This study has clearly shown ascorbic acid to be one component of food responsible for iron "leaching" from pots, pans, and processing equipment. The addition of ascorbic acid to foods during processing can be expected to affect the food's corrosive properties. Also, the addition of reduced iron either intentionally or from inadvertent corrosion processes can be expected to substantially accelerate the rate of vitamin C degradation in some foods.

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Registry No. Ascorbic acid, 50-81-7; iron, 7439-89-6.

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Detection, Isolation, and Identification of Impurities in Technical Thiofanox

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The detection, isolation, and identification of the impurities in the technical grade of the insecticide thiofanox are described. A ^{13}C NMR method was employed to detect impurities at about the 0.1% level or greater. The ^{13}C spectrum of very high signal-to-noise ratio of an impurity concentrate was used to guide subsequent separation and identification work. The sensitivity and reliability of this approach are discussed. Preparative and semipreparative high performance liquid chromatography was employed to isolate 39 nonvolatile components. These were identified on the basis of spectral data and by comparisons with authentic samples which were synthesized when necessary. Some stereochemical assignments are made on the basis of NMR chemical shifts. An additional 30 volatile components were isolated and identified by vacuum distillation and gas chromatography/mass spectrometry.

INTRODUCTION

The detection, identification, and quantification of impurities in technical pesticides are problems of scientific, economic, and social importance. Government regulations will continue to make it increasingly difficult for industry to register new pesticide products. The need to characterize technical pesticides in greater detail, and the increasing complexity of the chemical structures involved, necessitates the development of more sophisticated analytical methods than presently exist (Donaldson and Garrison, 1979; Fed. Regis., 1978; Libby and Freeberg, 1978).

Technical pesticides will generally be complex mixtures of components because of process variables, side reactions, and impurities in starting materials. It is reasonable to expect that many of the impurities will be similar in chemical structure to the major component. In some cases, the existence of geometrical isomers or diastereomers can further complicate characterization efforts.

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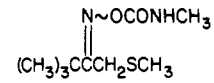
Thiofanox, 3,3-dimethyl-1-(methylthio)butane-2-one *O*-(*N*-methylcarbamyl)oxime, is a systemic insecticide which is one of a class of aldoxime and ketoxime carbamates, certain of which have excellent activity (Magee and Limpel, 1977). Preliminary work using high performance



thiofanox

liquid chromatography (HPLC) with ultraviolet (UV) detection has shown technical thiofanox to be a complex mixture containing, in some cases, seventy or more detectable components (Gertenbach et al., 1978). A large number of components is not necessarily unique to technical thiofanox. Similar analysis of other pesticide products might also reveal a surprisingly large number of impurities. Governmental pesticide registration regulations require that only those impurities present at or above some predetermined concentration need be pursued. Hence an analytical method is needed to approximately quantify impurities before they are identified. This eliminates expensive, additional work to pursue components substantially below the threshold level.

Gas chromatography (GC) has been used to estimate the number and concentration of unidentified impurities in mixtures. Unfortunately, the thermal stability of thiofanox (Corkins et al., 1982) and chemically related impurities is such that GC cannot be used reliably for such an estimation. HPLC with UV detection can be used with confidence to separate and detect the individual components. However, molar absorptivities can vary by orders of magnitude at a given wavelength, even among related compounds. Separation of undecomposed components can be obtained by using HPLC, but the concentrations of unidentified components cannot be determined.

One method that does not suffer from variations of absorptivity among components is nuclear magnetic resonance (NMR) spectroscopy (Leyden and Cox, 1977). Under proper conditions the area of an NMR peak is proportional to the number of nuclei giving rise to that signal. Although NMR is usually considerably lower in sensitivity than UV spectroscopy, recent advances (Farrar and Becker, 1971; Leyden and Cox, 1977) have improved NMR sensitivity to the point where components at the 0.1% level can be readily detected with ^{1}H Fourier transform (FT) NMR.

The favorable sensitivity of ^{1}H NMR is offset by the poor spectral resolution usually obtained for complex mixtures of similar compounds. This is due to the small ^{1}H chemical shift range and the complex multiplet structures often observed.

The large chemical shift range of the ^{13}C nucleus and the ability to observe all carbons as narrow singlets make proton-decoupled ^{13}C NMR an attractive technique for the study of mixtures. These advantages can outweigh the disadvantages of low sensitivity and somewhat variable peak intensities due to nuclear Overhauser and spin relaxation effects (Shoolery, 1977).

We report here the use of ^{13}C NMR to detect unidentified impurities at or greater than about 0.1% in technical thiofanox. The method is designed to serve as a screening technique to guide subsequent separation (using preparative HPLC) and identification work in the most profitable direction. It also serves as a method to determine when all impurities at or greater than 0.1% have been accounted for. It is not designed to serve as the primary tool for identification or quantitation, although ^{13}C NMR is used extensively in the subsequent identification work. The sensitivity and reliability of the method are discussed and a brief comparison is made to the alternative of high field ^{1}H NMR.

We also describe a strategy for isolation and identification of the nonvolatile components in the impurity concentrate. The investigation of the impurities in thiofanox initially involved ten samples of the technical material. Spectroscopic comparison by ^{13}C NMR indicated all samples to be qualitatively similar. One sample was then chosen for the separation work described below. Initially, the nonvolatile components were separated partially with preparative liquid-solid (LSC) and reversed-phase (RP) chromatography. Carbon-13 NMR was used to analyze fractions of the preparative chromatography in order to ascertain which ones were to be pursued further. The identities of the majority of the 39 impurities were confirmed by independent synthesis (reported as supplementary material).

Inherent in this liquid chromatographic method was the loss of any volatile components which may have been present in the technical material. In addition, ^{13}C NMR spectra of impurity concentrates suggested the presence of components which could not be accounted for by the nonvolatile impurities. In order to circumvent these problems, volatile components were isolated by a high vacuum technique and then analyzed by gas chromatography/mass spectrometry (GC/MS) and ^{1}H and ^{13}C FTNMR. The identities of the majority of the 30 volatile impurities were confirmed by independent synthesis or by comparison with commercial standards (reported as supplementary material).

RESULTS AND DISCUSSION

(I) Detection by ^{13}C NMR. (A) Factors Affecting ^{13}C NMR Sensitivity. Since the success of the present method depends heavily on optimization of the NMR sensitivity, it is advantageous to discuss the various factors affecting sensitivity as they relate to the present case. The goal of this initial stage was to develop a method for *detection* of all impurities at or above 0.1% in thiofanox. An accurate *quantitation* was not required at this point. Accurate quantitation can be performed by HPLC after identification. Hence, minor variations of sensitivity among components can be tolerated if a safety factor is present.

With the equipment available, it is necessary to work with the impurity rich "oil" (impurity concentrate) that is usually associated with technical thiofanox (see the Experimental Section). The enrichment factor upon going from technical material to impurity concentrate is variable, but is usually above five. Variations in this factor were taken into account by examining a number of samples. In our case, ten samples were examined for the overall study.

Many factors affect the ultimate signal–to–noise ratio obtained for a ^{13}C FTNMR spectrum (Becker et al., 1979; Mareci and Scott, 1977). Besides the more obvious ones of concentration, temperature, and magnetic field, there are the pulse repetition rate (relative to the spin lattice relaxation times (T_1's) of the system studied), the pulse width or flip angle, and the nuclear Overhauser enhancement (NOE). We do not intend to discuss all of these factors in great detail, since this has been done elsewhere (Becker et al., 1979; Farrar and Becker, 1971; Mareci and Scott, 1977; Shoolery, 1977). It will suffice for us to justify the conditions used in this study.

