JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Organophosphorus Pesticide Residues in Mexican Commercial Pasteurized Milk

JOSÉ HUMBERTO SALAS,† MARÍA MAGDALENA GONZÁLEZ,† MARIO NOA,‡ NORMA ALICIA PÉREZ,*,† GILBERTO DÍAZ,§ REY GUTIÉRREZ,† HÉCTOR ZAZUETA,§ AND ISIDRO OSUNA§

Departamento de Producción Agrícola y Animal, Universidad Autónoma Metropolitana-X. Calz. Del Hueso 1100. Col. Villa Quietud, C.P. 04960, México, D.F., Mexico; Departamento de Prevención, Facultad de Medicina Veterinaria, Universidad Agraria de la Habana, Km 23 y Carretera Tapaste, C.P. 32700, San José de las Lajas, Habana, Cuba; and Laboratorio de Estudios Ambientales, Facultad de Ciencias del Mar, Universidad Autónoma de Sinaloa, Paseo Klaussen s/n, Mazatlán, Sinaloa, Mexico

A study was conducted to measure residues of 13 organophosphorus (OP) pesticides, widely used as dairy cattle ectoparasiticides or in crops used for animal feed, in homogenized and pasteurized Mexican milk samples. Four different milk brands with high distribution were collected biweekly during a 12 month period ($n = 96$) in supermarkets. OP pesticide residues were measured by gas chromatography with a flame photometric detector. Approximately 39.6% of the samples contained detectable levels of OP pesticide residues. Eight samples contained residues exceeding established maximum residue limits (MRL), and the OP pesticides present in these samples were dichlorvos (five samples), phorate, chlorpyrifos, and chlorfenvinphos (one sample, respectively). Average residues of 13 OP pesticides measured were below established MRLs ranging between 0.0051 and 0.0203 ppm.

KEYWORDS: Organophosphorus pesticides; residues; pasteurized milk

INTRODUCTION

Organophosphorus (OP) pesticides can appear in milk due to several possible causes: (a) use of insecticides directly on dairy cattle for ectoparasite control; (b) pasture, forages, or animal feed manufactured from plant material that has been treated with insecticides; and (c) use of insecticides in stables or diary factories.

It is well-known that OP pesticides are less stable and persistent than organochlorine pesticides; however, there are several reports of their presence in cow's milk (*1*−*7*). Residues of diazinon, chlorpyrifos, and malathion in levels of 0.005−0.586 ppm (*8*−*10*), 0.059 ppm (*5*), and 0.110 ppm (*5*), respectively, have been reported in milk.

OP pesticides are widely used in Mexico in animal husbandry and in pasture, alfalfa, and crops for animal feed production. No reports were found in the literature about OP pesticide residue levels in milk produce in our country. Because monitoring studies to detect pesticide residue levels in milk are very important to determine dietary exposure, the aim of this work was to study the possible presence in milk of the most commonly used OP pesticides implicated in milk production in our country.

* Corresponding author (e-mail normaaperez@hotmail.com).
† Universidad Autónoma Metropolitana-X.
‡ Universidad Agraria de la Habana.
§ Universidad Autónoma de Sinaloa.

MATERIALS AND METHODS

Sampling. Three commercial brands (A−C), with wide national distribution, and one brand (D) produced and distributed by the government for people of low resources were selected. Sampling was done every 2 weeks over the course of a year, in supermarkets (brands A−C) and in government stores (brand D).

Analytical Method. The Ministry of Welfare, Health and Cultural Affairs, Leidschendam, The Netherlands, multiresidue method 5 (submethod 3) for the analysis of organophosphorus compounds in milk (*11*) was used. Milk (50 mL) was blended with ethyl acetate (100 mL). Sodium sulfate (50 g) was added, and the mixture was shaken and then allowed to stand for 2−3 min. The upper layer was decanted, and a 50 mL aliquot was evaporated to dryness in a rotating film evaporator (35 °C). The residue was dissolved in hexane (10 mL) and then extracted with 2 × 25 mL of acetonitrile, saturated with hexane. Combined acetonitrile phases were evaporated to dryness, as described above. The residue was dissolved in 2 mL of ethyl acetate.

Chromatographic Analysis. The OP pesticides analyzed were chlorfenvinphos, chlorpyrifos, coumaphos, diazinon, dichlorvos, dimethoate disulfoton, ethion, fenthion, malathion, mevinphos, parathion-methyl, and phorate.

