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Trabajo Original

Otros

Antioxidants, reactive oxygen species and oxidative damage associated to the presence of organochlorine pesticides in breast milk

Antioxidantes, especies reactivas de oxígeno y daño oxidativo asociado a la presencia de plaguicidas organoclorados en la leche materna

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Abstract

Introduction: Organochlorine pesticides (OCPs) can increase the production of reactive oxygen species (ROS), the effects of which can be countered by the antioxidant system, also present in breast milk.

Objective: To evaluate the indicators of oxidative stress in breast milk associated to the presence of OCPs, and their relationship with seafood intake.

Methods: Breast milk was collected from 108 lactating women. Indicators of oxidative stress (antioxidant enzyme activity, glutathione concentration, superoxide radical [$O_2^{\bullet-}$] production, lipid peroxidation and carbonylated protein concentration) were analyzed by spectrophotometry. OCPs concentrations were measured by gas chromatography.

Results and discussion: $O_2^{\bullet-}$ production had no significant relationship with OCPs concentrations. Significant correlations between OCPs concentrations and antioxidant enzyme activities (glutathione reductase [GR] activity and aldrin concentration [r = - 0.5], superoxide dismutase [SOD] activity and α-HCH concentration [r = 0.45]) were found. Oxidative damage had a low correlation with OCPs content ($r < 0.30$, $p < 0.05$). It is possible that OCP's levels are not sufficient to increase $O_2^{\bullet-}$ production, that production of ROS other than $O_2^{\bullet-}$ is increased, or that the antioxidant capacity is sufficient to avoid oxidative damage in breast milk.

Conclusion: Results from this study suggest that marine diet is not a determinant factor in the level of contamination by OCP's or in the oxidative damage in breast milk.

Key words:

Antioxidants. Breast milk. Marine diet. Organochlorine pesticides.

Resumen

Introducción: los plaguicidas organoclorados (POC) pueden incrementar la producción de especies reactivas de oxígeno (ERO). Tales efectos pueden ser contrarrestados por el sistema antioxidante, el cual se encuentra también en la leche materna.

Objetivo: evaluar los indicadores de estrés oxidativo en leche materna asociados a la presencia de POC y su relación con la ingesta de alimentos marinos.

Métodos: la leche materna fue colectada de 108 mujeres lactantes. Los indicadores de estrés oxidativo (actividad enzimática antioxidante, concentración de glutatión, producción de radical superóxido [$O_2^{\bullet-}$], concentración de peroxidación de lípidos y carbonilos proteicos) se analizaron por espectrofotometría. Las concentraciones de POC se midieron por cromatografía de gases.

Resultados y discusión: la producción de $O_2^{\bullet-}$ no presentó relación significativa con las concentraciones de POC. Se encontraron correlaciones significativas entre las concentraciones de POC y la actividad de las enzimas antioxidantes (actividad de glutatión reductasa [GR] y concentración de aldrín [r = - 0,5], actividad de superóxido dismutasa [SOD] y concentración de α-HCH [r = 0,45]). El daño oxidativo mostró baja correlación con el contenido de POC ($r < 0,30$, $p < 0,05$). Es posible que los niveles de POC no sean suficientes para incrementar la producción de $O_2^{\bullet-}$, ya sea que el incremento en la producción de ERO se deba a especies reactivas diferentes a $O_2^{\bullet-}$ o debido a que la capacidad antioxidante es suficiente para evitar el daño oxidativo en leche materna.

Conclusión: los resultados de este estudio sugieren que la dieta marina no es un factor determinante en el nivel de contaminación por POC, ni en el daño oxidativo presente en leche materna.

Palabras clave:

Antioxidantes. Leche materna. Dieta marina. Plaguicidas organoclorados.

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INTRODUCTION

Humans are exposed to a variety of xenobiotics, among them organochlorine pesticides (OCPs), which can be found in food, water, air and the environment (Xavier, 2004). Despite the benefits that OCPs have provided to human health (e.g. by controlling vector-borne diseases and pests in crops), and due to their toxicity, their use was banned in developed countries over 40 years ago, and 13 years ago in Mexico (37). Certain OCPs have been reported to be used for agriculture in the Baja California peninsula and, although the presence of OCPs has been reported in tissues from mammals in the Baja California peninsula (33,40), the level of contamination in humans is unknown.

