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Chlorpyrifos, Ethion, Fenitrothion, and Methidathion Residues in Chickens

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Chickens are exposed to pesticides by different ways. Feeds are composed of several vegetable products that suffer treatment to control parasites. This control may be made directly on the crop before the harvest or after, when feeds are transported or when they are stored in granaries. Another cause may be responsible for the contamination, the direct treatment on the chickens by external application of dusts or sprays, the oral administration in the feed and the use of pesticides in chicken-houses (Foster 1974, Fournier and Bonderef 1983) like chlorpyrifos used for control of termites (Leidy et al 1991).
In Portugal is important chicken's production and the portuguese population consumed, in 1991, 19.300kg *per capita* of this protein source. The chicken contamination with pesticide residues may affect the production and the consumption moreover that's very deleterious to human population.

There are methods for determination of organophosphorus pesticide residues in animal origin foods (Brown et al 1987, Holstege et al. 1991, Goodspeed and Chestnut 1991, Luke and Richards 1984). This paper reports the findings of a pesticide residue study in chicken muscle and skin samples examined between July and October of 1991. Fifteen chicken samples were collected from super-markets and groceries in Coimbra.The samples's collection was made at random in three sites for five days and the analysis were conducted for chlorpyrifos, ethion, fenitrothion and methidathion.

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., 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), Florisil (60-100 mesh, Fluka Chemika, USA) heated at 300ºC in a furnace for at least 3h, cooled in a desiccator prior to appropriate deactivation with water (6%) and used in the next 48h, were used. Pesticide standards were obtained from Dr. Ehrenstorfer (Augsburg, Fed. Rep. of Germany) with purity grade of 99.9%, 94.8%, 98.5% and 99.9% for chlorpyrifos, ethion, fenitrothion and methidathion, respectively. Stock solutions were prepared in n-hexane or acetone-n-hexane. Working solutions were prepared in n-hexane and contained 0.02 to 0.2 ng/µl for chlorpyrifos, 0.2 to 1.0 ng/µl for ethion, 0.01 to 0.2 ng/µl to fenitrothion and 0.1 to 0.8ng/µl to methidathion.

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A blender (Braun AG, Western Germany), a rotary vacuum evaporator (Heidolph VV 2001), mechanical shakers for separatory funnels (Agitelec, J. Toulemond, Paris; HS 501D Junke & Kunkel IKA-Labortechnik, Staufen-West Germany) and a gas chromatograph Hewlett Packard 5890 equipped with two nitrogen phosphorus detectors, two glass columns (6feet x 2mm i.d.) packed one with 10%OV-210 on 80/100 mesh Chromosorb WHP and another with 10%OV-101 on 80/100 mesh Chromosorb WHP were used. Operating conditions were: injector temperature 220ºC; column temperature 200º C; detector temperature 250ºC; carrier gas (helium) flow rate 24 ml/min.; hydrogen flow rate 3,6 ml/min.; air flow rate 100ml/min. A integrator Hewlett Packard 3390A was also used.

Each chicken sample was separated into muscle and skin and the bones were discarded. Each part were extracted, partitioned, and cleaned up using the procedure described for milk samples by Toyoda et al. 1990; Lino e Silveira, 1992 and by a modification of their method using Florisil for clean up.

Muscle (25g) or skin (2.5g) samples submitted to analysis were weighted in a beaker and covered with acetonitrile (25ml or 5ml) and the contents let stand for 15 min. After that period it was blended at high speed for 2 min. The supernatant was filtered with suction through paper filter into a Büchner funnel. The sample was rehomogenized twice with 70% acetonitrile-water (25ml or 5ml) for another 2 min. and filtered again. The kitasato and the filter cake were washed with the same solvent mixture. The filtrates and washings were quantitatively transferred into a 500ml or 250ml separatory funnel containing zinc acetate (5,981g) and water (100ml). After shaking 10min., the mixture was filtered to 1000ml or 500ml separatoty funnel containing 3% sodium chloride (200ml or 100ml) and dichloromethane (100 or 50ml). Then the separatory funnel was shaken during 5min., the dichloromethane phase passed through anhydrous sodium sulfate/wool glass and this was concentrated and eliminated in a rotary vacuum evaporator (≈35ºC) by adding n-hexane.

The n-hexane extract was purified passing through a adsorption chromatographic column (300 x 22mm i.d.), with a coarse sintered disc and stopcock, filled with 20g of Florisil deactivated with 6% of water, topped with 2cm of anhydrous sodium sulphate and pre-wetted with hexane. The elution was carried out with 250ml of acetone-n-hexane (6:94). The eluate was concentrated in a rotary vacuum evaporator at ≈35ºC to 1-5ml and injected into the gas chromatograph according with the reported gas chromatographic conditions.

