Dehydrochlorination of Some Organochlorine Pesticides in Freeze-Dried Egg and Egg Fat during Storage

Dehydrochlorination of α-HCH, γ-HCH, and pp′-DDT in freeze-dried egg or extracted egg fat can take place over a period of several days. Other, more stable, organochlorine pesticide residues are not affected. The technique for fat extraction influences subsequent decomposition.

During the course of a collaborative exercise among 11 laboratories on the determination of organochlorine pesticides in foodstuffs of animal origin, examination of spiked freeze-dried egg, and spiked egg fat, showed that a marked decomposition of α-HCH (α-hexachlorocyclohexane; α-BHC), γ-HCH (γ-hexachlorocyclohexane; γ-BHC; lindane), and pp′-DDT had taken place in the egg substrates over a period of a few days to some weeks. Since extracted egg fat may be stored during analysis and since the fate of organochlorine pesticides on freeze-dried egg may differ from that in the whole egg, some further investigations were undertaken to clarify the situation. Degradation of organochlorine pesticides during avian metabolism in vivo is well documented (Foster, 1974; Saha and Burrage, 1976; Walker and Jefferies, 1978) but leads to a much wider range of products than those in the present work.

EXPERIMENTAL SECTION

Preparation of Freeze-Dried Egg. Egg homogenate was spiked, as appropriate, with acetone solutions of the pesticides and rehomogenized before freeze-drying. The homogenized egg was freeze-dried over a period of 20–24 h by using a Virtis Freeze-Mobile, Model 10-145 MR-BA, during which the temperature of the sample rose slowly from −40 to +25 °C and the vacuum reached ~60 μmHg. Samples of spiked and control freeze-dried egg were each subdivided into suitable portions (5–20 g of fat) for circulation to laboratories in cleaned glass bottles having caps lined with aluminum foil.

Preparation of Egg Fat. Unless otherwise stated, fat was extracted from whole egg homogenate by the procedure described by Telling et al. (1977). Other extractions used were those of the AOAC (1975), using cold hexane, of the EEC Expert Committee on Methods of Residues Analysis (Heeschen, 1974), using Soxhlet extraction with hexane, and one using hexane–acetone (4:6) Soxhlet extraction; 4:1 anhydrous sodium sulfate–egg homogenate was used in both Soxhlet procedures and refluxing carried out for 6 h. Extracts were concentrated in a rotary evaporator.

Reaction of Egg Fat with Pesticide. For determination of whether degradation of the pesticides in egg fat took place, 1–5 g of fat were usually spiked with 1 mg kg^{-1} organochlorine pesticide in hexane so that the concentration of fat in hexane was ~50% and stood at room temperature out of direct sunlight in stoppered tubes for various times before analysis.

Analysis of Egg Substrate. The samples of fat and freeze-dried egg were analyzed by the Telling et al. (1977) method, but in one series of experiments this was checked by using an AOAC (1977) method. GLC was carried out on 0.9 m × 2 mm columns of 10% DC 200 on 80–100-mesh Gas-Chrom Q, 5% DEGS, or 5% OV-17 on 80–100-mesh Gas-Chrom Q. Nitrogen flow rates were ~50 mL min^{-1} and either ^{3}H or ^{63}Ni electron capture detectors were used.

Dehydrochlorination Products of HCH Isomers. γ-Pentachlorocyclohexene (γ-PCCH) (commercially unavailable) was prepared from γ-hexachlorocyclohexane (γ-HCH) as follows. Pure γ-HCH (1 g) was added to hexane (100 mL), methanol (50 mL), and 1% sodium hydroxide (100 mL) in a stoppered flask. The mixture was shaken continuously at room temperature until aliquots of the hexane layer showed, by using gas chromatography–mass spectrometry, that the expected PCCH had reached a maximum concentration. The hexane layer was separated, washed twice with water (500 mL), and dried on a column of anhydrous sodium sulfate. The solvent was evaporated on a rotary evaporator.

In addition to γ-PCCH, γ-HCH produced three trichlorobenzenes, of which the 1,2,4 isomer was by far the most abundant. The γ-PCCH was isolated in a manner similar to that used by Reed and Forgash (1970). The residual products were dissolved in warm hexane and eluted with hexane from a silica column (22 × 2 cm, Merck Kieselgel 0.2–0.5-mm particle diameter), and those fractions containing mainly γ-PCCH (100–225 mL) were combined, evaporated, and rechromatographed and the fractions containing only γ-PCCH retained. γ-PCCH was isolated as a liquid from which well-defined crystals slowly formed at low temperature. The crystals were washed with cold hexane and dried. The electron-impact mass and NMR spectra corresponded to those expected of pentachlorocyclohexene. The infrared spectrum corresponded almost entirely with that published by Kolka et al. (1954) for γ-PCCH. As only a single peak was developed on GLC using polar columns, the product was probably the pure 36/45 isomer (Kurihara et al., 1974).

Attempts to produce PCCH's from α-HCH and β-HCH failed: 1,2,4-trichlorobenzene was very rapidly produced from α-HCH with no detectable PCCH and practically no other trichlorobenzene isomers; β-HCH appeared very resistant to dehydrochlorination, as found by Cristol et al. (1951).

