Bull. Environ. Contam. Toxicol. (1985) 34:634–644

Bulletin of Environmental Contamination and Toxicology

Volatility of Ten Priority Pollutants from Fortified Avian Toxicity Test Diets

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Methods for conducting avian dietary toxicity tests were developed by DeWitt et al. (1962) and standardized by Stickel and Heath (1965) for use in pesticide investigations by the Fish and Wildlife Service. The Environmental Protection Agency (1978) adopted the basic method of Stickel and Heath as one of several tests for use in support of pesticide registration under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA, PL 92-516, enacted in 1972). With some modification, the same method was adopted by EPA (1982) for use under the Toxic Substances Control Act (TSCA, PL 94-469, enacted in 1976).

With a few important exceptions, most pesticides have low volatility. However, a significant number of the industrial chemicals under the jurisdiction of TSCA have sufficient volatility to affect availability in fortified test diets and consequently the test results. Although extremely volatile chemicals might evaporate from test diets, there is insufficient data to indicate what levels of volatility are of concern.

The stability of test substances over the duration of toxicity tests is important in evaluating the results and in making risk assessments. The Organization for Economic Cooperation and Development (1982) has developed ecotoxicity test guidelines that specify the need to maintain test substance concentrations within 20 percent of the nominal concentration. Volatility may be only one of a variety of factors influencing the fate of organic chemicals in test diets. Other mechanisms such as hydrolysis, adsorption, and photolysis should also be considered, but for many chemicals having significant vapor pressures, volatility is likely to be the major source of loss.

An estimation of the volatility of organic chemicals added to avian test diets could theoretically be made with models such as those used for volatility losses from water and soil (Lyman et al. 1982). However, many of the substrate-related factors affecting volatility from feed have not been determined, e.g., sorption, diffusion, particle size, surface area, and feed mixing. If a reasonable estimation of the volatility losses could be derived from simple physical properties, then the investigator could better judge what special procedures might need to be taken, such as frequency of test feed preparation, handling and storage of test feed, and how often to collect samples for chemical analysis. It also might be possible to estimate volatility losses and determine whether a dietary toxicity test would be practical for a particular chemical.

In this investigation the losses of 10 priority pollutants from avian test diets over a 96-hour period were measured and compared with vapor pressures, Henry's law constants, and octanol/water partition coefficients. Simulation of avian dietary test conditions was accomplished by performing a series of feed volatility tests in a temperature controlled chamber. Birds, cages, photoperiods, etc., were not used, but the air flow across the test feed was carefully controlled and the relative humidity was monitored. This approach allowed us to perform simple, safe, and repeatable volatility measurements. Simulating the birds perturbation of the feed, i.e., "pecking in the feed hopper", did not seem practical. Instead of using a commercial type feed hopper that would hold all of the feed in one common container, we divided the fortified feed into equal aliquots contained in glass beakers. These undisturbed beakers became our "standard test containers". We also demonstrated the effect of feed perturbation by increasing the surface area of the test container and stirring the feed.

MATERIALS AND METHODS

The test chemicals (Table 1), selected from the 129 EPA priority pollutants (Callahan and Slimak 1979), represent a broad range of vapor pressures (0.0001 to 1.97 mm Hg), water solubilities (1.69 to 16,000 µg/ml), and octanol/water partition coefficients (log Kow 1.58 to 4.18). Seven of the 10 chemicals were EPA analytical standards. The other three, HCE, NB, and 4-NP, were reagent grade (98% or better) and found to be free from significant impurities by GLC.

Commercially available game bird starter ration (Purina Game Bird Startena) of the "crumble" formulation (particle size 2 to 4 mm) was fortified with the desired test chemicals using 2% (v/w) table grade corn oil as a carrier. Corn oil is a common carrier for water insoluble compounds in avian as well as mammalian dietary toxicity tests (USEPA 1978). The appropriate amount of each chemical was dissolved in 10 ml corn oil in a volumetric flask. After mixing, 9 ml of the fortified corn oil was added dropwise to 450 g feed while mixing with a spatula. Care was taken to add the oil slowly and evenly and to continue mixing for 10 minutes after the corn oil had been added, obtaining what appeared to be a uniform coating on the feed particles. The remaining 1 ml of corn oil was saved for chemical analysis.

