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Article

Effect of pasteurization on the concentration of dichlorodiphenyltrichloroethane (DDT) and hexachlorocyclohexane (HCH) in bovine milk

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Abstract:

Dichlorodiphenyltrichloroethane (DDT) and hexachlorocyclohexane (HCH) are endocrine disruptors whose presence in milk entails a health risk. There is evidence that pasteurization decreases or increases the concentration of organochlorine pesticides in dairy products. The present research evaluated the effect of pasteurization at 63 °C 30 min^{-1} and 73 °C 15 sec^{-1} on the concentrations of DDT, HCH and their metabolites in bovine milk, in order to estimate the dietary exposure from human consumption of pasteurized milk. A completely randomized experimental design with two treatments was performed on 100 milk samples collected in Soledad de Doblado and Jamapa, Veracruz, Mexico. Pesticides were quantified by gas chromatography with an electron microcapture detector. Data were analyzed by single-factor analysis of variance (P<0.05), and means were compared with Tukey's test (P<0.05). The dietary exposure to pesticides was assessed based on the estimated daily intake (EDI) and average daily dose (ADD) in three population groups. Pasteurization at 73 ºC reduced the concentrations of p,p'-DDE, p,p'-DDD, o,p'-DDT, p,p'-DDT and total DDT by 30.94, 44.51, 3.18, 81.23, and 42.82 %, respectively, as well as the concentrations of β-HCH, γ-HCH and total HCH (by 85.68, 18.88, and 99.31 %, respectively). The EDI of total DDT by children, adults, and elderly people was lowest for consumption of milk pasteurized at 73 °C, and that of γ-HCH, for milk pasteurized at 63 °C. The DDP of total DDT decreased with pasteurization at 73 °C. The dietary exposure to DDT and HCH was higher in children.

Key words: DDT, HCH, Milk, Pasteurization, Dietary exposure, Children.

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Introduction

Since 1949, organochlorine pesticides (OCPs), such as dichlorodiphenyltrichloroethane (DDT) and hexachlorocyclohexane (HCH), have been widely used across the world, mainly in agricultural and public health programs for the prevention of pests, weeds and other pathogens in tropical countries(1). In Mexico, DDT was the insecticide used for malaria control; in Veracruz, it was routinely applied in endemic regions until 2003, and γ-HCH (lindane) was used for veterinary pest control(2), since this isomer is considered to be the active element of technical HCH with specific insecticidal properties(3,4). In May 2004, the use of these pesticides was restricted following the entry into force of the Stockholm Convention. The purpose of this international treaty was to protect human health and the environment from toxic, persistent, and bioaccumulative chemicals(5). However, in Mexico and other countries, lindane is used as a seed preservative, as an ectoparasiticide in livestock, and in lotions or soaps for the treatment of scabies and lice in humans(6). Due to their vapor pressure and partition coefficient, OCPs are persistent and mobile compounds present in the environment(7), and, because of their lipophilic nature, they bioaccumulate and biomagnify through the food chain(8,9). DDT and HCH are considered carcinogenic and endocrine disrupting compounds(4,10), whose residues have been reported in animals and humans(11). The half-life of OCPs can range from a few months to several years to decades. The estimated degradation of DDT in soil ranges between 4 and 30 yr(12).

Animal exposure to DDT and HCH can increase due to direct treatment with pesticides, inhalation of air, or ingestion of contaminated forage and feed(2). In bovine organisms, OCPs enter the liver after reabsorption and are slowly metabolized before being released into the circulatory system and, eventually, deposited in fat or eliminated through milk, through which they pass to the calf or to the human consumer(9,13). Several studies have demonstrated the presence of DDT and HCH in bovine milk, meat and tissues(9,14,15). Furthermore, bovine milk has been used as an indicator of the persistence of these pesticides due to animal feeding, air inhalation and intensive use in programs for the control of ectoparasites in cattle. Within this context, OCP levels in these foods have been monitored in order to estimate population exposure and potential health risks(16,17).

