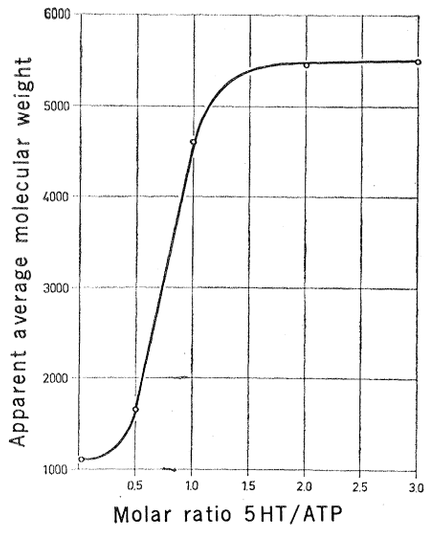

Fig. 2. Dependence of apparent molecular weight of mixtures of 5HT oxalate and ATP on their molar ratio at constant total concentration of the two solutes (10 percent by weight).

The solubility of 5HT is substantially increased, because that of 5HT oxalate used for the present experiments amounts only to about 6 percent.

The dependence of the apparent molecular weights of mixtures of 5HT and ATP on their molar ratio is presented in Fig. 2, the total concentration of the two solutes added together being 10 percent by weight. Evidently micelle formation is favored at molar ratios above 0.5 and reaches a plateau at a ratio of about 2 and more. Ratios higher than about 3 cannot be realized because of limited solubility of 5HT.

Micelles are also formed by constituents of the 5HT organelles (Fig. 1, curve 4), apparent molecular weights being even higher than those obtained with artificial mixtures of 2 moles of 5HT and 1 mole of ATP (Fig. 1, curve 3). Again a very pronounced concentration dependence of the apparent molecular weights is obvious. The following findings give evidence that these micelles are mainly composed of 5HT and ATP in a molar ratio of about 2. (i) 5HT and ATP are major constituents of the 5HT organelles, their molar ratio being about 2 to 3 (*7*). (ii) Artificial mixtures of 5HT and ATP in a molar ratio of 2 to 1 show very pronounced micelle formation (Fig. 1, curve 3), the concentration dependence of which is quite similar to that in the storage organelles of 5HT (Fig. 1, curve 4). Extrapolation of curve 4 in Fig. 1 to the concentration times 0 yields an apparent molecular weight of about 800 which is similar to that of a monomer consisting of one molecule of ATP and two molecules of 5HT (molecular weight, 859). (iii) The ultraviolet spectrum of the content of 5HT organelles corresponds to that of a mixture of 5HT and ATP in a molar ratio of about 2 (*13*).

The higher apparent molecular weights found with biological material as compared with those of artificial mixtures (compare curves 3 and 4 of Fig. 1) may be due to additional stabilizing factors. An influence of ions present and of temperature on micelle weight has been found in preliminary experiments.

Our results suggest that in the 5HT storage organelles in vivo, 5HT is fixed together with ATP in micelles. The micelle weights in the organelles may be very high since extrapolation of curve 4 in Fig. 2 to a concentration in vivo of about 45 percent 5HT plus ATP yields apparent molecular weights of several millions. Thus, micelle formation may explain the osmotic stability of the organelles. Micelles may be formed by vertical stacking of units of one ATP plus two to three 5HT molecules.

Micelle formation may be a general principle for the storage of compounds which form salts or complexes of mixed hydrophilic-hydrophobic character, such as aromatic monoamines plus ATP. We have evidence for the aggregation of catecholamines and ATP in the contents of granules of bovine adrenal medulla.

K. H. Berneis
M. Da Prada
A. Pletscher

F. Hoffmann-La Roche & Co. Ltd., Basle, Switzerland

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Degradation and Disappearance of ortho, para Isomer of Technical DDT in Living and Dead Avian Tissues

Abstract. *The* o,p′*-DDT in technical DDT is broken down to* p,p′*-DDT and then to 1,1-dichloro-2,2-*bis(p-*chlorophenyl)ethylene in living avian tissue. In the anaerobic conditions existing after death,* o,p′*-DDT is metabolized to 1,1-dichloro-2-*(o-*chlorophenyl)-2-*(p-*chlorophenyl)ethane. The absence of* o,p′*-DDT and metabolites in field specimens is ascribed to the rapid rate of breakdown and a masking of the 1,1-dichloro-2-*(o-*chlorophenyl)-2-*(p-*chlorophenyl)ethane residue during analysis by the relatively large amounts of 1,1-dichloro-2,2-*bis(p-*chlorophenyl)-ethylene.*

