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METABOLISM AND DISTRIBUTION OF p,p′-DDT DURING FLIGHT OF THE WHITE-CROWNED SPARROW, ZONOTRICHIA LEUCOPHRYS

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Introduction

DDT, other chlorinated hydrocarbons, and many of their metabolites are highly lipophilic and accumulate in fatty tissue. Avian species have proven to be particularly susceptible to deleterious effects from DDT use. The dehalohalogenation product, DDE, has induced eggshell thinning which caused dramatic declines of many top level predators such as the bald eagle, peregrine falcon, and brown pelican. Despite this drawback, DDT effectively controls vector-borne diseases carriers. Therefore, its use has continued in many tropical regions.

Migratory birds can bioaccumulate DDT and other lipophilic compounds in their overwintering grounds in these tropical regions, especially prior to migration when their lipid reserves are greatest. Once migration begins, birds experience high energetic demand, which depletes lipid stores, thus mobilizing stored contaminants. Birds, which experience cycles of high and low lipid stores due to migration, breeding, or adverse weather conditions, are susceptible to large pulses of contaminants [1-4]. We were particularly interested in the effects of staging and migratory flight on redistribution of DDT in migratory birds. This issue is especially relevant given the use of DDT for vector control in many tropical and neo-tropical areas, where migratory birds stage for transcontinental migrations. Also, toxicity manifests if threshold concentrations of organochlorine insecticides are exceeded in the brain [5, 6]. Therefore, there is a need to understand the metabolism and disposition of DDT during a migratory flight.

Laboratory experiments have demonstrated the relationship between lipid mobilization and energetic stress in fasted birds [7-9]. However, little work evaluated the

role of more strenuous exercise, such as flight, and its ability to impact contaminant storage and metabolism. Fasting is a simple stressor to introduce in laboratory studies. However, it may not be truly representative of metabolism and contaminant partitioning during a migratory flight. Avian flight in wind tunnels provides a more accurate measure contaminant movement during strenuous exercise. However, training birds to fly in a wind tunnel is difficult and limits studies to low sample sizes [10].

Gender-specific corticosterone responses to identical stimuli were found in breeding free-living white-crowned sparrows based upon breeding status [11] and ambient temperatures [12]. Additionally, strong social hierarchies and elevated corticosterone levels may occur in males grouped together. White-crowned sparrows, like many passerines, are sexually monomorphic. Minor morphometric differences such as wing length exist [13], but overlap between males and females precludes reliable use of this criterion. This makes genetic evaluations necessary if it is desirable to conduct gender specific studies.

This study was part of a larger research effort to evaluate effects of physical stress on circulating corticosteroid, DDT, and DDT transformation product concentrations. The portion of the study presented here, examined the metabolism and movement of *p,p'*-DDT in fasting female white crown sparrows that were sedentary or undergoing simulated migratory flight. This study was intended evaluate the extent to which stressors influenced DDT transformation and distribution among tissues.

Materials and Methods

Reagents

Analytical standards included *p,p′*-DDT, *p,p′*-DDD, *p,p′*-DDE, *p,p′*-DDMU, *p,p′*-DDNU and *p,p'*-DDOH (Aldrich Chemical, Milwaukee, WI). All solvents used were of pesticide analysis grade (VWR Scientific, West Chester, PA), including n-hexane, acetone, dichloromethane, methanol, and diethyl ether (99%). Anhydrous sodium sulfate (granular, 60-100 mesh) and florisil (60-100 mesh) (VWR Scientific, West Chester, PA) were baked overnight at 400 and 150ºC, respectively. Florisil was deactivated with 1.2% deionized water before use.

Animals

White-crowned sparrows (*Zonotrichia leucophrys*) were collected near Lubbock, Texas, during the early winter. Birds were captured with mist nets, transported to an outdoor aviary, using procedures described previously [15].

The local Audubon Society lists this species as abundant to the Lubbock, TX area through the first week in May, tapering down to absent by June. Therefore, sparrows were exposed and flown acutely fasted, and euthanized in mid-May, the latter portion of their natural migratory window.

A chromo-helicase-DNA-binding gene (CHD) on the avian Z and W chromosomes was amplified with polymerase chain reaction (PCR) to identify the sex of sparrows [15]. In this way, we limited the study population to female sparrows.

Flight Training

An open circuit suction type wind tunnel was used for flight training. The tunnel included a 2m long x 1.25 m high x 1 m wide working/observation section, partitioned from the tunnel and fan using ½" nylon netting. Air velocity was maintained at 16 km/hr. The chamber floor and netting were lined with 18 gauge bare copper wires, set 5 mm apart and carrying 0.05 to 0.1 mA. The current was sufficient to discourage perching without causing undue stress [16-18]. Flight training was conducted in this system over a six week period beginning in February (See Supporting information).

Experimental Design

This study required assessment of contaminant distribution and corticosteroid modulation under different stress scenarios. Thus, the experimental design is somewhat complex (Figure 1) in an effort to properly assess both toxicant and steroid behavior in the same organism with appropriate controls. The sheer number of birds needed for all stress and control groups (n=208) and the need to conduct all treatments in a single nocturnal cycle presented significant logistical challenges and limited sample sizes to some extent.

After completion of flight training, sparrows ingested 5 mg *p, p′*-DDT per kg per day in 70 to 110 µL of corn oil for three consecutive days. This particular dosing regimen was use to simulate contaminant loading during hyperphagia, in preparation for migratory flight. Half of the birds (n=108) were given the DDT solution and the other half (n=108) received an equivalent amount of corn oil (Figure 1). After dosing, all

birds rested for 1 day. On the next day (fifth day) birds were divided into three groups: unstressed (UNS), fasted only (FO), or fasted and flown (FF).

Test sparrows were transported to the wind tunnel facility and divided into three groups. The first group contained birds that were UNS and held in their cages with food and water. UNS birds were euthanized periodically throughout the experiment to evaluate hormonal fluctuations. Remaining sparrows fasted between 20 min and 9 hr before being euthanized. Fasted birds were divided such that half remained in their cages (FO) and half were flown (FF). Groups were paired such that when FF sparrows began their 20 min flights, food was removed from the cage of a corresponding FO group. After 20 min, the first FF groups and the corresponding FO groups of birds were euthanized.

