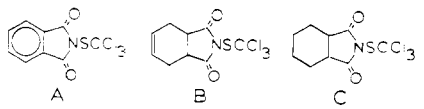

A B C

Figure 2. Structures of N—imide—$SCCl_3$ compounds

electronegativity of the latter structure. A reduction in the charge on $>N^-$ causes the free imide to be a stronger acid and causes the imide-$SCCl_3$ compound to be more reactive in nucleophilic reactions.

Among the imide-$SCCl_3$ compounds, *N*-(trichloromethylthio)cyclohexane-1,2-dicarboximide (Figure 2*C*) is exceptional. This aliphatic (cyclic) imide-$SCCl_3$ compound controls powdery mildew pathogens (*Erysiphe graminis*) and hydrolizes at a faster rate than captan (*17*). However, the free imide possesses fungitoxicity and acidity on a level with that of tetrahydrophthalimide, the imide of captan. Thus, the increase in reactivity of *N*-(trichloromethylthio)-cyclohexane - 1,2 - dicarboximide over that of captan must be a result of the condensation rather than be ascribed to the sum of the standard free energies of the two moieties and resonance in the imide moiety. Since NMR spectra have shown no rearrangement of hydrogen atoms of the cyclohexane ring during the condensation reaction, the increase in reactivity is not due to a change of hydrogen atoms on positions 1 and 2 from cis to trans relationship. The source of the increase in activity has yet to be determined.

Organic toxicants permeate fungal cells rapidly. To do so, the compounds must possess an oil-water partition coefficient suitable for penetrating the lipoid layers of cell membranes. Undoubtedly, the trichloromethyl group of trichloromethylthio compounds contributes to an oil-water partition coefficient suitable for these compounds to permeate fungal cells (*6*, *15*).

The lipophilic properties of compounds of the type R—$SCCl_3$ may be altered by change in the R-group. The higher level of standard free energy of phthalimide over that of tetrahydrophthalimide may contribute to the 10-fold increase in oil-water partition coefficient of folpet over that of captan (*15*). The increase in lipophilicity with change in imide group may be responsible, in part, for the greater fungitoxicity of folpet over that of captan to mildew pathogens. That carboxyl substitution to the benzene ring of trichloromethylthiobenzene sulfonate reduces fungitoxicity (*20*) suggests that lipophilicity of the carboxylated derivatives is reduced. The reduction in lipophilicity can occur from the hydrophilicity and ionization of the carboxyl substituent.

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THALLIUM ASSAY

X-Ray Emission Spectrographic Determination of Thallium in Biologic Materials

MARVIN GOLDMAN, R. P. ANDERSON, J. P. HENRY, and S. A. PEOPLES

School of Veterinary Medicine, University of California, Davis, Calif.

An x-ray emission method for the determination of trace concentrations of thallium in biologic materials is described. The ratio of emission intensity at the Tl Lβ line to an adjacent background line was constant in dried biologic materials whose scattering efficiency varied. In the presence of added Tl, the net peak-to-background ratio was linearly related to Tl concentration over a 5- to 1000-p.p.m. range. Nondestructive Tl determinations can be completed in about 10 minutes on dried biologic materials with a precision of ± 18 p.p.m. for single determinations.

THE usefulness of x-ray emission to determine thallium in geologic specimens has been demonstrated (*3*). This method has been adapted to study thallium toxicosis as a part of an ecological evaluation of this metal in the control of nuisance birds and provides for the rapid, nondestructive determination of Tl with minimal sample preparation.

Experimental

Apparatus and Reagents. The instrumental settings on the Norelco Universal x-ray spectrograph utilized in these studies are summarized in Table I. The measurement of the emission intensity of the L-characteristic lines of thallium is within the range of energies available using a LiF analyzing crystal and a tungsten target tube powered by a 50 KVP x-ray generator. The qualitative nature of the Tl spectrum in a light biologic matrix is shown in Fig-

ure 1. Calibration standards were prepared in which known amounts of thallium sulfate were admixed to biologic specimens as described below. The specimens and thallium additives are shown in Table II. Aliquots of urine from dogs fed thallium were dried in plastic planchets and compared with similarly prepared aliquots of control urine to which known concentrations of thallium had been added; the feces were dehydrated and ground to a powder and analyzed in the same manner as the tissue specimens.

Procedure. The effect of the matrix on thallium emission intensity is largely due to the x-ray scattering of the beam within the specimen. This can be seen by changes in the background continuum, and can produce considerable variability in this determination (*1*). The physical state of the specimen, including its surface, total mass, and density are also factors which involve not only scattering and background but attenuation of emission intensity as well. Calibration standards were prepared in which known amounts of thallium sulfate were admixed to biologic samples which had been dehydrated in a vacuum oven and ground to a powder of 60 to 200 mesh. The Tl_2SO_4 in aqueous solution was added to weighed aliquots of powdered samples, thoroughly mixed, redried, and repowdered so as to exert some degree of control over the physical state of the samples. Where high-fat content interfered with desiccation and grinding, prior solvent extraction was utilized.

