Effects of a Supplemented Deltamethrin and Piperonyl Butoxide Diet on Residues in Products of Animal Origin. 1. Feeding Study in Pigs

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The study of deltamethrin and piperonyl butoxide residues in tissues of pigs fed for almost all their lives with cereals (wheat) treated with these products after harvest at the maximum recommended rate showed no detectable residue of deltamethrin in kidney or liver (less than 0.002 mg/kg), maximum residue levels of 0.003 mg/kg in muscle, and 0.04 mg/kg in fat. No residues of piperonyl butoxide were detected above the following detection limits: 0.05 mg/kg for muscle, 0.1 mg/kg for kidney, 0.2 mg/kg for fat, and 0.5 mg/kg for liver.

INTRODUCTION

The insecticide properties of deltamethrin (Figure 1) (Tessier, 1982) were first reported by Elliott et al. (1974). Belonging to the family of light-stable, synthetic pyrethroids, its use in the protection of agricultural produce stored loose or in sacks has been extensively studied and defined worldwide (Mestres and Mestres, 1992). In France, it is recommended for use on stored cereal foodstuffs to control the following pests: corn moths, weevils, sylvan beetles, and flour beetles (Robbe-Durand, 1991). Deltamethrin's effect is often synergized by the use of piperonyl butoxide (PB) (Figure 2), which acts by inhibiting oxidases that participate in the metabolism of the product in the animal. Its use, therefore, enables the dose of the associated active ingredient to be reduced accordingly, while maintaining the activity. Deltamethrin can in this case be recommended at doses between 0.25 and 1 mg/kg (0.25–1 g/ton of foodstuff) for complete pest control. The FAO/WHO Joint Meeting on Pesticide Residues (JMPR) defined an acceptable daily intake (ADI) of 0.01 mg/kg for deltamethrin. The maximum residue limit (MRL) is concerned with the residue resulting from the use of a pesticide in circumstances designed to protect the food or food product from pest attack, in accordance with good agricultural practices. The Committee of the Codex Alimentarius ratified the MRL proposed by the JMPR. These limits are established on the basis of average adult body weight (60 kg) and daily consumption of the food concerned. The aim of the undertaken study was to furnish to the Committee of the Codex Alimentarius the data necessary for the establishment of MRL for deltamethrin in meat. An experiment on farm animals was therefore carried out at CEBIPHAR (Biological and Pharmaceutical Research Centre, Luynes, France) to determine the effect of a diet based on treated cereals on the residue levels of the active ingredients in animal tissues. In this study, the experimental animal was the pig, the choice of this species being justified by the possibility of a diet being able to contain up to 80% cereals.

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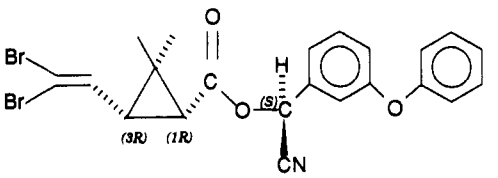

Figure 1. Stucture of deltamethrin [(S)-α-cyano-3-phenoxybenzyl (1*R*,3*R*)-3-(2,2-dibromovinyl)-2,2-dimethylcyclopropan-1-carboxylate].

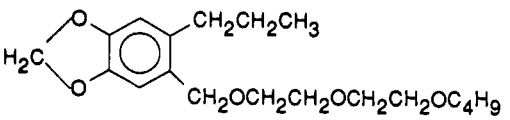

Figure 2. Stucture of piperonyl butoxide [5-[[2-(2-butoxyethoxy)ethoxy]methyl]-6-propyl-1,3-benzodioxole].

MATERIALS AND METHODS

Grain Treatment. At the CNCATA experimental station at La Brosse Monceau, Montereau, France, 6 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 of deltamethrin and 250 g/L of technical grade piperonyl butoxide). On the basis of the percentages of active ingredients in the preparation, the theoretical applied doses were 1.2 mg/kg of deltamethrin and 9.6 mg/kg of piperonyl butoxide. The various analyses carried out by the CEA laboratory at Pierrelate F.84 showed levels in the seeds of 1.11 ± 0.06 mg/kg of deltamethrin and 9.9 ± 1.0 mg/kg of piperonyl butoxide. After grinding, the flour contained these two compounds at levels of 0.95 ± 0.07 and 9.59 ± 0.37 mg/kg, respectively. It should be noted that 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 of deltamethrin had fallen to around 0.8 mg/kg and that of piperonyl butoxide from 9.5 to 7.5 mg/kg.

