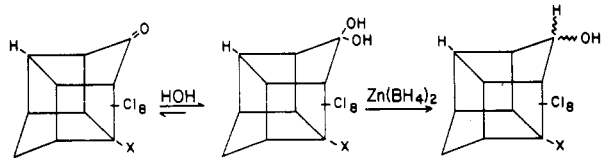
Scheme II



$Zn(BH_4)_2$ (Yoon et al., 1976) was used to reduce monohydro-CD (5) and dihydro-CD (6). Analysis of the products by GLC demonstrated only monohydro-CDOH (7) (RT = 8.3 min) or dihydro-CDOH (8) (RT = 5.7 min) and no other dechlorinated derivatives. The stereochemistry of monohydro-CDOH (7) is unknown. The CI mass spectra for these reduction products were characterized by the ion clusters; $(M + H)^+$, $[(M + H) - HOH]^+$, and $(M - Cl)^+$. These ions were found at m/e 455, 437, and 419 for monohydro-CDOH and m/e 421, 403, and 385 for dihydro-CDOH. The EI mass spectra demonstrated major fragments at m/e 149, 184 (base peak), and 218 for monohydro-CDOH and m/e 149 and 184 (base peak) for dihydro-CDOH. These data suggest that reduction of monohydro- and dihydro-CD with $Zn(BH_4)_2$ forms pure standards of monohydro- and dihydro-CDOH while avoiding further dehalogenation (Scheme II). The dechlorinated derivatives of CD appear more sensitive to the alkalinity of the reducing agent than CD itself. Consequently, the structure of the reactant in addition to the base strength of the reducing agent must be considered in the preparation of CDOH and its analogues.

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Distribution of Chlorinated Pesticides in Animal Feed Components and Finished Feeds

Frequency of occurrence and levels of selected organohalogens were monitored in animal feed components and animal feed over a 7-year period. Random samples collected for quality assurance were screened for lindane, β-BHC, aldrin, dieldrin, *p,p'*-DDE, *o,p'*-DDT, *p,p'*-DDD, *p,p'*-DDT, heptachlor epoxide, and heptachlor. Gas chromatographic analysis revealed average organochlorine contamination levels of less than 10 ppb.

Numerous examples of contamination of animals through animal feeds have been reported in the literature (Buck, 1970, 1975; Van Houweling et al., 1977). However, public awareness of this did not become acute until 1974 when polybrominated biphenyls (PBB) were associated with an animal feed accident in Michigan. The direct impact of this incident resulted in the destruction of 30 000 cattle and 6000 swine, as well as sheep, poultry, and dairy products. In addition, hundreds of millions of dolloars in lawsuits remain to be settled.

The total indirect impact of this and other incidents has yet to be measured. However, one of the outgrowths has been the difficulty of feed companies in obtaining and/or continuing to maintain liability insurance.

In an attempt to circumvent this problem, the University of Iowa, supported by a local feed company, developed a program for the monitoring of selected organohalogens, organophosphorus, and inorganic contaminants in the production of animal feeds. This paper presents the protocol and results of this program for organohalogen compounds which has been in existence for the last 7 years and represents a sale of more than 100 million dollars worth of feed products.

EXPERIMENTAL SECTION

General. A list of animal feed components, which represent the input into this company, is presented in Table I. The components are divided into four major categories: grains, meals, byproducts, and miscellaneous. The grain category comprises the largest portion of the feed. Although pesticides are widely used in grain production, residue levels in the harvested grain are relatively

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Table I. Components of Finished Animal Feeds

grains, grasses, and byproducts	meal products	animal byproducts	miscellaneous
corn	soybean meal	meat scraps	molasses
oats	bean meal	blood meat	sugar
alfalfa	bone meal	blood	bentonite
flax		tallow	oyster shells
middlings		fat	

low. Normally, significantly higher levels are found in the grain byproducts such as middlings, husks, and screenings (Sissons and Telling, 1979).

Meals such as fish, shrimp, and soybean are often used as meat protein substitutes. These products contain very little residual fat; thus lipophilic materials are not usually found at high concentrations.

Animal fats and tallows are added to feeds, particularly pelleted products. These byproducts have the highest probability of containing high concentrations of lipophilic-persistent contaminants.

Sampling. Quality assurance sampling of the finished feed was accomplished through the use of a series of automatic samplers in the grain bins and ducts. Samples were taken in a random manner over an 8-h shift as composite samples. Since a wide variety of feeds were produced in a given sampling period, the composite consisted of a heterogeneous mixture of pelleted feed products. Complete records were kept to account for the products going into the sample so that any determined contamination could be easily traced to the initial source.

