Retention of Vaporized Lindane by Plants and Animals

DAVID M. WHITACRE[1] AND GEORGE W. WARE[1]

Chickens, rats, mice, frogs, fish, and seven species of plants were exposed to vaporized lindane in a greenhouse cubicle for periods varying from 7 to 46 days. Nearly all animal tissues or organs and plants showed significant storage of lindane, including egg yolks from test chickens. Tableware and other inert surfaces bore varying amounts of lindane. The only test animals visibly affected by the vapor were the test fish, all of which died during the exposure.

Lindane, 99% gamma isomer of 1,2,3,4,5,6-hexachlorocyclohexane (also known as benzene hexachloride or BHC), in numerous formulations has been used to control insect pests and other arthropods since Slade (*10*) discovered its insecticidal properties in 1942. Volatilizing devices for lindane were available soon after this, and many types have since been used to disperse lindane to control flying and crawling insects in restaurants, poultry and dairy barns, grocery stores, and similar places. Recently, because their use may be harmful to humans, the U. S. Public Health Service was planning to recommend that the U. S. Department of Agriculture delete the registration of lindane vaporizers for nonprofessional use (*1*). Few investigators have examined the effects of lindane vapor on animals. This study explores that problem area and should contribute to needed data.

Slade (*10*) reported lindane solubility in water at 10 p.p.m., with 1 p.p.m. being sufficient to kill goldfish. Fulton, Nelson, and Smith (*4*) concluded that a lindane vapor concentration of 0.1 gram per 1000 cu. feet was generally sufficient for insect control. Sullivan (*11*) described a different dispersal method which involved passing hot air through a glass fiber air filter treated on both sides with lindane. Good insect control resulted, but some reservations regarding the potential vapor toxicity to humans were indicated.

Moore (*6*) reported that canaries showed poisoning symptoms, with one dying shortly after exposure to lindane vaporized from an electric thermal unit at a rate of 0.05 gram per 1000 cu. feet per day. Schulze, Sweetman, and Spear (*8*) found similar results of lindane poisoning in fish. They stated that illness presumably attributed to lindane was manifested by droopy fins, lethargic movements, hovering near the surface, and hypersensitivity. They concluded that lindane vaporizers should not be used where fish are housed.

Baker, Whitney, and Worden (*2*) published results of experiments involving the use of heat-volatilized insecticidal and acaricidal aerosols on small mammals, birds, and laboratory workers exposed from 178 to 386 days. The results indicated that mouse fur remained free of lindane, but budgerigars accumulated large amounts of the insecticide in their feathers and did not survive the highest concentration of 0.754 gram per 1000 cu. feet per day. No apparent effects on mammals or men were observed.

Ohio Agriculture Research and Development Center, Wooster, Ohio.

[1] Present address, Department of Entomology, University of Arizona, Tucson, Ariz.

Siakotos (*9*) reported that with three methods of application, vaporizing lindane was the most contaminating but the least persistent. He attributed this to the fact that sorbed lindane revaporized from food and other surfaces.

Materials and Methods

The primary objective of this project was to subject several species of animals and plants to thermally generated lindane, for designated lengths of time, while holding them in a chamber. Animals were sacrificed and plant samples taken periodically for tissue analysis to detect accumulated lindane residues. Settings of tableware and Petri dishes were also included in the experiment as surface samples on which vaporized lindane could collect and be measured.

A 12 × 13 foot greenhouse cubicle, of approximately 1750 cu. feet, with walls and ceiling constructed of glass panes in a wooden framework, was used as the test chamber. Concrete planting tables stood against three walls and supported the animal cages, plants, and other test materials.

The cubicle had no heat control and was ventilated for 2 hours every second day, to provide a generous interchange of air. During warm days the cubicle temperatures reached 90° F. or more, but night temperatures were reasonably constant.

To avoid contamination of the two adjacent greenhouse cubicles, large unseamed sheets of 0.006-inch polyethylene were used to cover the adjacent and front walls of the test chamber. Smaller strips were used to cover the planting tables. The edges were sealed with masking tape. Control animals, plants, and materials were kept under similar conditions in another cubicle separated from the test area by two cubicles.