The volatility of the test chemicals was measured in a series of nine tests. A minimum of three and as many as ten chemicals were used in each test. The first six tests were experimentally

Table 1. Physical properties of ten priority pollutants.

Chemical	Abbrev.[a]	Mol. wt.	Vapor press. (mm Hg)	Sol.[f] (μg/ml)	Log Kow[g]	-Log H[h]
Bis(2-Cl-ethyl) ether	B2CEE	143	1.30[b]	10,200	1.58	4.62
1,4-Dichlorobenzene	1,4-DCB	147	1.80[b]	79	3.39	2.36
1,2-Dichlorobenzene	1,2-DCB	147	1.97[b]	145	3.38	2.58
Hexachloroethane	HCE	237	0.72[b]	50	3.34	2.35
Nitrobenzene	NB	123	0.29[b]	1,900	1.85	4.61
Dimethylphthalate	DMP	194	0.014[c]	4,320	2.12	6.08
4-Nitrophenol	4-NP	139	0.0003[d]	16,000	1.91	8.46
2,4-Dinitrotoluene	2,4-DNT	182	0.0001[b]	270	2.01	7.05
Fluorene	Fluor	166	0.0044[e]	1.69	4.18	3.24
4-Cl-phenyl phenyl ether	4-CPPE	204	0.004[b]	3.3	4.08	3.49

[a] Abbreviated name used in this text.

[b] Values obtained from Callahan and Slimak (1979) and corrected to 30°C using method from Lyman et al. (1982).

[c] Value extrapolated to 30°C from vapor pressures (150 to 250°C) from Peakal (1974). A value of 0.013 mm was calculated using the modified Watson correlation, Method 2 (Lyman et al. 1982).

[d] Value extrapolated to 30°C from vapor pressures (120 to 186°C) from Timmermans (1965). A value of 0.0002 mm was calculated using the modified Watson correlation, Method 2 (Lyman et al. 1982).

[e] Value was calculated at 30°C using method from Lyman et al. (1982) using the vapor pressure of 18 mm at 161°C from Boublik et al. (1973). A value of 0.02 mm was extrapolated from vapor pressures (161 to 296°C) from Boublik et al. (1973). A value of 0.0004 mm was calculated using the modified Watson correlation, Method 2 (Lyman et al. 1982).

[f] Solubilities at temperatures between 20 and 25°C obtained from Callahan and Slimak (1979).

[g] Octanol/water partition coefficients obtained from Callahan and Slimak (1979).

[h] Henry's law constant (atm-m^3/mole).

identical except for the number, type, and concentration of test chemicals used. The seventh test demonstrated the effect of feed perturbation and surface area and the eighth test demonstrated the effect of using a water carrier instead of corn oil. The nominal concentrations used for each chemical in each test are shown in Table 2.

Table 2. Percent nominal feed concentration at the start of each test and after freezer storage.

Chemical	Test	Conc. (μg/g)	% ± SE[a] (time 0)	% ± SE[b] (freezer)
B2CEE	2	355	79 ± 3.2	91 ± 2.7
	5	62.0	90 ± 0.7	91 ± 0.9
	7	313	87 ± 0.2	---
	8	118	79 ± 4.4	74 ± 3.3
1,4-DCB	3	296	92 ± 1.7	81 ± 1.7
	6	55.0	63 ± 5.5	81 ± 5.6
	7	266	82 ± 0.4	---
1,2-DCB	2	312	76 ± 0.5	85 ± 3.0
	5	58.4	86 ± 1.5	77 ± 1.2
	7	265	80 ± 0.4	---
HCE	1	260	74 ± 1.4	75 ± 8.4
	4	47.0	76 ± 2.4	72 ± 2.5
	7	378	77 ± 0.1	---
NB	1	299	92 ± 2.5	85 ± 2.1
	3	330	108 ± 1.2	99 ± 1.2
	4	49.8	141 ± 6.8	135 ± 5.2
	6	57.2	75 ± 4.0	88 ± 4.6
	7	290	95 ± 0.5	---
	8	75.0	90 ± 7.6	84 ± 4.8
DMP	2	260	91 ± 3.3	105 ± 6.3
	5	68.4	103 ± 0.6	103 ± 3.1
	7	305	99 ± 0.6	---
	8	123	98 ± 18	91 ± 10
4-NP	1	278	107 ± 6.8	103 ± 6.2
	4	46.2	92 ± 2.5	122 ± 5.6
	7	310	138 ± 1.4	---
	8	111	226 ± 51	114 ± 16
2,4-DNT	3	296	132 ± 3.9	108 ± 2.7
	6	48.8	92 ± 4.1	100 ± 6.2
	7	336	100 ± 0.7	---
Fluor	3	308	107 ± 4.1	99 ± 2.2
	6	54.4	98 ± 1.6	101 ± 5.4
	7	294	98 ± 0.2	---
4-CPPE	2	281	84 ± 3.4	98 ± 6.1
	5	50.4	102 ± 1.4	99 ± 2.9
	7	354	100 ± 0.2	---

