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Organophosphorus Pesticide Residues in Cow's Milk: Levels of *cis*-Mevinfos, Methyl-Parathion, and Paraoxon

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Organophosphorus pesticides are probably a dairy's and milk's contamination source. Their presence is obviously caused by the animals ingestion of contaminated feeds (hay, forage, grazing ground etc.). The pesticide absorption by the skin can result from its use against parasites that infest animals and against stable insects which are other means of contamination. Other factors responsible for the presence of pesticides in dairy products are also the environmental contamination and accidents.

This chemical group of pesticides is widely employed in Portugal and in despite of some analysts (Mosha et al. 1991; Muan and Skaare 1986) had reported low levels of some of them in milk and blood after oral, dermal and intravenous administration due to its rapid metabolism in animals, we tried to study three of them in cow's milk samples.

This paper reports the findings of a pesticide residue study in cow's milk samples examined during the period between March and June of 1991. These samples (21) were taken up in the market and correspond to an area distributed by north, north-east and all the central zone as far as the Mondego Valley. Four samples were collected directly from the animals. Analyses were conducted for *cis*-mevinphos, methyl parathion and paraoxon.

MATERIALS AND METHODS

N-hexane, acetonitrile, dichloromethane and acetone pesticide grade (Carlo Erba, Italy), anhydrous sodium sulphate for residue analysis, zinc acetate-dihydrate G.R. and wool glass (Merck Darmstad, Germany), sodium chloride AR (M&B), water purified via Milli-Q (Millipore, Bedford, MA, USA), filter paper Whatman nº 4 (Maidstone, England) were used. Pesticides standards were obtained from Dr. Ehrenstorfer (Augsburg, Fed. Rep. of Germany) with purity grade of 99.2 and 99.8% for methyl-parathion and paraoxon, respectively. E-mevinphos was provided in isooctane solution containing 10ng/µl. Stock solutions were prepared in n-hexane or acetone-n-hexane. Work solutions were prepared in n-hexane and contained 0.1, 0.2, 0.4, 0.6, 0.8 and 1.0ng/µl for *cis*-mevinphos and methyl-parathion and 0.2 to 1.0ng/µl for paraoxon. A rotary vacuum evaporator (Heidolph VV 2001), a mechanical shaker for separatory funnels (Agitelec, J.Toulemond, Paris) and a gas chromatograph (Hewlett Packard 5890) equipped with two nitrogen phosphorus detectors, two glass columns (6feet x 2mm i.d.) packed one

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with 10% OV-210 on 80/100 mesh Chromosorb WHP and another with 10% OV - 101 on 80/100 mesh Chromosorb WHP,were used.The conditions were for temperature 220ºC for injector, 200ºC for columns and 250ºC for detectors and for gas flows 24,5ml/min to helium 60 carrier, 3,6ml/min to hydrogen and 110.0ml/min to air. A integrator (Hewlett PacKard 3390 A) was also used.

Twenty one cow's milk samples were collected from commercial circles and divided in six groups (M_1 to M_6) based in different trade-marks. In these groups, there are UHT skim milk (A), UHT low fat milk (B) UHT fat milk (C) and pasteurized day milk (BD) (CD). Four samples are whole milk and were collected directly from different producers (MP_1) (MP_2) (MP_3) (MP_4).

Milk samples submitted to analyse, milk samples free of pesticide residues, reagent blanks and milk samples fortified at two different levels for each chemical in study were extracted and purified according to the previously described method (Toyoda et al. 1990).Samples of 50ml of milk were placed into 250ml separatory funnel and extracted with 50ml acetonitrile by mechanical shaking for 10 min. The aqueous acetonitrile layer was transferred to 500ml separatory funnel. The other layer was extracted twice with 50ml 70% acetonitrile-water and filtered through paper to the same 500ml separatory funnel. After adding 100ml water and 5.981g zinc acetate the mixture was shaken for 10min and filtered to 1000ml separatory funnel containing 200ml 3% sodium chloride and 100ml dichloromethane. Then the separatory funnel was shaked during 5min, the dichloromethane phase passed through anhydrous sodium sulfate/wool glass, and the extract concentrated in rotary vacuum evaporator (≈ 35ºC). Dichloromethane was eliminated by several additions of n-hexane-acetone (4:1) and concentrated to 1-10ml. Suitable aliquots of the concentrated was injected into the gas chromatograph.

RESULTS AND DISCUSSION

The calibration plots for the standards *cis*-mevinphos, methyl-parathion and paraoxon were obtained plotting the mean peak area *versus* the relative concentration ranging between 0.1 and 1.0ng/µl, 0.2 and 2.0 and 0.1 and 1.0 ng/ µl, respectively. The plots were linear with a mean correlation coefficient of 0.994 for *cis* -mevinphos and paraoxon and 0.997 for methyl-parathion.

The recoveries for the three chemicals from fortified samples at each level are based on the peak areas. For *cis*-mevinphos the recoveries ranged from 104.7% to 99.7% (SD 1.7 to 4.2) in the samples fortified with 1.0 and 0.1ppm, for methyl-parathion from 100.4% to 98.7% (SD 3.1 to 3.3) when added of 2.0 and 0.2ppm and for paraoxon from 95.6% to 105.2% (SD 2.4 to 3.2) in samples fortified with 1.0 and 0.1ppm levels.