Test animals included six of each sex of the BALB/c strain of white Swiss laboratory mouse; all were young adults in good health, and caged in standard pair galvanized steel cages, provided with half-pint water bottles and metal dip tubes. Four each of young adult male and female laboratory rats were also used. The cages consisted of the standard pair rat cage with the aforementioned drinking apparatus. Females and males were separated except for two mated pairs of mice, one each in the test and control chambers.

Six laying white leghorn hens were housed, three each in the test and control cubicles, in three-unit battery cages provided with a standard laying ration and water.

Six adult leopard frogs were placed three each in two plastic refrigerator trays containing water, and were covered with wire-mesh tops. The trays were placed in

control and test chambers on day 7 of the experiment.

Nine guppies and 13 2½-inch carp were also used in this investigation. Three guppies and seven carp were contained together in a 5-gallon aquarium equipped with an electric aerator, and three carp and three guppies were placed in a 4-gallon glass cut-carboy without an aerator. Three carp and three guppies were used as controls and held in an unaerated 4-gallon carboy. The aquaria were cleaned, and fresh water was added every 2 weeks. Aquatic plants were present in each of the two unaerated cut-carboys.

To measure lindane accumulation in plants subjected to continuous vapor exposure, leaf samples were taken periodically from seven species of plants growing in a soil and sand mixture in standard, 8-inch earthenware pots. Only three plant species were held in the control chamber and were not introduced until the fourth day. Four plants were duplicated in the test chamber.

Surface samples consisted of porcelain dinner plates, porcelain cups, drinking glasses, and stainless steel tableware. Two plates were stacked to ascertain the amount of lindane that might accumulate on the lower plate.

Petri dishes were also used for surface samples, 11 each for the control and test series. These were stripped periodically with hexane to determine the lindane accumulation.

Except for the frogs, control plants, and one pair of mice, all materials were introduced into the test chamber at least 1 week before the initiation of the test.

The day vaporization began, all animals except fish and frogs were weighed and identified for comparison with weights taken at the termination of the experiment.

The vaporizing device was a Junior Vapo-Swat thermal generator (Vapor Chemical Co., River Junction, Mich.), designed to treat spaces less than 5000 cu. feet. It was heated by a chrome wire resistor element equipped with a fuse and a pilot light, and held a 7-gram lindane disk that fitted snugly into the thermal cup. The apparatus was suspended 6½ feet above the floor and 3 feet from the ceiling of the test chamber.

The duration of the test was 46 days. Test chickens were sacrificed on days 15, 30, and 46; mice on days 15, 30, and 37; rats on days 15 and 30; and frogs on days 8, 23, and 38. All test animals had control counterparts which were sacrificed at the same time. The test fish died between days 8 and 19, and were frozen immediately, while control fish were sacrificed for analysis on day 46. Hen eggs were gathered daily, identified, and refrigerated.

Approximately 2 grams each of liver, brain, fat, and kidneys or the total amount, when less then 2 grams, was taken from chickens, frogs, rats, and mice, weighed, and frozen in vials for extraction. Fish were wrapped in aluminum foil and frozen.

The extraction method varied with the type of tissue, and was chosen on the basis of recovery, clean samples, and ease of extraction. The method of de Faubert Maunder *et al.* (*3*) was used for extraction of hen tissues. Briefly, each sample was ground into a fine dry powder with anhydrous sodium sulfate, transferred to a 150-ml. beaker, and the mortar washed with 50, 20, 20, and 10 ml. of hexane into the beaker and allowed to simmer for 2 minutes. After cooling, the solution was decanted into a 100-ml. volumetric flask and brought to the mark. Suggested cleanup of the samples using dimethylformamide was unnecessary. A 25-ml. portion was refrigerated for later analysis by electron capture gas chromatography. Recovery studies on livers from two control hens indicated a recovery rate of 92.5 and 91.5%.

