The Effect of an Accidental Exposure of Broiler Chicks to Dieldrin

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An abnormally high death rate of two shipments of chicks introduced into a local poultry farm shortly after treatment of the poultry houses with dieldrin prompted an investigation by the State Veterinary Laboratory and the Agricultural Biochemistry Department of the University of Hawaii. The results of a pathological and chemical study of the affected birds are the substance of this report.

History of Incident: An 18% emulsifiable concentrate of dieldrin, diluted 1:50, was sprayed on all parts of six poultry houses three days before chicks were placed in the houses, to control a termite infestation. This was the first time dieldrin was used on this farm. The houses contained no litter material, feed, or drinking water at the time of the spray operation.

The first shipment of 8,320 day-old chicks placed in House No. 1 had a mortality of 4.4% during the nine-day period after receipt of the chicks; the greatest number dying on the fifth and

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sixth days (Table I). Although unusual neurological signs were present, the poultryman attributed the excessive loss (normal mortality is about 0.1% per week for a brood this size) to environmental stress factors associated with relatively unfavorable delivery conditions compounded with the stress of fowl pox vaccination on the day the chicks were received.

Five days later, a second shipment of day-old chicks was placed in House No. 2. The poultryman considered these chicks to be in excellent condition. Nevertheless, this group of 5,720 chicks had a mortality of 12.4% during the next nine days (Table I), and again the highest rate was on the fifth and sixth days.

Five days after the introduction of this second shipment into House No. 2, samples of live and dead birds were forwarded to the State Veterinary Laboratory. The majority of live chicks exhibited acute signs of central nervous system involvement - staggering and twisting of their heads and necks, falling on their sides and backs, fluttering their wings, and thrashing their legs violently in the air. Affected chicks succumbed within 5 to 10 minutes once the convulsive state was reached.

Pathology: Dead chicks only were submitted from the first shipment, and because they were badly decomposed, examination was limited to gross observations. Non-specific umbilical infection, peritonitis, and focal pustular dermatitis at the site of wing-web fowl pox vaccination were the only remarkable lesions found in one-third of the chicks examined.

TABLE I

Mortality Data on Chicks

House No.	Delivery Date	No. of Chicks Delivered	Mortality Day 1	2	3	4	5	6	7	8	9	Total Mortality	% Mortality
1	8-5-65	8,320	0	2	3	22	153	136	25	20	3	364	4.4
2	8-10-65	5,720	0	12	0	10	267	280	118	23	2	712	12.4
3	8-17-65	11,444	0	3	4	2	5	3	4	1	0	22	0.2
4	8-23-65	7,280	2	1	2	3	2	4	1	4	2	21	0.3
5	8-31-65	11,444	3	2	1	4	3	5	2	0	2	22	0.2
6	9-8-65	5,200	2	1	2	0	2	2	0	1	2	12	0.2

Similar observations were made on some of the chicks from the second shipment. However, the majority was unaffected by the lesions described above. The remarkable gross lesions found at necropsy, and confirmed by histologic studies, were congestion of the lungs and liver. Histologically, no organs, including the brain, revealed evidence of an infectious process nor any of the known neurological diseases affecting young chicks, such as Newcastle disease, avian encephalomyelitis or vitamin E deficiency (neuroencephalomalacia). Hemagglutination-inhibition tests for Newcastle disease were negative at this time and convalescent sera obtained from the affected groups three weeks later were also negative.

Discussion of Pathological Study: Umbilical and vaccinal infections are common in chicks raised under local conditions, but they have never been associated with disorders of the central nervous system; and, they were considered to be totally unrelated to the neurological syndrome described above.

Congestive changes of the lungs and liver may be related to numerous causes, including intoxications with chlorinated hydrocarbons (15). Brain lesions due to chlorinated hydrocarbons have been described as obscure or absent (10, 15). None of the lesions are pathognomonic for dieldrin intoxication. Therefore, analysis for pesticides was undertaken.

Pesticide Residue Analysis: Acetonitrile, hexane, and petroleum ether were redistilled. Ethyl ether, ethanol, potassium hydroxide,

and sodium sulfate were reagent grade. Florisil, 60/100 mesh (Floridin Co., Hancock, W. Va.) was heated 24 hours at 130°C prior to use. Gas chromatography analyses were made on a MicroTek MT 220 (columns 6' x 1/4" glass, 5% QF-1 on Gas Chrom Q; 10% DC-200 on Chromport XXX; inlet temp. 223°C, column 198°C, detector 193°C; flow rate 70 ml/min) and an Aerograph Hy-Fi 600-D (column 5' x 1/8" glass, 5% Dow-11 on Chromosorb P; oven temperature 200°C; flow rate 100 ml/min), using an electron capture detector and nitrogen carrier gas.

Procedure: Dead birds from the August 10 shipment, and samples of chickens taken from the houses at intervals over a period of weeks after the incident, were analyzed for pesticide residues. Chickens were obtained from another farm, which had no prior history of dieldrin usage, and were analyzed for pesticide residues.

