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Assessment of Polychlorinated Biphenyls in Some Imported Honey Sold in Warri, Delta State, Nigeria

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ABSTRACT: Even though honey has many health advantages, the presence of pollutants like polychlorinated biphenyls (PCBs) in it could be harmful to consumers' health. Thus, this study assessed polychlorinated biphenyls in imported honey sold in Warri, Delta State, Nigeria to establish the safety of the honey. Ten imported honey samples were purchased from superstores in Warri, Delta State. Honey samples were analyzed for PCBs using a gas chromatograph combined with a mass selective detector (GC-MSD). All the honey samples analyzed tested positive for PCBs. The concentrations of $\Sigma 28$ PCBs in the honey ranged from 0.13 to 2.70 ng/g. There was significant variation in the concentrations of PCBs among these honey samples. The toxic equivalency (TEQ) concentrations for the dl-PCBs in the honey samples ranged 3.69×10^{-5} to 4.3×10^{-2} ng/g. The TEQ concentrations in 40 % of the honey samples were above the European Commission's specified limits of 6.5×10^{-3} ng/g. The estimated daily intakes were above the maximum permissible daily intake of PCBs specified by the World Health Organization. The values of both the hazard index and total cancer risks from human intake of the honey samples were < 1 and 1×10^{-4} respectively in 60 % of the honey suggesting that there are no potential health risks via the honey consumption.

Keywords: Honey, PCBs, TEQ, Risk assessment, Warri

Introduction

Honey is a natural sweet substance produced by bees. Honey is a highly healthy food item because it contains minerals, proteins, lipids, carbohydrates *e.t.c.* (Kadri *et al.*, 2017). Several medicinal and therapeutic effects have been attributed to honey such as wound-healing, anti-inflammatory, cancer prevention, antioxidant capacity, antidiabetic, memory improvement, antimutagenic and skin healing and restoration (El-Nahhal, 2020; Ekakitie *et al.*, 2021a; Ekakitie *et al.*, 2021b; Ekakitie *et al.*, 2022). Given the nutritional and medicinal value of honey, guarantee of the safety of its consumption is crucial to consumer acceptance as its nutritional and health effects can be made of on effect if it is contaminated with PCBs and other toxic chemicals (Darko *et al.*, 2017; Tesi *et al.*, 2024).

PCBs are a group of organic compounds that involved two benzene rings and between 1 and 10 chlorine atoms substituted on each of the benzene rings (Tesi and Iniaaghe, 2020). There are 209 congeners of PCBs among which are 12 PCBs with no chlorine atom or only one chlorine atom at the ortho-position. The benzene rings of these 12 PCBs can rotate and can adopt the coplanar structure of the dioxins making them have the same toxicity as the dioxins and are known as dioxin-like PCBs (Baars *et al.* 2004). PCBs are chemically stable, resistant to heat and have found wide application in paints, coolants, lubricants, plasticizers, transformer insulators, electrical capacitors *e.t.c.* (Babalola *et al.*, 2017). The persistent and widespread nature of these compounds in the environment is due to

their hydrophobic and long-distance migration characteristics (Klees *et al.*, 2015). Moreover, they have a tendency to bioaccumulate in the food chain (Baqar *et al.*, 2017).

PCBs are extremely toxic to humans, animals and plants, and have been implicated as carcinogens, endocrine disruptors, and neurotoxins. Other toxicity effects include attention deficit/hyperactivity disorder (ADHD), autism, reproductive and infertility disorders, immuno and neurological disorders, carcinogenicity, low birth weight, diabetes, hypertension, cardiovascular disease and neurodevelopment disorders (Mitchell *et al.*, 2012; Jahnke and Hornbuckle, 2019). Because of these adverse consequences, the production, trade and application of PCBs is prohibited globally. For example, PCBs production and usage has been banned by the United Nations Stockholm Convention on Persistent Organic Pollutants. However, PCBs are still prominent in environmental matrices, such as soils, air, water, dusts and sediments, as a result of their persistence, discharges from aged equipment (Iwegbue *et al.*, 2020; Ierhievwie *et al.*, 2020), and unintentional contributions from industrial thermal processes such as secondary metal smelting (Jiang *et al.*, 2015), thermal wire reclamation, and co-incineration of sewage sludge in cement kilns (Liu *et al.*, 2013; Jin *et al.*, 2017; Sohail *et al.*, 2018). PCBs are also produced unintentionally as by-products from silicone-based adhesives, and pigment and paint production (Anezaki and Nakano, 2015; Jahnke and Hornbuckle, 2019).