Choice of Animal Subjects and Weighting Characteristics. Six male and six female piglets of the same race, Landrace hybrid, and of comparable weight were divided into two groups of three males and three females. Group 1 served as the control group; group 2 was fed flour prepared from the wheat treated as described above. Flour given to the control group was prepared from wheat of the same origin but nontreated. The average weights of males, control group, were, initial, 27.5 kg, at slaughter, 116.3 kg, and of the treated group, initial, 27.3 kg, at slaughter,

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Table I. Pig Feeding Program

duration of stage, weeks	quantity of flour, kg/day	quantity of dietary supplement, kg/day
2	0.600	0.300
2	0.800	0.400
2	1.000	0.500
2	1.200	0.600
2	1.400	0.700
2	1.600	0.800
until slaughter	1.800	0.900

99.6 kg. The average weights of females, control group, were, initial, 19.0 kg, at slaughter, 93.0 kg, and of the treated group, initial, 24.6 kg, at slaughter, 99.6 kg (De Laistre Banting, 1989).

Feeding Program. The feed consisted of two-thirds flour and one-third dietary supplement. Only the flour fed to the treated group contained the mixture of deltamethrin and piperonyl butoxide. The amounts of these products present are given above. Table I presents details of the feeding program.

Slaughter and Conservation of Samples. The animals were slaughtered when they had attained a weight of about 100 kg, which occurred between 131 and 140 days of controlled feeding. Samples of fat, muscle, liver, and kidney were taken, labeled, weighed, and immediately frozen. Transport to the laboratory and conservation in the laboratory were carried out at −18 °C. At analysis the samples were homogenized in an electric blender.

Chromatography. Analysis of deltamethrin was performed on two gas chromatographs, both equipped with an electron capture detector (ECD): HP 5840A, run isothermally at 240 °C, on a glass column (1.5 m length and 2 mm i.d.), packed with 2% SP-2110 and 1% SP-2510 DA on 100/120 Supelcoport; HP 5710A, used for the control of positive results using a wide-bore column HP 1 (10 m and 530 μm) at 240 °C also. 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.

For piperonyl butoxide, high-pressure liquid chromatography was performed with two C_{18} 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. Diethyl ether (DiEE) and 95% ethanol were freshly redistilled; petroleum ether (PE) and acetonitrile (ACN) were of pesticide analysis grade. Anhydrous sodium sulfate (analytical reagent grade) and Florisil 60/100 mesh (after activation at 190 °C) were conserved at 130 °C. A mixture of anhydrous sodium sulfate–celite 545 (2/1) was prepared before analysis. Sodium chloride was used in aqueous solution at 1.5%.

Deltamethrin (purity 99.9%) and piperonyl butoxide (purity >99%) were donated by Roussel-Uclaf, Division Agrovet, Romainville, France. Standard stock solutions were prepared by dissolving 50 mg in 100 mL of appropriate solvent. Working solutions were prepared at 0.1, 0.2, and 0.4 mg/L (for deltamethrin), and at 1.0, 2.0, and 4.0 mg/L (for PB) and stored at 4 °C.

Experimental Methods. After several methodologies were tested, a general scheme of operation was set up for the various substances to be analyzed: extraction of residues, purification of extract by partition and Florisil chromatography, and chromatographic analysis (GC or HPLC). 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, 1980; Marti-Mestres, 1984; Mestres and Mestres, 1985; Mestres et al., 1977, 1979, 1985) and for piperonyl butoxide by (Mestres and Susilo, 1979), in particular with regard to the solvents of extraction and elution. The various modifications are summarized in Table II. Two complete sequences are described below by way of example. The first describes the method for determination of deltamethrin in muscle and the second piperonyl butoxide in kidney.

