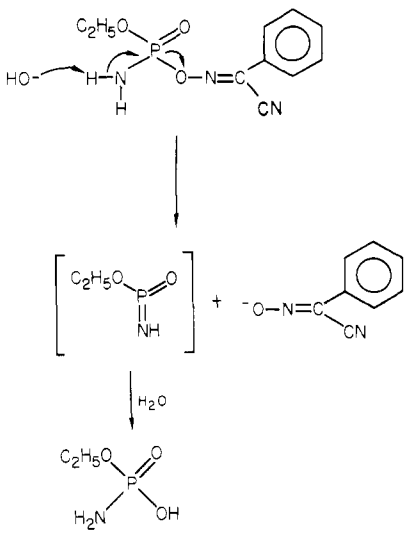

phosphorus center. Hydrolysis of **7** must take place by attack of hydroxide on the phosphorus but owing to their much larger rate constants, hydrolysis of **1** and **2** probably occurs by attack of hydroxide on the amido proton.

The α-cyanobenzaldoxime phosphoramidates were surprisingly strong inhibitors of AChE and activity appeared to be a function of both polar and steric effects. The relatively high anticholinesterase activity of the *N,N*-dimethyl analogue **7** was unexpected because of its low reactivity toward hydroxide ion.

The large differences in housefly and mouse toxicity observed for compounds **4**, **9**, and **10** cannot be rationalized in terms of differences in inhibition between housefly-head and mammalian AChE since the differences in enzyme inhibition were more or less the same with all compounds. The toxicity differences probably are attributable to differences in rates and routes of detoxication in houseflies and mice, as suggested for the related diethyl ester analogue phoxim (Vinopal and Fukuto, 1971).

The interesting toxicological properties of the α-cyanobenzaldoxime phosphoramidates justify further examination of compounds of this type as potential insecticides.

LITERATURE CITED

Ellman, G. L., Courtney, K. D., Andres, V., Jr., Featherstone, R. M., *Biochem. Pharmacol.* **7**, 88 (1961).
Fahmy, M. A. H., Khasawinah, A., Fukuto, T. R., *J. Org. Chem.* **37**, 617 (1972).
Fujita, T., Iwasa, J., Hansch, C., *J. Am. Chem. Soc.* **86**, 5175 (1964).
Fukuto, T. R., Metcalf, R. L., *J. Agric. Food Chem.* **4**, 930 (1956).
Fukuto, T. R., Metcalf, R. L., Winton, M. Y., March, R. B., *J. Econ. Entomol.* **56**, 808 (1963).
Hansch, C., in "Drug Design", Vol. I, Ariens, E. J., Ed., Academic Press, New York, N.Y., 1971, p 271.
Hansch, C., Deutsch, E. W., *Biochem. Biophys. Acta* **126**, 117 (1966).
Hansch, C., Steward, A. R., Iwasa, J., *Mol. Pharmacol.* **1**, 205 (1965).
Hollingworth, R. M., Fukuto, T. R., Metcalf, R. L., *J. Agric. Food Chem.* **15**, 235 (1967).
March, R. B., Metcalf, R. L., *Bull. Calif. Dept. Agric.* **38**, 93 (1949).
Mel'nikov, N. N., Prokof'eva, A. F., Krylova, T. P., Vladimirova, I. L., *Khim. Org. Soedin. Fosfora, Akad. Nauk SSSR, Old-Obshch. Tech. Khim.*, 267 (1967); *Chem. Abstr.* **69**, 2629h (1968).
Perrot, R., *C.R. Acad. Sci.* **199**, 585 (1934).
Quistad, G. B., Fukuto, T. R., Metcalf, R. L., *J. Agric. Food Chem.* **18**, 189 (1970).
Rorig, K., Johnston, J. D., Hamilton, R. W., Telinski, T. J., "Organic Synthesis", Collect. Vol. IV, Wiley, New York, N.Y., 1963, p 576.
Sanborn, J. R., Fukuto, T. R., *J. Agric. Food Chem.* **20**, 926 (1972).
Schonne, A., Braye, A., Bruylants, A., *Bull. Soc. Chim. Belges* **62**, 155 (1953).
Taft, R. W., Jr., in "Steric Effects in Organic Chemistry", Newman, M. S., Ed., Wiley, New York, N.Y., 1956, p. 556.
Traylor, P. S., Westheimer, F. H., *J. Am. Chem. Soc.* **87**, 553 (1965).
Vinopal, J. H., Fukuto, T. R., *Pestic. Biochem. Physiol.* **1**, 44 (1971).

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# Toxicity, Accumulation, and Depletion of Hexachlorobenzene in Laying Chickens

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Laying pullets were given seven consecutive daily oral doses of hexachlorobenzene (HCB) ranging from 1 to 100 mg/kg. At higher doses, HCB delayed the onset of full egg production but also appeared to offer protection against development of hemorrhagic fatty liver. Relative accumulation of HCB in fat was inversely related to dose, but elimination from fat was constant for residue concentrations from 3–800 ppm. Elimination half-time from fat was estimated at 24–27 days and more than half of the elimination was due to parent compound excreted in egg yolks. Other tissues generally paralleled fat, but skin became an increasingly significant reservoir and liver concentrations remained rather constant.

