Bull. Environ. Contam. Toxicol. (2006) 77:399–405

DOI: 10.1007/s00128-006-1079-7

Bulletin of Environmental Contamination and Toxicology

Pesticide Residues in Commercial Egg Samples in Sao Paulo City, Brazil, 2003–2005

C. H. P. Ciscato, A. B. Gebara, H. S. Spinosa

Reference Laboratory of Pesticide Residues in Food, Biological Institute, Av. Conselheiro Rodrigues Alves, 1252, CEP 04014-002, São Paulo, Brazil

Received: 20 January 2006/Accepted: 24 July 2006

The availability of food is important for maintaining the population, especially human beings. When the food supply is obtained in a balance manner it is possible to guarantee the carbohydrates, proteins besides mineral salts and vitamins to human development. Each being uses a different way to get the food. Humans, due to their capacity to adapt had their food habits changed including food productions techniques for animal and vegetables supply. Other factors such as industrialization, urbanization and recent globalization, has contributed to a change in food habits. Surveys have shown a decrease in the intake of rice and beans – Brazilian's basic diet - and an increase in the intake of food from animal sources, such as chicken egg, cheese and beef (Marchioni et al. 2002). Among the animal products the egg has great importance, due to its nutritional characteristic for humans. The egg, yolk and white parts, guarantee a 4% of energy and nutrients for human adults.

According to IBGE (Brazilian Geographical and Statistics Institute), in 2000, Brazil produced 2,515,773 dozen of eggs and a major part of these eggs was exported to many countries as Saudi Arabia, China, Japan and Netherlands. Brazil is the 3rd in egg production (FAO 2003) and *per capita* consumption are 94 chicken eggs/year.

Due to the necessity to produce eggs with enough nutrients, the poultry system production uses some chemical components as in feeding as controlling animal diseases and pests. These substances can contaminate the egg and display it to human.

Pests and diseases must be controlled for food production. In Brazil the pesticide average consumption is 3.2 kg of active substances per hectare and it is the 8th in world pesticide consumption. The pesticide employment beyond the advantage to agricultural and cattle production can cause serious problems to human and animal health. Besides, the inadequate use and physical-chemical characteristics of pesticides can collaborate to environmental problems and can be displayed for a long time. Therefore the presence of these substances on food must be controlled. Although eggs are an important Brazilian diet food, there are insufficient studies about pesticide residues in chicken eggs.

Correspondence to: C. H. P. Ciscato

This study reports the results from 2003 to 2005 of pesticide residues in chicken eggs from Sao Paulo markets.

MATERIALS AND METHODS

The present survey included 130 samples collected in Sao Paulo City, during the period from October 2003 through July 2005. All samples were taken in accordance with the guidelines of the Codex Alimentarius (2000).

The samples were analyzed for some insecticides, fungicides, herbicides, acaricides and some metabolites. The substances for spike studies were chosen according to MRL (Maximum Residue Levels) established by Brazilian Legislation and Codex Alimentarius in egg. It was used a gas chromatographic multiresidue method (Specht 1992). A 20 g of homogenized sample (yolk and white) was blended with acetone. Water was added beforehand in an amount that takes full account of the natural water content of the sample so that during extraction the acetone: water ratio remains constant at 2:1 (v/v). The extract was partitioned with dichloromethane in the presence of sodium chloride, resulting in separation of excess water. After evaporation the organic phase is cleaned up by gel permeation chromatography (BIO BEADS® S-X_3 polystyrene gel), using cyclohexane and ethyl acetate (v/v) as eluant. A supplemental cleanup was followed by silica gel deactivated 1.5%. The silica gel column was eluted with: hexane + toluene (65:35 v/v), toluene (100), toluene + acetone (95:5 v/v), toluene + acetone (80:20 v/v) and acetone (100). Each eluate (10 mL) was collected and then injected (1 µL) into a gas chromatograph for qualitative and quantitative analysis. The solvents used were pesticide residue analysis grade. The analytical standards were about 95 to 100% of purity obtained from Dr. Ehrenstorfer GmbH.

