**Organochlorine insecticide residues in raw milk sold**

**in Córdoba, Colombia**

**Residuos de insecticidas organoclorados presentes en leche cruda comercializada en el departamento de Córdoba, Colombia**

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**Abstract**

In this investigation organochlorine pesticide residues in raw milk from Dairy herds in Cordoba were determined. During the extraction procedure it was used acolumn of diatomaceous earth as eluting system and a mixture of n-hexane-acetone-ethyl acetate (4:2:1), followed by 5% methanol in hexane. For the determination we used a gas Chromatograph Perkin Elmer, Autosystem XL with electron capture detector, splitless injection mode, a capillary column Rtx-5 30m, 0.25 mm id and 0.25 µm film thickness. The recovery rates for certain pesticides were between 88.5 and 96%, the detection limits were defined between 0.01 and 0.04 ng/g, with relative standard deviations less than 6%. In the 63 samples tested p, p'-DDT, α-HCH, δ-HCH, aldrin, dieldrin, endrin, heptachlor, heptachlor epoxide and γ-chlordane were determined, establishing concentrations between 27.1 and 469.6 ng/g. The frequencies of occurrence ranged between 1.6 and 65.1% for heptachlor and p, p'-DDT, respectively. The older population that lives in the sub-regions: Middle Sinú, San Jorge and Savannas were exposed to high health risk associated with the concentration of α-HCH, aldrin and dieldrin in raw milk.

**Key words:** Cow milk, organochlorine pesticides, pesticide persistence, residues.

**Resumen**

En el estudio se determinaron residuos de plaguicidas organoclorados en leche cruda proveniente de hatos lecheros del departamento de Córdoba, Colombia. Durante el procedimiento de extracción se utilizó una columna de tierra de diatomeas y como sistema eluyente una mezcla de n-hexano-acetona-acetato de etilo (4:2:1), seguida de metanol al 5% en hexano. Para la determinación se usó un cromatografo de gases Perkin Elmer, Autosystem XL con detector captura de electrones, en modo de inyección ‘splitless’, una columna capilar Rtx-5 30 m, 0.25 mm di y 0.25 µm de espesor de película. El porcentaje de recuperación para los plaguicidas determinados se encontró entre 88.5 y 96%, los límites de detección se definieron entre 0.01 y 0.04 ng/g con desviaciones estándar < 6%. En las 63 muestras analizadas se determinaron p,p´-DDT, α-HCH, δ-HCH, aldrín, dieldrín, endrín, heptacloro, heptacloro epóxido y γ-clordano, estableciendo concentraciones entre 27.1 y 469.6 ng/g. Las frecuencias de aparición oscilaron entre 1.6 y 65.1% para heptacloro y p,p´-DDT, respectivamente. La población adulta mayor que habita en las subregiones Sinú Medio, San Jorge y Sabanas se encuentra expuesta a riesgo alto en la salud, asociado con la concentración de α-HCH, aldrín y dieldrín en leche cruda.

**Palabras clave:** Leche de vaca, persistencia de los plaguicidas, plaguicidas organoclorados, residuos.

**Introduction**

Pesticides are a group of chemical products used to control pests or to prevent and reduce their damage in crops, landscape and ani-mals. As such, they can be present in foods of daily and frequent consumption (Tadeo, 2008). The health effect of food intake with pesticide residues, transferred through the food chain, has been associated with altera-tions in the reproductive system. Langer (2003) found a significant relation between the presence of organochlorine insecticides and thyroid gland alterations. Goutner *et al*. (2001) reported residual levels of organo-chlorine pesticides and PCBs in gull´s eggs samples collected in 1997 and 1998, and established a temporal pattern of contami-nation by the presence of this type of residues in the environment on the Aegean sea.

Gas chromatography is useful to determine pesticides (Fong *et al*., 1999), however, frequently new variants are found in the extraction, cleaning and injection procedures. Valsamaki *et al*. (2006) developed a chromato-graphy method for multiresidual analysis of 20 organochlorine insecticides and eight PCBs analogs present in the matrix of chicken eggs, which was dispersed in florisil and eluted with in a dichlorometane-hexane (1:1) mix. In the other hand, in Greece Lambropoulou *et al.* (2006) proposed an useful analytic method to determine organochlorine residues in liver samples of different birds by using ultrasound while extraction and sulfuric acid for cleaning.