An acetone-hexane extraction was used for mice, rats, frogs, and fish. The sample organ or entire fish was ground with five times its weight of anhydrous sodium sulfate. This was extracted three times with a total of 25 ml. of redistilled acetone in the mortar. Five milliliters of distilled water and 10 ml. of hexane were added to the 60-ml. separatory funnel, and the layers were allowed to separate. The acetone layer was reextracted with two 5-ml. portions of hexane.

Recovery of lindane from mouse brain, liver, fat, and kidney was 86, 85, 83, and 86%, respectively. Fish were cut into small pieces and blended in a Waring microblender with 20 to 30 ml. of redistilled acetone for 5 minutes. The homogenate was then filtered through glass wool and extracted as described above.

The eggs were extracted by the method of Olney, Donaldson, and Kerr (*7*) using only the yolk. Each yolk was combined in a microblender with 2-ml.-per-gram aliquots of redistilled acetonitrile. After the mixture was blended for 5 minutes, another equal volume of acetonitrile was added and blended for 5 minutes, after which the extracted yolk was filtered and the lindane partitioned into hexane. Two trial extractions with this method resulted in 90 and 85% recoveries.

Table I. Residues in Eggs and Tissues from Chickens Exposed to Vaporized Lindane, P.P.M.[a]

	Day										
	4	8	12	15	18	23	27	30	37	41	46
Eggs											
1	0.003	0.074	0.35		0.65	1.40	0.90	1.03	1.32	0.82	1.20
2	0.0045	0.066	0.19		0.63	0.70	0.92	0.71	0.80	[b]	
Brain				0.016				0.57			0.11
Liver				0.125				0.52			0.68
Fat				1.18				2.76			11.0
Kidney				0.04				0.198			0.50

[a] No residues detectable in controls at any time.
[b] Chicken sacrificed for tissue analysis.

Samples from both test and control plants were taken each week beginning with day 7. Leaves were removed, weighed, and frozen in vials.

Every 4 or 5 days, the test and control Petri dishes were rinsed twice with 10-ml. volumes of hexane which were combined in screw-cap vials and refrigerated.

Tableware was sampled each week while the stacked plates, upturned glass, and cup were taken the second and fifth weeks. The plate, cup, and glass were each rinsed with two 10-ml. portions of hexane which were combined in vials and refrigerated. Each knife, fork, and soup spoon was rinsed with 7 ml. of hexane; the rinse solutions were combined and refrigerated. Two control sets of tableware were sampled on days 11 and 37.

Plants were extracted in the sample vials by standing overnight in 20 ml. of redistilled benzene. The leaves were removed and enough anhydrous sodium sulfate was added to dry the benzene, which was refrigerated.

Table II. Residues in Mice and Rats Exposed to Vaporized Lindane, P.P.M.[a]

	Day				Day	
	15	30	37		15	30
	MICE				RATS	
Female				Female		
Brain	Trace[b]	0.0	Trace[b]	Brain	0.113	0.08
Liver	0.17	0.117	0.09	Liver	0.064	0.04
Fat	0.71	0.29	0.85	Fat	1.10	1.35
Kidney	0.015	0.0	0.18	Kidney	0.09	0.156
Male				Male		
Brain	0.0	Trace[b]	Trace[b]	Brain	0.05	0.08
Liver	0.065	0.05	0.155	Liver	0.94	0.081
Fat	0.55	1.73	2.30	Fat	0.68	1.25
Kidney	0.0	0.127	0.36	Kidney	0.60	0.60

[a] No residues detectable in controls at any time. [b] Less than 0.005 nanogram peak.

Table III. Residues in Fish and Frogs Exposed to Vaporized Lindane, P.P.M.[a]

	Day[b]							
	8	11	15	17	18	19	23	38
				FISH				
Carp								
Aerated								
1	...	4.4	...	...	...	...	...	...
2	...	6.2	...	...	...	...	...	...
3	...	...	6.8	...	...	...	...	...
4	...	...	...	2.5	...	...	...	...
5	...	...	...	3.2	...	...	...	...
6	...	...	...	6.8	...	...	...	...
7	...	...	...	...	...	9.8	...	...
Unaerated								
1	3.1	...	...	...	...	...	...	...
2	...	4.2	...	...	...	...	...	...
3	...	4.8	...	...	...	...	...	...
Guppies								
Aerated								
1	...	6.5	...	...	...	...	...	...
2	[c]							
3	[c]							
Unaerated								
1	...	4.2	...	...	...	...	...	...
2	...	6.5	...	...	...	...	...	...
3	...	1.9	...	...	...	...	...	...
				FROGS				
Brain	...	...	...	...	3.7	...	4.3	55.5
Liver	...	...	...	...	6.5	...	26.0	53.0
Fat	...	...	...	...	10.0	...	7.5	None

[a] No residues detectable in controls at any time.
[b] Fish died on days indicated.
[c] Specimens not recovered.