The national production of bovine milk in 2018 was 11.923 billion liters, of which the state of Veracruz contributed 6.0 %. Mexico ranked eighth in milk production worldwide; notably, the European Union, considered as a geopolitical entity made up of 28 countries, ranked first in milk production[18,19]. In Mexico, per capita milk consumption during 2018 was 339 ml/person/day[18]. Because of its composition, milk is a complete and balanced food that provides a high content of nutrients in relation to its caloric content, and, therefore, its consumption should be considered necessary from infancy to old age[20]. Due to the importance of this product as a food, several researches have been developed with the purpose of reducing the content of OCPs in milk. Abd-Rabo *et al*[21] reported that pasteurization of buffalo milk at 73 °C caused a 23.07 % decrease in the initial concentration of p,p'-DDE and a 32.85 % decrease in p,p'-DDT, and a 30 % increase in the concentration of p,p'-DDD. Deiana and Fatichenti[22] reported a 6.5 % increase in the concentration of DDT and HCH in bovine milk pasteurized at 73 °C. In contrast, Abou-Arab[23] observed the decrease of γ-HCH (72.90 and 65.00 %) in bovine milk pasteurized at 63 and 72 ºC.

However, little research has been done to evaluate the health risk associated with the consumption of commercially marketed raw and pasteurized milk contaminated with these pesticides[24,25]. Abou-Arab *et al*[26] found that the estimated daily intake (EDI) of γ-HCH in raw, pasteurized and ultra-pasteurized milk was 0.28, 0.11, and 0.03 µg kg^{-1} bw d^{-1}, respectively, while the DDT metabolite with the highest EDI was o,p'-DDE (0.48 µg kg^{-1} bw d^{-1}) from raw milk, and o,p'-DDD (0.60 µg kg-1 bw d^{-1}) from pasteurized milk. Amir *et al*[17] evaluated the presence of OCPs in various feeds. However, the highest EDIs corresponded to raw milk, with 0.011, 0.074, and 0.103 µg kg^{-1} bw d^{-1}, respectively, for γ-HCH, total HCH, and total DDT. Miclean *et al*[27] reported total EDIs of HCH for women, men and children of 0.002, 0.002, and 0.012 µg kg^{-1} bw d^{-1}, and of DDT of 0.001, 0.002, and 0.008 µg kg^{-1} bw d^{-1}, respectively.

Based on the above, the objective of this research was to determine the effect of pasteurization at 63 °C 30 min^{-1} (slow, low-temperature) and at 73 °C 15 sec^{-1} (fast, high-temperature) on the concentration levels of DDT, HCH and their metabolites (p,p'-DDE, p,p'-DDD, o,p'-DDT, p,p'-DDT, α-HCH, β-HCH, γ-HCH, and δ-HCH) in bovine milk from the central agricultural zone of the State of Veracruz, Mexico, as well as to estimate the dietary exposure of population groups of children, adults, and elders to these pesticides contained in raw and pasteurized milk at these two temperatures, by means of EDI and PDD

Material and methods

Ten clinically healthy animals over two years old, with more than one calving that were milking in two production units (PU) located in the municipality of Soledad de Doblado (19º03' N, 96º24' W) and Jamapa (19º01' N, 96º13' W), Veracruz, Mexico, were selected.

The municipalities are characterized by a warm sub-humid climate with rainfall in the summer; the annual precipitation range is 900 to 1,100 mm and 1,100 to 1,300, respectively, and the annual temperature range is 24 to 26 ºC[28,29]. The study was conducted with the approval of the Bioethics and Animal Welfare Committee of the Faculty of Veterinary Medicine and Animal Husbandry. A total of 100 bovine milk samples (500 ml each) from the first milking of the same selected animals were collected every month during an annual cycle (2017-2018) and transported to the Toxicology Laboratory of the Faculty of Veterinary Medicine and Zootechnics of Universidad Veracruzana in accordance with NOM-243-SSA1-2010[30]. In each sampling, a pool of the samples was collected from both Pus, half of which were randomly assigned to slow pasteurization, and the rest, to fast pasteurization. Thus, 25 out of 100 samples were pasteurized at 63 °C 30 min^{-1}, and the other 25, unpasteurized, constituted the 63 °C control. Likewise, 25 samples were pasteurized at 73 °C 15 sec^{-1}, and 25 unpasteurized samples constituted the 73 °C control. Thus, the mixture of raw milk from different origins made at the collection centers and sold to the dairy industry in the area was simulated.

Milk pasteurization

The pasteurization process was carried out at the Toxicology Laboratory in accordance with the NOM-243-SSA1-2010 standard[30]. Briefly: 500 ml of raw milk was heated for slow pasteurization at 63 °C for 30 min, and 500 ml, for rapid pasteurization at 73 °C for 15 sec. At the end of each pasteurization, the samples were rapidly cooled to 4 °C and, once this temperature was reached, immediately centrifuged at 3,500 rpm to separate the fat, which was stored in amber-colored vials labeled and stored at -20 °C until analysis.