Wood shavings, used as a floor covering and also as bedding material for the chickens, are replaced in the houses with each new shipment of chicks. Shavings, feed samples, and scrapings and chips from the interior wooden walls were obtained from Houses 1, 2, and 3 at the time the incident occurred. Additional shavings and scrapings were obtained from all six houses, seven months later. The gross feed supply and the "clean" shavings stockpile, stored in an area remote from the houses, were also sampled.

The liver, kidney, and portions of the body fat and tissue were removed from each bird. One to 5 grams of tissue, an equal amount of anhydrous sodium sulfate, and 30 ml of hexane were mixed

in an Omnimixer, for 3 minutes, and the mixture was filtered. The tissue was extracted three additional times with 30 ml of hexane each time. The hexane extracts were combined, concentrated on a steam bath, the residue was transferred with petroleum ether to a 100-ml volumetric flask and made up to volume. Fifty ml of the extract was added to a Florisil column prepared as described by the FDA (5). The column was eluted with 200 ml of 6% ethyl ether in petroleum ether. The eluate was concentrated on a steam bath, and made to a suitable volume for gas chromatography. The remaining 50 ml of the extract was evaporated to dryness in a beaker, 20 ml of 25% potassium hydroxide in ethanol (freshly prepared) was added, the beaker was covered with a watch glass, and the mixture was gently refluxed for 30 min. on the steam bath. The mixture was cooled, transferred to a 250-ml separatory funnel, 10 ml hexane was added, and the mixture was shaken thoroughly. The aqueous phase was removed and was extracted three more times with 10 ml of hexane each time. The hexane extracts were combined, washed once with distilled water, dehydrated with sodium sulfate, and made to a suitable volume for gas chromatography. Feed components and ingested shavings were removed from the digestive tracts of some of the chickens and processed in the same manner. The 6% ethyl ether fraction was examined for lindane, aldrin, DDE, DDD, and DDT. The saponified fraction was examined for DDE and dieldrin. Infrared absorption analysis was used to confirm the identity of the dieldrin isolated from the tissues.

Shavings, poultry rations, and wood samples were refluxed 30 min. with acetonitrile. The extracts were partitioned with petroleum ether, and the ether fraction was subjected to the Florisil cleanup procedure, then analyzed for dieldrin.

Samples of tissues and shavings were fortified with dieldrin, to check the reliability of the analytical procedure. Recovery of the pesticide from tissue ranged from 91% to 99%; from the shavings, 85% to 100%.

Results: The dieldrin residues found in the chicks placed in House No. 2 on August 10, 1965 are given in Table II.

TABLE II

Dieldrin Residue in Tissues

From 5-Day-Old Chicks Obtained from House No. 2, Two Weeks After Treatment of House with Dieldrin[a]

Sample No.	Liver	Kidney	Body Tissue	Body Fat
	p.p.m.	p.p.m.	p.p.m.	p.p.m.
1	21.5	5.3	4.6	3.4
2	18.6	6.3	1.7	133.3
3	3.6	2.1	5.2	357.1
4	5.6	2.4	24.6	181.8
5	12.8	7.6	8.0	450.0
6	16.8	7.9	12.6	480.0
7	34.9	13.8	14.6	600.0
8	15.2	14.0	5.5	537.5
9	2.7	12.7	10.5	866.6
10	16.4	10.7	10.4	397.0
Mean	14.8	8.3	9.8	400.7
Minimum	2.7	2.1	1.7	3.4
Maximum	34.9	14.0	24.6	866.6
N=10				

[a] Each sample a composite of 2 chicks.

The chickens that survived exposure to dieldrin in House No. 2 showed relatively high residues in the body fat after 10 weeks of growth (Table III). The chickens placed in the houses at subsequent intervals of time after dieldrin treatment of the houses contained smaller but, in some cases, significant amounts of dieldrin residue. The feed components from the digestive tracts of the chickens contained dieldrin residues in the range of 3 to 20 p.p.m.; ingested shavings contained dieldrin in the range of 4 to 35 p.p.m. The one-week-old and nine-week-old chickens obtained from the uncontaminated farm contained no dieldrin residue. All of the chicken tissues contained lindane, DDE, and DDT, which ranged from 0.01 to 3.5 p.p.m.

TABLE III

Dieldrin Residue in Chicken Tissue

Age of Chickens	House No.	Sample No.	Liver	Kidney	Body Tissue	Body Fat
			p.p.m.	p.p.m.	p.p.m.	p.p.m.
10 Weeks[a]	2	1	1.42	0.81	0.80	16.20
		2	1.52	2.00	0.13	40.00
		3	1.00	0.67	0.18	12.50
8-15 Days[b]	1	1	0.11	0.04	0.02	1.86
		2	0.16	0.04	0.04	1.58
	2	1	0.11	0.03	0.06	1.52
		2	1.24	0.62	0.22	7.81
10 Weeks[c]	3	1	0.10	0.10	0.00	1.30
		2	0.04	0.36	0.04	0.00
		3	0.00	0.05	0.00	0.00
		4	0.06	0.01	0.01	0.22

[a] Obtained 2-1/2 months after treatment of house with dieldrin. Chickens were survivors of acute intoxication incident of August 10, 1965; see Tables I and II.