In addition, random samples were taken from feed ingredients and analyzed for organohalogen content. Samples of incoming feed components were analyzed the same day the shipment arrived at the plant, while finished feeds were analyzed the day before shipment to the customer.

Pesticide Analysis. Samples of materials were screened for lindane, β-BHC, aldrin, dieldrin, *p,p'*-DDE, *o,p'*-DDT, *p,p'*-DDD, *p,p'*-DDT, heptachlor epoxide, and heptachlor. The procedure was developed as a screening device, having the elements of speed, simplicity, and low cost. For samples having contaminant levels approaching U.S. Food and Drug Administration (FDA) limits, a more extensive workup and determination was employed after notification of the feed company.

The extraction procedure used has been previously described (Mills et al., 1963) for nonfatty foods. One hundred grams of sample is blended for 2 min with 200 mL of acetonitrile and 10 g of Celite. Subsequently, the suspension is filtered and transferred to a separation funnel containing 100 mL of petroleum ether. After the mixture is shaken, 10 mL of saturated sodium chloride solution and 600 mL of distilled water are added, and the mixture is rocked gently to avoid emulsions. After separation, the ether layer is washed (2×) with 100-mL portions of water, dried with anhydrous sodium sulfate, and concentrated under a stream of dry nitrogen. The sample is placed on a 1 × 10 cm Florisil column and eluted with 15% diethyl ether in petroleum ether. This is concentrated to a final volume of 10 mL.

Instrumentation. Chlorinated pesticide screening was accomplished by using a Tracor 222 gas chromatograph with a ^{63}Ni electron capture detector. The column for screening purposes was a 6 ft × 6 mm o.d., 5% OV-1 liquid phase on 80–100-mesh Chromosorb W. The column used for validation was a mixed 1.5% OV-17 and 1.95% OV-210 on 100–120-mesh Chromosorb W. The column temperature was maintained at 200 °C, the inlet was maintained at a temperature of 235 °C, and the detectors were operated at 265 °C. The nitrogen carrier gas was maintained at 50 mL/min.

The resolving power of the OV-1 column was not sufficient to separate lindane from β-BHC, *p,p'*-DDE from dieldrin, or *o,p'*-DDT from *p,p'*-DDD. For this reason, results are reported as a combination of the unresolved pesticides. The second confirmatory column was used only when action levels were observed.

RESULTS

Frequency. The data presented are based on a 7-year period involving approximately 6500 samples. Table II presents the relative frequency of observation of contam-

Table II. Relative Frequency of Contamination[a] (Percent)

	soybean meal (1073)[b]	corn (700)	oats (83)	alfalfa (98)	meat scraps (168)	middlings (106)	finished feeds (4315)
lindane + β-BHC	2	3	10	21	23	13	18
heptachlor	3	3	4	1	9	7	5
aldrin	2	4	8	2	5	3	6
heptachlor epoxide	1	2	8	21	11	2	7
p,p'-DDE + dieldrin	2	7	30	24	30	20	21
o,p'-DDT + *p,p'*-DDE	2	10	14	6	10	17	14
p,p'-DDT	<1	<1		3	5	2	3

[a] Percentage of total samples containing >1 ppb. [b] Number of samples are in parentheses.

Table III. Average Levels of Contamination[a] (ppb)[b]

	soybean meal	corn	oats	alfalfa	meat scraps	middlings	finished feeds
lindane + β-BHC	0.1 ± 1.2 (31)	0.1 ± 0.4 (5)	0.3 ± 1.3 (10)	1.1 ± 4.2 (36)	1.1 ± 3.5 (30)	0.8 ± 3.6 (34)	0.5 ± 2.4 (91)
heptachlor	0.1 ± 0.7 (15)	0.1 ± 0.8 (18)	0.1 ± 0.4 (3)	0 ± 0.3 (3)	0.3 ± 1.5 (13)	0.4 ± 1.7 (10)	0.2 ± 1.2 (29)
aldrin	0.1 ± 0.6 (11)	0.1 ± 0.3 (4)	0.2 ± 0.7 (4)	0 ± 0.2 (2)	0.1 ± 0.3 (3)	0.1 ± 0.5 (3)	0.1 ± 0.8 (22)
heptachlor epoxide	0.1 ± 0.8 (21)	0 ± 0.3 (5)	0.3 ± 1.1 (7)	0.5 ± 1.0 (5)	0.6 ± 2.8 (30)	0.1 ± 0.8 (6)	0.3 ± 1.3 (26)
p,p'-DDE + dieldrin	0 ± 0.3 (7)	0.1 ± 0.6 (8)	4.1 ± 18 (164)	0.6 ± 1.4 (8)	0.8 ± 1.7 (10)	0.7 ± 1.8 (11)	0.8 ± 2.8 (58)
o,p'-DDT + *p,p'*-DDD	0.2 ± 3.2 (83)	0.5 ± 2.3 (42)	2.9 ± 16 (139)	0.6 ± 3.9 (37)	0.6 ± 2.3 (19)	1.5 ± 5.5 (43)	1.1 ± 4.7 (88)
p,p'-DDT	0.1 ± 0.8 (16)	0 ± 0.4 (7)		0.6 ± 4.2 (37)	1.1 ± 7.8 (10)	0.6 ± 4.8 (44)	0.2 ± 2.6 (98)

