RESEARCH ARTICLE

Organochlorine pesticides and polychlorinated biphenyls in raw bovine milk from various dairy farms in Beja, Tunisia: contamination status, dietary intake, and health risk assessment for the consumers

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Abstract

We determined organochlorine pesticides (OCPs) and polychlorinated biphenyls (PCBs) in raw bovine milk taken directly from the milking equipment for different animals and various farms in Béja region, which is one of the largest milk producing areas in Tunisia. All milk samples were contaminated with one or more pesticides and exhibited measurable concentrations of some PCB congeners. The residue levels are generally marked by the predominance of dichlorodiphenyltrichloroethanes (DDTs) (median: 17.60 ng g^{-1} fat), followed by hexachlorobenzene (HCB) (median: 14.31 ng g^{-1} fat), PCBs (median: 4.71 ng g^{-1} fat), and hexachlorocyclohexanes (HCHs) (median: 0.77 ng g^{-1} fat). DDT/DDE ratios across the samples vary between 0.09 to 32.24 and exhibit the hypothesis of sustaining illegal use of the banned pesticide. The historical or recent use of OCPs and possible emission sources of PCBs identified near studied farms may lead to increased levels of these contaminants in produced milk. The estimated daily intake (EDI) values were several orders of magnitude below the tolerable daily intake (TDI) fixed by FAO/WHO, though recorded concentration in some samples exceeded the maximum residue limits (MRLs) fixed by the EU, especially for OCPs. According to health risk assessments, consumption of raw milk did not pose an obvious cancer risk or other health problems for local inhabitants.

Keywords Tunisia · Raw bovine milk · Organochlorine pesticides · Polychlorinated biphenyls · Dietary intake · Risk assessment

Introduction

PCBs and OCPs are two groups of persistent organic pollutants (POPs) that have been widely considered over the last several decades. The origin of their occurrence in the environment as well as their main physicochemical properties are well known and documented (Fiedler 2003; Robertson and Hansen 2015). Like other POPs, they are characterized by high lipophilicity, chemical persistence, and high bioaccumulation capacity through the food web, with a high risk of causing adverse effects on human health and the environment (Carpenter 2013; Harrad 2012; Rose and Fernandes 2013).

Between the 1940s and 1980s, OCPs and PCBs were extensively used in Tunisia for agricultural, industrial, and sanitary purposes. The legal import has been prohibited since 1984 and 1986, respectively, for OCPs (dieldrin, heptachlor, DDT, aldrin, chlordane, endrin, and toxaphene) and PCBs. The Stockholm Convention was signed by our country in 2001 and became legally binding in 2004. The existing situation is characterized by relatively high quantities (1407 tons estimated in 2003) of old stockpiles of obsolete pesticides, including principally HCH and DDT, and a large number of transformers or equipment containing PCBs are still used or presently stored in unsatisfactory conditions and dispersed through the country in 132 sites (ANGED 2010; APEK 2005). Moreover, forbidden pesticides are still in demand because of their low cost and effectiveness against

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pests, and investigations have revealed that compounds are still being illegally imported from nearby countries where their usage is not prohibited (USDA 2014). A number of studies, carried out in Tunisia, have documented the contamination levels of organochlorine compounds (OCs) in various environmental samples in Tunisia, including sediment (Barhoumi et al. 2016; Derouiche et al. 2004), water (Necibi and Mzoughi 2020), fish (Ben Ameur et al. 2013a), atmosphere (Barhoumi et al. 2018), and in human samples, like breast milk (Ben Hassine et al. 2012; Ennaceur et al. 2007, 2008), blood (Ben Hassine et al. 2014), and adipose tissue (Achour et al. 2017).

It is known that the main route of human exposition to OCs is through food intake, with a contribution of more than 90% of the total exposure, and fat foods of animal origin (fish, meat, milk, and dairy products) present approximately 80% of dietary exposure (Djien Liem et al. 2000). Among the studies carried out on human adipose tissues and breast milk collected in the coastal area (Bizerte region, Tunisia) have shown a clear correlation between the frequency of fish consumption and the levels of PCBs (Achour et al. 2017; Ennaceur et al. 2008). Therefore, a national survey is required to examine the intake of OCs due to dietary differences, with a strong focus on the more specific populations living in agricultural and rural zones, who consume a large amount of milk and dairy products compared to the coastal regions.

The Béja governorate is located in the northwest of Tunisia and has 306,300 inhabitants (a little less than 3% of the national population). It is characterized by a high annual amount of rainfall (between 350 and 1000 mm/year) and fertile soils. It is a territory with very appreciable natural resources, mountainous areas, grassy valleys, and green plains. Its agricultural land represents more than 91% of its area (Atlas Numérique du Gouvernorat de Béja 2016). The economic activity is focused essentially on agriculture, and placed it among the first governorates in the agronomic production of the country (Agence de promotion de l'industrie et de l'innovation 2017). Farming sheep and cattle are well developed here, which contributes to the increase in production and consumption of typical dairy products. This region produces 12.5% of Tunisia's cow milk, making it one of the largest milk production areas. The domestic market receives 20% of this production, while 8% is transformed into fresh milk-based products using the traditional process (LACTIMED 2015).

Milk and dairy products have been recognized as among vital components of a healthy and balanced human diet (Park 2009). These fat animal foods are a large part daily diet of the inhabitants in this region. For 83% of distributors surveyed during the study carried out by local agroclusters for typical and innovative Mediterranean dairy products: LACTIMED (2015), drinking milk continues to

be the largest-selling dairy product, followed by yogurt and cheese, which represents a small proportion of these sales. Moreover, most residents of the study area buy these foods from specialized shops (creameries), which are made from raw milk delivered from local farms. Consequently, bovine raw milk is probably one of the major food products potentially responsible for human exposure in this area and can significantly contribute to the dietary intake of the OCs and can cause chronic and acute toxicity.

Regular surveys and monitoring programs of these chemicals attached with risk assessments have been carried out and reported in many developed countries worldwide, and this is done in order to protect consumer health, improve the management of agricultural resources, and minimize economic losses (DAFF 2022; EFSA 2012; US-FDA 2022). But unfortunately, data on the state of contamination of milk with persistent organochlorine pollutants and the health risk assessment related to it consumption was not well considered, and this may be due to the paucity of data and lack of interest on the institutional level. To the best of our knowledge, the present work is the first study investigating the levels of OCPs and PCBs in raw milk collected in this region. The only available Tunisian data is published by Sabbah and Bouguerra (1997), who measured the levels of OCPs in milk samples. Therefore, the current study was designed to (i) screen the status of OCP and PCB residues in bovine fresh raw milk collected from local farms in Béja region (ii) monitor the possible variation pattern across the various studied farms (iii) assess the human dietary exposure to OCPs and PCBs in the study area and (iv) estimate cancer and noncancer health risks due to the consumption of the milk.

