# Phosphorus-31 Nuclear Magnetic Resonance Analysis of Technical Organophosphorus Insecticides for Toxic Contaminants

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The analysis of 11 insecticides by $^{31}P$ FT/NMR showed the major known toxic contaminants to be simple trialkyl phosphorothio- and -dithioic acid esters and the *S*-alkyl insecticide isomers. Small amounts of the bis derivatives and the dithiopyrophosphate were also detected. These contaminants included both byproducts from the synthesis as well as degradation products. In view of their toxicological properties, monitoring of all technical products for contaminants is indicated, especially for products stored for long periods of time prior to their use. $^{31}P$ FT/NMR provides a facile method for the simultaneous identification of all phosphorus-containing contaminants provided that adequate precaution is taken to validify the analytical condition.

It has been known for some time that low levels of toxic contaminants occur in technical-grade organophosphorus insecticides (Gysin and Margot, 1958). They are formed either as byproducts during manufacture or on degradation of technical products, especially when they are stored at elevated temperatures (Umetsu et al., 1977; Miles et al., 1979). The similarity of substitution at the phosphorus moiety and the use of common intermediates in the manufacture of organophosphorus insecticides result in the formation of several common toxic contaminants. Their presence would be expected to change the properties of the insecticide and may enhance the hazard associated with handling and its use (Baker et al., 1978; Diggory et al., 1977).

Both gas chromatography (GC) and high-performance liquid chromatography (HPLC) are extensively used to monitor technical organophosphorus pesticides and their formulations for contaminants. These techniques, however, have limitations since some contaminants may undergo thermal rearrangements or degradation on GC analysis (Miles et al., 1979) while the UV detector used in HPLC cannot analyze for simple phosphate esters (Cochane et al., 1979). $^{31}P$ FT/NMR with enhanced sensitivity through the use of higher magnetic fields, and quadrature detection permits the determination of phosphorus compounds in the low-ppm range (Gurley and Ritchey, 1976; O'Neill and Pringiner, 1976; Sojka and White, 1978). Although NMR is not as sensitive as GC or HPLC, its high specificity, minimal sample preparation, and rapid analysis time make it an attractive alternative for the determination of contaminants in technical-grade organophosphorus insecticides (Greenhalgh and Shoolery, 1978). In this study NMR is used to analyze a number of technical products for known toxic contaminants.

## MATERIALS AND METHODS

**NMR.** The $^{31}P$ spectra were recorded on a Bruker WM 250 FT/NMR spectrometer operating at 101.2 MHz. All spectra are a result of 200 transients with 90° pulses (15 $\mu$s), a 0.5-s accumulation time, and a 12-s recycle time with proton decoupling. Quadrature detection was to minimize pulse imperfections over the wide spectral window (Sojka and White, 1978). $T_1$ values were determined by the inversion recovery method from the slope of the linear log plot of signal intensity vs. time. Chemical shifts were measured with respect to the internal standard, triphenyl phosphate (TPP), at −17.11 ppm relative to 85% $H_3PO_4$.

**Sample Preparation.** Technical-grade insecticides (0.5 g) and TPP (6.25 mg) were dissolved in benzene-$d_6$ (2 g) and transferred to a 10 mm o.d. NMR tube. Chromyl acetonylacetonate (CAA) (1 mg) was added to each sample. Duplicate analyses were made. Concentration was determined by comparison of the peak area response relative to that of the internal standard in triplicate for each analysis and the results were averaged.