Hussen *et al*. (2007) developed a selective extraction procedure for multiresidual ana-lysis of organochlorine pesticides in soil from Ethiopia by using pressurized liquid (SPLE). This method make extraction and cleaning simultaneously which results in recovery percenttages above 80% for endosulfan, sulfate endosulfan, p,p-DDT and p,p-DDE. Kalyoncu *et al.* (2009) used gas chromato-graphy with electron capture detector to evaluate 18 organochlorine in fish tissue collected in the Konya market, Turkey, finding mainly DDT and HCH residues. Sum of average DDT concentrations varied between 0.0008 and 0.0828 µg/g. The low residual levels were not associated with health risk and food intake.

In Colombia, the use of organochlorine pesticides is restricted and forbidden since the 70´s (ICA, 2004). In Córdoba, organo-chlorine insectides were heavily used for pest control on cotton, corn, sorghum and plantain (CVS, 2002). Additionally, the development of intensive livestock favors production and commercialization of milk and its derivatives, these foods can be a vehicle for contaminants and residues like organochlorine insecticides (Pardio *et al*. 2003; Waliszewski *et al*. 2003; Zhong *et al*. 2003; Real *et al.* 2005). For the region of study Lans *et al*. (2008) found resi-dual levels of α-HCH, β-HCH, γ-HCH, aldrin y heptachlor epoxide in 0.112 µg/lt, 0.586 µg/lt; 0.112 µg/lt; 0.280 µg/lt and 0.323 µg/lt concentrations, respectively, in water from Ciénaga Grande in the low valley of Sinú river. The aim of this research was to deter-mine organochlorine pesticide residual levels in raw milk samples from commercialized milk in subregions of Córdoba.

**Materials and methods**

Córdoba is located at the Northwest of Colombia between 7° 22’ and 9° 26’ N and 76° 30’ E. The area of study consisted on ten towns belonging to four subregions: Jorge, Sinú Medio, Sabanas and Costanera (Map 1). Six samplings were done during one year in the wet, dry and transition weather picks in the zone.

**Equipment.**

For the measurements a gas chromatographer Perkin Elmer, Autosystem XL, equipped with electron capture detector ECD Ni63 serie 61N9060517, a computer with Turbochrom Navigator software for data reading. A GC/MS Thermo Trace Ultra was used to confirm the identification of the pesticides.

**Chromatography conditions.**

Injector and detector temperatures were 200 °C and 300 °C, respectively. Oven was programmed for an initial temperature of 100 °C for 15 °C/min until 160 °C (6 min), 3 °C/min until 250 °C (10 min). Helium and Nitrogen of high purity was used as carrier gas (12 psi) and make up gas (60 ml/min).

**Extraction, cleaning and analytic determination.**

1 ml of milk was passed through to a diatome earth column using a mix of solvents from n-hexane-acetone-ethyl acetate (4:2:1), followed by 5% methanol on hexane. Eluate was eva-porated by drying on a rotary evaporator at 70 °C, 60 rpm. 1.0 ml of *n*-hexane was added to the concentrated extract. For extract cleaning a pre-conditioned C-18 cartridge was used by eluting 6.0 ml of water-methanol (1:1) follo-wed by 6.0 ml of acetonitrile-ethyl acetate (3:2); cartridge was eluted with 6 ml of *n*-hexane. Eluate was evaporated to dryness; concentrated was diluted with 1.0 ml of *n*-hexane and 2 µl were injected in the chroma-tographer for analysis. (Wu-Hsiung and Li-Jun, 2000).

**Statistical analysis and quality control.**

Concentration values are shown as average ± standard deviation (SD) of the triplicates, normal distribution was evaluated by the Kol-mogorov-Smirnov´s test (Kolmogorov, 1933). When results differed from a normal distribution, non-parametric methods were used (Wilcoxon, 1945). To check that measu-rements were between the method parameters of validation, each 10 runs a standard sample and a previously read sample were measured by duplicate that were verified with the standard and sample control charts.

**Map 1.** Subregions in Córdoba-Colombia.

Source: CVS, 2002.

**Results and discussion**

Pesticides with higher frequency of appearance in milk samples were p,p’- DDT (65.1%), endrin (49.2%) and dieldrin (39.7%); heptachlor, δ-HCH and γ-chlordan with fre-quencies of 1.6%, 7.9% and 4.8 % respecti-vely (Figure 1). In similar studies done in Mexico, Real *et al*. (2005) found frequencies of appearance of de 67.7% for aldrin + dieldrin and 93.6% for endrin. These values were associated with the level of aldrin metabo-lization.