OCPs can be bioaccumulated in adipose tissue due to their lipophilic character (13). Pesticides affect receptors involved in the metabolism of carbohydrates, fats and proteins. They also induce a decrease in insulin production, affecting glycolysis and increasing the risk of type II diabetes (28). To avoid chronic disorders, the body has mechanisms to detoxify OCPs, namely, biotransformation or excretion, for example, via breastfeeding (13), for lactation is the main process to remove OCPs from the human body (8). Despite the fact that breast milk is the best food for infants during their first months of life, toxic compounds can be transferred through breast milk. Since 1950, pesticides have been detected in breast milk, including organochlorines such as dichlorodiphenyltrichloroethane (DDT) and its metabolites (4). Breast milk generally does not result in acute toxicity for infants because of the relatively low concentrations of pesticides reported in milk and the presence of molecules with detoxification function, such as antioxidants (4).

Pesticides may lead to increased generation of reactive oxygen species (ROS) and/or affect antioxidant enzyme activities (3), leading to an imbalance between pro-oxidant and antioxidant molecules, inducing oxidative stress (42). In order to counteract oxidative damage, the human body has developed an antioxidant system which can also be found in breast milk. The antioxidant system comprises antioxidants enzymes, such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR) and glutathione S-transferase (GST), as well as small molecular weight antioxidants, such as glutathione (GSH) (22).

In a previous study, we reported that maternal age and number of pregnancies increased lipid peroxidation (TBARS) in breast milk due to the increase in metabolism and the consequent production of ROS (9). The increasing age of mothers has been associated with an increase in the concentration of OCPs (6), while the parity has been negatively correlated with OCPs content (41). In some areas, consumption of food contaminated with OCPs, especially food with high fatty acid content, has been associated with increased concentrations of these contaminants in breast milk (8). Although intake of food of animal origin has been linked to an increase in the concentration of OCPs (11), some foodstuffs, such as seafood, additionally contain antioxidant molecules that help prevent oxidative damage caused by the xenobiotics present (23,31).

The toxicological evaluation in human milk may be a non-invasive indicator of population and environmental pollution (13). In addition, the assessment of antioxidant and/or detoxifying systems allows determining the defenses present in human milk which may contribute to maintain the breast milk quality. Therefore, the aim of this study was to evaluate ROS production, oxidative damage, and the antioxidant system in breast milk in association with OCPs, as well as their relationship with seafood (fish and shellfish) intake.

MATERIAL AND METHODS

STUDY GROUP

Samples were taken from 108 lactating women from La Paz, Baja California Sur, Mexico, who were within 1 to 2 weeks postpartum. Milk samples were taken by a volunteer nurse and were immediately refrigerated, transported to the laboratory, and stored at -80 °C until analysis. Informed consent, as well as a structured questionnaire, was collected. The questionnaire requested information on age, body weight, and height, as well as marine diet assessment of the women. Weight and height were used to calculate the body mass index (BMI = weight [kg]/height squared [m^2]). The assessment of women's feeding habits allowed to know their consumption of marine diet (no consumption of fish or shellfish, fish intake only, shellfish intake only, consumption of both fish and shellfish). The project and the informed consent were approved by the Baja California Sur Chapter of the National Academy for Bioethics, A.C. The study was carried out in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans.

SAMPLE ANALYSES

Prior to analyses, whole milk samples (500 µL) were homogenized and centrifuged at 905.6 x g for 10 minutes at 4 °C (Sorvall RT, Massachusetts, USA). Phenyl methyl sulfonyl fluoride (PMSF, 1 mM) was added to the recovered supernatant as protease inhibitor.

ANTIOXIDANT ENZYME ACTIVITY

The activity of SOD was determined following the inhibition of the reduction of nitroblue tetrazolium (NBT); one unit of SOD activity is defined as the amount of enzyme required to inhibit the reaction by 50% (43). Catalase activity was analyzed spectrophotometrically by registering the disappearance of H_2O_2 at 240 nm (1); one unit of CAT activity is defined as the quantity of enzyme required to reduce 1 µM H_2O_2 per minute. To determine the activity of GPx, H_2O_2 was used as substrate and the decrease

in the concentration of reduced nicotinamide adenine dinucleotide phosphate (NADPH) was measured in a coupled assay with GR (16,25); one unit of GPx activity is defined as the amount of enzyme that oxidizes 1 µM NADPH per minute. Glutathione S-transferase activity was measured by following the formation of the dinitrobenzene glutathione thioether complex formed by the conjugation of GSH with 1-chloro 2,4- dinitrobenzene (CDNB) (21); one unit of GST activity is defined as the amount of enzyme that catalyzes the conjugation of 1 µM CDNB. GR activity was determined by measuring the decrease in observed absorbance during the oxidation of NADPH to $NADP^+$ by oxidized glutathione (GSSG) (19,25); one unit of GR activity is defined as the amount of enzyme that oxidizes 1 µM NADPH per minute. In order to standardize the results, protein content (mg of protein mL^{-1}) in milk samples was measured following the method of Bradford (29), using the Bio-Rad© kit (Laboratories Hercules, CA) adapted to microplate. All samples were analyzed in triplicate and expressed in units of enzyme activity per milligram protein (U mg^{-1} of protein).

