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Excretion of Radioactivity Following the Intraperitoneal Administration of ^{14}C-DDT, ^{14}C-DDD, ^{14}C-DDE and ^{14}C-DDMU to the Rat and Japanese Quail

S. C. Fawcett,[1] P. J. Bunyan,[2] L. W. Huson,[1] L. J. King,[1] and P. I. Stanley[2]

[1]*Department of Biochemistry, University of Surrey, Guildford, Surrey GU2 5XH;*
[2]*Tolworth Laboratory, Ministry of Agriculture, Fisheries and Food, Tolworth, Surrey KT6 7NF, United Kingdom*

The recognition of the undesirable persistence of the organochlorine insecticide DDT and its metabolites in the environment has led to the phasing-out of its use in favour of less persistent alternatives. However considerable quantities of DDT are still used world-wide and residues are widely distributed through the environment. The principle environmental contaminant is the lipophilic metabolite DDE which accumulates in animal tissues and is considered to be responsible for eggshell thinning in predatory birds (COOKE 1973).

The metabolism of DDT in mammals has been extensively studied and a metabolic pathway in rats has been proposed (PETERSON & ROBISON 1964) which involves the initial reductive dechlorination of DDT to form DDD. The DDD is then dehydrochlorinated to DDMU which is converted through DDMS and a series of intermediates to DDA. The DDA appears to be largely excreted as conjugates with amino acids (JENSEN *et al.* 1957). DATTA (1970) suggested that in the rat DDT may also be metabolised through DDE to DDMU and then through a series of intermediates to DDA. The metabolism of DDT in other mammals appears to be similar to that in the rat with, for example, DDA being excreted by the hamster (GINGELL 1976), mouse (APPLE 1968) and dog (FINNEGAN 1949).

The metabolism of DDT in birds is less well understood. Pigeons appear not to excrete significant quantities of DDA (BAILEY *et al.* 1969a; SIDRA & WALKER 1980) but following intraperitioneal administration of ^{14}C–DDT to Japanese quail AHMED & WALKER (1979) reported that DDA was the major excretion product. The role of DDMU in the metabolic pathway for DDT in the pigeon has been questioned as BAILEY *et al.* (1972) reported that DDMU was not converted to DDMS. There is increasing evidence that the metabolic fate and the biochemical activity of DDE and DDMU is different in birds and mammals (BUNYAN *et al.* 1972; BUNYAN & PAGE 1973; DILLOWAY *et al.* 1981).

Abbreviations: ^{14}C-DDT, 1, 1-di(4-chlorophenyl)-2,2,2trichloroethane-ring-UL-^{14}C; ^{14}C-DDD, 1,1-di(4-chlorophenyl)-2,2-dichloroethane-ring-UL-^{14}C; ^{14}C-DDE,1,1-di (4-chlorophenyl)2,2-dichloroethylene-ring-UL-^{14}C;^{14}C-DDMU, 1,1-di(4-chlorophenyl)-2-chloroethylene-ring-UL^{14}C; DDA, di(4-chlorophenyl)acetic acid. DDMS, 1,1-di(4-chlorophenyl)-2-chloroethane.

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A major study is in progress to examine the metabolic fate of DDT in birds and mammals. The first phase of the study, which is reported here, has been to establish the rate of excretion of radioactivity following the intraperitioneal administration of ^{14}C–DDT, ^{14}C–DDE, ^{14}C–DDD and ^{14}C–DDMU to male rats and male Japanese quail. The proportion of the administered dose excreted as the unchanged compound has also been determined.

EXPERIMENTAL

Materials Pure DDT was prepared from technical DDT (ROHM HAAS) by the method of WEST & CAMPBELL (1950) with final recrystallization from ethanol to the constant melting point of 108–109°C. DDD was prepared from technical DDD (ROTHANE) by repeated recrystallization from methanol to the constant melting point of 109–110°C. DDMU and DDE were prepared from DDD and DDT respectively by the methods described by APPLE (1968) with final recrystallization from methanol to the constant melting points of 64 and 88°C respectively. DDT (ring-UL-^{14}C) was obtained from the Radiochemical Centre, Amersham, Bucks. DDD (ring-UL-^{14}C) was prepared by the method of FAWCETT *et al.* (1981). DDMU (ring-UL-^{14}C) was prepared by the method of BUNYAN *et al.* (1977). DDE (ring-UL-^{14}C) was synthesised from DDT (ring-UL-^{14}C) by chemical dehydrohalogenation in an alcoholic potassium hydroxide solution (2 g potassium hydroxide in 20 mL ethanol). The solution was stirred and allowed to reflux for 3 h. The DDE was then precipitated by pouring into cold water, extracted into diethyl ether and purified using preparative layer chromatography on silica gel G (type 60) plates (1 mm) run in chloroform:hexane (1:9).