Materials and Methods

Sample collection and storage

The cow milk samples were collected in late autumn 2016 from six farms located throughout Béja Governorate (Fig. 1). Organized sampling fiches were completed for each sample, including characteristics of dairy cows, water for drinking, and fodder. In addition, geographic data and anthropogenic activities around the farms were also collected to search possible for relationships between the contamination levels of milk and identifiable exposure factors.

Their herds were composed of more than 90% are Holstein-Friesians, including Holstein and Black Pied cows born in Tunisia. In farms 1–3, dairy cows are housed in free stalls, while in farms 4 to 6, tie-stall housing is used. Livestock feed consists of fodder (hay, straw, silage, and greenery) produced on their own land or purchased from the same region, supplemented with concentrate feeds (industrial and/ or farmers). The farmers store the animal feed in open-sided
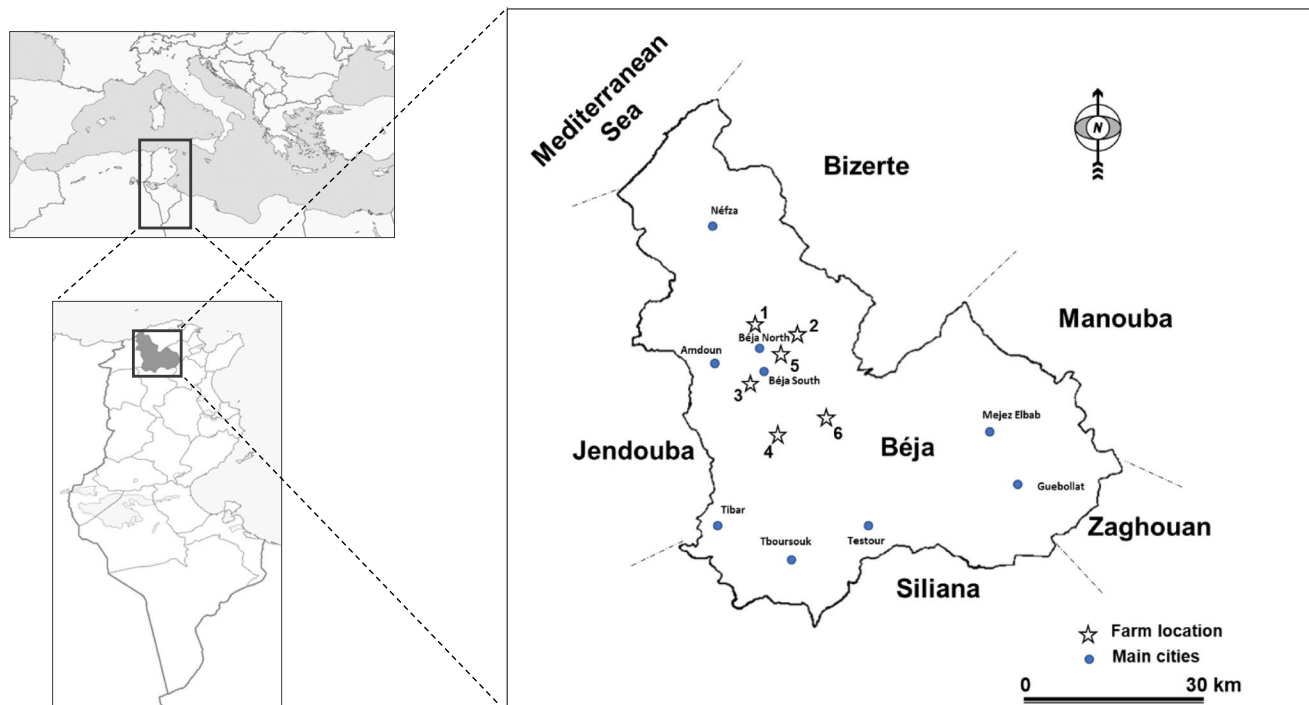
Fig. 1 Map of Béja, Tunisia, showing location of farms where raw bovine milk samples were collected

sheds or in the open air. Water from uncontrolled wells is mostly consumed by cows in addition to municipal water.

Three farms (1, 2, and 5) are located in the north of Béja City (over 100 thousand inhabitants), near an industrial zone within a radius of 5 km. According to Atlas Numérique du Gouvernorat de Béja (2016), this industrial site had 97 small units, where the food industry represents 46.4% of the total number of companies installed. There are other industries, including textiles, leather, chemicals, and electronics. Located in southwestern Beja City, farm 3 is close to a dump of old electrical and mechanical equipment and is influenced by another industrial unit that produces steam. Farm 6 is located in an agricultural area specializing in seed production and close to a municipal landfill and incinerator situated in El Maagoula, located a few kilometers to the southeast of Béja City. Farm 4 is far from urban and industrial sources of pollution but is influenced by agricultural inputs.

A total of 30 bovine milk samples (five per farm and one per animal) were taken directly from the milking equipment, placed in analytical-grade glass containers of 100 mL capacity with teflon-lined caps, kept cooled until they reached the laboratory, then frozen and stored at − 20 °C until chemical analysis.

Chemicals and analytes

Standard of selected pesticides (HCB, β-HCH, γ-HCH, p,p′-DDE, p,p′-DDD, and p,p′-DDT) were purchased from Polyscience Corporation Analytical Standards (Niles, IL, USA). Individual standard solutions of each compound were prepared in hexane at 1000 mg mL^{-1} except β-HCH which is dissolved in acetone. The PCB analytical mixture solution was obtained from Supelco (CIL, USA) containing twelve congeners (PCB 18, − 28, − 31, − 52, − 44, − 101, − 149, − 118, − 153, − 138, − 180, and − 194) at 10 mg mL^{-1} in heptane. PCB 15 and − 209, for use as surrogate standards, as well as internal standard of PCB 155, were purchased by Cluzeau Info Labo (Sainte-Foy La Grande, France, purity 99%) and dilute in hexane to suitable concentrations. The working standard solutions were obtained by further dilution in n-hexane to appropriate concentration levels. All of these solutions are stored in dark at 4 °C ad left for 1 h at ambient temperature before use.