In Britain a nationwide survey of organochlorine residues in wild birds since 1961 was conducted (*1–3*) and showed the presence of compounds derived from DDT in the majority of samples analyzed. Of these compounds the one most commonly found has been *p,p′*-DDE (*4*). This compound was found in 816 (97.1 percent) out of 840 predatory bird samples (livers and eggs) examined (*2*). The other commonly occurring compounds were *p,p′*-DDT (27.0 percent) and *p,p′*-DDD (27.0 percent). The residue of *p,p′*-DDT would result from consuming prey which had recently fed on food contaminated with technical DDT [*p,p′*-DDT forms 65 to 73 percent of technical DDT (*5*)]. The *p,p′*-DDE is the main metabolite produced on degradation of *p-p′*-DDT in the living avian body (*6, 7*). A small percentage [0.17 to 4.0 percent (*5*)] of *p,p′*-DDD occurs in technical DDT, and it is also used in small quantities as an insecticide (Rhothane). However, the most

likely source of the majority of this material is the breakdown of *p,p'*-DDT after death (*7*).

The main constituent of technical DDT besides *p,p'*-DDT is *o,p'*-DDT. Although this material makes up 19 to 21 percent (*5*) of all the technical DDT used, it has only been found in six samples out of over 1000 analyzed for the Nature Conservancy and then only at a maximum concentration of 0.2 part per million (ppm). Because *o,p'*-DDT has an estrogenic effect on birds (*8*) the reason for its nonappearance in wildlife samples was studied. Klein *et al.* (*9*) found evidence for the isomeric conversion of *o,p'*-DDT to *p,p'*-DDT in the living rat, and Ecobichon and Saschenbrecker (*6*) suggest that a similar pathway occurs in birds. We examined this breakdown pathway in several living avian tissues and studied the rate of breakdown and whether a different pathway occurs after death.

To study the degradation of *o,p'*-DDT in living birds, seven homing pigeons (*Columba livia*) were each fed a single dose of 250 mg of *o,p'*-DDT in a gelatin capsule. Food was withheld overnight and for 2 hours after administration of the dose in an attempt to prevent vomiting. However, all birds vomited and in most cases stopped feeding. Although the health of the birds deteriorated after receiving the dose, their condition had returned to normal by 72 hours. The birds were killed by cervical dislocation at intervals of 2, 4, 24, 48, 72, 120, and 240 hours after the treatment and were dissected immediately after death. Samples of liver (1.5 g), fat (1 g), and breast muscle (2 g) were ground to a dry granular mix with sand and anhydrous sodium sulfate and extracted immediately with 200 ml of distilled *n*-hexane. The hexane extract was then filtered through 10 g of anhydrous sodium sulfate and evaporated to 10 ml (water bath, 70°C). The filtrate was used for analysis without further purification. Specimens were analyzed with gas-liquid chromatography (*10*), and two columns with different retention times (*11*) were used for more certain identification of the DDT isomers present. As a second test of identification, all residues were confirmed by thin-layer chromatography (*12*). Standard solutions of pure *o,p'*-DDT were analyzed by gas-liquid chromatography and found to be quite stable.

Table 1. The liver, breast muscle, and fat contents of seven pigeons at various intervals after dosage of 250 mg of *o,p'*-DDT. No metabolites were found in liver. Residues measured in parts per million (wet weight); ND, none detected.

Hours after dosage	Liver	Breast muscle			Fat		
	o,p'-DDT (ppm)	*o,p'*-DDT (ppm)	*p,p'*-DDT (ppm)	*p,p'*-DDE (ppm)	*o,p'*-DDT (ppm)	*p,p'*-DDT (ppm)	*p,p'*-DDE (ppm)
2	3.10	5.80	ND	ND			
4	4.21	4.39	ND	ND			
24	2.50	3.09	ND	ND			
48	2.00	5.00	0.91	0.22	50	ND	2.5
72	ND	2.49	.31	.20			
120	ND	2.00	.40	.20	74	ND	3.0
240	ND	ND	ND	ND	2.55	3.49	2.8

