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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EXPERIMENTAL SECTION

Chemicals. CF (2,3-dihydro-2,2-dimethyl-7-benzofuranol methylcarbamate; CAS Registry No. 1563-66-2), HOCF [2,3-dihydro-2,2-dimethyl-3,7-benzofurandiol 7-(methylcarbamate); 16655-82-6], and COCF [2,2-dimethyl-7-[[(methylamino)carbonyl]oxy]-3(2*H*)-benzofuranone; 16709-30-1] were used. Details for chemical sources, instrumentation, and GC–MS operation were described previously (Chapman and Robinson, 1977; Robinson and Chapman, 1980).

Methods. *Sample Preparation.* Recovery tests were performed with the three carbamate standards mixed in aqueous acid media at 0.02 ppm. In each run, 1.0 mL of acetonitrile containing 10 μg each of CF, HOCF, and COCF was added to 500 mL of 0.25 N HCl, except where other acid concentrations are indicated. Several studies were made using these test solutions: (method a) without being heated, they were successively extracted 1–4 times with 100 mL of chloroform in the absence or the presence of 400 g of NaCl to saturate the aqueous phase; (method b) others were boiled under reflux for various periods (0.5–4 h) and then cooled, salt saturated, and extracted with three 100-mL portions of chloroform; (method c) solutions at several HCl concentrations (0–2.5 N) were refluxed for 1 h, cooled, salted, and extracted as in part b.

During studies at varied pH, the air above the refluxing solution was replaced with nitrogen and, on completion, the liquid was cooled under N_2 with protection from atmospheric CO_2 by using a soda–lime tube. The pH was measured immediately, and this value was taken as the pH existing during reflux. When the starting pH was 4.5 or lower, no measurable change occurred on boiling, but, predictably, there were slight pH increases (0.1–0.3 unit) for very dilute hydrochloric acid due to elimination of dissolved CO_2; acid blanks and carbamate solutions exhibited identical pH behavior on boiling.

The chloroform extracts from all experiments were dried with sodium sulfate, filtered, reduced in volume (in vacuo at 40 °C), and solvent exchanged to 10 mL of dry benzene. Aliquots (1 mL) were derivatized for GC by adding 60 μL of heptafluorobutyric anhydride and 10 μL of pyridine and storing at room temperature overnight. Separate aliquots of the spiking standards in acetonitrile were similarly solvent exchanged to benzene, derivatized in the same way, and used as the basis for calculating recoveries.

Assays. For GC (OV-17; 160 °C; isothermal) the derivatized sample was water washed, in the same vial, with three 1-mL portions of water; 1 μL of the benzene layer (containing 1 ng of each carbamate at full recovery) was assayed by measuring the GC peak heights at the appropriate retention times of the selected fragment ion (*m/e* 228) and comparing the three responses in the recovered samples with those of the standards.

All experiments were run in duplicate; at least two replicate vials were assayed for each of the experimental duplicates and three to four injections were assayed from each vial. The coefficient of variation on all of the mean values reported ($N \geq 8$) was less than 5%.

RESULTS AND DISCUSSION

Extraction from the Acid Hydrolysate. It is remarkable that little attention has been paid to the fundamental practice of "salting out" with NaCl to maximize extractive recoveries of these carbamates following the acid hydrolysis. In the recovery of Baygon (closely related to CF) from milk, Stanley and Thornton (1972) used NaCl with chloroform extraction after sulfuric acid hydrolysis, but, with CF, only Williams and Brown (1973) report increased recovery with the use of salt, although at a different stage in their procedure. Otherwise NaCl has been used only in cleanup methods involving solvent partitioning [e.g., Holden (1973) and Lawrence and Leduc (1977)]. Cook et al. (1969) have recommended dichloromethane, without mention of salt, for extraction of the acid hydrolysate of vegetable materials and have pointed out (Nelsen and Cook, 1980) the need for a relatively polar solvent to ensure quantitative recovery of HOCF. We have found chloroform to be fully effective, but both solvents equally require the aid of salt for total recovery of HOCF. The data in Table I show that without use of salt, there is a 20% loss of HOCF even with four successive chloroform extractions whereas after salt saturation only two extractions would probably suffice to recover all three of the compounds quantitatively.

Table I. Effect of Repetitive Extraction and Salt Saturation on Recovery of CF, HOCF, and COCF from Nonboiled 0.25 N HCl[a]

no. of $CHCl_3$ extractions[b]	recovery, %					
	nonsalted			salt saturated		
	HOCF	CF	COCF	HOCF	CF	COCF
1	34	97	96	92	94	97
2	52	98	97	106	99	100
3	66	99	98	100	98	99
4	82	98	98	97	98	98

[a] Method a. [b] Identical results were obtained with dichloromethane.

Table II. Effect of Reflux Time on Recovery of CF, HOCF, and COCF from 0.25 N HCl[a]

duration of reflux, h	recovery, %		
	HOCF	CF	COCF
0.5	98	92	99
1.0	99	87	95
2.0	93	80	91
4.0	89	71	85

[a] Method b.