Hexachlorobenzene (HCB) is a chlorinated hydrocarbon dispersed in the environment and in animal feeds (Burns and Miller, 1975; Yang et al., 1976). HCB has a low acute toxicity, but causes a variety of ill effects during and after prolonged exposure (Booth and McDowell, 1975).

Feeding studies in poultry indicate that concentrations up to 100 ppm HCB do not produce notable ill effects, but residue accumulation is quite marked (Avrahami and Steele, 1972a,b; Reed et al., 1977a,b). The following study was undertaken using a short-term, more intensive dosing

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regimen in order to determine the effects on toxicity and to permit evaluation of residue depletion patterns.

## MATERIALS AND METHODS

**Experimental Design.** Forty-five 20-week-old white leghorn pullets (hiline cross) were obtained from a commercial source, divided into 15 groups of three each, and wing-banded for identification. Six groups were designated to be terminated 50 days following the first of seven daily oral doses of 0, 1, 5, 10, 14, or 100 mg of HCB/kg body weight. Nine additional groups receiving 1, 10, or 100 mg of HCB/kg (three groups of three birds/dose) were designated for biopsy and intermediate kill dates. The 14 mg/kg group was to be dosed with 25 mg/kg, but an error in dose measurements resulted in a total of $[(4 \times 5 \text{ mg/kg}) + (3 \times 25 \text{ mg/kg})/7] \simeq 14$ mg/kg.

Purified HCB was weighed into gelatin capsules, basing the dose on pullet weights 3 days after arrival. A small amount of olive oil (0.2 mL) was added to each capsule to aid in dispersion of HCB. After a 1-week acclimation, the pullets were given the encapsulated HCB every morning for 7 consecutive days.

Fresh water and weighed quantities of a standard layer ration determined to be free from chlorinated hydrocarbon contamination were provided ad libitum. The birds were housed in banks of individual layer cages and egg production was monitored for all birds designated to survive 6 weeks or more after the final dose.

For birds scheduled for termination on days 43 and 71, all eggs were collected the week prior to termination. Consecutively dated eggs were analyzed for HCB residues.

Four days prior to the 71-day termination, 24-h total excrement was collected from each bird.

Twenty-four hours after the final dose (day 1), three birds each from the 1, 10, and 100 mg/kg dose groups were terminated by cervical dislocation and exsanguination. Birds were examined for gross pathological changes and major organs and tissues were weighed and divided into 2.00-g aliquots for residue analysis. Forty-three days after the final dose, three birds from each of the six dose levels were terminated. Other groups of three birds from dose levels of 1, 10, and 100 mg/kg were similarly terminated 15 and 71 days following the final dose. Eight days following the final dose, dissectable abdominal fat biopsies (0.5 g) were obtained surgically from the birds to be killed 1 week later. Two biopsy samples (29 and 57 days) were obtained from each bird scheduled to be terminated 71 days after the final dose.

Liver fat content was determined on birds killed on days 15 and 43. Blood samples were drawn from wing veins into heparinized capillary tubes for determination of packed cell volume for birds killed on day 43.

**Materials.** Ninety-seven percent hexachlorobenzene (Aldrich Chemical Co.) was washed with warm absolute ethanol and recrystallized three times from hot benzene. The product was shown to be of greater than 99.5% purity by electron-capture gas–liquid chromatography.

Ottawa silica sand, granular $Na_2SO_4$, and NaCl were washed in boiling distilled acetone, dried by vacuum filtration, and baked at 200 °C for 4 h. Concentrated $H_2SO_4$ and 9 N aqueous NaOH were extracted with hexane. Alumina (Fisher Scientific Co. A540) was activated at 800 °C for 8 h and deactivated just prior to use by the addition of distilled water (5% w/w). Silica gel (Grace Chemical 950) was washed with hexane and activated at 120 °C. The silica gel was also deactivated by the addition of distilled water (5% w/w).

All solvents used were either distilled in all-glass apparatus or were of "pesticide grade" quality.

All analytical glassware and glass wool plugs were rinsed with distilled acetone before use.

**Methods.** Gas–liquid chromatography was performed using a Varian Aerograph Model 2700 equipped with a tritium electron-capture detector. HCB was analyzed on a 0.9 m × 6 mm o.d. × 2 mm i.d. glass column packed with 10% QF-1 on 80/100 mesh Gas-Chrom Q. The column head pressure was kept between 1.5–1.8 atm of $N_2$ carrier gas with injector, column, and detector temperatures set at 210, 175, and 225 °C, respectively. Quantitation was accomplished by using sequential standards in the range 5.00–40.0 ppb at an electrometer setting of $32 \times 10^{-10}$. Samples were appropriately diluted to fall within the linear range of the standard curve and peak heights were measured to the nearest 0.5 mm. Reagent blanks were simultaneously analyzed with each batch of samples, demonstrating that HCB contamination was generally 5 ppb or less per sample. The detection limit was generally 0.5 ppb or less, but the working detection limit was elevated to 10 ppb because of the background level.