Disparity existed between the flight and fasted times for birds that flew for greater than 20 min. Sparrows tired after 15 min of flight and began to land in the tunnel. Therefore, after the first 20 minute flight, flight intervals were reduced to 15 minutes. after which time birds were returned to their cages, provided water, and allowed to rest for at least 45 min. Following this schedule and using the space constraints of the wind tunnel, it took 4 hr of fasting to attain 1 hr of flight and 9 h of fasting for 2.5 h of flight. Fasting and flight times listed in Figure 1 are nominal.

Upon flight or fast completion, birds were immediately decapitated. For each FF cohort that completed their designated flight time, a corresponding FO cohort was similarly euthanized. Blood was collected and plasma prepared and stored for hormone analysis [15]. Dissections were completed within 10 min of decapitation, and all tissues

were frozen in liquid nitrogen to prevent postmortem metabolism. Tissues were stored at -20°C until processed for residue analysis.

Residue Analysis

Tissues were homogenized with 30 g of anhydrous sodium sulfate before static extraction with 1:1 hexane acetone in an accelerated solvent extractor (Dionix ASE 200). Extracts were purified with 1.2% deactivated florisil before quantitation with a Hewlett Packard 6890 gas chromatograph equipped with a 5973 mass selective detector (Avondale, PA) operated in the selected ion mode. Recoveries for DDT (74±4%), DDD (76±5%), DDE (73±4%), DDη (68±3%), DDμ (75±4%), and DDOH (65±5%) were based on 80 spiked chicken livers. One spiked chicken liver was analyzed with each batch of tissues. The limit of detection for each analyte was 25 ng/g wet weight).

Statistical Analysis

In general, residue data departed from normal distribution according to the Shapiro-Wilk test for normality. Statistical tests were performed on log transformed residues, which maximized normality of these data. Two-way analysis of variance (2-way ANOVA) was used to compare mean residue concentrations for birds receiving DDT doses and vehicle control with residues determined in unstressed, fasted, and flown treatments. The total time that each bird was stressed was not considered for this analysis. An analysis of covariance (ANCOVA) was used to determine effects of dose (DDT or vehicle), time fasted, time flown, and combinations thereof on residues in

tissues. UNS, FO, and FF sparrow cohorts experienced the same treatment at time 0. Therefore, these three cohorts were combined for the time 0 residue data when evaluating temporal distributions of DDT and metabolites in sparrows. Statistical analyses were performed using JMP Start Statistics (SAS Institute, Cary, NC) using $P \leq 0.05$ to indicate significance. Weight loss data were regressed for FF and FO groups separately using time fasted as the dependent variable, since in the FF group the time fasted was highly correlated with time flown.

Results

Body Weights

Flight and fasting times lowered sparrow body weights as demonstrated in Figure XX. Flight caused more weight loss than did fasting ($p<0.01$).

Mean Tissue Residues

It should also be noted that no quantifiable residues of DDμ, DDη, and DDOH were found in sparrow tissues. While considering mean contaminant residues in each tissue type (Table 1), it should be noted that mean concentrations encompass samples from sparrows s that were stressed for varying amounts of time. For instance, the FO group contains birds which fasted from 0 min to 9 hr and the FF group contains sparrows which flew from 26 min to 2.5 hr while fasting from 30 min to 10 hr. Residue means were significantly higher ($p<0.0001$) in all tissues of birds dosed with DDT (Table 2) compared with those which received corn oil vehicle only (hereafter referred to as vehicle birds: VB). Treatment effects were found in all tissues except muscle ($p \leq 0.05$). DDE

concentrations in tissues of dosed-FO and dosed-UNS groups were 40-80% of concentrations in the dosed-FF group, but approximately three times greater than concentrations in VBs (Table 1). DDT and DDD concentrations followed a similar pattern in the brain, kidney and subcutaneous adipose (Table 1). Dose x treatment interactions occurred for DDT and DDD in brain and DDD in muscle because concentrations in VBs were significantly lower than in dosed birds and did not change among treatment groups. However, muscle from dosed FF birds showed a 1 to 3-fold increase in DDD concentration compared to other treatments (unstressed and fasting).

Mean percentages of DDT, DDD, and DDE in abdominal adipose tissue of dosed birds contained approximately 70% of the DDT (Table 3), 55% of the DDE, and 45% of the DDD found in the analyzed tissues of these birds. Brain and kidney contained the least amounts, generally less than 10% of total DDT, DDD, and DDE. Liver contained the greatest percentage of DDD, approximately twice the amount of either DDT or DDE, regardless of stress.

Stress did not influence the percentage of DDT, DDD, or DDE in the abdominal adipose. However, in other tissues percentages of DDT, DDD, and DDE were lowest in the UNS birds and highest in the FF sparrows. DDE in liver was unique in that the greatest percentage, 36%, was in the unstressed birds and least in the fasted-only sparrows, 19.4%.

Treatment Effects Over Time on Tissue Residue Concentrations

Among the fasted-only sparrows, each parameter of the model was significant for DDT, DDD, and DDE in all tissues ($p \leq 0.0299$) with the exception of DDD in brain ($p=0.1294$: Table 4). The significant result was driven by differences in DDT, DDD, and DDE concentrations in tissues of dosed and vehicle birds. Residues were significantly greater in all tissues of dosed birds ($p \leq 0.0203$), with the exception of DDD ($p \leq 0.1183$) and DDE ($p \leq 0.0610$) in brain. The lack of significance ($p \leq 0.05$) during time fasted indicates that DDT, DDD, and DDE concentrations did not change relative to the length of time fasted. Lacking that difference, there could be no interactions between dose (DDT or vehicle birds) or time fasted.

Among birds in the FF group dose, time fasted and time flown, affected DDT, DDD, and DDE concentrations in tissues ($p \leq 0.0366$), with exceptions to this significance emerging for DDE in abdominal adipose ($p \leq 0.0626$), subcutaneous adipose ($p \leq 0.0613$), and liver ($p \leq 0.0652$: Table 5). Similar to FO birds, differences between the FF groups were primarily driven by dose. DDT and DDD were greater ($p \leq 0.0416$) for all tissues except brain in dosed birds relative to the VBs. Unlike results for FO sparrows, dosing alone was insufficient to increase DDE concentrations in any FF tissues or DDT and DDD in brain above concentrations observed in VBs. With regard to the effect of time flown, DDT concentrations increased in brains ($p \leq 0.0373$) and kidneys ($p \leq 0.0260$), but DDT concentrations in livers were negatively associated with time flown ($p \leq 0.0110$). DDD concentrations similarly increased with time flown in sparrow brains ($p \leq 0.0094$) and kidney ($p \leq 0.0211$), and DDE accumulated in the kidney ($p \leq 0.0075$). Again, similar to the FO birds, fasting did not influence residues in any of the tissues from FF birds.

