of the samples analysed ranged from 0.699 WHO-TEQ pg/g fat to 0.758 WHO-TEQ pg/g fat for dioxins and 0.562 WHO-TEQ pg/g fat for dioxin-like PCBs.

Congener profile of PCDDs/PCDFs and dioxin-like PCBs in palm oil samples

Figure 2A shows the congener profile of PCDDs/PCDFs in palm oil and PKO samples collected at refinery A. Congener profile of PCDDs/PCDFs in palm oil and PKO supply chain products of refinery A were mostly dominated by octa-chlorinated dibenzo-p-dioxins (octaCDD). OctaCDD in the sample accounted for more than 80% of the total PCDDs/PCDFs concentration in the samples analysed. Samples from both palm oil and PKO products had a similar pattern of PCDDs/PCDFs congeners. Besides the presence of octaCDD, other PCDDs/PCDFs congeners in the samples comprised 1,2,3,4,6,7,8-heptaCDD > octa chlorinated dibenzofurans > 1,2,3,4,6,7,8-heptaCDF. As for palm product samples from refinery B, octaCDD congener were also the dominant congener in all samples analysed. Figure 2B shows the congener profile of PCDDs/PCDFs in palm oil and PKO samples collected at refinery B. OctaCDD congener in all palm products samples analysed from refinery B constitutes about 74% to 85% of the total concentration. However, the congener profiles of PCDDs/PCDFs in the sample were different between palm products analysed from refinery A and refinery B. In addition, the congener profile was also found to be slightly different between palm oil and PKO products collected from refinery B. For palm samples collected from palm oil products, the congener profile comprised octaCDD > 1,2,3,4,6,7,8-heptaCDD > 2,3,7,8-tetraCDF > 1,2,3,7,8,9-hexaCDD. In the meantime, the congener profile of PCDDs/PCDFs from palm samples collected from PKO supply chain products included octaCDD > 2,3,7,8-tetraCDF > 1,2,3,7,8-pentaCDD > 1,2,3,4,6,7,8-heptaCDD > 2,3,4,7,8-pentaCDF.

Congener profile pattern of dioxin-like PCBs in palm products from refinery A were more varied between each samples. Generally, 2,3,4,4',5-penta chlorinated biphenyl (PCB114) contributed the highest concentration in the most of palm samples analysed from palm oil and PKO products of refinery A. However in some of the samples, other congeners also contributed to the highest concentration as well. As shown in Figure 3A, PCB157 and PCB169 contributed the highest concentration in RBD palm oil and RBD palm olein, respectively. Meanwhile for PKO supply chain products, PCB169 contributed the highest concentration in CPKO and BPKO samples. The varied profile pattern of dioxin-like PCBs in the samples suggested that the contamination was not contributed by a single source but instead the contamination of dioxin-like PCBs in the samples may come from various sources. The congener profile of dioxin-like PCBs in palm oil and PKO supply chain products of refinery B were dominantly influenced by PCB114 and PCB189. Figure 3B shows the congener profile of dioxin-like PCBs in palm oil and PKO supply chain products of refinery B. For palm oil supply chain products, the congener profiles for all samples were dominated by PCB114 followed by PCB189 and PCB123. On the other hands, in PKO supply chain products, the congener profiles for all samples were dominated by PCB189 followed by PCB114 and PCB123. This observation indicated that the contamination of dioxin-like PCBs in the palm oil supply chain products may originate from one single source based on the similar profile observed.

4. Conclusions

The concentration of dioxins and dioxin-like PCBs were found to be below the maximum levels imposed by European Commission directive. This study can alleviate the concern on the presence of dioxins and dioxin-like PCBs in palm products. Hence, palm oil products are safe for human consumption.