Determination of Deltamethrin in Muscle. The first step was an extraction: the sample provided was homogenized, and 30 g was taken for analysis. Fifteen grams of a mixture of anhydrous sodium sulfate–Celite 545 (2/1) was added and the whole extracted with 3 × 100 mL of DiEE–PE (50/50) for, respectively 10, 10, and 5 min. 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 ACN and subsequently washed with 2 × 20 mL of PE saturated with ACN, which was discarded. The ACN phase was then diluted with 160 mL of aqueous 5% NaCl and extracted with 2 × 70 mL of DiEE–PE (50/50). The organic phases were combined, filtered through anhydrous sodium sulfate, and evaporated to dryness. The third step was chromatography over Florisil. Preparation of Florisil: After activation at 190 °C for 4–5 days, the Florisil was conserved at 130 °C. Florisil + 5% water was prepared 18–24 h before use and kept in stoppered bottles. It must not be kept longer than 3 days. Five grams of Florisil (5% water) was placed between two 2.5-cm layers of anhydrous sodium sulfate in a column (2.1 cm i.d.). The column was washed with 25 mL PE which was rejected. Eluate 1 (E1): The residue from partition was taken into 10 mL of PE and placed on the column. The residue flask was rinsed out with a further 25 mL of PE which was also added to the column. The eluate thus obtained was rejected. Eluate 2 (E2): The column was then eluted with 70 mL PE–DiEE (80/20). The residue flask was always rinsed with this mixture before elution. The eluate was evaporated to 5 mL. This eluate contains the deltamethrin residues. Eluate 2 (2–4 μL) was injected to perform the last step: GC analysis.

Before commencing the study, we calculated the retention time of deltamethrin (Figure 3) by injections of deltamethrin standard solution and matrix with deltamethrin. For the column 2% SP-2110 and 1% SP-2510 DA at 240 °C the retention time was: 11.30 ± 0.10 min. Then we checked the linearity of detector response (Massart et al., 1988; Miller and Miller, 1988) to increasing amounts of deltamethrin, over the range 0.25–1.25 ng. An analysis of variance on data allowed a linear regression between peak height and concentration of deltamethrin to be calculated. The regression formula appears as $y = bx + a$, where y is peak height, x is nanograms of deltamethrin, the slope (b) was 8.137, the intercept (a) was −0.6361, the determination coefficient (r^2) was 0.923, and the standard estimation error ($s_{y/x}$) was 0.474. Further calculations show that the standard deviation for the intercept is $s_a = 0.557$, the standard deviation for the slope is $s_b = 0.839$, and the use of the appropriate t value for 9 degrees of freedom ($t = 2.262$) gives as 95% confidence limits for the intercept and slope $a = -0.636 \pm 1.260$ and $b = 8.137 \pm 1.897$.

The limit of detection was estimated after injection of ca. 3

Table II. Different Analytical Procedures Employed for the Control of Deltamethrin and Piperonyl Butoxide Residues

tissue	sample size,[a] g	extraction	partition	extraction[d]	chromatography/Florisil E 1 (PE) + E 2 (PE–DiEE)	analysis
				Deltamethrin		
muscle	30	PE–DiEE	ACN/EP	H_2O/PE–DiEE	35 mL + 70 mL (80/20)	GC
fat	20	ACN			35 mL + 75 mL (80/20)	GC
kidney	20[b]	PE–DiEE	ACN/EP	H_2O/PE–DiEE	35 mL + 75 mL (80/20)	GC
liver	20	ACN	ACN/EP	H_2O/PE–DiEE	30 mL + 60 mL (80/20)	GC
				PB		
muscle	20	EP–DiEE	ACN/EP	H_2O/PE–DiEE	35 mL + 150 mL (80/20)	HPLC
fat	15	ACN			50 mL + 75 mL (70/30)	HPLC[e]
kidney	20	ACN		H_2O/PE–DiEE	50 mL + 75 mL (70/30)	HPLC[e]
liver	15[c]	ACN		H_2O/PE–DiEE	50 mL + 75 mL (70/30)	HPLC[e]

[a] In all cases 15 g of sodium sulfate–Celite mixture (2/1) is added to the sample. [b] 15 mL of 95% ethanol added. [c] This liver sample is left to macerate for 2 h with 3 mL of formaldehyde before extraction. [d] PE–DiEE mixture (50/50). [e] Eluate 2 is evaporated to dryness, taken into 2 × 5 mL of ACN. Combined ACN washed with 3–4 mL of PE saturated with ACN, then used for final analysis.