Redistribution of DDT, DDD, and DDE in sparrows was measured as increased toxicant concentrations in organs with increasing time stressed. Vehicle birds had background concentrations that infrequently exceeded instrument detection limits. Therefore, vehicle birds' metabolite concentrations were not dependent upon, and did not increase, with time stressed. This relationship is demonstrated in the dose x time flown interaction in DDT and DDD in brain. Similarly, fasting was ineffective in mobilizing metabolites into the kidney and liver. This finding directly contrasts effects of flying. The difference in slopes caused by fasting and flying for DDT, DDD, and DDE in kidney and DDT in liver indicated significant interactions in time flown x time fasted.

DDE and DDD concentrations in liver increased with increasing time fasted and flown (Figures 2&3). However, the increase due to time flown caused a much more dramatic effect when fasting times were short. With short fasting times, flight time rapidly decreased DDT concentrations in the liver (Figure 4). However, at increased fasting times, flight increased DDT residue in liver, though at a much reduced rate. The pattern for DDT in the liver ($p=0.0376$) and kidney ($p=0.0425$) of dosed flown birds is distinctly different. DDT in the kidney increased with increased flight time regardless of the amount of time fasted (Figure 5). Fasting appears to increase DDT concentration initially and decreases concentration as fasting time progresses. The effect appears to be more dramatic with shorter flight times.

Discussion

DDT Metabolism

The absence of DDμ, DDη and DDOH in sparrow tissue does not readily support DDT metabolic pathways that have been hypothesized by prior research. Based upon rat studies, Peterson and Robison [19] suggested that DDT was metabolized into DDE and DDD. DDE and DDD were then metabolized into DDμ which cascaded through a series of metabolites including DDMS, DDη, DDOH, finally to be excreted as DDA (Peterson and Robison, 1964). Based on mice and hamster studies, Gold and Brunk [20] have since described a pathway in which DDT is primarily converted into DDD which is transformed directly into DDA and excreted. DDμ and DDE exist as minor pathways at best, the former a derivative of DDD and the latter of DDT. Fawcett and King [21] injected rats and Japanese quail (*Coturnix japonica*) intraperitoneally with ^{14}C- labeled DDT, DDD, DDE, and DDμ and traced the pharmacokinetics of each compound. Their findings contradict the previous authors, showing the metabolism of DDT to DDA does not generate DDμ as an intermediate product. Rather, DDA is produced from DDμ, but DDμ is not a direct metabolic product of DDT. They also found the metabolic pathways are similar between rats and quail. However, the clearance of metabolites is much quicker in rats, indicating quail have a much poorer ability to metabolize DDT and the other metabolites into hydrophilic DDA. Quail were able to excrete DDμ as rapidly as the rat. In addition, rats produced DDOH as a metabolite of DDT unlike the quail.

There are several explanations accounting for the lack of DDμ, DDη, and DDOH in our study. First, metabolites may have been present but not at concentrations sufficient for detection. Sparrows in our study received 5 mg DDT/kg bird mass. Other metabolite

studies dosed animals with 100 ppm DDT or greater. Since our sparrows received 20 times less DDT, metabolite quantities may have been below method or instrument detection levels. Secondly, species-specific metabolism of DDT has been recorded for mammals [22]. It is therefore possible that the white-crowned sparrow transforms DDT to these metabolites sparingly if at all. Thirdly, the missing metabolites may have present only fleetingly as intermediate compounds that were quickly metabolized and/or excreted.

Residue Mobilization in the Fasted-only Sparrows

In the fasted-only sparrows, analyte concentrations in all tissues were different between the dosed and VB groups, with the exception of DDD in brain. All of these differences can be attributed to the DDT dose that birds received. Although there was a positive correlation between residues in tissues and time fasted, fasting for 9 hr did not significantly increase concentrations. During periods of fasting, fat deposits will be depleted and the concentration of lipophilic residues within these deposits will increase [1, 4, 23].

The classic model of fasting involves 3 phases [24]. Phase I involves a rapid loss in body mass from carbohydrate metabolism. Body mass loss is slowed in phase II as triglycerides, having a high mass specific energy content, become the major source of fuel. Phase III begins with another rapid loss of body mass as lipids become exhausted and protein catabolism becomes the major source of energy. The length of phase I is

variable and depends on individual bird species and their ability to tolerate extended fasts. Barn owls (*Tyto alba*) are in phase I for less than a day [25], quail for 2-3 d [26], and penguins (*Pyoscelis adeliae*) up to a week [27]. Based upon their body size, white-crowned sparrows are probably intolerant to long periods of fasting ([28]). In addition, increasing DDT, DDD and DDE concentrations in lipid pools indicates utilization of the pools for energy [1]. Mobilization of lipid reserves defines stage II of the classic model of fasting.

Nutritional status in birds, as measured by lipid reserves, is critical in the distribution of lipophilic contaminants such as DDT and its metabolites. Contaminant mobilization from fat depots into other tissues, including the brain, increased during periods of rapid lipid utilization [1, 4, 29-32]. Therefore, it is more likely that if fat reserves had been more thoroughly depleted, DDE and DDD concentrations in the brain of dosed sparrows would have been higher compared to the concentration observed in sparrows in the VB group.

Residue Mobilization in Flown Sparrows

The sizeable fractions of DDT, DDE, and DDD determined in abdominal adipose suggest that these toxicants had moved into this reasonably stable lipid depot by the time that flight or fasting stresses began. Contaminant movement into the brain, which has a more stable lipid pool, is well known to be impaired by the blood brain barrier. Thus retention of DDT in the abdominal adipose is clear indication of the extent to which the dose had come to equilibrium in the tissues.

FO and FF groups are similar in that whole model significance is driven by the dose. Unlike fasting, flying affected residues in brain, kidney, and liver tissues (Figures 2-5). There is clear indication that time flying increased the absolute concentration of DDT and its metabolites in tissues of dosed sparrows, and that this trend is followed to a lesser extent for birds that fasted without physical stress (Table 1). This is an important aspect of the data interpretation and can easily become lost in the various increases and decreases in analyte concentrations within each tissue. Even though, time stressed in the flown birds can be separated into time fasted and time flown, these variables were correlated. In general, birds that fasted for short periods flew for short intervals. Similarly, birds that fasted for longer periods, flew for longer intervals. Therefore, treatments in the FF groups are not completely independent.