**Chemicals.** Triphenyl, trimethyl, and triethyl phosphates were purchased from Aldrich Chemicals, Milwaukee, WI. Technical-grade organophosphorus pesticides (94–98% pure) were obtained from the Chemical Standards Laboratory, Agriculture Canada, Ottawa, Ontario, Canada, and the various manufacturers. *O,O,O*-Trimethyl phosphorothioate (OOOTMPT), *O,O,S*-trimethyl phosphorothioate (OOSTMPT) (Hilgetag et al., 1960), *O,O,S*-trimethyl phosphorodithioate (OOSTMPDT) (Umetsu et al., 1981), and its *O,S,S*-trimethyl isomer OSSTMPDT (Aldridge et al., 1979) and all the triethyl analogues (Umetsu et al., 1981), tetraalkyl pyrophosphates (Toy, 1950), the dithio analogues (Toy, 1951), paraoxon, and *S*-ethyl parathion were synthesized as referenced or by standard synthetic procedures and characterized by MS and $^1H$ NMR. The fenitrothion metabolites, methyl bis(4-nitro-3-tolyl) phosphorothioate, *S*-methyl fenitrothion, and fenitrooxon had previously been prepared in this laboratory (Greenhalgh and Marshall, 1976). *S*-Methyl malathion was a gift of Cheminova, DK-7620, Lemvik, Denmark. Organophosphorus compounds are readily absorbed dermally and in general are toxic (e.g., paraoxon and TEPP). Samples should be prepared in a fume hood and appropriate safety measures observed when handled.

## RESULTS AND DISCUSSION

Quantitation by FT/NMR necessitates prior knowledge of a number of factors related to both instrument and compound. These include the nuclear Overhauser effect (NOE), relaxation time ($T_1$), and analyte response relative to the internal standard at different analyte concentrations.

The NOE effect, which enhances signal intensities of some nuclei, is proportional to the relative contribution of the proton-phosphorus dipole–dipole ($T_{DD}$) interaction to the overall $T_1$. For phosphites, where hydrogen is directly attached to phosphorus, the signal can be enhanced as much as 100% (Yeagle et al., 1975). The average NOE enhancement of phosphate and thiophosphate esters is only approximately −6% (Gurley and Ritchey, 1976); the values for fenitrothion and some of its contaminants at 32.2 MHz were of the same order (Greenhalgh and Shoolery,

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Table I. Accuracy and Reproducibility of $^{31}$P FT/NMR Analysis of a Known Standard Relative to Internal Standard[a]

| known, % w/w | calculated, % w/w,[b] for compound | | |
|---|---|---|---|
| | F | SMF | FO |
| 1 | 1.12 (0.04) | 1.20 (0.02) | 1.16 (0.05) |
| 0.5 | 0.52 (0.02) | 0.53 (0.01) | 0.54 (0.04) |
| 0.1 | 0.10 (0.05) | 0.11 (0.03) | 0.11 (0.03) |
| 0.05 | 0.042 (0.009) | 0.042 (0.008) | 0.043 (0.011) |

[a] 0.25% w/w TPP. [b] F = fenitrothion; SMF = *S*-methyl fenitrothion; FO = fenitrooxon. Standard deviation ($n$ = 5) is shown in parentheses.

1978). Chemical shift anisotropy dominates the overall relaxation time leading to a shorter $T_1$ at high fields; hence the $T_{DD}$ contribution is relatively less. This fact together with the presence of paramagnetic ions in the solution should reduce the NOE contribution still further. The NOE's were measured and found to be within the experimental error under the conditions of the analysis.

The relaxation times ($T_1$) for fenitrothion and the contaminants in a technical sample were determined both with and without the relaxing agent. In the latter case $T_1$ values ranged from 0.9 to 7.5 s, with OOOTMPT having the longest $T_1$. This value was reduced to 2.8 s by the addition of 1 mg of CAA.

For analysis, 25% solutions of the analyte in benzene-$d_6$ were used to reduce viscosity of the sample and provide an adequate lock signal. Triphenyl phosphate was selected as the internal standard because it is easily purified and has a chemical shift (−17 ppm) upfield of all the contaminants. The accuracy and reproducibility of the method were determined by using a standard mixture of fenitrothion, *S*-methyl fenitrothion, and fenitrooxon as representative compounds. The concentration of this standard was varied from 1 to 0.05% w/w, and the response was measured relative to the TPP internal standard (0.25% w/w). The results (Table I) show an average relative error for the three compounds of 16, 6, 8, and −15% w/w, respectively, for the standard mixture concentrations of 1, 0.5, 0.1, and 0.05% w/w. Accuracy appears to be related to the relative concentrations of the analyte and internal standard. The error increasing as the difference in the concentrations gets larger.

