Bull Environ Contam Toxicol (2012) 89:882–887
DOI 10.1007/s00128-012-0764-y

Organochlorine Pesticide Residues in Bovine Milk from Organic Farms in Chiapas, Mexico

Rey Gutiérrez · Jorge Luis Ruíz · Rutilio Ortiz · Salvador Vega · Beatriz Schettino · Alberto Yamazaki · María de Lourdes Ramírez

Received: 22 March 2012 / Accepted: 26 July 2012 / Published online: 5 August 2012

Abstract Thirty six samples of bovine milk were collected from Chiapas State, Mexico between January 2011 and December 2011 with the intention of identifying and quantifying organochlorine pesticide residues in organic farms. The analyses were done using gas chromatography with an electron capture detector (Ni^{63}). In general the values found in raw milk were lower than the permissible limit proposed by FAO/WHO/Codex Alimentarius 2006. Average concentrations for alpha + beta HCH were 3.62 ng/g, gamma HCH 0.34 ng/g, heptachlor + epoxide 0.67 ng/g, DDT and isomers 1.53 ng/g, aldrin + dieldrin 0.77 ng/g, and endrin 0.66 ng/g (only present in samples from farm 2). The organic milk from Chiapas has shown low concentrations of pesticide residues in recent years and satisfies international and national regulations for commercialization.

Keywords Pesticides · Residues · Organic milk · Warm climate

Organochlorine insecticides are important environmental pollutants as they are applied widely in agriculture, livestock, forestry, and used for domestic and industrial activities. Physicochemical properties of these compounds, especially their high lipophylicity, facilitate their absorption and storage of in human and animal bodies (Kampire et al. 2011). Residues in milk, which is one of the most widely used foodstuffs containing lipids, can be a quantitative and qualitative index for the presence of these toxins in animal bodies. Accumulation has the potential to adversely affect the food chain (Bulut et al. 2011).

Many developed countries have banned the use of some types of organochlorine, for example the USA which banned the use of DDT in 1970. Environmental studies have found that pesticide residues such as DDT, PCBs, and other organochlorine compounds continue to be present in humans and other mammals around the world many years after production and use have been limited. Due to their persistence, it is difficult to eliminate the organochlorines by simply avoiding their use, as many years are required for them to disappear from the environment (Subir and Mukesh 2008). These pesticides are also still being used in some parts of the world due to their potent and wide spectrum effects against harmful organisms. The main health hazards associated with exposure to these compounds are abdominal pains, diarrhea, hypertension, respiratory diseases, and dysfunctioning of the reproductive system, pre/post T natal damage, carcinogenesis and mutagenesis (Aktar et al. 2009).

The intake of contaminated feed and fodder by animals is the main source of entry of pesticides to the bodies of animals, which ultimately results in the contamination of milk, meat, etc. consumed by humans (Hernández et al. 2010) and inevitably humans also can be contaminated. Milk is considered as a nearly complete food since it is a good source of protein, fat and major minerals. Also, milk is one of the main constituents of the daily diet, especially for vulnerable groups such infants, school age children and older people (Kampire et al. 2011). Certain environmental chemicals, including pesticides termed

R. Gutiérrez · R. Ortiz · S. Vega (✉) · B. Schettino · M. de Lourdes Ramírez
Laboratorio de Análisis Instrumental, Departamento de Producción Agrícola y Animal, Universidad Autónoma Metropolitana Unidad Xochimilco, Calzada del Hueso 1100, Colonia Villa Quietud, 04960 Coyoacán, Mexico
e-mail: bkygualito@gmail.com; svega@correo.xoc.uam.mx

J. L. Ruíz · A. Yamazaki
Facultad de Medicina Veterinaria y Zootecnia, Universidad Autónoma de Chiapas, Chiapas, Mexico

as endocrine disruptors, are known to elicit their adverse effects by mimicking or antagonising natural hormones in the body and it has been postulated that their long-term, low-dose exposure is increasingly linked to human health effects such as immune suppression, hormone disruption, diminished intelligence, reproductive abnormalities and cancer (Aktar et al. 2009).

Recently organic production has increased in many countries. In Mexico organic milk production has been established in Chiapas State where many individual farms exist and the sale of organic milk and other milk based products is seen as economically advantageous, in particular export to other countries. Organic milk production has been carried out for 20 years in Chiapas State but in spite of this, some studies have shown the presence of contaminants such as organochlorine pesticide residues in soil, water, vegetables and milk. This work describes the presence of organochlorine pesticide residues in organic milk of cattle from four farms in Chiapas.