In Table I are the ^{13}C T_1's for thiofanox and several related compounds. The values span a wide range but will generally fall into two groups: (1) protonated carbons, with

Table I. ^{13}C T_1's (s) and NOE's for Thiofanox and Related Compounds[a]

carbon	$(CH_3)_3CC(NOCONHCH_3)CH_2SCH_3$ 1[b]	$(CH_3)_3CC(NOH)CH_2SCH_3$ 5	$(CH_3)_3CC(NOCONHCH_3)CH_2S(O)CH_3$ 3	$(CH_3)_3CC(NOCONHCH_3)CH_2S(O)_2CH_3$ 4
C=N	32 (2.3)			
C=O	31 (2.3)			
C_{quat}	30 (2.9)			
$3 \times CH_3$	2.1 (3.0)	1.7	1.6	1.6
CH_2	1.5 (3.4)[c]	1.1	1.0	1.0
NCH_3	5.0 (3.1)[c]		3.3	3.9
SCH_3	6.2 (3.4)	5.7	2.1	1.9

[a] At 22.6 MHz and 35 °C. Samples were 10 wt% solutions in $CDCl_3$. Estimated accuracy of T_1's and NOE's, ±15%. [b] NOE's in parentheses. [c] Assignments based on T_1's.

short T_1's, and (2) nonprotonated carbons, with long T_1's (Allerhand et al., 1971; Wehrli, 1976). The nonprotonated carbon T_1's are usually 5–15 times those of protonated carbons in the same molecule. It is not possible to simultaneously optimize the pulse repetition rate for a wide range of T_1's. Maximum intensity can be obtained for all carbons but at the expense of long delays and reduced sensitivity per unit time. One possibility is the reduction of the ^{13}C T_1's (and hence pulse repetition rate) by the addition of a paramagnetic relaxation reagent such as $Cr(acac)_3$ (Gansow et al., 1972; Shoolery, 1977). This option is not available to us since transition metal complexes are known to catalyze the decomposition of thiofanox (Corkins et al., 1982). The other option is to use a reduced flip angle and pulse repetition rate which constitute a good compromise (Becker et al., 1979). We have used a 5-s repetition rate and 55° flip angle, which we believe is a good choice given the data in Table I. It should be pointed out that the T_1's in Table I are probably longer than the comparable values for the samples of the impurity concentrate, due to viscosity effects.

The approximate NOE's for all carbons of thiofanox are also in Table I. As expected, most carbons possess the maximum value of 3 (Allerhand et al., 1971). Values greater than 3 represent experimental error. The C=O and C=N carbons have somewhat reduced, but substantial NOE's. Major variations of NOE's (more than about 30%) are not expected for molecules of the type listed in Table I at low magnetic fields. The added sensitivity from the NOE compensates for minor variations that may exist.

Early in the study we investigated the use of high field ^1H NMR for detection of impurities in thiofanox. As previously mentioned, ^1H NMR is attractive from a sensitivity standpoint. Figure 1 is the upfield region of a 300-MHz ^1H NMR spectrum (obtained in the continuous wave mode) of a thiofanox impurity concentrate. The spectrum consists of several characteristic regions: 0.8–1.5 ppm, CCH_3 especially *tert*-butyl; 1.8–2.5 ppm, CH_3CO and SCH_3; 2.8–3.0 ppm, NCH_3; 3.2–3.5 ppm, SCH_2. Several additional resonances are seen in the downfield portion of the spectrum (not shown).

Although resonances due to impurities are seen in Figure 1, the similarity of the compounds and the small shift range limit the utility of ^1H NMR at 300 MHz. Higher magnetic fields may improve the situation somewhat. Subsequent work confirms that many of the minor resonances in Figure 1 each arise from several impurities, particularly in the *tert*-butyl region. Such is not the case for ^{13}C NMR. In addition, significant shift changes due to solvent effects can be a problem in ^1H NMR studies of this type, where it is necessary to accurately match shifts of pure standard and compound in a mixture.

(B) Detection of the Impurities. To establish an approximate sensitivity or cutoff level, it is essential to have quantitative data for one or more known impurities, containing both protonated and nonprotonated carbon types, in one or more samples. For this purpose we have used several compounds known to be impurities from the early history of the insecticide (Gertenbach et al., 1978). These compounds are (methylthio)pinacolone (**8**), bis(methylthio)pinacolone (**9**), dimethylurea (**10**), and a nitrile compound (**16**). (Compounds are numbered as in Tables IV and V.) These impurities have many well-resolved, easily assignable resonances of different carbon types, and they occur over a range of concentrations. The approach we have taken here is to quantitate these impurities in the technical material and then correlate these quantitative data with detectability of resonances in the impurity concentrate.

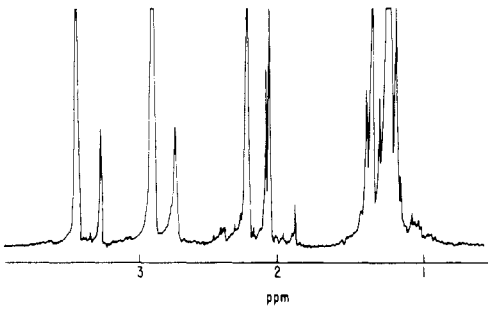


Figure 1. Upfield region of the 300-MHz ^1H NMR spectrum of a thiofanox impurity concentrate.

Figure 2 is the ^{13}C spectrum of the impurity concentrate of one sample studied. The spectrum is divided into appropriate expanded spectral plots. Using quantitative results for the above-mentioned compounds, we have established approximate detection levels. The horizontal arrows next to the peaks of interest correspond to 0.1% *in the technical* material. Clearly, in all cases 0.1% can be detected. In most cases, substantially less than 0.1% can be seen. This also applies to both protonated and nonprotonated carbons. Hence, it is felt that if the presence of all peaks in the high sensitivity ^{13}C NMR spectrum of a thiofanox impurity concentrate are rationalized, all impurities at or above 0.1% will be accounted for. Since the ^{13}C NMR spectra of all of the ten impurity concentrates are very similar, only one need be pursued in detail.

Figure 2 is divided into four sections approximately representing (a) carbonyls, (b) unsaturated carbons, (c) nitriles and highly deshielded aliphatic carbons, and (d) aliphatic carbons. Over 100 peaks can be identified in the ^{13}C spectrum of the impurity concentrate. Table II lists the peaks from Figure 2 and the impurities which can be assigned to these peaks. The impurities were identified by subsequent chromatographic and spectroscopic work (see below).

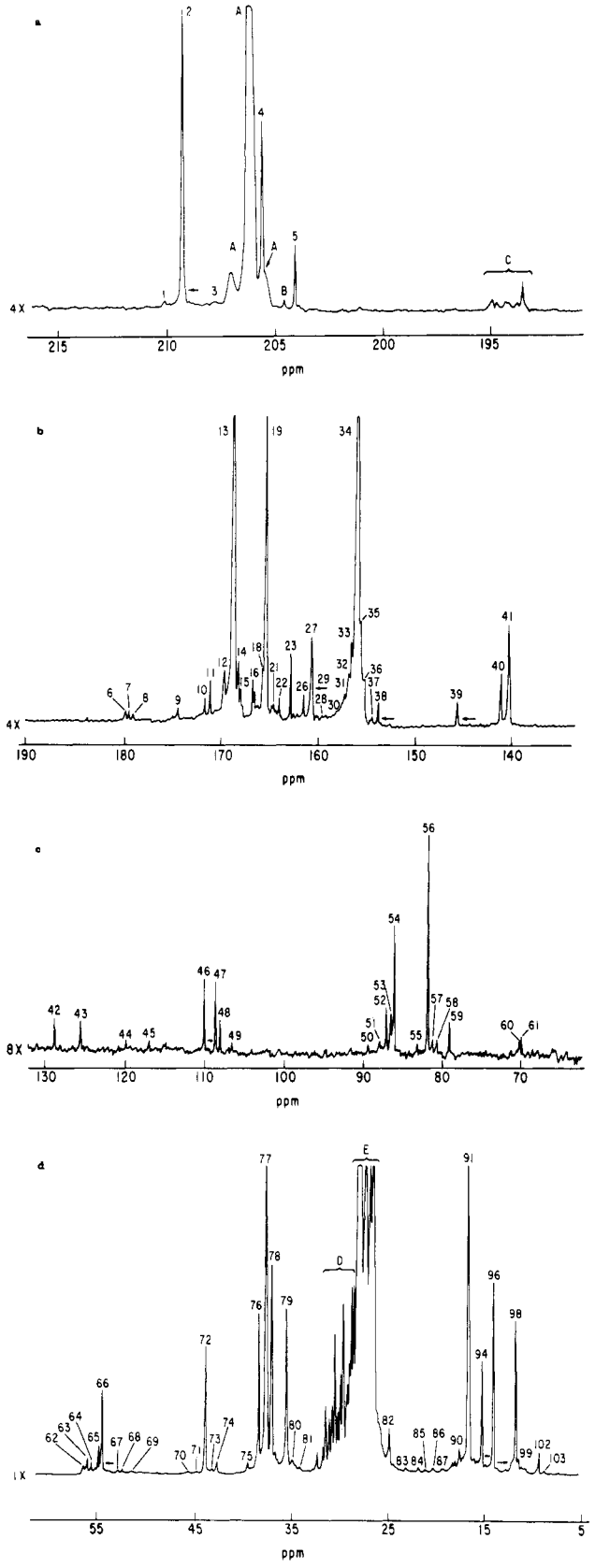


Figure 2. 22.6-MHz ^{13}C NMR spectrum of a thiofanox impurity concentrate: (a) 191–217 ppm, (b) 136–191 ppm, (c) 65–132 ppm, (d) 5–60 ppm. No peaks occurred in regions not shown.