Five replicate analyses were carried out for each concentration represented in Table I. The precision decreased with increasing concentration due to the reduction in S:N, which at the lowest concentration was 20:1.

The $^{31}$P chemical shifts of some toxic contaminants reported to be present in technical organophosphorus insecticides are shown in Table II. It contains only nonspecific contaminants resulting from the use of common intermediates in the synthesis. As can be seen, these compounds are readily differentiated by their chemical shift. In addition, there are contaminants specific to each insecticide, whose chemical shifts are well separated from the parent compound, e.g., the bis (58.21 ppm), *S*-methyl (26.95 ppm), and oxon (−5.49 ppm) derivatives of fenitrothion (66.1 ppm).

The mode of action of these toxic contaminants varies considerably. *S*-Alkyl isomers and oxon analogues of insecticides, together with pyro compounds, e.g. sulfotepp, are potent cholinesterase inhibitors. Other contaminants like the bis derivatives, e.g., ethyl bis(4-nitrophenyl) phosphate reputed to be present in parathion, are active against neurotoxic esterases (Johnson, 1982). Simple phosphorothioate and dithioate esters have been reported to potentiate the mammalian toxicity of malathion and

Table II. Toxicity and $^{31}$P Chemical Shifts of Some Common Organophosphorus Contaminants

| compound | $LD_{50}$, mg/k[a] | δ, ppm[b] |
|---|---|---|
| *O,O,O*-trimethyl phosphorothioate | | 73.96 |
| *O,O,S*-trimethyl phosphorothioate | 15 (26 days)[c] | 31.11 |
| *O,S,S*-trimethyl phosphorodithioate | 25 (6 days)[d] | 57.14 |
| *O,O,S*-trimethyl phosphorodithioate | 660[d] | 99.5 |
| *O,O,O*-triethyl phosphorothioate | 750 (mouse)[e] | 68.59 |
| *O,O,S*-triethyl phosphorothioate | 45-90 (8 days)[e] | 27.25 |
| *O,O,S*-triethyl phosphorodithioate | | 95.41 |
| *O,O,O,O*-tetramethyl pyrophosphate | 1.7 | 3.0 |
| *O,O,O,O*-tetramethyl dithiopyrophosphate | | 57.87 |
| *O,O,O,O*-tetraethyl pyrophosphate | 1.1[e] | −13.05[f] |
| *O,O,O,O*-tetraethyl monothiopyrophosphate | 4.2 (mouse)[e] | 54.64-14.8[d] |
| *O,O,O,O*-tetraethyl dithiopyrophosphate | 8.0[g] | 53.44 |

[a] Oral rat. [b] In benzene-$d_6$, relative to 85% $H_3PO_4$. [c] Mallipudi et al. (1979). [d] Umetsu et al. (1981). [e] Margot and Gysin (1957). [f] Crutchfield et al. (1974). [g] Toy (1951).

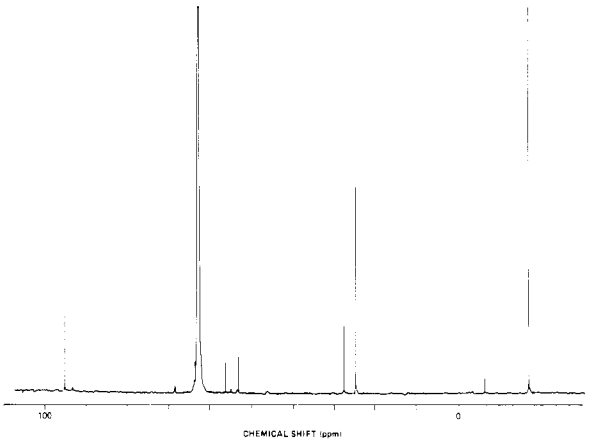


**Figure 1.** $^{31}$P FT/NMR spectrum of a technical-grade parathion sample.

phenothate (Pellegrini amd Santi, 1972). More recently, OOSTMPT and OOSTMPDT have been reported to produce delayed mortality to rats (Umetsu et al., 1981; Hammond et al., 1980). The effect of these contaminants in technical-grade insecticides is to increase toxicity. Pure malathion (99.2%) has an $LD_{50}$ of 12 500 mg/kg, but when contaminated with 0.05% isomalathion or dithioate ester, the $LD_{50}$'s are decreased to 4400 and 3100 mg/kg, respectively.