The eggs were hard-boiled and yolk weights determined. Proportional aliquots of three yolks for the 43-day termination birds and two yolks for the 71-day termination birds were pooled for each chicken. Yolk (5.00 g) was thoroughly ground with 50 g of $Na_2SO_4$ and packed into a glass wool plugged 19 mm i.d. × 300 mm glass column with a 250-mL reservoir. The column was eluted with 250 mL of hexane into a 300-mL flask. The solvent was removed by flash evaporation to a volume of 3–5 mL. Ten milliliters of hexane was added to the flask, followed by 10 mL of hexane-washed concentrated $H_2SO_4$. The sample was swirled, and after 30 min, the sample was carefully transferred to a 500-mL separatory funnel containing 50 mL of hexane and 50 mL of 5% aqueous NaCl. The flask was rinsed with 50 mL of the NaCl solution and transferred to the separatory funnel followed by a hexane rinse of 50 mL. After the funnels cooled to room temperature, 41 mL of 9 N aqueous NaOH was very slowly added to the separatory funnel followed by gentle swirling. After the mixture cooled again, the pH of the aqueous layer was tested to see that it was slightly basic (pH 7.4–7.8). The aqueous layer was then extracted by the original volume of hexane followed by 2 × 75 mL of hexane. The hexane was dried over $Na_2SO_4$ and concentrated to 1–2 mL by flash evaporation. The sample was then quantitatively transferred to a 10-mL Kuderna-Danish concentrator tube and diluted to 10.0 mL with hexane. One (1.00) milliliter of the extract was chromatographed on 2 g of 5% deactivated alumina dry packed into a 6 mm i.d. × 200 mm glass wool plugged column with a 50-mL reservoir. The extract was adsorbed onto the column before the addition of 11 mL of hexane. The eluate volume was then adjusted for GLC analysis.

Emulsion formation must be avoided during the extraction in order to achieve reasonable recovery. Samples amended to 1–50 ppm HCB yielded an extraction efficiency of 92.5 ± 5.1% when emulsions were avoided. With emulsion formation, the recovery was only 52.2 ± 23.1% in the same concentration range.

Total 24-h feces were mechanically mixed and two distal 5.0-g samples were separately mixed with 50 g of sand–$Na_2SO_4$ (1:1). The mixture was added to the top of an additional 30 g of sand–$Na_2SO_4$ in a 19 mm × 300 mm column and eluted with 250 mL of hexane. The hexane extract was washed with 50 mL of 5% NaCl, dried over $Na_2SO_4$, and concentrated to 3–5 mL. The concentrated extract was then cleaned by alumina column chromatography as with the eggs.

Table I. Influence of Seven Daily Doses of HCB on Various Parameters in Layer Chickens Terminated 43 Days after Last Dose[a]

| parameter | daily HCB, mg/kg | | | | | |
|---|---|---|---|---|---|---|
| | control | 1 | 5 | 10 | 14 | 100 |
| body weight, g | 1572 ± 3 | 1510 ± 67 | 1465 ± 229 | 1544 ± 136 | 1538 ± 114 | 1595 ± 66 |
| feed consumption, g bird$^{-1}$ day$^{-1}$ | 129 ± 30 | 130 ± 11 | 112 ± 14 | 131 ± 22 | 135 ± 27 | 119 ± 27 |
| liver weight, % body | 2.64 ± 0.38 | 2.10 ± 0.07[c] | 2.30 ± 0.45 | 2.38 ± 0.28 | 2.19 ± 0.28[c] | 2.35 ± 0.04 |
| liver fat,[b] % f.w. | 14.1 ± 7.6 | 11.2 ± 3.3 | 17.8 ± 9.1 | 12.0 ± 7.1 | 14.1 ± 6.8 | 15.0 ± 4.4 |
| liver pathology[e] | | | | | | |
| fatty | ++ | ++ | ++ | ++ | +± | + |
| friable | NN | ± | NN | ± | ± | NN |
| hemorrhage | + | + | + | ± | ± | ++ |
| packed cell volume, % | 23.5 ± 0.5 | 27.9 ± 1.9[d] | 26.7 ± 2.1[d] | 25.8 ± 2.3[c] | 25.4 ± 0.8 | 27.1 ± 0.8[d] |

[a] Data expressed as mean ± SD for $n = 3$. [b] Two separate determinations from each bird. [c] Significantly different from control at $p < 0.03$ by Tukey's Multiple Means Test. [d] Significantly different from control at $p < 0.003$. [e] Not notable (NN), mild (+), moderate (++), or severe (+++) as a group average; (±) indicates some birds with NN.

Table II. Time-Dependent Development of Apparent Fatty Liver in Layer Chickens Receiving Lower Doses of HCB[a]

| | 1 mg/kg of HCB | | | 10 mg/kg of HCB | | | 100 mg/kg of HCB | | |
|---|---|---|---|---|---|---|---|---|---|
| | 1[b] | 15 | 71 | 1 | 15 | 71 | 1 | 15 | 71 |
| body weight, g | 1454 ±153 | 1557 ±14 | 1603 ±170 | 1508 ±35 | 1468 ±151 | 1565 ±71 | 1385 ±99 | 1459 ±264 | 1690 ±516 |
| liver weight, % body | 2.38 ±1.13 | 2.34 ±0.08 | 3.35 ±0.12 | 2.26 ±0.26 | 2.34 ±0.40 | 3.16 ±0.52 | 2.15 ±0.16 | 2.06 ±0.15 | 3.00 ±0.70 |
| liver fat % f.w. | | 18.2 ±4.0 | | | 9.0 ±3.7 | | | 7.2 ±0.2 | |
| liver pathology[c] | | | | | | | | | |
| fatty | NN | ++ | ++ | NN | + | ++ | NN | ± | + |
| friable | NN | + | +++ | NN | NN | NN | NN | NN | NN |
| hemorrhage | NN | NN | +++ | NN | NN | NN | NN | NN | + |
| swollen | NN | NN | +++ | NN | NN | NN | NN | NN | NN |

[a] Values are mean ±SD for $n = 3$. [b] Time (days) following last of seven daily doses. [c] NN = not notable. Otherwise, mild (+), moderate (++), or severe (+++) as a group average; ± indicates some birds with NN.