One way in which honey is contaminated by PCBs is through the environment where bees forage. The foraging area of bees is approximately 7 km² and includes various environments, plants and foods (Sereviciene *et al.*, 2022). When going from flower to flower and foraging for nectar, bees also come in contact with PCBs contaminated air, water, soil, leaves etc (Sereviciene *et al.*, 2022; Solayman *et al.*, 2021). During production of honey, Bees transfer the PCBs into the honey. Thus, honey may be considered a bio-indicator of environmental pollution (Iwegbue *et al.*, 2015). This probably explains why the evaluation of the presence and occurrence of PCBs in bees and bee products have been and is being undertaken by scholars all over the world such as in Brazil (Mohr *et al.*, 2015; Dos Santos *et al.*, 2021), Portugal, Spain, Morocco and Slovenian (Mohr *et al.*, 2015), Lebanon (Al-Alam *et al.*, 2018) and Turkey (Sari *et al.* (2021).

In order to ascertain the safety of honey consumed in Delta State in particular and Nigeria in general; and also contribute to the general understanding of the risks and benefit of consuming honey, the knowledge of the concentrations of contaminants in honey is needed. Studies on the contamination and risks of contaminants in honey in Nigeria are few. The available studies on honey in Nigeria focused on metals, PAHs and pesticides (Tesi *et al.*, 2024; Iwegbue *et al.*, 2016; Iwegbue *et al.*, 2015; Kpomah and Okunoja, 2022). Although residues of PCBs have been detected in crops, food items and environmental samples in Nigeria such as in water, sediment, and fish of Wupa River, Nigeria (Okoh *et al.*, 2022); in dusts and soils from an urban environment in the Niger Delta of Nigeria (Ossai *et al.*, 2023) and in canned sardines (Tesi and Iniaighe, 2020), there are no studies on PCBs contamination of honey in Delta State in particular and in Nigeria in general from the literature search. It is on this basis this study was initiated to determine the concentrations of PCBs in imported honey sold in Warri, Delta State, Nigeria. Specifically, this study aims to; (i) determine the concentrations of PCBs in imported honey sold in Warri, Delta State; (ii) compare the PCBs concentrations in the honey with others in literature; (iii) evaluate the dietary intake of PCBs in the honey and (iv) assess the human health risk associated with the consumption of PCB contaminated honey sold in Warri, Delta State.

Materials and methods

Sample collection: Ten imported honey samples were purchased from supermarkets in Warri, Delta State, Nigeria.

Chemicals/reagents: All chemicals and reagents used were of analytical grade. The PCB standard solution containing 28 PCBs congeners (PCB8, PCB18, PCB28, PCB44, PCB52, PCB60, PCB77, PCB81, PCB101, PCB105, PCB114, PCB118, PCB123, PCB126, PCB128, PCB138, PCB153, PCB156, PCB157, PCB167, PCB169, PCB170, PCB180, PCB185, PCB189, PCB195, PCB206, and PCB209) was used for calibration (AccuStandard Inc., CT, USA). Dichloromethane and n-hexane were obtained from Aldrich (USA). The mixed standard of surrogate ¹³C₁₂-labelled PCBs was obtained from Cambridge Isotope Laboratories Inc. (MA, USA). Anhydrous sodium sulphate, alumina and silica gel were obtained from Merck (Darmstadt, Germany).

