Bull. Environ. Contam. Toxicol. (1990) 45:375-381

Bulletin of Environmental Contamination and Toxicology

Distribution and Elimination of 14C-Ethion in Laying Hens and Eggs after Oral Exposure

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The organophosphorus compound (OP), ETHION (0,0,0',0'-tetraethyl S,S'-methylene bis-(phosphorodithioate)) is used both as an insecticide and as an acaricide (Smith 1975). Domestic animals are usually exposed to ethion during dipping or spraying to remove ectoparasites. Poultry may be exposed to ethion if their feed is contaminated. Such exposures of poultry to pesticides have been documented by Keating (1977). Although OPs as a group are less persistent than organochlorines (Osweiler et al. 1985) the problem of residues may still exist if proper withdrawal periods are not observed after exposure to OPs. Thus poultry meat or eggs from hens exposed to ethion may be a source of ethion to man. There is therefore a need to ensure that proper withdrawal periods are followed before consuming poultry products from exposed birds. It was the purpose of the present investigation to study the distribution and elimination of ethion in poultry tissues and eggs after oral exposure.

MATERIALS AND METHODS

Ten Rhode Island Red laying hens, aged 24-26 weeks and weighing between 1.5-2.1 kg were used in the study. The birds were housed in two cages and were provided with water and feed ad libitum. The feed consisted of a commercial standard laying hens mash ("DLG Total 9" from Dansk Landbrugs Grovvareselskab) supplemented with oyster shells.

(14C-methylene)Ethion, with a specific activity of 2 μCi/mg was dissolved in glycerol formal and administered orally to each bird at the dose of 5 mg/kg. The dose of 5 mg/kg was chosen on the basis of pilot experiments where 10 mg/kg caused diarrhea in laying hens. After drug administration the birds were observed for signs of toxicity.

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Eggs were collected from the cages in the morning and afternoon. They were individually separated into egg-white and -yolk, each of which was liquidized by shaking with glass balls. Faeces were collected daily, oven-dried at 105°C for 24 hours and weighed; they were then ground into fine powder and mixed thoroughly.

Two hens were killed on each of days 1, 3, 7, 15 and 21 after dosing and liver, kidney, skeletal muscle (pectoral muscle), heart, brain and abdominal fat collected. Blood was collected from each of the two birds during killing and on days 5 and 10 from the other hens as pooled samples. All tissues and plasma separated from blood were stored at -20°C.

The concentration of unchanged ethion in egg-white, -yolk and plasma was measured by gas chromatography. Ethion was extracted from the plasma or egg-white by an equal volume of hexane containing parathion as internal standard. The hexane extract from each egg-white was concentrated five times. The hexane extracts were analysed on a Hewlett-Packard 5890A gas chromatograph with a nitrogen phosphorus detector (NPD). Chromatographic conditions were as follows: A Hewlett-Packard standard packed glass column 6'x2 mm internal diameter, packed with chromosorb W-HP 100/120 coated with 3% SE-30. Carrier gas: Helium with a flow rate of about 25 mL/min. Temperatures: Injector 240°C, column 220°C and detector 290°C. Injection volume 4 μL. Retention times: Parathion 2.2 min, ethion 4.9 min. Recovery rate for ethion in plasma was 94±5% and 90±2% in egg-white. The detection limit for ethion was 3 ppb.

Unchanged ethion was extracted from egg-yolk by a modification of the method described by Muan and Skaare (1986) for extraction of malathion from milk. The final hexane extract (1 mL) was not clean enough for gas chromatography. Impurities were removed by passing through a column with 2 g aluminium oxide (Brockmann) activated by heating at 800°C for 4 hours and partly deactivated by addition of 5% water (Holden and Marsden 1969). Recovery studies showed that 75 mL of hexane was required to elute the ethion from the column. The eluate was concentrated and then used directly for gas chromatography. The recovery rate of ethion from the egg-yolk was 69±4%.

