Effects of a Supplemented Deltamethrin and Piperonyl Butoxide Diet on Levels of Residues in Products of Animal Origin. 2. Feeding Study in Poultry

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Feeding of chickens for almost all their lives with cereals treated with deltamethrin and piperonyl butoxide at the maximum recommended rates did not result in detectable residues of piperonyl butoxide in muscle, fat, skin, or liver. Deltamethrin residues were about 0.003 mg/kg in fat, 0.01mg/kg in skin, and less than 0.002 mg/kg in muscle and liver. No detectable residues of either deltamethrin or piperonyl butoxide were found in eggs from laying hens fed for 12 weeks on the same diet. Analyses were performed by gas chromatography with electron capture detector (for deltamethrin) and high-pressure liquid chromatography with UV detector (for piperonyl butoxide), after appropriate solvent extraction and cleanup.

Keywords: *Deltamethrin; piperonyl butoxide; residues; poultry*

INTRODUCTION

Deltamethrin ((*S*)-α-cyano-3-phenoxybenzyl (1*R*,3*R*)-3-(2,2-dibromovinyl)-2,2-dimethylcyclopropane-1-carboxylate), a synthetic pyrethroid insecticide, generally applied in association with piperonyl butoxide (5-[[2-(2-butoxyethoxy) ethoxy]methyl]-6-propyl-1,3-benzodioxole) as a synergist, is used to improve the control of several pests on stored cereal foodstuffs (Mestres and Mestres, 1992). Its properties have been described, in particular against corn moths, weevils, sylvan beetles, and flour beetles (Robbe-Durand, 1991).

Several data have been published about the potential transfer of these two compounds to animal meat following the ingestion of treated feed, particularly in lactating dairy cows (Akhtar et al., 1986, 1992), rats (Ruzo et al., 1978), and mice (Ruzo et al., 1979). Our first sudy (Marti-Mestres et al., 1993) of deltamethrin and piperonyl butoxide (PB) residues in tissues of pigs fed almost all their lives with treated wheat showed no detectable residue of deltamethrin in kidney or liver, maximum residue levels of 0.003 mg/kg in muscle, levels of and 0.04 mg/kg in fat. No residues of piperonyl butoxide were detected above the following detection limits. The effect of deltamethrin on domestic fowl (laying hens, chicks) has been reported by several authors (Akhtar et al., 1985; Zunyi et al., 1989; Saleh et al., 1986), after short term (1–3 days) administration of relatively high doses of deltamethrin (7.5–20 mg/kg of body weight).

In our study, chicks and laying hens were fed for up to 70 and 84 days, respectively, on flour made from wheat treated with approximately 1 mg/kg deltamethrin and 10 mg/kg piperonyl butoxide which correspond to the maximum recommended rates of treatment (Robbe-Durand, 1991). Consecutive analyses were performed on eggs and chicken liver, muscle, skin, and fat.

The aim of this study was to furnish to the Committee of the Codex Alimentarius supplementary data for the establishment of MLRs (maximum residue limits) in poultry meat and eggs. In this study, the experimental animals were chicks and laying hens, the choice of this species being justified by the possibility of the diet being able to contain up to 70% cereals.

MATERIALS AND METHODS

Solvent extraction and cleanup of samples have been previously described in part 1 of this study (Marti-Mestres et al., 1993). We develop here only those procedures that differ significantly from the reported method.

Grain Treatment. Six tons of wheat var. Centurion were divided in two. One part was treated on a fully automatic seed-treatment line, operating capacity 30 tons of cereal/h, with K-Othrin seed EC 25 PB (containing 25 g/L deltamethrin and 250 g/L technical grade piperonyl butoxide); the other part was not treated. Both parts were individually ground into flour in a rotating hammer will (Gondar 35 CV 3T/h) without additional processing. Trials carried out by Roussel Uclaf (De Wilde, 1990) on whole flour showed that both deltamethrin and piperonyl butoxide are highly stable during long periods of storage at ambient temperatures. After 12 months the initial value of approximately 1 mg/kg deltamethrin had fallen to around 0.8 mg/kg and that of piperonyl butoxide from 9.5 to 7.5 mg/kg. Levels of deltametrin and PB in grains and flour were verified prior to the study (De Wilde, 1990).