The highest levels of average concentration were 469.6 ng/g for α-HCH, 47.17 ng/g for p,p´-DDT and 38.3 ng/g for δ-HCH (Table 1). In contrast, Zapata *et al.* (1996), Prado (1998), Waliszewski *et al*. (2003), Real *et al*. (2005) and Vallejo (1993) in Nicaragua, Mexico and Colombia, respectively, found total DDT and (α+β)-HCH concentrations to be superior to the ones found in this study. Bioavailability of these kind of residues in Córdoba was associated with the pesticide dose use of 50 kg/ha in cotton when the recommendation by the Colombian Institute on Agriculture (ICA) was 2 kg/ha (CVS, 2002). A similar situation was found in Jordania for excessive use of HCH and DDT during the agricultural pro-cesses in India (Salem *et al*., 2009).

**Figure 1**. Appearance frequency of organochlorine pesticides in milk samples in regions of Córdoba, Colombia.

Additionally, there were found maximum concentrations of 36.6 ng/g of aldrin, 48.7 ng/g of dieldrin and 47.1 ng/g of endrin (Table 1). The concentrations in the milk samples from the Sinú Medio subregion follo-wed this trend endrin > dieldrin > aldrin and, they were associated with the aldrin meta-bolism level in soils (Casarett *et al*., 2008). Similarly, in Colombia, Vallejo (1993) found concentrations of 100 ng/g for aldrin and 40 ng/g for dieldrin. In Mexico, Prado (1998) found concentrations of 250 ng/g (aldrín + dieldrín) and 50 ng/g of endrín.

Maximum concentration of heptachlor in raw milk was 27.1 ng/g, of heptachlor epo-xide was 65.3 ng/g and of γ-clordano was 2.6 ng/g (Table 1). In similar studies with this compound Vallejo (1993) found in Colombia higher values (170 ng/g) and in Nicaragua Zapata *et al*. (1996) found 63 ng/g. In Me-xico, Prado (1998) found in milk 150 ng/g of heptachlor + heptachlor epoxide, and Real *et al*. (2005) found 90 ng/g for the same mix.

**Metabolization level.**

For the analyzed samples coming from Sinú Medio and Costanera, the average value of the concentration ratio δ-HCH/α-HCH was 0.16 and 0.28 respectively which indicates a use close in time of α-HCH that is associated with slow metabolization. Average concentrations of α-HCH in milk samples taken in Sinú Medio for both periods of study were different (P > 0.05), which suggest a recent use of that insecticide in this subregion. These results are contrasting with the findings in Costanera subregion. Concentration ratio of diel-drin/aldrin found in samples from San Jorge (1.55 ng/g), Sinú Medio (1.20 ng/g), and Sabanas (1.19 ng/g) subregions indicate absence of a recent use of aldrin and a medium level of metabolization. No diffe-rences were found (P > 0.05) between the average concentration of this type of insec-ticide between periods of study; this suggests a lower use of aldrin in these subregions.

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| **Cuadro Table 1.** Organochlorine pesticide concentrations in ng/g in row milka from different subregions in Córdoba, Colombia. |
| **Subregion** | **p.p'- DDT** | **α-HCH** | **δ-HCH** | **Aldrin** | **Dieldrin** | **Endrin** | **Hepta-****chlor** | **Hep.-epoxide** | **γ-Chlordane** |
| San Jorge | 47.2 ± 29.0 | 260.2 ± 112.6 | NR | 31.4 ± 6.1 | 48.7 ± 24.9 | 37.6 ± 12.2 | NR | 61.7 ± 18.6  | NR |
| Sinú Medio | 36.5 ± 12.2 | 232.8 ± 139.9 | 38.2 ± 5.4 | 33.5 ± 9.2 | 40.3 ± 10.6 | 47.1 ± 19.3 | 27.1 ± 0.5 | 50.2 ± 4.7 | 40.8 ± 11.4 |
| Sabanas | 34.3 ± 7.5 | 469.6 ± 7.2 | NR | 36.6 ± 14.8 | 43.5 ± 11.5 | 37.9 ± 10.7 | NR | 60.1 ± 12.4 | NR |
| Costanera | 37.1 ± 9.2 | 122.3 ± 32.7 | 34.9 ± 1.0 | NR | 30.7 ± 6.2 | 37.2 ± 4.5 | NR | NR | NR |
| a. Milk average density: 1.032 g/ml; Average fat percentage in row milk: 4.0%. \* Milk fat base. |

**Risk with consumption of organochlorine contaminated milk.**

Daily food intake (CDA) proposed by Codex Alimentarious (2005) were adjusted according to the average body weight of an adult person living in the Atlantic Coast of Colombia (Cas-tilla *et al*., 2010). To qualify the risk asso-ciated with consumption of raw and conta-minated milk with organochlorine pesticides, the daily possible intake (CDP) was compared with the admissible one (CDA), and it was concluded that for CDP values < 30% of CDA the associated risk is low; whereas for CDP values between 30 and 100% of CDA the risk is medium and, CDP values > 100% the risk is high (Majul *et al*., 2004) (Table 2).