The doses to be administered were prepared by mixing radiolabelled and unlabelled compound to give the required specific activity. The purity of the doses was checked using gas-liquid chromatography with electron capture detection and was found to be >99%. The contamination of the administered doses with DDE was found to be <0.5% for the DDT and <0.3% for the DDD and DDMU.

Silica gel G (type 60) (MERCK) was obtained from Anderman and Co., East Molesey, Surrey. Metapol HC 100 was purchased from Durham Chemical Distributors Ltd., Birtley, Tyne & Wear. Soluene 350 and Dimilume 30 were purchased from the Packard Instrument Co. Inc., Downers Grove, Illinois, U.S.A. Scintol S was purchased from Koch-Light Laboratories Ltd., Colnbrook, Bucks. All other chemicals were of the highest grade commercially available.

Animal Experiments Male TAS strain Wistar rats, 6–7 weeks old, weighing 170–240 g and male Japanese quail (*Coturnix coturnix japonica*) from a highly inbred colony, 4 weeks old and weighing 75–100 g were supplied by the Tolworth Laboratory, Ministry of Agriculture, Fisheries and Food.

Each of the radiolabelled compounds was dissolved in corn oil at 35°C and administered to 3 rats and 3 quail by intraperitioneal injection. The injected volume was 0.25–0.5 ml, the specific activity of the compounds dosed was 0.1–0.3 μCi/mg and the dose levels used were approximately 200 mg/kg body weight. The rats were individually housed in shielded metal metabolism cages and the quail were caged separately over glass plates. The animals were kept at 21°C and 65% relative

humidity and allowed food (41B powdered diet, Oxoid Ltd., for the rats and turkey starter crumbs. Spillers Ltd., for the quail) and water *ad libitum*. The urine and faeces from each rat and the excreta from each quail were collected daily and stored at -20° C.

Determiniation of excreted radioactivity A small volume (0.25 mL) of each urine sample, 1 mL of water and 12 mL scintillant (8.25 g PPO, 0.5 L Metapol HC 100 and 1L toluene) were added to a glass vial. The vials were counted in an NE 8310 Scintillation Counter (Nuclear Enterprises, Beenham, Reading) which gave efficiencies of 70–80%.

A representative 500 mg sample of each of the daily faeces collection was air dried at 45–50°C to constant weight. Two 20–40 mg samples were placed in glass vials and 100 µL water and 1 mL Soluene 350 were added to each. The digestion was allowed to proceed overnight at 50°C, the vials were cooled and 2 drops of hydrogen peroxide were added followed by 12 mL of Dimilume 30 scintillant. The vials were counted by the NE 8310 Scintillation Counter which gave efficiencies of 60–70%. Some 200 mg samples of dried faeces were also combusted using a Packard Tricarb Sample Oxidizer. The samples, in scintillant (60 mL Scintol S, 100 mL 2-methoxymethanol and 1 L toluene) were counted using a Packard Tricarb Scintillation Counter which gave efficiencies of 90–95%. The results indicated that there was good agreement between the radioactivity determined in the faeces by both the digestion and combustion method. This justified the routine use of the digestion method to estimate the radioactivity in the faeces.

RESULTS

The mean values from the three animals in each experimental group for the amount of radioactivity excreted daily are shown for the rat in Fig. 1 and for the Japanese quail in Fig. 2. The mean values for the rat represent the combined excretion in urine and faeces but the proportion excreted in the urine was low (the mean values for the animals dosed with DDT, DDE, DDD and DDMU were 1.7, 1.0, 6.3 and 2.9% of the dose excreted in 24,56,17 and 17 days respectively). The time taken to excrete 50% of the administered radioactivity (and the 95% interval estimate) has been calculated by linear interpolation (SNEDECOR & COCHRAN 1967) over that part of the curve covering the 50% point. The excretion of radioactivity by the Japanese quail following administration of ^{14}C–DDE was very slow and in this case the time taken to excrete 50% of the dose was calculated by extrapolation. The times taken to excrete 50% of the administered dose are shown in Table 1.