(C) Preparative Chromatography and ^{13}C NMR. After obtaining the high signal-to-noise ^{13}C spectrum of the impurity concentrate, it was necessary to separate chromatographically and identify the impurities responsible for the peaks in Figure 2. Since one goal of this work was to be able to pursue only those impurities at or above

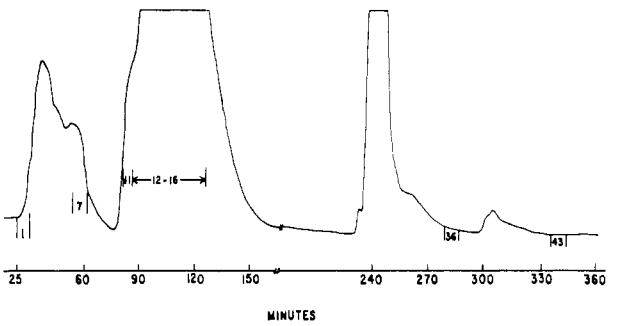


Figure 3. Preparative LSC chromatogram of a thiofanox impurity concentrate.

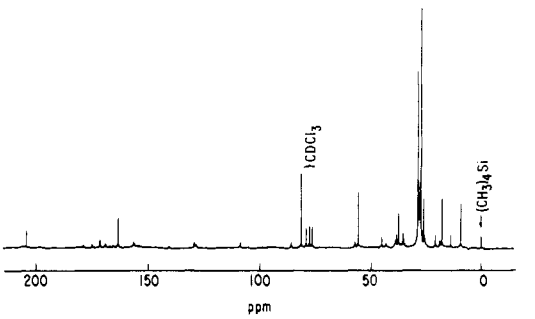


Figure 4. 22.6-MHz ^{13}C NMR spectrum of cut 11 of the preparative LSC chromatogram in Figure 3.

0.1%, at least the first stage of chromatography must be linked to the total impurity concentrate spectrum. To conveniently scan the chromatographic cuts, we have used preparative scale liquid chromatography for the initial separations.

The complexity of the samples dictated the use of both LSC and RP separations to insure that the entire polarity range of the components was encompassed. In addition, the previously cited limitations of UV detection mandated that all preparative runs be collected in a continuous series of cuts. This precaution prevented inadvertently missing a component only because the chromatogram appeared to be at or near base line.

The impurities were arbitrarily defined as low, medium, and high polarity components. The preparative runs were usually made with the objective of optimizing the resolution in two of these three categories. However, the LSC and RP mobile phase examples listed in the Experimental Section represent efforts to maximize the probability of eluting all of the sample from the column.

Figure 3 shows a preparative LSC run of a 50-g sample of an impurity concentrate during which three mobile phases were employed. The effluent was collected in a series of 45 continuous cuts. The weights of these are listed in Table III. Cuts 12–16 represent the region during which the major components (the *E* and *Z* isomers of thiofanox) were collected.

Carbon-13 NMR spectra were taken for most of the cuts in Table III as well as for the cuts of the corresponding RP separation. Some cuts with weights of 0.05 g or less were not examined. In most cases the ^{13}C NMR spectra indicated that the preparative HPLC cuts were mixtures of 2–10 major components requiring further separation. Often, a single impurity appeared in several consecutive cuts. In two cases impurities were completely resolved.

Figure 4 is the ^{13}C spectrum of cut 11. This cut was particularly rich in impurities, as judged by peak matching to the total spectrum. Further separations work was performed on cut 11 (see below). While ^{13}C NMR could again be used to follow the initial chromatography, this

Table II. Peak Assignments in the ^{13}C NMR Spectrum of a Thiofanox Impurity Concentrate[a]

peak no. (Figure 2)	assignments: carbon	assignments: compound	peak no. (Figure 2)	assignments: carbon	assignments: compound
1	C=O	17	56	CH(O)S	22
2	C=O	8	57	CH_2O	37
3	C=O	18, 45	58		not identified[e]
A	C=O	acetone-d_6	59	$CHCl_3$	33
4	C=O	9	60	$CHS(O)_2$	30[c]
B		artifact[b]	61		
5	C=O	22	62	OCH_3	23
C		artifact[b]	63	OCH_2	13, 14
6		not identified[c]	64		
7		not identified[e]	65	$CH_2S(O)_2$	4
8	C=N	52		OCH_3	22, 24
9	C=O	19	66	CHS_2	9
10	C=N	13, 20	67	$CH_2S(O)$	3
11	C=N	19	68	$CH_2S(O)$	6
12	C=N	23, 25, 31[d]		$CH_2S(O)_2$	7
13	C=N	1	69	C_{quat}	37
14		not identified[e]	70	C_{quat}	18, 28
15		d	71	C_{quat}	4, 7, 17
16	C=N	14, 26, 29	72	CH_2S	8
17				C_{quat}	9
18	C=N	27	73	C_{quat}	17, 18
19	C=N	2	74	C_{quat}	22
20	C=N	29, 30	75	C_{quat}	3, 6, 30
21			76[g]	C_{quat}	8
22	C=N	3, 32	77[g]	C_{quat}	1
23	C=N	5	78[g]	C_{quat}	2
24	C=N	4, 37	79	CH_2	2
25			80	CH_2	27
26	C=N	21	81	CH	26
27	C=O	10		C_{quat}	32
28	C=N	28		CH_3	36
29	C=N	6		CH_2	50
30	C=N	7	D	CD_3	acetone-d_6[h]
	C=O	13	E		i
31	C=O	11	82	$(CH_3)_3$	52
32	C=O	31		CH_3C=O	38
33	C=O	25, 26, 27, 32		$(CH_3)_3$	40
34	C=O	1, 2	83	$(CH_3)_2C$	37
35			84	CH_3	12
36	C=O	4, 20, 23, 28, 29, 30	85		not identified[e]
37			86	SCH_3	26
38	C=O	16	87	CCH_3	27
39	C=N	16		SCH_3	35
40	C=N	15	88	SCH_3	23, 30
41[f]	CH=N	21	89		
42	C=C	52	90	SCH_3	5, 19, 20, 29, 52
43	C≡N	34	91	SCH_3	1
44		not identified[a]	92	SCH_3	26, 35
45	C≡N	35	93		
46	C≡N	15	94	SCH_3	8
47	C≡N	16	95	SCH_3	27, 50
48	C=C	52	96	SCH_3	2, 49
49		not identified[e]	97	SCH_3	18, 28
50		not identified[e]	98	SCH_3	9
51		not identified[e]	99	SCH_3	31[e]
52			100	SCH_3	39
53	CH(O)S	23, 24, 29	101	SCH_3	17
54			102	SCH_3	22
55		not identified[e]	103	CH_3C=N	37

[a] Structures can be found in Tables IV and V. [b] These are images of strong peaks in the upfield region of the spectrum due to imperfect quadrature detection. [c] These peaks do not appear in any other thiofanox impurity concentrate spectrum studied. [d] All or part spinning side band of nearby strong peak. [e] Will probably be accounted for by one or more tentatively identified impurities (Table V) whose ^{13}C spectrum has not yet been obtained, based on expected ^{13}C shifts. [f] These peak positions are solvent and concentration dependent, and the peaks in the standards are shifted by about 1 ppm from the positions here. Compound 21 was confirmed by spiking the cut in question with the standard compound. [g] The quaternary carbons of the *tert*-butyl groups of many impurities occur in this region. [h] In addition, resonances from 5, 11, 20, 26, 39, and 43 occur in this region. No attempt was made to assign individual peaks in the region. [i] The *tert*-butyl methyls, NH methyls, and peaks of many other impurities, as well as (*Z*)-thiofanox, occur in this region.

was not the case for further separations on any other preparative HPLC cuts. In these latter cases, ^1H FTNMR, FT infrared spectroscopy (IR), and mass spectrometry (MS) were used. After identification and confirmation by independent synthesis, the ^{13}C spectrum was matched to that of the impurity concentrate.