The organic solvents used were pesticide quality and were obtained from Fluka (Buchs, Switzerland). Sulfuric acid and sodium oxalate were purchased from Biotechnica (Tunis, Tunisia). High-purity florisil adsorbent (60–100 mech) was obtained from Fluka, activated at 650 °C for 8 h, cooled, and then heated at 130 °C for 5 h and preserved in desiccators until use. Anhydrous sodium sulfate (analytical grade, Fluka) was baked at 300 °C and stored in an oven at 130 °C. All glassware was washed with detergent, rinsed with water, heated overnight at 300 °C, and rinsed with hexane before use.

Sample preparation and instrumental analysis

The stored milk samples were left to defrost at ambient temperature and were then shaken vigorously for 5 min. Ten milliliters of milk sample was taken, spiked with a known concentration of PCB 15 and −209 as surrogates, and OCs were extracted according to the method described by (Moreno Frias et al. 2004) with some modifications. Initially, 1 mL of methanol and 0.1 g of sodium oxalate were added to each aliquot of 10 mL sample and vortexing for 1 min. Ten milliliters of n-hexane:diethyl ether (1:1 *v/v*) was added to the mixture, agitated for 2 min, and centrifuged for 5 min at 2500 rpm. The organic phase was collected, and the aqueous phase was extracted twice with n-hexane: diethyl ether (1:1 *v/v*). The hexane layers are combined in the same flask, filtered on anhydrous sodium sulfate, and then evaporated to 5 mL. One milliliter of this solution was taken for fat content determination using gravimetric method. The remaining extracts were first purified using 2 mL of sulfuric acid and then with adsorption chromatography according to the method (Ennaceur et al. 2008). A glass column (0.5 cm i.d.) containing a wad of silane-treated glass wool at the bottom, filled with 2 g of activated florisil, and topped with 1 g of anhydrous sodium sulfate. The organochlorine compounds were eluted with 30 mL of n-hexane:dichloromethane (9:1 *v/v*). The eluted was evaporated to near dryness, fortified with internal standard (PCB 155) and the final volume was adjusted to 0.5 mL in n-hexane.

A Hewlett-Packard 6890 plus gas chromatograph equipped with ^{63}Ni electron capture detector (GC-ECD) operated by HP Chemstation software was used for target analytes determination. The final sample extract (1 μL) was injected in the pulsed-splitless mode (0.6 min) onto SLB5-MS (30 m × 0.25 mm i.d. × 0.25 μm thickness) with hydrogen as carrier gas at a flow rate (constant flow) of 0.9 mL min^{-1}. The temperatures of injector and detector were set at 250 and 300 °C, respectively. The oven temperature program was 100 °C (1 min) to 200 °C at 10 °C min^{-1}, increased to 220 °C at 1 °C min^{-1}, then ramped to 280 °C at 5 °C min^{-1} and held for 10 min. A second SPB-608 fused silica column 30 m × 0.32 mm ID × 0.25 μm film thickness was used for confirmation of detected analytes.

Statistical analysis

Database management and statistical analysis were performed with Microsoft Excel (version 16.04339) and XLSTAT (version 2016.02.27444; Addinsoft). Data under detection limits were set to zero for statistical analysis. According to the Shapiro–Wilk test ($p < 0.05$) and even after the log, square root, or cube root, the data significantly deviated from normal distribution, thus nonparametric statistics were used. To characterize the distribution of examined contaminants in analyzed milk samples, concentrations are expressed with a median, the 25th, 75th, and 95th percentiles, range, and frequency of detection. The values of mean and standard deviation are also given as indicator values for comparison purposes. All *p*-values below 0.05 were considered statistically significant.

Quality control

Identification of examined OCs in all samples was confirmed; their concentrations were measured using surrogate standards (to monitor analyte recovery) and internal standards (to correct for injection errors) and were expressed on a fat weight basis unless otherwise specified.

The procedures of quantification and validation were performed following some parameters of the CEAEQ method MA. 400—BPCHR 1.0 (CEAEQ 2015). A complete quantitative calibration for all target analytes (concentrations ranging between 1 to 200 ng mL^{-1}) was conducted, and randomly selected single calibration points were checked before, in the middle, and at the end of analyzing each series of samples. The linearity was verified, and the deviation of the average relative response factors (RRF) of each compound was less than 15%. To ensure the quality of the data, procedural blanks were regularly performed after every ten samples to check for interferences or contamination from solvent, glassware, equipment, and instrumentation. No quantifiable amounts of target compounds were detected in the blanks. The average surrogate recovery in spiked milk samples at two fortification levels in triplicate (1 et 0.1 ng mL^{-1}) was higher than 75% with relative standard deviations (RSD) lower than 10%. Relative recoveries of PCB congeners ranged between 88 and 102%, and for OCP, they were 91–105%. Inter- and intra-day variability was calculated by analyzing fortified samples within the same day (repeatability) and on different days (intermediate precision), respectively, and were always < 15%. The limit of detection (LOD) and quantification were estimated as the concentration of analyte in the sample, producing a peak with a signal-to-noise ratio (S/N) of 3/1 and 10/1, respectively. The corresponding LOD and LOQ for individual OCPs and PCBs were 0.01 to 0.09 ng g^{-1} fat and 0.02 to 0.24 ng g^{-1} fat.

Estimation of dietary intake and risk assessment

Human health risk assessment is the process of determining if exposure to a chemical, at any dose, could cause an increase in the incidence of adverse health impacts. It can be assessed using a variety of approaches. The most widely used are estimation of daily intake (EDI) and comparison with the tolerable/acceptable daily intake (TDI/ADI) fixed in many guidelines, and application of the

model derived from US-EPA to estimate the carcinogenic and noncarcinogenic risks.

The estimated daily intakes (EDI) were calculated by the following equation (Pardio et al. 2003) (1):

$$EDI = \frac{DCR \times C_{fat} \times C_A}{BW} \qquad (1)$$

where EDI is the estimated daily intake (ng kg^{-1} day^{-1}), DCR is the daily consumption rate (g d^{-1}), C_{fat} is the fraction of fat in each sample C_A is the 50th percentile concentration of target compounds (ng g^{-1} fat) in samples (or 95th percentile concentration for high exposure), and BW is the mean body weight for adults (kg). The consumption data are founded from the consumption database of the National Institute of Nutrition and Food Technology (2013). About 300 g of milk are consumed daily by an adult weighing 70 kg. To evaluate the risk of exposure to the examined contaminants via raw milk consumption were assessed according to the approaches described by US-EPA (US-ATSDR 2022). Noncarcinogenic health risks were evaluated by calculating hazard quotient (HQ) using Eq. (2):

$$HQ = \frac{EDI}{RfD} \qquad (2)$$

where EDI is the estimated daily intake and RfD is recommended reference dose (US-EPA 2005).