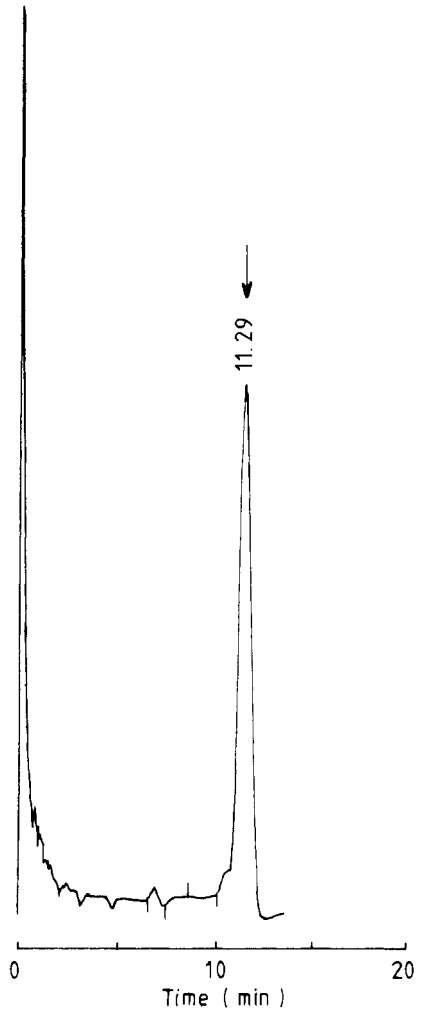

Figure 3. Chromatogram of deltamethrin 2.3 μL × 0.4 mg/L (column 2% SP-2110, 1% SP-2510 DA, 240 °C).

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 Feshes 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 blank sample, and established the minimum level at which the analyte can be reliably detected. Thus, for each matrix, the detection limit was calculated, and they are summarized in Table III.

Determination of Piperonyl Butoxide in Kidney. The first step was an extraction. The sample provided was homogenized, and 20 g was taken for analysis. Fifteen grams of a mixture of anhydrous sodium sulfate–Celite 545 (2/1) was added and the whole extracted with 100, 75, and 75 mL of ACN for 10, 10, and 5 min, respectively. The ACN phases were combined, filtered, and reduced to about 30 mL in a rotary evaporator, water bath at 30 °C. The second step was a partition. The ACN phase recovered was diluted in 200 mL of 1.5% aqueous NaCl solution and then extracted with 100, 75, and 75 mL of PE–DiEE (50/50). The organic phases were combined, filtered through anhydrous sodium sulfate, and evaporated to about 5 mL. The third step was a chromatography over Florisil: 5 g of Florisil (5% water) was placed between two 2.5-cm layers of anhydrous sodium sulfate in a column (2.1 cm i.d.). The column was washed with 25 mL of PE which was rejected. Eluate 1: The residue from partition was taken into 10 mL of PE and placed on the column. The residue flask was rinsed out with a further 40 mL of PE which was also added to the column. The eluate thus obtained was rejected. Eluate 2: the column was then eluted with 75 mL of PE–DiEE (70/30). The residue flask was always rinsed with this mixture before elution. The eluate was evaporated to dryness. This eluate contains the piperonyl butoxide residues. The residue was taken in 5 mL of ACN and washed with 3 mL of PE saturated with ACN. The ACN phase was used for final analysis. The last step was HPLC analysis: The operating conditions and equipment are described above. Before commencing the study, we checked the linearity of UV detector response to increasing amounts of PB, over the range 1–5 mg/L. The retention time was calculated as for deltamethrin. For PB the retention times were 5.5 ± 0.05 min at 0.7 mL/min (flow rate) and 7.5 ± 0.05 min at 1 mL/min.

An analysis of variance on data allowed a linear regression between peak height and concentration of PB to be calculated. The regression formula appears as $y = bx + a$, where y is peak height, x is mg/L of PB, b is the slope, and a is the intercept, with $a = 0.294$, $b = 0.110$, $r^2 = 0.994$, and $s_{y/x} = 0.499$. Further calculations show that the standard deviation for the intercept $s_a = 0.343$, the standard deviation for the slope $s_b = 0.003$, and the use of the appropriate t value for 9 degrees of freedom ($t = 2.262$) gives as 95% confidence limits for the intercept and slope $a = -0.294 \pm 0.775$ and $b = 0.1103 \pm 0.007$. The limit of detection was calculated as for deltamethrin and evaluated and summarized in Table IV.