[a] Represents all samples, including those < 1 ppb. [b] Data format: average ± standard deviation (maximum level observed).

ination greater than 1 ppb for feed components and finished feed. As expected, corn and soybean meals show the lowest frequency of contamination incidence. Since these represent a large proportion of the final product, they tend to dilute any residues in the final product. In comparison, oats and alfalfa have an increased contamination incidence from heptachlor epoxide and dieldrin (and *p,p'*-DDE). As expected, wheat middlings and meet scraps have the highest incidence of contamination. Table III presents the average concentration observed in each of the media. As can be seen, soybean meal and corn generally contain lower levels of pesticides than the other feed components. In addition, the tendency for pesticide residues to accumulate in animal fatty tissue can be observed.

Distribution. Analysis of the data for finished feeds indicates the following. The concentrations of heptachlor and heptachlor epoxide rarely exceeded 10 ppb (w/w), and contamination levels were infrequent at 1–10 ppb. Levels of aldrin rarely exceeded 1 ppb while *p,p'*-DDT was rarely observed at detectable levels. The combinations of *o,p'*-DDT and *p,p'*-DDD and of *p,p'*-DDE and dieldrin represent major contributions to the total amount of organohalogen pesticide contaminant in feeds. The concentrations observed for these contaminants were generally less than 20 ppb.

Summary. Data have been presented with respect to an operational program for monitoring organochlorine pesticides in animal feeds. Although no incidents of high levels of contaminants have been detected and verified, the program is successful in that the quality of the animal feed has been assured prior to and during manufacture. In addition, detection and immediate correction of the presence of a high level contaminant are assured.

Evaluation of the data associated with levels of contaminants indicates that low levels of organochlorine pesticides are present in animal feed components and finished feeds. These concentrations are appreciably below the action limits defined by the U.S. Food and Drug Administration. In addition, comparison of the data from the period 1972–1975 with the data from 1976–1980 indicates that both the frequency of observation and the average levels observed for these pesticides are decreasing (Sissons and Telling, 1979).

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Sources of Loss in Residue Assays for Carbofuran and Its Metabolites

The stability of carbofuran and its two principal metabolites 3-hydroxycarbofuran and 3-ketocarbofuran in boiling hydrochloric acid was determined at varied acid concentrations and reflux times. Carbofuran undergoes 10–20% degradation when refluxed for 1 h at pH 0.8 in 0.25 N HCl, a commonly used medium for conjugate hydrolysis. The pH range within which all three materials are stable during this treatment is narrow (1.7–2.7, corresponding to 0.02–0.002 N HCl); 3-ketocarbofuran is stable between pH ~0.7 and pH 3, while 3-hydroxycarbofuran survives in the range of ~0.7–4. Recovery of the latter compound by chloroform or dichloromethane extraction is quantitative only when the acid hydrolysate is salt saturated. Losses were measured from 0.02-ppm solutions by using gas chromatography–chemical ionization mass spectrometry and selected ion monitoring. The significance of the findings is discussed in relation to procedures used in residue and metabolite studies.

Carbofuran (CF) (see Chemicals, below) is an anticholinesterase systemic insecticide of the *N*-methylcarbamate type (Cook, 1973). Metabolic processes produce two carbamate derivatives, 3-hydroxycarbofuran (HOCF) and 3-ketocarbofuran (COCF), which are of regulatory interest since they still possess considerable toxicity (Metcalf et al., 1968). Normally HOCF is the major carbamate metabolite found in plants and in most animals, largely as a conjugate; smaller amounts of COCF are more often found in soils together with unchanged CF. Reviews of major studies of CF and its metabolites—chemistry, toxicology, and residue determination—are available (Kuhr and Dorough, 1976; FAO M-84, 1977; NRCC, 1979).

In residue analysis of vegetables, milk, and meat, CF, HOCF, and COCF are often determined in the same analytical sample following liberation of the conjugated components of HOCF (and associated phenolic residues) by hydrolysis in hot acid in which, ideally, all of the carbamates should be stable (Cassil et al., 1969; Cook et al., 1969). This report describes the stability and recovery of CF, HOCF, and COCF from hydrochloric acid solutions of varied pH and heated under reflux for 0.5–4 h.

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