To assess the overall potential effects posed by the different OCs, the HQs calculated for OCPs and PCBs were summed and expressed as the hazard index (HI) with Eq. (3):

$$HI = \sum_{i=1}^{n} HQ \qquad (3)$$

In cases where HI values did not exceed one, it was assumed that no chronic risk was likely to occur.

From the US-EPA (1997) exposure factor handbook, Lifetime Cancer Risk (LCR) is calculated as follows:

$$LCR = EDI \times CSF \qquad (4)$$

where CSF is the slope factor of the contaminant via oral exposure route CSF.

The total cancer risk can be calculated from:

$$TCR = \sum_{i=1}^{n} EDI_i \times CSF_i \qquad (5)$$

where EDI$_i$ is the estimated daily intake of compound/group of compounds and CSF$_i$ is the corresponding value of the cancer slope factor.

US-EPA uses a risk level of 10^{-6} as the point at which risk management decisions can be considered. Risk management decisions most frequently used are in the total cancer risk range of 10^{-6}–10^{-4}.

Results and discussion

Residue levels and contamination status

The global result of OCP and PCB concentrations in raw bovine milk samples are summarized in Table 1 and expressed in nanograms per gram of extracted fat (ng g^{-1} fat). All samples studied were positive for one or more residues. The highest detection frequencies were obtained in the following descending order of PCBs > DDTs > HCB = HCHs. These results show ubiquitous contamination by these chemicals in this food item and possible accumulation and health implications for the consumers, despite their prohibition of use in Tunisia before 1990. As regards the residue levels of OCs, the analyzed kinds of milk are generally marked by the predominance of DDTs, followed by HCB, PCBs, and HCHs. The results are consistent with those of recent studies in Tunisia finding DDT and HCB predominant in human biological media, including breast milk (Ben Hassine et al. 2012), blood (Ben Hassine et al. 2014), and adipose tissues (Achour et al. 2017).

Organochlorine pesticides

All milk samples were contaminated with one or more pesticides. The total OCP concentrations ranged from 0.87 to 141.08 ng g^{-1} fat, with a median value of 35.20 ng g^{-1} fat. The relative contribution of the different pesticides detected compared to the sum of pesticides in contaminated samples of milk is presented in Fig. 2. The median concentration of DDTs was 17.60 ng g^{-1} fat, which contributes approximately 53% to the sum-OCPs. The frequency of detection of DDT and its metabolite following this order in the investigated matrix: DDE > DDD > DDT. p,p'-DDE was found to be the major organochlorine contaminant (median 14.45 ng g^{-1} fat), detected in 73.3% of samples and accounted for about 58% of total DDTs. p,p'-DDT was detected only in six milk samples, with concentrations ranging from nd to 39.5 ng g^{-1} fat and represented 17% of DDTs. It is well-known that the concentration ratio of p,p'-DDT/p,p'-DDE could be used to distinguish between current and historical exposure to DDT (Ahlborg et al. 1995). DDT/DDE ratios below one indicate high persistence in the environment and ongoing biomagnification, while those above one indicate both increasing exposure and ongoing biomagnification to DDT (Ozcan and Aydin
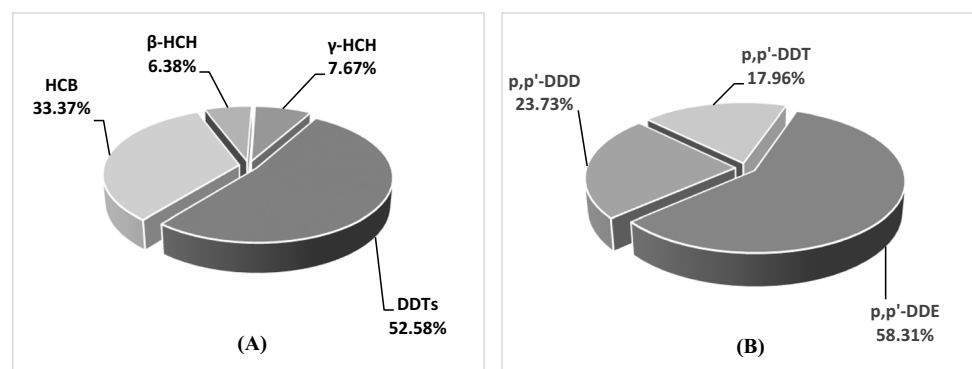
Table 1 Summary statistics of organochlorine compound concentrations (ng g^{-1} fat) in raw bovine milk samples collected from five farms in Béja, Tunisia

	Frequency (%)	Min	Percentiles				Max	Mean (SD)	MRL EU	% > MRL
			25th	50th	75th	95th				
Lipid %	100	3.9	4.1	4.5	4.8	5.0	5.2	4.6 (0.17)		
HCB	76.7	<LOD *	0.56	14.31	22.90	41.69	91.38	16.34 (19.22)	5 [1]	60.0
β-HCH	40.0	<LOD	0.00	0.00	1.54	12.80	27.00	3.13 (6.34)	10 [1]	20.0
γ-HCH	56.7	<LOD	0.00	0.52	0.73	27.07	36.50	3.76 (9.41)	10 [1]	13.3
HCHs [a]	76.7	<LOD	0.50	0.77	11.22	28.55	36.50	6.88 (11.02)		
p,p'-DDE	73.3	<LOD	0.14	14.45	22.38	41.90	82.40	15.02 (18.37)		
p,p'-DDD	36.7	<LOD	0.00	0.00	6.03	21.70	72.67	6.11 (14.36)		
p,p'-DDT	20.0	<LOD	0.00	0.00	0.00	10.81	39.50	2.29 (7.07)		
DDTs [b]	93.3	<LOD	7.47	17.60	26.02	84.89	101.80	25.75 (28.30)	40 [1]	13.3
OCPs [c]	100	0.87	21.87	35.20	73.90	125.13	141.08	48.98 (38.28)		
PCB 44	73.3	<LOD	0.01	0.14	0.40	0.90	1.83	0.30 (0.40)		
PCB 101	66.7	<LOD	0.00	0.35	0.52	0.85	9.38	0.61 (1.68)		
PCB 149	73.3	<LOD	0.01	0.05	0.12	0.28	0.41	0.09 (0.10)		
PCB 118	80.0	<LOD	0.11	0.22	0.53	1.10	5.24	0.47 (0.95)		
PCB 153	80.0	<LOD	0.39	0.79	1.58	12.81	18.38	2.54 (4.61)		
PCB 138	93.3	<LOD	0.48	0,92	2.12	9.79	30.47	2.84 (5.90)		
PCB 180	90.0	<LOD	0.34	0.66	1.69	11.58	17.92	2.38 (4.34)		
PCB 194	53.3	<LOD	0.00	0.11	0.53	3.17	5.40	0.59 (1.33)		
PCBs [d]	100	2.19	3.58	4.71	12.84	27.68	42.66	9.83 (9.87)		
ndl-PCB$_6$ [e]	100	1.49	2.67	4.04	11.13	26.94	31.25	8.38 (8.47)	40 [2]	00.0