Choice of Animal Subjects. *Chicks.* A total of 160 chicks, poulet de chair ja 657 (males and females), were divided into two equal groups (treated and control), ringed, and weighed. Each group was housed separately. The initial weights and their evolution during the experiment are given in Table 1.

Laying Hens. A total of 90 laying hens, Isabrown, were divided into two equal groups (treated and control). At the beginning of the study, the hens were aged 20 weeks and weighed approximately 2 kg.

Feeding Program. The feed consisted of 70% flour and 30% dietary supplement (Soya, eggshell, calcium bicarbonate, methionin, lerbek, premix). Flour given to the control group was prepared from wheat of the same origin but nontreated. (De Laistre Banting, 1990). The amounts of these products

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Table 1. Average Weight of Chicks

days from start of study	control group (g)	treated group (g)
d0	41 (±3)	41 (±3)
d14	214 (±35)	198 (±25)
d28	548 (±88)	584 (±70)
d49	1239 (±177)	1153 (±150)
d70	1602 (±445)	1588 (±274)
d73	1661 (±455)	1579 (±223)
d77	1989 (±233)	1820 (±437)
d84	2156 (±448)	2092 (±529)
d91	2178 (±349)	2036 (±386)

Table 2. Retention Times

column	conditions	deltamethrin (min)	PB (min)
2% SP-2110, 1% SP-2510 DA	240 °C	11.30 (±0.10)	
	230 °C	16.35 (±0.15)	
wide bore HP 1 C18	240 °C	11.58 (±0.10)	
	0.7 mL/min (flow rate)		7.5 (±0.05)
	1 mL/min (flow rate)		5.5 (±0.05)

present are given above. Animals were fed *ad libitum*, corresponding to the intake of about 120 g/day for hens and about 60 g/day for chicks. Under these conditions, the intake varied between 0.05 and 0.1 mg of deltamethrin and 0.5 and 1 mg of PB/kg of body weight.

Slaughter and Conservation of Samples. *Chicks.* After 14, 28, 49, and 70 days, 10 chicks were taken from each group and slaughtered. After 70 days the treated feed was no longer given, both groups receiving the control feed. In order to detect possible detoxification, further chicks were slaughtered after 73, 77, 84, and 91 days. Each time, tissues from the 10 animals were pooled together and analyzed as one sample for each tissue type, since during the early weeks of the study the body weight of the chicks was insufficient to provide an adequate individual sample. Samples of fat, muscle, liver, and skin were taken, labeled, weighed, and immediately frozen. Transport and conservation in the laboratory were carried out at −18 °C. At analysis the samples were homogenized in an electric blender.

Laying Hens. A total of 30 eggs from each group were taken at the beginning of the study and again after 7, 14, 28, 56, and 84 days. After 84 days the treated feed was no longer given, both groups receiving the control feed. In order to detect possible detoxification, further chicks were slaughtered after 91, 98, and 112 days. The eggs were immediately conditioned and frozen. In this study eggs from each group were pooled and analyzed as one sample.

Chromatography. Analysis of deltamethrin was performed on two gas chromatographs, both equipped with electron capture detectors (ECD): HP 5840A, run isothermally at 230 or 240 °C on a glass column (1.5 m length and 2 mm i.d.) packed with 2% SP-2110 1% and SP-2510 DA on 100/120 Supelcoport, and HP 5710A, used for the control of positive results using a wide bore column HP 1 (10 m and 530 μm i.d.) at 240 °C. Detectors and injectors were maintained at 300 and 275 °C, respectively. Carrier gas was argon/methane (90/10) with a flow rate of 70 mL/min.

