Environ Monit Assess (2015) 187: 467
DOI 10.1007/s10661-015-4694-0

Inverse correlation among organochlorine pesticide levels to total lipid serum contents: a preliminary study in Veracruz, México

Mario Caba · Enrique Meza · Stefan M. Waliszewski · Carmen Martínez-Valenzuela

Received: 15 December 2014 / Accepted: 16 June 2015 / Published online: 27 June 2015

Abstract Organochlorine pesticides, due to their hydrophobic nature and persistence, accumulate in tissues rich in lipids, which had been used as a biomarker for environmental pollution. In humans, organochlorine pesticides are continuously circulating and equilibrating among body compartments. The objective of the study was to evaluate the concentrations of organochlorine pesticides in blood serum and compare their levels to the total lipid contents in Veracruz, México inhabitants. Our hypothesis is that concentrations of organochlorine pesticides will increase just as lipid concentrations. Levels of organochlorine pesticides were divided in ascending tertils according to their total lipid content. The linear trend model applied surprisingly reveals that the average level of all organochlorine pesticides decreases as the lipid concentration increases. From one tertil to the next β-HCH, it shows a decrease of -3.19 mg kg^{-1} on lipid basis, pp.′DDE levels decrease by -3.70 mg kg^{-1} on lipid basis and pp.′DDT levels decrease -1.13 mg kg^{-1} on lipid basis. We conclude that the levels and the orderly sequence of organochlorine pesticide distributions in the blood serum maintain an inverse relationship to total lipid blood serum concentrations.

Keywords Organochlorine pesticides · Blood serum lipids · Trends · Humans

M. Caba (✉) · E. Meza · S. M. Waliszewski
Centro de Investigaciones Biomédicas, Universidad Veracruzana, Xalapa, Ver, México
e-mail: mcaba@uv.mx

C. Martínez-Valenzuela
Universidad de Occidente, Los Mochis, Sin, México

Introduction

The consumption of chemical substances, especially pesticides, has drastically increased worldwide in the last decades. The general human population is predominantly exposed to organochlorine pesticides through foodstuffs including fish, meat, dairy products, and eggs (ATSDR 2002, Smith and Gangolli 2002). Pesticides are among the most toxic substances delivered to the environment, since they show high chemical stability, resistance to metabolism, and high lipid solubility. These features make them harmful for humans and other living organisms and justify the need for their control in the environment and food webs (El-Amrani et al. 2012). In the Veracruz region, the use of organochlorine pesticides as a vector control in the past four decades was a practice widely used, resulting in high persistence in foods and human tissues (Waliszewski et al. 1997, 2001, 2012).

Due to the lipophilic properties and resistance to the metabolic reactions, these pesticides are transported through the food chain with strong biomagnification at higher trophic levels (Daley et al. 2014). Humans, being at the top of the food chain, accumulate and concentrate organochlorine pesticides in tissues and fluids rich in lipids, serving them as sensitive biomarkers of environmental pollution. These studies have been very useful to evaluate concentrations of these contaminants in specific populations worldwide and their relationship with several pathologies (Eskenazi et al. 2009), thus contributing to a better understanding of their impact to environmental pollution.

Chemicals, just as organochlorine pesticides, first pass through a diffusion barrier that may be a mucus or biological membrane, to reach circulating fluids; the relative solubility of such chemicals in water may act as a surrogate for lipids. Also, another important factor is molecular size, that may simulate partitioning and diffusion processes in tissues containing lipids, affecting their tendency to bioconcentrate (Katagi 2010). Thereafter, the organochlorine pesticides enter the circulatory system and are transported through the lipid and lipoprotein components of blood serum and deposit in adipose tissue according to the partition coefficients between blood and adipose tissue lipids, which occurs on the people exposed (Daley et al. 2014; Dirtu et al. 2013; Malarvannan et al. 2013; Herrero-Mercado et al. 2011). It is considered that these lipophilic substances are in a state of equilibrium across body compartments: blood, fat, organs (Porta et al. 2009). Consequently, the determination of their levels in serum and adipose tissues are being considered as indicators of exposure in monitoring studies.