Analytical determinations

The determination of the ethereal content of milk was performed using the Gerber method[31], and the concentration levels of DDT, HCH and their metabolites in milk fat were estimated with the modified Murphy methodology[32]. All chemicals used in the analyses were Merck's (Darmstadt, Germany), J.T. Baker's, and the Sigma-Aldrich Company's (St. Louis, MO, USA) analytical grade. Each sample was analyzed in triplicate, and the results were expressed as µg kg^{-1} lipid base. The chemical residues from the analyses stored in amber gallons were collected by the EcoEntorno S.A. de C.V. company, hired by Universidad Veracruzana for the collection of hazardous waste.

Chromatographic analysis

The concentration of DDT, HCH and their metabolites were quantified on an Agilent-Hewlett-Packard 6890 Plus gas chromatograph with a 7683 autosampler and a Ni-63 electron microcapture detector. The operating conditions were as follows: injector in splitless mode, a purge flow of 60 mL min^{-1}, a purge time of 0.80 min, and a temperature of 250 ºC, a HP-608 column measuring 30 m in length x 530 µm in diameter and 0.5 in µm film thickness, with a constant ultrapure nitrogen flow of 2 ml min^{-1}. The oven was programmed with the following temperature ramp: initial temperature from 80 ºC to 180 ºC at 30 ºC min^{-1}, and from 280 ºC to 10 ºC min^{-1}, sustained during 2.7 min. The run time was 19.03 min; the detector temperature was 320 °C, with a constant nitrogen flow of 30 mL min^{-1}, and an injection volume of 1 µL.

Linearity, limits of detection and quantification

Detector linearity was determined by linear regression analysis, performed by ChemStation software from five points on the calibration curves for each pesticide. Calibration was carried out prior to sample analysis using OCP standards purchased from ChemService (Chem Service, Inc., West Chester, PA, USA) and Supelco (Supelco Park, Bellefonte, PA, USA). Qualitative and quantitative analyses were performed by comparing the retention times and peak area of the sample, respectively, with the calibration reference standards. The limit of detection (LOD) and limit of quantification (LOQ) were calculated for each pesticide according to Su(33). Fortified milk samples with a recovery rate of 91-99 % were used to validate this method. The LOD for DDT and HCH ranged from 0.0004-0.00036 and 0.00002-0.00031µg kg^{-1}, respectively, and the LOQ ranged from 0.001 µg kg^{-1} lipid base.

Assessment of dietary exposure to pesticides

Dietary exposure to DDT, HCH and their metabolites was assessed by the estimated daily intake (EDI) and average daily dose (ADD) according to Pandit and Sahu(34). The risk was estimated in three population groups, children, adults, and elders. The EDI was reported as µg pesticide kg^{-1} bw (body weight) day^{-1} and was calculated with the following formula: $EDI=C_a \times F \times I$; where Ca= mean level of organochlorine pesticide residues in milk samples (µg kg^{-1} lipid base), F= fat content in milk samples (%), I= milk intake in milliliters per kilogram body weight per day (ml kg^{-1} bw day^{-1}). The daily milk intake (339 ml per person day^{-1}) used to calculate the EDIs was based on the per capita consumption of milk in Mexico during 2018, and the average weights per person were 25 kg in children[35], 70 kg in adults[36], and 51 kg in elders[37].

The average daily dose (ADD) is the average dose rate in a specific exposure period expressed in mass-time units (µg kg^{-1} d^{-1}) estimated using the following formula: *ADD*=C_m×F× I; where Cm= maximum concentration of organochlorine pesticides in milk samples (µg kg^{-1}), F= fat content in milk samples (%), I= milk intake (mL kg^{-1} bw d^{-1}). The following daily milk intake (mL) and recommended weights (kg) were considered for pesticide ADD in three main population groups: children (480 mL/25 kg)[35], adults (240 mL/70 kg)[38], and elders (310 mL/51 kg)[37].

Statistical analysis

A completely randomized experimental design was employed, with the source of variation being the method of slow and rapid pasteurization of milk to evaluate its effect on the concentration levels of DDT, HCH and their metabolites (p,p'-DDE, p,p'-DDDD, o,p'-DDDT, p,p'-DDDT,α-HCH, β-HCH, γ-HCH, and δ-HCH) in milk. The statistical model of the experimental design was as follows:

$$Y_{ij} = \mu + \tau_i + \varepsilon_{ij}$$

Where:

$\boldsymbol{Y_{ij}}$ is the response variable (OCP concentration) of the ijth experimental unit (each milk sample) (i and j denote factor level and replication at factor level, respectively);

$\boldsymbol{\mu}$ is the effect of the overall mean;

$\boldsymbol{\tau_i}$ is the effect of the ith treatment (slow and fast pasteurization);

$\boldsymbol{\varepsilon_{ij}}$ is the effect of the experimental error associated with the ith experimental unit.