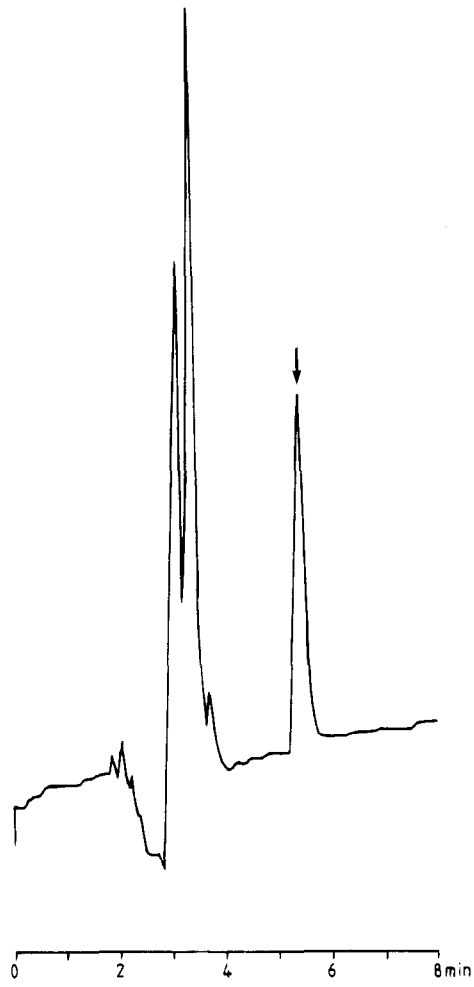

Figure 1. Chromatogram of piperonyl butoxide: 1 mg/L; two columns in series (25 cm × 5 μm), C18; UV, 290 nm; flow rate, 1.0 mL/min.

For piperonyl butoxide (Figure 1), high-pressure liquid chromatography was performed with two C18 columns (25 cm × 5 μm) in series, connected to a Chromatem 380 pump and a Shimadzu SPD.6A UV detector set at 290 nm. The mobile phase was acetonitrile−water (85−15) with a flow rate of 0.7−1.0 mL/min.

Reagents. Reagents have been previously described in part 1. In addition, freshly redistilled methanol and potassium hydroxide (analytical reagent grade) were used. Deltamethrin (purity 99.9%) and piperonyl butoxide (purity 99%) were used to prepare working solutions.

Identification of Peaks and Quantification Method. The peaks were identified by direct comparison of their retention times with those of authentic samples. We calculated the retention time of deltamethrin by injections of deltamethrin standard solution and of matrix with deltamethrin or PB. They are listed in Table 2. Following this we checked the linearity of detector response (Massart et al., 1988; Miller and Miller, 1988), summarized in Table 3.

Table 3. Linearity of Detector Response to Deltamethrin and Piperonyl Butoxide

column	deltamethrin		PB
	2% SP2110, 1%SP2510DA		C18
conditions	240 °C	230 °C	1 mL/min
range used (ng)	0.10−1.25	0.10−1	10−200
y(peak height) = bx(ng) + a	$y = 8.137x + 0.636$	$y = 8.865x + 0.197$	$y = 0.110x + 0.294$
determination coef, r^2	0.923	0.958	0.994
standard deviation, S_a	0.557	0.331	0.343
standard deviation, S_b	0.839	0.559	0.003
slope, 95% confidence limits (with the appropriate t value)	$b = 8.137 \pm 1.897$	$b = 8.865 \pm 1.230$	$b = 0.110 \pm 0.007$
intercept, 95% confidence limits (with the appropriate t value)	$a = -0.636 \pm 1.260$	$a = 0.197 \pm 0.728$	$a = -0.294 \pm 0.775$

Table 4. Recovery Rates Tested from Different Tissues Analyzed in Duplicate for Deltamethrin and in Triplicate for Piperonyl Butoxide[a]

	muscle (mg/kg)	fat (mg/kg)	skin (mg/kg)	liver (mg/kg)	eggs (mg/kg)
			Deltamethrin		
	81% (±4) for 0.01	95% (±6) for 0.01	100% (±15) for 0.01	100% (±11) for 0.01	75% (±6) for 0.01
	97% (±5) for 0.03	95% (±5) for 0.02	100% (±10) for 0.015	100% (±10) for 0.02	100% (±5) for 0.05
			100% (±8) for 0.02		95% (±5) for 0.1
limit of detection	0.002	0.002	0.002	0.002	0.002
			Piperonyl Butoxide		
	70% (±5) for 0.4	75% (±6) for 0.6	75% (±8) for 0.5	70% (±10) for 0.66	72% (±6) for 0.1
					75% (±4) for 1
limit of detection	0.05	0.2	0.1	0.5	0.025

[a] Data obtained by GC/ECD on column 2% SP-2110, 1% SP-2510 DA, 240 or 230 °C, and confirmed on column wide bore HP 1, 240 °C, for deltamethrin and by HPLC, UV detection at 290 nm, two columns in series (C18, 25 cm), for piperonyl butoxide; $\bar{x}$ (s, $n = 6$) for deltamethrin and $\bar{x}$ (s, $n = 9$) for piperonyl butoxide (three injections for each replicate).