PCB extraction and analysis: The United State Environmental Protection Agency method 3550C (USEPA, 2015) was used to extract the PCBs from the honey. A mass of 10 g of the honey sample was mixed with the same amount of anhydrous Na₂SO₄, until the mixture becomes free-flowing. A 30 mL of n-hexane/dichloromethane (DCM) (1:1 v/v) was added to the resulting material and placed in an ultra-sonic bath, and sonicated at 30°C for 30 minutes. The

organic extract was filtered and the process was repeated two more times by sonication of the residue with a fresh mixture of n-hexane/dichloromethane each time as described above. The extracts were combined and reduced to 1 mL by using a rotary evaporator. The concentrated extract was cleaned-up or purified in a multi-layer column containing 4.0 g of anhydrous Na₂SO₄, 4.0 g of alumina and 4.0 g of silica gel packed from top to bottom. The elution of PCBs from the column was carried out with a 30 mL aliquot of a 1:1 n-hexane/DCM mixture and the eluate was concentrated to 2 mL under a slow flowing stream of pure nitrogen gas. A gas chromatograph (6890N Agilent technologies) coupled with a mass spectrometry (Agilent 5975B) (GC–MS) was used to quantify the PCBs concentrations in the samples.

Quality control and assurance: Quality control was assured using method blanks, matrix spiked samples and surrogate ¹³C12-labelled PCBs. Method blanks were carried out by applying the analytical procedure without sample. Percentage recoveries were calculated after re-analyzing already analyzed samples spiked with known concentrations of the standard PCBs mixture and the surrogate ¹³C12-labelled PCBs. The PCB congeners were not detected in the blanks. The recoveries of the spiked samples ranged from 86.2 to 103% while the recoveries of the surrogate ¹³C12-labelled PCBs varied between 89.1 and 91.4%.

Estimation of daily intake: Daily intake was estimated from the formula:

$$\text{Daily intake (ng kg}^{-1} \text{ bw day}^{-1}) = \frac{C \times IR}{BW} \quad (1)$$

where, C is the concentrations of the PCBs in the honey. IR is the ingestion rate. The IR was obtained from the per capita honey consumption of 0.5 kg per annum per person in Nigeria which amount to 1.4 g/day and a BW of 60 kg for adult and 15 kg for children (Kpomah and Okunaja, 2022; Iwegbue et al., 2016).

Evaluation of PCBs toxic equivalency (PCBs-TEQs): Toxic equivalency (TEQ) concentrations give information on the toxicity of the dioxin-like PCBs. In this study, the TEQs of the dioxin-like PCBs was evaluated using equation (2).

$$TEQ = \sum TEF_i \times C_i \quad (2)$$

where, C_i is the measured concentration of the 12 dioxin-like PCBs in the honey sample and TEF_i is the toxic equivalency factor of the dioxin-like PCB congeners for humans and animals (Van den Berg et al., 2006).

Estimation of non-carcinogenic risk: The non-cancer risk associated with PCBs via consumption of the honey was assessed as hazard index (HI). This was evaluated using equations (3) and (4) (USEPA, 2022). The hazard quotient (HQ) for the individual 12 dioxin-like PCBs were computed. Thereafter, the HI was obtained by adding up the HQs based on dose additivity (Tesi et al., 2024; Mukiibi et al., 2021) as expressed in equations 3 and 4.

$$HQ = \left[\frac{C \times IR \times EF \times ED}{BW \times AT_{nc}} \times 10^{-6} \right] / RfD \quad (3)$$

$$HI = HQ1 + HQ2 + HQ3 + \dots + HQ12 \quad (4)$$

where, RfD = oral reference dose (USEPA, 2012), EF = exposure frequency (day/yr) = 350; ED = exposure duration = 6 and 30 years for children and adults respectively (USEPA, 2011); AT_{nc} = averaging time for non-carcinogenic risk = ED x 365. HI value greater than 1 indicates the presence of non-carcinogenic risk while HI value less than 1 indicates the absence of non-carcinogenic risk (USEPA, 2022).