A Hewlett-Packard model 5890 GC system equipped with a flame photometric detector and a phosphorus filter (526 nm) was used. The capillary column was a 25 m, 0.2 mm i.d., 0.25 μm, HP-1 (100% methyl silicone). The GC conditions were as follows: helium carrier gas flow, 1 mL/min; temperature program, from 40 °C (2 min) to 170 °C (30 °C/min) and finally to 280 °C (3 °C/min). A three-point external standard calibration using the standard mixture at 1, 5, and 10 μg/mL

10.1021/jf020942i CCC: $25.00
Published on Web 06/18/2003

Table 1. Summary of Analytical Results

pesticide	% recovery (CV)[a]	MDL[b] (ppm)	no. of samples > MDL	residue level range (ppm)	residue mean[c] (ppm)	95th percentile[d] (ppm)	MRL[e] (ppm)
dichlorvos	63.0 (15.4)	0.014	6	0.0146–0.2994	0.0203	0.0146	0.020
mevinphos	60.2 (16.7)	0.016	1	0.0160–0.0354	0.0162	0.0160	ne
phorate	75.1 (12.8)	0.014	2	0.0481–0.1728	0.0160	0.0140	0.050
dimethoate	81.1 (9.1)	0.012	1	0.0120–0.0161	0.0120	0.0120	ne
diazinon	83.6 (9.8)	0.013	0	<MDL	0.0130	0.0130	0.020
disulfoton	33.0 (10.6)	0.0050	1	0.0050–0.0107	0.0050	0.0050	ne
parathion-methyl	60.0 (5.0)	0.0050	4	0.0068–0.0253	0.0050	0.0050	ne
malathion	47.2 (22.1)	0.019	1	0.0190–0.0271	0.0019	0.0019	ne
fenthion	75.5 (11.9)	0.013	0	<MDL	0.0013	0.0013	0.050
chlorpyrifos	94.4 (6.6)	0.0090	1	0.0133	0.0090	0.0090	0.010
chlorfenvinphos	98.9 (5.0)	0.0080	3	0.0117–0.2063	0.0080	0.0080	0.008
ethion	43.3 (13.8)	0.0090	0	<MDL	0.0090	0.0090	0.020

[a] Coefficient of variation. [b] Method detection limit. [c,d] Calculated by assigning the MDL to the samples with nondetectable residues or with residue levels under the MDL. [e] Mexican maximum residue limits in milk; ne, not established in milk.

of each pesticide was performed initially to establish the GC-FPD linear range. The injection volume was 1 μL.

Quality Assurance. The efficiency of the analytical method used was determined prior to milk sample analysis. The method detection limit (MDL) was estimated on the basis of the results of 10 replicate analyses of a milk sample spiked at a level of 0.05 μg/mL of each OP pesticide standard. The MDL was calculated with the following formula: MDL $= t_{(0.99)} \times$ SD, where $t_{(0.99)}$ is the Student's one-tailed t value at the 99% confidence level and with $(n - 1)$ degrees of freedom and SD is the standard deviation of replicate analyses (*12*).

Peak areas of standards and extracts of spiked samples, run under identical conditions, were compared to determine percentage recoveries. Precision was determined from these data by calculating the coefficient of variation (CV) of each OP pesticide under study.

During milk sample analysis, a milk control free from OP pesticide residues and a fortified milk sample were included in each sample set.

RESULTS AND DISCUSSION

The method used demonstrated acceptable performance for the OP pesticide residue analysis. The detector response was linear in the range tested. The MDLs for 13 OP pesticides under study ranged between 0.0050 and 0.019 ppm (**Table 1**), which allowed compound detection under or near maximum residue levels (MRLs) established in our country (*13*), which are similar to those established in the Codex Alimentarius. No interfering peaks with the same retention times were found in blank milk samples (**Figure 1**); the selectivity and sensitivity of the flame photometric detector for compounds containing phosphorus resulted in easily interpreted chromatograms. In several samples there were unidentified peaks suggesting the presence of other OP pesticides. Recoveries of OP pesticides averaged 75.26% (ranging between 60.04 and 98.89%) except for disulfoton, ethion, and malathion, which had mean recoveries of 40% (**Table 1**). In multiresidue methods it is very difficult to obtain satisfactory results for all of the compounds analyzed (*11*), so the method would be useful for detecting with confidence 10 of the 13 OP pesticides under study. Coefficients of variation of recovery data ranged from 5.0 to 22.1%, with an average of 11.4%. Each pesticide recovery including the lower ones was taken into account for calculating MDLs and residue levels.