With the concentrations obtained, a one-way ANOVA (P<0.05) was performed to evaluate the effect of pasteurization at 63 and 73 °C on the mean concentration levels of DDT, HCH, and their metabolites in raw bovine milk. Significant differences between the means were determined by Tukey's test (P<0.05) using the Minitab v.17.0 statistical software.

Results and discussion

Concentration levels of DDT and HCH in raw milk

According to the results (Table 1), the concentrations of p,p'-DDE, p,p'-DDDD, o,p'-DDDT, and total DDT (15.736 ± 8.133, 9.849 ± 5.271, 15.237 ± 9.006 and 26.480 ± 13.880 µg kg^{-1} lipid base, respectively) of the raw milk used as control pasteurized at 63 ºC were higher (P<0.05) than those of the control milk pasteurized at 73 ºC (3.829 ± 2.172, 4.904 ± 3.493, 3.734 ± 1.526, and 8.920 ± 7.340 µg kg^{-1} lipid base, respectively.

However, the concentrations of total DDT in the control milks did not exceed the Maximum Residue Limit (MRL) of 50 µg kg^{-1} (lipid basis) established by the Food and Agricultural Organization/World Health Organization[39].

Table 1: Mean and standard deviation of DDT, HCH and their metabolites (µg kg^{-1} lipid base) in control raw milk and pasteurized milk

Pesticide	Treatment at 63 ºC 30 min^{-1}		Treatment at 73 ºC 15 seg^{-1}	
	Control milk (n=25)	Pasteurized milk (n=25)	Control milk (n=25)	Pasteurized milk (n=25)
p,p'-DDE	15.736 ± $8.133^{a,x}$	10.673 ± 5.682^{b}	3.829 ± $2.172^{a,y}$	2.644 ± 2.023^{a}
p,p'-DDD	9.849 ± $5.271^{a,x}$	8.334 ± 4.771^{a}	4.904 ± $3.493^{a,y}$	2.721 ± 1.333^{a}
o,p'-DDT	15.237 ± $9.006^{a,x}$	7.559 ± 4.015^{b}	3.734 ± $1.526^{a,y}$	3.615 ± 1.831^{a}
p,p'-DDT	13.441 ± $7.905^{a,x}$	13.484 ± 6.673^{a}	11.351 ± $6.485^{a,x}$	2.130 ± 1.566^{a}
DDT total	26.480 ± $13.880^{a,x}$	20.170 ± 16.05^{a}	8.920 ± $7.340^{a,y}$	5.100 ± 3.210^{a}
α-HCH	0.270 ± $0.154^{a,x}$	0.355 ± 0.137^{b}	0.310 ± $0.111^{a,x}$	0.543 ± 0.151^{b}
β-HCH	0.401 ± $0.276^{a,x}$	0.919 ± 0.062^{b}	1.438 ± $0.376^{a,y}$	0.206 ± 0.113^{b}
γ-HCH	0.511 ± $0.338^{a,x}$	0.702 ± 0.236^{a}	1.187 ± $0.204^{a,y}$	0.963 ± 0.122^{a}
δ-HCH	0.424 ± $0.229^{a,x}$	1.504 ± 2.379^{a}	0.523 ± $0.346^{a,x}$	1.504 ± 2.379^{a}
HCH total	0.552 ± $0.368^{a,x}$	1.067 ± 1.076^{a}	1.241 ± $0.830^{a,x}$	0.684 ± 0.452^{a}

DDT total= p,p'-DDE + p,p'-DDD + o,p'-DDT + p,p'-DDT; HCH total= α-HCH + β-HCH + γ-HCH + δ-HCH.

[a,b] Values with different letters indicate a significant difference (P<0.05) between columns of the same treatment.

[x,y] Values with different letters indicate a significant difference (P<0.05) between the controls of the two treatments.