Liver fat content was determined gravimetrically by homogenization and extraction of duplicate 5-g samples with chloroform–methanol (2:1).

Residue analysis of tissues was accomplished by a modification of the method of Hansen et al. (1977), in which hexane is substituted for benzene–methanol. The extracts were cleaned by alumina–silica gel chromatography. The mean recovery efficiency for the described method is 99 ± 4% for fat and 94 ± 12% for tissues with lower lipid content as determined from replicate analysis of samples seeded with 0.1, 0.5, 1.0, and 10.0 ppm. Efficiency of tissues other than fat were lower (84%) between 1 and 10 ppm and near 100% at lower concentrations.

## RESULTS AND DISCUSSION

**Dose.** The oral doses administered are about tenfold higher than the dose a chicken (1.5 kg) would receive consuming 100–150 g/day of feed contaminated to a comparable level; thus, 1 mg/kg dose ≃ 10 ppm in the feed.

**Performance.** HCB had no effect on body weight (Tables I and II) or feed consumption (Table I). Contrary to anticipated results, livers from control birds were somewhat larger than those from HCB-dosed birds killed on day 43. Gross observations indicated that the livers from birds receiving the higher HCB doses were apparently somewhat less fatty, but quantitative determination of liver fat content revealed no differences (Table I). The differences in liver fat were quite significant on day 15 when the manifestations of fatty liver were first becoming apparent (Table II), but the characteristic severely swollen and friable liver is generally not fully apparent until the birds are near 30 weeks of age. Unfortunately, this was an unanticipated phenomenon and insufficient data were collected, but at the 71-day necropsy (birds were 32-weeks old) the livers from hens having received only 7 × 1 mg/kg of HCB were distinctly and invariably more severely affected than those receiving the higher doses. Liver weight, although showing a tendency to remain lower in HCB-treated birds, is not a reliable parameter since both HCB and fat deposition tend to cause hepatomegally. It appears that HCB may have some prophylactic activity towards development of hemorrhagic-fatty liver, a common syndrome in confined laying hens (Wolford and Polin, 1975; Garlich et al., 1975) and this activity overrides the potential HCB-induced liver enlargement seen in other species. Microsomal induction by HCB (Grant et al., 1974) may help by increasing catabolism of fatty acids and steroids or HCB may interfere with fat synthesis as does Aroclor 1254 (Gamble and Kling, 1976).

There were no other differences in relative organ weights of birds killed on day 50 and the only gross pathology observed other than the livers were polycystic kidneys in one control and one 7 × 10 mg/kg bird. Packed cell volume was somewhat lower in control birds (Table I) and may have been related to the hemorrhages associated with the fatty liver problem.

Onset of full egg production was delayed in groups receiving 10, 14, or 100 mg/kg of HCB (Table III). A similar delay was observed by Avrahami and Steele (1972b), but they hesitated to attribute it to dietary HCB because of low experimental numbers. Our independent observation of the same phenomenon indicates that HCB does, indeed, delay egg production, but final egg production is not affected. This was the only apparent adverse effect of HCB.

Table III. Mean (±SD) Weekly Egg Production for Layer Chickens Receiving Various Oral Doses of HCB for 7 Days

| | daily HCB, mg/kg | | | | | |
|---|---|---|---|---|---|---|
| time[a] | control ($n$ = 3) | 1 ($n$ = 6) | 5 ($n$ = 3) | 10 ($n$ = 6) | 14 ($n$ = 3) | 100 ($n$ = 6) |
| 1 | 1.3 ± 1.5 | 1.3 ± 2.8 | 2.7 ± 3.0 | 1.8 ± 2.8 | 0 | 0.3 ± 0.8 |
| 2 | 5.3 ± 2.1 | 2.7 ± 3.2 | 4.7 ± 1.5 | 2.0 ± 3.1 | 1.7 ± 2.1 | 0.7 ± 1.2 |
| 3 | 5.7 ± 0.6 | 4.0 ± 2.6 | 6.0 ± 1.0 | 3.0 ± 2.5 | 3.3 ± 2.3 | 1.7 ± 1.6 |
| 4 | 6.3 ± 1.5 | 5.3 ± 1.8 | 6.3 ± 0.6 | 5.8 ± 1.9 | 6.0 ± 2.6 | 4.7 ± 2.5 |
| 5 | 6.3 ± 0.6 | 6.5 ± 0.5[b] | 6.7 ± 0.6 | 6.3 ± 1.2[b] | 8.0 ± 0.0 | 5.8 ± 2.1[b] |
| 6 | 7.0 ± 1.0 | 6.8 ± 0.8 | 6.3 ± 0.6 | 6.3 ± 0.5 | 6.3 ± 0.6 | 5.8 ± 1.1 |

[a] Weeks following 1-week dosing period. [b] Three of these birds were biopsied during the week.