[a] Two samples were analyzed at the start of each test.

[b] Four samples were analyzed, 2 each removed from the freezer at 48 and 96 hours.

Each volatility test was conducted in a modified laboratory drying oven (inside dimensions 24.3 x 55.9 x 21 cm). The seal around the oven door and small openings along the sides and bottom were sealed with duct tape. Ambient air was pulled through the oven with laboratory vacuum. The air entered the bottom of the oven through two 2.54-cm diameter holes and passed through two overlapping aluminum baffles located 7.6 cm off the bottom of the oven. The air flowed out the top of the oven through a 0.64-cm diameter glass tube fitted with a rubber stopper, then through a flow meter, and finally through an activated carbon filter which trapped volatile organics. The air flow was maintained at 0.60 ± 0.02 m^3/hr. The temperature was held at 30 ± 1°C. The relative humidity (RH) was monitored three times per day either inside the oven (tests 7 and 8), or in the air immediately surrounding the oven (tests 1-6). The range of RH was 32 to 40% (tests 1-7) and 23 to 40% (test 8).

The oven and flow system was characterized for uniform mixing and temperature by conducting a gravimetric test with distilled water. The water was placed in 12 beakers set in a 3 x 4 grid pattern inside the oven. An additional water-filled beaker was placed in each of the four corners of the oven. After 118.5 hours, the weight loss for each beaker was measured, resulting in a percent standard deviation of 8.1% for those beakers located within the grid. The four beakers in the oven corners had a mean evaporation rate 27% higher, but since all feed volatility test containers were placed within the grid pattern, the flow and temperature differences within the chamber were assumed insignificant.

In tests 1-6, 450 g feed was fortified and mixed. Ten 25-g aliquots of feed were weighed into 50-ml beakers and immediately placed in the test oven in a 2 x 5 grid. The feed in each beaker was 5.1 cm deep and 1.3 cm from the top of the beaker. Two additional 25-g aliquots were placed in 60-ml powder bottles, covered with 35 ml of pesticide grade acetonitrile, and stored in a refrigerator. These initial feed samples represented the start of each test (time 0). The remaining 150 g of feed was stored in the freezer at -22°C. Two randomly selected beakers were removed from the test oven at 6, 24, 48, 72, and 96 hours. Each 25-g feed sample was transferred to a powder bottle, covered with 35 ml of acetonitrile, stored in the refrigerator, and analyzed within one week. At the 48 and 96-hr sampling periods, two 25-g samples were removed from the freezer and treated identically to the other samples. All of the tests were restricted to 96 hours to conform to time constraints of a five-day work week.

To determine the effect of feed perturbation and surface area, test 7 was performed using both beakers and 10-cm petri dishes to contain the 25-g feed aliquots. Six beakers were placed in a 2 x 3 grid in the middle of the oven and six petri dishes were arranged with the first three in front and the second three behind the six beakers. The surface area of the 10-cm petri dish is 6.93 times that of the test beakers. The 25 g of feed in the petri dish formed a layer one or two particles deep (4 to 6 mm). In addition to the increased surface area, the petri dish samples were stirred twice daily with a glass capillary pipet. The petri dish samples represented an extreme case, relative to the static beaker tests, of high surface area and feed perturbation. Two samples were randomly collected at 24, 48, and 96 hours for both the beaker and petri containers.