As shown in Table 2, these concentrations detected in raw milk were lower than those recorded in raw bovine milk in the previous study conducted by Pardío *et al*[40] in the state of Veracruz. Comparing our data with the concentrations of DDT metabolites detected in raw milk from Brazil[24], Colombia[14] and Romania[27], the levels detected in the 63 ºC and 73 ºC control milks in the present study were up to 26 and 11 times higher, respectively. However, in Egypt, the concentration of p,p'-DDDT in raw milk was twice as high as that found in the controls in this research. The β-HCH and γ-HCH concentrations (1.438 ± 0.376 and 1.187 ± 0.204 µg kg^{-1} lipid base, respectively) of the 73 °C control milk were (*P*<0.05) higher than those of the 63 °C control concentrations (0.401 ± 0.276 and 0.511 ± 0.338 µg kg^{-1} lipid base, respectively). Although the total MBM of the 73 °C control milk was 2.24 times higher than that of the 63 °C control, it was not significantly different (*P*>0.05). The variation in OCP concentrations among the controls may have been due to the indistinct variations in their levels in the milk of each PU throughout the annual cycle. This variation may be attributed to the combination of zootechnical management practices in production units and diets formulated with contaminated ingredients, the effect of kinetics due to the physicochemical properties of each isomer, metabolic activities related to lipid mobilization, and, possibly, a lack of homogeneity in the concentration of metabolites due to the remobilization of lipids and contaminants during milk synthesis, as well as to the application load of the pesticide, since it can be deposited or absorbed from the atmosphere to the surface of the pasture, being affected by temperature, rain, wind and weather[2].

The α-HCH, β-HCH, γ-HCH and total HCH metabolites present in the 63 °C and 73 °C controls did not exceed the MRLs established by FAO/WHO, of 50, 20, 10 and 100 µg kg^{-1} (lipid basis), respectively[39]. As shown in Table 2, the concentrations of α-HCH, β-HCH and γ-HCH metabolites evaluated in the present study were lower than those reported by Pardío *et al*[40] in Veracruz, Mexico. The levels of γ-HCH reported in milk from Tlalixcoyan, Veracruz, Mexico, were 250.3 times higher than those reported in control milk at 63 ºC (0.511 ± 0.338 µg kg^{-1} lipid base), and 107.76 times higher than those reported in control milk at 73 ºC (1.187 ± 0.204 µg kg^{-1} lipid base). The concentration of γ-HCH in Egypt was 62.5 times higher than that reported in raw milk at 63 ºC, and 26.9 times higher, in the control milk at 73 ºC. However, the concentration of α-HCH recorded in the Colombian milk was similar to that observed in the control milk at 73 ºC (0.310 ± 0.111 µg kg^{-1} lipid base).

Table 2: Organochlorine pesticide residues (µg kg^{-1} lipid base) in raw milk reported in other studies

Location/Country	p,p'-DDE	p,p'-DDD	p,p'-DDT	o,p'-DDT	α-HCH	β-HCH	γ-HCH	δ-HCH	Reference
Medellín, Veracruz/Mexico	39.000	ND	89.000	26.000	13.000	23.000	49.000	NA	Pardío *et al*[40]
Paso San Juan, Veracruz/Mexico	18.000	ND	49.000	ND	13.000	17.000	22.000	NA	Pardío *et al*[40]
Tlalixcoyan, Veracruz/Mexico	24.000	ND	36.000	ND	31.00	69.000	128.000	NA	Pardío *et al*[40]
Rio Grande do Sul/Brazil	0.011	0.000	ND	0.000	0.002	ND	0.006	ND	Heck *et al*[24]
Cairo/Egypt	12.000	6.000	24.000	3.000	NA	NA	32.000	NA	Abou-Arab *et al*[26]
Sabanas, Córdoba/Colombia	ND	ND	0.034	ND	0.469	ND	ND	ND	Díaz *et al*[14]
Rumania	0.011	0.000	0.000	0.000	0.001	0.010	0.002	0.001	Miclean *et al*[27]

NA= not analyzed; ND= not detected.

It should be noted that organochlorine pesticides and their residues are highly lipophilic and persistent in nature, and, therefore, they are easily concentrated in milk fat[26]. This implies that, although OCPs were banned for agricultural use in the early 1970s in most countries, their residues still persist. Due to their persistence, the detected concentrations of DDT, HCH and their metabolites in the raw milk of the present study are probably due to the loads of DDT applied during its previous legal use, which has caused the contamination of cattle grazing areas in the state of Veracruz. Likewise, γ-HCH continues to be used for the control of ectoparasites in livestock[2]. In fact, these pesticides continue to be applied in various parts of the world because of their potent and broad-spectrum effects against harmful organisms[41].