RESULTS AND DISCUSSION

Recovery Rates. For deltamethrin, spiked samples (0.01–0.066 mg/kg) were usually left for 2–4 h before analysis to ensure even distribution of the active ingredient (ai) after solvent evaporation. The recovery rates for the different pig tissues analyzed in duplicate according to the methodology described above are summarized in Table III with percent recovery ranging from 75 to 100%. Chromatograms are shown in Figures 4 and 5. PB analysis recoveries were performed in triplicate as indicated in Table IV; recoveries for each matrix were about 80%.

The results of this study are summarized in Table V and Figures 6 and 7. 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 and kidney (<0.002 mg/kg). In all muscle samples analyzed, low levels were detected (0.003 mg/kg for males and 0.001–

Table III. Deltamethrin Recovery Rates Tested from Different Tissues in Duplicate[a]

	muscle, mg/kg	fat, mg/kg	kidney, mg/kg	liver, mg/kg
	100% ± 7 for 0.0266	99% ± 8 for 0.010	80% ± 6 for 0.020	87% ± 7 for 0.010
	99% ± 9 for 0.0666	100% ± 6 for 0.020	81% ± 5 for 0.012	75% ± 5 for 0.015
	99% ± 7 for 0.0666[b]	80% ± 5 for 0.050		81% ± 7 for 0.026
limit of detection	0.002	0.002	0.002	0.002

[a] Data obtained by GC/ECD on column 2% SP-2110, 1% SP-2510 DA, 240 °C, and confirmed on column wide-bore HP 1, 240 °C. [b] This sample was left for 24 h for comparison.

Table IV. Piperonyl Butoxide Recovery Rates Tested from Different Tissues in Triplicate[a]

	muscle, mg/kg	fat, mg/kg	kidney, mg/kg	liver, mg/kg
	80% ± 4 for 0.66	77% ± 6 for 0.66	80% ± 8 for 0.5	81% ± 9 for 0.50
limit of detection	0.05	0.2	0.1	0.2

[a] Data obtained by HPLC, UV detection at 290 nm on column C_{18} 2 × 25 cm.

Table V. Levels of Deltamethrin and Piperonyl Butoxide Residues in Tissues of Pigs (Mean Values ± SD)[a]

	muscle, mg/kg	fat, mg/kg	kidney, mg/kg	liver, mg/kg
		Deltamethrin		
control group, males	<0.002	<0.002	<0.002	<0.002
control group, females	<0.002	<0.002	<0.002	<0.002
treated group, males	0.003 ± 0.001	0.015 ± 0.01	<0.002	<0.002
treated group, females	0.002 ± 0.001	0.03 ± 0.01	<0.002	<0.002
		PB		
control group, males	<0.05	<0.2	<0.1	<0.5
control group, females	<0.05	<0.2	<0.1	<0.5
treated group, males	<0.05	<0.2	<0.1	<0.5
treated groups, females	<0.05	<0.2	<0.1	<0.5

[a] Data obtained by GC/ECD on column 2% SP-2110, 1% SP-2510 DA, 240 °C, and confirmed on column wide-bore HP 1, 240 °C, for deltamethrin and by HPLC, UV detection at 290 nm on column C_{18} 2 × 25 cm for PB.