Estimation of carcinogenic risk: The cancer risk associated with PCBs via consumption of the honey was assessed as total cancer risk (TCR). This was evaluated using equations (5) and (6) (USEPA, 2022). The incremental lifetime cancer risk (ILCR) for the individual 12 dioxin-like PCBs was computed. Thereafter, the TCR was obtained by adding up the ILCRs based on dose additivity (Tesi et al., 2024; Mukiibi et al., 2021) as expressed in equations 5 and 6.

$$ILCR = \frac{C \times IR \times EF \times ED \times CF \times CSF}{BW \times AT_{ca}} \quad (5)$$

$$TCR = ILCR1 + ILCR2 + ILCR3 + \dots + ILCR12 \quad (6)$$

where, CSF = cancer slope factor (USDOE, 2011); AT_{ca} is the averaging time for carcinogens = LT x 365 (USDOE, 2011); LT = Life expectancy of an average Nigerian = 55 years (WHO, 2018). TCR value greater than 1 × 10⁻⁴ indicates the presence of carcinogenic risk while TCR less than 1 × 10⁻⁴ indicates the absence of carcinogenic risk (USEPA, 2022).

Statistical analysis: The statistical analysis of data was done with the IBM-SPSS software (Version 23). Analysis of variance was used to determine if there was significant variation in the concentrations of PCBs in the honey.

Results and Discussion

PCBs concentrations in the honey samples: PCBs concentrations in the honey samples are shown in Table 1. The concentrations of $\Sigma 28$ PCBs in the honey samples ranged from 0.14 to 2.70 ng/g. ANOVA showed significant variation in the concentrations of the PCBs in the honey samples. The observed significant variation might be due to the environment where bees forage or via during production of honey (Sereviciene *et al.*, 2022). It was not possible to compare with previous studies in Nigeria since these were not available. However, as shown in Table 2, the concentrations of PCBs obtained in this study were similar to those reported by Mohr *et al.* (2015) for Brazilian, Portuguese, Spanish, Moroccan and Slovenian honeys. However, PCBs in honey from this study were lower than those reported by Sari *et al.* (2021) and dos Santos *et al.* (2021).

Table 1: PCBs concentrations (ng/g) in honey samples

	IH1	IH2	IH3	IH4	IH5	IH6	IH7	IH8	IH9	IH10
PCB8	0.03	0.18	0.03	0.14	ND	0.01	0.27	0.17	0.03	ND
PCB18	ND	ND	ND	ND	ND	ND	ND	0.04	0.27	ND
PCB28	ND	0.07	ND	0.12	ND	ND	ND	ND	ND	ND
PCB44	0.1	ND	0.09	0.26	ND	ND	ND	ND	ND	0.11
PCB52	0.04	ND	0.02	ND	0.31	ND	ND	ND	ND	ND
PCB66	0.08	ND	ND	ND	0.04	0.03	ND	ND	0.19	ND
PCB77	0.07	ND	0.06	0.02	ND	0.04	ND	ND	ND	ND
PCB81	0.68	0.01	0.07	0.09	0.05	0.31	ND	0.02	ND	ND
PCB101	ND	ND	ND	ND	0.03	0.02	ND	ND	ND	ND
PCB105	0.05	ND	ND	ND	0.02	0.03	0.02	ND	ND	ND
PCB114	ND	ND	0.04	0.06	ND	0.02	ND	0.05	0.16	ND
PCB118	0.33	ND	0.03	ND	ND	0.01	0.02	0.03	0.07	ND
PCB123	ND	0.08	0.04	0.06	0.10	0.04	0.06	0.06	0.05	0.01
PCB126	0.11	0.05	ND	0.02	0.03	0.32	0.43	0.04	0.21	0.02
PCB128	0.04	ND	0.09	ND	0.06	0.12	0.01	0.32	ND	ND
PCB138	0.01	ND	ND	ND	0.07	ND	ND	0.01	0.10	ND
PCB153	ND	ND	0.05	ND	0.01	ND	0.02	ND	0.18	ND
PCB156	0.01	ND	0.03	ND	ND	ND	ND	ND	ND	ND
PCB157	0.04	ND	0.05	ND	0.14	ND	ND	ND	ND	ND
PCB167	0.07	ND	0.07	ND	0.21	ND	ND	0.05	ND	ND
PCB169	ND	ND	ND	ND	0.11	ND	ND	ND	ND	ND
PCB170	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
PCB180	ND	ND	0.02	ND	0.06	ND	ND	0.04	ND	ND
PCB187	ND	ND	0.01	ND	0.16	ND	ND	ND	0.05	ND
PCB189	0.72	ND	0.07	ND	0.24	ND	ND	ND	ND	ND
PCB195	0.32	ND	0.04	ND	0.05	ND	ND	0.03	ND	ND
PCB206	ND	ND	ND	ND	0.26	ND	ND	0.02	ND	ND
PCB209	ND	ND	0.02	ND	ND	ND	ND	ND	ND	ND
TOTAL	2.70	0.39	0.83	0.77	1.95	0.95	0.83	0.88	1.31	0.14

