from about 60 to about 40 percent. These findings show that, even in the desert lizard *Sauromalus*, cutaneous evaporation is a major avenue for water loss. This is contrary to the common belief that reptilian skin is essentially impermeable to water.

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Residues of DDT in Brains and Bodies of Birds That Died on Dosage and in Survivors

Abstract. *Residues of 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)-ethane (DDT) and 1,1-dichloro-2,2-bis(*p*-chlorophenyl)-ethane (DDD) in brains of cowbirds* (Molothrus ater) *killed by dietary dosage of DDT were similar in birds that died after various lengths of time on dosage and in birds that died of delayed effects after as much as 40 days on clean food. Residues of DDT and DDD, but not of 1,1-dichloro-2,2-bis-(*p*-chlorophenyl)-ethylene (DDE), were much lower in survivors 112 days after dosage. The relative importance of DDT and DDD in brains could not be determined, but DDE appeared not to be critical. Residues in brains of cowbirds were similar to those reported for robins, sparrows, eagles, and white rats. Residues in livers and carcass remainders (with the possible exception of DDD in the liver) appeared unsuitable for diagnosing the cause of death.*

Probabilities that deaths of birds in the field are due to pesticides are difficult to establish because neither times of exposure nor dosages are known. For residues to be most useful in diagnosing the cause of death, levels in dead animals should be similar regardless of either dosage or time on dosage before death. This has been true of residues of DDT found in brains of sparrows (*Passer domesticus*) studied experimentally (*1*) and in laboratory rats (*Rattus norvegicus*) (*2, 3*). Correlative evidence has appeared in data on residues in robins (*Turdus migratorius*) collected tremoring or dead after DDT treatments for the control of Dutch elm disease (*1, 4*). However, little attention has been given to the relations between the metabolites involved, to the residues present in acute versus delayed mortality, or to comparisons between residues in the brain and in other portions of the body.

These relations are shown here for a group of experimental cowbirds that had been fed DDT in their diet. Comparisons are made of residues in birds dying at various times during dietary dosage, in those dying after dosage, and in the survivors. General validity of the conclusions is discussed by comparing various other species for which data could be obtained.

In our study, 27 cowbirds were fed a diet containing 500 parts per million (ppm) of *p,p'*-DDT. The DDT was dissolved in cottonseed oil and mixed into the diet of turkey-starter crumbles. Thirteen birds in one cage were fed toxicant until all had died, a period of 12 days. In another cage, 14 birds were fed toxicant for 8 days, at which time seven had died. The seven remaining birds were given clean food for the duration of the experiment. Four of the seven died after 2, 9, 40, or 93 days on clean diet; three survived for 112 days and were killed. Severe tremoring, typical of DDT poisoning, was observed in four of the birds that died while on the toxic diet and in the one bird that was dead after 9 days on clean food. Deaths of the other birds were not observed.

Autopsy of all birds showed conditions common to cowbirds succumbing to DDT poisoning: Fat was essentially gone from the visible storage sites; there was no muscular emaciation (pectorals and other muscles were full and firm); and the gall bladder was full.

Brains, livers, and remainders (remaining parts of the specimen exclusive of the digestive tract, which was dissected out and discarded) of 17 male cowbirds were analyzed (*5*) for DDT residues—11 individually, the remaining 6 as a pool.

DDT and DDD levels in the brains of dead cowbirds showed no time-related trends. They were similar in birds that died after varying times on dosage and in those that died after 2 to 40 days on clean food. DDT plus DDD averaged 66 ± 6 ppm wet weight, with a range of 35 to 99 ppm. The effects of DDT cannot be separated from those of DDD, for brain levels of the two were similar and both were always present. Brains of birds that died 9 and 40 days after dosage contained more DDE than the brains of birds that died during dosage did. Brains of survivors, killed after 112 days, contained more DDE than most birds that died on dosage. Hence, DDE appears not to be critical in this series. Brains of birds that died contained less DDE than either DDD or DDT, a relation that was reversed in survivors. It is clear that more and more DDE was being produced from residues elsewhere in the body.

Quantities of DDT residues in brains of cowbirds were remarkably similar to those reported for a variety of other species, when expressed comparably and with allowance for differences in chemical methodology. In Table 1, data for the cowbirds in this study, robins (*1, 4, 6*), sparrows (*1*), eagles (*Haliaetus leucocephalus*) (*1*), white rats (*2, 3*) are compared. It appears that similar brain levels of DDT and DDD combined may be associated with DDT-induced mortality over a wide range of species, mammals as well as birds.

Despite these similarities, the residue levels in the brains of individuals that died from DDT poisoning varied widely (Table 1). Thus we cannot set a single level that is positively diagnostic of death. It is of practical value, however, to recognize the range within which lethal levels fall. When a level falls within this range, it seems entirely fair to say that the animal was at least seriously endangered by the poisoning. The probability that an animal was killed by DDT obviously is far greater at high residue levels than at low ones.