The tableware and Petri dish extracts required only the addition of sodium sulfate before analysis.

A Model 15 Barber-Colman gas chromatograph equipped with a tritium source electron capture detector and a single point recorder with 1/8-inch-per-minute chart speed was used for the analyses. The U-shaped borosilicate glass column, 6 feet × 1/4-inch i.d., was packed with 5% Dow 11 on Chromosorb W. Prepurified nitrogen, maintained at 100 ml. per minute and passed through a molecular sieve, was the carrier gas.

Results and Discussion

The results indicate that lindane residues were present in every animal and plant exposed to the vapor. Generally the residue in an animal or plant and the duration of exposure were correlated.

Residues in egg yolks and chicken tissues are shown in Table I. Eggs from only two of the three test chickens were selected, since only two were laying regularly. Tissue residues generally increased with length of exposure.

Residues in mice and rats are shown in Table II. In the experiment one female control rat and one female test mouse died, probably because of the high temperature in the cubicles resulting from direct sunlight.

Residues in the fish and frog tissues are shown in Table III. Guppies and carp were the only test species affected in their behavior by vapor contact, since abnormal behavior was never observed in the controls. The test carp showed characteristics of poisoning on day 7, primarily hyperactivity, resulting many times in random swimming and collision with the aquarium wall. Symptoms of terminal poisoning were floating on their sides and swimming downward when touched only to drift slowly to the surface. Following these reactions the fish usually died within 24 hours. The residues in frog tissues generally increased with length of exposure. Of the various animals tested in this investigation, frogs had the greatest residues. This may be explained by the frogs' refusal to eat, thus lowering their metabolism and excretory rates, which may have lowered the metabolism and excretion of lindane.

Plant residues are shown in Table IV. Of the plants analyzed, the wheat and red kidney bean showed the

Table IV. Residues on Plants Exposed to Vaporized Lindane, P.P.M.[a]

	Day										
	7	11	15	17	21	26	30	33	37	40	44
Red kidney bean 1	5.2	...	9.7	...	21.4	...	48.0	...	70.0	...	17.0
Red kidney bean 2	...	8.7	...	15.0	...	31.0	...	40.0	...	202.0	...
Cucumber 1	4.0	...	14.5	...	19.6	...	42.0	...	...	...	...
Cucumber 2	3.6	...	16.1	...	...	...	...	...	...	...	...
Wheat 1	4.3	...	14.9	...	8.3	...	35.0	...	19.7	...	319.0
Wheat 2	...	24.2	...	26.0	...	30.0	...	66.5	...	342.0	...
Tomato 1	6.5	...	12.2	...	17.0	...	25.0	...	41.0	...	138.0
Tomato 2	...	8.9	...	18.0	...	17.0	...	22.0	...	124.0	...
Fava bean	5.7	...	17.9	...	16.0	...	33.0	...	33.4	...	98.0
Potato	1.9	...	6.1	...	7.1	...	19.5	...	31.0	...	91.0
Begonia	3.0	...	4.0	...	4.1	...	7.3	...	19.0	...	18.0

[a] No residues detectable in controls at any time.

