CONCLUSION

In the present work, evidence has been presented for an equilibrium between nitrous acid, N^{α}-acetyltryptophan, and its nitrosated form. The equilibrium constant is equal to 255.6 M^{-1}; the formation of the NacNOTRP is an exothermic process with $\Delta H_0 = -54000$ J·mol^{-1} in the range of 6.5–42.9 °C. A standard molar entropy can be given equal to −140 J·mol^{-1}·K^{-1}. Thus, tryptophan reactivity is well defined over a large range of pH and temperature.

This helps to determine the cause of the release of nitrite by cured meat and nitrosated myofibrillar proteins, after washing with water (Noël et al., 1981). This can be explained by a shift of the equilibrium caused by the removal of free nitrous acid from the solution. In this case, nitrosotryptophan acts as a reserve of nitrite that is progressively released when the free nitrite concentration decreases in the medium. As it can be seen in the Figure 1 the equilibrium is shifted toward the nitrosotryptophan at low pH. This is in agreement with the results of Noël et al. who showed that labile bound nitrite was in larger amount at a lower pH. Furthermore, this equilibrium explains the "transnitrosation" reaction from nonheme protein tryptophyl residues toward the myoglobin as described by Ito et al. (1983).

Heme groups, which are very reactive toward nitrite, bind to free nitrite. This causes the shift of the equilibrium until the nitrosotryptophan is exhausted. This new transfer mechanism of nitrite from a site to another site of the protein or of another molecule is to be added to the free-radical mechanism suggested by Buglass et al. (1974) in the case of the aromatic amines nitrosation. The mechanism provides the chemical basis for a better classification of the different states of nitrite in cured meat. Usually, nitrite is classified into free and bound fractions. Bound nitrite should be now divided in two forms: irreversibly and reversibly bound. Reversibly bound nitrite can be nitrosotryptophan, but other unknown species cannot be excluded. Usually nitrite extraction from cured meat products is carried out under basic conditions to avoid nitrite destruction. Equilibrium constant variation as a function of the pH provides an additional justification to this method: at basic pH the equilibrium is shifted toward nitrite releasing. Then, the AOAC method of nitrite assay takes in account nitrite reversibly bound on the tryptophan.

ABBREVIATIONS USED

NacTRP = *N*-acetyltryptophan
NacNOTRP = *N*-acetyl-N^1-nitrosotryptophan
OD = optical density
ϵ = molar extinction coefficient for NacNOTRP
K_{app} = apparent equilibrium constant

Registry No. L-NacTRP, 1218-34-4; NacNOTRP, 58332-35-7; NO_2^-, 14797-65-0.

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Persistence and Distribution of Cypermethrin, Deltamethrin, and Fenvalerate in Laying Chickens

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Persistence and distribution of cypermethrin, deltamethrin, and fenvalerate in laying chickens were studied following oral administration of a single-dose mixture containing 10 mg/kg of body weight of each pyrethroid. Residues of the parent compounds were analyzed in fat, skin, blood, heart, brains, liver, kidney, ovary, egg yolk, and egg white 1, 2, 3, 5, 7, and 14 days after oral administration. Residues of the parent compounds in the brains were much higher and more persistent than other tissues investigated. Fenvalerate was the most persistent in all tissues followed by cypermethrin and deltamethrin.

The potential tranfer of pesticides from agricultural uses and from pesticide residues in processed agricultural products used as feed to livestocks and poultry is greatly emphasized. Cypermethrin [α-cyano(3-phenoxybenzyl) 3-(2,2-dichloroethenyl)-2,2-dimethylcyclopropanecarboxylate], deltamethrin [(*S*)-α-cyano-3-phenoxybenzyl *cis*-(1*R*,3*R*)-2,2-dimethyl-3-(2,2-dibromoethenyl)-2,2-dimethylcyclopropanecarboxylate], and fenvalerate [cyano-(3-phenoxybenzyl) 4-chloro-α-(1-methylethyl)benzeneacetate] (Figure 1) are among the synthetic pyrethroid