The critical question is how low a level may be taken to represent serious danger and possible death from DDT. From all available data, including

Table 1. Residues in brains of animals killed by DDT.

Species	Treatment	DDT + DDD (ppm wet weight)			DDT + DDD (ppm lipid weight)	
		No. of specimens	Mean	Range	Mean	Range
Male cowbirds*	Dietary dosage (500 ppm)	17	66 ± 6	35 to 99	887 ± 82	476 to 1306
Male white rats†	Single oral dose (150 mg/kg)	4		35 to 52	737 ± 73	524 to 848
Bald eagles‡	Dietary dosage	5	74 ± 6	58 to 86		
Tremoring male robins§	Dutch elm disease control, Wis.	11	43 ± 13	17 to 68	665 ± 73	218 to 1149
Tremoring male robins‖	Dutch elm disease control, N.H.	13	58 ± 11	31 to 188	1251 ± 323	334 to 4910
Tremoring male robins¶	Dutch elm disease control, Mich.	11	92 ± 10	62 to 173		
Robins¶	Dietary dosage	8	82 ± 6	61 to 116		
House sparrows¶	Dietary dosage (100 to 300 ppm)	20	109 ± 8	56 to 181		

* Present study; electron capture gas chromatography. † Studies cited in (*2*) and (*3*); modified Schechter-Haller method (*12*), presumably measuring DDT + DDD. ‡ Patuxent Center, unpublished data, (*7*); Schechter-Haller method (*13*), read at wavelength 596–600, presumably measuring DDT, DDD, and an unknown amount of other substances. DDE, however, is read at 540 and thus does not enter into colorimetric readings used here. § Study cited in (*6*); electron capture gas chromatography. ‖ Study cited in (*4*); electron capture gas chromatography. ¶ Study cited in (*1*); Schechter-Haller method (*13*) read at wavelength 596–600, presumably measuring DDT, DDD, and an unknown amount of other substances.

some not presented here, we believe that 30 ppm of DDT and DDD combined in the brain is a useful approximation of the lower limit. Deaths with tremors do occur when brain residues are below 30 ppm, but they are so rare that only two are represented in data given here; some, at least, appear to represent complicating factors (*8*).

The necessity for caution in interpretation is emphasized by the fact that animals in weakened condition may succumb with lower brain concentrations than otherwise would be expected. For example, residues in two male rats that died after a dietary dose of 200 ppm of DDT for 3 months, followed by up to 10 days of starvation, were 205 and 905 ppm (lipid base), whereas eight survivors of the same program contained 90 to 236 ppm, with an average of 164 ppm; thus there was an overlap of concentrations between those that died and those that lived (*9*).

Residues in livers or remainders of dead birds appear to be less suitable than those in brains for interpreting cause of death. Both DDT and DDE tended to increase with time on dosage before death. In the liver, DDT increased both during dosage and up to 40 days after dosage, as if there were a continuous supply of toxicant, first dietary, and then from material stored in the body. DDT in the remainders followed a pattern consistent with this supposition, increasing during dosage but declining after dosage. DDT levels in livers of birds that survived for 112 days were very low; those in remainders were still high, but lower than in dead birds.

Throughout both the dosage period and the period after dosage, DDE increased in remainders of birds that died; it increased in livers during the period after dosage. Remainders of survivors had greater quantities of DDE than those of dead birds. Livers of survivors contained more DDE than those of many of the dead birds, but less than those of birds that died in the period after dosage.

DDD, however, did not show an upward trend in livers and remainders of birds in this series that died, which suggests its possible utility in diagnosis of death. In livers and remainders of another group of cowbirds, however, where more birds died after fewer days on dosage, upward trends of DDD with time were apparent (*7*). Despite this trend, quantities of DDD in livers were of a lower magnitude in the survivors than in the dead birds; hence

Table 2. Residues of DDT and metabolites in cowbirds killed by DDT and in survivors. Mean values for birds that died are based on 17 birds, including those analyzed in a pool of 6. Standard errors were computed from the 11 individual analyses only. Data for survivors are from individual analyses of three birds killed after 112 days on clean food.

Chemical	Residues in dead birds				Residues in survivors			
	ppm wet weight		ppm lipid weight		ppm wet weight		ppm lipid weight	
	Mean	Range	Mean	Range	Mean	Range	Mean	Range
				Brain				
DDD	37 ± 11	25 to 65	496 ± 48	241 to 862	3	2 to 3	38	30 to 50
DDT	29 ± 11	10 to 40	391 ± 47	135 to 556	1	0.3 to 2	15	4 to 28
DDE	5 ± 2	2 to 26	72 ± 26	24 to 326	6	5 to 8	92	65 to 121
				Liver				
DDD	406 ± 68	154 to 751	4972 ± 454	2838 to 7024	26	24 to 27	369	300 to 472
DDT	9 ± 8	1 to 92	98 ± 88	12 to 997	2	1.5 to 2.4	29	19 to 47
DDE	38 ± 20	12 to 247	472 ± 214	138 to 2658	40	34 to 47	574	497 to 664
				Remainders				
DDD	189 ± 16	79 to 240	4153 ± 506	1261 to 6851	18	10 to 23	185	85 to 250
DDT	245 ± 50	25 to 520	4902 ± 697	1822 to 9994	46	20 to 69	458	199 to 751
DDE	24 ± 5	6 to 68	622 ± 264	204 to 3338	102	85 to 123	1003	721 to 1223

the level of DDD in the liver might be useful as a diagnostic criterion.