Table V. Residues on Tableware and Petri Dishes Exposed to Vaporized Lindane, μg. per Object and Ng. per Sq. Cm., Respectively[a]

	Day													
	7	11	17	21	23	25	31	35	37	38	41	44	45	46
Petri dishes	4.5	13.5	22.6	8.0	...	16.0	6.8	23.8	...	52.0	57.0	68.0	...	54.0
Tableware														
Plate	4.0	4.8	100.0	...	1280.0	...	...	...	128.0	...	...	...	200.0	...
Glass	0.22	0.64	0.02	...	14.0	...	...	...	0.90	...	...	...	3.2	...
Silverware	0.82	3.0	0.80	...	4.4	...	...	...	4.0	...	...	...	15.2	...
Cup	0.34	0.90	0.32	...	100.0	...	...	...	2.8	...	...	...	3.2	...
Stacked tableware														
Plate	...	0.9[b]	...	...	...	...	...	...	2.0[b]	...	...	...	...	...
Glass	...	0.08[c]	...	...	...	...	...	...	0.34[c]	...	...	...	...	...
Silverware	...	3.4	...	...	...	...	...	...	4.4	...	...	...	...	...
Cup	...	0.01[c]	...	...	...	...	...	...	0.24[c]	...	...	...	...	...

[a] No residues detectable in controls at any time.
[b] Beneath the test plate taken on the same day.
[c] Inverted position.

greatest residue on day 44. One aspect to be considered when expressing leaf residues in parts per million is the relative area per unit weight. For example, the begonia is much heavier per unit leaf area than wheat or red kidney bean and had a much lower residue.

Lindane residues on the Petri dishes and tableware are shown in Table V. The increase in residue shows a linear relationship with time. However, residues that accumulated on such surfaces also appeared to be dependent upon the temperature within the chamber. Two reasons can be given: As the temperature within the chamber rose above 90° F. the lindane pellet liquefied and vaporized at an increased rate, resulting in increased atmospheric lindane. Lindane vapor that has condensed or solidified as crystals on the surfaces in the chamber will revaporize slowly at normal room temperatures, but at an increased rate on warm days. These factors would promote a higher lindane deposition on surfaces as the chamber cools and the vapor condenses on the exposed surfaces.

The basis for the stacked plate test is the restaurant procedure of stacking and considering the covered plates to be clean even after long storage periods. The same restaurant practice was used when glasses and cups were stored in an inverted position before determining the amount of lindane that accumulated on the inner unexposed surface.

Conclusions

Previous investigators who tested thermal generators (*2*, *5*, *11*) recommended that vaporizers should not be used outside the restrictions set by law. It was felt that these units, if used outside the range of original intention, could cause unwarranted toxic effects.

Although lindane residues in the organs of the test animals, with the exception of fish, were below levels that proved to be toxic, the length of exposure was relatively short. Since the effects of low concentrations of insecticide residues in animals or humans, over extended periods of time, are not known with certainty, unfortunate effects could develop. As is the case with pesticides in general, applied in any manner, no absolute statement can be made with respect to safe usage, and therefore extreme caution must be exercised when using thermal vaporizers around living organisms, other than the target arthropods.

Literature Cited

(1) *Agr. Chem.* **21,** 15 (1966).
(2) Baker, A. J., Whitney, G. F. H., Worden, A. W., *Lab. Practice* **26,** 3 (1959).
(3) de Faubert Maunder, M. J., Egan, H., Godly, E. W., Hammond, E. W., Roburn, J., Thomson, J., *Analyst* **89,** 168 (1964).
(4) Fulton, P. A., Nelson, R. H., Smith, F. F., *J. Econ. Entomol.* **43,** 223 (1950).
(5) Hays, W. J., Jr., Chem. Specialties Mfrs. Assoc., Proc. 39th Mid-Yr., pp. 95–7, May 1953.
(6) Moore, S., *J. Econ. Entomol.* **45,** 1092 (1952).
(7) Olney, C. E., Donaldson, W. E., Kerr, T. W., *Ibid.*, **55,** 477 (1962).
(8) Schulze, Faith, Sweetman, H. L., Spear, P. J., *Ibid.*, **45,** 1094 (1952).
(9) Siakotos, A. N., *Ibid.*, **49,** 212 (1956).
(10) Slade, R. E., *Chem. Ind.* (*London*) **40,** 314 (1945).
(11) Sullivan, W. N., *J. Econ. Entomol.* **44,** 125 (1951).

Received for review October 17, 1966. Accepted February 10, 1967.