The concentration of 14C-ethion (ethion and metabolites expressed as ethion equivalents) in tissues, plasma, egg-white, -yolk and faeces was determined by liquid scintillation counting (Gyrd-Hansen et al. 1984). The following modifications were made: Plasma

was counted in OptiPhase HiSafe (FSA Laboratory Supplies) scintillation cocktail. The faeces (20 mg) were prewetted with 100 µL water and required 48 h for complete dissolution with 1 mL Soluene-350 (Packard). To digest the yolk (400 µL), 2 mL Soluene-350 was used while 1 mL was used for the same amount of egg-white.

RESULTS AND DISCUSSION

The dose of 5 mg/kg did not produce any clinical signs of toxicity in the hens and egg-laying was not disturbed. This was expected in view of the results of the pilot experiments. The oral LD50 of ethion is 27 and 65 mg/kg in female and male rats, respectively (Gaines 1969).

Only traces of unchanged ethion were detected in plasma, egg-white and yolk. In plasma unchanged ethion (0.028 ppm) was detected on day 1 only, while in egg-white 0.001 ppm was detected on both days 1 and 2. In egg-yolk unchanged ethion was detectable on days 2 and 3 at 0.013 and 0.004 ppm, respectively.

Figure 1 shows the average concentration of 14C-ethion (parent compound + metabolites) in plasma, egg-white and -yolk. The concentration of 14C-ethion in egg-white on day 1 was about 4-5 times lower than in plasma. This was expected because the oviduct always contains sufficient water soluble proteins for about 2 eggs (Oades and Brown 1965). Since egg-white takes about 12-14 hours to form (Ionova and Zhecheva 1977) the 0.1 ppm ethion residues found in egg-white on day 1 originates from the part of the egg-white, which was secreted within the first few hours after ethion administration.

Regarding the delay between the appearance of the drug in plasma and in egg-yolk (fig. 1), Warren and Conrad (1939) showed that in laying hens the ova mature within 9-10 days. This time is measured from the time of increased growth rate of the ovum until the egg is laid. Using this time as reference it would be expected that if 14C-ethion was deposited in the maturing eggs immediately upon feeding, ethion-positive yolks should be obtained in a day or two and continue to be positive for 9-10 days after ethion had disappeared from the blood. Corresponding findings in laying hens have been recorded by Raica et al. (1956) after feeding tetracyclines, by Blom (1975) and Geertsma et al. (1987) using sulfonamides and by Ionova and Zhecheva (1977) after feeding erythromycin, tylosin and chloramphenicol.

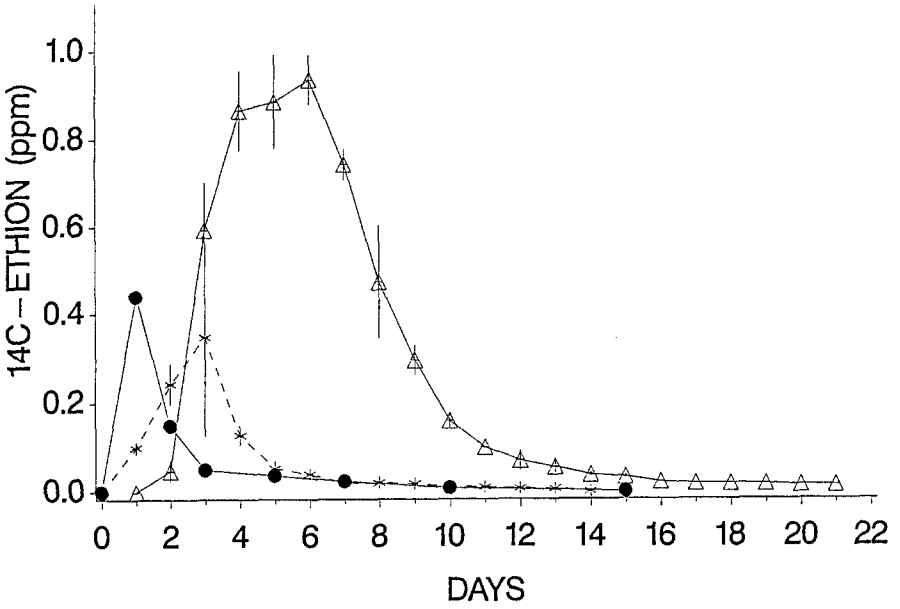

Figure 1. Average concentration of 14C-ethion in plasma, egg-white and -yolk. Plasma: ● ; egg-white: *; egg-yolk: △ . Egg-white and -yolk: day 1-15 n=4; day 16-21 n=2.