The ubiquitous nature of DDE in the environment and metabolic processes explain the fact that dosed birds from dosed FF birds contained DDE concentrations in adipose tissue and liver were not different from those in the corresponding VB groups (Table 1), Sparrows in this study were taken from the wild and can be assumed to carry background DDE concentrations. The *ad libitum* feeding would allow birds to retain the fat stores and thus lipophillic contaminants, which existed upon capture. Furthermore, lipid stores are thought to be used for energy early in flight which could release recently DDE from recently deposited lipid thereby returning DDE concentrations in the overall lipid stores to predosing concentrations. Plasma free fatty acids resulting from lipid hydrolysis increase rapidly within the first hour of flight [33, 34]. Therefore, the FF group should more rapidly mobilize lipid pools relative to the FO group. Also

concentrations exhibited high variance for the dosed FF group. Background DDE concentrations in adipose tissues tended to increase more rapidly in the flown birds relative to fasted sparrows, but not significantly. Increased metabolic rate during flight may also have increased DDE transformation to products that were not in our suite of analytes.

This is the first study to demonstrate that flying time enhances the movement of chlorinated pesticides into the brain above and beyond fasting-induced stress. DDT and DDD concentrations in the brain increased with time flown but not with fasting time. These data effectively demonstrate the potential for brief flights to enhance the mobilization of DDT and its metabolites across the blood brain barrier.

Implications of differential transfer based on fasting or physical activity are significant for chlorinated hydrocarbon transfer to critical organ tissues. This is especially true of the brain, which is highly susceptible to impairment when chlorinated hydrocarbons cross the blood brain barrier. The mobilization of DDT and its metabolites into brain tissue is important because DDT and its metabolites are neurotoxic [35]. As few as 10 mg/kg DDT or 20 mg/kg DDD in the brain are diagnostic of death. In contrast, adipose tissue may contain hundreds of mg/kg residues with no adverse effects [35]. Therefore, flight stress should be incorporated into any evaluation of DDT mobilization during migration.

DDT, DDD, and DDE concentrations also increased in the kidney relative to time flown. Kidneys filter large amounts of blood and may receive 20% or greater of the

cardiac output. However, they are not typically fatty organs and do not accumulate lipophilic contaminants. Clearance rates of DDT, DDD, and DDE are not significantly different from muscle, liver, brain, heart gonad, or abdominal adipose [36]. Therefore, increased concentrations of DDT and its metabolites in the kidneys of exercised sparrows may result from increased plasma concentration and increased cardiac output.

DDT in the liver represents the only analyte that was negatively correlated with time flown. Lipid in the liver represents a readily available lipid pool which should release organochlorine compounds upon energetic stress. Also, liver and muscle are more metabolically active and have the greater ability to metabolize DDT [37]. The role of increased mobilization is clearly demonstrated by the fact that DDT removal from sparrow liver was greater for birds experiencing longer flight time relative to fasting time. Increased metabolite:DDT ratios have been observed in livers of grackles dosed with DDT [38]. In addition, induction of liver cytochrome P450 enzymes by chlorinated hydrocarbons has been observed in other avian species [39, 40] and mammals [41, 42].

Our results clearly demonstrate that physical activity transforms and mobilizes DDT from lipid reserves, allowing movement into highly perfused organs, specifically liver and brain. These data indicate the need to incorporate actual physical activity when evaluating the effect of migratory stress on contaminant mobilization and distribution in birds. Simple caloric stress does not represent the situation experienced by migrating wildlife in the environment. Mobilization of DDT into the brain is significant as a potential route for neurotoxicity, which would be particularly detrimental during migration.

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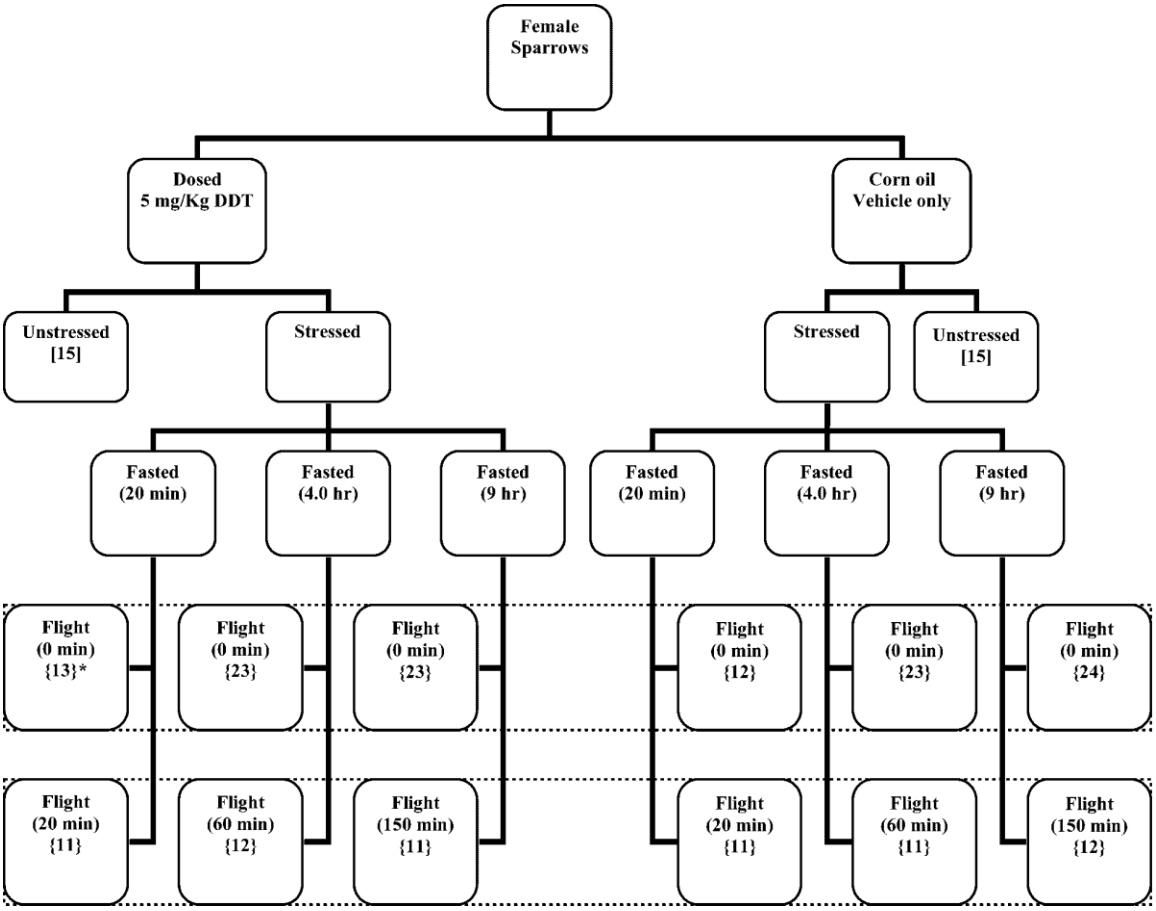


Figure 1. Dosing paradigm for white-crowned sparrows. Fasted and flown groups comprised of birds in respectively labeled levels. Flight and fasting times are nominal. *: {N} = sample size

Table 1. Mean DDT, DDD, and DDE concentrations[a] in selected tissues from stressed white-crowned sparrows.