Materials and Methods

Bovine milk samples (36) were collected from four organic farms in the Centro region of Chiapas. This region is 320 m above sea level and the climate is warm and humid all year, with high precipitation from June to November (1 932 mm average precipitation). The months with the highest temperatures are May and June (25°C average temperature).

Samples were taken fresh in the morning in glass bottles, kept in ice in a cool box during transport and stored in a refrigerator in the laboratory before analysis. Sampling was carried out monthly from January 2011 to December 2011 with representative samples taken from each farm except during holiday times in July, August and September. The analysis was performed within 24 h of collection of the samples.

A 250 mL aliquot of milk sample was transferred to a suitable container and milk fat was obtained with by extraction with a detergent solution in a warm water bath (Frank et al. 1975). 50 mg of fat was weighed and dissolved in hexane (HPLC grade) and the samples cleaned by passing through a chromatographic column. This column (20 × 300 mm) was prepared in hexane with 5 g of activated florisil deactivated with deionized water (1.25 %) and topped with anhydrous sodium sulfate. The sample was eluted with a mixture of hexane-ether (9:1 and 8:2 v/v). The organic extract containing the organochlorine pesticides was concentrated in a rotary evaporator to 3 mL and transferred to a vial for GC analysis according USEPA (1981). Finally the volume was made up with isooctane and analysis carried out in splitless mode.

The 16 pesticides analysed in this study are on the priority list of the US EPA. The qualitative and quantitative analyses were done by gas chromatography with an electron capture detector (ECD Ni^{63}). The column used was an HP-5 fused silica capillary column with dimensions 30 m × 0.25 mm id × 0.25 μm film thickness; the temperature programming was from 90 to 300°C, injector temperature at 250°C and detector temperature of 300°C. The carrier gas was helium at a flow rate of 1 mL/min through the column and 30 mL/min make up. The identification of peaks and quantification was done based on external standard solutions.

Results and Discussion

Concentrations of the organochlorine pesticides are recorded in Table 1. HCH isomers are the most abundant, followed by epoxide of heptachlor + heptachlor, and DDT. There was considerable variability of concentration of these compounds probably caused by environmental conditions in the area, different feedstuffs used by the farmers, and control of pests in health campaigns for both humans and animals. Further differences between the organic farms in terms of other individual organochlorine pesticides and total concentration were also evident.

Figure 1 shows total concentration of organochlorine compounds and it can be clearly seen that beta HCH was the most abundant residue on all four organic farms (farm 1: 2.1 ng/g to farm 3: 3.7 ng/g). Heptachlor + epoxide of heptachlor were found in all four farms with values from 0.2 ng/g (farm 2) to 1.4 ng/g (farm 1) but did not surpass the permissible limit of 6 ng/g. DDT and/or its metabolites were found in all farms, in spite of the fact that cultural practices of organic farming of livestock were established 20 years ago; values were less than 20 ng/g, the safe limit according to FAO/WHO/Codex Alimentarius (2006). It is likely that the presence of DDT alone in milk samples was probably due to a recent fumigation in adjacent areas to control malaria vectors as there were only low concentrations of the degradation compounds such as DDE and DDD.

Organic farms 2 and 4 showed a large number of pesticide residues (13 and 12 respectively) while there were a lower number of pesticide residues in farm 1 (8).

The organochlorine pesticide residues are classified according to chemical structure in Table 2. Alicyclics compounds were dominant in all farms, the percentage of total compounds ranging from 48.2 % (farm 2) to 59.7 % (farm 1). It is probable that HCH and their isomers are pesticides that are still in use in this region and contaminate the land of the organic farmers. In second place were cyclodienic compounds with 6.4 % (farm 4) to 38.1 % (farm 1), and