The residues found in the liver, fat, and breast muscle at increasing intervals after administration of the dose are given in Table 1. There was a gradual disappearance of *o,p'*-DDT in all three tissues. Disappearance was most rapid in the liver, where about half of the *o,p'*-DDT had gone after 48 hours, and there was no detectable residue 72 hours after dosage. In the breast muscle, *o,p'*-DDT was still present, though diminished, at 120 hours and in fat, at 240 hours after treatment. The complete absence of any metabolites in the liver is noteworthy, because this is the organ where metabolism and detoxication is most likely to take place. In the breast muscle no metabolites were found until 48 hours after treatment, although there was evidence of a diminution of the original *o,p'*-DDT content. This situation is very different from that occurring after the consumption of *p,p'*-DDT. When two pigeons were each fed a 100-mg single dose of *p,p'*-DDT and then killed 24 hours later, both showed the rapid metabolism of *p,p'*-DDT and the early appearance of its metabolites in the liver. The liver of one pigeon contained 6.07 ppm of *p,p'*-DDT plus 1.07 ppm of *p,p'*-DDE and the other contained 5.10 ppm of *p,p'*-DDT plus 1.18 ppm of *p,p'*-DDE.

The metabolites of *o,p'*-DDT, when they appeared in breast muscle and fat, were found to be *p,p'*-DDT and *p,p'*-DDE, thus confirming that the breakdown route in birds is similar to that in mammals (*9*). An isomeric conversion of *o,p'*-DDT to *p,p'*-DDT is followed by the normal breakdown of the latter to *p,p'*-DDE. It has also been shown that isomeric conversion can occur in tissues other than the fat stores (*9*).

We tested the possibility that *o,p'*-DDT would be metabolized more rapidly in the presence of *p,p'*-DDT, as would be the case when an animal consumed technical DDT. A pigeon was fed 200 mg of *p,p'*-DDT plus 50 mg of *o,p'*-DDT (the ratio in technical DDT) and killed 24 hours later. The liver content (ppm) was *o,p'*-DDT, 1.04; *p,p'*-DDT, 6.57; and *p,p'*-DDE, 1.07. The amount of *o,p'*-DDT present after 24 hours suggests that its breakdown would not be more rapid when mixed with *p,p'*-DDT. Neither this bird nor the two birds fed *p,p'*-DDT contained *p,p'*-DDD in the liver when they were analyzed immediately after death. This confirms the findings of Jefferies and Walker (*7*) that this material originates in the liver mainly after death.

We studied the degradation after death and rate of disappearance of *o,p'*-DDT with the 2- and 4-hour birds from the in vivo experiment. Samples of liver were removed at intervals of 1, 2, 6, 24, and 48 hours after death, and the incision was closed after each sampling. Also two samples of breast muscle were removed from each bird, one at 0 (different from those reported in Table 1) and the other at 48 hours after death. The bodies were maintained at 22°C during the sampling period.

There was a very rapid decrease in the amount of *o,p'*-DDT in the liver starting immediately after death (Table 2). Breakdown in the breast muscle was much slower. The breakdown product formed was found to be *o,p'*-DDD, which increased in amount as the *o,p'*-DDT decreased and, unlike the metabolites in vivo, was found in the liver as well as the breast muscle. The production of a metabolite within an hour

Table 2. The liver and breast muscle contents of the birds killed at 2 hours (bird 1) and 4 hours (bird 2) after dosing with *o,p'*-DDT (see Table 1), measured at increasing intervals after death. Residues measured in parts per million (wet weight).

Hours after death	Bird 1		Bird 2	
	o,p'-DDT	*o,p'*-DDD	*o,p'*-DDT	*o,p'*-DDD
	Liver			
0	3.10	0	4.21	0
1	2.77	.32	3.96	.31
2	2.65	.57	3.70	.55
6	1.45	.89		
24	0.88	1.75	2.52	3.04
48	.56	2.47	1.12	3.58
	Breast muscle			
0	5.87	0	4.17	0
48	3.40	1.1	2.93	1.0

of death and the loss of over half the *o,p'*-DDT content from the liver of the 2-hour bird in 6 hours shows that a more rapid breakdown occurs after death than in vivo (Table 1). However the exponential form of the curve suggests that a small percentage of the *o,p'*-DDT may remain in the liver for some days after death.