Effect of pasteurization on DDT and HCH concentration levels

As shown in Tables 1 and 3, after pasteurization at 63 ºC, the concentration levels of the metabolites p,p'-DDE and o,p'-DDDT (10.673 ± 5.682 and 7.559 ± 4.015 µg kg^{-1} lipid base, respectively) decreased (P<0. 05) with respect to the levels of the control (raw) milk by 32.17 and 50.39 %, respectively, and only p,p'-DDDT increased (P>0.05) in concentration, by 0.31 %, with respect to the control. Therefore, the concentration of total DDT (20,170 ± 16,050 µg kg^{-1} lipid base) decreased (P>0.05) by 29.83 % with respect to that of the control (26,480 ± 13,880 µg kg^{-1} lipid base). On the other hand, the levels of α-HCH and β-HCH

(0.355 ± 0.137 and 0.919 ± 0.062 µg kg^{-1} lipid base, respectively) increased (P<0.05) by 31.48 and 129.11 %, respectively; however, the increase in the levels of γ-HCH, δ-HCH and total HCH was not significant (P>0.05). The α-HCH and γ-HCH isomers can isomerize to β-HCH. The stability of β-HCH, its tendency to accumulate in human and animal tissues over time, its rapid (525-fold) bioconcentration in man, and its slower elimination, represents a risk with respect to its chronic toxicity. This metabolite is the most toxic, followed by α-, γ-HCH and δ-HCH, due to its longer biological half-life (of 7-8 yr) in the body (ATSDR)[42].

Table 3: Variation (%) of the concentration of DDT, HCH and their metabolites due to the pasteurization effect of bovine milk in different studies

Type of sample/Provenance	PEST	Pasteurization 63 °C x 30 min^{-1}		Pasteurization 73 °C x 15 seg^{-1}		Reference
		Decrease	Increase	Decrease	Increase	
Buffalo milk/Egypt	p,p'-DDE	NA	NA	23.07	---	Abd-Rabo *et al*[21]
	p,p'-DDD	NA	NA	---	30	
	p,p'-DDT	NA	NA	32.85	---	
Bovine milk/Italy	DDT total°	NA	NA	---	6.5	Deiana y Fatichenti[22]
	HCH total▫	NA	NA	---	6.5	
Bovine milk/Egypt	γ-HCH	72.90	---	65.00	---	Abou-Arab [23]
Bovine milk/Mexico	p,p'-DDE	32.17	---	30.94	---	Present studio
	p,p'-DDD	15.38	---	44.51	---	
	o,p'-DDT	50.39	---	3.18	---	
	p,p'-DDT	---	0.31	81.23	---	
	DDT total	29.83		42.82	---	
	α-HCH	---	31.48	---	75.16	
	β-HCH	---	129.11	85.68	---	
	γ-HCH	---	37.37	18.88	---	
	δ-HCH	---	257.24	---	187.57	
	HCH total▫	---	93.29	99.31	---	

PEST= pesticide; NA= not analyzed; DDT total= p,p'-DDE + p,p'-DDD + o,p'-DDT + p,p'-DDT; ▫HCH total= α-HCH + β-HCH + γ-HCH + δ-HCH.

Pasteurization at 73 ºC reduced (P<0.05) the concentration levels of DDT and its metabolites in milk with respect to the control milk from 3.18 % (o,p'-DDDT) to 81.23 % (p,p'-DDDT). A significant (P<0.05) increase in α-HCH and δ-HCH levels (0.543 ± 0.151 and 1.504 ± 2.379 µg kg^{-1} lipid base) was observed at 75.16 and 185.57 %, respectively, and there was a significant (P<0.05) decrease in β-HCH (0.206 ± 0.113 µg kg^{-1} lipid base), by 85.68 %. The observed decrease in the concentrations of p,p'-DDE, p,p'-DDDD, o,p'-DDDT and total DDT in pasteurized milk at 63 °C compared to the control concentration, except for p,p'-DDDT, could be attributed to the isomerization of p,p'-DDE and p,p'-DDDD to p,p'-DDDT by heating. However, the concentrations of HCH and all its metabolites were increased by pasteurization at 63 °C, and α-HCH and δ-HCH metabolites, by pasteurization at 73 °C. Organochlorine pesticides can be degraded by photolysis, hydrolysis, oxidation and reduction, temperature, and pH. The retention of pesticides will depend on the physicochemical properties of the pesticide molecule, as well as of the feed(43). However, the concentration of total DDT in control and pasteurized milk at 63 and 73 °C did not exceed the FAO/WHO MRL of 50 µg kg^{-1} (lipid basis)(39). Likewise, the metabolites α-HCH, β-HCH, γ-HCH and total HCH present in milk pasteurized at 63 ºC and 73 ºC did not exceed the MRLs of 50, 20, 10 and 100 µg kg^{-1} (lipid basis), respectively, established by FAO/WHO(39). However, the δ-HCH metabolite, whose levels increased in both pasteurizations, is structurally related to carcinogenic HCHs (ATSDR)(42).