For identification and quantification of organochlorines, pyrethroids, and some fungicides an ECD was used. Chromatographic conditions were: capillary column 30m x 0,32mm i.d. and 0.25µm film thickness (SPB™ 608); temperature program: 90°C to 210°C (30°C/minute), hold 2 minutes, 250°C (10°C/minute), hold 14 minutes; splitless injection system at 220°C, hold 2 minutes; detector temperature 300°C; gas flow rates: carrier gas (nitrogen) 1mL/minute on column and 29mL/minute make up. Peak identities were confirmed on alternative capillary columns with different phases and polarities (PTE™5 and SPB™1).

A NPD was used to determine organophosphorus and carbamates compounds and the chromatographic conditions were: capillary column 30m x 0.32mm i.d and 0.25µm film thickness (PTE™5); temperature program: 80°C to 200°C (30°C/minute), hold 2 minutes, 230°C (15°C/minute), hold 6 minutes, 250°C (5°C/minute), hold 2 minutes; splitless injection system at 200°C, hold 2 minutes; detector temperature: 300°C; gas flow rates: carrier gas (nitrogen) 1mL/minute and 29mL/minute make up, hydrogen: 4,5mL/minute, air 175mL/minute. For confirmation were used capillary columns 30m x 0.32mm i.d and 0.25µm film thickness (SPB™20 and HP™1).

Some polar organophosphorus pesticides as acephate, monocrotophos and methamidophos were analyzed by using FPD detection with megabore column 30m x 0.53mm i.d. and 0.25µm film thickness (DB^{TM} 1701); temperature program: 200°C/10minutes, on column injection system at 220°C, hold 2 minutes, detector temperature: 220°C; gas flow rates: carrier gas (nitrogen) 5mL/minute and 25mL/minute make up, hydrogen 140mL/minute, air 1: 80mL/minute and air 2: 175mL/minute. For confirmation was used megabore column 30m x 0.53mm i.d. and 0.25µm film thickness (DB^{TM}5).

Residue concentrations were quantified with a calibration curve, generated from peak area obtained by the results of standard injections. The detection limit was 0.001 mg/kg for almost organohalogenates, 0.01 – 0.05 mg/kg for organophosphorus, organonitrogenous and other molecules studied. The analytical method used was validated and tested in spiked sample analysis, including internal laboratory checks with blank analysis. Recoveries generally ranged from 70 to 120%. Limits of quantification were set at 0,01mg/kg for the most of pesticides analyzed. In every analytical series, blank and spiked samples containing different types of pesticides were analyzed routinely to monitor method performance. The fortification tests were performed in two levels.

RESULTS AND DISCUSSION

The levels were chosen at MRL, and below or above according to pesticide characteristics. The study was conduced in triplicate and the standard deviation and median values are presented in the Table 1.

Table 1 Pesticide recoveries in egg samples (white and yolk)

Pesticides	MRL Brazil mg/kg	MRL Codex mg/kg	Fortification levels mg/kg	Recovery median %	Standard deviation	LOQ mg/kg
acephate		0,10	0.1	60	14.142	0.03
			1.0	60.5	14.849	
alachlor			0.01	110	14.142	0.003
			0.2	136.4	2.050	
bifenthrin		0.01	0.01	100	0	0.003
			0.1	83.5	9.192	
carbaryl	0.5	0.5	0.25	82.5	3.535	0.075
			0.5	118.4	11.105	
cypermethrin		0.05	0.05	68.4	0	0.015
			0.1	129	0	
chlorpyrifos		0.05	0.05	97.2	18.172	0.015
			0.1	81.8	19.445	
DDT and isomers		0.1				
op'DDD			0.01	78	29.698	0.003
op'DDD			0.1	65	8.485	