GLUTATHIONE CONCENTRATION

To quantify the GSH content, the method of Griffith (20) as modified by Hermes-Lima and Storey (25) was used. The method is based on the reaction of GSH with 5'5-dithiobis 2-nitrobenzoic acid (DTNB) which generates a yellow product. This product can be detected by spectrophotometry at 412 nm. The results were expressed in nmol of glutathione equivalents (GSH-Eq) per milligram of protein.

SUPEROXIDE RADICAL PRODUCTION

Endogenous $O_2^{\bullet-}$ production was quantified by an indirect and discontinuous photocolorimetric method following the reduction of ferricytochrome *c* to ferrocytochrome *c*. Data were expressed in nmol $O_2^{\bullet-}$ per milligram of protein per minute.

OXIDATIVE DAMAGE

Lipid peroxidation levels in milk samples were quantified as the concentration of thiobarbituric acid reactive substances (TBARS). The concentration of malondialdehyde (MDA), a crystalline pink pigment produced when hydroperoxides and lipid aldehyde products of lipid peroxidation react with thiobarbituric acid (TBA), was quantified at 532 nm (34). The results were expressed as nmoles per milligram of soluble proteins. Oxidative damage to proteins was quantified by calculating the content of protein carbonyls (45). The concentration of the complex formed between protein carbonyl derivatives and 2, 4-dinitrophenyl hydrazine (DNPH) was measured. Results are expressed in µmol of protein carbonyls per milligram of soluble proteins.

ORGANOCHLORINE PESTICIDE (OCPs) CONCENTRATIONS

Twenty one pesticides were quantified following an adaptation to the method described by Gardner et al. in 2003 (18) and Ávila and Gemio in 2011 (7). OCPs from each whole milk sample (approximately 1 g) were extracted with HPLC grade solvents (hexane, dichloromethane) purchased from Acros (New Jersey, USA) and sulfuric acid obtained from Sigma Chemical Co. (St Louiss, MO, USA). Then they were purified by using a florisil and anhydrous sodium sulfate (Sigma Chemical, New Jersey, USA). Finally, 1 µL of sample was injected into a gas chromatograph (Network GC System, Mod. 6890 N; electron capture detector, Mod. G2397-65505, automatic Palo Alto CA, USA) with a capillary column (30 m x 0.025 mm x 0.25 µm, 5% phenylmethyl silicone, HP-5MS Agilente Wilmington, DE, USA). To calculate the recovery efficiency during the procedure, 1, 7, 8 trichlorodibenzo-p-dioxin (TriCDD) and 1, 2, 3, 4-tetrachlorodibenzo-p-dioxin (TetraCDD) (Accu Standar, New Haven, C.T., USA) were used as standards. The recovery of the process was calculated to be 80%. For the calculations, a calibration curve was performed for each pesticide (1 to 0.001 ppm). The results are expressed in ppm (mg L^{-1}). The detection limits range from 0.0016 to 0.003 ng mL^{-1}, depending on the individual pesticide.

STATISTICAL ANALYSES

OCPs were clustered by chemical structure as hexachlorocyclohexanes (HCHs; this group included α-, β-, γ- and δ-isomers), chlordanes (β and δ-isomers), cyclodienes (aldrin, dieldrin, endrin, endrin andehyde, endrin cetone, and endosulfan I and II), dichlorodiphenyltrichloroethanes (DDTs; this group included DDT, *pp*-DDE and *pp*-DDD metabolites), heptachlors (heptachlor and, β-heptachlor epoxide), hexachlorobenzene, and methoxychlor (30). For those OCPs that had concentrations below the detection limit (DL), and in order to calculate the descriptive statistics, a distributional method named probability plot was used to extrapolate the missing data (24,30).

Distribution of the data for the oxidative stress indicators was assessed by a *Kolmogorov-Smirnov* normality test ($p < 0.01$). Because the data did not have a normal type distribution, descriptive statistics are presented as median, range and percentiles (25^{th} and 75^{th}). Spearman correlation matrices were performed to evaluate relationships between oxidative stress indicators and OCPs concentrations. When OCPs concentration was below the DL, the half value of the specific DL for each OCP (0.0008-0.0015) was used to complete the database for multivariate analyses (14). The principal component analysis (PCA) was employed in order to reduce the number of variables, according to their contribution to explain data variability. In addition, PCA allowed understanding distributional patterns between women according to pesticide concentrations, endogenous antioxidant defenses, and oxidative damage (30,44). In the multivariate analysis, only those pesticides detected in at least 30% of the data were analyzed (24). Significant factor loadings were considered when ≥ 0.5.