The method precision was analysed by repeated determinations of prepared milk samples. Intra-assay coefficient variation (CV) of five samples on two days ranged from 3.1% to 3.4% for methyl parathion, from 1.6% to 4.3% for *cis*-mevinphos and from 2.4% to 3.2% for paraoxon. All the studied parameters reveal the method safety and reliability for such purpose (Campmany et al. 1990). Figure 1.A. shows a representative chromatogram of the 1.0 ng *cis*-mevinphos, methyl-parathion and paraoxon standards with retention times of 2.39, 10.21, and 17.27 minutes, respectively. The chromatograms of two fortified milk sample are shown in Figure 1.B and C, respectively.

The detection limit for this method to the organophosphorus in study based on 50ml

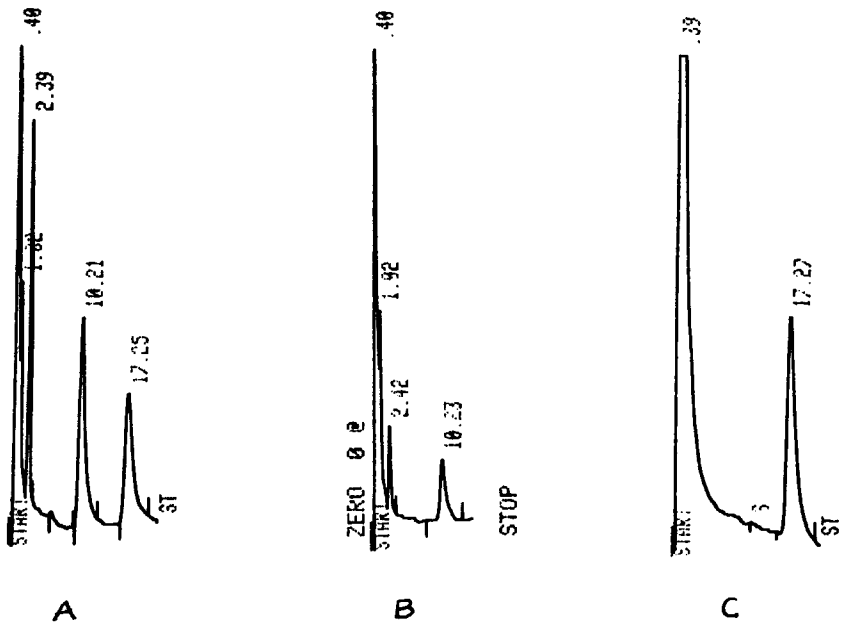

Figure 1. A. Standards of *cis*- mevinphos, methyl-parathion and paraoxon at 1.0ng.
B. Milk sample fortified with *cis* -mevinfos at 1.0ppm and metyl-parathion at 2.0ppm (0.2µl).
C. Milk sample fortified with paraoxon at 1.0ppm(2.0µl)

of sample, concentrated to 1ml and injection of 2µl was 0.5ppb for *cis*-mevinphos, 1.0ppb for methyl-parathion and 1.0ppb for paraoxon.

Some modifications were made to the Toyoda et al. (1990) method. The contact for 30 min. of the dichloromethane phase with the anhydrous sodium sulfate was suppressed and substituted by the passage of that phase by sodium sulphate and wool glass because in a previous work (*Lino C.M. and Noronha da Silveira M.I. (1991) Resíduos de pesticidas organofosforados em leite. I. Teores de diazinão, malatião, paratião e fosfamidão. 3º Congresso Nacional de Ciências Farmacêuticas, Lisboa, Maio*) we observed low recoveries of some organophosphorus pesticides. Also the dichloromethane was eliminated to avoid pernicious efect on the detector glass rubidium bead with an instable baseline and decrease of its life (Ambrus et al. 1981; Committee on Analytical 1985; Kolb et al. 1977; Luke et al. 1981; Winnett and Silver 1981). Its substitution was processed by n-hexane-acetone [4:1].

In general, the necessity to purify the samples is reduced when the N/P detector is used, but in this particular substrate it is necessary because of its free fatty acids content. Milk fat contains 25 to 30% palmitic acid, 12% stearic acid, 11% miristic acid and 23% oleic acid, among others (Luquet 1985). The fatty acids' elimination was made adding zinc acetate to form insoluble zinc carboxylates. Their removal prevents some adverse effects on the gas chromatographic column and on the detector (Adachi et al. 1984).