The faeces have been extracted with hexane and methanol and work is in progress to identify by mass spectrometry the compounds present in the extracts. Preliminary studies have established the proportion of the dose excreted as the unchanged compound (Table 2).

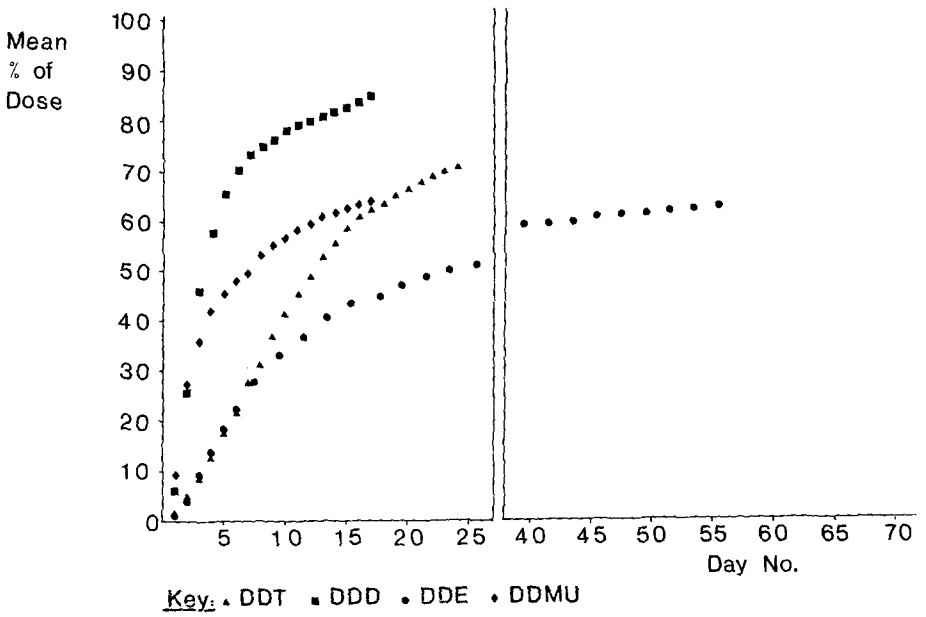

FIG.1. The excretion of radioactivity after I.P. dosing male Wistar rats with ^{14}C-DDT, ^{14}C-DDD, ^{14}C-DDE and ^{14}C-DDMU.

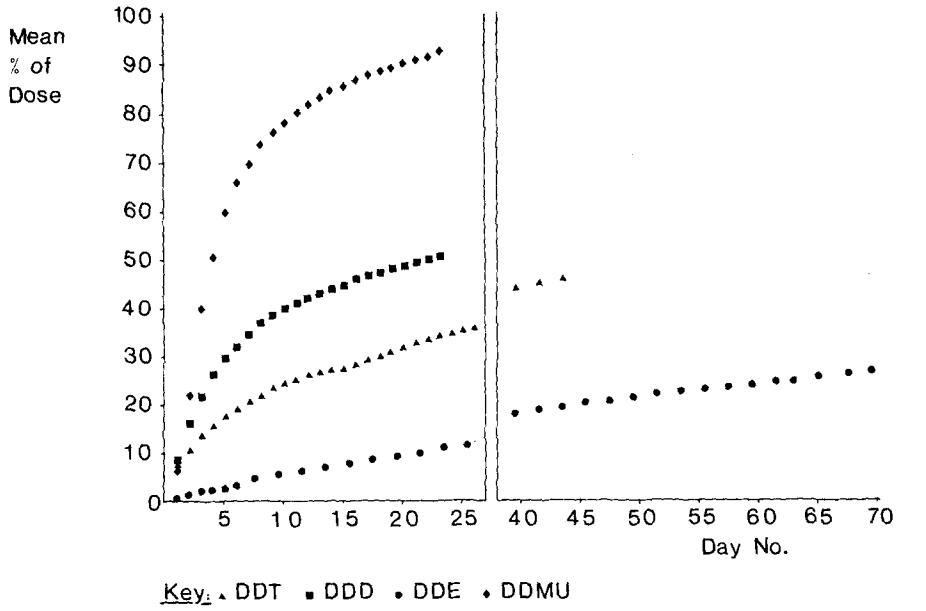

FIG.2. The excretion of radioactivity after I.P. dosing male Japanese quail with ^{14}C-DDT, ^{14}C-DDD, ^{14}C-DDE and ^{14}C-DDMU.