Blood serum is a good sample to assess human exposure to organic pollutants (Mueller et al. 2008; Wang et al. 2011). Compared to other samples such as milk that can only be obtained from the female population during the lactation period and the adipose tissue obtained during surgeries only, blood samples are more easily collected. Furthermore, blood can cover a wide range of ages of both genders and health conditions. Thus, blood serum is considered a good matrix to assess concentrations of pollutants in large populations (Wang et al. 2013). However, a limitation for low levels or no detection of some residual compounds in serum samples compared to the adipose tissue has been reported (Whitcomb et al. 2005). The relation of structure activity is that the biological activity of a chemical in the human body and the environment relates to its molecular structure and physical-chemical properties. Thereby, these characteristics would explain different concentrations of some organochlorine pesticides determined in serum, compared to adipose tissue (Herrero-Mercado et al. 2011). Then, we decided to explore whether the organochlorine pesticide concentrations can depend on the blood serum lipid levels in a monitored population.

With the aim of contributing to a better understanding of organochlorine compounds in relation to body's lipids, the purposes of this study were monitoring the concentrations of various organochlorine pesticides in the blood serum and comparing their levels to the total lipid concentrations of blood serum by using a linear trend model. Our hypothesis is that concentrations of organochlorine pesticides will increase just as lipid concentrations.

Materials and methods

Sampling

One hundred fifty blood serum samples representing those that remained after clinical analyses were obtained randomly from clinical analysis in laboratories, of Veracruz inhabitants as a case study. The serum samples were stored in glass jars, immediately frozen and kept at −25 °C until analyzed.

Analytical technique

Chemical analyses of organochlorine pesticides were performed according to a previously detailed method (Waliszewski and Szymczynski 1991; Waliszewski et al. 2004, 2012). Blood serum samples were placed in a 25-mL tube with stopper, weighed, and acetic acid in a ratio of 1:1 was added. Samples were left for 30 min to hydrolyze and release the pesticides from blood's endogenous substances. Thereafter, organochlorine pesticide residues were extracted three times with 10-mL portions of a mixture of hexane and acetone (9:1). The extracts were collected in a 100-mL separatory funnel and washed twice with 25-mL of distilled water to remove traces of acetic acid, acetone, and water-soluble substances. The organic phase was collected in a 30-mL tube with stopper, and 2 mL of concentrated sulfuric acid was added. The contents were vigorously shaken for about 1 min and left for 3 min to ensure good phase separation. The hexane phase was passed through a sodium sulfate layer to a 50-mL round-bottom flask. The sodium sulfate was rinsed several times with hexane. The extract with rinse was rotary evaporated to a few drops. The concentrate was transferred quantitatively with hexane to a 1-mL calibrated tube, and the final volume was adjusted to 1.0 mL for qualitative and quantitative gas chromatography determinations. Gas chromatography was conducted with a Varian model 3400CX (Palo Alto, CA, USA) equipped with a ^{63}Ni electron-capture detector (ECD). The operating conditions were as follows: the capillary chromatography column from J&W Scientific (Folsom, CA, USA) was a DB-608 with a 30-m, 0.32-mm inner diameter (i.d.), and 0.83-μm film thickness; the temperature program was 193 °C (7 min) to 250 °C at 6 °C/min, hold for 20 min; the carrier gas was nitrogen at 6.3 mL/min and a 1 μL aliquot was injected in a splitless mode.

All samples were analyzed for HCB; α-, β-, γ- HCH; pp.′DDE; pp.′DDT; and op′DDT. The minimum detection limits for organochlorine pesticide residues were 0.05 mg kg^{-1} on lipid basis. To determine the quality of the method, a recovery study was performed on ten spiked replicates of blank cow blood samples, which presented contamination levels below the detection limits. The fortification study, performed at 1.0 mg kg^{-1} on lipid basis, showed mean recovery values from 85 to 92 %. The standard deviation and coefficient of variation were below 10, indicating an excellent repeatability of the method. Concentrated sulfuric acid used in the cleanup step degrades the ubiquitous phthalate esters, interfering in the GC-ECD identification of organochlorine pesticides, allowing their accurate determination (Waliszewski and Szymczynski 1991).