Recoveries of chlorpyrifos, ethion, fenitrothion and methidathion were determined at fortifications levels shown in Table 1 usig muscle and skin chickens. Fortifications were made directly onto the muscle or skin homogenate prior to the blending operations and the fortified samples (n=5) were then carried through the procedure as outlined.

RESULTS AND DISCUSSION

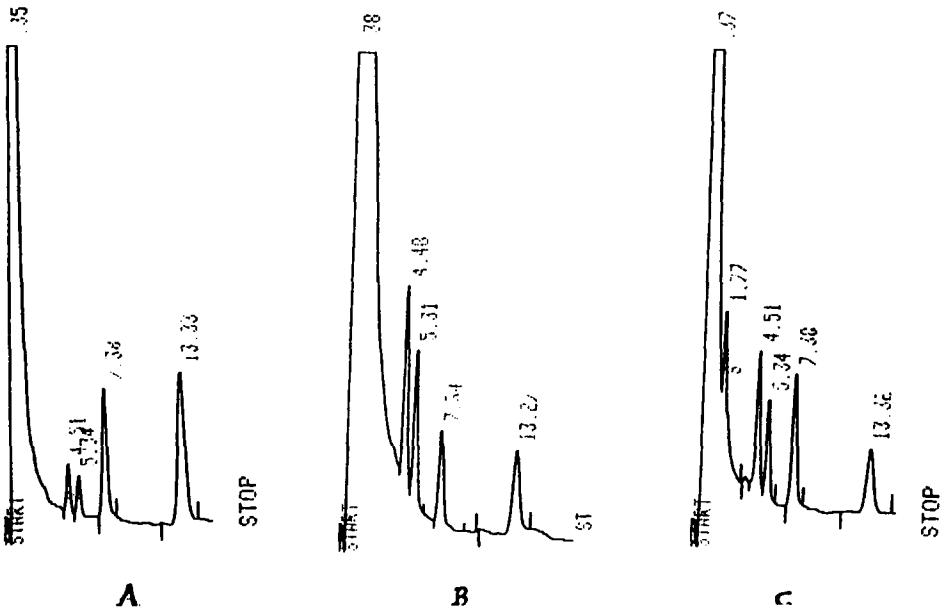
The calibration plots for the standards of chlorpyrifos, ethion, fenitrothion and methidathion were obtained by plotting the mean peak area *versus* their relative concentration ranges , on 10% OV-101 column, from 0.04 to 0.2ng, 0.2 to 1.0ng, 0.01 to 0.2ng and 0.1 to 0.8ng, respectively. Mean correlation coefficients of 0.998, 0.992, 0.997 and 0.960 for, respectively chlorpyrifos, ethion, fenitrothion and methidathion were obtained. With the column packed with 10%OV-210, used for confirmation, the standard calibrations are plotted ranging the concentration between

Table 1. - Recovery of organophosphorus pesticides in chicken.

PESTICIDE	MUSCLE Addition Level (µg/kg)	Recovery ± SD (c.v. %)
Clorpyrifos	6.6	91.89 ± 5.09 (5.54)
	66.0	95.11 ± 5.57 (5.86)
Ethion	39.6	82.42 ± 6.96 (8.44)
	78.6	94.70 ± 7.04 (7.44)
Fenitrothion	6.6	113.46 ± 7.02 (6.18)
	66.0	111.23 ± 8.45 (7.60)
Methidathion	6.6	96.09 ± 5.64 (5.87)
	66.0	94.16 ± 7.83 (8.32)

PESTICIDE	SKIN Addition Level (µg/kg)	Recovery ± SD (c.v. %)
Clorpyrifos	19.0	105.24 ± 11.19 (10.63)
	163.0	85.28 ± 4.54 (5.32)
Ethion	100.0	91.22 ± 7.73 (8.47)
	195.7	92.66 ± 5.62 (6.07)
Fenitrothion	19.0	105.38 ± 8.95 (8.50)
	163.0	106.80 ± 8.12 (7.60)
Methidathion	38.0	95.22 ± 8.47 (8.90)
	326.1	91.94 ± 5.39 (5.86)

Mean of 5 replicate recoveries ± standard derivation (c.v.%)



A B C

Fig. 1. A. standards of fenitrothion (0.04ng), chlorpyrifos (0.04ng), methidathion (0.2ng) and ethion (0.3ng).
B. muscle sample fortified with fenitrothion (0.163ng), chlorpyrifos (0.167ng), methidathion (0.145ng) and ethion (0.15ng).
C. skin sample fortified with fenitrothion (0.133ng), chlorpyrifos (0.121ng), methidathion (0.204ng) and ethion (0.134ng).