Table 2: Comparison of PCBs in honey from this study with others previously reported

Country	No. of honey samples	No. of analyzed	PCBs Concentrations Range (ng/g)	References
Nigeria	20	28	0.13-2.7	This Study
Brazil	16	20	0.513-3.267	Mohr <i>et al.</i> (2015)
Portugal	4	20	1.073-2.210	Mohr <i>et al.</i> (2015)
Spain	10	20	0.458-2.439	Mohr <i>et al.</i> (2015)
Morocco	3	20	0.955-1.496	Mohr <i>et al.</i> (2015)
Slovenian	4	20	0.505-0.626	Mohr <i>et al.</i> (2015)
Lebanon	18	22	BDL	Al-Alam <i>et al.</i> (2018)
Turkey	7	50	105 \pm 31.5	Sari <i>et al.</i> (2021)
Turkey	7	50	112 \pm 21.6	Sari <i>et al.</i> (2021)
Brazil	90	11	27.0-531	Dos Santos <i>et al.</i> (2021)

Estimated of daily intake: The estimated dietary intake of PCBs in the honey is shown in Figure 1. The estimated daily intake values ranged from 0.01 to 0.25 ng/kg bw/day for child intake and 0.003 to 0.06 ng/kg bw/day for adult intake. The maximum permissible daily intake of PCBs specified by WHO is 0.001 to 0.004 ng/kg bw/day (WHO, 2000; Van Leeuwen *et al.* 2000). However, daily intake values of PCBs in the honey samples were above the WHO specified range for both children and adults.