Table 1 Pesticide recoveries in egg samples (white and yolk) – continued

Pesticides	MRL Brazil mg/kg	MRL Codex mg/kg	Fortification levels mg/kg	Recovery median %	Standard deviation	LOQ mg/kg
pp'DDD			0.01	83	21.213	0.003
			0.1	66.5	6.293	
op'DDE			0.01	103	5.657	0.003
			0.1	101	4.243	
pp'DDE			0.05	85	23.193	0.005
			0.1	99.5	4.384	
op'DDT			0.05	87.9	6.293	0.006
			0.1	94.1	27.365	
pp'DDT			0.05	78.4	1.909	0.008
			0.1	85.4	10.182	
deltamethrin			0.02	94.4	6.222	0.006
			0.2	92.2	3.182	
demethon-S-methyl sulphoxide		0.05	0.05	80.2	5.303	0.015
			0.10	92.0	9.949	
diazinon		0.02	0.02	100.7	27.223	0.006
			0.2	101.3	22.981	
fenvalerate			0.01	121.3	1.838	0.003
			0.02	119.5	8.485	
dicofol		0.05	0.05	101.2	12.374	0.010
			0.1	111.8	11.525	
dieldrin		0.05	0.05	97.5	32.173	0.010
			0.1	77.7	1.343	
dimethoate		0.05	0.05	120.5	0.707	0.015
			0.1	116.5	19.092	
disulfoton		0.02	0.02	71.3	1.626	0.01
			0.20	70.7	0.363	
endosulfan and isomers alpha endosulfan			0.04	91.0	8.202	0.010
			0.1	88.8	0.707	
beta endosulfan			0.04	87.0	11.313	0.012
			0.1	77.0	0.012	
endosulfan sulphate			0.04	117.1	6.767	0.010
			0.1	73.5	1.415	
endrin			0.05	93.3	11.455	0.010
			0.1	89.8	0.707	
ethion			0.2	82.5	43.133	0.06
			0.4	57.6	0.212	

Table 1 Pesticide recoveries in egg samples (white and yolk) – continued

Pesticides	MRL Brazil mg/kg	MRL Codex mg/kg	Fortification levels mg/kg	Recovery median %	Standard deviation	LOQ mg/kg
fenpropathrin		0.01	0.01	107.4	10.535	0.003
			0.1	95.5	6.363	
heptachor		0.05	0.02	77.8	2.050	0.006
			0.1	85.8	4.688	
heptachlor epoxi			0.02	90.3	14.566	0.006
			0.1	89.1	1.767	
gamma HCH		0.01	0.01	79.2	7.353	0.003
			0.05	95.5	13.435	
alpha HCH			0.01	80.0	15.839	0.003
			0.05	110.3	10.818	
beta HCH			0.01	79.3	1.131	0.003
			0.05	125.4	2.262	
metidathion		0.02	0.01	104.2	0.424	0.003
			0.02	114.2	8.202	
monocrotophos		0.02	0.1	57.5	10.606	0.03
			1.0	111.0	115.556	
pirimiphos methyl		0.05	0.05	87.4	21.849	0.015
			0.1	93.4	47.305	
profenophos		0.02	0.1	101.5	48.790	0.03
			0.5	41.0	28.991	
quintozene		0.03	0.01	84.0	22.627	0.003

In Brazil, only 2, 4-D, cyromazine, carbaryl, ethion and thidiazuron have MRL established in egg and in this study, using a multiresidue method it was possible to analyze only carbaryl and ethion. For the others as 2, 4-D and thidiazuron it is necessary a specific method for herbicides and also for cyromazine. The organochlorine pesticides were studied even if they have been banned in Brazil since 1985, but residues from these products could be still found. The main reason could be attributed to their chemical nature and environmental persistence.

130 samples were analyzed and one was positive for alachlor a regular herbicide used in corn production in Brazil. The quantity found was 0.01 mg/kg on egg sample. Possibly characteristics such as UV radiation stability and its permanence into soil after 42 to 70 days could result alachlor to be found in plant and its parts such as seeds, and as a consequence to be found in chicken eggs. Although only one sample was positive to pesticide residues this result is important to food quality control and consumer health. It helps to emphasize the risk studies on pesticide residues on animal and vegetable food.

Pesticide residues in animal products have been studied in many countries and the concern about food contamination is an important subject for Latin America.

Argote-Rivero et al. 1984, in Havana – Cuba showed that all samples collected from the market were contaminated by DDT and isomers. In Mexico, Albert et al. 1988, verified that heptachlor epoxi, DDT and DDE were the pesticides most frequent in egg samples. In Brazil, Vieira et al. 2001 have conduced in Rio de Janeiro, at a *Leishamnia* area a pesticide residues study for DDT and isomers. The organochlorine pesticide was used until 1990 for *Leishmania* vector combat. The authors showed that DDT and isomers were present in almost soil and egg samples analyzed (82.0%). For other developing countries organochlorine pesticides are the most frequent pesticides found in egg samples (El-Hoshi et al. 2000, Kahunyo et al. 1988, Kessabi et al. 1990, Muganbi et al. 1989 and Singh et al. 1988).