Technical-grade samples of the dialkyl aryl phosphorothioates (diazinon, fenchlorphos, fensulfothion, fenitrothion, methyl parathion, and parathion), the trialkyl phosphorodithioates (dimethoate, ethion, and malathion), and two dialkyl aryl phosphorodithioate (methidathion and phosalone) were analyzed in triplicate by $^{31}$P FT/NMR. The spectra of parathion and malathion are shown in Figures 1 and 2, respectively. The types of toxic contaminants in parathion are typical for both the diethyl and dimethyl esters of this series. The main contaminant is *S*-ethyl parathion (24.64 ppm), with smaller amounts of the bis derivative (57.19 ppm), OOSTEPT (26.61 ppm),

Table III. $^{31}P$ Chemical Shifts and Concentrations of Some Toxic Contaminants in Technical Dialkyl Aryl Phosphorothioate Insecticides[a]

| | diazinon | | dasanit | | parathion | | | ronnel | | fenitrothion | | Me-parathion | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| contaminant[b] | δ | % w/w | δ | % w/w | δ | % w/w | contaminant | δ | % w/w | δ | % w/w | δ | % w/w |
| OOSTEPDT | | | | | 95.15 | 0.51 | OOSTMPT | | | | | 99.87 | 0.10 |
| OOOTEPT | 68.71 | 0.41 | 68.56 | 1.47 | 68.57 | 0.42 | OOOTMPT | | | 73.70 | 0.48 | | |
| bis | 56.29 | 0.18 | | | 56.17 | 0.95 | bis | 59.25 | 0.04 | 58.26 | 0.56 | 58.30 | 0.02 |
| SULFOTEPT | 53.61 | 0.34 | | | 53.19 | 0.11 | SULFOTMPP | | | 57.18 | 0.21 | | |
| OOSTEPT | 27.40 | 0.12 | 27.56 | 0.06 | 27.61 | 0.41 | OOSTMPT | 31.47 | 0.05 | 31.18 | 0.33 | 30.95 | 0.06 |
| *S*-ethyl | 26.34 | 0.09 | 27.94 | 0.16 | 24.64 | 1.24 | *S*-methyl | 27.53 | 0.15 | 26.62 | 1.29 | 27.53 | 0.01 |
| oxon | | | -6.12 | 0.35 | -6.54 | 0.08 | oxon | -4.84 | 0.07 | -4.41 | 0.03 | -4.81 | 0.03 |

[a] Chemical shift in ppm relative to 85% $H_3PO_4$. [b] OOSTEPDT/OOSTMPDT = *O,O,S*-triethyl/trimethyl phosphorodithioate; OOOTEPT/OOTMPT = triethyl/trimethyl phosphorothioate; bis = bis derivative; SULFOTEPP/SULFOTMPP = *O,O,O,O*-tetraethyl/tetramethyl dithiopyrophosphate; OOSTEPT/OOSTMPT = *O,O,S*-triethyl/trimethyl phosphorothioate; *S*-ethyl/*S*-methyl = *S*-ethyl/methyl isomer; oxon = oxon derivative.