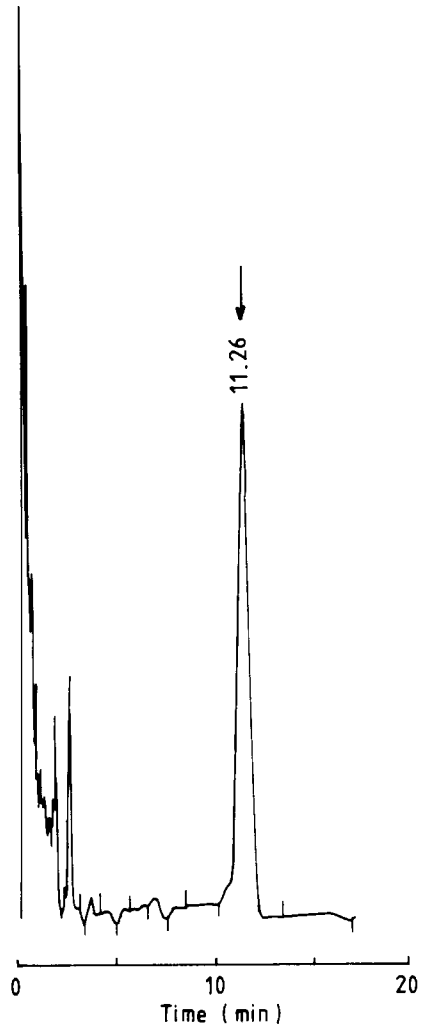

Figure 4. Chromatogram of control pork muscle sample, spiked at 0.066 mg/kg deltamethrin [injection of 2.5 μL of eluate 2 (4.75 mL) from 30 g of muscle; column 2% SP-2110, 1% SP-2510 DA, 240 °C].

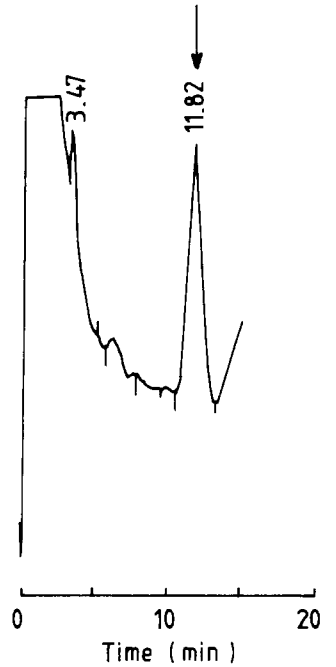

Figure 5. Chromatogram of control pork fat sample, spiked at 0.05 mg/kg deltamethrin [injection of 2.9 μL of eluate 2 (4.6 mL) from 20 g of fat; column wide-bore HP 1, 240 °C].

0.003 mg/kg for females) (Figure 6). These values are too low to make any conclusions regarding the influence of sex on the residue levels. However, we were able to discern a difference between the sexes for deltamethrin levels in fat: male average level, 0.015 ± 0.01 mg/kg; female average level, 0.03 ± 0.01 mg/kg. No residues of piperonyl butoxide were detected above the limits of detection in any tissue. The animal experiments carried out at the Biological and Pharmaceutical Research Centre, CEPHIBAR, for which we performed the analytical study concerning the search for residues of deltamethrin and piperonyl butoxide in the tissues of pigs fed for 131–140 days with food loaded with these two chemicals at the maximum recommended doses, allow us to conclude the following: As was shown by the analyses carried out by the CEA laboratory at Pierrelatte and by Roussel Uclaf at Romainville, direct treatment of wheat grains by a mixture of deltamethrin and piperonyl butoxide at the respective theoretical doses of 1.2 and 9.6 mg/kg resulted in a flour containing 0.95 mg/kg of deltamethrin and 9.5 mg/kg of piperonyl butoxide. These chemicals are very stable in flour conserved at ambient temperatures, since after a year the levels were still 0.8 and 7.5 mg/kg, respectively. Daily ingestion of this diet, at the rate of 0.6–1.8 kg/day per animal as a function of their growth, did not lead to accumulation of this insecticide or the synergist. The levels of PB remained always below the limit of determination, 0.05–0.5 mg/kg, depending on the tissue, indicating that it is effectively metabolized. Its metabolim has been described (*The Agrochemicals Handbook*, 1989) in mammals and insects and involves oxidative attack on the carbon atom of the methylenedioxy group and oxidative degradation of the side chain; elimination is as the glucoside or amino acid derivative. Accordingly, it seems that there is little probability that concurrent use of PB will lead to an accumulation of deltamethrin in mammals. Analysis of deltamethrin in samples of liver and kidney gave in each case negative results, i.e., below the limit of detection, 0.002 mg/kg. Detectable residues were found mainly in the fat—0.015 ± 0.01 mg/kg in males and 0.03 ± 0.01 mg/kg in females. In muscle, levels were near the limit of