*LOD = limit of detection. [a] HCHs = β-HCH + γ-HCH. [b] DDTs = p,p'-DDT + p,p'-DDE + p,p'-DDD. [c] OCPs = sum of detected organochlorine pesticides. [d] PCBs = sum of all detected congeners. [e] ndl-PCB$_6$ = PCB 28 + PCB 52 + PCB 101 + PCB 138 + PCB 153 + PCB 180. [1] Cited from Commission Regulation (EU) (2017); [2] Cited from EFSA (2012)

2009). This ratio across the samples, when the two chemicals were detected, vary between 0.09 and 32.24, suggesting the presence of a continuing source of use of DDT, despite the lengthy period of time since this pesticide was banned in Tunisia (1981) (APEK 2005). Moreover, the presence of p,p'-DDT alone in two samples with levels of the two principal metabolites (DDE and DDD) at sublimit of detection may confirm this result. This finding was also deducted in recent studies that measured these compounds in human adipose tissue (Achour et al. 2017), human milk (Ben Hassine et al. 2012), and sediments collected from Bizerte, the neighboring of Béja region (Barhoumi et al. 2014). The authors suggest that illegal usage in some suburban agriculture and leakage from the old stockpiles deposited under unsatisfactory conditions may be responsible. Despite these findings cited above, the mean concentration of p,p'-DDE and p,p'-DDT reported by Sabbah and Bouguerra (1997) in Tunisia (89.6 and 6 ng g^{-1} fat, respectively) could be an indication of declining contamination status.

Fig. 2 Relative contribution of **A** HCB, β-HCH, γ-HCH, and DDTs to total OCPs and **B** DDT and its metabolites to total DDTs

(A)

β-HCH 6.38%
γ-HCH 7.67%
HCB 33.37%
DDTs 52.58%

(B)

p,p'-DDD 23.73%
p,p'-DDT 17.96%
p,p'-DDE 58.31%

When comparing the sum of DDT residues recorded in this study (mean: 25.75 ng g^{-1} fat) with the most recent international studies, the data show that were significantly lower than Ethiopia (mean: 269–477 ng g^{-1} fat) (Gebremichael et al. 2013), Egypt (mean: 223 ng g^{-1} fat) (Abou Donia et al. 2010), India (mean: 156.77 ng g^{-1} fat) (Aslam et al. 2013) and Uganda (mean: 52 ng g^{-1} fat) (Kampire et al. 2011), comparable with those in raw milk samples from Mexico (mean: 26.48 ng g^{-1} fat) (Pardío Sedas et al. 2021), Jordan (mean: 27 ng g^{-1} fat) (Salem et al. 2009) and Pakistan (mean: 24.82 ng g^{-1} fat) (Fazal et al. 2022), but higher than DDTs levels in Poland (mean: 9.20 ng g^{-1} fat) (Pietrzak-Fiećko 2018), Turkey (mean: 12.22 ng g^{-1} fat) (Aydin et al. 2019), Mexico (mean: 0.44–2.44 ng g^{-1} fat) (Gutierrez et al. 2012) and China (mean: 2–3 ng g^{-1} fat) (Zhang et al. 2017).

As the second most predominant OCP, HCB was detected in 76.7% of milk samples and accounted for approximately 33% of total OCPs with a median value of 14.31 ng g^{-1} fat (Fig. 2). This current finding indicates that HCB contamination sources still exist in this area and we cannot eliminate the hypothesis of a secret use of this chemical in the agriculture as a fungicidal treatment for seeds and olive tree (Sabbah and Bouguerra 1997). In addition, the amount of HCB may be attributed not only to its prior legal or illegal use but also to its release from industrial processes at high temperatures, such as industrial processes, incineration, and water or wastewater treatment (Thomsen et al. 2009; Van Birgelen 1998). It may also exist as an impurity in several formulations of pesticides or chlorinated solvents (Erdogrul et al. 2005). This present result was also observed in a recent study in Tunisia, which recognized that HCB was among the most concentrated OCP after the p,p'-DDE in many humans (Achour et al. 2017; Ben Hassine et al. 2012, 2014) and environmental samples (Barhoumi et al. 2014; Ben Ameur et al. 2013a, b). On comparing our results with those obtained by Sabbah and Bouguerra (1997) during 1994 in Tunisia, we observed that contamination by HCB has fallen about four times in milk. Furthermore, the comparison of HCB amount (mean: 16.34 ng g^{-1} fat) in raw milk from Béja region with worldwide studies shows that is relatively higher than those reported from Brazil (mean: 0.52 ng g^{-1} fat) (Avancini et al. 2013), Turkey (mean: 0.30 ng g^{-1} fat) (Bulut et al. 2011), Tibet (mean: 1.00 ng g^{-1} fat) (Pan et al. 2014), and Spain (median: 2.22–3.67 ng g^{-1} fat) (Luzardo et al. 2012) but extremely lower than those reported from Egypt (mean: 150 ng g^{-1} fat) (Abou Donia et al. 2010).

For HCHs, the γ- and β-isomers were detected in 56.6 and 40% of the collected raw milk, respectively. It was shown that this group of contaminants has the lowest concentration (median: 0.77 ng g^{-1} fat; mean: 6.88 ng g^{-1} fat) and accounts for about 14% of total OCPs. This is due to their lower potential of bioaccumulation (Loganathan and

Kannan 1994) and the rapid evaporation to the atmosphere after their application (Ramesh et al. 1991). Among the HCHs measured, γ-HCH was more abundant isomer in the analyzed samples. When compared with data from similar studies around the world, concentrations of HCHs (mean: 6.88 ng g^{-1} fat) in raw cow milk in Beja were similar to those in Tibet (mean: 4.46 ng g^{-1} fat) (Pan et al. 2014) and were slightly lower than those in Uganda (mean: 26 ng g^{-1} fat) (Kampire et al. 2011) and were much lower than those in Turkey (mean: 63.6 ng g^{-1} fat) (Aydin et al. 2019), Jordan (mean: 83 ng g^{-1} fat) (Salem et al. 2009), Romania (mean: 66.3–131.0 ng g^{-1} fat) (Rusu et al. 2016), Egypt (mean: 165 ng g^{-1} fat) (Elsharkawy 2015), and India (mean: 349.65 ng g^{-1} fat) (Aslam et al. 2013).