OP pesticide residues were found in four milk brands analyzed. Approximately 29% (milk A), 45% (milk B), 38% (milk C), and 50% (milk D) of milk samples tested contained OP pesticide residues. The numbers of samples exceeding the MRLs established in our country were one (milks A and D), four (milk B), and two (milk C). In each brand there were one or several OP pesticide residues over the MRL. The OP pesticides present in violative levels were chlorfenvinphos (0.236 ppm), chlorpyrifos (0.013 ppm), dichlorvos (0.066−0.299 ppm), and phorate (0.048−0.173 ppm).

When total results ($n = 96$) were analyzed, detectable residues of 84% of positive samples were at levels below the corresponding established Mexican limits. **Table 1** summarizes the residue level ranges (>MDLs) found, the means, and 95th percentiles calculated by assigning the MDLs to samples with nondetectable residues and to the positive samples with levels under MDLs. Mean levels and 95th percentiles of pesticides analyzed were under or close to MRLs established in our country.

The presence of chlorfenvinphos and dichlorvos may be explained due to their use as ectoparasiticides in dairy cattle. Dichlorvos is also used for insect control in barns and silos. The presence of chlorfenvinphos residues in raw milk has been observed by Kituyi et al. (*7*). No reports of the presence of dichlorvos in milk were found in the literature. This OP pesticide is considered of low persistence, with security intervals ranging between 5 and 12 h. However, Repetto et al. (*14*) considered that sometimes the stability of this compound is underestimated because they have found that dichlorvos residues may last for 20 days in rat tissues (blood, liver, muscle, and adipose tissue).

In the Mexican *Veterinary Drug Dictionary* (*15*) there are no indicated withholding periods between the pesticide application and milking. The lack of this information may generate the presence of these residues in milk. The Codex Committee on Veterinary Drugs (*16*) states the necessity of giving sufficient and exact information to the users of these substances in order to observe good veterinary practices and avoid toxic residues in milk.

Chlorpyrifos and phorate residues may be present in milk because of their use in crops destined for animal feed such as alfalfa, sorghum, soy, and maize. The presence of these OP pesticides contrasts with the opinion of Blüthgen and Heeschen (*17*), who considered that metabolic breakdown of OP pesticide is rather quick and that there is only a rare chance to observe their residues in milk. Chlorpyrifos is one of the OP pesticides with higher use in our country (*18*). El-Hoshy (*5*) has detected the presence of chlorpyrifos residues in raw milk. Bolles et al. (*19*) considered that chlorpyrifos residues are rarely detected in food items purchased by consumers, for example, market milk. This could possibly be true in the so-called first world, but our results show that in developing countries the presence of this OP pesticide may be of concern and confirms the

