The Excretion of Activity Following the Administration of 1,1-di(4-chlorophenyl)-2-chloroethylene-ring-UL-^{14}C (^{14}C-DDMU) to Japanese Quail

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INTRODUCTION

DDT and to a lesser extent DDD were until recently used extensively in agriculture and horticulture as insecticides. The metabolism of DDT was first investigated in the rabbit (WHITE, 1945) but it was only in 1964 that a complete metabolic pathway was proposed for the conversion of DDT to DDA in the rat (PETERSON and ROBISON 1964). This involved the initial conversion of DDT to DDE or DDD and the subsequent conversion of DDD to DDA by a series of alternate dehydrohalogenation and hydrogenation stages with a final oxidation step to DDA.

DDE has attracted considerable attention recently because it is the principle DDT related residue found in the environment. It is known to be stable in many organisms, and in birds has been associated with eggshell thinning. (COOKE, 1973). DDE was not thought to be involved in the production of DDA from DDT, until DATTA (1970) proposed a pathway for the conversion of DDE to DDA in the rat in which the production of DDMU was the first step.

The metabolism of DDT has not been as extensively studied in birds as in mammals. ABOU-DONIA and MENZEL (1968) proposed a complete pathway based on the chromatographic isolation and spectrographic identification of ten metabolites after feeding DDT to 1-day old chicks. They reported that 4,4'-dichlorobenzophenone was the principal metabolite found when the White Leghorn chicks or fertile eggs were exposed to ^{14}C DDE. LAMBERTON *et al* (1975) did not detect the metabolite 4,4'-dichlorobenzophenone following the feeding of DDE to Japanese Quail indicating a different metabolic route to that of the chicks. BAILEY *et al* (1969) found no DDA or 4,4'-dichlorobenzophenone when any of the DDT metabolites proposed for the rat (PETERSON and ROBISON, 1964), were administered to pigeons. The sequence of transformations appeared to be similar as far as DDMU but the latter compound was not converted to DDMS (BAILEY *et al* 1972).

Abbreviations: DDT, 1,1-di(4-chlorophenyl)-2,2,2-trichloroethane; DDD, 1,1-di(4-chlorophenyl)-2,2-dichloroethane; DDA, di(4-chlorophenyl)acetic acid; DDE, 1,1-di(4-chlorophenyl)-2,2-dichloroethylene; DDMS, 1,1-di(4-chlorophenyl)-2-chloroethane.

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BUNYAN *et al* (1972) found DDE to be a more potent inducer of hepatic microsomal enzymes in rats and Japanese quail than DDT. Further studies showed that DDMU caused more elevation of hepatic microsomal protein, cytochrome P_{450}, aniline hydroxylase and N-ethyl morphine demethylase levels in the quail than DDE, although the latter gave rise to greater tissue residues. In the rat where tissue residue levels of DDE and DDMU were lower than those in the quail, DDE caused greater changes in hepatic microsomal enzyme levels than DDMU. In the quail changes caused by DDMU were larger than those observed following the ingestion of comparable levels of any other metabolite of DDT in the rat or the quail. From this evidence it has been suggested (BUNYAN and PAGE, 1973) that DDT may be metabolised differently in birds than in mammals. In the former DDT may give rise to a highly active liver inducer through a pathway involving DDMU. More recent work (STANLEY *et al*, in press) has demonstrated that effects, such as the elevation of the cytochrome P_{450} level in quail, caused by the ingestion of DDMU occur very rapidly and at a low dietary level.

It therefore appeared that an investigation of the rate and nature of the excretion products of DDMU in the Japanese quail could provide an insight into the mechanism by which DDMU causes induction of hepatic enzymes in birds. For this purpose DDMU (ring-UL-^{14}C) of high purity was prepared, and administered to Japanese quail. This publication reports on the studies to determine the rate of excretion.

EXPERIMENTAL

Materials DDT was prepared from technical DDT (Rohm Haas) by the method of WEST and CAMPBELL (1950) with final recrystallisation from ethyl alcohol to constant melting point 108-109°C (uncorr). Glc analysis gave only one peak. DDT (ring-UL-^{14}C) was obtained from the Radiochemical Centre, Amersham, Berks (England). The silica gel (60, F_{254} Merck) thin layer plates were supplied by BDH Chemicals Ltd. All other chemicals were of the highest grade commercially available.