Table IV. Mean HCB Residues[a] in Tissues of Laying Hens 1 or 15 Days following Administration of Seven Daily Oral Doses

| | 1 mg/kg[b] | | 10 mg/kg[b] | | 100 mg/kg[b] | |
|---|---|---|---|---|---|---|
| tissue | 1[c] | 15[c] | 1[c] | 15[c] | 1[c] | 15[c] |
| abdominal fat | 25 ± 5 | 19 ± 4 | 197 ± 76 | 117 ± 50 | 804 ± 304 | 596 ± 60 |
| subcutaneous fat | 18 ± 3 | 17 ± 3 | 158 ± 25 | 145 ± 70 | 809 ± 218 | 519 ± 208 |
| skin | 5.0 ± 2.4 | 9.3 ± 1.8 | 27 ± 3 | 53 ± 27 | 250 ± 161 | 218 ± 131 |
| caecum | 3.1 ± 1.3 | 1.3 ± 0.5 | 12 ± 8 | 23 ± 24 | 174 ± 122 | 64 ± 35 |
| proventriculus | 2.6 ± 1.3 | 2.6 ± 3.1 | 18 ± 19 | 16 ± 9 | 78 ± 54 | 56 ± 60 |
| small intestine | 1.1 ± 0.8 | 2.3 ± 1.0 | 20 ± 5 | 15 ± 0.1 | 65 ± 6 | 69 ± 60 |
| crop | 0.5 ± 0.7 | ND[d] | 1.0 ± 0.6 | ND | 32 ± 22 | ND |
| duodenum | 0.2 ± 0.0 | 0.5 ± 0.0 | 5.9 ± 3.5 | 2.3 ± 1.4 | 8.2 ± 4.0 | 7.8 ± 0.7 |
| gizzard | 0.4 ± 0.4 | 0.1 ± 0.1 | 1.2 ± 0.8 | 1.9 ± 2.1 | 6.5 ± 2.0 | 3.1 ± 0.7 |
| liver | 2.1 ± 2.0 | 2.1 ± 1.6 | 12 ± 9 | 3.5 ± 1.5 | 24 ± 10 | 17 ± 3 |
| pancreas | 0.3 ± 0.1 | 0.8 ± 0.7 | 3.0 ± 0.2 | 3.7 ± 2.4 | 24 ± 22 | 12 ± 6 |
| kidney | 0.3 ± 0.1 | 0.4 ± 0.1 | 2.5 ± 1.5 | 2.0 ± 0.6 | 16 ± 3 | 8.0 ± 4.4 |
| heart | 0.4 ± 0.1 | 0.2 ± 0.0 | 8.6 ± 2.8 | 1.8 ± 0.8 | 14 ± 7 | 10 ± 4 |
| spleen | 0.3 ± 0.1 | 0.2 ± 0.0 | 2.2 ± 1.0 | 0.8 ± 0.5 | 13 ± 8 | 4.3 ± 1.2 |
| brain | 0.4 ± 0.1 | 0.3 ± 0.1 | 2.8 ± 0.8 | 2.0 ± 1.2 | 9.3 ± 4.8 | 9.3 ± 4.8 |
| lung | 0.3 ± 0.1 | 0.4 ± 0.2 | 1.7 ± 0.3 | 2.0 ± 0.8 | 9.4 ± 2.2 | 4.6 ± 1.4 |
| oviduct | 0.6 ± 0.4 | 0.2 ± 0.1 | 1.3 ± 0.4 | 0.7 ± 0.8 | 3.9 ± 1.1 | 3.9 ± 0.3 |
| leg muscle | 0.2 ± 0.2 | 0.2 ± 0.1 | 0.8 ± 0.2 | 1.1 ± 0.3 | 3.0 ± 0.2 | 5.4 ± 4.9 |
| breast muscle | ND | 0.03 ± 0.02 | ND | 0.2 ± 0.1 | ND | 0.6 ± 0.2 |
| blood | 0.2 ± 0.1 | 0.2 ± 0.2 | 2.1 ± 1.4 | 1.4 ± 0.8 | 4.3 ± 1.4 | 4.2 ± 1.4 |

[a] Mean ppm HCB (wet weight) ± SD for $n$ = 3. [b] Daily oral dose. [c] Time (days) following last of seven daily doses. [d] ND = not determined.

Table V. Mean HCB Residues[a] in Tissues of Laying Hens 43 Days following Seven Daily Oral Doses

| tissue | 0[b] | 1[b] | 5[b] | 10[b] | 14[b] | 100[b] |
|---|---|---|---|---|---|---|
| abdominal fat | 1.5 ± 0.3 | 9.7 ± 2.5 | 28.7 ± 3.2 | 68 ± 34 | 70 ± 17 | 253 ± 35 |
| subcutaneous fat | 1.2 ± 0.2 | 8.6 ± 1.8 | 25.3 ± 3.0 | 68 ± 35 | 73 ± 23 | 215 ± 34 |
| skin | 0.2 ± 0.1 | 4.4 ± 3.2 | 7.0 ± 4.3 | 19 ± 9 | 14 ± 5 | 93 ± 22 |
| liver | 0.2 ± 0.1 | 0.6 ± 0.3 | 3.5 ± 3.5 | 3.0 ± 1.0 | 7.1 ± 5.1 | 16 ± 6 |
| caecum | 0.04 ± 0.01 | 0.16 ± 0.06 | 0.9 ± 0.6 | 2.0 ± 1.4 | 2.6 ± 3.1 | 5.5 ± 2.8 |
| leg muscle | 0.009 ± 0.004 | 0.11 ± 0.08 | 0.35 ± 0.33 | 0.9 ± 1.0 | 0.6 ± 0.4 | 1.5 ± 0.7 |
| breast muscle | 0.004 ± 0.001 | 0.05 ± 0.02 | 0.13 ± 0.06 | 0.13 ± 0.06 | 0.09 ± 0.04 | 0.6 ± 0.1 |
| blood | 0.03 ± 0.01 | 0.05 ± 0.01 | 0.25 ± 0.01 | 0.74 ± 0.55 | 0.61 ± 0.17 | 1.8 ± 0.3 |
| egg yolk[c] | 0.3 ± 0.1 | 1.6 ± 0.4 | 6.2 ± 3.2 | 20 ± 9 | 25 ± 3 | 54 ± 11 |