Sparrows dosed with corn oil vehicle only

Unstressed	Abdominal Adipose			Brain			Kidney			Liver			Muscle			Subcutaneous Adipose		
	DDD	DDE	DDT	DDD	DDE	DDT	DDD	DDE	DDT	DDD	DDE	DDT	DDD	DDE	DDT	DDD	DDE	DDT
mean[b]	0.056	0.197	0.068	0.023	0.021	0.023	0.034	0.034	0.025	0.021	0.035	0.025	0.024	0.029	0.022	0.038	0.127	0.062
95% LCL[c]	0.039	0.087	0.036	0.019	0.019	0.018	0.023	0.024	0.021	0.015	0.022	0.015	0.019	0.021	0.018	0.032	0.057	0.032
95% UCL	0.081	0.447	0.130	0.028	0.023	0.029	0.049	0.047	0.031	0.029	0.056	0.042	0.030	0.041	0.026	0.044	0.279	0.118
n[d]	15	15	15	15	15	15	10	10	10	15	15	15	15	15	15	15	15	15
Fasted																		
mean	0.041	0.138	0.048	0.021	0.022	0.021	0.025	0.033	0.029	0.022	0.030	0.026	0.021	0.033	0.021	0.037	0.120	0.038
95% LCL	0.038	0.080	0.039	0.019	0.020	0.019	0.023	0.027	0.022	0.017	0.022	0.018	0.019	0.023	0.018	0.033	0.065	0.034
95% UCL	0.045	0.238	0.059	0.023	0.025	0.023	0.027	0.040	0.038	0.028	0.041	0.038	0.023	0.047	0.023	0.041	0.220	0.043
n	28	28	28	28	28	28	28	28	28	26	26	26	27	27	27	28	28	28
Flown and Fasted																		
mean	0.055	0.340	0.066	0.021	0.025	0.021	0.029	0.049	0.031	0.020	0.058	0.021	0.021	0.037	0.023	0.043	0.351	0.056
95% LCL	0.047	0.186	0.054	0.019	0.020	0.019	0.022	0.034	0.021	0.017	0.031	0.018	0.016	0.022	0.015	0.038	0.161	0.041
95% UCL	0.063	0.622	0.081	0.022	0.031	0.022	0.038	0.071	0.045	0.023	0.109	0.024	0.028	0.060	0.034	0.050	0.764	0.076
n	18	18	18	17	17	17	18	18	18	18	18	18	18	18	18	18	18	18

DDT= 1,1,1-trichloro- bis-(4chlorophenyl) ethane; DDE= 1,1-dichloro-2,2-bis (4 chlorophenyl) ethane; DDD=1,1-dichloro-2,2-bis (4 chlorophenyl) ethane.

[a] (mg/Kg wet wt); [b] Geometric means within each treatment without considering time stressed for individual sparrows.

Sparrows dosed with DDT

Unstressed																		
	DDD	DDE	DDT	DDD	DDE	DDT	DDD	DDE	DDT	DDD	DDE	DDT	DDD	DDE	DDT	DDD	DDE	DDT
mean	0.402	0.455	3.714	0.024	0.032	0.062	0.132	0.088	0.709	0.242	0.111	0.765	0.099	0.059	0.414	0.413	0.585	11.059
95% LCL	0.130	0.192	0.783	0.019	0.022	0.028	0.085	0.047	0.280	0.080	0.045	0.302	0.047	0.027	0.196	0.230	0.269	6.460
95% UCL	1.247	1.081	17.619	0.031	0.046	0.137	0.206	0.164	1.794	0.735	0.272	1.937	0.206	0.130	0.875	0.743	1.273	18.931
n	10	10	10	10	10	10	4	4	4	10	10	10	10	10	10	10	10	10
Fasted																		
mean	0.637	0.519	7.585	0.026	0.032	0.081	0.074	0.050	0.463	0.196	0.066	0.407	0.171	0.072	0.795	0.541	0.416	12.921
95% LCL	0.394	0.337	4.050	0.022	0.026	0.054	0.054	0.037	0.331	0.109	0.045	0.232	0.126	0.047	0.588	0.300	0.233	5.984
95% UCL	1.030	0.799	14.204	0.031	0.039	0.119	0.102	0.066	0.647	0.351	0.097	0.715	0.232	0.110	1.074	0.975	0.744	27.900
n	32	32	32	32	32	32	31	31	31	31	31	31	31	31	31	32	32	32
Flown and Fasted																		
mean	0.925	0.985	13.173	0.043	0.051	0.229	0.154	0.096	0.847	0.366	0.130	0.882	0.198	0.089	0.793	1.127	1.343	28.654
95% LCL	0.385	0.428	5.017	0.031	0.036	0.154	0.103	0.076	0.568	0.167	0.073	0.444	0.163	0.051	0.643	0.634	0.686	14.179
95% UCL	2.221	2.265	34.584	0.060	0.072	0.343	0.230	0.121	1.262	0.806	0.229	1.753	0.240	0.153	0.978	2.003	2.631	57.906
n	20	20	20	20	20	20	20	20	20	19	19	19	20	20	20	20	20	20

[c] LCL and UCL represent the lower and upper confidence levels, respectively.[d] *n* = number of birds.

Table 2. Results from 2-way analysis of variance spell out examining the effects of dose (DDT or vehicle only) and treatment (unstressed, fasted, or flown) on mean DDT, DDD, and DDE concentrations in white-crowned sparrows.