(D) Future Refinements of the Methodology. Although the ^{13}C NMR method described here was developed because of the thermal instability of thiofanox and related compounds, it is obviously not restricted to thermally unstable materials. The method does provide a relatively quick estimate of the number and concentration of im-

Table III. Weights of Cuts Recovered from an LSC Separation of a 50-g Sample of Impurity Concentrate

cut no. (Figure 3)	weight, g	cut no. (Figure 3)	weight, g
1	0.1	23	0.1
2	0.3	24	0.1
3	0.9	25	<0.05
4	0.7	26	0.1
5	0.7	27	<0.05
6	1.3	28	<0.05
7	1.8	29	<0.05
8	0.3	30	0.1
9	<0.05	31	0.4
10	1.8	32	1.7
11	1.3	33	0.5
12	7.5	34	0.2
13	8.5	35	0.4
14	10.1	36	0.2
15	8.6	37	0.1
16	4.6	38	0.2
17	3.0	39	0.2
18	1.8	40	0.1
19	1.2	41	0.1
20	0.5	42	0.3
21	0.1	43	0.4
22	0.1	44	0.3
		45	0.1

purities without the need to search for optimum conditions as in a chromatographic technique.

The major limitation of the method as applied here is the need to use an impurity-rich concentrate. Such a concentrate should usually be obtainable by solvent extraction or zone refining. It might also be possible to directly detect impurities at 0.1% in the technical insecticide itself, without the concentration step. Commercial ^{13}C NMR spectrometers based on wide-bore cryomagnets have sensitivity substantially improved over that of the instrumentation used in this study. Such systems employ large volume sample tubes (20 mm o.d.) and, for a study of the present type, would require about 5 g of sample. Hence 0.1% (approximately 5 mg) should be detectable on such equipment, without the need for relaxation reagents. A preliminary ^{13}C spectrum of a technical thiofanox sample (91.6% assay) acquired on a Bruker WH-180 instrument using 20-mm sample tubes confirms the above contention (Komoroski, 1978). Commercial NMR spectrometers available today operate at frequencies up to 500 MHz for ^{1}H and 125 MHz for ^{13}C. In addition, modern spectrometers have much improved electronic designs which provide increased sensitivity beyond that due to increases in magnetic field. The increased spectral resolution from high field operation is an additional benefit for the study of complex mixtures. This is particularly true for ^{1}H NMR. Other recent developments in NMR which are as yet untested for the analysis of complex mixtures are two-dimensional and multiple-quantum NMR.

Another possible refinement is the addition of an internal area standard of known T_1(s) and NOE(s). This approach would allow for direct quantification of various components in a complex mixture without the necessity of chromatographic separation and purification.

(II) Separation and Identification of Nonvolatile Impurities. (A) Chromatography. Figure 5 illustrates the complexity of the samples involved in this study. This analytical chromatogram of the impurity-rich concentrate was obtained under conditions in which the more nonpolar components eluted from the column first. Conditions similar to this analytical separation were used for the preparative chromatography. The general scheme for the purification of the large scale preparative cuts is outlined in Figure 6. In a few instances the cuts were sufficiently

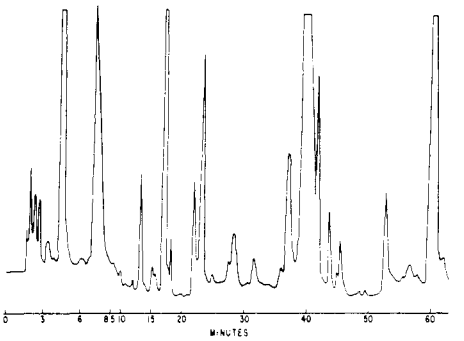


Figure 5. LSC analytical chromatogram of the nonpolar components of a thiofanox impurity concentrate.

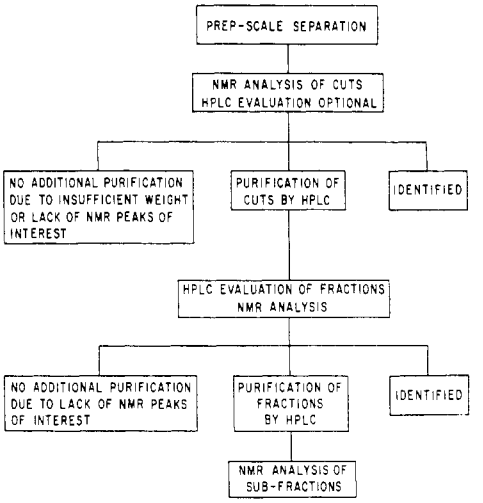


Figure 6. General scheme for the purification of the large scale preparative cuts.

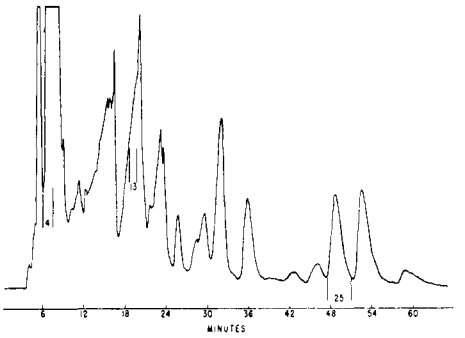


Figure 7. LSC semipreparative chromatogram of cut 11.

pure to permit direct, unambiguous identification. However, NMR and HPLC analyses generally showed that the cuts were mixtures ranging from a few to more than a dozen components.

The results of a typical LSC preparative run are summarized as follows. A series of 45 cuts was collected from a preparative run of a 50-g sample. The initial cuts were highly concentrated in compounds 8 and 9 (Table IV). The first cut contained, in addition to 9, a component identified as compound 17, whereas by the seventh cut essentially pure 8 was collected. NMR analysis indicated that cut 11, which represented approximately 2–3% of the original sample, contained several peaks of interest (Figure 4). The major components, the isomers of thiofanox, compounds 1 and 2, eluted during cuts 12–16.

Replicate, injections of cut 11 were made on an 8-mm i.d. semipreparative column. Figure 7 shows one of these runs. Figure 8 illustrates a reinjection on an analytical column of fraction 4, obtained from cut 11, and indicates

Table IV. Compounds Identified in Technical Thiofanox

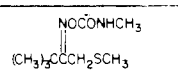
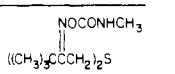
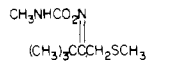
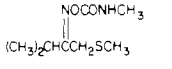
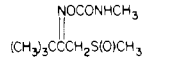
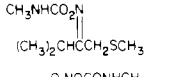
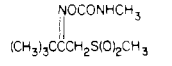
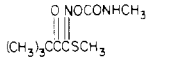
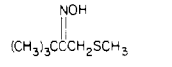
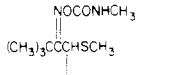
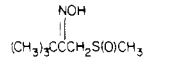
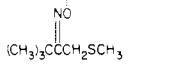
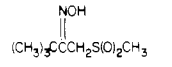
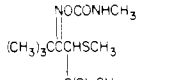
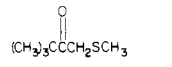
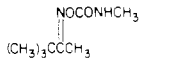
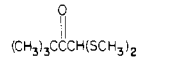
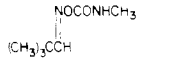
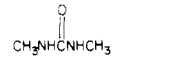
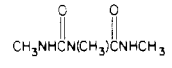
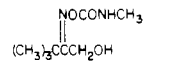
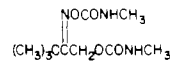
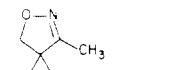
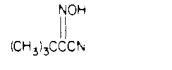
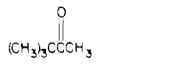
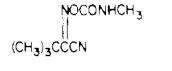
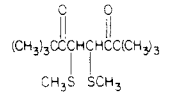
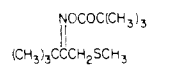
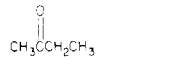
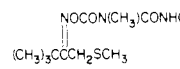
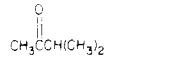
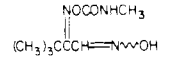
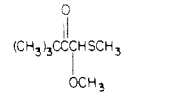
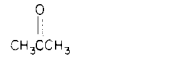
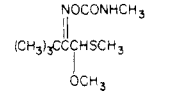
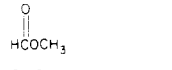
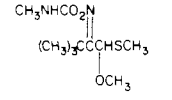
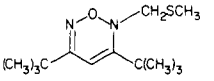
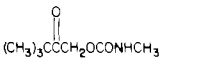
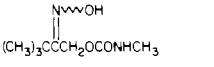
compd	structure	compd	structure
1		25	
2		26	
3		27	
4		28	
5		29	
6			
7		30	
8		31	
9		32	
10		33	$CHCl_3$
11		34	$(CH_3)_3CCN$
12	CH_3SSCH_3	35	CH_3SCH_2CN
13		36	$(CH_3)_3CCl$
14		37	
15		38	
16		39	$(CH_3)_3CSCH_3$
17, 18 (2 diastereomers)		40	$(CH_3)_2CHCH_3$
		41	$(CH_3)_2C{=}CH_2$
		42	CH_3SCH_3
		43	$(CH_3)_3COH$
		44	CH_2Cl_2
19		45	
20		46	
21		47	CCl_4
		48	CH_3NCS
		49	$CH_3SCH_2SCH_3$
22		50	$CH_3SCH_2CH_2SCH_3$
		51	
23		59	
		60	CH_3OH
24		61	CH_3Cl
		66	$(CH_3)_2CHCN$