In most of the developed countries organochlorine pesticides were forbidden since 1970s. Some monitoring studies showed that these pesticides could be, even now, found in almost animal products as egg (Frank et al. 1990, in Canada, Kan-Do Office 1995, in The USA, Juhler et al. 1995, in Denmark). Noble et al. 1990, studied the feed used for poultry and related that pesticides could be present on egg if they were present on feed. Pesticide residues monitoring studies are very important to guarantee the food safety consumption.

Acknowledgments. We thank the FAPESP (Fundação de Amparo a Pesquisa do Estado de Sao Paulo) that sponsored part of the work. This work is part of a PhD thesis presented by Cláudia H.P. Ciscato to Department of Pathology of the Faculty of Veterinary Medicine of the University of Sao Paulo.

REFERENCES

Argote-Rivero T, Arias-Verdes JA, Izquierdo-Perez L, Fernandez-Garcia A (1988) Levels of organochlorine pesticides in eggs, City of Havana, 1984. Rev. Cubana de Higiene y Epidemiologia 26:27-31

Albert LA, Rendon-von-Osten J (1988) Contamination by organochlorine compounds in some foodstuffs from a region of Mexico. Rev. Saúde Pública 22:500-506

Codex Alimentarius (2000) Portion of commodities to which MRLs Apply and which is analyzed. Information Division – FAO/WHO

El-Hoshi SM, Nazem AM (2000) Some chemical contaminants of public health hazard in dried egg. J Assoc Vet Med 42:25-35

FAO – Major food and agricultural commodities and producers On line: July, 2003 http://www.fao.org/es/ess/top/commodity.jsp?lang=EN&commodity

Frank R, Braun HE, Stonefield KI, Rasper J, Luyken H (1990) Organochlorine and organophosphorus residues in the fat of domestic farm animal species, Ontario, Canada 1986-1988. Food Add Contam 7:629-636

Juhler RK, Lauridsen MG, Christensen MR, Hilbert G (1999) Pesticide residues in selected food commodities: results from the Danish National Pesticide Monitoring Program 1995-1996. J Assoc Off Anal Chem Int 82: 337-358

Kan-Do Office and Pesticide Team (1995) Accumulated pesticide and industrial chemical findings from a ten-year study of ready-to-eat foods J Assoc Off Anal Chem Int 78:614-631

Kanhunyo JM, Froslie A, Maitai CK (1988) Organochlorine pesticide residues in chicken eggs: a survey. J Toxicol Environ Health 24:543-550

Kessabi M, Abdennebi E, Laraje R, Lhafi A (1990) Contamination of eggs, poultry liver and bovine liver and kidney by chlorinated pesticides in Morocco. Sci Tot Environ 90:283-287

Marchioni DML, Zaccarelli EM (2002) Transição nutricional. Rev Higiene Alimentar 96: 16-22

Mugambi JM, Kanja L, Maitho TE, Skaare JU, Lokken P (1989) Organochlorine pesticide residues in domestic fowl (*Gallus domesticus*) eggs from central Kenya. J Sci Food Agric 48:165-176

Noble A (1990) The relation between organochlorine residues in animal feeds and residues in tissues, milk and eggs: a review Australian. J Exp Agric 30:145-154

Specht W (1992) Organochlorine, organophosphorus, nitrogen-containing and other pesticides. In: Manual of Pesticide Residue Analysis DFG Deutsche Forschungsgemeinschaft, Pesticides Commission, VCH, Weinheim (Germany) – VCH Publishers Inc. New York, NY (USA), Vol. I, p.317-322

Singh PP, Chawla RP (1988) Insecticide residues in total diet samples in Punjab India. Sci Tot Environ 76:139-146

Vieira ED, Torres JP, Malm D (2001) DDT environment persistence from its use in a vector control program: a case study. Environ Res 86:174-82