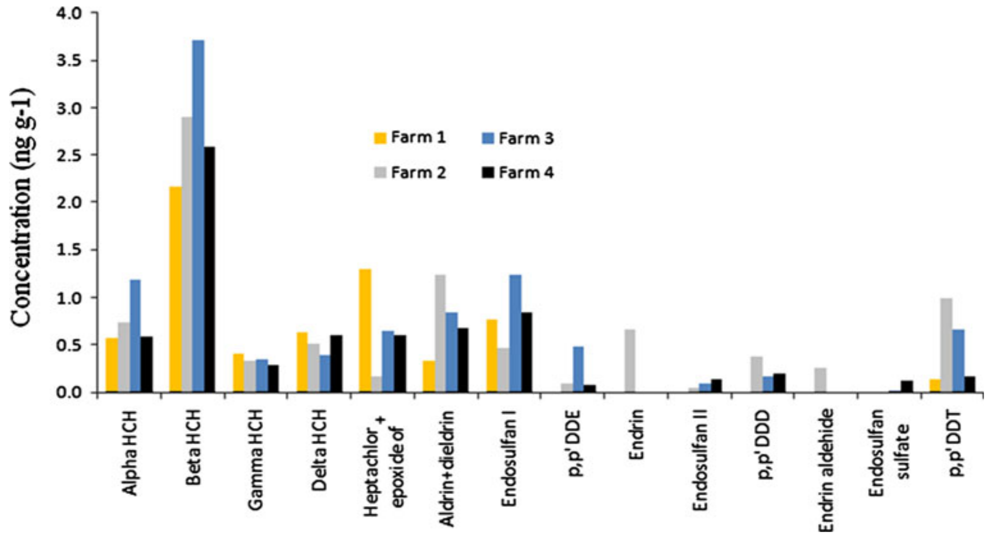

Fig. 1 Concentration of organochlorine pesticide residues in organic milk from Tecpatan, Chiapas

Table 1 Concentrations of organochlorine pesticide residues (ng/g) from organic milk from the Centro region

Compounds	Farm 1	Farm 2	Farm 3	Farm 4
Alpha HCH	0.58 ± 0.73	0.73 ± 0.85	1.20 ± 1.39	0.59 ± 0.65
	LD − 1.81[a]	LD − 2.27	LD − 4.08	LD − 1.77
Beta HCH	2.16 ± 2.21	2.90 ± 2.99	3.71 ± 3.23	2.59 ± 2.69
	LD − 5.73	LD − 6.87	LD − 9.11	LD − 6.44
Gamma HCH	0.40 ± 0.62	0.33 ± 0.67	0.34 ± 0.35	0.29 ± 0.51
	LD − 1.82	LD − 2.02	LD − 0.97	LD − 1.48
Delta HCH	0.62 ± 0.82	0.51 ± 0.65	0.40 ± 0.65	0.61 ± 1.12
	0.06 − 2.21	LD − 2.08	LD − 2.00	LD − 3.49
Heptachlor + epoxide of	1.30 ± 2.60	0.16 ± 0.36	0.64 ± 1.24	0.60 ± 1.18
	LD − 5.84	LD − 1.10	LD − 3.76	LD − 3.03
Aldrin + dieldrin	0.33 ± 0.87	1.23 ± 2.46	0.85 ± 1.78	0.68 ± 1.77
	LD − 1.18	LD − 7.03	LD − 5.25	LD − 5.35
Endosulfan I	0.77 ± 2.18	0.47 ± 0.67	1.24 ± 1.21	0.85 ± 1.19
	LD − 2.81	LD − 1.83	LD − 2.83	LD − 2.96
p,p′ DDE	–	0.60 ± 0.71	0.48 ± 0.86	0.08 ± 0.25
		LD − 1.83	LD − 2.44	LD − 0.76
Endrin	–	0.66 ± 1.98	–	–
		LD − 5.95		
Endosulfan II	–	0.05 ± 0.16	0.91 ± 0.27	0.13 ± 0.40
		LD − 0.49	LD − 0.82	LD − 1.20
p,p′ DDD	–	0.38 ± 0.86	0.17 ± 0.36	0.20 ± 0.56
		LD − 2.54	LD − 0.99	LD − 1.70
Endrin aldehide	–	0.26 ± 0.77	–	–
		LD − 2.30		
Endosulfan sulfate	–	–	0.02 ± 0.06	0.12 ± 0.33
			LD − 0.18	LD − 0.99
p,p′ DDT	0.14 ± 0.42	1.00 ± 2.99	0.66 ± 1.37	0.16 ± 0.49
	LD − 1.26	LD − 8.96	LD − 3.98	LD − 1.47
Total concentration	6.30	9.28	10.62	6.9