Polychlorinated biphenyls

We found that all milk samples exhibited measurable concentrations of some target PCB congeners except PCB 18, 28/31, and 52. As seen in Table 1, PCBs (defined as the sum of all detected congeners) concentrations varied between 2.19 and 42.66 ng g^{-1} fat with a median value of 4.71 ng g^{-1} fat. The distribution profiles of individual PCB congeners in the analyzed samples are presented in Fig. 3. The contamination status is marked by the predominance of PCBs 153, −138, and −180 and account for approximately 79% of total PCBs detected in the milk samples. The dominance of these non-dioxin-like congeners (ndl-PCB) is related to their high lipophilicity, highly resistant to metabolic attack, and their abundance in commercial mixtures. Indeed, it has been generally considered that congeners having chlorine atoms in positions 2, 4, and 5 on one or both nuclei or having no vicinal hydrogen atoms in meta-para position are poorly metabolized (De Voogt 1996). This contamination profile of
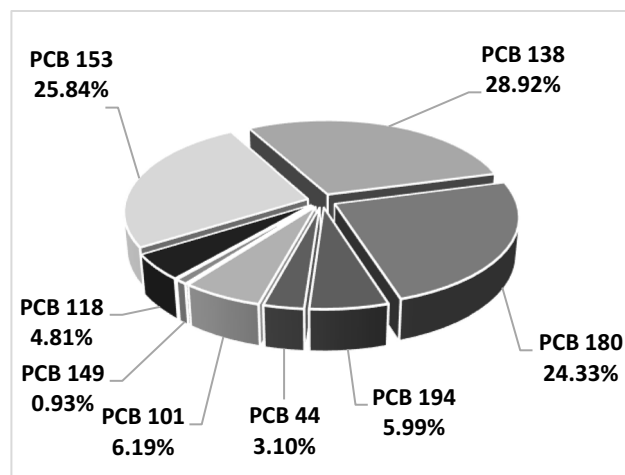

Fig. 3 Relative contribution of individual PCB congener to total detected PCBs in raw bovine milk

raw milk collected in Béja region is similar to those reported by EFSA (2012) in different European countries for milk and dairy products. This European agency recommended the use of six ndl-PCB indicators (PCB 28, −52, −101, −138, −153, and −180) to monitor these chemicals in food of animal origin and in human tissue. It has been proposed that ndl-PCBs indicators represent around 50% of all the PCB congeners that can be quantified in these matrices. The sum of the six ndl-PCB concentrations recorded in this study ranged from 1.49 to 31.25 ng g^{-1} fat, with a median of 4.04 ng g^{-1} fat, respectively. This level was numerically similar to the levels in raw bovine milk samples collected from different countries in Europe, such as Belgium (median: 4.72 ng g^{-1} fat) (Cimenci et al. 2013), Italia (median: 6.74 ng g^{-1} fat) (Lorenzi et al. 2016) and France (median: 3.0 ng g^{-1} fat) (Arnich et al. 2009). Moreover, it has been recognized that PCB 118 (dioxin-like PCB: dl-PCB) was among the most commonly detected and quantified congeners in milk (EFSA 2012). In our study, it is detected in 80% of samples with concentrations ranging from nd to 5.24 ng g^{-1} fat and contributes approximately 3% of the PCBs.

Comparison of contamination profile between farms

Contamination of milk with persistent organic pollutants is multifactorial: it is dependent on environmental factors, factors specific to the farming system (fodder and potentially contaminated soil, lactation stage, and health status of the herd), and the ability of the animals to metabolize and biotransform the pollutants ingested. Many studies demonstrate

that dairy ruminants are exposed to OCs mainly via ingestion of contaminated fodder or soil (Rychen et al. 2014, 2008). It has been revealed that pasture can be polluted from the deposition of contaminants from the atmosphere to both the vegetation and soil (Thomas et al. 1998). Moreover, the high levels of pollutants such as PCBs and OCPs can be a consequence of the formulation of animal feeds, particularly if they contain fishmeal (Jacobs et al. 2004). The distribution of total concentration of HCB, HCHs DDTs, and PCBs in raw milk samples collected in the different investigated farms are presented in Fig. 4. The highest total HCB values were found in milk samples collected in farms 5 (132.56 ng g^{-1} fat) and 6 (136.78 ng g^{-1} fat). It may be due to the historical use of this pesticide in this area and also a possible increase, as mentioned before, through waste incineration near El Maâgoula, located a few kilometers southeast of Béja City. The other farms display similar contents of total HCB in the milk samples, and varied from 42.87 to 69.51 ng g^{-1} fat. The high levels of total DDTs were detected on farms 1, 2, and 3. These levels ranged from 154.17 to 191.49 ng g^{-1} fat. These breeding units are surrounded by fields that are used to cultivate cereals, and marked by a historical input of pesticides and exposure of animals during grazing may explain these high levels. The minimum values of total HCHs were recorded in a farm situated in rural area (farm 4: 2.74 ng g^{-1} fat; farm 6: 5.01 ng g^{-1} fat), while the other farms followed almost an equal trend of HCHs distribution ranging from 39.51 to 64.95 ng g^{-1} fat. The high values might result from recent use in the urban zone against heat lice and scabies or as vector control by veterinarians in
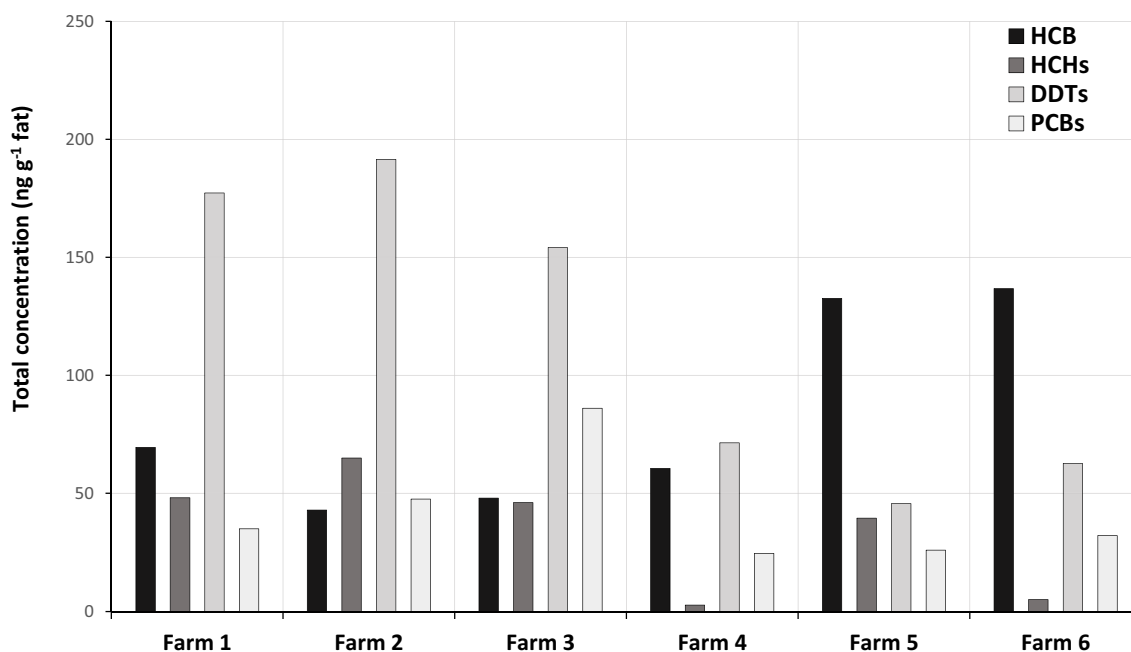