Table IV. $^{31}P$ Chemical Shifts and Concentrations of Some Toxic Contaminants in Technical Trialkyl and Dialkyl Aryl Phosphorodithioates[a]

| | ethion | | phosalone | | | dimethoate | | malathion | | methidathione | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| contaminants[b] | δ | % w/w | δ | % w/w | contaminants | δ | % w/w | δ | % w/w | δ | % w/w |
| OOSTEPDT | 95.29 | 3.45 | 95.25 | 0.07 | OOSTMPDT | 99.81 | 6.13 | 99.85 | 0.68 | 99.79 | 0.25 |
| OOOTEPT | 68.71 | 0.39 | | | OOOTMPT | | | 73.88 | 0.08 | | |
| oxon | 65.85 | 0.12 | | | *S*-methyl | 57.20 | 1.68 | 57.10 | 0.24 | | |
| | | | | | | | | 55.91 | | | |
| SULFOTEPP | 53.81 | 0.23 | | | OOSTMPT | 29.98 | 0.90 | 31.82 | 0.16 | 30.40 | 0.04 |
| OOSTEPT | 27.01 | 0.04 | | | oxon | | | 27.42 | 0.12 | | |

[a] Chemical shift in ppm relative to 85% $H_3PO_4$. [b] OOSTMPDT/OOSTEPDT = *O,O,O*-trimethyl/triethyl phosphorodithioate; OOOTMPT/OOOTEPT = *O,O,O*-trimethyl/triethyl phosphate; oxon = oxon derivative; OOSTEPT = *O,O,S*-triethyl phosphorothioate.

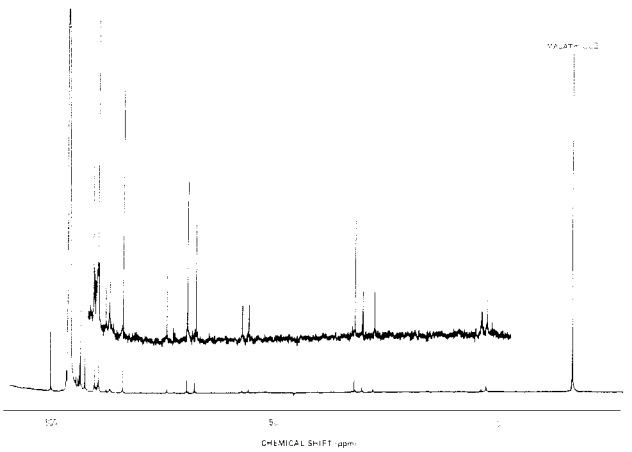

**Figure 2.** $^{31}P$ FT/NMR spectrum of a technical-grade malathion sample.

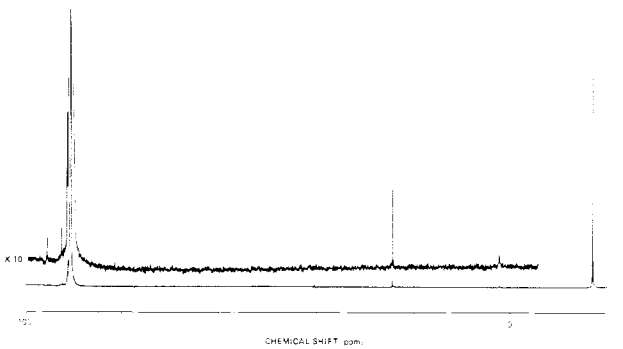

**Figure 3.** $^{31}P$ FT/NMR spectrum of a technical-grade phosalone sample.

sulfotepp (53.19 ppm), paroxon (-6.54 ppm), and some OOSTEPDT (95.15 ppm). The latter contaminant is derived from the precursor diethyl phosphorodithioic acid. The spectrum of malathion is complex, showing the presence of numerous phosphorus contaminants, with OOSTMPDT (99.85 ppm) being present in the largest amount. Other major contaminants include bis(dimethylthiophosphoryl) sulfide (84.3 ppm) and dimethyl phosphorothioic acid (69.5 ppm). The mixed malathion esters, homomalathion and the dimethylthiophosphoryl polysulfides, occur in the range 89–95 ppm. Other toxic contaminants present include OOOTMPT (73.88 ppm) and OOSTMPT (31.92 ppm). *S*-Methyl malathion occurs as a doublet (57.10 and 55.91 ppm) due to diastero isomerism in the molecule. The chemical identity of the contaminants malaoxon, *S*-methyl malathion, OOSTMPD, OOSTMPT, bis(dimethylthiophosphoryl) sulfide, and dimethyl phosphorothioic acid was confirmed by addition of pure standards and other compounds by comparison with the chemical shift values reported by Wayne et al. (1982).