HCH hexachlorocyclohexane, *p,p′-DDE* dichlorodiphenyldichloroethylene or 1,1-bis-(4- chlorophenyl)-2,2-dichloroethene, *p,p′-DDD* dichlorodiphenyldichloroethane or 1-chloro-4-[2,2- dichloro-1-(4-chlorophenyl)ethyl]benzene

[a] Low and high value of each compound found in all samples, – not detected

Table 2 Organochlorine compounds in organic milk from Tecpatan, Chiapas

Compounds	Concentration (ng/g)			
	Farm 1	Farm 2	Farm 3	Farm 4
Alicyclics (isomers of HCH)	3.76 (59.7[a])	4.47 (48.2)	5.65 (53.2)	4.08 (59.1)
Aromatics (DDT and isomers)	2.40 (38.1)	1.98 (21.3)	1.31 (12.3)	0.44 (6.4)
Cyclodienics	0.14 (2.2)	2.83 (30.5)	3.66 (34.5)	2.38 (34.5)
Total concentration	6.30	9.28	10.62	6.90

[a] Percent of total concentration

finally aromatics compounds with values from 2.2 % (farm 1) to 34.5 % (farm 1). The order of values for organic farms were farm 3 (10.62 ng/g) > farm 2 (9.28 ng/g) > farm 4 (6.90 ng/g) > farm 1 (6.30 ng/g). Generally the lowest concentrations, were seen on farms 1 and 4, probably influenced by good production practices.

We tried to understand the behavior of pesticide residues through the dry and wet season in the region, and found a trend in number and concentration of pesticide residues in the zone. In the dry season (Fig. 2), the beta HCH concentrations were greatest followed by aldrin + dieldrin, endosulfan, heptachlor + epoxide of heptachlor and p,p'DDT.

According to high temperature records in the zone (approx 30°C) on warm days the pesticides residues are available to vegetation, water or in the air because of evaporation in the environment and through solubility in vegetation as food for livestock.

Figure 3 shows that in the wet season the number and concentration were slightly lower except for HCH and its isomers, which maintained similar concentrations (except for farm 3 where the value was 5.5 ng/g). The rainy season did not influence significantly the presence of organochlorine residues in raw milk.

The presence and concentration of HCH, DDT and other organochlorine pesticide residues in the organic milk indicate

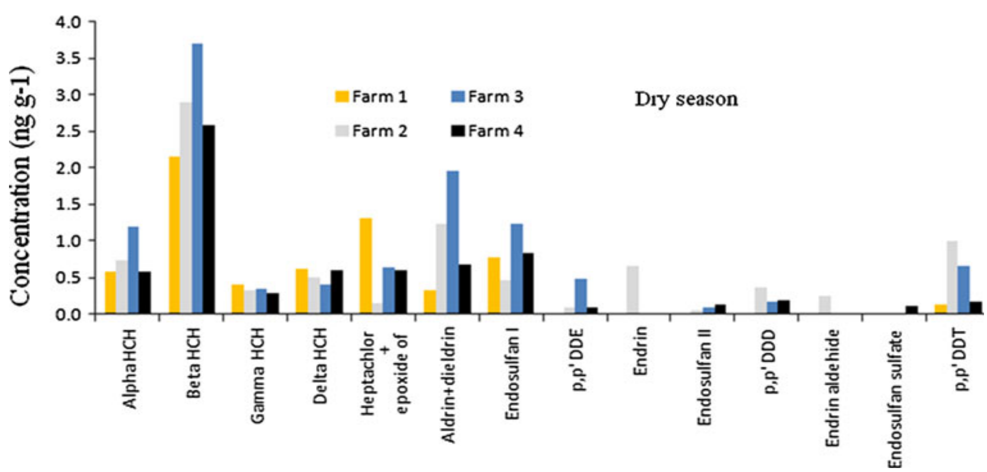

Fig. 2 Concentration of organochlorine pesticide residues in organic milk in the dry season (January to May)