The results of pasteurization at 63 and 73 °C differ with those found by Abou-Arab(23), who reported a decrease of 72.9 and 65.0 %, respectively, in the concentration of γ-HCH in bovine milk, whereas in the present study this metabolite increased by 37.37 % with pasteurization at 63 °C, but decreased by 18.88 % with pasteurization at 73 °C. In Egypt, Abd-Rabo *et al*(21) reported that the metabolites p,p'-DDE and p,p'-DDDT decreased in milk pasteurized at 73 °C. The present study shows a similar decrease in p,p'-DDE, but an increase in p,p'-DDDT. The decrease in p,p'-DDD (44.51 %) observed in this study contrasts with the increase reported by these authors (30 %). In Italy, Deiana and Fatichenti(22) reported that the concentrations of total DDT and HCH increased by 6.50 % in bovine milk pasteurized at 73 °C, while in the present study they decreased by 42.82 and 99.31 %, respectively. These results indicate that pasteurization at 73 °C 15 sec^{-1} reduces the concentration of most of the OCP analyzed. It is important to note that milk is considered a staple product in human nutrition. The benefits of bovine milk are not limited to its nutritional value; these are a factor in the prevention of pathologies such as cardiovascular diseases, certain types of cancer, arterial hypertension, and bone or dental diseases(20). Hence the importance of reducing the concentration of these contaminants in milk by pasteurization, given that their presence entails a risk to public health.

Estimation of dietary exposure to DDT and HCH through human consumption of bovine milk

Estimated daily intake (EDI) of DDT and HCH

In order to protect public health, OCP intake limits have been established for milk and other foods that should not be exceeded. The Acceptable Daily Intake (ADI) of total DDT is 20 µg

kg^{-1} bw d^{-1} recommended by FAO/WHO[39], and 0.5 µg, recommended by the EPA[44]; the for γ-HCH (lindane), it is 8 µg[39]. Thus, the EDI was calculated for total DDT and the metabolite γ-HCH from the concentration detected in the milks studied for three population groups: children, adults and the elderly.

Table 4 shows that in the children's group, the highest EDI was calculated in the 63 °C control milk, which did not exceed the acceptable values recommended by FAO/WHO (20 µg kg^{-1} bw d^{-1}), but was 28.06 times higher than the levels recommended by EPA (0.5 µg). In the adult group, the highest concentration also occurred in the 63 °C control milk, without exceeding the FAO/WHO recommended values; however, exceeded the EPA acceptable values by 10 times. The EDI of the elderly group was higher for the 63 °C control milk, exceeding the EPA acceptable values by 13.74 times. Abou-Arab *et al*[26] estimated total DDT EDIs of 0.394 and 0.113 µg kg^{-1} bw d^{-1} for adults and 0.475 and 0.135 µg for children, likewise γ-HCH EDIs were 0.280 and 0.113 µg for adults and 0.336 and 0.135 µg for children from consumption of raw and pasteurized (63 °C) milk, respectively, from local markets in Cairo, Egypt. Accordingly, the EDIs in the present study were lower than those estimated in adults and children consuming raw and pasteurized milk in Egypt, because the concentrations of DDT and HCH in that area were higher than those reported in the present investigation.

Table 4: Estimated daily intake (EDI) (µg kg^{-1} bw d^{-1}) of total DDT and γ-HCH for children, adults and the elderly estimated on consumption of raw (control) and pasteurized milk

Population group	Treatment 63 ºC x 30 min^{-1}		Treatment 73 ºC x 15 sec^{-1}		ADI (µg kg^{-1} bw d^{-1})
	Control milk	Pasteurized milk	Control milk	Pasteurized milk	
Total DDT					20[39] 0.05[44]
Children	14.03	10.68	4.72	2.70	
Adults	5.00	3.81	1.62	0.96	
Elders	6.87	5.23	2.31	1.32	
γ-HCH					8[39]
Children	0.27	0.37	0.62	0.50	
Adults	0.04	0.06	7.81	0.09	
Elders	0.08	0.11	0.19	0.16	

ADI= acceptable daily intake; FAO/WHO, 1997[39]; EPA[44].
Total DDT= p,p'-DDE + p,p'-DDD + o,p'-DDT + p,p'-DDT.