Total serum lipids were determined colorimetrically with phosphovanillin according to the method recommended by Hycel de Mexico using a commercial kit applied by clinical laboratories (Anonymous 2011). Reference values of the method for Mexican populations are 4.0–8.5 g L^{-1} (Anonymous 2011).

Statistical

Statistical calculations were conducted by using statistical software Minitab version 15. Concentrations of organochlorine pesticide (milligrams per kilogram on lipid basis) were expressed as arithmetic means (X) ± standard deviations (SD). Then, the resulting concentrations were used to determine the relation between the organochlorine pesticide level and the total lipid contents. To this purpose, the total population was divided into small subgroups in ascending tertils (50 participants in each tertil) according to the total lipid content. To evaluate differences and trends among subgroups, the linear trend model, calculating smooth of data by averaging consecutive observations in a series to provide forecasts was applied. The trend model is as follows:

$$Y_t = \beta_o + \beta_1 t + e_t$$

($\underline{Y}$ trend, β_o, β_1: slope of the line, represents the average change from one to the next result, e_t: the error term).

Results and discussion

There are still unresolved issues about toxicokinetics of organochlorine pesticides in humans. Identification of blood concentration of these pesticides by total serum lipids is frequently applied, on the premise that lipophilic substances are in a state of equilibrium across body compartments such as blood, fat, and organs (Porta et al. 2009). During our analysis, only the presence of β-HCH, pp.′DDE, and pp.′DDT was detected, and among them, pp.′DDE showed the higher concentration (11.84 ± 8.07 mg kg^{-1} on lipid basis), which is consistent since it has a longer half-life in human adipose tissue and can remain in the body during the whole lifetime. This is followed by β-HCH concentration (5.30 ± 5.17 mg kg^{-1} on lipid basis). Meanwhile, DDTs, such as insecticide pp.′DDT, show lower concentrations (1.96 ± 1.78 mg kg^{-1} on lipid basis) and its isomer op′DDT was not detected among analyzed blood samples.

To evaluate our hypothesis that concentrations of organochlorine pesticides will increase just as lipid concentrations, the organochlorine pesticide levels obtained from randomly selected people were grouped into ascending tertils (50 participants in each group) according to the total lipid blood levels. The values obtained are shown in Table 1 and Fig. 1. Analyzing the mean and standard deviation of mean, levels of all organochlorine pesticides, and their fluctuation among tertils, results show surprisingly that pesticide levels decrease as the lipid concentration increases (Fig. 1).

To evaluate the trend of organochlorine pesticide concentrations when blood serum lipids increase, the linear trend analyses model was applied. The results of mean values and mean absolute deviations (MAD) are showed in Fig. 2. The statistical model applied showed that β-HCH concentration decreases by 11.99 mg kg^{-1} on lipid basis when the total blood lipid concentration increases, and the negative trend indicates an evident average decrease of −3.19 mg kg^{-1} on lipid basis from one tertil to the next. Similarly, pp.′DDE levels have an average of 19.24 mg kg^{-1} on lipid basis with a negative trend of −3.70 mg kg^{-1} on a lipid basis decrease from one tertil to the next, as the lipid level from the blood serum increases. While the result of the pp.′DDT trend

Table 1 Organochlorine pesticide level in serum samples (mean ± SD) levels (mg kg^{-1} on lipid basis) divided into tertils according to total lipid mean levels (g/L)

Tertils	β-HCH	pp′DDE	pp′DDT	ΣDDT	Lipids g/L^{-1}
1	9.06 ± 6.68	15.76 ± 10.47	3.20 ± 2.46	16.55 ± 11.20	357 ± 59
2	5.09 ± 4.20	11.41 ± 6.07	1.82 ± 0.93	11.94 ± 6.44	490 ± 39
3	2.67 ± 2.24	8.36 ± 4.84	0.93 ± 0.65	9.01 ± 5.66	723 ± 172

also shows that their concentration among tertils decreases by 4.25 mg kg^{-1} on lipid basis as the lipid concentration of the blood serum increases, this average result is less pronounced, −1.13 mg kg^{-1} on lipid basis among tertils in comparison to β-HCH and pp.′DDE. The behavior of DDT total (Σ-DDT) was dominated by pp.′DDE and their concentrations, resulting in an analogous trend to the pp.′DDE results. Statistical calculations indicate an average decrease of 20.04 mg kg^{-1} on the lipid basis with −3.77 mg kg^{-1} on the lipid basis decrease among each tertil.