Data of lactating women were grouped according to seafood consumption (no consumption of fish or shellfish [n = 25], only fish intake [n = 39]), only shellfish intake [n = 2], consumption of both fish and shellfish [n = 42]). A discriminant analysis (DA) was used to determine if variables (oxidative stress indicators and OCPs concentrations) allow discriminating or separating the groups defined *a priori*. The Wilks' Lambda and squared Mahalanobis distances statistics were used to evaluate the significance in group separation (44,46). All statistics were considered as significant when $\alpha \leq 0.05$. Statistical analyses were run in Statistica v.8 and R v.3.0.2 (35).

RESULTS

The women that participated in this study were, on average, 27 years old (range 15-44 years old) with a mean BMI of 30 kg m^{-2} (range 19-49 kg m^{-2}). Results for activity of antioxidant enzymes, concentration of endogenous antioxidants and oxidative damage in breast milk of lactating women are presented in table I. Twenty one different OCPs were found in the milk of 108 women. Median, rank, percentiles (25th and 75th) and frequency of the concentration of OCPs detected in breast milk are shown in table II. The pesticides most frequently observed were dichlorophenyl dichloroethylene (DDE) in 108 women (100%), β-hexachlorocyclohexane (β-HCH) in 64 women (60.2%), aldrin in 56 women (53.8%), β-chlordane in 55 (51%), γ-chlordane in 51 women (47.2%), and dieldrin in 51 women (47.2%) (Fig. 1). Significant ($p < 0.05$) correlations among OC pesticide concentrations and oxidative stress indicators were found and are presented in table III. The main correlations found were between the activity of GR and the concentration of aldrin (*Spearman's correlation coefficient* r_s = - 0.5), as well as between SOD activity and α-HCH concentration ($r = 0.45$).

The PCA generated three principal components (PCs) explaining 38.5% of the total variance in the data (16.3%, 12.4% and 9.8%, respectively) (Fig. 2). The factor loadings describing the correlations between the PCs obtained with the original variables are shown in table IV. The first PC was positively correlated with the activity of SOD, as well as with hexachlorocyclohexanes and cyclodienes (aldrin, endrin aldehyde) concentration. The second PC was negatively correlated with chlordanes and dieldrin content. The third PC was negatively correlated with the activity of GPx and γ-chlordane and heptachlor concentration. PC1 and PC2 were plotted; the data grouped to the positive side of the PC1 axis corresponded to milk samples with higher SOD activity and higher hexachlorocyclohexanes and cyclodienes (aldrin, endrin aldehyde) concentration. In this plot, samples with the higher chlordane and dieldrin content congregated to the negative values of the PC2 axis (Fig. 2). When PC1 and PC3 were plotted, samples with the higher GPx activity and higher γ-chlordane and heptachlor concentrations accumulated to the negative values of the PC3 axis (Fig. 2). The outcomes of the discriminant analysis showed two canonical discriminant functions for women grouped by seafood consumption (Fig. 3). However, the analysis did not show a significant separation between groups with the set of variables used as predictors (OCPs concentrations and oxidative stress indicators) (Wilks' Lambda = 0.44; $F_{(69,245)} = 1.14$; $p = 0.23$).

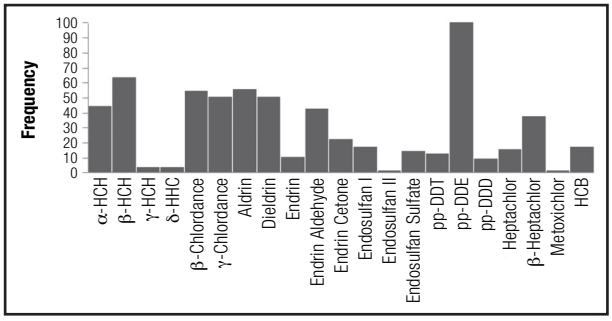

Figure 1.

Frequency of organochlorine pesticides detected in breast milk samples from 108 lactating women from La Paz, Baja California Sur.

Table I. Indicators of oxidative stress measured in breast milk of women from La Paz, Baja California Sur (n = 108)

	Median	Range	25th percentile	75th percentile
TBARS nmol mg^{-1} protein	231.755	3.089 - 2066.284	102.986	474.521
Protein carbonyls (µmol mg^{-1} protein)	21.690	0.569 - 99.236	6.428	47.525
$O_2^{\bullet-}$ (nm min^{-1} mg^{-1} protein)	0.009	0.0003-0.160	0.004	0.017
GPx (U mg^{-1} protein)	0.063	0.0003 - 10.203	0.016	0.207
SOD (U mg^{-1} protein)	198.172	1.652 - 2117.956	39.977	775.454
GR (U mg^{-1} protein)	0.017	0.0003 - 7.324	0.005	0.051
GST (U mg^{-1} protein)	0.002	0.000 - 0.070	0.0008	0.006
CAT (U mg^{-1} protein)	0.253	0.0000 - 4.578	0.105	0.488
GSH (nmol mg^{-1} protein)	21.948	1.420 - 253.428	12.180	38.348

TBARS: Thiobarbituric acid reactive substances; $O_2^{\bullet-}$: Superoxide radical; GPx: Glutathione peroxidase; SOD: Superoxide dismutase; GR: Glutathione reductase; GST: Glutathione S-transferase; CAT: Catalase; GSH: glutathione.