[a] Mean ±SD ppm HCB (wet weight) for $n$ = 3. [b] Daily oral dose of HCB (mg/kg) for seven consecutive days. [c] Pooled samples from three consecutive eggs for each hen; week prior to termination (days 36-40 following final dose).

**Residues.** Residues accumulated primarily in the fat and skin of the chickens (Tables IV, V, and VI). At 1 day after the final dose, the relative accumulation in fat (HCB in fat/daily dose) decreased with increasing dose (22.5, 17.8, and 8.1 for 1, 10, and 100 mg/kg, respectively). This decrease was also observed in other tissues and indicates a rate-limiting factor in absorption at the higher doses. Limited dissolution of HCB was probably the major factor, since HCB crystals have been observed in the gastrointestinal tract of other animals administered high doses (Gralla et al., 1977). During the first 2 weeks following dosing, the walls of the caeca, proventriculus, and small intestine also contained high concentrations of HCB relative to other tissues (Table IV). Leg muscle contained higher HCB concentrations than breast muscle (Table IV), but it is difficult to draw conclusions due to the variability of the leg muscle samples.

Fat, muscle, blood, and egg yolk residues declined with time, but mean HCB concentrations in livers remained high or actually increased (Tables IV, V, and VI). The concentrations in livers were quite variable and generally increased with increasing liver fat in any given group of three birds; thus, it is likely that the high fat content of laying chicken livers permits a high equilibrium concentration of HCB.

Skin was a belated reservoir for HCB, generally not reaching maximum concentrations until 1–8 days following the final dose (Table IV). At the highest dose, skin remained a significant reservoir and the HCB concentration was approaching that of fat 71 days after the final dose (Table VI).

Since fat is the most significant reservoir for HCB, more samples were generated by using biopsy techniques and by analyzing two separate dissectible fat reservoirs at

Table VI. Mean HCB Residues[a] in Tissues of Laying Hens 71 Days following Seven Daily Oral Doses

| tissue | 1[b] | 10[b] | 100[b] |
|---|---|---|---|
| abdominal fat | 2.9 ± 1.0 | 23.4 ± 2.8 | 129 ± 2 |
| subcutaneous fat | 3.5 ± 0.6 | 24.2 ± 0.8 | 134 ± 10 |
| skin | 1.8 ± 1.6 | 7.1 ± 6.1 | 122 ± 47 |
| liver | 0.9 ± 0.2 | 4.2 ± 1.8 | 24 ± 6 |
| caecum | 0.16 ± 0.03 | 0.5 ± 0.2 | 3.7 ± 1.0 |
| leg muscle | 0.03 ± 0.01 | 0.34 ± 0.34 | 0.9 ± 0.1 |
| breast muscle | 0.04 ± 0.03 | 0.08 ± 0 | 0.2 ± 0.1 |
| blood | 0.02 ± 0.01 | 0.35 ± 0.29 | 1.0 ± 0.1 |
| egg yolk[c] | 0.65 ± 0.06 | 4.82 ± 0.09 | 25.2 ± 3.2 |
| excrement[d] | <0.005 | 0.018 ± 0.005 | 0.13 ± 0.02 |

[a] Mean ±SD ppm HCB (wet weight) for $n = 3$; $n = 2$ for 100 mg/kg group. [b] Daily oral dose of HCB (mg/kg) for 7 consecutive days. [c] Pooled samples from two consecutive eggs; week prior to termination. [d] Duplicate grab samples from mixed 24-h feces from each hen; collected 4 days prior to termination.

Table VII. Estimation of Daily Percent Body Burden of HCB Excreted in Egg Yolks

| | 1 mg/kg | | 10 mg/kg | | 100 mg/kg | |
|---|---|---|---|---|---|---|
| parameter | 43 day | 71 day | 43 day | 71 day | 43 day | 71 day |
| body burden,[a] mg | 1.35 | 0.45 | 10.2 | 3.6 | 35.1 | 19.6 |
| excretion in eggs,[b] mg | 0.020 | 0.007 | 0.234 | 0.057 | 0.581 | 0.304 |
| % excreted[c] per day | 1.48 | 1.58 | 2.25 | 1.58 | 1.65 | 1.56 |

[a] Average concentration in fat on day of termination (mg/kg) × 1.5 kg body weight × 10% body fat. [b] Concentration in egg yolk during week prior to termination (mg/kg) × yolk weight (0.013 kg) × (no. eggs laid during week/7 days). [c] Excretion in eggs/body burden × 100%.