Current results of human blood serum lipid and organochlorine pesticide concentration comparisons and trend analysis reveal a decrease of organochlorine pesticide concentrations when the serum blood lipid levels increase. Therefore, biological activity of organochlorine pesticides in the human body can be related to its molecular structure and physical-chemical properties. Our results are in concordance with that of Malarvannan et al. (2013), Dirtu et al. (2013), and Herrero-Mercado et al. (2011), indicating a potential steady redistribution of these pesticides among body compartments and low metabolic rate (Kim et al. 2011; Gyalpo et al. 2012). Recently, De Roos et al. (2012) showed that indicators of greater adiposity (such as weight, body mass index, fat mass percent, subcutaneous abdominal fat, intra-abdominal fat, waist circumference, and hip circumference) were related to lower plasma concentrations of most organochlorine pesticides. In accordance, the current results indicate that in the human body, organochlorine pesticide concentrations dilute when blood lipid concentration increase

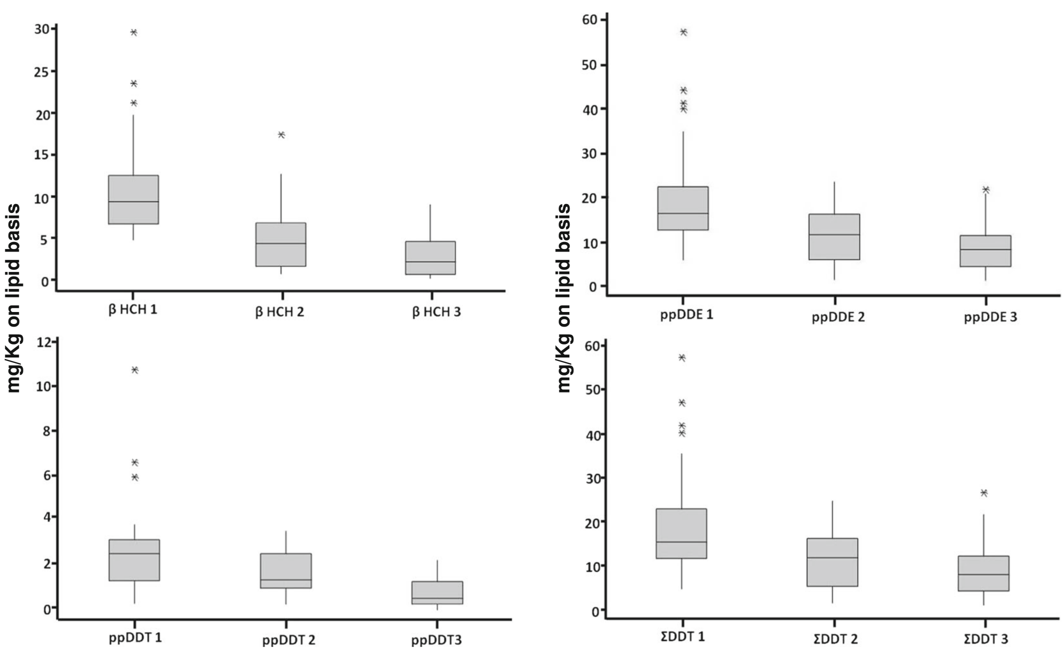

Fig. 1 Behavior of β-HCH, pp.′DDE, pp.′DDT, and ΣDDT mean ± SD and 95 % confidence intervals (CI) of means (mg kg^{-1} on lipid basis) among ascendant tertils. Values were presented in Table 1