Table 5. Levels of Deltamethrin and Piperonyl Butoxide Residues in Tissues of Chicken and Eggs Analyzed in Duplicate[a]

		muscle (mg/kg)	fat (mg/kg)	skin (mg/kg)	liver (mg/kg)	eggs (mg/kg)
			Deltamethrin			
treated group	d14	<0.002	0.002 (±0.001)	0.01 (±0.001)	<0.002	<0.002
	d28	<0.002	0.002 (±0.001)	0.008 (±0.001)	<0.002	<0.002
	d49	<0.002	<0.002	0.01(±0.001)	<0.002	<0.002
	d70	<0.002	0.003 (±0.001)	0.01 (±0.001)	<0.002	<0.002
	d73	<0.002	<0.002	0.01 (±0.001)	<0.002	<0.002
	d77	<0.002	0.003 (±0.001)	0.005 (±0.001)	<0.002	<0.002
	d84	<0.002	<0.002	0.005 (±0.001)	<0.002	<0.002
	d91	<0.002	<0.002	0.005 (±0.001)	<0.002	<0.002
control group (thoughout time of experiment)		<0.002	<0.002	<0.002	<0.002	<0.002
			Piperonyl Butoxide			
treated group (thoughout time of experiment)		<0.05	<0.2	<0.1	<0.5	<0.025
control group (thoughout time of experiment)		<0.05	<0.2	<0.1	<0.5	<0.025

[a] Data obtained by GC/ECD on column 2% SP-2110, 1% SP-2510 DA, 240 or 230 °C, and confirmed on column wide bore HP 1, 240 °C, for deltamethrin and by HPLC, UV detection at 290 nm, two columns in series (C18, 25 cm), for piperonyl butoxide; x (s, $n = 6$); three injections for each replicate.

The limit of detection was estimated after injection of ca. 3 μL of eluate 2 once the optimum conditions for deltamethrin analysis had been identified. For determination of this limit, we chose the methodology using, as recommended by USP (1990) and Freshes and Timme (1980), the signal to noise ratio of 3/1, by comparing test results from samples with known concentration of analyte with those of a blank sample, and established the minimum level at which the analyte could be reliably detected. For each matrix, detection limits were calculated and are summarized in Table 4.

Experimental Methods. However, the different natures of the active ingredients, and of the sample matrices, led us to make adjustments to the published operating conditions for deltamethrin (Marti-Mestres et al., 1993; Marti-Mestres, 1984; Mestres and Mestres, 1985; Mestres et al., 1985) and piperonyl butoxide (Mestres and Susilo, 1980), in particular with regard to the solvents of extraction and elution.

Determination of Deltamethrin. About 20 g of homogenized sample was accurately weighed; 15 g of a mixture of anhydrous sodium sulfate–Celite 545 (2–1) was added (for eggs 30 mL of 95% ethanol was added to the sample) and the whole extracted with 3 × 100 mL (10, 10, and 5 min) of petroleum ether–diethyl ether (50–50, v/v) for muscle, skin, or eggs and 3 × 100 mL of acetonitrile for fat and liver. The organic phases were combined, filtered, and evaporated to dryness in a rotary evaporator, water bath at 30 °C. The second step was a partition: the residue was taken into 40 mL of acetonitrile and subsequently washed with 2 × 20 mL of petroleum ether saturated with acetonitrile which was discarded. Acetonitrile was then evaporated to dryness. A supplementary step was necessary for eggs only. The acetonitrile phase was then diluted with 160 mL of aqueous 5% NaCl and extracted with 2 × 70 mL of petroleum ether–diethyl ether (50–50, v/v). The organic phases were combined, filtered through anhydrous sodium sulfate, and evaporated to dryness. The third step was chromatography over 5 g of florisil (deactivated with 5% water), placed between two 2.5 cm layers of anhydrous sodium sulfate in a column (2.1 cm i.d.). The