The number of contaminants present in dialkyl aryl phosphorothioates varied; the known toxic ones found are listed in Table III. The major contaminants detected in the dimethyl esters were the *S*-methyl isomers, OOSTMPT, and the bis derivatives. Although the *S*-ethyl isomers are present in all three diethyl esters of this series, it is only the dominant contaminant in the case of parathion. The simple trialkyl phosphorothioate esters OOSTEDT, OOOTEPT, and OOSTEPT were present in all three insecticides, while sulfotepp was found in two samples.

The samples of methidathion and phosalone, two insecticides in the trialkyl and dialkyl aryl phosphorodithioate series, were relatively pure. The spectrum of phosalone (Figure 3) shows only trace amounts of contaminants.

The main toxic contaminants in the trialkyl phosphorodithioate series are the non-insecticide-specific trialkyl

phosphorothio- and -dithioic acid esters (Table IV). Dimethoate contained 6.13% OOSTMPDT and ethion contained 3.45% OOSTEPDT. The spectra of dimethoate and ethion showed numerous unidentified phosphorus contaminants to be present in the technical material. It was not possible to speculate on the nature of all the contaminants detected in the spectra of the technical products nor on their possible source. This was principally due to the lack of standards to effect confirmation, especially for the specific contaminants, and the fact that the history of the technical products was not known.

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**Registry No.** OOOTMPT, 152-18-1; OOSTMPT, 152-20-5; OSSTMPDT, 22608-53-3; OOSTMPDT, 2953-29-9; OOOTEPT, 126-68-1; OOSTEPT, 1186-09-0; OOSTEPDT, 2524-09-6; *O,O,O,O*-tetramethyl pyrophosphate, 690-49-3; SULFOTMPP, 51120-35-5; *O,O,O,O*-tetraethyl pyrophosphate, 107-49-3; *O,O,O,O*-tetraethyl monothiopyrophosphate, 645-78-3; SULFOTEPP, 3689-24-5; ronnel, 299-84-3; fenitrothion, 122-14-5; methyl parathion, 298-00-0; diazinon, 333-41-5; dasanit, 115-90-2; parathion, 56-38-2; dimethoate, 60-51-5; malathion, 121-75-5; methidathion, 950-37-8; ethion, 563-12-2; phosalone, 2310-17-0.