Table 2. - Pesticide residues detected in muscle and skin chicken samples (µg/kg)

	Ethion *		Methidathion *	
Sample	Muscle	Skin	Muscle	Skin
6	40.55 (± 5.728)	622.75 (±28.91)		
7			3.25(±0.127)	199.04 (±16.9)
8	51.37 (±3.026)	381.3 (±78.26)		
13	31.315(±1.676)	1796.8 (±105.783)		
14	46.575(±7.474)	1522.0 (±8.485)		
15			4.41(±0.566)	1205.0 (±21.213)

* Mean of two determinations and standard deviation

0.02 and 0.1ng for chlorpyrifos, 0.02 and 0.2ng for fenitrothion and similar concentrations used for 10% OV-101 column for another two chemicals. The correlation coefficients are, in this case 0.927, 0.983, 0.996 and 0.994 for chlorpyrifos, ethion, fenitrothion and methidathion, respectively.

The recoveries, the standard deviation and the coefficient variation to the four chemicals from fortified chicken muscle and fortified chicken skin samples at each level are based on the peak areas and are shown in Table 1. Figure 1.A. shows a representative chromatogram of 0.04ng of fenitrothion, 0.04ng of chlorpyrifos, 0.2ng of methidathion and 0.3ng of ethion in a 10% OV-101 column with retention time of 4.51, 5.34, 7.38 and 13.33 and relative retention time to parathion (trr=5.26) of 0.85, 1.01, 1.40 and 2.53, respectively. The chromatograms of fortified muscle and skin samples are shown in Figure 1.B and C, respectively.

The detection limit of the used method was to fenitrothion, clorpyrifos, methidathion and ethion 3.2, 2.5, 1.3 and 4.4 μg/kg for muscle and 5.6, 2.2, 4.0 and 4.5 μg/kg for skin/chicken respectively.

In the fifteen analysed samples we didn't detect fenitrothion and chlorpyrifos residues in muscle or skin chicken but we detected ethion residues in four samples and methidathion in two samples in both tissues. The detected levels are presented in Table 2. In the same sample we can observe that skin had higher pesticide levels than the muscle. The residue's proportions between skin and muscle chicken samples oscilated between 7.42(sample no 8) and 273 (sample no 15) times. This phenomena may be attributed to the liposolubility of these chemicals making possible greater burden in skin chicken as a result of its highest levels in fat.The skin's samples has higher fat levels than the muscle tissue (minimun value of muscle fat = 1.04% / maximum value of skin fat = 45.26% ;mean % muscle fat /skin fat (n=15)=3.89 / 36.55)

In poultry, Foster (1974) reported the presence of residues of chlorpyrifos in skin and fat of chickens and turkeys fed with 100ppm for 28 days, residues of methidathion in eggs of that avians fed with 50ppm during 30 days, but ethion residues weren't detected in eggs, fat or tissues of poultry after feeding at a level of 0.5ppm for 8 weeks.

Mosha et al.(1990) after oral administration of (14C-methylene)ethion to laying hens at doses of 5 mg/kg found the highest levels on the day one.The concentrations oscillated between 0.008 ppm in skeletal muscle and 3.0 ppm in liver.The highest levels found in egg-yolk than in egg-white is a probable consequence of the high lipidic solubility of ethion.Also in goat milk about 97% of the ethion was retained in cream and 3% in skim milk, which is in accordance with the lipophilic caracteristics of the ethion (log Kow=10000) (Mosha et al.1991).

The lipid contents and the growth of the chicken's body may change the concentration of the contaminants that reflects the contamination of the breeding female through the eggs and the residue levels on the food (Becker and Sperveslage 1989). Also, some analists (Perry et al. 1990) found in the skin of the birds, in the coastal plain of Israel, higher levels of DDE than in muscle because that tissue contains large amounts of fat where that chemical is easily stored.

Levels up to 70 μg of organophosphorus pesticides were found per red tailed hawks; the highest levels of parathion were followed by diazinon, methidathion and chlorpyrifos. Those birds were exposed to organophosphorus during the winter

dormant spray season (Wilson et al. 1991). Also in 43% of tissue samples of loggerhead shrikes were detected chlorpyrifos, diazinon and ethyl parathion (Blumton et al. 1990)

The maximum residue limits (MRL) established by FAO/WHO (Codex 1987) for organophosphorus compounds in poultry are 0.1mg/kg, 0.2mg/kg, 0.05mg/kg and 0.02mg/kg for chlorpyrifos, ethion, fenitrothion and methidathion, respectively. In muscle tissues the levels of ethion and methidathion are below of those limits but in skin they are above in all the samples.

The established ADI (Codex 1987) is 0.005mg/kg body weight for methidathion and 0.0005mg/kg body weight (temporary ADI) for ethion. For adults and children the ADI for methidathion is not exceeded but the same isn't observed for ethion. A daily ingestion of 100g of chicken by adults (≈ 50 - 60kg) in some samples (no. 13 - 14) is near or exceed the ADI and by children, with body weight lower than 30kg, exceed the ADI, for ethion, in the four detectable samples.

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