Table V. Compounds Tentatively Identified in Technical Thiofanox

compd	tentative structure
52	
53a	
53b	
54, 55 (2 diastereomers)	
56	
57a	
57b	
58a	
58b	
62	CH_3OCH_3
63	$CH_3CH_2CH_2OH$
64	$(CH_3)_2CHOH$
65	$CH_3CH_2CH_2CN$
67	$CH_3CH_2CH_2CH_2SH$
68	
69	$(CH_3CH_2CH_2CH_2S)_2$

that relatively pure material was realized from the semipreparative operation. Fraction 13 has been tentatively identified as compound **52** (Table V). Fractions 4 and 25 were identified as compounds **22** and **23**, respectively (Table IV). The purification of cut 11 ultimately led to the identification of five impurities as well as the tentative identification of compound **52**.

In rarer instances the fraction did require additional purification. Although these fractions were usually approximately 85–90% pure, the presence of the minor interfering components sometimes presented difficulties in the interpretation of the NMR data. These fractions were subjected to reinjection onto analytical columns and collected as subfractions. Except for the additional time required to complete these tasks, the acquisition of the subfractions created no undue problems.

The rationale of collecting the cuts as a continuous series was reinforced by at least two of the cuts taken while the chromatogram appeared to be at base line. Cut 36 was identified as compound **14** while cut 43 was approximately 100% compound **10**. These cuts represented roughly 0.4 and 1.0% of the sample, respectively.

(B) Confirmed Identifications. The structures of a number of nonvolatile impurities in technical thiofanox are illustrated in Table IV. Also listed are the structures of (*Z*)- and (*E*)-thiofanox. The identities of all the compounds in Table IV were confirmed by independent syn-

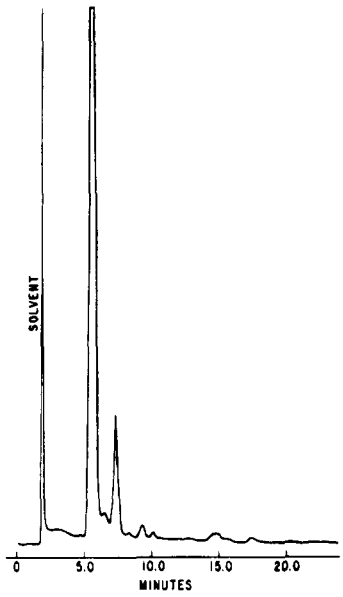


Figure 8. LSC analytical chromatogram of fraction 4 from cut 11.

thesis or the acquisition of commercially available compounds. Spectral and other data for authentic samples appear as supplementary material.

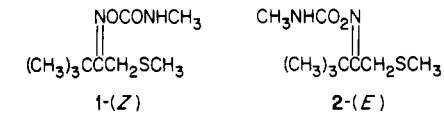
The initial identification of a given impurity was made by one or more techniques, depending on how an impurity was first encountered. Reasonably pure fractions and subfractions were identified by using ^1H NMR or electron impact MS. In several cases, the initial identification was made by using the ^{13}C NMR spectrum of a preparative HPLC cut or a semipreparative fraction. In most cases, more than one type of spectroscopy was employed before synthesis of the proposed structure was carried out.

Proton NMR chemical shifts of authentic samples and chromatographic fractions usually agreed to 0.02 ppm or better for nonexchangeable hydrogens. For ^{13}C NMR, the agreement was usually 0.2 ppm or less. The ^{13}C chemical shift of the azomethine carbon of compound **21** varied over about 1.5 ppm among the standard sample and several fractions in which **21** was the major component. The presence of **21** in the fractions in question was confirmed by MS and IR and by spiking with standard sample and subsequently reacquiring the ^1H and ^{13}C NMR spectra.

The ^{13}C NMR method of Section I was used to guide the separation by directing efforts to the preparative HPLC cuts of greatest importance. Subsequent separations of the HPLC cuts usually could only be followed by ^1H NMR due to the amounts of samples available. Since it was sometimes difficult to relate the ^1H spectrum of a fraction to the ^{13}C spectrum of the preparative cut containing that fraction, more compounds were identified than were necessary to account for all the peaks in the ^{13}C spectrum of the impurity concentrate. In addition, fractionation of preparative HPLC cuts often yielded pure fractions of minor impurities which could be readily identified by ^1H NMR, although they were not critical for peak matching to the total spectrum.

(C) Tentative Identifications. In Table V are the tentative structures of some additional impurities isolated from technical thiofanox. In the Supplementary Material we also list the spectral data used to arrive at the tentative structures. For **53**, **57**, and **58**, we have proposed two possible structures, the first of which we consider the more likely. Confirmation of the structures in Table V must await independent synthesis.

(D) Stereochemical Assignments. The identification of impurities which were oximes or oxime derivatives was complicated by the possible presence of both *E* and *Z* isomers of the same impurity. For example, thiofanox itself exists as a mixture of isomers, where the predominant and most insecticidally active (Magee and Limpel, 1977) isomer is assigned the *Z* configuration. Clearly, the



presence of *E*–*Z* isomerism can substantially increase the number of impurities to be identified. Both ^{13}C and ^1H NMR chemical shifts of several isomeric pairs have been compared with previous results in the literature for ketoximes. Finally, chemical shift comparisons have been used to confirm or assign the stereochemistry of a number of impurities found in technical thiofanox.

Proton NMR chemical shifts have been used previously to establish configuration in oximes and related compounds (Karabatsos and Taller, 1968; Karabatsos et al., 1963; Lustig, 1961). The α-protons syn to the hydroxy group in oximes occurred at lower field than the corresponding protons in the anti position (Karabatsos and Taller, 1968). Shift differences at the β-position were generally smaller than at the α-position and were variable in sign.

In Table VI are the ^1H NMR chemical shifts of the *E* and *Z* isomers for (methylthio)pinacolone oxime, thiofanox, and four thiofanox derivatives. As expected, substantial differences are seen between *E* and *Z* isomers of all resonances except *N*-methyl (Karabatsos and Taller, 1968). The α-methylene protons syn to oxygen appear downfield of their position when anti to oxygen for the compounds in Table VI. The reverse is seen for the methine proton of the bis(*S*-methyl) compounds in Table VI. Consistent differences between isomers are also seen for β-(*tert*-butyl) and γ-(*S*-methyl) protons.

Carbon-13 NMR has also been used to study the configuration of oximes (Hawkes et al., 1974; Levy and Nelson, 1972). Roberts and co-workers (Hawkes et al., 1974) studied a wide range of ketoximes and found that syn α-carbons appear at higher field by about 3–9 ppm than the same carbons in the anti configuration. Changes of several ppm were seen for β-carbons. The imine carbon was not a useful probe of oxime configuration, since the *E*–*Z* shift difference was usually less than 1 ppm.