LITERATURE CITED

Aldridge, W. N.; Miles, J. N.; Mount, D. L.; Verschoyle, R. D. *J. Agric. Food Chem.* **1979**, *27*, 130–137.
Baker, E. L.; Zack, M.; Miles, J. W.; Alderman, L.; Warren, M.; Dobbin, R. D.; Miller, S.; Teeters, W. R. *Lancet* **1978**, 31–33.
Cochrane, W. P.; Lanouette, M.; Greenhalgh, R. *J. Assoc. Off. Anal. Chem.* **1979**, *62*, 1222–1230.
Crutchfield, M. M.; Dungan, C. H.; Letcher, J. H.; Mark, V.; Van Wazer, J. R. In "Topics in Phosphorus Chemistry"; Grayson, M.; Griffiths, E. J., Eds.; Interscience-Wiley: New York, 1974; Vol. 5, pp 321–376.
Diggory, H. J.; Landrigen, R. J.; Latimer, K. P.; Ellington, A. C.; Kimbrough, R. D.; Liddle, J. A.; Cline, R. E.; Smrek, A. L. *Am. J. Epidermiol.* **1977**, *106*, 145–153.
Greenhalgh, R.; Marshall, W. D. *J. Agric. Food Chem.* **1976**, *24*, 788–713.
Greenhalgh, R. J.; Shoolery, N. *Anal. Chem.* **1978**, *50*, 2039–2042.
Gurley, T. W.; Ritchey W. M. *Anal. Chem.* **1976**, *48*, 1137–1140.
Gysin, H.; Margot, A. *J. Agric. Food Chem.* **1959**, *6*, 900–903.
Hammond, P. S.; Badawy, S. M. A.; Marsh, R. B.; Fukuto, T. R. *Pestic. Biochem. Physiol.* **1980**, *18*, 90–100.
Hilgetag, G.; Lehnman, G.; Feldheimm, W. *J. Prakt. Chem.* **1960**, *12*, 1–5.
Johnson, M. K. *Vet. Hum. Toxicol.* **1982**, *24*, 240.
Mallijudi, N. M.; Umetsu, N.; Toia, R. F.; Talcott, R. E.; Fukuto, T. R. *J. Agric. Food Chem.* **1979**, *27*, 463–466.
Margot, A.; Gysin, H. *Helv. Chim. Acta* **1957**, *40*, 1562–1574.
Miles, J. W.; Mount, D. L.; Steignor, M. A.; Teeters, W. R. *J. Agric. Food Chem.* **1979**, *27*, 421–415.
O'Neill, I. K.; Pringiner, M. A. *Anal. Chem.* **1976**, *49*, 588–590.
Pellegrini, G.; Santi, R. *J. Agric. Food Chem.* **1972**, *20*, 944–949.
Sojka, S. A.; White, P. A. *Anal. Chem.* **1978**, *50*, 585–587.
Toy, A. D. F. *J. Am. Chem. Soc.* **1950**, *73*, 2065–2071.
Toy, A. D. F. *J. Am. Chem. Soc.* **1951**, *73*, 4670–4674.
Umetsu, N.; Grose, F. H.; Attahgaru, R.; Abu-Gl-Hay, S.; Fukuto, T. R. *J. Agric. Food Chem.* **1977**, *25*, 946–953.
Umetsu, N.; Mallipundi, N. M.; Toia, R. F.; March, R. B.; Fukuto, T. R. *J. Toxicol. Environ. Health* **1981**, *7*, 481–497.
Wayne, R. S.; Stockton, G. W.; Wilson, L. A. *Pestic. Chem., Proc. Int. IUPAC Congr. Pestic. Chem.* **1982**, in press.
Yeagle, P. L.; Hutton, W. C.; Martin, R. B. *J. Am. Chem. Soc.* **1975**, *97*, 7175–7177.

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# 2-Acetylpyridine Thiosemicarbazones as Inhibitors of Ecdysis in *Oncopeltus fasciatus*: Structure–Activity Relationship Study

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Effects of structural variations of [1-(2-pyridinyl)ethylidene]hydrazide of 1-pyrrolidinecarbothioic acid (**23**) on ecdysis of *Oncopeltus fasciatus* were examined in 50 related compounds. Marked ecdysis-inhibiting effects were elicited by substituted thiosemicarbazones of 2-acetyl-, 2-propionyl-, and 2-butyrylpyridine but 2-formyl, 2-phenylacetyl, and 2-benzoyl derivatives were inactive. Essential for activity was the thiocarbonyl moiety: analogues containing C=O or C=NH groups were inactive. Substitutents on the thiosemicarbazone portion of the molecule had variable effects: the 1-piperidinecarbothioic acid analogue of **23** exceeded the activity of **23**, and five other analogues were as active as **23**. Hydrogenation of the exocyclic C=N in **23** did not reduce its biological activity.

Recently, the broad spectrum of biological activity of thiosemicarbazones was extended to include inhibition of ecdysis in certain insects (Kelly et al., 1982). Although ecdysis and the entire molting process in insects can be disrupted by juvenoids (Sláma et al., 1974), ecdysteroids (Kelly et al., 1981), or chitin synthesis inhibitors (Mitsui et al., 1980), physiological and biochemical effects of thiosemicarbazones suggest that their mechanism of action is different from those proposed for the other groups of compounds (Redfern et al., 1981, 1982). Because Kelly et al. (1982) have shown that various thiosemicarbazones of 2-acetylpyridine differed greatly in their effects on the large milkweed bug, *Oncopeltus fasciatus* (Dallas), we synthesized a series of variously substituted thiosemicarbazones and determined their biological effects in that species. Herein we report results of structure–activity

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