[b] Obtained eight months after treatment of house with dieldrin.

[c] Obtained seven months after treatment of house with dieldrin.

All of the feeds, shavings, and wall scrapings from the houses contained dieldrin residue (Table IV). The feed and shavings samples obtained from the area remote from the houses did not contain any dieldrin residue.

TABLE IV

Dieldrin Residue (p.p.m.) in Feed, Shavings, and Wall Scrapings from Poultry Houses

	Date of Sample Collection	House No. 1	2	3	4	5	6
Feed	8-19-65	0.75	0.91	0.74	*	*	*
Shavings	8-19-65	32.6	44.8	20.6	*	*	*
	3-10-66	3.0	1.8	1.1	1.0	1.2	0.6
Wall Scrapings	8-19-65	232.1	332.1	118.0	*	*	*
	3-10-66	6.4	6.1	16.3	6.9	5.4	26.8

* No sample available.

Discussion: The large amounts of dieldrin residue found in the chicks of the August 10, 1965 shipment suggest a correlation to the high mortality rate of this shipment. The maximum daily death rate that occurred between the fifth and seventh days after initial exposure to the pesticide for both the August 5 and August 10 shipments corresponds to the observations of Sherman and Ross (14), Shellenberger, et al. (11), and Azevedo, et al. (2) in their studies on the effect of chlorinated pesticides on chicks, quail, and pheasants. The correlation is strengthened by the pathological observations concerning the symptoms displayed by the dying chicks. These symptoms were essentially the same as those

described by Sherman and Rosenberg (12, 13) in their toxicity studies employing aldrin and dieldrin, and to those described by Rosenberg and Adler (9) for DDT intoxication. In this study, the amounts of DDT, DDE, and lindane found were trace quantities, calculated on the whole-chicken basis, and may have been present in the eggs (6, 7).

The data in Table IV show that the feed and shavings placed in the houses absorbed dieldrin from the environment. It has also been shown that the chickens ingested dieldrin-contaminated shavings. It can be assumed, therefore, that the chickens absorbed dieldrin by at least three different routes, feed intake, ingested shavings, and bodily contact with the shavings, the floors, and the walls.

A third shipment of 11,000 chickens, received one week after the August 10 shipment, was placed in House No. 3. No drastic losses were experienced with this group or with three subsequent groups, each received at intervals of approximately one week later for each group. The data in Table IV indicate that House No. 3 was not as thoroughly saturated with dieldrin as were Houses 1 and 2. Also, more than two weeks had lapsed from the time the houses were treated with dieldrin to the date that chicks were placed in House No. 3. The dieldrin concentration in this environment had apparently decreased to a point where the daily intake by the chicks was not sufficient to be fatally toxic. Houses No. 3 and 5 contained asphalt floors; the other houses had wooden floors. The

asphalt floors were not sprayed with dieldrin, and as a result only run-off material could have contaminated the floor surface. This, also, could partially explain the lower toxicity environment in these houses. Unfortunately, shavings and wall samples of Houses Nos. 4, 5, and 6 were not collected at the earlier date, and no comparable data were obtained.

Chickens placed in the houses five and eight months later continued to absorb dieldrin from this confined environment (Table III). Wall scrapings obtained seven months after treatment of the houses contained appreciably smaller residues of dieldrin (Table IV). However, seven months after treatment, the environment contained enough dieldrin to impart residue to the shavings, and probably to the feed rations, and hence provided a continuous source for dieldrin absorption by the chickens.

Pesticide residue problems created by the misuse of a pesticide have been well documented in the recent literature. Ivey (8) reported lindane residues of 131 p.p.m. in the fat of chickens after one week and 97 p.p.m. after 16 weeks following the application of lindane to a poultry house. Stadelman and Liska (16) placed chickens in coops that had been sprayed 7 days earlier with a 5% DDT solution, and found that a sufficient amount of the pesticide penetrated the skin of the birds to deposit DDT residue in the body fat. Gannon, et al. (6) reported high residues of dieldrin in the fat of hens after a 12-week feeding experiment; and Cummings, et al. (4) noted that the decline of dieldrin is so

gradual in contaminated hens that it would not be practical for commercial poultry operators to hold the hens for egg production. These facts illustrate why the poultry associations (1) have recommended that dieldrin should not be used on a poultry farm. Also, following the report of the Food and Drug Administration on the finding of residues of DDT, DDD, and lindane, and benzene hexachloride in a number of egg samples, the pesticide control officials of the United States Department of Agriculture directed that instructions for use in and around poultry houses be dropped from government-registered labels for seven pesticides, lindane, DDT, DDD, benzene hexachloride, toxaphene, perthane, and dieldrin (3).

Dieldrin residues can persist for months in the area of application, and although the amount of residue may decrease markedly, over a subsequent period of time, poultry can continue to absorb the pesticide if they are confined in such an environment.

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