		Whole model[a]	Dose	Treatment	Dose*treatment[b]
Abdominal Adipose					
	DDT	<0.001[c]	<0.001	0.364	0.487
	DDD	<0.001	<0.001	0.404	0.520
	DDE	0.002	<0.001	0.040	0.822
Brain					
	DDT	<0.001	<0.001	0.007	0.003
	DDD	<0.001	<0.001	0.039	0.010
	DDE	<0.001	<0.001	0.013	0.224
Kidney					
	DDT	<0.001	<0.001	0.161	0.196
	DDD	<0.001	<0.001	0.003	0.126
	DDE	<0.001	<0.001	0.001	0.460
Liver					
	DDT	<0.001	<0.001	0.395	0.130
	DDD	<0.001	<0.001	0.620	0.407
	DDE	0.002	<0.001	0.020	0.858
Muscle					
	DDT	<0.001	<0.001	0.105	0.088
	DDD	<0.001	<0.001	0.196	0.029
	DDE	0.003	<0.001	0.498	0.964
Subcutaneous Adipose					
	DDT	<0.001	<0.001	0.114	0.095
	DDD	<0.001	<0.001	0.048	0.190
	DDE	<0.001	<0.001	0.002	0.989

DDT= 1,1,1-trichloro- bis-(4chlorophenyl) ethane; DDE= 1,1-dichloro-2,2-bis (4 chlorophenyl) ethane; DDD=1,1-dichloro-2,2-bis (4 chlorophenyl) ethane

[a]Mean values do not take the time stressed for each individual into account. Therefore, there is no time effect.
[b]Dose*Treatment indicates level of interaction between effects.
[c]*p* values are tabulated.

Table 3 - Percentages of DDT, DDD, and DDE in abdominal adipose, brain, kidney and liver in white-crowned sparrows (*Zonotrichia leucophrys*) dosed with DDT

	Abdominal adipose			Brain			Kidney			Liver		
	DDD	DDE	DDT	DDD	DDE	DDT	DDD	DDE	DDT	DDD	DDE	DDT
Unstressed												
Mean[a]	40.87	52.46	71.08	3.34	6.15	1.78	4.60	5.80	3.93	51.18	35.59	23.22
SE[b]	11.35	7.29	7.47	1.24	0.65	0.39	0.92	1.57	1.17	12.76	7.39	7.00
95% LCL[c]	18.62	38.16	56.43	0.90	4.88	1.02	2.81	2.71	1.64	26.17	21.10	9.50
95% UCL	63.13	66.76	85.72	5.77	7.42	2.54	6.40	8.88	6.21	76.20	50.08	36.93
n[d]	7	7	7	7	7	7	7	7	7	7	7	7
Fasted												
Mean	50.49	64.82	74.87	6.65	10.97	4.41	5.52	4.79	3.71	37.34	19.42	17.01
SE	5.55	4.32	5.71	2.01	1.87	2.42	1.45	0.70	0.72	5.42	3.75	4.92
95% LCL	39.60	56.36	63.68	2.71	7.31	−0.32	2.68	3.43	2.29	26.72	12.08	7.37
95% UCL	61.37	73.28	86.05	10.59	14.62	9.15	8.36	6.16	5.13	47.95	26.76	26.65
n	28	28	28	28	28	28	28	28	28	28	28	28
Fasted and flown												
Mean	38.19	57.03	68.14	6.45	12.96	5.32	8.86	6.21	7.24	46.50	23.81	19.29
SE	7.02	6.41	7.02	1.92	3.37	2.27	3.39	1.62	2.86	6.98	5.00	5.02
95% LCL	24.42	44.47	54.38	2.69	6.35	0.88	2.21	3.03	1.64	32.81	14.01	9.46
95% UCL	51.96	69.58	81.91	10.21	19.57	9.76	15.52	9.38	12.84	60.18	33.60	29.13
n	18	18	18	18	18	18	18	18	18	18	18	18

[a] Geometric means within each treatment without considering time stressed for individual sparrows.
[b] Standard error.
[c] LCL and UCL represent the lower and upper confidence levels, respectively.
[d] n ¼ number of birds.
DDT ¼ 1,1,1-trichloro-bis(4-chlorophenyl)ethane; DDE ¼ 1,1-dichloro-2,2-bis(4-chlorophenyl)ethylene; DDD ¼ 1,1-dichloro-2,2-bis(4-chlorophenyl)ethane.

Table 4. Analysis of covariance analysis of DDT, DDD, and DDE residues in fasted White-crowned Sparrows (*Zonotrichia leucophrys*)

	Whole model	Dose	Time fasted	Dose × time fasted
Abdominal adipose				
DDT	<0.001	0.0001	0.935	0.710
DDD	<0.001	0.0001	0.411	0.719
DDE	0.002	0.0068	0.764	0.875
Brain				
DDT	<0.001	<0.001	0.375	0.336
DDD	0.129	0.118	0.166	0.723
DDE	0.008	0.061	0.402	0.645
Kidney				
DDT	<0.001	<0.001	0.050	0.708
DDD	<0.001	<0.001	0.840	0.254
DDE	0.030	0.020	0.790	0.530
Liver				
DDT	<0.001	<0.001	0.771	0.460
DDD	<0.001	<0.001	0.708	0.872
DDE	0.004	0.015	0.228	0.969
Muscle				
DDT	<0.001	<0.001	0.534	0.059
DDD	<0.001	<0.001	0.744	0.288
DDE	0.006	<0.001	0.882	0.147
Subcutaneous adipose				
DDT	<0.001	<0.001	0.950	0.881
DDD	<0.001	<0.001	0.700	0.401
DDE	0.004	0.003	0.487	0.394

The p values[a] are tabulated. Whole model values indicate significance of dose (DDT or vehicle) and length of time fasted on residue concentrations within each tissue. Dose and time fasted are effect tests. Dose x time fasted indicates level of interaction between effects.

DDT - 1,1,1-trichloro-bis(4-chlorophenyl)ethane; DDE - 1,1-dichloro-2,2- bis(4-chlorophenyl)ethylene; DDD - 1,1-dichloro-2,2-bis(4-chlorophenyl) ethane.