Risk associated with human health for presence of p,p´-DDT, δ-HCH, heptachlor y heptachlor epoxide residues in milk samples in the subregions studied was consideredlow, except for samples of San Jorge region that had heptachlor epoxide. A high risk for presence of α-HCH, aldrin and dieldrin in milk samples from San Jorge, Sinú Medio and Sabana was determined, these results are contrasting to the ones obtained by Castilla *et al*.(2010) for the same analytes. Similarly, Heck *et al.*, (2007) found lower concentrations to the ones obtained in this research and absence of health risk due to their presence in milk samples consumed by children in Rio Grande do Sul in Brazil.

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| **Table 2.** Health risk by presence of organochlorine pesticides in row milk samples collected in subregions of Córdoba, Colombia |
| **Pesticide** | **(CDP/ -adjusted CDA)\*100 by region** |  |
| **San Jorge** | **Sinú Medio** | **Sabanas** | **Costanera** |
| DDT | 11.4 (b)\* | 8.8 (b) | 8.2 (b) | 8.9 (b) |
| α-HCH | 156.3 (a) | 140.0 (a) | 282.4 (a) | 73.5 (m) |
| δ-HCH | nr | 23.0 (b) | nr | 5.2 (b) |
| Aldrin | 151.0 (a) | 161.1 (a) | 176.1 (a) | nr |
| Dieldrin | 234.3 (a) | 193.8 (a) | 209.2 (a) | 147.7 (a) |
| Endrin | 90.4 (m) | 113.3 (a) | 91.2 (m) | 89.5 (m) |
| Heptachlor | nr | 2.9 (b) | nr | nr |
| Heptachlor-epoxide | 59.4 (m) | 16.1 (b) | 57.8 (m) | nr |
| γ-Chlordane | nr | 39.3 (m) | nr | nr |
| \* Risk: (a) high; (m) medium; (b) low; nr: not registered. |

**Conclusions**

* The organochlorane pesticides p,p´-DDT, α-HCH, δ-HCH, aldrin, dieldrin, endrin, heptachlor, heptachlor epoxideand γ-chlordan were found in raw milk samples in the subregions of San Jorge, Sinú Me-dio, Sabanas and Costanera, in Córdoba, Colombia. Samples taken in Sinú Medio showed a higher variability in contami-nants, opposite to what was found in Costanera.
* In the milk samples analyzed there were no detectable analytical signs in: β-HCH, γ-HCH, α-Clordano, p,p’- DDD, endosulfan I, endosulfan sulfate, endrin aldehyde.
* The risk associated with organochlorine pesticide presence in raw milk consumed in different subregions of Córdoba (Colom-bia), is qualified as high due to the presen-ce of α-HCH, aldrin and dieldrin in sam-ples from San Jorge, Sinú Medio and Sabanas.

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**References**

Casarett, L.; Klaassen, C.; and Doull, J. 2008. Casarett and Doull's toxicology: the basic science of poisons. McGraw-Hill Professional. p. 150 - 165.

Castilla, P. Y.; Alvis, E. L.; and Alvis, G. N. 2010. Exposición a organoclorados por ingesta de leche pasteurizada comercializada en Cartagena, Colombia. Rev. Salud pública 12(1):14 - 26.

Codex Alimentarius. 2005. Pesticide residues in food. Maximum residue limits. Second edition, CODEX Alimentarius. Joint FAO/WHO Food Standards Programme. Vol 2B. Roma.

CVS (Corporación Autónoma Regional de los Valles del Sinú y del San Jorge). 2002. Plan de Gestión Ambiental Regional. PGAR 2002 – 2012. En: Corporación Autónoma Regional de los Valles del Sinú y del San Jorge (ed.). Noviembre, Montería.

Fong, W. G.; Moye, H. A.; Seiber, J. N.; and Toth, J. P. 1999. Pesticides residues in foods: techniques and regulations. Munich: John Wiley & Sons.

Goutner, V.; Albanis, T.; Konstantinou, I.; and Papakonstantinou, K. 2001. PCBs and organochlorine pesticide residues in eggs of audouin's gull (*Larus* *audouinii*) in the North-Eastern Mediterranean. Mar pollut. bull. 42:377 - 388.