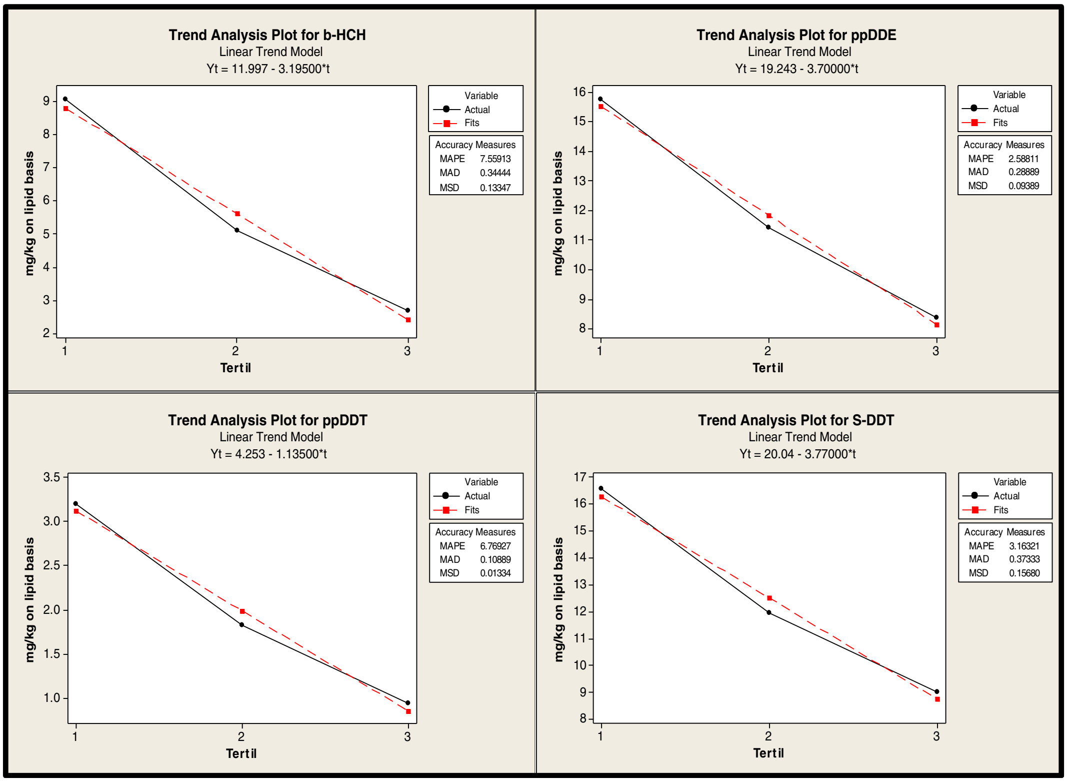

Fig. 2 Trend levels among tertils of β-HCH, pp.′DDE, pp.′DDT, and ΣDDT (mg kg^{-1} on lipid basis) according to ascendant lipid concentrations. *MAPE*—mean absolute percentage error which measure the accuracy of fitted series values and express accuracy as a percentage, *MAD*—mean absolute deviation which measure the accuracy of fitted series values and helps conceptualize the amount of error, *MSD*—mean squared deviation which measure accuracy of fitted series values

and the abovementioned process seems to be a dominant factor regulating the levels of these pollutants in the blood compartment. That is to say, as the blood lipid increase, there is a drop in organochlorine pesticide concentrations.

Organochlorine pesticide elimination is restricted by the types of pesticide and organism for which bioamplification is likely to occur. As an example, bioamplification occur during weight loss events, but the extent of this effect depends on the rate of pesticide elimination and the loss of the partitioning capacity (De Roos et al. 2012; Chevrier et al. 2000; Hue et al. 2006; Pelletier et al. 2002). The process will be maximized for organochlorine pesticides showing hydrophobicity, because the process inversely correlates with chemical elimination and for pesticides that undergo little or no metabolic biotransformation (Daley et al. 2014). This assumption correlates to the results of the current study, indicating that increases in levels of organochlorine pesticides released to serum from human fat deposits might be of concern, suggesting that mobility of fats and their metabolism could possibly cause the reaccumulation of organochlorine pesticides in fat deposits. The resulting increase in the internal exposure to these contaminants may adversely affect health, as metabolism and/or elimination of these compounds are altered (Pelletier et al. 2002). Despite the fact that absolute levels of organochlorine pesticides are no different between human organs and fat deposits (Waliszewski et al. 2003), the present finding suggests that a higher level of selected pesticides in blood serum lipids seems to predispose them to selective accumulation, maintaining an inverse relationship to total blood serum lipid concentrations. Further studies must be carried out to identify the effects of changes in parameters such as weight loss, body mass index, and other fat mobilizing status for a better understanding of this inverse relationship among organochlorine pesticides and blood serum lipids.