Table II. Organochlorine pesticide concentrations (mg L^{-1}) measured in breast milk of women from La Paz, Baja California Sur (n = 108)

Pesticide	Median	Range	25th percentile	75th percentile	Frequency (%)
α-HCH (1)	0.0110	0.0030 - 0.390	0.0110	0.0118	41.67
β-HCH (2)	0.0024	0.0005 - 0.0305	0.0024	0.0053	60.19
δ-HCH (2)	0.0072	0.0072 - 0.0214	0.0072	0.0072	3.70
γ-HCH (1)	0.0049	0.0049 - 0.0129	0.0049	0.0049	3.70
β-chlordane (1)	0.0034	0.0021 - 0.0080	0.0021	0.0038	50.93
γ-chlordane (1)	0.0039	0.0008 - 0.0099	0.0026	0.0048	47.22
pp-DDD (1)	0.0026	0.0021 - 0.0172	0.0026	0.0026	9.26
pp-DDE (1)	0.0875	0.0150 - 1.630	0.0790	0.1123	100.00
pp-DDT (1)	0.0058	0.0058 - 0.080	0.0058	0.0058	12.04
Aldrin (1)	0.0086	0.0043 - 0.9933	0.0043	0.0098	52.78
Dieldrin (1)	0.0024	0.0024 - 0.0046	0.0024	0.0033	47.22
Endrin (1)	0.0013	0.0011 - 0.0136	0.0013	0.0013	10.19
Endrin aldehyde (2)	0.0462	0.0030 - 0.6155	0.0180	0.0225	38.89
Endrin ketone (2)	0.0114	0.0114 - 0.0624	0.0114	0.0114	21.30
Endosulfan I (2)	0.0051	0.0041 - 0.2211	0.0051	0.0051	15.74
Endosulfan II (1)	0.0057	0.0057 - 0.0157	0.0057	0.0057	1.85
Endosulfan sulfate (1)	0.0049	0.0049 - 0.0203	0.0049	0.0049	13.89
Heptachlor (2)	0.0029	0.0024 - 0.0186	0.0029	0.0029	14.81
β-heptachlor epoxide (1)	0.0019	0.0014 - 0.0202	0.0019	0.0021	35.19
Metoxichlor (2)	0.0067	0.0067 - 0.0310	0.0067	0.0067	1.85
HCB (1)	0.0014	0.0010 - 0.015	0.0014	0.0014	16.67

α-, β-, γ-, δ-HCH's: Hexachlorocyclohexanes; α-, β-, γ- and δ-isomers; β-, γ-chlordanes: Chlordanes β and γ isomers; p,p-DDD: p,p dichloro diphenyl dichloroethane; pp- DDE: p,p-dichloro diphenyl dichloroethylene; pp-DDT: pp-dichloro diphenyl trichloroethane; (1): Detection limit = 0.0016 mg L^{-1}; (2): Detection limit = 0.0030 mg L^{-1}.

Therefore, indicators of oxidative stress and OCPs concentrations are not sufficient to explain differences between groups.

DISCUSSION

Concentrations of OCPs have been reported in human milk due to breastfeeding being considered a form of detoxification of the human body. The presence of OCPs may result in metabolic changes, and increase the risk of many diseases (8,28). The government of Mexico signed the Stockholm Convention (2001), which displays, among other things, the banning of persistent organic pollutants (POPs), including OCPs (37). The outcomes in breast milk reflect the mother´s pollutant load, and may yield information on local pollution in the study area (12).

According to the results of this study (Table I), the only OCPs detected in 100% of the samples analyzed was DDE, a metabolite of DDT. The concentrations found for DDT, DDD and DDE were 0.0058, 0.0026 and 0.0875 mg L^{-1}, which are comparable with those in other studies (10,32). In milk samples from Polish women, values of DDT, DDD and DDE of 0.005, 0.0009 and 0.0211 mg L^{-1}, respectively, were reported (10), while in Hindu women, they were reported to be 0.078, 0.210 and 0.672 mg L^{-1}, respectively (32). Higher values for the DDE/DDT ratio are characteristic of a high prevalence of DDE in the environment and its constant bioaccumulation. On the other hand, lower DDE/DDT ratio is taken as an indicator of recent exposure to DDT (11). The DDE/DDT ratio for this study was calculated to be 15.1, indicating that DDT exposure is not current, and is comparable to an earlier study in Mexico in which a value of 6.14 was reported (47). At that time, exposure to DDT was still recent. Developed countries, like the United Kingdom and New Zealand, show values of 24.2 and 24.1, respectively, while developing countries, such as Thailand and Indonesia, show values of 3.16 and 4.75, respectively (11,12). Therefore, given the ban on DDT use, a continuous decrease in the concentration of DDT in breast milk is expected over time, as suggested in earlier studies.