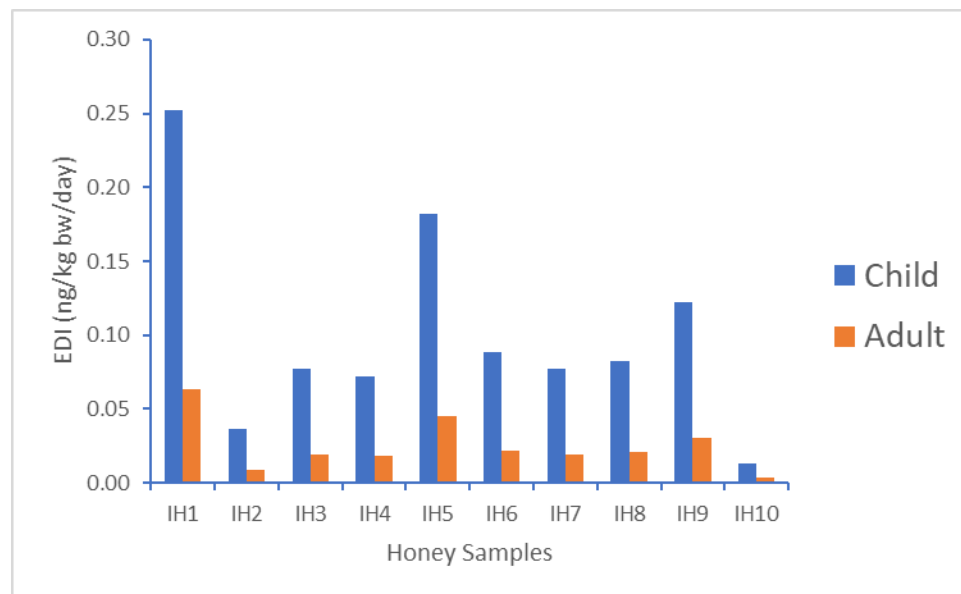


Figure 1: Estimated dietary intake of PCBs in the honey

Evaluation of PCBs toxic equivalency (PCBs-TEQs): Toxic equivalency (TEQ) concentrations give information on the toxicity of the dioxin-like PCBs. The TEQs of the dioxin-like PCBs in honeys are shown in Table 3. The total TEQ (TTEQ) concentrations for the dl-PCBs in the honey samples ranged from 3.69×10^{-5} to 4.30×10^{-2} ng/g. A TEQ value greater than 0.0065 ng/g is unacceptable (European Commission, 2011). The TEQ values in 40 % of the samples were above the 0.0065 ng/g stipulated by the European Commission.

Table 3: TEQs of dioxin-like PCBs in honeys

	PCB77	PCB81	PCB105	PCB114	PCB118	PCB123	PCB126	PCB156	PCB157	PCB167	PCB169	PCB189	TEQ
IH1	7.00E-06	2.04E-04	1.50E-06	0.00E+00	9.90E-06	0.00E+00	1.10E-02	3.00E-07	1.20E-06	2.10E-06	0.00E+00	2.16E-05	.12E-02
IH2	0.00E+00	3.00E-06	0.00E+00	0.00E+00	0.00E+00	2.40E-06	5.00E-03	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	.01E-03
IH3	6.00E-06	2.10E-05	0.00E+00	1.20E-06	9.00E-07	1.20E-06	0.00E+00	9.00E-07	1.50E-06	2.10E-06	0.00E+00	2.10E-06	.69E-05
IH4	2.00E-06	2.70E-05	0.00E+00	1.80E-06	0.00E+00	1.80E-06	2.00E-03	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	.03E-03
IH5	0.00E+00	1.50E-05	6.00E-07	0.00E+00	0.00E+00	3.00E-06	3.00E-03	0.00E+00	4.20E-06	6.30E-06	3.30E-03	7.20E-06	.34E-03
IH6	4.00E-06	9.30E-05	9.00E-07	6.00E-07	3.00E-07	1.20E-06	3.20E-02	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	.21E-02
IH7	0.00E+00	0.00E+00	6.00E-07	0.00E+00	6.00E-07	1.80E-06	4.30E-02	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	.30E-02
IH8	0.00E+00	6.00E-06	0.00E+00	1.50E-06	9.00E-07	1.80E-06	4.00E-03	0.00E+00	0.00E+00	1.50E-06	0.00E+00	0.00E+00	.01E-03
IH9	0.00E+00	0.00E+00	0.00E+00	4.80E-06	2.10E-06	1.50E-06	2.10E-02	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	.10E-02
IH10	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	3.00E-07	2.00E-03	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	.00E-03

Non-carcinogenic and carcinogenic risks of PCBs in the honey: The hazard index and total cancer risks values computed for the PCBs in the honey is displayed in Figures 2 and 3 respectively. The hazard index values for child ranged from 0.005 to 5.498 while those of adult ranged from 0.001 to 1.370. Moreover, the total cancer risk values for child ranged from 4.29×10^{-7} to 5.00×10^{-4} while those of adults ranged from 5.91×10^{-8} to 6.89×10^{-5} . With the exception of four samples for children exposure, the hazard index values were < 1 . Also, the total cancer risk values were $< 1 \times 10^{-4}$ in all the samples except for the four samples for children exposure. This indicates that there is no adverse risk associated with the PCBs from the intake of 60 % of the honey samples whereas there is adverse risk for children from PCBs in 40 % of the honey samples.