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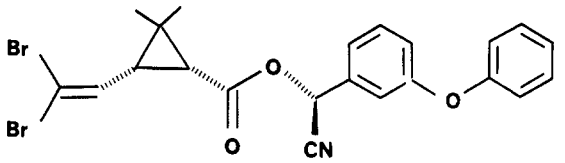
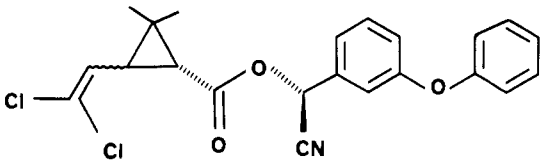
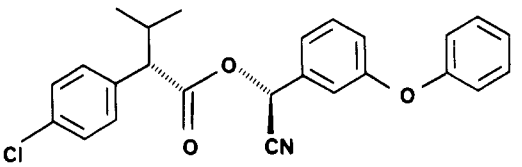


Deltamethrin



Cypermethrin



Fenvalerate

Figure 1. Structures of pyrethroids examined.

insecticides that are being used extensivily in Egypt for pest control on numerous field crops.

Persistence, metabolism, and distribution of synthetic pyrethroids in rats, mice, plants, and soil have been thoroughly investigated (Ruzo and Casida, 1977; Ruzo et al., 1978; Shono et al., 1979; Miyamoto, 1981; Kaufman et al., 1981; Marei et al., 1982; Cole et al., 1982). However, the literature is lacking information regarding the persistence and distribution of pyrethroid insecticides in laying chickens. We now report a comparative study on the persistence and distribution of cypermethrin, deltamethrin, and fenvalerate in chicken tissues and eggs following oral administration.

MATERIALS AND METHODS

Chemicals. Cypermethrin (cis–trans mixture) was provided by Shell Co. (Wadi El-Nile, Mohandiseen, Cairo, Egypt). Deltamethrin was provided by Roussel-Uclaf (El-Saraya El-Kobra, Cairo, Egypt). Fenvalerate was provided by Sumitomo Co. (Scientific Office, Abu El-Feda, Zamalek, Cairo, Egypt).

Treatment of Chickens. White leghorn (Dokki 4) laying hens weighing 1.45–1.68 kg were used in these studies. Each chicken was housed in a laying cage with food and water and maintained in a greenhouse at 24–35 °C. The dose was prepared by dissolving equal amounts of cypermethrin, deltamethrin, and fenvalerate in cottonseed oil to a final concentration of 15 mg/mL of each pyrethroid. Each chicken was weighed and treated with a single dose of the pyrethroid mixture (10 mg of each pyrethroid/kg) through a stomach tube through the mouth into the gizzard. Control chickens were treated only with pure cottonseed oil. After treatment, chickens were returned to their cages where they were maintained at 24–35 °C housing under extended daylight from 5 p.m. to 2 a.m. Three chickens were sacrificed by cervical dislocation after 1, 2, 3, 5, 7, and 14 days after treatments. Eggs were collected daily.

Extraction of Tissues, Organs, and Eggs. Fat, skin, meat, brains, liver, heart, kidney, ovary, and blood of sacrificed chickens were removed and stored frozen for subsequent analysis. Residue of the parent pyrethroids was extracted as follow.

Body fat and skin were separately extracted in hexane as described by Gaughan et al. (1978). Samples (20 g) were homogenized and extracted in five 30-mL portions of hexane. The combined hexane extract was dried over anhydrous sodium sulfate, concentrated by evaporation to a total volume of 20 mL, and extracted with acetonitrile (3 × 40 mL). The combined acetonitrile extract was dried over anhydrous sodium sulfate and evaporated to dryness; the residue was transferred to small vial with hexane and adjusted to a final volume of 3 mL.

Liver, heart, kidney, and meat (20 g each) were separately extracted in a mixture of hexane–acetone (1:2; 4 × 50 mL solvent). Extracts were washed several times with water to remove the acetone. The hexane layer was dried over anhydrous sodium sulfate and evaporated under nitrogen to a total volume of 3 mL.

Brains (2.3 g) were extracted in acetonitrile in the presence of anhydrous sodium sulfate as described by Marei et al. (1982). The extract was filtered and evaporated to dryness, the residue was transferred to small vials with hexane, and the total volume was adjusted to 3 mL in hexane.

Blood samples were extracted in hexane–acetone (1:2); the extracts were washed with water, dried over anhydrous sodium sulfate, and evaporated to final volume of 3 mL.