Heck, M. C.; Sifuentes dos Santos, J.; Bogusz Jr. S.; Costabeber, I.; and Emanuelli, T. 2007. Estimation of children exposure to organochlorine compounds through milk in Rio Grande do Sul, Brazil. Food Chem. 102:288 - 294.

Hussen, A.; Westbom, R.; Megersa, N.; Mathiasson, L.; and Björklund, E. 2007. Selective pressurized liquid extraction for multi-residue analysis of organochlorine pesticides in soil. J. Chromatogr. 1152:247 - 253.

ICA (Instituto Colombiano Agropecuario). 2004. Restricciones, prohibiciones y suspensión de registros de plaguicidas de uso agrícola en Colombia. En: agrícola, S. P. y. R. (ed.). Bogotá.

Kalyoncu, L.; Agca, I.; and Aktumsek, A. 2009. Some organochlorine pesticide residues in fish species in Konya, Turkey. Chemosphere 74:885 - 889.

Kolmogorov, A. 1933. Sulla determinazione empirica di una legge di distributione, Giornale dell’ Istituto Italiano degli Attuari 4:83 - 91.

Lambropoulou, D.; Konstantinou, I.; and Albanis, T. 2006. Coupling of headspace solid phase microextraction with ultrasonic extraction for the determination of chlorinated pesticides in bird livers using gas chromatography. Anal. Chim. Acta 573:223 - 230.

Langer, P. 2003. Possible effects of polychlorinated biphenyls and organochlorinated pesticides on the thyroid after long-term exposure to heavy environmental pollution. Joem 45:526.

Lans, C. E.; Marrugo Negrete J. L.; and Díaz Pongutá, B. 2008. Estudio de la contaminación por pesticidas organoclorados en aguas de la Ciénaga Grande del Valle Bajo del río Sinú. Temas Agrarios 13:49.

Majul, E. M.; Morón-Jiménez, M. J.; and Ramón, A. N. 2004. Estimación de la ingesta diaria potencial de nitritos en productos cárnicos de mayor consumo en adolescentes. Julio – septiembre. Respyn 5(3)17 - 26.

Pardio, V. T.; Waliszewski, K. N.; Landin, L. A.; and Bautista, R. G. 2003. Organochlorine pesticide residue in cow’s milk from a tropical region of Mexico. Food Addit. Contam. 20:259 - 269.

Prado G. 1998. Residuos de plaguicidas organoclorados en leche pasteurizada comercializada en Ciudad de México. Arch. med. Vet. 55 - 66.

Real, M.; Ramírez, A.; Pérez, E.; and Noa, M. 2005. Residuos de plaguicidas organoclorados en leche cruda y pasteurizada de la zona metropolitana de Guadalajara, México. Rev. SaludAnim*.* 27:48 - 54.

Salem, N. M.; Ahmad, R.; and Estaitieh, H. 2009. Organochlorine pesticide residues in dairy products in Jordan. Chemosphere 77:673 - 678

Tadeo, L. J. 2008. Analysis of pesticides in food and environmental samples. Madrid, España. INIA 177 - 200.

Vallejo, M. C. 1993. Residualidad de los plaguicidas en los alimentos. En: Universitario, F. N., (ed.). Toxicología y seguridad de los alimentos. Bogotá, Colombia. Primera ed. 151 p.

Valsamaki, V.; Boti, V.; Sakkas, V.; and Albanis, T. 2006. Determination of organochlorine pesticides and polychlorinated biphenyls in chicken eggs by matrix solid phase dispersion. Anal. Chim. Acta. 573:195 - 201.

Waliszewski, S. M.; Villalobos-Pietrini, R.; Gómez-Arroyo, S.; and Infanzon, R. M. 2003. Persistent organochlorine pesticide levels in cow’s milk samples from tropical regions of México. Food Addit. Contam. 20:270 - 275.

Wilcoxon, F. 1945. Comparaciones individuales por métodos que alinean. Bol. biomét. 1:80 - 83.

Wu-Hsiung, Ho; and Li-Jun, Yu. 2000. Determination of organochlorine pesticides in milk by electron capture capillary gas chromatography. J Chin. Chem. Soc*.* 47(3):469 - 473.

Zapata, M.; Santamaría, R.; Alvarez, I.; Salazar, V.; and Müller, U. 1996. Organochlorine pesticide residues in cow's milk, Nicaragua. Bol. Oficina Sanitaria Panamericana 120:483.

Zhong, W.; Xu, D.; Chai, Z.; and Mao, X. 2003. Survey of organochlorine pesticide in retail milk from Beijing, PR China. Food Addit. Contam. 20:254-258.