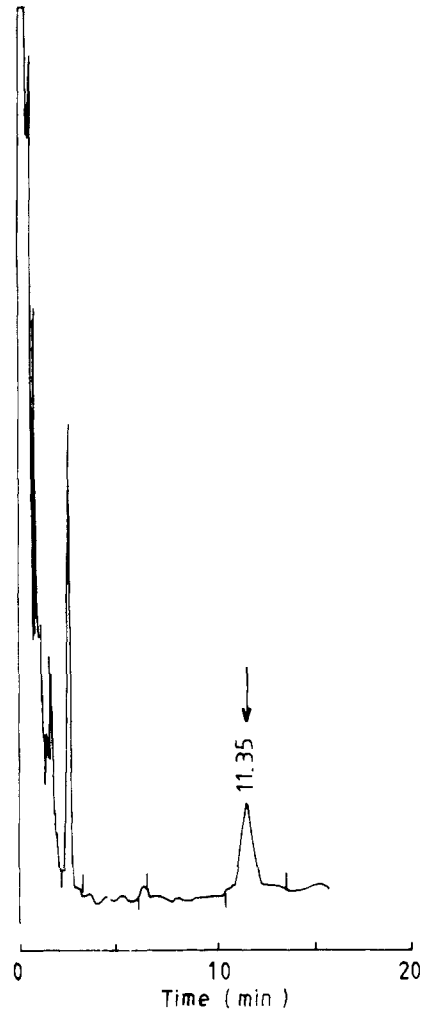

Figure 6. Chromatogram of treated pork muscle sample [injection of 4.15 μL of eluate 2 (2.15 mL) from 30 g of muscle; column 2% SP-2110, 1% SP-2510 DA, 240 °C].

Figure 7. Chromatogram of control pork fat sample [injection of 2.5 μL of eluate 2 (4.8 mL) from 20 g of fat; column wide-bore HP 1, 240 °C].

detection (0.002 mg/kg), being, respectively, 0.003 ± 0.001 mg/kg in males and 0.002 ± 0.001 mg/kg in females.

Akhtar et al. (1992) published a study on deltamethrin residues in milk and tissues of lactating dairy cows. After 28 days of feeding with a standard dairy ration containing 2 or 10 mg/kg (largely superior to the 1 mg/kg used in our study), the residues were determined in various tissues. The order of relative concentration reported was renal fat > subcutaneous fat > forequarter muscle > hindquarter muscle > liver > kidney, which is quite comparable to our own results. The residue levels were also of the same order of magnitude—0.027–0.042 mg/kg for fat and traces (close to detection limit) for muscle and liver.

All of these results suggest that deltamethrin and PB residues obtained after prolonged feeding regimes are not at levels currently considered to be of toxic concern. Our data were used to establish for the Codex Alimentarius Commission (1992) (step 6) an MRL of 0.5 mg/kg in meat (in meat fat).

LITERATURE CITED

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* (3), 235-253.

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

De Laistre Banting, A. Internal Report, E 307; Cebiphar, Luynes, France, 1989.

De Wilde, G. Internal Report, partie 2; Roussel Uclaf, Paris, France, 1990.

Elliott, M.; Farnham, A. W.; Jane, N. F.; Needham, P. H.; Pulman, D. A. Synthetic Insecticide with a New Order of Activity. *Nature* **1974**, *248*, 710–711.

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

Marti, G. Thèse d'Université, Université Montpellier I, France, 1980.

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

Massart, D. L.; Vandeginste, B. G. M.; Deeming, S. N.; Michotte, Y.; Kaufman, L. Regression Methods. In *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, G.; Espinoza, Cl.; Chevalllier, Ch. *Med. Nutr.* **1986**, *22*, 182–184.

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

Mestres, R.; Susilo, H. *Trav. Soc. Pharm. Montpellier* **1980**, *40*, 277–284.

Mestres, R.; Chevallier, Ch.; Espinoza, Cl.; Cornet, R. Photostable pyrethroid insecticide residues in Citrus fruit. *Proc. Int. Soc. Citric.* **1977**, *2*, 429–432.

Mestres, R.; Espinoza, Cl.; Chevallier, Ch.; Marti, G. *Trav. Soc. Pharm. Montpellier* **1979**, *39*, 329–336.

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*; Horwood: Chichester, England, 1988; 226 pp.

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

Tessier, J. Stucture, Synthesis and Physical-Chemical Properties of Deltamethrin. In *Deltaméthrin monograph*; Lhoste, J., Ed.; Roussel Uclaf: Paris, 1982; Chapter 2, pp 37–65.

The Agrochemicals Handbook; Royal Society of Chemistry: Cambridge, England, April 1989.

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

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