Eggs were analyzed by separating egg whites from egg yolks and extracted separately in acetonitrile as described by Hicks et al. (1972).

Cleanup of Extracts. Extracts were cleaned up by chromatography on silica gel columns covered with a layer of anhydrous sodium sulfate and packed with hexane. Pyrethroids were eluted in a mixture of hexane–acetone (9:1). A 100-mL portion of the eluent were collected and evaporated to a final volume of 1 mL (Goughan et al., 1978).

Gas Chromatographic Analysis. Gas chromatography was carried out on the Packard Model 427 chromatograph with a ^{63}Ni electron capture detector (ECD) and coiled glass column (200 cm × 3 cm i.d.) packed with 3% OV101 on 80–100-mesh DMCS-treated Chromosorb W. The column temperature was 240 °C; injector and detector temperatures were 280 and 300 ° C, respectively. Nitrogen was used as the carrier gas at 20 mL/min. An on-line integrator provided the retention time and area of each peak; 2 μL of each sample was analyzed twice with and without adding known amounts (100 ng/g of sample) of deltamethrin as an internal standard.

RESULTS AND DISCUSSION

Analytical Methods. The 3% OV101 packed column used for routine analysis for pyrethroids residue in chicken tissues and organs gave good resolution for the pyrethroids under investigation with a single well-resolved peak for each pyrethroid, except for fenvalerate which appeared as two partially resolved peaks (Figure 2). The linear electron capture detector used provided excellent proportionality of the amount of compound injected to the peak area over the entire range of peak areas involved in the present study. High recovery values (82–99%; Table I) are obtained on extraction of pyrethroids from chicken tissues and organs at fortification levels of 10 ppb, 50 ppb, and 1 ppm. Pyrethroid levels as low as 10 ppb can be detected and measured with no interference in the analytical region.

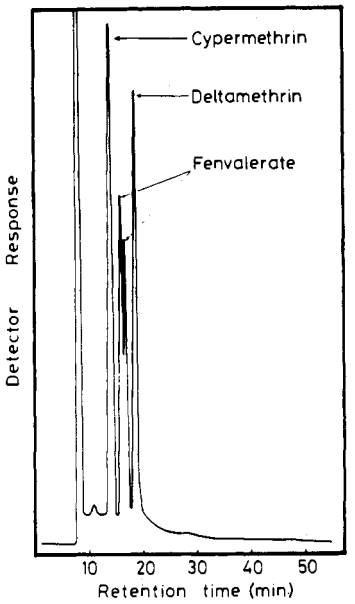


Figure 2. GLC analysis of pyrethroid mixture on OV-101 packed column. ECD relative response: cypermethrin, fenvalerate 1.01; deltamethrin, 1.25.

Pyrethroid Persistence and Distribution in Tissues and Organs following Oral Administration. Examination of tissues and organs of chickens following oral administration of pyrethroid mixture revealed that fenvalerate was the highest in concentration and most persistent in all of the examined samples. Cypermethrin was second, and deltamethrin was the lowest in concentration and the least persistent.

In all tissues and organs except blood, the level of the pyrethroid started out relatively low the following day after treatments and then increased gradually, reaching a maximum value 3–5 days after treatment. The pyrethroid level then sharply decreased for the following 9 days, except in the brains where the pyrethroid level continuously increased throughout the period of experiment (Figure 3).

Blood analysis revealed a high residue level of pyrethroid the first day following oral treatment, being as high as 3–4 ppm; the residue level then decreased sharply to a minimum value of 0.03–0.04 ppm at the end of the experiment (Figure 3).

Pyrethroid residue (parent compounds) in the brains was the highest among the examined tissues and started out at 1.1–1.5 ppm 24 h after treatments and continued to increase for the following 2 weeks, reaching values as high as 2.8–3.9 ppm.