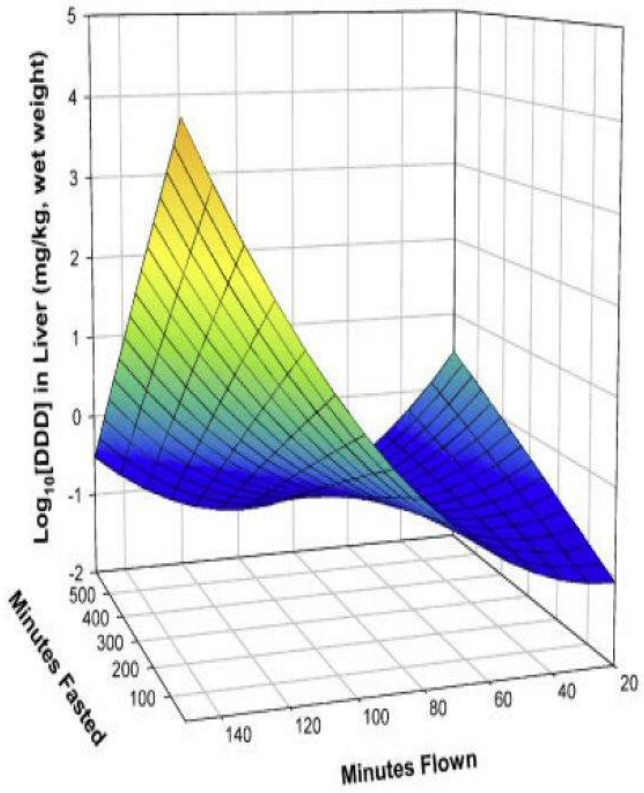
[a]p - probability of accepting a false hypothesis.

Table 5 - Analysis of covariance of DDT, DDD, and DDE in fasted and flown white-crowned sparrows

	Whole model	Dose	Time flown	Time fasted	Dose× time flown	Dose× time fasted	Time flown× time fasted	Dose × time flown ×time fasted
Abdominal adipose								
DDT	<0.001	0.002	0.948	0.792	0.772	0.532	0.741	0.998
DDD	<0.001	0.042	0.855	0.937	0.972	0.660	0.415	0.781
DDE	0.063	0.475	0.477	0.456	0.680	0.963	0.649	0.414
Brain								
DDT	<0.001	0.079	0.037	0.973	0.011	0.667	0.140	0.046
DDD	<0.001	0.610	0.009	0.401	0.006	0.436	0.077	0.059
DDE	0.001	0.924	0.133	0.711	0.079	0.717	0.404	0.323
Kidney								
DDT	<0.001	<0.001	0.026	0.889	0.667	0.862	0.036	0.932
DDD	<0.001	0.003	0.021	0.868	0.793	0.560	0.014	0.586
DDE	0.005	0.510	0.007	0.528	0.733	0.334	0.008	0.957
Liver								
DDT	<0.001	<0.001	0.011	0.953	0.168	0.261	0.006	0.234
DDD	<0.001	<0.001	0.314	0.524	0.652	0.797	0.068	0.278
DDE	0.065	0.142	0.725	0.551	0.081	0.640	0.913	0.097
Muscle								
DDT	<0.001	<0.001	0.300	0.597	0.422	0.863	0.348	0.424
DDD	<0.001	0.003	0.392	0.134	0.199	0.106	0.204	0.069
DDE	0.037	0.604	0.6503	0.220	0.292	0.143	0.526	0.646
Subcutaneous adipose								
DDT	<0.001	<0.001	0.148	0.687	0.110	0.470	0.385	0.148
DDD	<0.001	<0.001	0.131	0.591	0.228	0.450	0.134	0.134
DDE	0.061	0.381	0.070	0.979	0.692	0.998	0.229	0.820

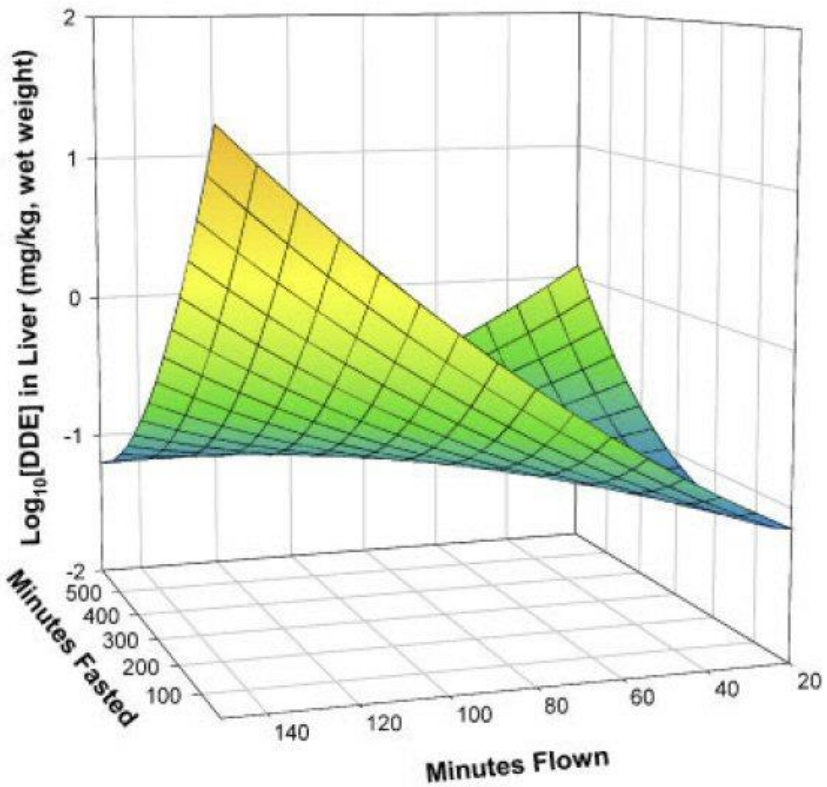
The p values[a] are tabulated. Whole model values indicate significance of dose group, length of time flown, and length of time fasted on residue concentrations within each tissue. Dose, time flown, and time fasted are effect tests. Dose x time flown, dose x time fasted, time flown x time fasted, and dose x time flown x time fasted indicate level of interaction between effects.
DDT - 1,1,1-trichloro- bis-(4chlorophenyl) ethane; DDE - 1,1-dichloro-2,2-bis (4 chlorophenyl) ethylene; DDD - 1,1-dichloro-2,2-bis (4 chlorophenyl)
ethane.
[a]p - probability of accepting a false hypothesis.

The p values[a] are tabulated. Whole model values indicate significance of dose (DDT or vehicle) and length of time fasted on residue concentrations within each tissue. Dose and time fasted are effect tests. Dose x time fasted indicates level of interaction between effects.
DDT - 1,1,1-trichloro-bis(4-chlorophenyl)ethane; DDE - 1,1-dichloro-2,2- bis(4-chlorophenyl)ethylene; DDD - 1,1-dichloro-2,2-bis(4-chlorophenyl) ethane.
[a]p - probability of accepting a false hypothesis.