Because breakdown of *o,p'*-DDT to *o,p'*-DDD has not been recorded before and both materials have identical retention times on both of the columns used, a second confirmatory technique [dehydrochlorination of *o,p'*-DDD to its olefin *o,p'*-DDMU (*4*)] was used in addition to thin-layer chromatography. A portion of the liver test solution was reduced to zero volume and 10 ml of 2.5 percent alcoholic potassium hydroxide was added. The mixture was allowed to stand for 4 hours at 37°C and was then shaken with 50 ml of distilled water and 5 ml of *n*-hexane. The hexane was used for analysis in gas-liquid chromatography. This was repeated separately with test solutions of commercially prepared *o,p'*-DDD and *o,p'*-DDT to test the peak positions. Saponification of pure *o,p'*-DDD produced only one peak, that of *o,p'*-DDMU, and pure *o,p'*-DDT was changed to *o,p'*-DDE. Peaks corresponding to *o,p'*-DDMU and *o,p'*-DDE were produced by saponification of the test liver solution, thus proving the original constituents to be *o,p'*-DDT and *o,p'*-DDD rather than *p,p'*-DDE. If the metabolite had been *p,p'*-DDE there would have been no change upon saponification.

The question of whether this breakdown after death depends on anaerobic conditions, as in *p,p'*-DDT (*7*), was tested in the following manner. A pigeon liver was divided into four 2.5-g portions, and each was finely sliced and placed in a flask containing 25 ml of buffer (*p*H 7.4; NaOH and NaH_2PO_4). The *o,p'*-DDT (100 μg) was added to two flasks and then oxygen was passed over the liquid surface of one and nitrogen over the surface of the other for 24 hours at 22°C. The other two flasks received the same treatment but with *p,p'*-DDT added. Samples (5 ml) were removed from each flask at the start and after periods of 2, 8, and 24 hours of incubation. These samples were then analyzed to determine the content of DDT and DDD. The aerobic samples showed no change to DDD with either *o,p'*-DDT or *p,p'*-DDT. The anaerobic samples showed an increasing amount of DDD with time. At 0 hour, there was no DDD present and at 2, 8, and 24 hours of incubation the ratios of DDD to DDT (in micrograms) were 0.007, 0.16, and 0.54 with *o-p'*-DDT and 0.01, 0.21, and 0.83 with *p,p'*-DDT, respectively. Thus the anaerobic conditions which exist in a carcass are necessary for the postmortem breakdown of *o,p'*-DDT as well as that of *p,p'*-DDT.

During life *o,p'*-DDT is broken down by isomeric conversion to *p,p'*-DDT and then to *p,p'*-DDE. After death there is no evidence of isomeric conversion, and the *o,p'*-DDT remaining is broken down by reductive dechlorination to *o,p'*-DDD. No further metabolites were found. The reasons for the lack of evidence of *o,p'*-DDT in field specimens of avian liver are now clear. (i) The *o,p'*-DDT fraction is only one-fifth of the total DDT consumption and so, even in optimum conditions, is only likely to be present in small amounts compared with the concentrations of *p,p'*-DDT and its metabolites. (ii) The rate of disappearance of *o,p'*-DDT and its conversion to *p,p'*-DDT after the time of original consumption is fairly rapid, and there is no detectable residue after 3 days. This means that it is unlikely to occur in monitored specimens, for in most instances birds obtaining sublethal doses of technical DDT would have lost the *o,p'*-DDT before analysis. (iii) After death the breakdown of *o,p'*-DDT is much more rapid than that occurring during life. (iv) The breakdown product formed after death is less likely to be found because, in the two columns usually employed in these tests, any residues of *o,p'*-DDD would be hidden (as the retention times are similar) by the much greater quantities of *p,p'*-DDE present from *p,p'*-DDT and *o,p'*-DDT breakdown during life. Our tests have shown that *p,p'*-DDE is present in undiminished quantities in avian liver 10 days after death.

These factors ensure that a bird dying of technical DDT poisoning after approximately 24 to 48 hours and then remaining in the field or laboratory for a further 2 to 3 days before analysis would be unlikely to show any *o,p'*-DDT content. The small amounts remaining would be untraceable because of the dilutions necessary to measure the very much greater peaks of the *p,p'*-isomers of DDT, DDE, and DDD.

M. C. French
D. J. Jefferies
Monks Wood Experimental Station, Nature Conservancy, Abbots Ripton, Huntingdonshire, England

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11. Two 76-cm glass columns were used, one packed with Diatomite CQ coated with Apiezon Epikote and the other with Diatomite CQ coated with silicone Epikote. Relative retention times of the DDT isomers, *o,p'*-DDMU, *o,p'*-DDE, *p,p'*-DDE, *o,p'*-DDD, *o,p'*-DDD, *p,p'*-DDT on these columns were respectively, 64, 76, 100, 100, 132, and 176 on the silicone, and 68, 84, 121, 121, 161, and 239 on the Apiezon with HEOD (dieldrin) as the standard at 100.
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