In Table VII are the ^{13}C chemical shifts of the same compounds as in Table VI. The α-methylene (and methine) carbons show the same behavior as observed previously for oximes (Hawkes et al., 1974). However, very little change is seen for either the quaternary or methyl carbons of the *tert*-butyl group. Comparison cannot be made with previous data in this latter case since Roberts and coworkers (Hawkes et al., 1974) observed only one isomer for ketoximes with an α-quaternary carbon. Consistent *E*–*Z* shift differences are seen for the *S*-methyl groups. The chemical shifts of the imine carbon or carbons on the carbamate side chain are not usable for configurational assignments.

On the basis of the data in Tables VI and VII, it is possible to make configurational assignments for a number of other impurities found in technical thiofanox. For the *E*–*Z* pair **23** and **24**, we make the assignment shown on the basis of the *tert*-butyl and *S*-methyl proton shifts. This assignment is consistent with the α-methylene shifts in Table VI but not the α-methine shift of the bis(*S*-methyl) compounds.

Table VI. ^{1}H NMR Chemical Shifts of Some *E*–*Z* Pairs[a]

	N⁓OH ‖ $(CH_3)_3CCCH_2SCH_3$		N⁓OCONHCH$_3$ ‖ $(CH_3)_3CCCH_2SCH_3$		N⁓OCONHCH$_3$ ‖ $(CH_3)_3CCCH_2S(O)CH_3$		N⁓OCONHCH$_3$ ‖ $(CH_3)_3CCCH_2S(O)_2CH_3$		N⁓OCONHCH$_3$ ‖ $(CH_3)_3CCCH(SCH_3)_2$		N⁓OH ‖ $(CH_3)_3CCCH(SCH_3)_2$	
group	*E*[b]	*Z*	*E*	*Z*	*E*[b]	*Z*	*E*[b]	*Z*	*E*[b]	*Z*[e]	*E*[b]	*Z*[b]
CH_2 or CH	3.21	3.40	3.27	3.44	3.75 (15.6)[c]	3.83 (12.6)[c]	4.06	4.27	4.57	4.33	4.55	4.26
SCH_3	2.06	2.25	2.08	2.25	2.72	2.83	3.04	3.26	2.06	2.30	2.04	2.29
tert-butyl	1.35	1.20	1.38	1.24	1.35	1.26	1.38	1.26	1.39	1.29	1.37	1.24
NCH_3[d]			2.94 (4.9)	2.92 (4.9)	2.90 (5.0)	2.92 (5.0)	2.91 (5.0)	2.93 (5.0)	2.91 (4.8)	2.91 (4.9)		
NH			6.22	6.26	5.69	6.19	5.97	6.22	6.27	6.06		
OH	9.45	9.70									9.64	9.74

[a] In ppm downfield from internal $(CH_3)_4Si$. [b] Prepared during the course of another study. [c] AB quartets. Coupling constants (Hz) in parentheses. [d] Doublets. Coupling constants (Hz) in parentheses. [e] Described in supplementary material.

Table VII. ^{13}C NMR Chemical Shifts of Some *E*–*Z* Pairs[a]

	N⁓OH ‖ $(CH_3)_3CCCH_2SCH_3$		N⁓OCONHCH$_3$ ‖ $(CH_3)_3CCCH_2SCH_3$		N⁓OCONHCH$_3$ ‖ $(CH_3)_3CCCH_2S(O)CH_3$		N⁓OCONHCH$_3$ ‖ $(CH_3)_3CCCH_2S(O)_2CH_3$		N⁓OCONHCH$_3$ ‖ $(CH_3)_3CCCH(SCH_3)_2$		N⁓OH ‖ $(CH_3)_3CCCH(SCH_3)_2$	
group	*E*[b]	*Z*	*E*	*Z*	*E*[b]	*Z*	*E*[b]	*Z*	*E*[b]	*Z*[d]	*E*[b]	*Z*[b]
C=N	160.4	163.6	165.9	168.7	163.6	165.6	162.6	161.6	165.0	167.5	159.5	162.7
C=O			155.7	155.9	155.1	154.9	154.8	154.7	155.6	154.7		
C_{quart}	36.6[c]	37.2	37.5	37.8	37.8	37.8	38.0	37.9	38.0	37.8	37.0	37.4
3 × CH_3	27.7	28.3	28.1	28.3	27.8	27.5	28.0	27.5	27.9	27.9	27.5	28.3
NCH_3			27.6	27.6	27.5	27.7	27.8	27.7	27.9	27.2		
CH_2 or CH	36.3[c]	25.8	36.1	26.8	58.2	53.6	57.6	53.6	54.4	50.4	54.0	51.0
SCH_3	14.6	17.3	14.7	17.2	39.0	41.0	40.9	45.0	12.2	16.4	12.4	17.3

[a] In ppm downfield from internal $(CH_3)_4Si$. [b] Prepared during the course of another study. [c] Assignments ambiguous. [d] Described in supplementary material.

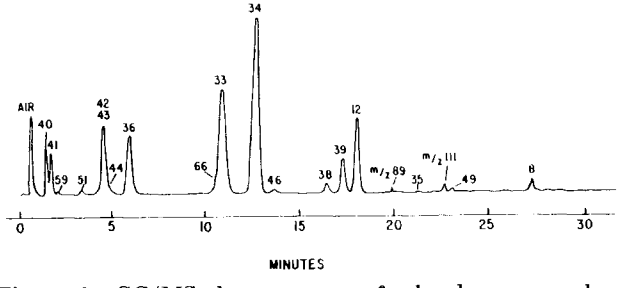


Figure 9. GC/MS chromatogram of a headspace sample of volatile impurities in technical thiofanox. (Numbers are compound numbers (Table IV).)

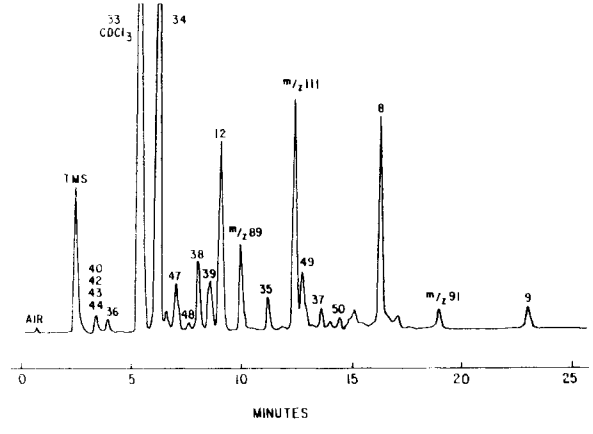


Figure 10. GC/MS chromatogram of a liquid sample of volatile impurities in technical thiofanox. (Numbers are compound numbers (Table IV).)

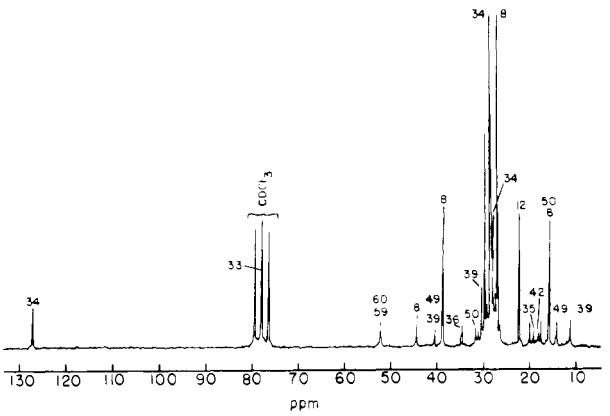


Figure 11. 22.6-MHz ^{13}C NMR spectrum of volatile impurities in technical thiofanox. (Numbers are compound numbers (Table IV).)