Test 8 was identical to the first six tests except water was used as the carrier and only four of the more water soluble chemicals (B2CEE, NB, DMP, and 4-NP) were used.

Chemical analysis was performed on all of the feed samples from each test as well as those samples stored in the freezer and the fortified corn oil and water carriers. Each feed sample was soxhlet extracted with pesticide grade acetonitrile for 4 hours and brought up to a known volume, usually 100 ml. A small aliquot of the extract was cleaned up on a commercially available 500-mg octadecyl reverse phase chromatography column. One ml of acetonitrile was added to wet the column, followed by 1 ml of sample extract, and elution with two 1-ml volumes of acetonitrile. The final volume was adjusted to 3.0 ml in a calibrated centrifuge tube and analyzed on a Hewlett-Packard 5880A gas chromatograph equipped with a 12-meter SE-54 chemically bonded fused silica capillary column and FID and Ni63 EC detectors. Sample extracts were measured down to 1 μg/ml without further cleanup.

In order to calculate a total mass balance, the 250 g of activated charcoal that was used to trap volatile organics was removed and divided into two 125 g sections and each was soxhlet extracted serially with acetonitrile, toluene, and hexane. The extracts were analyzed directly without additional cleanup.

Approximately 90% of the sample extracts analyzed on the gas chromatograph were injected and quantitated two or more times. A measure of instrument precision over the entire range of sample concentrations and test chemicals was calculated from the mean of the 566 individual percent standard deviations obtained from the replicate injections, resulting in an overall standard deviation of 4.0%. Since two individual test containers located randomly within the oven were collected at each time period and analyzed separately, an indication of sample analysis precision was obtained by determining the mean of the percent standard deviations for the 273 duplicate feed samples which was 6.5%.

Each fortified corn oil carrier was analyzed separately (before adding the carrier to the feed) for each volatility test. The measured percent recoveries of the nominal carrier concentrations for all tests and chemicals ($n = 32$) resulted in a mean percent recovery of 99.2% (83.2-110%).

RESULTS AND DISCUSSION

A measure of extraction efficiency for the feed samples was obtained by analyzing the fortified feeds at the start of each test. These results and the results from samples stored in the freezer are shown in Table 2. Mean recoveries for the samples collected at the start of each test range from 75.5% for HCE to 112% for 4-NP. There appeared to be little change in concentrations when the samples were kept frozen for up to 96 hours, indicating a possible approach to experimental dietary toxicity tests using volatile compounds. The feed could be prepared in batch form, divided into daily test ration quantities, frozen, and randomly removed daily as the test proceeds.

All of the feed concentration data were normalized to a relative concentration scale (0 to 100%) by dividing the measured concentration at a given time by the initial measured concentration (C_o). Thus, plots of log $\%C_o$ vs time were used to calculate first order rate constants and half-lives for each chemical. The normalized feed concentrations used to calculate the half-lives for each chemical in the first seven beaker tests are summarized in Table 3. Table 4 shows similar data for the petri dish and water carrier tests. Note that tests 7 and 8 are not replicated, thus the normalized concentrations are not mean values as in the beaker tests. For the beaker tests (Table 3), the normalized concentration at each time period was obtained from a minimum of two and a maximum of five separate volatility tests. Each test chemical was tested at both high (ca. 300 µg/g) and low (ca. 50 µg/g) initial feed concentrations and the mean normalized concentrations shown in Table 3 represent data from at least two different initial test feed concentrations. The resulting low standard errors suggest concentration independence.

Table 3. Normalized feed concentrations (percent C_o)[a] at each sample time for the beaker tests.