Table 1. Time (days) taken by rats and Japanese quail to excrete 50% of the radioactivity administered as ^{14}C–DDT, ^{14}C–DDD, ^{14}C–DDE and ^{14}C–DDMU.

Compound administered	Rat	Japanese quail
DDT	12.1 ± 0.7*	47.7 ± 3.3
DDD	3.4 ± 1.4	21.3 ± 2.1
DDE	23.7 ± 4.8	128.9 ± 8.5
DDMU	6.4 ± 2.6	4.3 ± 2.2

*95% interval estimate

Table 2. The percentage of the dose of DDT, DDD, DDE and DDMU recovered unchanged from the excreta collected during the time taken to excrete 50% of the administered radioactivity.

Compound administered	Rat	Japanese quail
DDT	0.4	11.8
DDD	1.0	2.5
DDE	5.2	34.8
DDMU	3.8	0.9

DISCUSSION

The results of the present study permit, for the first time, the strict comparison of the rates of excretion of DDT, DDD, DDE and DDMU administered intraperitoneally to rats and Japanese quail. The rat excreted the radioactivity administered as DDT, DDD and DDE substantially faster than the Japanese quail. This agrees with the results of other workers (AHMED & WALKER 1979; BAILEY *et al.* 1969a, b; BISHARA *et al.* 1972; GINGELL & WALLCAVE 1974; SEIBER 1976; SIDRA & WALKER 1980), which indicated that rats and other mammals tested were able to excrete DDT, administered orally or by injection, faster than the Japanese quail, pigeon, chicken and other birds examined.

The rat and Japanese quail excrete DDMU relatively rapidly and at a similar rate. This finding suggests that the apparent differences in the rates of excretion of DDT by birds and mammals probably arise from differences in the conversion of DDT to DDD and DDE or in the degradation of these metabolites to DDMU.

The Japanese quail differs from the rat in excreting substantial amounts of unchanged DDT, DDD and DDE. This probably reflects the inability of the Japanese

quail to readily metabolise these compounds. In particular, the long 'half-life' for DDE in the Japanese quail and the excretion during the 'half-life' of 35% of the dose as unchanged DDE indicates that DDE metabolism is very slow in this species. This slow degradation of DDE may be responsible for the high proportion of administered DDT recovered as DDE in tissues or excreta in experiments with the Japanese quail (AHMED & WALKER 1979) and the pigeon (SIDRA & WALKER 1980). The relatively slow elimination of DDE by birds compared with mammals is consistent with the accumulation of high residues of DDE in predatory birds. This has not been experienced to the same degree with predatory mammals.

One of the urgent problems facing research workers in this field is to identify the presently unknown route of metabolism of DDE in birds. The present study shows that most DDE excreted by the Japanese quail is eliminated as the parent compound and it is, therefore, not surprising that most workers have failed to identify any metabolites of DDE in birds although ABOU-DONIA & MENZEL (1968) reported 4, 4-dichlorobenzophenone in chicks exposed to ^{14}C–DDE. LAMBERTON *et al.* (1975) in a detailed study involving the administration of ^{14}C–DDE to Japanese quail could not find 4, 4-dichlorobenzophenone or any other metabolite in the tissues or excreta. SUNDSTROM *et al.* (1975) reported the excretion of phenolic metabolites of DDE by the rat, seal and guillemot but later work in the rat (SUNDSTROM 1977) indicated that the phenolic metabolites accounted for only a small proportion (5%) of the administered DDE.

The limited ability of the Japanese quail to metabolise DDE probably results from the fact that DDE is a less potent inducer of hepatic microsomal enzymes than DDMU in this species (BUNYAN & PAGE 1973). In contrast, DDE is a more active inducer in the rat which has a greater ability to metabolise DDE than the Japanese quail (BUNYAN & PAGE 1973).

The mechanism of excretion of the lipophilic compounds DDT, DDD and DDE unchanged by the rat and Japanese quail has not been established. JENSEN *et al.* (1957) identified free DDE and DDT in the bile of rats dosed intraveneously with DDT and excretion in the bile could explain the presence of these compounds in the faeces. It has been noted in monkeys that highly lipophilic compounds like hexachlorobenzene can be transported in the lymph and by diffusion from the lymph into the gut may occur in the faeces (MULLER *et al.* 1978). It is not known whether a similar mechanism operates in birds.

Further work is in progress to identify the radioactive compounds present in the tissues and excreta of the rats and Japanese quail following administration of ^{14}C–DDT, ^{14}C–DDD, ^{14}C–DDE and ^{14}C–DDMU.

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