Synthesis DDMU (ring-UL-^{14}C) was synthesised from DDT (ring-UL-^{14}C). The reaction involved the catalytic conversion of DDT (ring-UL-^{14}C) (500 mg, 30 µCi) to DDD (ring-UL-^{14}C) (ZIMMER and KLEIN, 1972). The conditions were modified by the addition of small amounts of the copper bronze catalyst during the reaction to ensure a high conversion of DDT (ring-UL-^{14}C) to DDD (ring-UL-^{14}C). The latter was purified using a silica gel column, (32 x 2 cm, 25 g, 100-200 mesh) activated at 120°C and deactivated with distilled water (5%) (HOLDEN and MARSDEN, 1969). The column effluent was monitored using the Beckmann Scintillation Counting System (LS 200), utilising a scintillant comprised of xylene and 2,5-diphenyloxazole (0.3%, w/w) which gave counting efficiencies of > 90%. Impurities were eluted with

hexane (100 ml) and the DDD (ring-UL-^{14}C) was then eluted with hexane : diethyl ether (90:10, v/v, 100 ml). the conversion to DDD (ring-UL-^{14}C) was 93%. only the fraction chromatographically pure by glc and tlc was used for conversion to DDMU (ring-UL-^{14}C).

Thin layer chromatograms were monitored radiologically using the Berthold Radiochromatogram Scanner (LB2732). Gas-liquid chromatographic (glc) separations were executed on a Pye Series 104 gas chromatograph equipped with an electron capture (ec) detector (^{63}Ni). The column (glass, 1.52 m long, 0.4 cm internal diameter) contained OV-101 (2%)/OV-210 (2%) on epicoated diatomite (100-120 mesh) and was used at an oven temperature of 175°C, detector temperature of 300°C and injection temperature of 190°C. The ec detector in its pulsed mode had a pulse space of 500 µs and an attenuation of 2.2×10^2.

Pure DDD (ring-UL-^{14}C) was dehydrohalogenated to DDMU with 0.35M alcoholic sodium hydroxide. (0.056 g NaOH, H_2O(4 ml), EtOH(40 ml)). The preparation which was chromatographically pure contained DDMU (ring-UL-^{14}C)(262.5 mg, 21.02 µCi) corresponding to a specific activity of 0.08 µCi/mg.

Bird experiments 3-week-old female Japanese quail *(Coturnix coturnix japonica)* from a highly inbred colony were individually caged over glass plates and maintained on the standard laboratory diet of turkey starter crumbs (Spillers Ltd) and water *ad libitum* at 21°C and 56% relative humidity. The light regime ensured that no eggs were layed.

Two 12-week-old female quail (1 and 2) were each injected intraperitoneally with DDMU (ring-UL-^{14}C)(49.97 mg, 4.39 µCi) in corn oil (0.51 ml). A further two quail (3 and 4) were dosed orally with DDMU (ring-UL-^{14}C)(44.42 mg, 4.36 µCi) in corn oil (0.25 ml) using gelatine capsules.

Excreta from each of the 4 quail was collected separately for each 24 h period and kept at -20°C before analysis.

Residue analysis After 15 days the quail were sacrificed and dissected. Aliquots of tissue were weighed and freeze dried. Excreta were air dried for 24 h at room temperature. Active residue levels in both faeces and tissues were measured using the plastic bag combustion technique developed by GUPTA (1968) as modified by LEWIS (1972).

Tissue residues of DDMU (ring-UL-^{14}C) were determined by glc in the usual way (BUNYAN and PAGE, 1973).

RESULTS AND DISCUSSION

During the 4 days following dosing, birds 1 and 2 excreted 9% (0.39 µCi) and 6% (0.28 µCi) respectively of the injected DDMU. During the same period birds 3 and 4 excreted 70% (2.54 µCi) and 78% (2.86 µCi) respectively of the ingested DDMU. Over the 15 day period birds 1 and 2 excreted 61% (2.67 µCi) and 37% (1.64 µCi) respectively, while birds 3 and 4 excreted 87% (3.22 µCi) and 88% (3.23 µCi) respectively (Figure 1). Hexane extraction of the excreta collected during the initial 4 days from birds 3 and 4 gave a solution which was shown to contain predominantly active DDMU by glc and tlc. LAMBERTON *et al* (1975) studied the excretion of activity, following the administration of ^{14}C-DDE by intubation to Japanese quail. They reported a similar excretion pattern and suggested that this represented the unabsorbed dose. The total activity excreted by each bird is consistent with the residues found in the tissues at the end of the experiment (Table 1). Previous work (BAILEY *et al* 1969) estimated a half-life for DDMU of 28 days in pigeons fed at 1000 ppm. The present work suggests a half-life of approximately 15 days in the quail given a single interperitoneal dose.