Previous studies have reported a relationship among age increase and OCPs concentrations, such as HCHs and DDT (r = 0.362 and r = 0.342 respectively) (6). On the other hand, other studies have found no correlation between age and OCPs (8). In this study, no significant relationship was found between age of mothers and OCPs concentrations.

Pesticides may lead to increased generation of ROS and/or affect antioxidant enzyme activities (3,5). In this study, $O_2^{\bullet-}$ was measured as an indicator of ROS production in breast milk; however, no significant correlation between $O_2^{\bullet-}$ and the concentration of pesticides was found. Probably, the OCPs concentrations are not high enough to cause significant production of $O_2^{\bullet-}$, or OCPs induce increased production of ROS other than $O_2^{\bullet-}$ (not quantified in this study), in breast milk. The OCPs affect lipid metabolism and are associated with increased lipid peroxidation (5). A positive relationship between OCPs (α-HCH, γ-HCH and the total HCH) and MDA content in human placenta, as well as a negative relationship between OCPs and GSH content, have been reported (2). The results obtained in this study showed a low correlation between indicators of oxidative damage to lipids (TBARS levels) and the concentration of OCPs (Table III). This may be due to the OCPs concentrations not being high enough to cause significant oxidative damage to lipids and/or antioxidant defenses effectively precluding lipid peroxidation in breast milk.

Other studies show significant correlations between antioxidant enzyme (SOD, GPx, GST and GR) activities and OCPs (α-HCH, γ-HCH) concentrations in tumors of women with breast cancer (27). In this study, correlations were found between antioxidant enzyme activities and pesticide concentrations (Table III), SOD activity was correlated with α-HCH and cyclodienes content (r = 0.45 and r = 0.39, respectively), GPx activity was correlated with concentrations of α-HCH and chlordanes (r = 0.35 and r = - 0.36, respectively), and GR activity was correlated with aldrin concentration (r = - 0.50). These results, combined with those of the principal component analysis (Table IV), suggest that the main antioxidant enzymes that neutralize ROS produced by OCPs are SOD and GPx. GR may also play an important role in the regeneration of GSH from GSSG, due to the fact that GSH content has also been correlated with the concentration of some OCPs (endrin aldehyde, DDE and DDTs sum) (Table III).

Pesticides commonly induce oxidative stress via different pathways; namely, by products of pesticide metabolism, by mitochondrial dysfunction, or through impaired antioxidant defenses (38). The first two pathways are generally associated to acute or current exposure, whereas upon prolonged exposure, pesticides generally affect the antioxidant system (38). Initially, OCPs or the products of their detoxifying metabolism can affect the activity of SOD, which transforms $O_2^{\bullet-}$ into H_2O_2. Subsequently, H_2O_2 is converted to H_2O by GPx (22). GPx activity depends on GSH concentration, which acts as a hydrogen donor and is oxidized to GSSG in the process (22). GSH is also required in the detoxifying reaction catalyzed by GST. GSH is necessary for both detoxification of pesticides and neutralization of ROS, which tends to decrease GSH concentration (15). To maintain the concentration of GSH, the enzyme GR acts reducing GSSG to GSH, allowing recycling and reuse of this low molecular weight antioxidant (15,38). Overall, the enzymatic and non-enzymatic antioxidant defenses act in concert to facilitate

Table III. Spearman correlation coefficients between oxidative stress indicators, body mass index (BMI), and organochlorine pesticides measured in breast milk of women from La Paz, Baja California Sur (n = 108, p < 0.05)

	α-HCH	β-HCH	δ-HCH	Σ HCH	β-chlordane	γ-chlordane	Σ chlordane	Endrin	Aldrin	Dieldrin	End. aldeh.	Σ cyclodienes	DDE	DDD	DDT	Σ DDT's	Σ Heptachlors
BMI		0.20		0.23													
TBARS				0.20			- 0.18	0.08	- 0.23					0.26			
PC			0.19						0.26								
GPx	0.35	0.20				- 0.32	- 0.36	0.26	- 0.29	- 0.23			- 0.21				
SOD	0.45	0.23				- 0.30	- 0.36	0.28	- 0.29			0.39		0.32	0.24		0.25
GR	0.39	0.21			- 0.20	- 0.32	- 0.37		- 0.50				- 0.28	0.28	0.19		
CAT	0.19																
GSH	0.25										0.23		- 0.26			- 0.21	