The confirmation of the analytical results was made using a low polarity

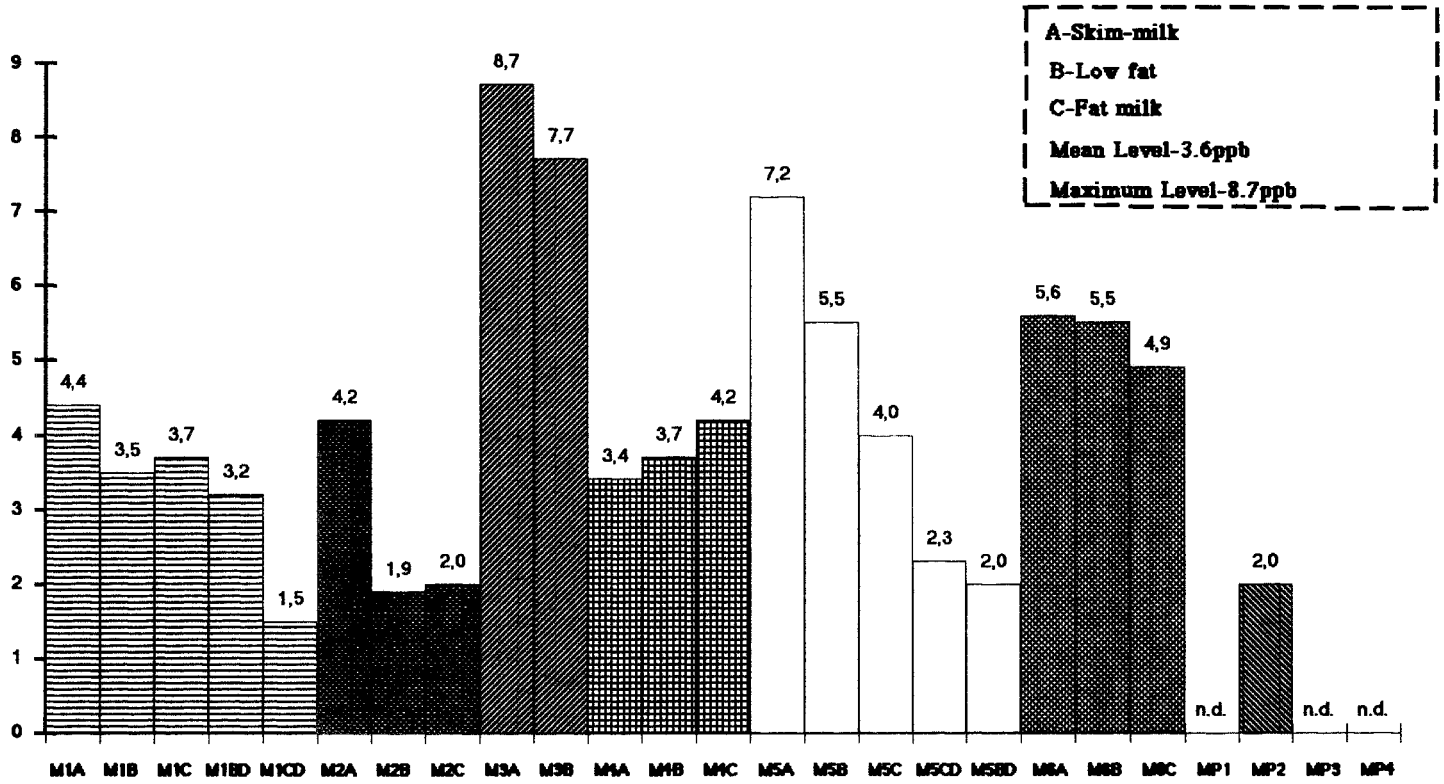

Figure 2 - Paraoxon concentration (ppb) in cow's milk commercialized in north-east and central zone of Portugal (March to June, 1991).

chromatography column (10%OV-101) to avoid false positives or negatives (Thier et al. 1989; Lino and Noronha da Silveira 1987).

Residues of *cis*-mevinphos and methyl-parathion were not detected in the twenty-five samples subjected to analysis. We observed that 22 of 25 samples had detectable paraoxon levels (Figure 2). This concentration ranged from 1.5 to 8.7ppb with an average for all samples of 3.6ppb. In a general view, among the six groups of the commercialized samples the paraoxon concentration was higher in the skim milk, than in the other milks, except in the group 4 (M_4) where fat milk had the highest levels. The samples that hadn't detectable paraoxon levels were the ones collected directly from the producer.

Paraoxon is the oxigen analogue of parathion and is a strong cholinesterase inhibitor which is very poisonous to mammals (oral LD_{50} rats=2.5mg/kg). It is formed by oxidative desulfuration of the thiophosphoryl group. In mammals the hepatic microsomal mixed function oxidase system is responsible for this conversion (Eto 1977).

Two other organophosphorus pesticides, *cis*-mevinphos and methyl-parathion, are subject to rapid metabolism in mammals. *Cis*-mevinphos is subject to two different detoxication reactions. One is conjugation with glutathione S-alkyltransferase that may act as a methyl group acceptor catalyzing the demethylation of some organophosphorus methyl esters. This reaction also occurs for methyl-parathion. The hydrolysis of carboxyester linkage in the particular case of mevinphos isn't important because the carboxyesterases play an insignificant part.With methyl-parathion also can take place another detoxication mechanism-the oxidative dearylation-resulting dimethylthiophosphoric acid and p-nitrophenol (Eto 1977).

The Codex Committee for Pesticide Residues (1987) does not establish the maximum residue limits (MRLs) for paraoxon perhaps due to its high toxicity for human beings and for this reason the MRL for paraoxon should be zero.

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