Generally, brains showed the highest residue level (maximum at 2.8–3.9 ppm) followed by heart (maximum at 0.8–1.0 ppm), kidney (maximum at 0.5–1.0 ppm), fat and skin (maximum at 0.1–0.2 ppm), liver (0.10–0.18 ppm), and ovary (0.03–0.09 ppm), and in meat the level was below 0.01 ppm). In all of the analyzed tissues and organs fen-

Table I. Recovery Analyses of Pyrethroids from Chicken Tissues and Organs[a]

tissue, organ	recovery, % deltamethrin	cypermethrin	fenvalerate
fat	84 ± 12	97 ± 10	98 ± 3
skin	84 ± 14	97 ± 10	98 ± 5
brain	82 ± 10	99 ± 5	92 ± 10
heart	82 ± 11	82 ± 5	91 ± 8
kidney	90 ± 9	89 ± 8	91 ± 8
liver	82 ± 10	83 ± 12	82 ± 10
blood	82 ± 12	91 ± 7	93 ± 6
ovary	82 ± 10	89 ± 11	84 ± 10
egg white	88 ± 8	91 ± 10	89 ± 10
egg yolk	82 ± 12	88 ± 12	94 ± 6

[a] Average and standard deviation for triplicate analyses at fortification level of 0.010–1.0 ppm.

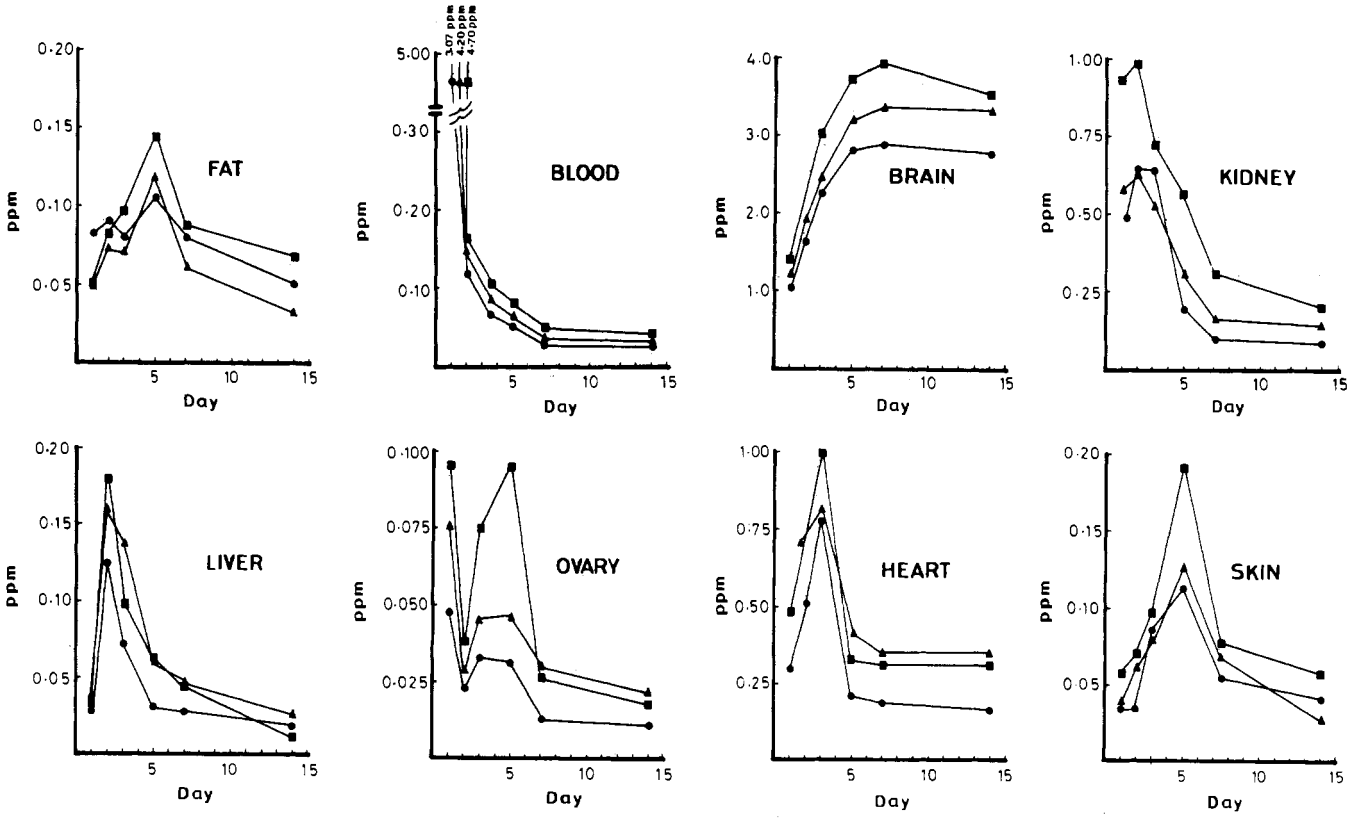


Figure 3. Pyrethroid levels in tissues and organs of chickens treated orally at 10 mg/kg (mean for three chickens with individual results averaging 10–15% deviation from the mean): ■, fenvalerate; ▲, cypermethrin; ●, deltamethrin.