BMI: Body mass index; TBARS: Thiobarbituric acid reactive substances; PC: protein carbonyls; GPx: Glutathione peroxidase; SOD: Superoxide dismutase; GR: Glutathione reductase; CAT: Catalase; GSH: Glutathione; α-HCH: α-hexachlorocyclohexane; β-HCH: β-hexachlorocyclohexane; δ-HCH: δ-hexachlorocyclohexane; Σ HCH's: Sum of hexachlorocyclohexanes; Σ chlordanes: Sum of chlordanes; End. aldeh: Endrin aldehyde; Σ cyclodienes: Sum of cyclodienes; DDE: p,p-dichloro diphenyl dichloroethylene; DDD: p,p-dichloro diphenyl dichloroethane; pp-DDT: p,p-dichloro diphenyl trichloroethane; Σ DDT´s: Sum of p,p-dichloro diphenyl trichloroethane and its metabolites; Σ heptachlors: Sum of heptachlors.

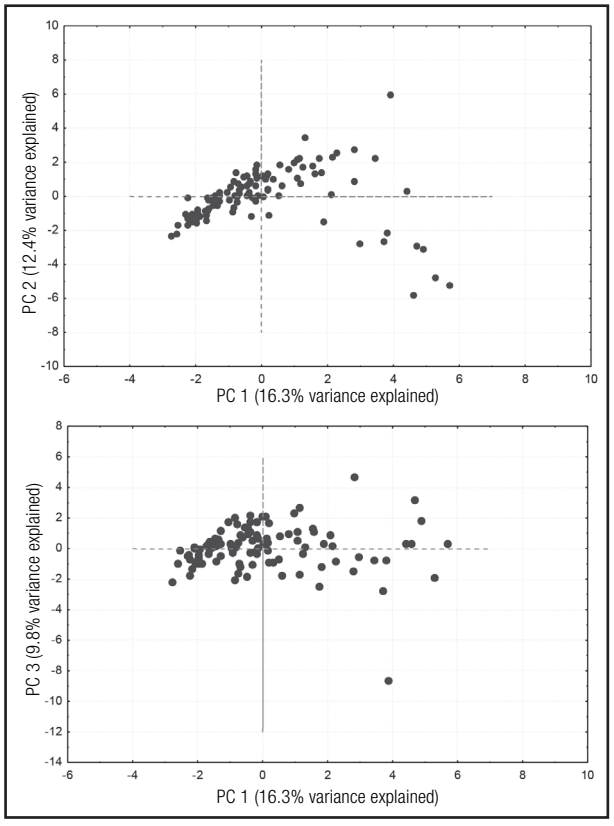

Figure 2.

Plot of sample scores from the principal components analysis (PCA) of the concentration of organochlorine pesticides and oxidative stress indicators measured in lactating women of La Paz, Baja California Sur. Line in plot without any statistical significance. A. Plot of principal component 1 *vs.* principal component 2. B. Plot of principal component 1 *vs.* principal component 3.

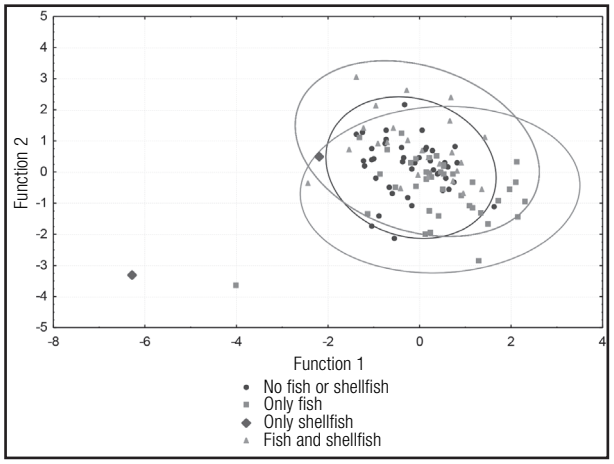

Figure 3.

Canonical scores plot in two canonical discriminant functions of the concentration of organochlorine pesticides and oxidative stress indicators measured in lactating women grouped according to seafood consumption (no consumption of fish or shellfish [n = 25], only fish intake [n = 39], only shellfish intake [n = 2], consumption of fish and shellfish [n = 42]).