termination. Averaging the terminal fat samples and including the biopsies, a log linear relationship is apparent for concentration of HCB in fat vs. time after the final dose (Figure 1). The plots of the three dose levels were very nearly parallel (Figure 1), so that, even though absorption processes were saturable, elimination rates remained constant within the range of doses administered. The plots were linear over the 70-day monitoring period and a half-life of elimination of 24–27 days can be estimated. This rate would appear to remain constant between concentrations in fat of 3 and 800 ppm since the lines are parallel and the lower dose levels may be considered continuations of the next higher level; however, the monitoring period included an interval of less than 3

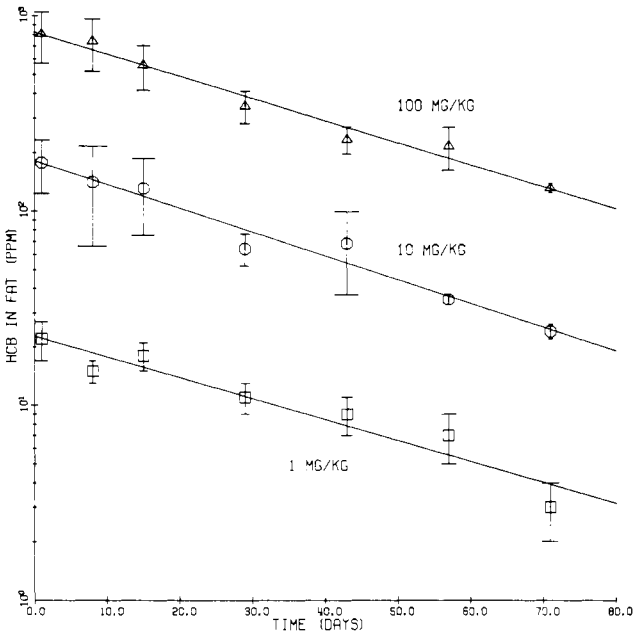


**Figure 1.** Disappearance of HCB from the fat of laying chickens at various times after seven consecutive daily doses of the indicated magnitude. Each point is mean ±SD for $n = 3$, except 100 mg/kg on day 71 where $n = 2$.

half-lives, so the elimination rate may change at fat concentrations less than 3 ppm HCB. In fact, the slope of the 1 mg/kg line is slightly less than the higher doses so that a decline in the rate of elimination may occur at fat concentrations less than 25 ppm HCB.

It will be shown in subsequent considerations that egg yolk is the major route of HCB elimination in laying chickens; therefore, reduced egg production in the higher dose groups during the early weeks (Table III) may have masked a potentially greater rate of elimination during the earlier phase of the elimination curves (Figure 1). The elimination rate may be considered to be independent of dilution by growth (Hansen and Welborn, 1977).

Mean residues in egg yolks were generally 15–30% as high as concentrations in the body fat (Tables V and VI). A conservative average egg yolk weight was 13 g; thus, chickens excreted the following approximate amounts of HCB in eggs during the week prior to termination: 0.14, 0.50, 1.64, 2.56, and 4.07 mg for dose levels of 1, 5, 10, 14, and 100 mg/kg, respectively. The body burden of HCB was estimated from average fat concentrations assuming a 1.5-kg weight and a body composition of 10% fat. After calculating the daily amount of HCB excreted in eggs, a surprisingly constant fraction of the body burden (1.68 ± 0.28%) was excreted daily in egg yolks (Table VII). Because of the limited data and crude estimates, caution

Table VIII. Relationship of Eggs Laid and Residues of HCB in Individual Chickens 50 or 78 Days following the First of Seven Consecutive Daily Doses of HCB

| | 1 mg/kg[a] | | | 10 mg/kg[a] | | | 100 mg/kg[a] | | |
|---|---|---|---|---|---|---|---|---|---|
| group | eggs[b] | yolk[c] | fat[c] | eggs[b] | yolk[c] | fat[c] | eggs[b] | yolk[c] | fat[c] |
| 50 day | 32 | 2.1 | 8.1 | 30 | 10.8 | 37 | 22 | 49 | 208 |
| | 13 | 1.5 | 7.7 | 18 | 21.5 | 62 | 15 | 47 | 220 |
| | 10 | 1.3 | 11.6 | 6 | 27.8 | 106 | 9 | 67 | 273 |
| 78 day | 53 | 0.6 | 2.5 | 57 | 4.9 | 22 | 42 | 23 | 127 |
| | 49 | 0.7 | 3.2 | 45 | 4.8 | 26 | 29 | 28 | 136 |
| | 44 | 0.6 | 3.9 | 38 | 4.8 | 23 | | | |

[a] Daily oral dose of HCB received for 7 consecutive days. [b] Total eggs laid from last day of dosing to termination day. [c] HCB residue (ppm fresh weight): yolks are pooled samples of three and two consecutive eggs collected during the week prior to termination for 50-day and 78-day groups, respectively; fats are average of terminal subcutaneous and abdominal cavity samples.

is required to prevent overinterpretation; nevertheless, a half-life of elimination in eggs of about 41 days (0.693/percentage of body burden excreted daily in egg yolks) can be estimated. Comparing this to the half-life of elimination from fat of 24–27 days (Figure 1), it becomes obvious that at least 50% of the decline in HCB residues is due to elimination of parent compound in egg yolk. Other routes of HCB disappearance include metabolism and fecal excretion of parent compound. Metabolism was not evaluated, but the average weekly excrement totaled 0.85 kg/bird, so that total fecal excretion of HCB by the end of the experiment (Table VI) was only 3.8 to 5.2% of the excretion in egg yolks [100% × (concentration in feces × 0.85 kg)/(concentration in yolks × 0.013 kg × no. eggs laid)].