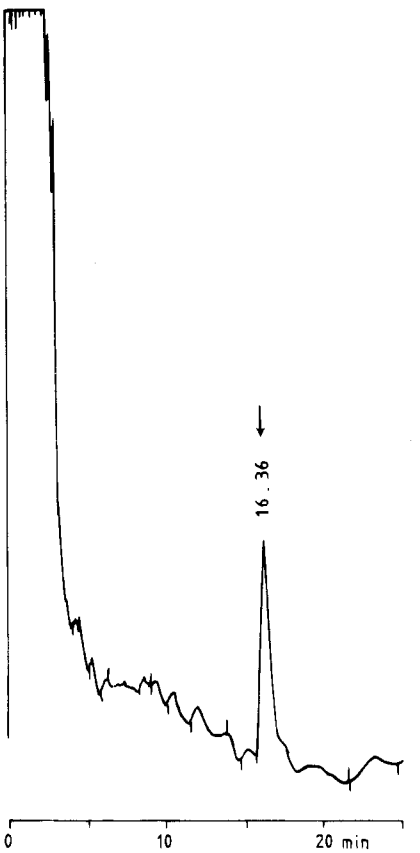

Figure 2. Chromatogram of control chicken liver sample, spiked at 0.012 mg/kg deltamethrin (injection of 3.8 μL (3.7 mL of eluate 2) from 20 g of muscle (column, 2% SP-2110, 1% SP-2510 DA, 230°C)).

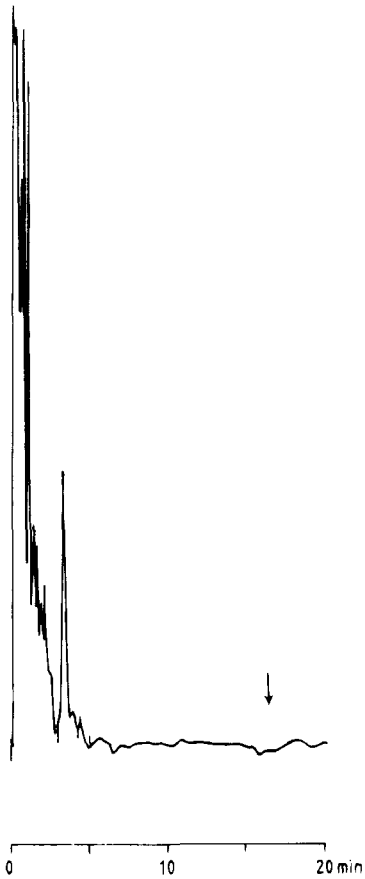

Figure 3. Chromatogram of treated eggs sample d84 (injection of 4.3 μL (5 mL of eluate 2) from 20 g of egg (column 2% SP-2110, 1% SP-2510 DA, 240 °C)). Deltamethrin residues < 0.002 mg/kg.

column was washed with 25 mL of petroleum ether. The residue from partition was taken into 10 mL of petroleum ether and placed on the column. The residue flask was rinsed out with a further 25 mL of petroleum ether which was also added to the column. The eluate 1 thus obtained was rejected. The column was then eluted with 70 mL of petroleum ether–diethyl ether (80–20, v/v). This eluate 2 which contains the deltamethrin residues was evaporated down to 5 mL. Gas chromatography analysis was realized on eluate 2.

Determination of Piperonyl Butoxide. About 20 g of homogenized sample was accurately weighed. For eggs and muscle, a saponification step (with 100 mL of methanol and 10 g of potassium hydroxide under reflux) was recommended. After removal of methanol by distillation, the aqueous phase was extracted by 100, 75, and 50 mL of petroleum ether–diethyl ether (50–50, v/v) prior to concentration to 5 mL. For fat, skin, and liver, extraction (100, 75, and 75 mL) was performed with acetonitrile followed by a partition of the acetonitrile phase diluted with aqueous sodium chloride solution by a mixture of petroleum ether–diethyl ether (50–50, v/v). For liver the sample was left to mix for 2 h with 3 mL of formaldehyde prior to extraction. Cleanup on florisil was then perfomed as for deltamethrin followed by HPLC on eluate 2.

RESULTS AND DISCUSSION

Recovery Rates. For deltamethrin, spiked samples (0.01–0.03 mg/kg) were usually left for 2–4 h before analysis to ensure even distribution of the active ingredient after solvent evaporation. The recovery rates for the different poultry tissues analyzed in duplicate according to the methodology described above are summarized in Table 4 with percent recovery ranging from 75 to 100%. Chromatogram is shown in Figure 2. Piperonyl butoxide analysis recoveries were performed