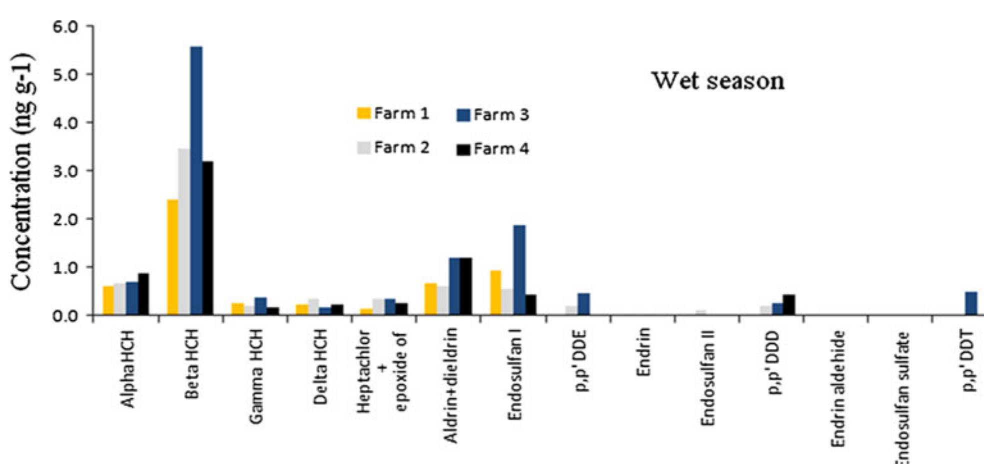

Fig. 3 Concentration of organochlorine pesticide residues in organic milk for the wet season (June to November)

that though the frequency and level has considerably decreased over the years, particularly in warm regions like Chiapas, contamination still exists albeit at a low level. The most important reason for this could be that most of the organochlorine pesticides compounds are persistent environmental contaminants and degrade slowly, this degradation being dependent on environmental factors such as soil type, organic matter and others environmental variables (Bulut et al. 2011).

It will require much more time for these persistent compounds to be completely eliminated from the environment. However, some of the compounds like DDT, lindane, etc. are not totally banned but restricted in their use, as a result of which animals may still be exposed to these compounds for many years to come and complete elimination is a distant possibility (Ocampo-Camberos et al. 2010).

HCH is a very popular and cheap insecticide used for management of a variety of insect pests in a wide range of crops in Mexico as a substitute for DDT and other pesticides. The transfer coefficient of endosulfan from feed to milk is much less but residues of it could still be found in milk samples.

The mean sum of DDT and isomers in the four farms was 1.53 ng/g of milk fat in this study (Table 1) and was below the value of 50 ng/g (fat basis) set by the FAO/WHO/Codex Alimentarius (2006). Few studies have investigated organochlorine pesticide residues in raw cow's milk. In comparison to another study made in Mexico by Waliszewki et al. (2003) we saw lower DDT concentrations. The global tendency for concentrations of DDT to reduce in the environment with time is due to chemical and biological degradation after bans in many countries and its use only in specific health programs to control some vectors such as malaria.

Considering the isomers of HCH beta HCH showed high concentrations in both seasons in comparison to other compounds analyzed, with values of 2.16 ng/g (farm 1) to 3.71 ng/g (farm 3) based on fat. In second place was alpha HCH with values of 0.58 ng/g (farm 1) to 1.20 ng/g (farm 3). The sum of alpha and beta HCH was below the permissible value of 100 ng/g (according to FAO/WHO/Codex Alimentarius). These isomers were found in 90 % of raw milk samples but did not represent a risk to human or animal health. In Mexico, the application of HCH has been restricted to special uses in the livestock and agriculture industries (Real et al. 2005).

Mean values for lindane (gamma HCH) were 0.29 ng/g (farm 4) to 0.40 ng/g (farm 1) in the current study and were lower than those reported by Radzyminska et al. (2008) and Ashnagar et al. (2009), who detected mean values of 2 and 7 ng/g of fat respectively. Further, our values are lower than those detected by Waliszewki et al. (2003) and Sharma et al. (2007) who detected lindane at 30 and 36 ng/g of fat respectively in raw milk. The values found were below the permissible limit of 10 ng/g (according to FAO/WHO/Codex Alimentarius). The frequency of presence of lindane in samples from the organic farms was below 44 % and did not represent a risk with respect to consumption of this product.

Levels of aldrin plus dieldrin were 0.33 ng/g (farm 1) to 1.23 ng/g (farm 2), below the permissible value of 6 ng/g, as stated in Codex Alimentarius.

In some studies in Mexico concerning raw bovine milk values of endrin greater than the value of 0.8 ng/g of FAO/WHO/Codex Alimentarius have been found, although in our study it was only present in farm 2 (0.66 ng/g).