Finally, there is a general correlation between the total number of eggs laid up to a given time and the concentration of HCB in eggs and body fat (Table VIII). The differences are more subtle in birds with lower residue burdens and more extensive data may be required to firmly establish the relationship. In addition, it may be possible that decreased egg production resulted from a higher body burden of HCB, but there is little doubt that egg yolk is the major excretory route for HCB in laying chickens and there is a relationship between HCB residues and egg production.

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LITERATURE CITED

Avrahami, M., Steele, R. T., *N.Z. J. Agric. Res.* **15**, 476 (1972a).
Avrahami, M., Steele, R. T., *N.Z. J. Agric. Res.* **15**, 482 (1972b).
Booth, N. H., McDowell, J. R., *J. Am. Vet. Med. Assoc.* **166**, 591 (1975).
Burns, J. W., Miller, F. M., *Arch. Environ. Health* **30**, 44 (1975).
Gamble, W., Kling, D., *Fed. Proc., Fed. Am. Soc. Exp. Biol.* **35**, 1625 (1976).
Garlich, J. D., Olson, J. D., Huff, W. E., Hamilton, P. B., *Poult. Sci.* **54**, 806 (1975).
Gralla, E. J., Fleischman, R. W., Luthra, Y. K., Hagopian, M., Baker, J. R., Esber, H., Marcus, W., *Toxicol. Appl. Pharmacol.* **40**, 227 (1977).
Grant, D. L., Iverson, F., Hatina, G. V., Villeneuve, D. C., *Environ. Physiol. Biochem.* **4**, 159 (1974).
Hansen, L. G., Welborn, M. E., *J. Pharm. Sci.* **66**, 497 (1977).
Hansen, L. G., Wilson, D. W., Byerly, C. S., Sundlof, S. F., Dorn, S. B., *J. Toxicol. Environ. Health* **2**, 557 (1977).
Reed, D. L., Booth, N. H., Bush, P. B., Goetsch, D. D., Kiker, J., *Poult. Sci.* **56**, 908 (1977a).
Reed, D. L., Bush, P. B., Booth, N. H., Kiker, J. T., Goetsch, D. D., Farrell, R. L., *Toxicol. Appl. Pharmacol.* **42**, 433 (1977b).
Wolford, J. H., Polin, D., *Poult. Sci.* **54**, 374 (1975).
Yang, R. S. H., Mueller, W. F., Grace, H. K., Golberg, L., Coulston, F., *J. Agric. Food Chem.* **24**, 563 (1976).

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# Distribution and Metabolic Fate of *trans*- and *cis*-Permethrin in Laying Hens

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Radiocarbon from $^{14}$C-carbonyl- and $^{14}$C-methylene-labeled preparations of (1*RS*)-*trans*- and (1*RS*)-*cis*-permethrin, administered to laying hens for 3 consecutive days at 10 mg/kg for each dose, is largely eliminated from the body within 1 day after the last dose, a portion as $^{14}CO_2$. The excreta contain all and the eggs most of the following compounds identified by thin-layer cochromatography with authentic standards and specific enzymatic hydrolysis: the unmetabolized pyrethroids; *cis*-permethrin hydroxylated at the 4′-position, at the methyl group trans to the carboxyl, and at both of these sites; the dichlorovinyl acids and their derivatives hydroxylated at the trans or cis methyl group; phenoxybenzyl alcohol, phenoxybenzoic acid and their 4′-hydroxy derivatives; sulfate, glucuronide, taurine, and other conjugates of these alcohols and acids. Residues of unmetabolized *trans*- and *cis*-permethrin in fat are 0.15 and 0.93 ppm, respectively, at 7 days after the last dose, and in eggs they reach peak levels of 0.3 and 1.2 ppm, respectively, at 3–4 days after the last dose.

The distribution and metabolic fate are defined for permethrin (Gaughan et al., 1977) and many other pyrethroids (Miyamoto, 1976) in rats and for permethrin in cows (Gaughan et al., 1978) but not for any pyrethyroid in hens. Permethrin is highly effective for housefly control and, when used as a direct spray on poultry, for northern fowl mite control (Burroughs Wellcome Co., 1978). This insecticide and its metabolites might also enter the meat and eggs of hens as a result of ingesting feed contaminated with permethrin residues.

The present investigation considers the metabolism of (1*RS*)-*trans*- and (1*RS*)-*cis*-permethrin in orally treated hens and the tissue and egg residues of the permethrin isomers and their metabolites.

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MATERIALS AND METHODS

**Chemicals.** Four [$^{14}$C]permethrin preparations with specific activities of 1.0 to 1.3 mCi/mmol and radiochemical purities of >99% were used: (1*RS*)-*trans*-permethrin (*t*-per) and (1*RS*)-*cis*-permethrin (*c*-per) each labeled in the carbonyl group of the acid moiety and the

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