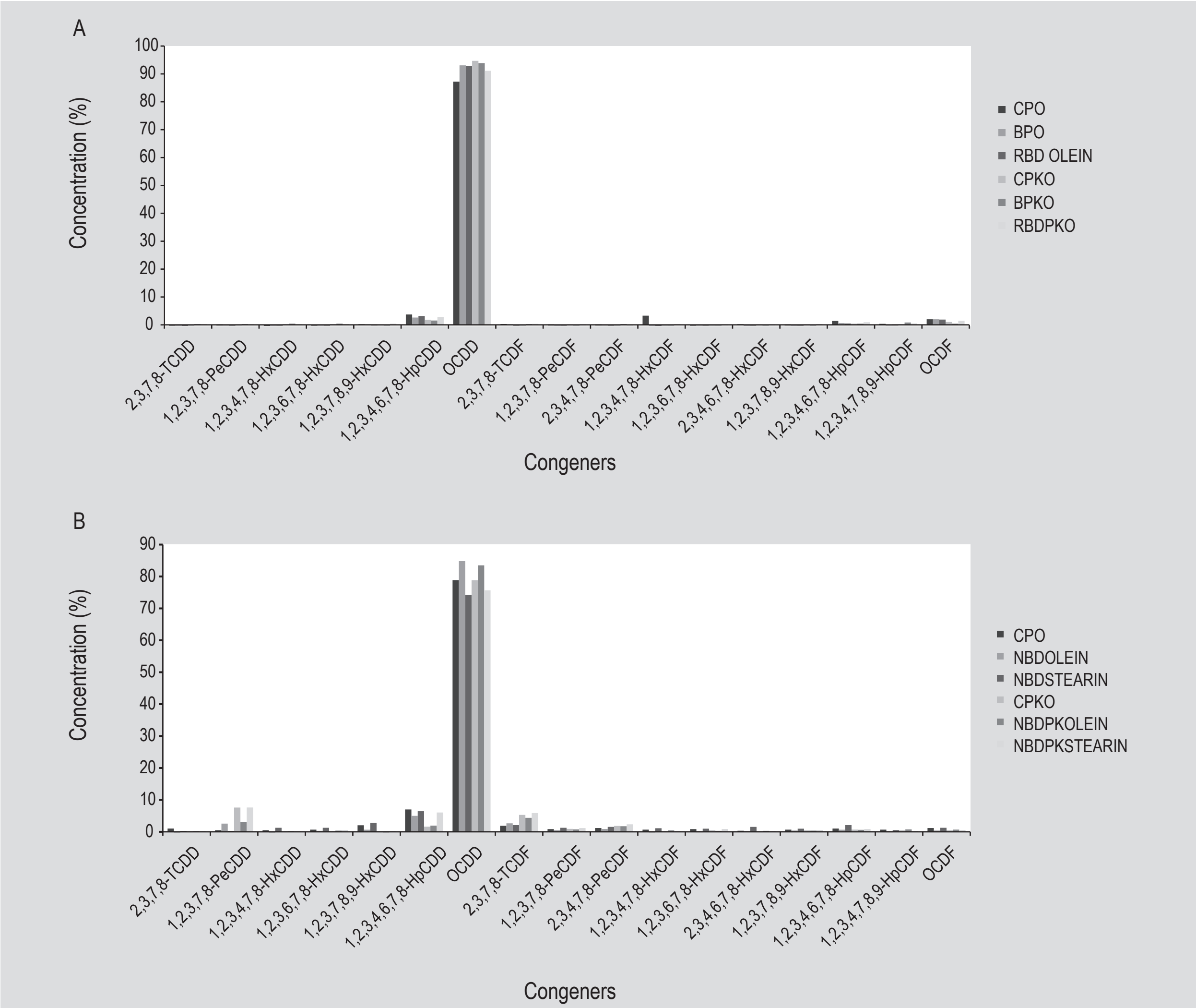

Figure 2. Congener profile of dioxins in palm oil and palm kernel oil supply chain samples collected at (A) refinery A and (B) refinery B. BPKO = bleached palm kernel oil; BPO = bleached palm oil; CPKO = crude palm kernel oil; CPO = crude palm oil; NBD olein = neutralised bleached deodorised olein; NBD PK olein = neutralised bleached deodorised palm kernel olein; NBD PK stearin = neutralised bleached deodorised palm kernel stearin; NBD stearin = neutralised bleached deodorised stearin; RBD olein = refined bleached deodorised olein; RBD palm kernel oil = refined bleached deodorised palm kernel oil.

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Supplementary material

Supplementary material can be found online at http://dx.doi.org/10.3920/QAS2012.0221.

Table S1. World Health Organization-toxic equivalency (WHO-TEQ) concentration (in pg/g) of polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDDs/PCDFs) and dioxin-like polychlorinated biphenyls (PCBs) in palm oil products collected from premise A.

Table S2. World Health Organization-toxic equivalency (WHO-TEQ) concentration (in pg/g) of polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDDs/PCDFs) and dioxin-like polychlorinated biphenyls (PCBs) in palm oil products collected from premise B.