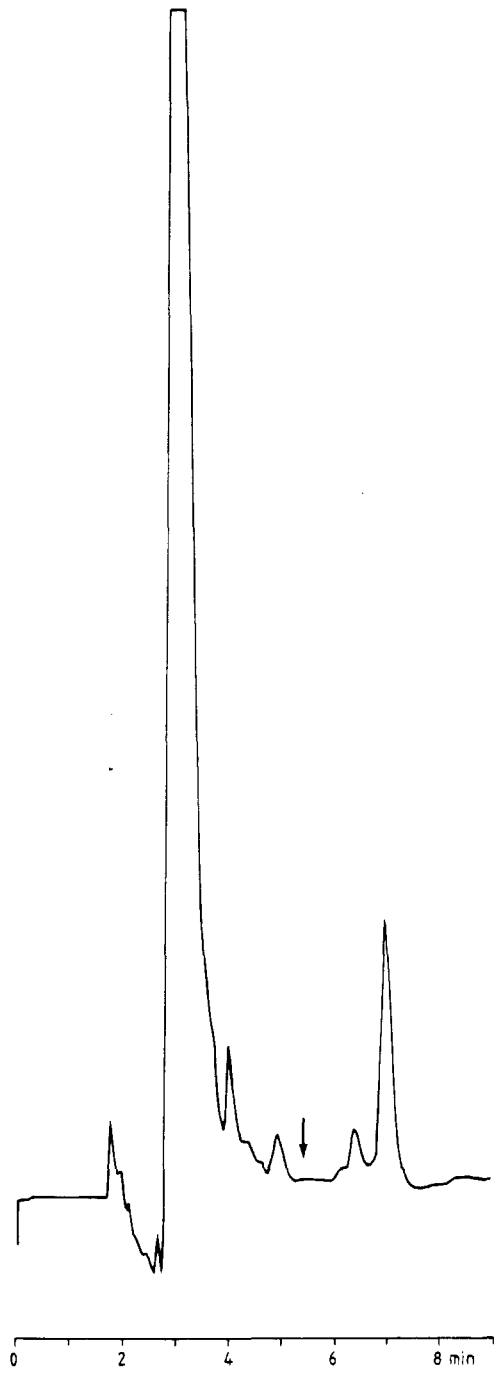

Figure 4. Chromatogram of chicken muscle sample, injection of 20 μL of eluate 2 (5.4 mL from 25 g of muscle; two columns in series (25 cm × 5 μm), C18; UV, 290 nm; flow rate, 1.0 mL/min). Piperonyl butoxide residues < 0.05 mg/kg.

in triplicate, according to the methodology describe above. As indicated in Table 4, recoveries for each matrix were about 75%.

The results of this study are summarized in Table 5. The analysis of the tissues of the control animals revealed no residues above the limits of detection. For the treated animals, deltamethrin levels were always inferior to the limits of detection in liver, muscle, and eggs (<0.002 mg/kg) for each step of the experiment. Figure 3 shows an example. In all fat samples analyzed, low levels were detected (0.002–0.003 mg/kg). The case of skin residues must be considered separately. The values obtained were suprisingly high, possibly due to an external contamination of the tissue from flour adhering to the feathers and down, which was difficult

Table 6. Levels of Deltamethrin Residues in Feathers Analyzed in Duplicate[a]

		down feathers, deltamethrin (mg/kg)
treated group	d28	0.015 (±0.005)
	d49	0.040 (±0.005)
control group	d49	<0.005
limit of detection		<0.005

[a] $\bar{x}$ (s, $n = 6$); three injections for each replicate. Data obtained by GC/ECD on column 2% SP-2110, 1% SP-2510 DA, 240 °C, for deltamethrin.

to remove completely from the skin samples. In order to check this hypothesis, we analyzed separately three batches of feathers (Table 6). The results showed that the control feathers from days 28 and 49 contained respectively 2 and 4 times more residues than the skin samples, which tends to support the hypothesis. No residues of piperonyl butoxide were detected above the limits of detection in any tissue, Figure 4.

Some studies have been previously carried out to determine the metabolic fate of deltamethrin, in particular in leghorn hens (Akhtar et al., 1986). After the oral administration of 7.5 mg of deltamethrin-^{14}C/hen/day on each of three consecutive days, 83% of the dose was eliminated during the first 24 h. Tissue residues were generally low. A more recent study (Zunyi et al., 1989) confirmed this result and led to the conclusion that, in laying hens, the rapid excretion of deltamethrin prevents accumulation of residues in major tissue compartments, after the ingestion of relatively high doses over a short period. Egg yolk contained up to 0.5 mg/kg, while a similar study (Saleh et al., 1986) gave slightly lower values (0.3 mg/kg in yolk).