In the present experiment the egg-yolk was positive for ethion residues for about 20 days. This was expected in view of the 14C-ethion residues which were detected in plasma to the level of 0.01 ppm on day 10.

From day 5 onwards plasma and egg-white concentrations of 14C-ethion residues were equal indicating an equilibrium between the two compartments. In the egg-yolk the residue levels were higher than in either plasma or egg-white from day 3 onwards. The higher concentration in egg-yolk, which has high lipid content, is a probable consequence of the high lipid-solubility of ethion and possibly its lipophilic metabolites such as ethion monooxon and dioxon (Selim 1985a). This would agree with Blom (1975) that drug deposition in the egg-yolk is influenced by the lipid-solubility of the drug. Also, once a chemical compound has been deposited in the egg-yolk it stays there i.e. no exchange with blood takes place after the yolk material has been formed (Geertsma et al. 1987).

Table 1 shows the concentration of 14C-ethion residues in tissues of hens killed at various times after the oral administration of 14C-ethion. The liver and the kidney had the highest concentration on day one followed by heart and brain. Fat and skeletal muscle had the lowest levels. The low 14C-ethion concentrations

Table 1. Tissue concentrations of 14C-ethion (ethion and metabolites expressed as ppm ethion) in hens dosed with 5 mg/kg ethion orally.

DAY+	HEN NO.	PLASMA	LIVER	KIDNEY	SKELET. MUSCLE	FAT	HEART	BRAIN
1	1	0.39	2.3	2.02	0.08	0.17	0.30	0.30
	2	0.49	3.0	2.70	0.16	0.15	0.44	0.32
3	3	0.05	0.30	0.31	0.05	0.12	0.13	0.16
	4	0.05	0.37	0.42	0.05	0.07	0.17	0.14
7	5	0.03	0.13	0.13	0.06	0.04	0.11	0.08
	6	0.02	0.12	0.14	0.04	0.06	0.10	0.07
15	7	N.D.	0.08	0.06	0.06	0.09	0.08	0.07
	8	N.D.	0.08	0.06	0.04	0.08	0.07	0.06
21	9	N.D.	0.02	0.02	0.02	0.02	0.03	0.03
	10	N.D.	0.03	0.02	0.03	0.02	0.03	0.02

+: Day after drug administration; N.D.: Not detectable

seen in fat indicate that the distribution of 14C-ethion is governed by factors other than lipid-solubility. The distribution pattern agrees with that obtained by Bodden & Zietlow (1985) after administration of gelatin capsules containing 2 mg/kg b.wt. 14C-ethion to hens for 10 days. Similar results were obtained by Selim (1985b) in rats after oral administration. A corresponding, comparatively high rate of disappearance from the body tissues has been demonstrated with other OP compounds - e.g. sulfprofos in hens (Clark et al. 1979) and dioxathion and chlorpyrifos in cattle (Palmer et al. 1977) - and is ascribed to the relatively rapid metabolism and the efficient excretion of the metabolites (Smart 1987; Palmer et al. 1977).

The total amount of 14C-ethion excreted in faeces from the hens was 69% of the dose. Most of this (58%) was recovered within 24 hours. The amount excreted is lower than the total of 93% found in urine and faeces from rats within the first 7 days after similar exposure to ethion (Selim 1985b).

FAO/WHO 1985 has recommended for poultry meat and eggs an MRL (Maximum Residue Limit) of 0.2 ppm. The MRL includes the parent compound, all metabolites and drug-based products (FAO/WHO 1989). Based on the MRL for poultry meat and eggs and the total residue levels observed in the present investigation it would take 7

days for carcases and approximately 10 days for the eggs to attain levels lower than the MRL.

Acknowledgments

The study was sponsored by the Danish International Development Agency (DANIDA). Unlabelled ethion was generously supplied by Cheminova, Denmark.

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Received March 20, 1990; accepted May 21, 1990.