TABLE 1

Activity Present in the Tissues from the Quail Dosed with ^{14}C-DDMU

	Total Activity (nCi)			
Tissue	Bird 1	Bird 2	Bird 3	Bird 4
Pectoral muscle	8.0	20.0	0.6	2.0
Skeletal muscle	120.0	130.0	0.6	3.0
Liver	20.0	9.0	1.0	1.3
Gut	90.0	n.a	n.a	2.0
Lungs	2.0	n.a	n.a	1.5
Kidney	10.0	n.a	n.a	1.5
Heart	1.0	n.a	n.a	0.5
Brain	0.5	n.a	n.a	n.d
Oviduct	4.0	n.a	n.a	n.d
Feathers	240.0	380.0	n.a	7.0
Skin	110.0	190.0	7.0	30.0
Skeleton	n.d	n.d	n.d	n.d

n.a. = not analysed

n.d. = none detected

TABLE II

Residue Levels in the Liver and Pectoral Muscle of Quail Dosed with ^{14}C-DDMU

	DDMU Residue Level in the Liver		DDMU Residue Level in the Pectoral Muscle	
Quail	µg/g(dry wt)	µg/g(wet wt)[a]	µg/g(dry wt)	µg/g(wet wt)[a]
1	52.5	18.8	9.0	2.8
2	24.3	7.6	17.5	4.6
3	2.4	0.7	2.8	0.8
4	6.5	1.9	2.8	0.8

A compound with the retention time of DDE was found in the livers of quail 3 and 4. Calculated on a dry wt basis as DDE this represented 5.0 µg/g and 4.2 µg/g respectively.

[a] Residues calculated

Quail 1 and 2 were injected; 3 and 4 were orally dosed.

The injected birds (1 and 2) accumulated higher residues in all body tissues than those dosed orally (3 and 4). Examination of Table I shows that the total activity remaining in bird 1 at the end of the experiment (605 nCi) was approximately 12 times greater than that in bird 4 (49 nCi). The skeletal muscle of bird 2 had approximately 200 times the activity found in the same tissue of bird 3. Similarly, bird 1 had in the liver, 20 times the activity found in that of bird 3, although the measured DDMU residue only differed by a factor of 3 (Table II). One possible explanation of this is the presence of an active metabolite of DDMU in the more highly induced liver (Table III) of the injected bird. A significant amount of activity was found in the oviduct and brain of bird 1 but none was detected in the same tissues from bird 4. The highest level of activity was found in the growing feathers of bird 1 which contained approximately 5% of the injected dose. This was considerably more than the amount found in the feathers of bird 4.

TABLE III

Relative Liver Weights (RLW) for Quail Dosed with ^{14}C-DDMU

Quail	Body wt (g)	Liver wt (g)	Liver wt/100 g Body wt. RLW
1	115	5.6	4.8
2	145	4.8	3.3
3	130	4.6	3.5
4	120	3.7	3.0

Quail 1 and 2 were injected; 3 and 4 were orally dosed.

Control birds had a RLW of 2.5 ± 0.2

Whilst analysis (Table II) of liver indicated the presence of 2.4 and 6.5 ppm DDMU (dry wt) in birds 3 and 4 respectively, a residue of the retention time of DDE was also detected. None of this residue was found in the livers of the injected birds (1 and 2) nor in the pectoral muscles of any of the birds. BAILEY *et al* (1969) reported low levels of a similar residue in pigeons fed DDMU treated food although the residue detected in significant amounts was DDMU. The apparent DDE residues were found at tissue levels of < 0.5 ppm and were thought to have arisen from impurities in the DDMU. It would appear improbable that this residue is DDE.