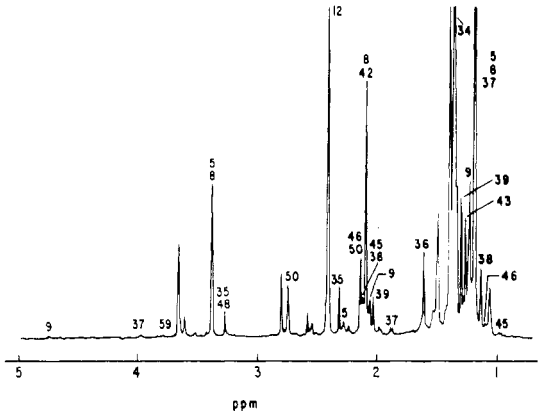
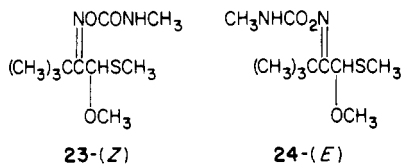


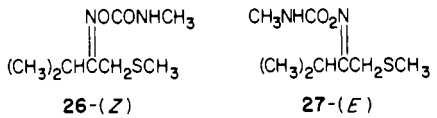
Figure 12. Upfield 90-MHz ^{1}H NMR spectrum of volatile impurities in technical thiofanox. (Numbers are compound numbers (Table IV).)

The pair **26** and **27** can be assigned as the *Z* and *E* isomers, respectively, on the basis of shift differences in



23-(*Z*) **24**-(*E*)

the pair for both the α-methylene and the α-methine protons. Assignments in the ^{13}C spectrum of a mixture of **26** and **27** were made on the basis of the trends mentioned above and a single-frequency, off-resonance decoupling experiment (Abraham and Loftus, 1978).



26-(*Z*) **27**-(*E*)

Configurational assignments can be made for a number of other impurities even though both isomers were not detected in technical thiofanox. Based on the ^{1}H and ^{13}C chemical shift trends seen for the compounds in Tables VI and VII, we can assign the *Z* configuration to the following impurities: **6**, **7**, **20**, **25**, **29** (by comparison to **1**), and **30**. For **13**, **14**, **15**, **16**, **19**, **21**, and **28**, the *Z* configuration is a reasonable assignment as is the *E* assignment for **31** and **32**. These latter assignments cannot be made with absolute confidence due to differences between the compounds of interest and those in Tables VI and VII. No configurational assignments were made for the tentatively identified impurities in Table V or the aldoxime moiety of **21** (Table IV).

(III) Separation and Identification of Volatile Impurities. Figures 9 and 10 illustrate typical GC chromatograms of headspace and liquid samples, respectively, of the volatile components in technical thiofanox. Table IV includes all the volatile impurities identified in the technical material by GC/MS. The presence of many of these compounds was confirmed by ^{13}C and ^{1}H NMR (Figures 11 and 12, respectively). In addition, previously identified compounds **5**, **8**, **9**, and **12** were also detected by GC/MS and NMR. Peaks were observed in the ^{1}H and ^{13}C NMR spectra of the volatile impurities which were not identified, but which probably arose from other impurities detected by GC/MS.

Table V includes the tentative structural assignments of other volatile components in technical thiofanox. Although reference samples of these compounds were available, a positive identification could not be made with the available data. Four components with apparent molecular ions at m/z 89, 91, 111, and 146 were also observed sometimes. Insufficient data were available, however, for any structural assignments.

CONCLUSIONS

We have developed a ^{13}C NMR method for screening technical pesticides to determine the number and concentration of the major impurities. The method has been applied to the impurities at or above 0.1% in thiofanox and has been used successfully to guide subsequent separation and identification work. Improvements in NMR instrumentation should make the approach taken here easier and more direct and reliable, eliminating the need for using an impurity concentrate.

Over 70 components (including stereoisomers) were positively or tentatively identified in technical thiofanox.

EXPERIMENTAL SECTION

(A) Detection by ^{13}C NMR. Technical thiofanox (Diamond Shamrock Corporation), as usually produced, consists of two phases. The crystalline component appears to be pure thiofanox. The liquid component, which is about 75% thiofanox, is substantially enriched in impurities. The liquid components of ten technical thiofanox samples, with total assay values (*E* and *Z* isomers) ranging from 92–97 wt %, were examined as approximately 75 vol % solutions in acetone-d_6.

Natural abundance proton decoupled ^{13}C NMR spectra were acquired at 35 °C on a Bruker WH-90 (22.6 MHz) Fourier transform spectrometer with quadrature detection capability. Samples were run in 10-mm o.d. NMR tubes. The 6024-Hz spectra were acquired in 16384 channels with a 5-s pulse delay and 55° radiofrequency (rf) pulses. A 90° rf pulse was 14.5-μs. Between 30 and 60 K transients were usually accumulated for spectra of thiofanox impurity concentrates, with 1-Hz line broadening present from digital exponential filtering for signal-to-noise enhancement. Chemical shifts were referenced to internal $(CH_3)_4Si$. All chromatographic cuts and standard compounds were run in $CDCl_3$ by using somewhat the same conditions as above, except that far fewer transients were accumulated per spectrum.

Carbon-13 T_1's were measured with the inversion–recovery technique (Vold et al., 1968), with the repetition rate equal to five times the longest protonated carbon T_1 or three times the longest nonprotonated carbon T_1. NOE's were measured with a gated decoupling technique (Freeman et al., 1972).

(B) Chromatography. All preparative separations were run on a Jobin-Yvon Chromatospac 100. The detector was an Altex Model 153 equipped with a preparative cell and a 280-nm filter. The liquid-solid chromatography (LSC) was performed on 40–63 μm silica gel 60. The column packing used for the reversed-phase chromatography (RP) was Lichroprep RP-8, 25–40 μm. Both packings were purchased from MCB Manufacturing Chemists, Inc. Recovery of the cuts was facilitated by use of a Buchi Rotavapor R.

Typical preparative schemes are outlined below. In attempts to optimize resolution, the initial mobile phase composition (v/v/v) was often replaced by one or two others of increased strength. The mobile phases noted below were specific parameters utilized in two experiments during the course of this work.

sample sizes	15–50 g
column packing	0.5–1.5 kg
flow rate	60–80 mL/min
mobile phase	
(LSC) initial	16 L hexanes/*n*-BuCl/EtOH (80/15/5)
intermdte	5 L hexanes/*n*-BuCl/EtOH (60/20/20)
final	4 L *n*-BuCl/EtOH (50/50)
(RP) initial	10 L H_2O/MeOH/THF (60/35/5)
intermdte	5 L H_2O/MeOH/THF (40/50/10)
final	4 L MeOH

The volumes per cut were 300–500 mL. The Rotavapor water bath was maintained at approximately 40 °C for the LSC cuts and 50–60 °C for the RP cuts. Generally this operation removed most but not all of the solvent. When present, the residual solvent posed no major problems in the NMR technique.

The preparative cuts, which were shown by NMR to contain species of interest but were in need of further purification, were fractionated on both semipreparative and analytical columns. The instruments and components used were a Spectra-Physics Model 8000 liquid chromatograph with a Model 8310 fixed wavelength (254 nm) detector, Waters Associates M-6000/M-6000A pumps, 660 programmer, and U6-K injector, Varian Models 8500 and 5020 liquid chromatographs and Models 635 and VUV-10 variable wavelength detectors, an Altex Model 153 fixed wavelength (254 nm) detector, Valco Model CV-6-UHPa-N60 injection valves, and a Milton-Roy Minipump.

The columns used were a Varian Si-10 (8 mm i.d. × 50 cm), Whatman Partisil-10 PAC, and Partisil-5 ODS (each 4.6 mm i.d. × 25 cm), EM Laboratories Lobar Si-60, 40–64 μm (25 mm i.d. × 31 cm), and an in-house slurry packed 10 μm Lichrosorb Si-60 (4.1 mm × 25 cm). All solvents were HPLC grade.

(C) Isolation of Volatile Impurities. Technical thiofanox impurity rich oils were subjected to a vacuum less than 4 mmHg at room temperature for approximately 1 h. Volatile components were collected in a trap cooled to −196 °C with liquid nitrogen.

(D) Spectroscopy. ^1H NMR spectra of chromatographic fractions were recorded on a Bruker WH-90 spectrometer (90 MHz). All samples, which were usually 0.1–1 mg, were dissolved in $CDCl_3$ with $(CH_3)_4Si$ for shift reference. The NMR tubes were 5-mm o.d. Some typical accumulation conditions were as follows: spectral width, 1165 Hz; size of transform, 8192; pulse delay, 5–10 s; 90° radio frequency pulse, 4.7 μs; broadening due to exponential multiplication for sensitivity enhancement, 0.5 Hz; number of transients, variable depending on sample concentration. Spectra were usually accumulated for 5–30 min each. ^1H NMR spectra of the authentic samples were recorded on a Bruker WH-90 spectrometer (90 MHz) or on a Perkin-Elmer R-24B spectrometer (60 MHz) in $CDCl_3$ with $(CH_3)_4Si$ as an internal standard. ^{13}C NMR spectra of the authentic samples were recorded on a Bruker WH-90 spectrometer in acetone-d_6 or, where indicated, in $CDCl_3$ with $(CH_3)_4Si$ as an internal standard.