Chemical	6 hr[b]	24 hr[c]	48 hr[b]	72 hr[c]	96 hr[c]
B2CEE	84 ± 6.5	71 ± 4.4	62 ± 3.0	58 ± 4.4	51 ± 4.1
1,4-DCB	82 ± 1.8	59 ± 1.9	40 ± 2.9	33 ± 3.7	21 ± 2.9
1,2-DCB	82 ± 8.3	63 ± 2.9	43 ± 4.2	37 ± 4.3	24 ± 1.2
HCE	77 ± 3.3	54 ± 5.3	44 ± 1.2	30 ± 4.1	18 ± 2.0
NB	94 ± 2.4	78 ± 2.1	72 ± 1.0	65 ± 2.0	61 ± 1.4
DMP	98 ± 12	95 ± 3.6	93 ± 5.7	93 ± 3.6	89 ± 1.2
4-NP	96 ± 0.6	90 ± 5.0	94 ± 6.6	83 ± 7.1	79 ± 4.1
2,4-DNT	95 ± 8.6	95 ± 2.7	90 ± 2.2	95 ± 8.0	90 ± 5.5
Fluor	98 ± 1.8	96 ± 2.0	93 ± 3.1	97 ± 5.8	92 ± 3.4
4-CPPE	98 ± 13	96 ± 5.0	94 ± 8.5	94 ± 4.3	91 ± 2.1

[a] Percent C_o is the measured concentration divided by the initial concentration.

[b] All values are means from two separate volatility tests, except for NB (n = 4).

[c] All values are means from three separate volatility tests, except for NB (n = 5).

The experimentally determined half-lives were greater than the 96-hour test period for all but three of the most volatile chemicals (1,4-DCB, 1,2-DCB, and HCE). Since the half-lives were quite large in some cases, thus subject to experimental error, another volatility parameter (96-hr % loss) was calculated for

Table 4. Normalized feed concentrations (percent C_o)[a] at each sample time for the petri dish and water carrier tests.

Water Carrier					
Chemical	6 hr	24 hr	48 hr	72 hr	96 hr
B2CEE	82	70	61	55	47
NB	87	82	78	75	66
DMP	88	96	88	96	72
4-NP	154	101	81	93	64

Petri Dish					
Chemical	6 hr	24 hr	48 hr	72 hr	96 hr
B2CEE	---	12	---	11	10
1,4-DCB	---	<1	---	<1	<1
1,2-DCB	---	<1	---	<1	<1
HCE	---	<1	---	<1	<1
NB	---	21	---	18	14
DMP	---	85	---	86	68
4-NP	---	85	---	88	82
2,4-DNT	---	92	---	98	87
Fluor	---	90	---	94	78
4-CPPE	---	90	---	95	80

[a] Percent C_o is the measured concentration divided by the initial concentration.

each chemical. The 96-hr % loss represents the percent of the initial concentration that is lost during the 96-hour test period and was calculated from the 96-hr $\%C_o$ value obtained from the regression data (log $\%C_o$ vs time) for each chemical. The experimental half-lives ($t\frac{1}{2}$), 96-hr % loss, and correlation coefficients (r^2) calculated from linear regressions of log $\%C_o$ vs time are shown in Table 5. For the beaker tests, greater variance in the regression data shows up in some of the chemicals having longer half-lives due to the variability in measuring very small changes in concentrations over the 96-hour test.

Nine of the 10 chemicals tested in the stirred petri dish experiment had greater volatility losses than in the static beaker tests (Table 5). Three chemicals (1,4-DCB, 1,2-DCB, and HCE) had negligible concentrations remaining after 24 hours, thus half-lives could only be estimated at less than 3 hours. The half-life and 96-hr % loss of 4-NP were approximately the same for both the static and stirred volatility tests. Static beaker half-lives for six of the less volatile chemicals averaged 2.6 times those of the stirred tests, while the three most volatile chemicals had half-lives approximately 15 times the estimated values in the stirred tests. Although the data from the single water carrier test may be inconclusive, the experimental half-lives and 96-hr % loss agree within a factor of two with those of the corn oil tests.

Table 5. Volatility rates of ten priority pollutants from test feed.