Carbamate Stability. The hydrolysis rate of carbofuran at a number of pHs, usually at or near room temperature, has been reported [Metcalf et al., 1968; p 129, Figure 7, of NRCC (1979); Ferreira and Seiber, 1981]. Similar quantitative information regarding the hydrolytic action of hot hydrochloric acid on CF, HOCF, and COCF is not readily available; this is surprising considering the wide usage of this medium in carbamate residue procedures.

Recoveries reported for CF and its metabolites are erratic (57–139% of theoretical value) and are usually low [pp 20–22, Table I, of NRCC, (1979)]. Robinson and Chapman (1980) reported a consistent loss of 15–20% of CF from 0.002-ppm standard solutions (based on aqueous volume) subjected to acid reflux (1 h in 0.25 N HCl) compared to that from nonboiled controls, and this loss was seen to occur equally in the presence of potato, onion, and turnip extractives. Apparently unidentified sources of loss occur in most of the procedures.

Duration of Heating. In the present data, Table I confirms the complete recovery of CF from salted nonboiled 0.25 N HCl while progressive loss over reflux times of 0.5–4 h is seen in Table II; even at 30 min CF is reduced by an average 8%. HOCF and COCF survive the 1-h reflux well, but their degradation is apparent by 2 h and is quite significant at 4 h.

We have observed repeatedly that the CF loss in 1 h appears to be slightly larger with a more dilute original solution of carbamate, as exemplified by the 15–20% loss

Table III. Effect of pH on Recovery of CF, HOCF, and COCF Refluxed One Hour in HCl[a]

pH during reflux	approximate acid concn, N	recovery, %		
		HOCF	CF	COCF
<<0.1	2.5	27	28	31
0.5	0.4	87	80	87
0.8	0.25	99	87	95
1.7	0.02	99	98	101
2.7	0.002	101	101	96
3.3		98	98	80
3.8		100	100	47
4.4		91	95	<5
4.8		75	85	0
5.5		41	67	0
~7	0	0	0	0

[a] Method c.

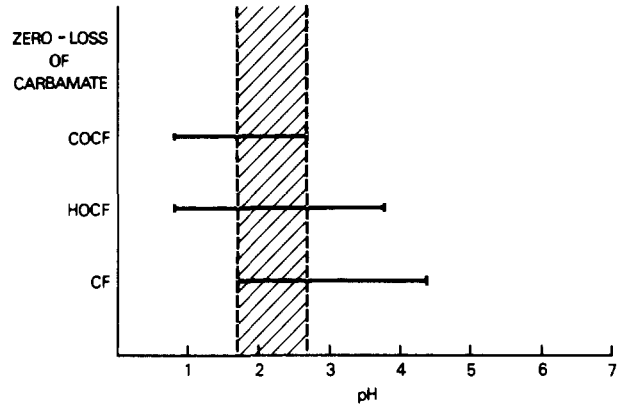

Figure 1. Zero-loss pH range common to CF, HOCF, and COCF subjected to 1-h reflux in HCl.

at 0.002 ppm (cited earlier) as compared to the 10–15% loss at 0.02 ppm in the present work. In the same examples, the data for HOCF and COCF show a similar trend, so this may be due to fixed procedural physical losses reflected as a larger percentage at the lower level.

So that loss due to boiling could be avoided, CF could be preextracted for separate determination from the nonboiled acid–crop blend before conjugate hydrolysis and the released HOCF recovered afterward as is done in the ethoxylation procedure of Nelsen and Cook (1980), but this is not an attractive alternative in the present case. It would seem preferable to seek milder, yet adequate, conditions for conjugate cleavage.

Acid Concentration. When refluxed in HCl for 1 h, all three carbamates are recovered quantitatively only when the pH lies between 1.7 and 2.7 (Table III; Figure 1) which approximates the concentration range of 0.02–0.002 N HCl. Thus, the widely used 0.25 N HCl hydrolysis medium is too concentrated to allow full recovery of CF from a 1-h reflux, while loss of COCF occurs when the acid concentration goes below 0.002 N (pH 2.7). For conjugate hydrolysis in metabolite studies, some procedures have utilized even more concentrated acid with longer boiling periods: e.g., 16 h on the steam bath with 0.12–0.5 N HCl (Metcalf et al., 1968); 0.5 N HCl refluxed for 2 h (Ashworth and Sheets, 1972); 1 N HCl heated for 2 h (Marshall and Dorough, 1977). Although in these cases the free carbamates had already been preextracted, a consideration of the data of Tables II and III suggests that significant degradation would occur to any HOCF released through glucoside hydrolysis under these conditions.

Summary. Clear solutions of carbamates have been used in the absence of complicating crop extractives, together with the high selectivity and reproducibility afforded by GC–MS ion monitoring, to reveal some possible sources of loss in carbofuran residue procedures. The results may help to explain the reported low and varied recoveries, particularly of CF and HOCF, from more complex systems involving biological materials.

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