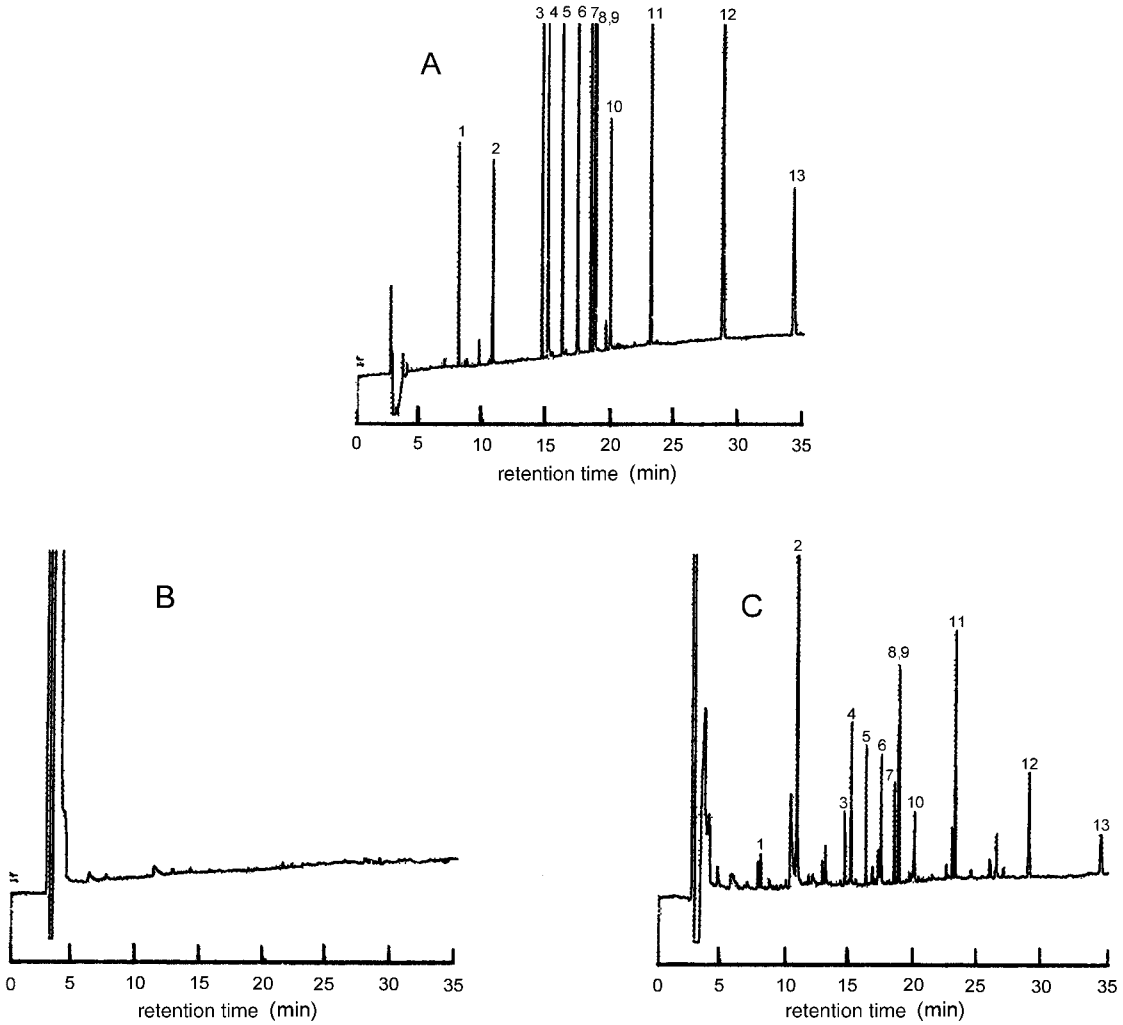

Figure 1. Chromatograms of (A) standard mixture (1 μg/mL each) [(1) dichlorvos, (2) mevinphos, (3) phorate, (4) dimethoate, (5) diazinon, (6) disulfoton, (7) parathion-methyl, (8) malathion, (9) fenthion, (10) chlorpyrifos, (11) chlorfenvinfos, (12) ethion, (13) coumaphos], (B) control milk, and (C) milk fortified at 50 ppb.

necessity of further studies in the stability and transfer of OP pesticide residues from animal feed to milk.

In the Mexican Official Catalog for Registered and Recommended Pesticides Uses (*13*), there are no indicated security intervals for the pesticide phorate, which could favor the presence of this residue in foodstuffs and consequently in milk. The presence of this pesticide in milk would be of concern because of its very high toxicity.

For the five samples with the highest violative OP pesticide residues, daily intakes were calculated and compared to acceptable daily intakes (ADI) established for each pesticide, to determine possible dietary risk. Two cases were assumed: a 10 kg child and a 60 kg adult, drinking 500 mL of milk daily. Dichlorvos (three samples), chlorfenvinphos (one sample), and phorate (one sample) were 1.18, 2.45, 3.75; 5.5; and 17.28 times, respectively, over the ADI for the child case. For the adult case, only the sample with phorate residues exceeded 2.88 times the ADI.

The presence of some samples with OP pesticide residues over the MRL and ADI values could be a possible risk to consumers health, especially children. It is of concern that homogenized pasteurized milk presents violative residues, considering that it is bulk milk mixed from several producers, which should present reduced residue levels and that the pasteurization treatment should also decrease them (*5*, *20*).

Blüthgen and Heeschen (*17*) stated that OP pesticide residues in milk are low and MRL violations are unlikely to occur (especially for ectoparasiticides), However, the situation seems to be different in developing countries, where handling of these highly toxic substances is not always done in accordance with good agricultural and veterinary practices, in addition to the lack of regular food-monitoring programs.

To protect consumers' health it would be necessary for milk producers, dairy industries, universities, and authorities, in developing countries, to collaborate to try to reduce toxic residues in such an important food as milk.

LITERATURE CITED

(1) Johnson, J. C.; Bowmann, M. C.; Leuck, D. B.; Knox, F. E. Persistence of Phosvel in corn silage and effect of feeding dairy cows the treated silage. *J. Dairy Sci.* **1971**, *54*, 1840−1947.

(2) Yess, N. J.; Houston, M. G.; Gunderson, E. L. *Food and Drug Administration Pesticide Residue Monitoring of Foods: 1978−1982*; *J. Assoc. Off. Anal. Chem.* **1991**, *74*, 265−272.