We felt it would be of interest to study the effect of amounts corresponding to the maximum permitted residue levels ingested along with the feed over a long period. Our results show that no accumulation occurred under these conditions (after ca. 3 months). Our data were used to establish MRLs of deltamethrin for the Codex Alimentarius Commission (1992) step CXL of 0.01 mg/kg in poultry meat and 0.01 mg/kg in eggs.

LITERATURE CITED

Akhtar, M. H.; Danis, C.; Trenholm, H. L. Metabolim, distribution and excretion of deltamethrin by leghorn hens. *J. Agric. Food Chem.* **1985**, *33*, 610–617.

Akhtar, M. H.; Danis, C.; Trenholm, H. L. Fate of [^{14}C] deltamethrin in lactating dairy cows. *J. Agric. Food Chem.* **1986**, *34*, 753–758.

Akhtar, M. H.; Danis, C.; Trenholm, H. L.; Hartin, K. E. Deltamethrin residues in milk and tissues of lactating dairy cows. *J. Environ. Sci. Health* **1992**, *B27*, 235–253.

Codex Alimentarius Commission. Guide to Codex maximum limits for pesticides residues. FAO-WHO Joint Office, April 1992; Part 2, CX/PR 2, pp 144–145.

De Laistre Banting, A. Internal Report E 308; Cebiphar: 37230 Luynes, France, 1990.

De Wilde, G. Internal Report part 2; Roussel Uclaf: France, 1990.

Feshse, H.; Timme, G. Quantitative residue analytical reliability: Beatitude through application of latitude. *Residue Rev.* **1980**, *73*, 27–47.

Marti-Mestres, G. Thèse ès-Sciences Pharmaceutiques, Université Montpellier I, France, 1984.

Marti-Mestres, G.; Cooper, J. F.; Mestres, J. P.; De Wilde, G.; Wynn, N. Effects of supplemented deltamethrin and piperonyl butoxide diet on residues in products of animal origin. 1. Feeding study in pigs. *J. Agric. Food Chem.* **1993**, *41*, 2416–2420.

Massart, D. L.; Vandeginste, B. G. M.; Deeming, S. N.; Michotte, Y.; Kaufman, L. Regression Methods. *Chemometrics a textbook*; Elsevier Science Publishing: New York, 1988.

Mestres, G.; Mestres, R. Crop Grouping. A survey for its possibilities through experimental data for deltamethrin registration. *Arch. Environ. Contam. Toxicol.* **1985**, *14*, 321–323.

Mestres, R.; Susilo, H. Study and determination of piperonyl butoxide residues in the cereal products. *Trav. Soc. Pharm. Montpellier* **1980**, *40*, 277–284.

Mestres, R.; Mestres, G. Deltamethrin: Uses and Environmental Safety. *Rev. Environ. Contam. Toxicol.* **1992**, *124*, 1–18.

Mestres, R.; Francois, Cl.; Causse, C.; Vian, L.; Winnet, G. Survey of exposure to pesticides in greenhouses. *Bull. Environ. Contam. Toxicol.* **1985**, *35*, 750–756.

Miller, J. C.; Miller, J. N. *Statistics for analytical chemistry*; Ellis Horwood: Chichester, England, 1988; p 226.

Robbe-Durand, P. *Index phytosanitaire*; Acta: Paris, France, 1991; p 488.

Ruzo, L. O.; Unai, T.; Casida, J. E. Decamethrin metabolim in rats. *J. Agric. Food Chem.* **1978**, *26*, 918–925.

Ruzo, L. O.; Engel, J.; Casida, J. E. Decamethrin methabolites from oxidative, hydrolytic and conjugative reaction in mice. *J. Agric. Food Chem.* **1979**, *27*, 725–731.

Saleh, M. A.; Ibrahim, N. A.; Soliman, N. A.; El Sheimy, M. K. Persitence and distribution of cypermethrin, deltamethrin and fenvalerate in laying chickens. *J. Agric. Food Chem.* **1986**, *34*, 895–898.

USP. XXII, NF XVII. *Validation of compendial methods*; Mack Printing: Easton, PA, 1990; pp 1740–1712.

Zunyi, X.; Zhisha, C.; Wenfan, Z. Metabolim, distribution and excretion of deltamethrin in hens. *Dongwu Xuebao* **1989**, 35, 164–169.

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