Fig. 4 Total HCB, HCHs, DDTs, and PCBs concentrations in raw milk samples from the different farms

these studied farms (Li 1999). The relatively high concentrations of PCBs are observed in farm 3 (86.10 ng g^{-1} fat) and could relate local anthropogenic sources especially the municipal garbage landfill and the old industrial unit using boilers for the generation of steam. There was no important difference between the total PCB levels reported in milk samples taken from farms 1, 2, and 5, which may be impacted by industrial and urban activities, and those from farm 4, which is located in a rural area. It is noteworthy that despite the presence of possible sources of contamination close to the studied farms, the Kruskal–Wallis analysis showed that there are no statistically significant differences in the amounts of HCB, DDTs, HCHs, and PCBs in the different farms ($p > 0.05$). This may be due to the small sample size or similar cattle feeding system, but the historical use, volatilization, as well as redistribution of these persistent pollutants in the different compartments of the environment over the years may explain also this result. Additionally, atmospheric transportation from neighboring regions, such as Bizerte and Grand Tunis, could also contribute to this finding. Barhoumi et al. (2018) have proven that there are relatively high levels of HCB, γ-HCH, p,p'-DDE, p,p'-DDT, and PCBs in the aerosols of Bizerte. These areas are highly populated and characterized by large-scale industrial activity. Furthermore, old pesticide stockpiles and transformers containing PCBs are still being used or are being stored in unsatisfactory conditions in these regions (UNEP 2007).

Tolerance limits and health risk assessment

The obtained residues of OCs were firstly compared to the strict maximum residual limits (MRLs) fixed by the European Union (EU) (Commission Regulation (EU) 2017; EFSA 2012). It should be noted that exceeding the MRLs is not legal for trade but does not necessarily imply a risk to health. The percentage of raw milk samples exceeding the limit was 20% for β-HCH and 13.3% for lindane (Table 1).

DDT residues exceeded the MRL in only four samples, and HCB exceeded its MRL in 60% of samples. For PCBs, the European Community fixed two threshold values, one for non-dioxin-like (sum of the six ndl-PCB indicators) and the other for PCB-like (dl-PCB). For non-dioxin-like congeners, the LMR is fixed at 40 ng g^{-1} fat for the sum of the six ndl-PCB indicators in milk. In this study, the determined levels of this group ranged between 2.67 and 31.25 ng g^{-1} fat and were lowering the recommended limit.

To understand the magnitude of exposure and possible health risk associated with OCs via raw milk consumption, the estimated daily intakes (EDI) were calculated and compared with tolerable/acceptable daily intake (TDI/ADI) and EPA toxicity references (RfD and CSF). The upper values of EDI based on 95th percentile measured concentrations of DDTs and lindane were far below the ADIs set in many guidelines (JMPR-FAO/WHO 2003; US-EPA 1996; Van Oostdam et al. 1999) (Table 2). The 95th EDI value calculated for the HCB is 25 and 40 times lower than the ADI value established, respectively, by EFSA (EFSA 2006) and Health Canada (Van Oostdam et al. 1999). For ndl-PCB indicators, estimated 95th EDI value is lower than the TDI of 10 ng kg^{-1} day^{-1} fixed by the Netherlands National Institute for Public Health and the Environment (RIVM) and adopted by EFSA (EFSA 2005).

In addition, EDI data of OCs associated with milk intake for adults are not available in Tunisia, and obtained values were compared with those measured in fish (Ben Ameur et al. 2013a) and mullet (Ben Ameur et al. 2013b) collected from Bizerte region (North of Tunisia). The EDI values in our study for HCB and DDTs were much higher (3–4 orders of magnitude), for PCBs were lower (2–4 orders of magnitude), and comparable for lindane than those obtained for the fish species. The intake of fatty foods (fish, dairy, and meat) might play a crucial role in OCPs and PCBs exposure levels. For several socio-economic reasons and frequency of consumption, the EDI obtained for raw milk may be the

Table 2 Acceptable or tolerable daily intake (ADI/TDI), estimated daily intake (EDI), and health risk assessments due to OCs for adults from Beja through drinking raw milk

	ADI/TDI (ng kg^{-1} day^{-1})	EDI (ng kg^{-1} day^{-1})		RfD (ng kg^{-1} day^{-1})	CSF (mg kg^{-1} day^{-1})$^{-1}$	Hazard quotient		Hazard index		Cancer risk (10^{-6})		Total cancer risk (10^{-6})	
		50th	95th			50th	95th	50th	95th	50th	95th	50th	95th
HCB	170 [1]; 270 [2]	2.45	7.15	800	1.60	0.003	0.009			3.93	11.43		
γ-HCH	300 [3]; 5000 [4]	0.09	4.64	300	1.30	0.000	0.015			0.11	6.03		
DDTs	10,000 [4]; 20,000 [2]	3.02	14.55	500	0.34	0.005	0.029			1.02	4.95		
PCBs	1000 [2]; 1500 [5]	0.81	4.75	20	2.0	0.040	0.237			1.61	9.49		
NDL-PCB[6]	10 [6]	0.69	4.62										
OCs								0.050	0.291			6.68	31.90

RfD, oral reference dose (USEPA 2005); *CSF*, cancer slope factor (USEPA 1997); *50th*, 50th percentile measured concentrations; *95th*, 95th percentile measured concentrations. ADI/TDI cited from [1] (EFSA 2006), [2] (Van Oostdam et al. 1999), [3] (USEPA 1996), [4] (JMPR-FAO/WHO 2003), [5] (ATSDR 2002), and [6] (EFSA 2005)

major part of the total daily intake of these contaminants for the local population. If we regard the obtained data, we can generally assume that global intake of OCs stays always lower than recommended ADI, indicating that there is no potential health risk associated with these foods' consumption in Tunisia.