(3) Noa, M.; Hernández, J.; Alfonso, H. A Monitoreo de plaguicidas en algunas cuencas lecheras de Cuba. Parte 2. Plaguicidas organofosforados. *Rev. Salud Anim.* **1992**, *14*, 165−170.

(4) Abd-Alla, E. A. M.; Sayed, A. F.; Ahmed, N. S. Incidence of some organophosphorous pesticide residues in buffalo milk in Giza Governorate. *Egypt. J. Dairy Sci.* **1991**, *19*, 243−248.

(5) El-Hoshy, S. M. Insecticide residues in milk and influence of heat treatments and bacterial fermentation as safeguard against these pollutants. *J. Assiut Med. Vet.* **1997**, *37*, 141−155.
(6) Spradbery, J. P.; Tozer, R. S. The efficacy of diazinon impregnated ear tags against buffalo fly and resulting weight gains and diazinon residues in meat and milk. *Aust. Vet. J.* **1996**, *73*, 6−10.
(7) Kituyi, E. N.; Wandiga, S. O.; Jumba, I. O. Occurrence of chlorfenvinphos residues in cow's milk sampled at a range of sites in western Kenya. *Bull. Environ. Contam. Toxicol.* **1997**, *58*, 969−975.
(8) El-Kohly, A. F.; Afifi, A. M.; Ragab, A. A.; El-Baroty, G. S. Contamination of buffalo milk with residues of diazinon insecticide after spraying animals. *Vet. Med. J. Giza* **2000**, *48*, 7−11.
(9) Szerletics, T. M.; Soos, K.; Vegh, E. Determination of residues of pyrethroid and organophosphorus ectoparasiticides in foods of animal origin. *Acta Vet. Hung.* **2000**, *48*, 139−149.
(10) Abo-El, N.; Kholif, A. M.; Abou-Arab, A. A. K.; El-Ashry, M. A. Effect of diazinon spraying to control the external parasites on the productive performance of dairy animals. 2. Some blood serum parameters of lactating buffaloes and Freisian cows. *Egypt. J. Dairy Sci.* **1994**, *22*, 275−285.
(11) *Analytical Methods for Pesticides*, 6th ed.; Ministry of Welfare, Health and Cultural Affairs: Leidschendam, The Netherlands, 1996.
(12) Baynes, R. E.; Bowen, J. M. Rapid determination of methyl parathion and methyl paraoxon in milk by gas chromatography with solid phase extraction and flame photometric detection. *J. AOAC Int.* **1995**, *78*, 812−815.
(13) CICOPLAFEST Catálogo Oficial de Plaguicidas. Secretaría de Agricultura, Ganadería y Desarrollo Rural, Secretaría del Medio Ambiente, Recursos Naturales y Pesca, Secretaría de Salud y Secretaría de Comercio y Fomento Industrial, México D.F., 1999.
(14) Repetto, M.; Martínez, D.; Sanz, P. Actualización de la toxicología de los plaguicidas. In *Toxicología Avanzada*; Repetto, M., Ed.; Ediciones Díaz de Santos: Spain, 1996.
(15) *Directorio de Medicina Veterinaria, Nutrición y Zootecnia*, 1st ed.; Mercadeo Estadístico: México D.F., 1997.
(16) CODEX. Consideraciones generales sobre los métodos de análisis para el control de residuos. In *Residuos de Medicamentos Veterinarios en los Alimentos*; FAO/OMS: Rome, Italy, 1995; Vol. 3, Sect. 3.
(17) Blüthgen, A.; Heeschen, W. H. Principles for the toxicological evaluation of the residues. In *Monograph on Residues and Contaminants in Milk and Milk Products*; International Dairy Federation: Brussels, Belgium. 1997.
(18) INE. Características de peligrosidad ambiental de plaguicidas. Manual de Trabajo. Instituto Nacional de Ecología. Secretaría del Medio Ambiente, Recursos Naturales y Pesca (SEMARNAP), México D.F., 2000.
(19) Bolles, H. G.; Dixon, W. H. E.; Peterson, R. K.; Tomerlin, J. R.; Day, Jr., E. W.; Oliver, G. R. U.S. market basket study to determine residues of the insecticide chlorpyrifos. *J. Agric. Food Chem.* **1999**, *47*, 1817−1822.
(20) Deiana, P.; Fatichenti, F. Pesticide residues in milk processing. *Ital. J. Food Sci.* **1992**, *4*, 229−245.

Received for review September 9, 2002. Revised manuscript received April 15, 2003. Accepted April 22, 2003.

JF020942I