IR spectra of chromatographic fractions were recorded on a Nicolet 7199 Fourier transform infrared spectrophotometer. Operating parameters varied little among samples. Some typical conditions were as follows: mirror velocity, 2 cm/s; gain, 1; number of scans, 100; points, 16384. The Happ-Genzel apodization function was used for all spectra. The general experimental procedure for the handling of each type of sample is as follows: (1) For oil and liquid samples, all spectra were obtained as smears or capillary films on KBr plates (4 mm × 25 mm). A background spectrum of the plate was first obtained so that any contaminants on the plate could be cancelled out. (2) All solid samples were run as KBr pellets. No background spectra were used due to the difficulties in making reproducible reference KBr pellets. IR spectra of the authentic samples were recorded on a Perkin-Elmer 283 spectrophotometer with KBr pellets or KBr plates.

Mass spectra of the authentic samples were recorded on a Varian-MAT CH7 spectrometer at 70 eV. Field desorption, chemical ionization, and high resolution mass spectra were obtained at the University of Illinois on a Varian 311 or Varian 731 spectrometer. Gas chromatography/mass spectrometry of the volatile impurities employed the Varian-MAT CH7 mass spectrometer and a Varian Aerograph 1740 gas chromatograph fitted with a Biemann-Watson separator. A Varian-MAT SS166 data system, interfaced with the mass spectrometer, acquired and stored the mass spectral data. Samples were injected onto a 10-ft glass column packed with 20% SP 2100/0.1% Carbowax 1500 on 100–120 mesh Supelcoport. Temperature programming was 10 min at 30 °C for headspace samples, then 30–190 °C at 10 °C/min. Liquid samples were held 5 min at 30 °C and then programmed as above.

UV spectra of the authentic samples were recorded on a Cary 118C spectrophotometer in CH_3OH.

(E) Physical Properties. Melting points were determined on a Thomas-Hoover Unimelt capillary melting point apparatus and are reported uncorrected. All boiling points are reported uncorrected. Elemental analyses were obtained on a Perkin-Elmer 240 elemental analyzer or at Galbraith Laboratories, Knoxville, TN.

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Registry No. **1**, 75013-97-7; **2**, 75013-98-8; **3**, 96758-78-0; **4**, 96758-79-1; **5**, 96758-80-4; **6**, 96758-81-5; **7**, 96758-82-6; **8**, 39199-12-7; **9**, 39195-75-0; **10**, 96-31-1; **11**, 816-00-2; **12**, 624-92-0; **13**, 96758-83-7; **14**, 96758-84-8; **15**, 96758-85-9; **16**, 96758-86-0; **17**, 96758-87-1; **18**, 96758-88-2; **19**, 96758-89-3; **20**, 96758-90-6; **21**, 96758-91-7; **22**, 96758-92-8; **23**, 75562-48-0; **24**, 96758-93-9; **25**, 96758-94-0; **26**, 96758-95-1; **27**, 96758-96-2; **28**, 96758-97-3; **29**,

96758-98-4; **30**, 73940-61-1; **31**, 96758-99-5; **32**, 96759-00-1; **33**, 67-66-3; **34**, 630-18-2; **35**, 35120-10-6; **36**, 507-20-0; **37**, 96759-01-2; **38**, 75-97-8; **39**, 6163-64-0; **40**, 75-28-5; **41**, 115-11-7; **42**, 75-18-3; **43**, 75-65-0; **44**, 75-09-2; **45**, 78-93-3; **46**, 563-80-4; **47**, 56-23-5; **48**, 556-61-6; **49**, 1618-26-4; **50**, 6628-18-8; **51**, 67-64-1; **52**, 96759-02-3; **53a**, 96759-03-4; **53b**, 69412-78-8; **54/55** (isomer I), 96759-04-5; **54/55** (isomer II), 96759-14-7; **56**, 96759-05-6; **57a**, 96759-06-7; **57b**, 96759-07-8; **58a**, 96759-08-9; **58b**, 96790-92-0; **59**, 107-31-3; **60**, 67-56-1; **61**, 74-87-3; **62**, 115-10-6; **63**, 71-23-8; **64**, 67-63-0; **65**, 109-74-0; **66**, 78-82-0; **67**, 109-79-5; **68**, 124-68-5; **69**, 629-45-8; (*Z*)-1,1-bis(methylthio)-3,3-dimethylbutan-2-one *O*-(*N*-methylcarbamyl)oxime, 73926-58-6; methyl 3,3-dimethyl-2-oxobutanehydroximidothioate, 96759-09-0; 3,3-dimethyl-2-oxobutanehydroximidoyl chloride, 96759-10-3; 3-methyl-1-(methylthio)butan-2-one oxime, 96759-11-4; 3-methyl-1-(methylthio)butan-2-one, 39199-24-1; 1-chloro-3-methylbutan-2-one, 17687-63-7; 1-chloro-3,3-dimethylbutan-2-one *O*-(*N*-methylcarbamyl)oxime, 96790-93-1; 1-chloro-3,3-dimethylbutan-2-one oxime, 24046-91-1; 1-cyano-2,2-dimethylpropanal oxime, 96758-85-9; 2,2-dimethylpropanal oxime, 637-91-2; 3,3-dimethyl-1-hydroxybutan-2-one oxime, 96759-12-5; 1-acetoxy-3,3-dimethylbutan-2-one, 38559-25-0; 1,3-dimethylurea, 96-31-1; 1,1-dichloro-3,3-dimethylbutan-2-one, 22591-21-5; 3,3-dimethylbutan-2-one, 75-97-8; 3,3-dimethyl-1-(methylsulfonyl)butan-2-one, 61524-35-4; 3,3-dimethyl-1-(methylthio)butan-2-one, 39199-12-7; (*E*)-3,3-dimethyl-1-(methylthio)butan-2-one oxime, 96759-13-6; 1-chloro-3,3-dimethylbutan-2-one, 13547-70-1; (*Z*)-3,3-dimethyl-1-(methylthio)butan-2-one oxime, 39195-82-9; thiofanox, 39196-18-4.

Supplementary Material Available: Syntheses and spectral data (46 pages). Ordering information is given on any current masthead page.

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A Rapid Method for the Determination of Olaquindox in Poultry Feeds by Derivative UV Spectrophotometry

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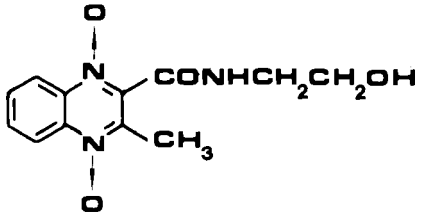
A simple method is described for the determination of the growth-promoting agent, Olaquindox, in poultry feeds. After filtration of the aqueous feed extract, the drug is directly quantified by 2nd derivative UV spectrophotometry. Recoveries from samples, fortified at levels 20–150 ppm, ranged between 96–103%. The proposed method is particularly recommended for everyday analysis of a large number of samples.

Olaquindox [2-[*N*-(2-hydroxyethyl)carbamoyl]-3-methylquinoxaline 1,4-dioxide] (1) is a growth-promoting agent added to pig and broiler chick diets at levels of 10–150 mg/kg.



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The initially described spectrophotometric method (Knapstein, 1977), for the determination of Olaquindox in mixed feeds, is rather time consuming, since it involves a prior cleanup of the crude feed extract by thin-layer chromatography to eliminate interfering substances. On the other hand, methods of analysis based on high-pressure liquid chromatography (HPLC) are faster but they still require liquid–liquid partition of the extract, before injecting it on to the column (Rückemann et al., 1979; Bories, 1979). A rapid HPLC procedure, not employing extract purification, has been reported recently (Thente and Anderson, 1982); however, HPLC column clogging should be expected after a certain number of injections.

In a study, conducted in our laboratory, on the performance evaluation of broiler chicks fed with Olaquindox, a method capable of estimating various levels of this additive in feeds has been developed that permits the direct quantitation of Olaquindox in the crude feed extract by second derivative UV spectrophotometry.

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