References

Anonymous (2011). http://www.spinreact.com.mx/public/instructivo/QUIMICA%20CLINICA/LIQUIDOS/1001270.pdf. Accessed November 20, 2011.

ATSDR (Agency for Toxic Substances and Disease Registry) (2002). Toxicological Profile for DDT, DDE, and DDD. http://www.atsdr.cdc.gov/toxprofiles/tp.asp?id/81&tid=20.

Chevrier, J., Dewailly, E., Ayotte, P., Mauriege, P., Despres, J. P., & Tremblay, A. (2000). Body weight loss increases plasma and adipose tissue concentrations of potentially toxic 460 pollutants in obese individuals. *International Journal of Obesity and Related Metabolic Disorders*, *24*(10), 1272–1278.

Daley, J. M., Paterson, G., & Drouillard, K. G. (2014). Bioamplification as a bioaccumulation mechanism for persistent organic pollutants (POPs) in wildlife. *Review of Environmental Contamination and Toxicology*, *227*, 107–155.

De Roos, A. J., Ulrich, C. M., Sjodin, A., & McTiernan, A. (2012). Adiposity, body composition, and weight change in relation to organochlorine pollutant plasma concentrations. *Journal of Exposure Science & Environmental Epidemiology*, *22*(6), 617–624.

Dirtu, A. C., Dirinck, E., Malarvannan, G., Neels, H., Van Gaal, L., Jorens, P. G., & Covaci, A. (2013). Dynamics of organohalogenated contaminants in human serum from obese individuals during one year of weight loss treatment. *Environmental Science & Technology*, *47*(21), 12441–12449.

El-Amrani, S., Pena-Abaurrea, M., Sanz-Landaluze, J., Ramos, L., Guinea, J., & Cámara, C. (2012). Bioconcentration of pesticides in zebrafish eleutheroembryos (Danio rerio). *Science of the Total Environment*, *425*, 184–190.

Eskenazi, B., Chevrier, J., Goldman, R. L., Anderson, H. A., Bornman, M. S., Bouwman, H., et al. (2009). The pine river statement: human health consequences of DDT use. *Environmental Health Perspectives*, *117*, 1359–1367.

Gyalpo, T., Fritsche, L., Bouwman, H., Bornman, R., Scheringer, M., & Hungerbühler, K. (2012). Estimation of human body concentrations of DDT from indoor residual spraying for malaria control. *Environmental Pollution*, *169*, 235–241.

Herrero-Mercado, M., Waliszewski, S. M., Caba, M., Martínez-Valenzuela, C., Gómez-Arroyo, S., Villalobos-Pietrini, R., et al. (2011). Organochlorine pesticide gradient levels among maternal adipose tissue, maternal blood serum and umbilical blood serum. *Bulletin of Environment Contamination and Toxicology*, *86*(3), 289–293.

Hue, O., Marcotte, J., Berrigan, F., Simoneau, M., Dore, J., Marceau, P., et al. (2006). Increased plasma levels of toxic pollutants accompanying weight loss induced by hypocaloric diet or by bariatric surgery. *Obesity Surgery*, *16*(9), 1145–1154.

Katagi, T. (2010). Bioconcentration, bioaccumulation and metabolism of pesticides in aquatic organisms. *Reviews of Environment Contamination and Toxicology*, *204*, 1–149.

Kim, M. J., Marchand, P., Henegar, C., Antignac, J. P., Alili, R., Poitou, C., Buillot, J. L., Basdevant, A., Le Bizec, B., Barouki, R., Clement, K. (2011). Fate and complex pathogenic effects of dioxins and polychlorinated biphenyls in obese subjects before and after drastic weight loss. *Environmental Health Perspectives*, 119(3), 377–383.