RESULTS AND DISCUSSION

Degradation in Freeze-Dried Material. Egg homogenate spiked with α-HCH, γ-HCH, pp′-DDE, and pp′-DDT was analyzed in four different laboratories between 3 days and 5 weeks after freeze-drying, with the results given in Table I. It is clear that under these conditions α-HCH was totally lost, γ-HCH mostly lost, and pp′-DDT partly (~40%) lost. The loss of pp′-DDT was mostly accounted for in terms of increased pp′-DDE. Further experiments on similar but not identical homogenates indicated 60–90% loss of γ-HCH during freeze-drying. Again, some γ-HCH remained together with the decomposition products, γ-PCCH and 1,2,4-trichlorobenzene.

Analyses of commercial freeze-dried egg samples from a number of countries by a UK food manufacturer (H. J. Heinz Co. Ltd., 1977) show little or any pp′-DDT and a preponderance of pp′-DDE: this may be attributed to avian metabolism in the whole egg or subsequent degradation on freeze-drying. Certainly our results indicate that organochlorine pesticide residue determinations in whole eggs are not necessarily a reliable indication of those in the freeze-dried material.

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Table I. Degradation of Organochlorine Pesticides in Freeze-Dried Egg

laboratory	0.20 mg kg^{-1} α-HCH added[a]	0.62 mg kg^{-1} γ-HCH added[a]	1.54 mg kg^{-1} total pp′-DDT added[a] as pp′-DDE and pp′-DDT	0.79 mg kg^{-1} pp′-DDE added[a]	0.62 mg kg^{-1} pp′-DDT added[a]
	mg kg^{-1} of pesticide remaining when				
A	0.00, 0.00	0.04, 0.05	1.12, 1.09	0.68, 0.63	0.36, 0.39
B	0.00, 0.00	0.05, 0.06	1.49, 1.50	0.99, 1.00	0.39, 0.39
C		0.03, 0.02	1.69, 1.49	1.15, 1.01	0.41, 0.37
D	0.01, 0.00	0.05, 0	1.42, 1.41	0.94, 0.94	0.37, 0.36
mean	0	0.04	1.42	0.92	0.38
SD		±0.02	±0.02	±0.18	±0.02

[a] Duplicate determinations in each laboratory.

Table II. Degradation of Organochlorine Pesticides in Various Egg Fat Samples and with Time As Determined by Recovery of Added Pesticide

egg sample	days between spiking and analysis	recovery, % α-HCH[a]	γ-HCH[a]	pp′-DDT[a]
A	1	9, 9	58, 65	59, 62
	7	1, 1	12, 15	74, 74
	30	5, 0	8, 0	60, 58
B	4	0, 0	19, 17	48, 48
C	4	0, 0	8, 7	54, 51
D	4	1, 1	11, 11	43, 49

[a] Duplicate determinations.

Table III. Degradation of Various Organochlorine Pesticides in Egg Fat over 4 Days As Determined by Recovery of Added Pesticide

pesticide	% recovery immediately after spiking[a]	4 days after spiking[a]
α-HCH	93, 92	0, 0
β-HCH	62, 63	65, 63
γ-HCH	94, 93	12, 11[b]
hexachlorobenzene	94, 95	85, 85
dieldrin	97, 97	103, 99
heptachlor epoxide	95, 97	102, 96
pp′-DDT	97, 97	66, 59[c]
pp′-DDE	100, 99	105, 101
pp′-TDE	100, 100	74, 71[d]
pp′-TDE olefin	100, 103	100, 110

[a] Duplicate determinations. Blank values were all <0.04 mg kg^{-1}. [b] Approximately 10% was also recovered as γ-PCCH. [c] Approximately 40% was also recovered as pp′-DDE. [d] Approximately 20% was also recovered as TDE olefin.

Degradation in Fat. Fat extracted from whole fresh eggs from a number of different sources by the Telling procedure was spiked at ~1 mg kg^{-1} level and the extent of degradation of α-HCH, γ-HCH, and pp′-DDT determined after up to 30 days (Table II). The loss of pp′-DDT was in each case wholly accounted for as pp′-DDE, within experimental error. When ethanol was used in place of acetone in the Telling fat-extraction procedure and compared with the standard procedure in examining the decomposition of α-HCH, the recovery of pesticide after 4 days was very similar (<1%). Acetone could therefore not be responsible for the losses.

Recovery of a number of organochlorine pesticides from a common sample of egg fat immediately after spiking (1 mg kg^{-1} level), to demonstrate the efficiency of the analysis, and after 4 days standing at room temperature was as in Table III. It is clear that those pesticides which will readily undergo a simple dehydrochlorination reaction

Table IV. Decomposition Products of α-HCH and γ-HCH in Egg Fat

	% found when 10 mg kg^{-1} α-HCH added[a]	100 mg kg^{-1} α-HCH added[a]	100 mg kg^{-1} γ-HCH added[a]
α-HCH	5, 4	15, 11	
γ-HCH			50, 44
γ-PCCH			37, 30
1,2,4-trichlorobenzene	93, 96	80, 82	17, 20
total	98, 100	95, 93	104, 102

[a] Duplicate determinations.