In certain areas, the federal and state government sprays some organochlorine pesticides in wetland areas to prevent the increase of vectors such mosquitoes. This can directly hit non-target vegetation, or can drift or volatilize from the treated area and contaminate air, soil, and non-target plants (KaushiK et al. 2011). Some pesticide drift occurs during every application, even from ground equipment. Drift can account for a loss of 2 %–25 % of the chemical being applied, which can spread over a distance of a few yards to several hundred miles. As much as 80 %–90 % of an applied pesticide can be volatilized within a few days of application (Aktar et al. 2009).

In general organic production of milk in Chiapas has low concentrations of pesticide residues and satisfies the international regulations for milk production. In spite of good intentions to avoid synthetic compounds, it is impossible to avoid low concentrations of organic and inorganic compounds in organic food due to applications of these organochlorine compounds in the past. This fact has been considered by national and international regulations in validation of the organic brands of many agriculture and livestock products.

We suggest periodic monitoring of organochlorine pesticide residues in the environment to assess trends of environmental contamination by these chemicals. We hope that good agricultural practices (GAP) will be maintained by these dairy farmers engaged in organic production in order to keep milk free of excessive contamination by pesticides.

Acknowledgments We thank UAM Xochimilco Campus for financial support of this research and the organic farmers in Chiapas together with the Universidad Autónoma de Chiapas, Facultad de Medicina Veterinaria y Zootecnia for their contributions to the study.

References

Aktar W, Sengupta D, Chowdhury A (2009) Impact of pesticides use in agriculture: their benefits and hazards. Interdiscip Toxicol 2:1–12

Ashnagar A, Naseri NG, Farmad MC (2009) Determination of organochlorine pesticide residues in cow's milk marketed in Ahwaz city of Iran. Int J Pharm Tech Res 1:247–251

Bulut S, Akkaya L, Gök V, Konuk M (2011) Organochlorine pesticide (OCP) residues in cow's, buffalo's, and sheep's milk from Afyonkarahisar region. Turkey Environ Monit Assess 181:555–562

FAO/WHO/Codex Alimentarius (2006) Codex maximum limits for pesticides residues. Codex Alimentarius Commission, FAO and WHO, Rome

Frank R, Smith EH, Braun HE, Holdrinet M, McWade JW (1975) Organochlorine insecticides and industrial pollutants in the milk supply of the Southern Region of Ontario. Canada J Milk Food Technol 38:65–72

Hernández M, Vidal JV, Marrugo JL (2010) Plaguicidas organoclorados en leche de bovinos suplementados con residuos de algodón en San Pedro, Colombia. Revista de Salud pública 12:982–989

Kampire E, Kiremire BT, Nyanzi SA, Kishimba M (2011) Organochlorine pesticide in fresh and pasteurized cow's milk from Kampala markets. Chemosphere 84:923–927

KaushiK CP, Rai SH, Gulati D, Kaushik A (2011) Changing patterns of organochlorine pesticide residues in raw bovine milk from Haryana. India Environ Monit Assess 182:467–475

Ocampo-Camberos L, Rosiles-Martínez R, Tapia-Pérez G, Sumano-López H (2010) Elimination kinetics of lindane at three doses in cow's milk fat. Agrociencia 44:461–469

Radzymińska M, Smoczyński SS, Kopeć M (2008) Persistent organochlorine pesticide, lead, cadmium, nitrate (V) and nitrate (III) in polish milk and dairy products. Polish J Environ Stud 17:95–100

Real M, Ramírez A, Pérez E, Noa M (2005) Residuos organoclorados en leche cruda y pasteurizada de la zona metropolitana de Guadalajara. México Rev Salud Anim 27:48–54

Sharma HR, Kaushik A, Kaushik CP (2007) Pesticide residues in bovine milk from a predominantly agricultural state of Haryana. India Environ Monit Assess 129:349–357

Subir KN, Mukesh KR (2008) Organochlorine pesticide residues in bovine milk. Bull Environ Contam Toxicol 80:5–9

USEPA (United States Environmental Protection Agency) (1981) Manual for analytical quality control for pesticides and related compounds in human and environmental samples. Second revision. EPA-600/2 81-059

Waliszewki S, Villalobos PR, Gómez AS, Infanzón RM (2003) Persistent organochlorine pesticides levels in cow's milk samples from tropical regions of Mexico. Food Addit Contam 20:270–275