Quantitative relations of metabolites differed among tissues as well as between dead and living birds (Table 2). In both living and dead birds, DDD residues reached their highest concentrations in the liver, and DDT, in the remainders, while DDE was at its highest concentration in the livers of dead birds and in the remainders of survivors.

It seems appropriate to add together the DDT and DDD residues, for both are commercial insecticides that are known to affect birds. Furthermore, DDD may be formed from DDT under widely varying natural conditions (*10*), including postmortem decomposition of animals (*11*). Since most animals are stored for weeks or years before being analyzed, it is possible that much of the DDT is converted to DDD after death.

The amount of DDT converted after death may vary considerably from organ to organ. For this reason, we cannot state that the concentration of metabolites found in different parts of the body (Table 2) reflects the condition in life. However, the differences are so great that they probably reflect life processes in large part. Certainly the avian liver converts a great part of the DDT to DDD, and it is logical to assume that this is not solely a postmortem effect.

DDE did not appear as a result of postmortem decomposition (*11*). This metabolite of DDT is formed rather slowly and is lost slowly; often it is found in large amounts in apparently healthy animals. Probably much of it is picked up from food organisms in which it has already been metabolized to DDE. Little is known about the effects of DDE in vertebrates, but this compound is not a commercial pesticide and is generally reported to be of low toxicity, a view that is in harmony with our findings.

The validity and usefulness of DDT residues in the brain for indicating death from DDT poisoning is strengthened by extension to additional species of birds, and particularly by the consistency of quantities in birds dying at various times after dosage; brain residue levels are seen to be similar in birds and certain mammals. Residues in livers and remainders appear to be more variable and time-related than those in brains and hence are of less value in diagnosing mortality. The concentration of DDE was low in dead specimens but was the highest metabolite in survivors. The relative importance of DDT and DDD was not apparent.

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Association between Potassium Concentration and Serological Type of Sheep Red Blood Cells

Abstract. *When red blood cells from 115 sheep were classified for the presence or absence of antigenic factor M and for high (as opposed to low) potassium concentration levels, the cells of the 22 M-negative sheep were low in potassium.*

Gene *M* is responsible for the presence of the M antigenic factor of sheep red cells and the corresponding M-positive phenotype; *mm* sheep have M-negative red cells (*1*). Gene Ka^L, for low concentration of red-cell potassium (low K), is dominant to its allele ka^h so that ka^hka^h sheep are of the high-potassium (high K) type (*2*).

Table 1. Classification of red cells from 115 sheep for antigenic factor M and potassium type (low or high).

M class	Low		High
	$Ka^L/?$	Ka^Lka^h	ka^hka^h
Negative	22	0	0
Positive	22	26	45

Table 2. Genotypes and phenotypes for the M red-cell factor and for red-cell potassium concentration in sheep of three breeds and their crosses.

M negative	M positive		
mm	M(m)*	*M(m)**	*M(M)**
Low K	Low K	Low K	High K
$Ka^L(Ka^L)$*	$Ka^L(ka^h)$*	Ka^Lka^h	ka^hka^h
	Cheviot		
3	2	3	3
	Scottish blackface		
3	6	3	7
	Welsh mountain		
0	4	2	6
	Cheviot × Scottish blackface		
3	4	9	7
	Cheviot × Welsh mountain		
10	4	3	6
	Scottish blackface × Welsh mountain		
3	2	6	16

* Predicted genotype

The red cells from 115 sheep of three breeds and their crosses were classified for the M factor with an ovine antiserum (isoimmune) in a hemolytic test (*1*) and for high or low-potassium type from estimation of the red-cell potassium concentration by flame photometry (*3*) (Table 1). With no prior information to indicate an association between M types and potassium types, four different phenotypes would be expected. However, all of the 22 M-negative bloods were from sheep of the low-potassium type; none was classified as M-negative, high potassium. Furthermore, all 26 sheep previously known by progeny tests to be heterozygous (Ka^Lka^h) for low potassium were M-positive, suggesting that all M-negative sheep are homozygous (Ka^LKa^L) for low potassium.

The association between the M-negative (*mm*) genotype and the apparently homozygous (Ka^LKa^L) low-potassium genotype suggests that the relation between the notation for the possible genotypes and the corresponding phenotypes is as follows:

Genotype	Phenotype
$Ka^LKa^L = mm$	Low K, M-negative
$Ka^Lka^h = Mm$	Low K, M-positive
$ka^hka^h = MM$	High K, M-positive