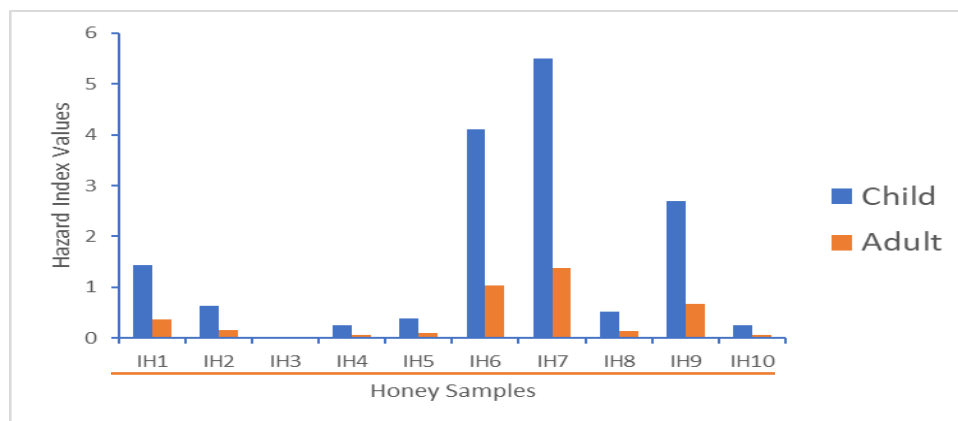


Figure 3: Hazard index values of PCBs in the honey

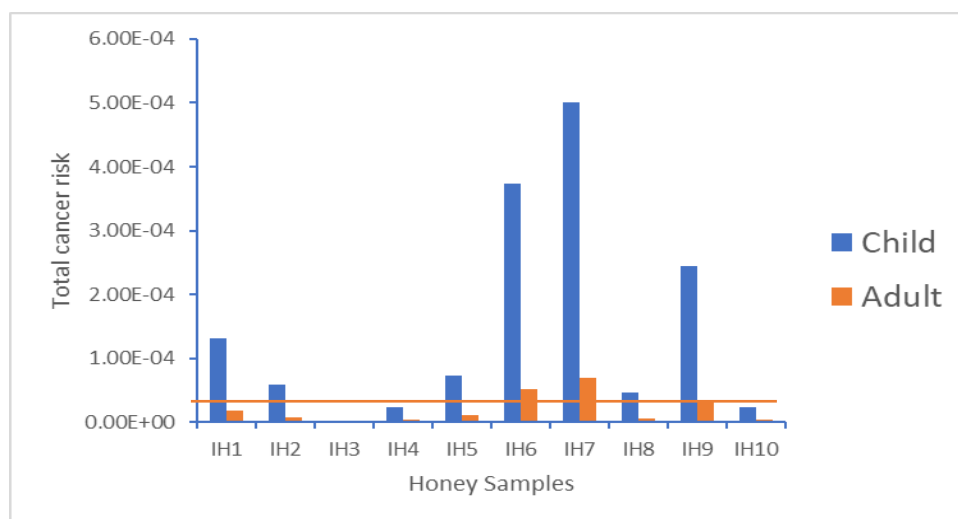


Figure 4: Total cancer risk values of PCBs in the honey

Conclusion

This study has shown that the honey samples analyzed tested positive for PCBs though at low PCBs concentrations. There was significant variation in the concentrations of the PCBs among the different imported honey samples. The toxic equivalency (TEQ) concentrations for the dioxin-like PCBs in 40 % of the honey samples were above the European Commission's specified limit. The health risk assessment indicated that there are no potential health risks from the intake of 60 % of the honey samples whereas there are risks associated with intake of 40 % of the honey samples.

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