Table V. Decomposition of γ-HCH and pp′-DDT in Egg Fat Extracted in Various Ways As Determined by Recovery of the Added Pesticide

cleanup	% of recovery extraction: hexane hot[a]	hexane cold[a]	acetone–hexane hot[a]	acetone–hexane cold[a]
recovery of γ-HCH				
by Telling et al.	91, 94	43, 39	94, 92	29, 33
by AOAC	82, 77	31, 26	67, 77	26, 27
recovery of pp′-DDT				
by Telling et al.	98, 98	88, 88	96, 96	79, 82
by AOAC	88, 74	72, 59	70, 88	66, 68

[a] Duplicate determinations.

degrade while the others do not. It may be noted that the accuracy of the method of analysis was not at all good with β-HCH in this case.

Degradation Products. In order to prove that α-HCH and γ-HCH were being dehydrochlorinated, it was necessary first to determine blank values and recoveries by the Telling method for those compounds likely to be found in egg fat as degradation products: recovery of γ-PCCH, pentachlorobenzene, the three tetrachlorobenzenes, 1,2,4-trichlorobenzene, 1,3,5-trichlorobenzene, and 1,2-dichlorobenzene were >80% at 1 or 10 mg kg^{-1} levels; blanks were low. Water-soluble degradation products were not sought.

The decomposition of α-HCH and γ-HCH in egg fat was determined over 3–4 days as follows at high spiking levels to allow the products to be determined (Table IV). No other products could be detected except for possible traces of 1,2,3- and 1,3,5-trichlorobenzene in the fat spiked with γ-HCH. The pattern of decomposition produced was al-

most exactly the same as that produced by sodium hydroxide dehydrochlorination, and the total recoveries suggest that no other types of reactions were occurring.

Influence of Extraction Procedure. Fat, extracted by the four different procedures referred to under Experimental Section, was examined for its ability to subsequently induce the γ-HCH and pp'-DDT degradations over 4 days and the products were examined after both the Telling and AOAC cleanup procedures (Table V).

It appears, therefore, that the degradation is induced by some heat-labile factor extractable from egg substrates. The losses were almost entirely accounted for by identification and quantification of the byproducts listed above, although the AOAC method leads to lower figures overall.

When "active" egg fat was heated by gentle refluxing in hexane for 4 h and then tested for its ability to decompose α-HCH at room temperature during 6 days, about two-thirds of the facility to induce degradation had been lost. Washing the active egg fat with dilute acid (0.1 N) completely destroyed its ability to degrade α-HCH and no active constituent could be recovered by neutralizing the acid wash.

The heat-labile factor responsible for the degradations could well be a natural component of hen's eggs. Preliminary tests showed that, although some amines may be capable of dehydrochlorinating α-HCH (and not pp'-DDE) in nonpolar solvents, potential mediators from eggs such as phosphatidylethanolamines or trimethylamine, derived from poultry feed, were unlikely to be responsible. The observed effect is unlikely to arise from an artifact in the analytical method or be of microbial origin.

It is concluded that, when analyzing eggs for organochlorine pesticide residues, the initial fat extract should not be stored, particularly if obtained by using solvents at room temperature, but immediately cleaned-up for the pesticide(s) to be determined by GLC. Analysis of freeze-dried material may likewise not correspond with the original levels of certain organochlorine pesticides in the fresh eggs because of dehydrochlorination-type reactions in situ.

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Importance of Nootkatone to the Aroma of Grapefruit Oil and the Flavor of Grapefruit Juice

The influence of nootkatone on the aroma of cold-pressed grapefruit oil and the flavor of grapefruit juice flavored with either the oil, limonene, or nootkatone in limonene was studied. Nootkatone had more effect on the aroma of the oil than on the flavor of juice flavored with the same oil. The aroma of oil with a naturally high nootkatone level was usually distinguishable from that of other samples. The frozen concentrated juice used in this study contained nootkatone at slightly above its threshold level prior to addition of oil despite its apparent low oil content. The aroma and taste panel results suggest that several other components of grapefruit oil are essential to good grapefruit aroma and flavor in addition to nootkatone.

Since MacLeod and Buigues (1964) first reported nootkatone as a flavor impact compound in grapefruit, nootkatone content has been suggested as a quality index standard in grapefruit oil (MacLeod, 1966), and synthetic nootkatone has been used in some grapefruit-flavored beverages (Shaw, 1978). As a result of their personal experiences with synthetic nootkatone, several food flavorists have raised the question to us as to what extent nootkatone influences grapefruit flavor and whether it is necessary for good grapefruit flavor.

MacLeod and Buigues (1964) stated that in sugar solutions the flavor threshold of nootkatone was 20–40 ppm and that the odor was detectable below 10 ppm. Berry et al. (1967) found a flavor threshold for nootkatone of 1 ppm in water and 6 ppm in grapefruit juice. Haring et al. (1972) reported an odor threshold of 0.8 ppm in water and 30 ppm