Table IV. Factor loadings from the principal components analysis (PCA) of the organochlorine pesticide concentrations and oxidative stress indicators measured in breast milk of women from La Paz, Baja California Sur (n = 108)

	Factor 1	Factor 2	Factor 3
Variance explained (%)	16.3	12.4	9.8
TBARS	0.255	0.195	- 0.006
Protein carbonyls	- 0.277	- 0.309	0.170
$O_2^{\bullet-}$	- 0.071	0.111	- 0.248
GPX	0.365	0.401	*- 0.508*
SOD	0.701	0.149	- 0.176
GR	0.109	0.134	0.201
GST	0.000	0.24w 7	- 0.095
CAT	0.144	0.313	- 0.144
GSH	0.295	- 0.126	- 0.284
α-HCH	*0.647*	- 0.580	0.066
β-HCH	0.187	0.124	- 0.099
Σ HCH´s	*0.653*	- 0.571	0.064
β-chlordane	- 0.401	- 0.410	- 0.181
γ-chlordane	- 0.359	- 0.459	*- 0.540*
Σ chlordanes	- 0.490	*- 0.585*	- 0.519
Aldrin	*- 0.542*	- 0.023	- 0.260
Dieldrin	- 0.254	*- 0.509*	- 0.295
Endrin aldehyde	*0.516*	- 0.320	- 0.095
Σ cyclodienes	*0.752*	- 0.564	- 0.052
DDE	0.158	- 0.065	0.281
Σ DDT´s	0.204	- 0.101	0.275
β-heptachlor epoxide	0.267	0.437	*- 0.609*
Σ heptachlor	0.359	0.195	*- 0.646*

TBARS: Thiobarbituric acid reactive substances; $O_2^{\bullet-}$: Supereoxide radical; GPx: Glutathione peroxidase; SOD: Superoxide dismutase; GR: Glutathione reductase; CAT: Catalase; GSH: Glutathione; α-HCH: α-hexachlorocyclohexane; β-HCH; β-hexachlorocyclohexane; δ-HCH: δ-hexachlorocyclohexane; Σ HCH's: Sum of hexachlorocyclohexanes; Σ chlordanes: Sum of chlordanes; End. aldeh: Endrin aldehyde; Σ Cyclodienes: Sum of cyclodienes; DDE: p,p-dichloro diphenyl dichloroethylene; DDD: p,p-dichloro diphenyl dichloroethane; pp-DDT: p,p-dichloro diphenyl trichloroethane; Σ DDT's: Sum of p,p-dichloro diphenyl trichloroethane and its metabolites; Σ heptachlors: Sum of heptac.

maintenance of the stability of nutritional molecules in breast milk. However, in this study no significant association of GST activity with OCPs concentrations was found.

The main route of exposure of OCPs in the human body is through diet, especially via the intake of products of animal origin with high fat content (8). Generally, seafood has been linked to

high concentrations of OCPs (17). Previous studies in the southwestern Gulf of California reported the presence of 13 different OCPs (aldrin, dieldrin and δ-HCH [100%], endosulfan 1, endrin, heptachlor epoxide and methoxychlor [80%]) in fish with high demand for human consumption (39). Seafood has a high content of antioxidants that may prevent and reduce the oxidative damage which may be caused by xenobiotics present (23,31). In this study, the oxidative stress indicators and OCPs concentrations were not good predictors of the type of diet ingested by women in Baja California Sur. This suggests that the consumption of fish and shellfish is not a determining factor in the level of contaminants or in the oxidative stress in breast milk. We suggest other factors such as intake of other feeds and occupational exposure may be involved. In addition, consumption of seafood has benefits such as their protein, antioxidant, vitamin, and mineral content (23,36,48). These molecules help to prevent or mitigate oxidative damage that might arise as a result of the production of ROS generated by the presence of OCPs in human breast milk. Therefore, it is important to continue evaluating the potential influence of the consumption of fish and shellfish on the antioxidant system in breast milk of women from the Baja California peninsula, given the culinary preference in this region.

CONCLUSIONS

OCPs were banned due to their toxicity and persistence in the environment. However, 100% of the individuals in this study showed traces of their metabolites (DDE) in human breast milk. The calculated DDE/DDT ratio suggests that this contamination is not recent. The concentration of OCPs can increase the production of ROS and may cause oxidation of lipids and proteins, molecules which are vital to the developing child. However, in this study, the production of ROS did not show a significant relationship with the concentration of OCPs. Furthermore, oxidative damage to lipids and proteins had a low correlation with OCPs content. It is possible that the activity of antioxidant enzymes, mainly SOD, GPx and GR, as well as the concentration of non-enzymatic antioxidants such as GSH, preclude oxidative damage to proteins and lipids in breast milk. In this study, marine diet was not a determining factor in the level of contamination or in the oxidative stress status of breast milk of women from the Baja California peninsula. The effect of other factors such as intake of other feeds and occupational exposure should be addressed in future studies.

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