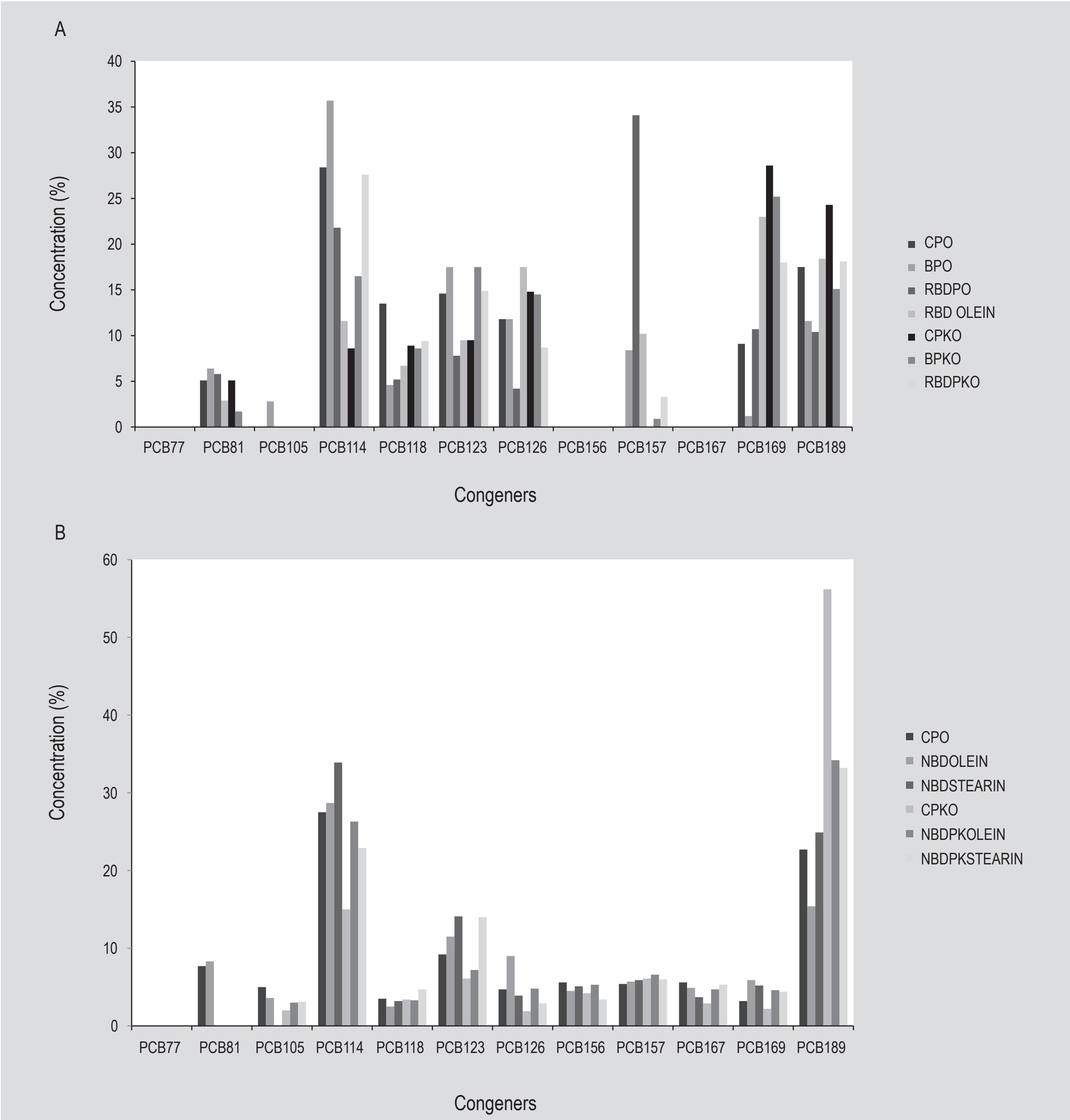

Figure 3. Congener profile of dioxin-like polychlorinated biphenyls (PCBs) in palm oil and palm kernel oil supply chain samples collected at (A) refinery A and (B) refinery B. BPKO = bleached palm kernel oil; BPO = bleached palm oil; CPKO = crude palm kernel oil; CPO = crude palm oil; NBD olein = neutralised bleached deodorised olein; NBD PK olein = neutralised bleached deodorised palm kernel olein; NBD PK stearin = neutralised bleached deodorised palm kernel stearin; NBD stearin = neutralised bleached deodorised stearin; RBD olein = refined bleached deodorised olein; RBD palm kernel oil = refined bleached deodorised palm kernel oil; RBD palm oil = refined bleached deodorised palm oil.

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