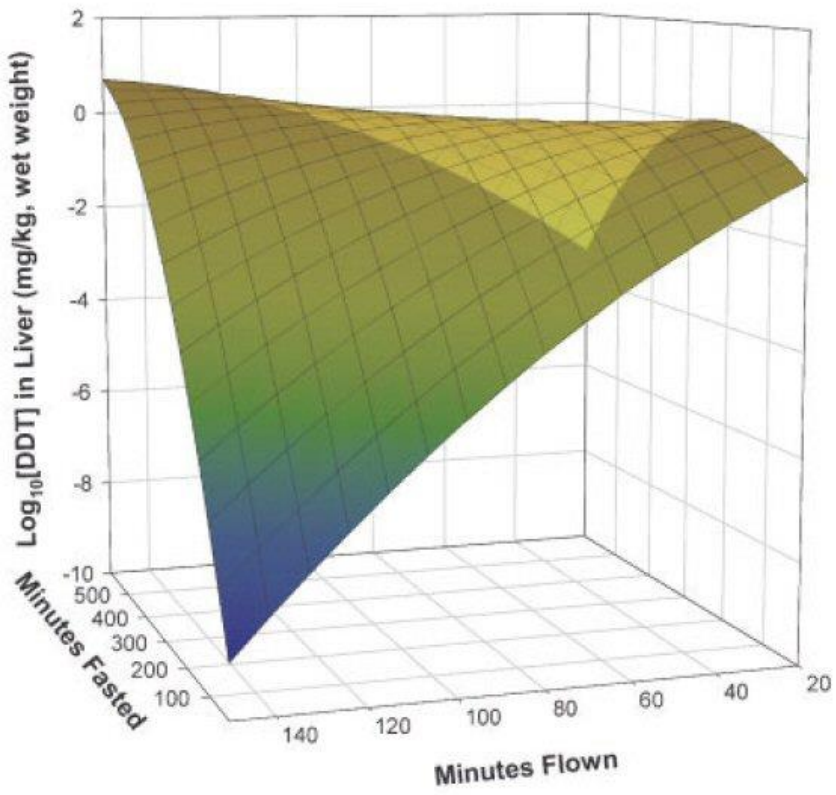
Model Parameters	Parameter Estimates	*p* value
Whole Model	NA	0.1215
Intercept	-1.0246	<0.0001
Flight	-0.0107	0.3077
Fasting	0.0045	0.0393
Fasting×Flight	-0.0001	0.1706
Fasting×Fasting	0.0001	0.9642
Flight×Flight	0.0003	0.0847

Figure 2. Model of fasting and flight effects on DDD concentration in liver of flown white-crowned sparrows dosed with DDT. Whole model estimates significance of model and *P*-values represent the probability of accepting a false hypothesis. Model based on 23 residue data entries.



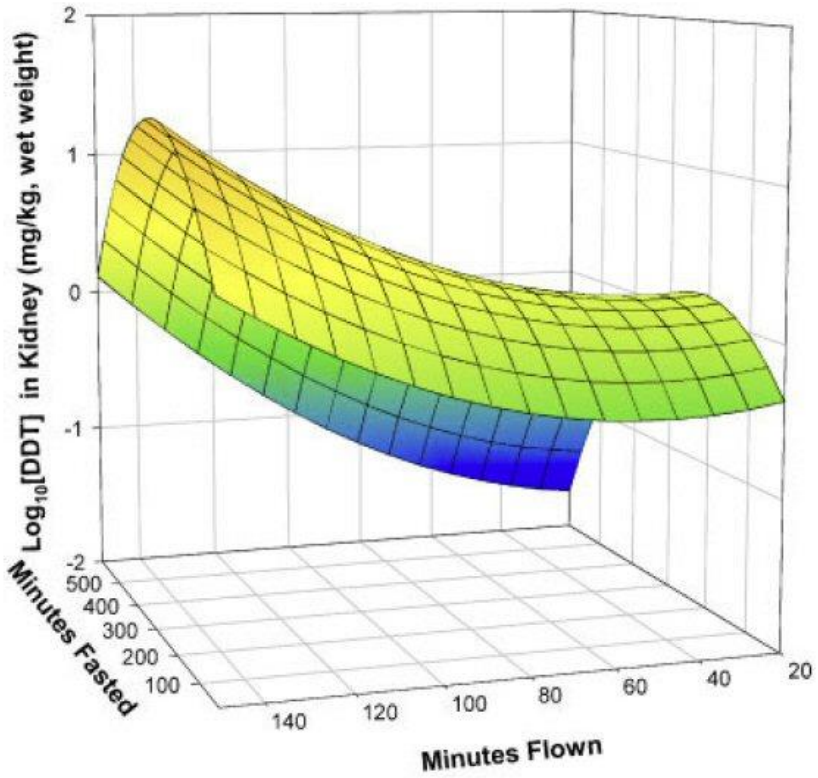
Model Parameters	Parameter Estimates	*p* value
Whole Model	NA	0.0915
Intercept	-1.3493	<0.0001
Flight	0.0115	0.1054
Fasting	-0.0007	0.6069
Fasting×Flight	-0.0001	0.1938
Fasting×Fasting	0.0001	0.1952
Flight×Flight	0.0001	0.6156

Figure 3. Model of fasting and flight effects on DDE concentration in liver of flown white-crowned sparrows dosed with DDT. Whole model estimates significance of model and *P*-values represent the probability of accepting a false hypothesis. Model based on 23 residue data entries.



Model Parameters	Parameter Estimates	*p* value
Whole Model	NA	0.0376
Intercept	-0.2269	0.3738
Flight	-0.0324	0.0533
Fasting	0.0066	0.0502
Fasting×Flight	0.0002	0.0860
Fasting×Fasting	-0.0001	0.0460
Flight×Flight	-0.0002	0.4545

Figure 4. Model of fasting and flight effects on DDT concentration in liver of flown white-crowned sparrows dosed with DDT. Whole model estimates significance of model and *P*-values represent the probability of accepting a false hypothesis. Model based on 23 residue data entries.



Model Parameters	Parameter Estimates	*p* value
Whole Model	NA	0.0425
Intercept	-0.3325	0.0450
Flight	-0.0087	0.3825
Fasting	0.0054	0.0115
Fasting×Flight	0.0001	0.8132
Fasting×Fasting	-0.0001	0.0725
Flight×Flight	0.000	0.5912

Figure 5. Model of fasting and flight effects on DDT concentration in kidney of flown white-crowned sparrows dosed with DDT. Whole model estimates significance of model and *P*-values represent the probability of accepting a false hypothesis. Model based on 23 residue data entries.