Malarvannan, G., Dirinck, E., Dirtu, A. C., Pereira-Fernandes, A., Neels, H., Jorens, P. G., et al. (2013). Distribution of persistent organic pollutants in two different fat compartments from obese individuals. *Environment International*, *55*, 33–42.

Mueller, J. F., Harden, F., Toms, L. M., Symons, R., & Furst, P. (2008). Persistent organochlorine pesticides in human milk samples from Australia. *Chemosphere*, *70*, 712–720.

Pelletier, C., Doucet, E., Imbeault, P., & Tremblay, A. (2002). Associations between weight loss induced changes in plasma organochlorine concentrations, serum T(3) concentration, and resting metabolic rate. *Toxicological Sciences*, *67*(1), 46–51.

Porta, M., Jariod, M., López, T., Pumarega, J., Puigdomènech, E., Malats, N., et al. (2009). Correcting serum concentrations of organochlorine compounds by lipids: alternatives to the organochlorine/total lipids ratio. *Environment International*, *35*, 1080–1085.

Smith, A. G., & Gangolli, S. D. (2002). Organochlorine chemicals in seafood: occurrence and health concerns. *Food and Chemical Toxicology*, *40*, 767–779.

Waliszewski, S. M., & Szymczynski, G. A. (1991). Persistent organochlorine pesticides in blood serum and whole blood. *Bulletin of Environment Contamination and Toxicology*, *46*, 803–809.

Waliszewski, S. M., Pardio, V. T., Waliszewski, K. N., Chantiri, J. N., Aguirre, A. A., Infanzon, R. M., & Rivera, J. (1997). Organochlorine pesticide residues in cow's milk and butter in Mexico. *Science of the Total Environment*, *208*(1), 127–132.

Waliszewski, S. M., Aguirre, A. A., Infanzon, R. M., Silva, C. S., & Siliceo, J. (2001). Organochlorine pesticide levels in maternal adipose tissue, maternal blood serum, umbilical blood serum, and milk from inhabitants of Veracruz, Mexico. *Archives of Environmental Contamination and Toxicology*, *40*(3), 432–438.

Waliszewski, S. M., Gómez-Arroyo, S., Infanzón, R. M., Villalobos-Pietrini, R., & Hart, M. M. (2003). Comparison of organochlorine pesticide levels between abdominal and breast adipose tissue. *Bulletin of Environment Contamination and Toxicology*, *71*(1), 156–162.

Waliszewski, S. M., Gómez-Arroyo, S., Carvajal, O., Villalobos-Pietrini, R., & Infanzón, R. M. (2004). Uso del ácido sulfúrico en las determinaciones de plaguicidas organoclorados. *Internacional de Contaminación Ambiental*, *20*(4), 185–192.

Waliszewski, S. M., Caba, M., Herrero-Mercado, M., Saldariaga-Noreña, H., Meza, E., Zepeda, R., et al. (2012). Organochlorine pesticide residue levels in blood serum of inhabitants from Veracruz, Mexico. *Environmental Monitoring and Assessment*, *184*, 5613–5621.

Wang, N., Shi, L., Kong, D., Cai, D., Cao, Y., Liu, Y., & Pang, G. (2011). Accumulation levels and characteristics of some pesticides in human adipose tissue samples from southeast China. *Chemosphere*, *84*(7), 964–971.

Wang, H. S., Chen, Z. J., Wei, W., Man, Y. B., Giesy, J. P., Du, J., et al. (2013). Concentrations of organochlorine pesticides (OCPs) in human blood plasma from Hong Kong: markers of exposure and sources from fish. *Environment International*, *54*, 18–25.

Whitcomb, B. W., Schisterman, E. F., Buck, G. M., Weiner, J. M., Greizerstein, H., & Kostyniak, P. J. (2005). Relative concentrations of organochlorines in adipose tissue and serum among reproductive age women. *Environmental Toxicology and Pharmacology*, *19*(2), 203–213.