Previous workers concerned with the metabolism of DDT and its metabolites have predominantly used the oral route for administration of these compounds. Treated food, single capsulated dose, intubation, or a combination of these techniques have been used so that it is difficult to compare previous results with the present study. STANLEY *et al* (in press) studied the time course of liver induction in quail fed DDMU at 100 ppm and found that the level of hepatic microsomal enzymes, such as cytochrome P_{450} reach a maximum after approximately 3 days. The relative liver weight (rlw) and liver lipid mobilisation also reach a maximum after 3 days, but it is suggested that the absolute maximum of xenobiotic metabolising capability is achieved after about 10 days on diet. This may explain the relatively slow rate of excretion of activity from the injected birds in the present study during

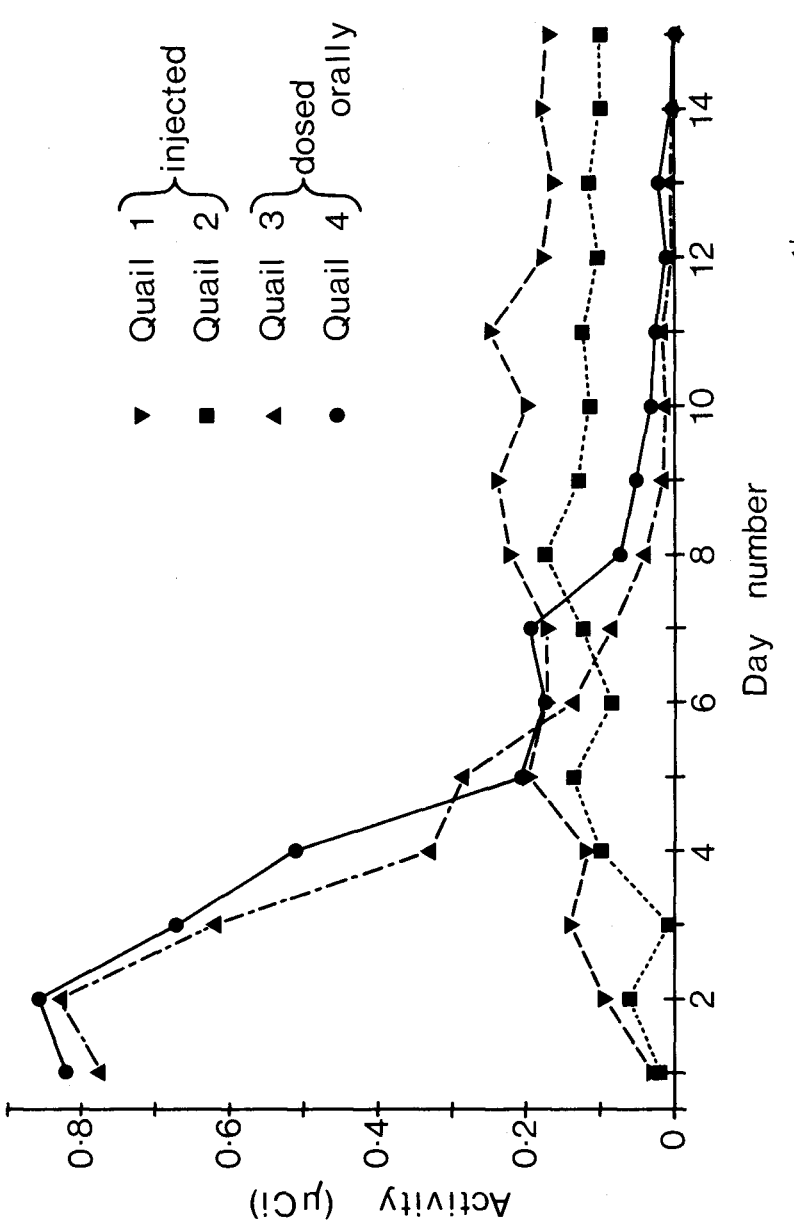

Figure 1. Activity Excreted by the Quail Dosed with ^{14}C-DDMU

the initial period. The excretion rate reached a maximum after 8 days (Figure 1) and then remained constant.

Detailed analysis of the excreta by chemical and radiological techniques is in progress including a search for possible conjugation products. The possibility of the presence of phenolic metabolites of DDMU is also being explored following the report by SUNDSTROM *et al* (1975) of a phenolic metabolite of DDE excreted by the rat, the seal and the guillemot.

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