Chemical	$t_{1/2}$[a] (hr)	96 hr % loss	r^2[b]
		Beaker Test	
B2CEE	111	51	0.92
1,4-DCB	45.2	79	0.98
1,2-DCB	49.9	76	0.98
HCE	42.5	81	0.98
NB	137	42	0.94
DMP	660	11	0.92
4-NP	314	20	0.86
2,4-DNT	918	9.7	0.52
Fluor	1080	7.4	0.59
4-CPPE	816	8.9	0.92
		Stirred Petri Dish	
B2CEE	35.4	93	0.60
1,4-DCB	<3[c]	>99	---
1,2-DCB	<3	>99	---
HCE	<3	>99	---
NB	40.7	88	0.70
DMP	219	28	0.76
4-NP	459	18	0.58
2,4-DNT	772	9.8	0.39
Fluor	367	18	0.61
4-CPPE	424	16	0.56
		Water Carrier[d]	
B2CEE	99.2	54	0.94
NB	198	33	0.89
DMP	333	20	0.45
4-NP	170	29	0.65

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[a] $t_{1/2}$ is the experimentally determined half-life.

[b] r^2 for regression of log $\%C_0$ vs time.

[c] Half-lives were estimated at < 3 hours for 1,4-DCB, 1,2-DCB, and HCE since less than 1% of these chemicals remained after 24 hours.

[d] Only those chemicals having high water solubilities (> 1900 μg/ml) were tested.

A mass balance for the entire series of tests was calculated by adding the amount of each chemical recovered from the test feed and the activated charcoal trap and dividing by the total amount of each chemical initially measured in the fortified feed. Percent recoveries for all ten chemicals ranged from 87% for 4-NP to 98% for 4-CPPE (mean = 93%).

Figure 1 shows the correlation between the 96-hr % loss in the static beaker tests and log vapor pressure for each chemical (r^2 = 0.74). Regression analysis of the same data using half-lives instead of 96-hr % loss resulted in r^2 = 0.60 (r^2 = 0.86 if 4-NP is not included). In aqueous systems, Henry's law constants, which define chemical partitioning between an air/water interface, are commonly used in volatility modeling (Lyman et al. 1982). The half-lives in water can theoretically be shown to be proportional to the inverse of Henry's law constants assuming the phase exchange coefficients are constant for each chemical (Lyman et al. 1982). The poor correlation between $t_{\frac{1}{2}}$ vs 1/H (r^2 = 0.01) and 96-hr % loss vs log H (r^2 = 0.39) suggests that the Henry's law constants based on aqueous solubilities are not applicable to the oil/air interface in the fortified feeds. Log Kow also shows a poor correlation (r^2 = 0.01) with 96-hr % loss.

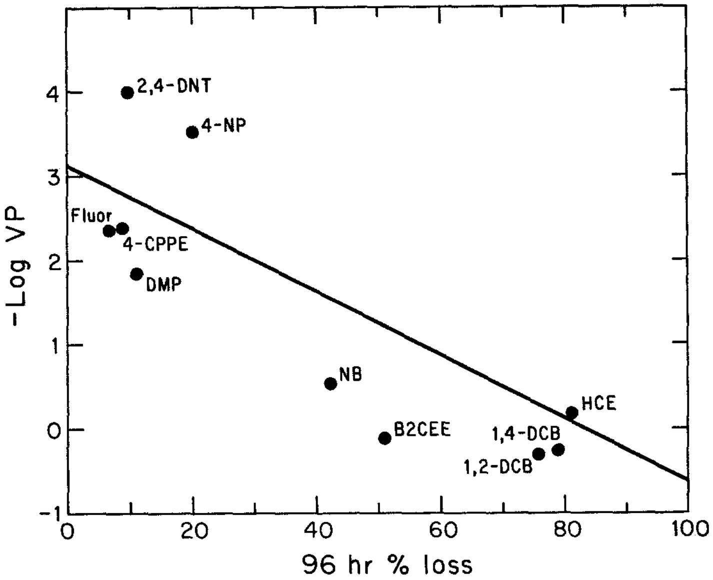

Fig. 1. Measured 96-hour % loss vs -log vapor pressure for each chemical in the static beaker tests (r^2 = 0.74).

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Received June 13, 1984; accepted July 30, 1984.