On the other hand, the comparison with worldwide studies shows that obtained EDI based on 95th percentile value for DDTs (14.55 ng kg^{-1} day^{-1}) were lower than those reported EDI in milk samples from Turkey (4780 ng kg^{-1} day^{-1}) (Aydin et al. 2019), Iran (150 ng kg^{-1} day^{-1}) (Bayat et al. 2011), Ethiopia (79 ng kg^{-1} day^{-1}) (Gebremichael et al. 2013), Egypt (46 ng kg^{-1} day^{-1}) (Abou Donia et al. 2010), India (35 ng kg^{-1} day^{-1}) (Nag and Raikwar 2008), Iran (26 ng kg^{-1} day^{-1}) (Ashnagar et al. 2009), similar to those from Poland (9 ng kg^{-1} day^{-1}) (Radzymińska et al. 2008) and Romania (3 ng kg^{-1} day^{-1}) (Georgescu et al. 2011). The EDIs of HCB and lindane were with respective values of 2.45 and 0.09 ng kg^{-1} day^{-1} were lower than those obtained in Iran (HCB: 90 ng kg^{-1} day^{-1}; lindane: 111 ng kg^{-1} day^{-1}) (Bayat et al. 2011) and higher than in Spain (HCB: 0.53 ng kg^{-1} day^{-1}; lindane: 0.01 ng kg^{-1} day^{-1}) (Luzardo et al. 2012). Concerning EDI for PCBs, our central tendency based on the median (0.81 ng kg^{-1} day^{-1}) was lower than estimated in other countries, such as Turkey (1140 ng kg^{-1} day^{-1}) (Aydin et al. 2019), Iran (260 ng kg^{-1} day^{-1}) (Bayat et al. 2011), close to Italy (0.78 ng kg^{-1} day^{-1}) (Lorenzi et al. 2016) and higher than Belgium (0.1 ng kg^{-1} day^{-1}) (Cimenci et al. 2013).

The health risk assessments for dietary exposure to OCs by raw milk consumption for the local inhabitants were calculated using EPA approach (Table 2). As for the noncancer risks, the values of hazard quotient (HQ) for PCBs and OCPs based on their 50th and 95th percentile concentrations were lower than 0.040 and 0.237, respectively. The cumulative risk assessment (HI) remains less than unity even if using the higher exposure levels, indicating that the exposure to OCPs and PCBs via raw milk intake is unlikely to pose any noncarcinogenic effects to consumers. In contrast, the lifetime cancer risks (LCR) of examined compounds were all exceed one in one million except for lindane with median concentration. The total cancer risks (TCR) associated with milk consumption based on the 50th and 95th percentile levels were 6.68 and 31.90, respectively. The obtained results indicate that the intake of these compounds by only the studied food item can cause carcinogenic effects in the human body. In addition, the contribution of LCR to TCR based on central tendency reflected highest share of HCB (59%) followed by PCBs (24%), and DDTs (15%). The results indicate that these OCs may be of particular concern. This finding is also mentioned by Ben Ameur et al. (2013a), who studied health risk assessments related to these contaminants via consuming fish collected in the north of Tunisia.

Moreover, we can be seen a disagreement between the EPA and WHO approaches (EDI/ADI), which also covers carcinogenic effects, concerning the possible cancer risk. This supports further the idea that a risk level of 10^{-6} is a highly conservative approach (Vogt et al. 2012). The highest value in our study is well below the EPA threshold of 10^{-4}, which indicates a relatively low cancer risk (US-EPA 2009), even though it is necessary to monitor the milk as well as feeds and fodder intended for animal consumption regularly.

Conclusion

Located in the northwest of Tunisia, Béja is ranked among the first governorates in agricultural production, and it is one of the largest milk production areas. Raw milk is among the most accessible and frequent sources of animal fatty food for local inhabitants. In order to evaluate the contamination status with lipophilic contaminants such as OCPs and PCBs and to assess the potential health risks related to its consumption, raw bovine milk was collected from the milking equipment for different animals and various farms located in the studied region.

All analyzed samples showed measurable residue of examined OCs, and the contamination profile is marked by the predominance of DDTs, followed by HCB, PCBs, and HCHs. Comparison of the level of OCPs in our study with the only data available in Tunisia for more than 20 years shows a decreasing trend of these prohibited chemicals in cow milk samples. However, the presence of p,p'-DDT in some samples supported the hypothesis of an active and continuous source of its illegal use in this region. This finding is in accordance with recent studies investigated in Tunisia that measured these compounds in human biological media. The contamination status of raw milk with PCBs is marked by the predominance of congeners − 153, − 138, and − 180, and the recorded concentrations and accumulation patterns are similar to those that are found in European countries. The results indicate that historical or recent use of OCPs and possible sources of emission of PCBs in the vicinity of the studied farms may contribute to the increase in levels of these contaminants in produced milk, despite the fact that there are no statistical significant differences.

The dietary intake levels of examined OCPs and PCBs by local consumers of row milk were far below the acceptable or tolerable daily intake (TDI/ADI) established by many international guidelines, despite the fact that in some case level of OCPs exceeded the strict maximum residual limits (MRLs) fixed by the European Union (EU). The results of the health-risk assessment (carcinogenic and noncarcinogenic effects) revealed that there is no indication of potential health risks associated with this food consumption in Béja region, even if using a high level of contamination.

Therefore, these pollutants should be preferentially managed as priority contaminants in Tunisia, and is a necessity to define the contamination routes and increase the efforts to reduce human exposure through food consumption. Thus, it remains necessary to launch a national program to monitor these contaminants in a large number of different food products of animal origins, drinks, potable water, aerosol, and house dust to clarify the variation of human exposure, accumulation levels, and exposure sources across the most representative region of our country.

Declarations

Ethical approval and consent to participate Consent to participate is not applicable.

Consent for publication The participant has consented to the submission of the study to the journal.

Competing interests The authors declare no competing interests.

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