The highest γ-HCH EDI in the three population groups occurred in the 73 °C control milk. However, none of the EDIs exceeded the acceptable value recommended by FAO/WHO (8 µg kg^{-1} bw per day). Pardío *et al*[40] reported that the EDI for infants and adults from consumption of raw milk from Tlalixcoyan, Veracruz, Mexico, contaminated with γ-HCH was 0.666 and 0.021 µg kg^{-1} bw d^{-1}, respectively. However, the EDIs for infants and adults from consumption of raw milk contaminated with total DDT were highest for milk from Medellín, Veracruz, Mexico with values of 0.530 and 0.017 µg kg^{-1} bw d^{-1}, respectively. In a recent study, Miclean *et al*[27] reported EDIs of total HCH in raw milk for women, men and children of 0.002, 0.002 and 0.012 µg, respectively; for total DDT they were 0.001, 0.002 and 0.008 µg for women, men and children, respectively, lower than those estimated for total DDT in children in the present study for the consumption of pasteurized milk at 63 and 73 °C and raw milk (respective controls). Comparing the results of Pardío *et al*[40] with those of the present study, it is observed that exposure to γ-HCH and total DDT through the consumption of raw milk has increased in the area. The above indicates an increase in contamination over time due to the continued use of γ-HCH in livestock in the region and the high DDT loads sprayed in the past that resulted in contaminated pastures near urban and suburban areas where DDT was sprayed for malaria control[2]. However, 30 to 37 % of the unpasteurized fluid milk of the national production is destined to the production of artisanal cheeses[45], of the production in the state of Veracruz, 50 % of the unpasteurized fluid milk in storage is sold to local cheese dairies for the production of cheese and other artisanal dairy products that are marketed in the main urban areas of the state[46]. As a consequence, consumers of milk produced in this agricultural zone of Veracruz are exposed to dietary levels of OPs that are higher than exposure levels in developed countries, where the use of these pesticides was banned many years ago[40]. This risk could be reduced if the milk were pasteurized at 73 °C 15 sec^{-1} to reduce the concentration of most of the OCPs analyzed. As shown in Table 3, according to the literature consulted, very few studies have been conducted on slow and rapid pasteurization and the estimation of the respective dietary intake. Monitoring the levels of these OCPs in milk and other foods is essential to ensure that the MRLs and ADIs recommended by FAO/WHO are not exceeded.

Estimated average daily dose (ADD) of DDT

The ADD is a prediction of the daily intake of residues of a pesticide based on the estimation of residue concentrations in food and on the available food consumption data for a given population[47]. For total DDT, the Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO) have established the ADD of 48 µg kg^{-1} d^{-1}[45]. In the case of HCH, there is no established ADD.

The highest estimated ADDs in children, adults and elders, from 63 ºC control milk, were 142.01, 71.00 and 91.72 µg kg^{-1} d^{-1}, respectively; these values exceed the FAO/WHO recommended limit (48 µg) by 2.9 times in the children’s group, 1.4 times among the adults, and 1.9 times among elderly people. After pasteurizing this milk at 63 ºC, the calculated ADDs in the three population groups (114.06, 57.03 and 73.66 µg kg^{-1} d^{-1}, in children, adults and elders, respectively) also exceeded the recommended limit. Notably, the estimated ADD for consumption of control milk at 73 ºC in children was 59.48 µg, which is higher than the limit recommended by FAO/WHO (48 µg). However, once the milk was pasteurized, the ADD value was 6.15 times lower (9.67 µg) than that of the control milk (9.67 µg). The ADD calculated in adults and elders for the control milk (29.74 and 38.41 µg, respectively) and for milk pasteurized at 73 ºC (18.08 and 23.35 µg, respectively) remained below the limit recommended by FAO/WHO and were lower than those estimated for the consumption of raw milk used as control at 63 ºC. These results contrast with those reported by Pardío *et al*[40] who estimated lower daily doses for children and for adults (4,068 and 2,339 µg, respectively) who consumed raw milk from Medellín, Veracruz, Mexico. It should be noted that there are certain population groups that are more vulnerable to the effects of these pesticides, such as the child population –especially those with some degree of malnutrition–, and the female population of childbearing age, particularly during pregnancy, given that there is evidence of their hormonal and lipid disruption activity[48]. The effects of OCP exposure to human health through food is a problem that deserves more attention. The results obtained indicate their presence in raw and pasteurized milk and an increase in ADDs, making it essential to review the MRLs and seek alternative pest control methods in order to improve food safety and protect public health.

Conclusions and implications

The pasteurization process of milk at 73 ºC reduces the concentrations of the DDT and its metabolites, as well as most of the concentrations of HCH metabolites. Therefore, the thermal process under these conditions represents a favorable alternative for the reduction of dietary exposure to these pesticides through human consumption of pasteurized milk at 73 ºC.

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