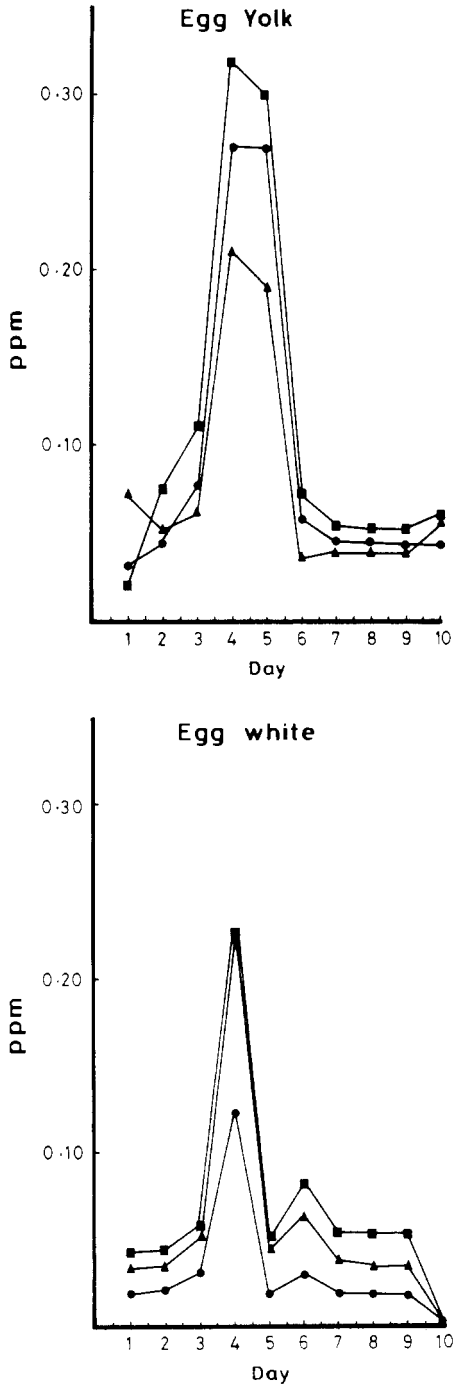


Figure 4. Pyrethroid levels in eggs (mean for three eggs): ■, fenvalerate; ▲, cypermethrin; ●, deltamethrin.

valerate was the highest in concentration followed by cypermethrin and deltamethrin.

Pyrethroid Levels and Distribution in Eggs. Eggs were collected daily following the oral administration of the pyrethroid mixtures. Egg whites and egg yolks were separated and analyzed separately as described in Materials and Methods. Residues in eggs were relatively low, averaging 0.05–0.55 ppm and peaking 4–5 days after dosing. The appearance of maximum residue levels in eggs after ca. 5 days has also been reported for dermal and oral administration of permethrin to laying hens (Hunt et al., 1979; Gaughan et al., 1978). Maximum residue levels of pyrethroids in egg yolks were 0.27, 0.21, and 0.32 for deltamethrin, cypermethrin, and fenvalerate, respectively. In egg whites maximum residue levels were 0.12, 0.23, and 0.23 for deltamethrin, cypermethrin, and fenvalerate. As in the other tissues and organs, fenvalerate was the highest in its residue in both egg whites and egg yolks and it was also the most persistent among the examined pyrethroids (Figure 4). Residues in egg yolks are higher than residues in egg whites. Since egg white (albumin) consists primarily of protein formed in the oviducal tissues (Gilbert, 1971), the low levels of residue in that fraction are understandable. The yolk substances, on the other hand, are formed in the liver and transported via the blood to the ovary, allowing potentially more contact with absorbed pyrethroids (Staiger et al., 1982).

Registry No. Cypermethrin, 52315-07-8; deltamethrin, 52918-63-5; fenvalerate, 51630-58-1.

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