Table I. Instrumental Settings

Counter (voltage)	Gas flow (1550 VDC)
X-ray tube target, voltage, current	Tungsten, 50 kv., 45 ma.
Analyzing crystal	LiF
X-ray path	Air
Collimators, entrance, exit	2 inches × 5 mil, 1 inch × 20 mil
Baseline, window	6.0 volts, integral
Tl Lβ_1 Bragg angle, 2θ	29.15°
Background 2θ angle for Tl measurements	27.5°
Peak and background measurements	Time to record 128,000 counts

Table II. Replicate Determination of Net Peak to Background Ratios on Dried Bird Tissue Containing Known Amounts of Thallium

	Net (P/B) (10^3)		
μg. Tl/Gram	A	B	C
5	5	5	−7
10	8	11	2
25	18	23	28
49	47	43	40
98	88	92	80
244	236	228	232
489	471	470	465
987	950	915	934

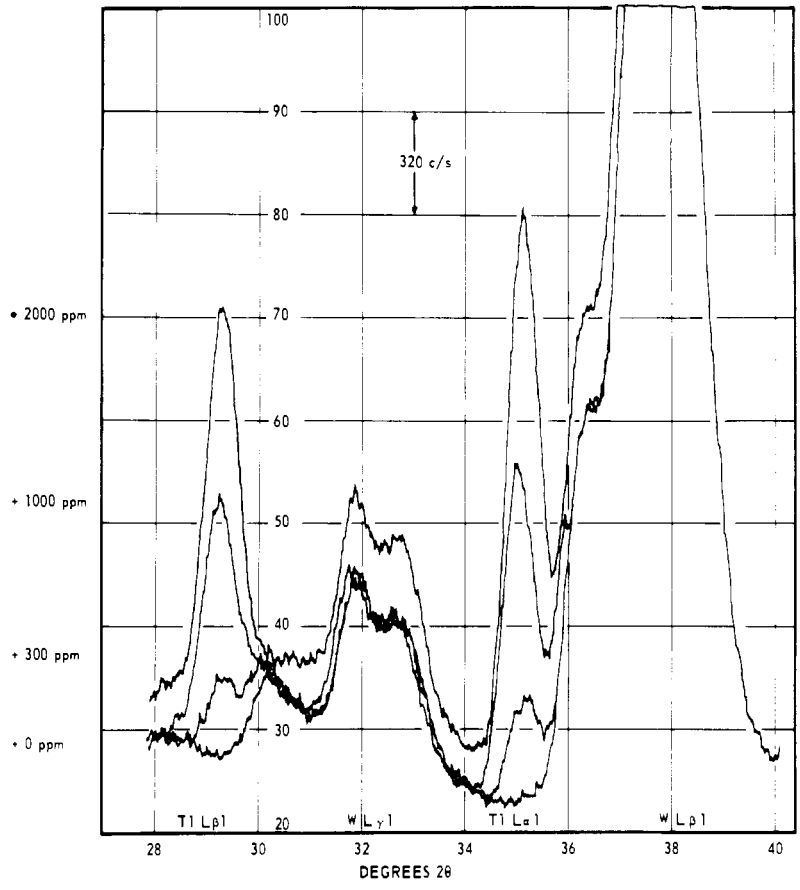

Figure 1. Spectral scan of dried ground bird tissue with additions of Tl

Note relation of x-ray tube target Wl. spectrum to that of the Tl L emission indicating minimal W interference on the Tl Lβ energy

The net counting rate was not constant in all samples for a given concentration of thallium owing to their inherent differences in composition. However, variations in the background continuum for the biologic media tested showed a constant ratio of counting rate at 29.15° 2θ to that at 27.50° 2θ. This proportionality was utilized in constructing a standard calibration curve for thallium-containing specimens. Day to day fluctuations in instrumental factors were minimized insofar as possible, and with care were kept to within 5%. The use of a peak-to-background ratio additionally minimized such variability, since changes in x-ray power output would affect both adjacent wavelength intensities in a similar manner. In avoiding the use of absolute net count rate, reproducibility was consistently maintained.

The thallium concentration in the sample was measured as the peak-to-background ratio of counts (P/B) from which were subtracted the peak-to-background ratio of nonthallus spectra—i.e., background counting rates at the two angles shown in Table I.

Results and Discussion

A thallium reference curve is shown in Figure 2 drawn from the data in Table II. A linear-regression analysis of the data demonstrated a high correlation between the thallium concentration and net peak-to-background ratio. The straight line which best fit the data was computed by the method of least squares such that

$$\mu g.\ Tl \text{ per gram specimen (dry)} = \text{p.p.m.} = \frac{\text{net P/B} + 2.315}{0.9508}$$

An analysis of variance of the data provided an index of detection sensitivity. In this instance, sensitivity is defined by the 95% confidence interval for a single measurement of P/B. Thus, as in Figure 2, 95% of the time a single measurement of P/B will fall within a ±18-p.p.m. wide band. The use of this statistic suggests that the lower limit of quantitation is based on the 2σ variance for a single measurement—i.e., 18 μg. Tl per gram—and that as the Tl concentration of the specimen increases, the per cent error will diminish. The minimal quantitation limit for all 24 measurements, using the same reasoning and assuming equal standard deviations for replicate analyses, would be about 3 μg. Tl per gram.

Since the measurements were all performed on dry specimens, the detection limits expressed on a wet-tissue

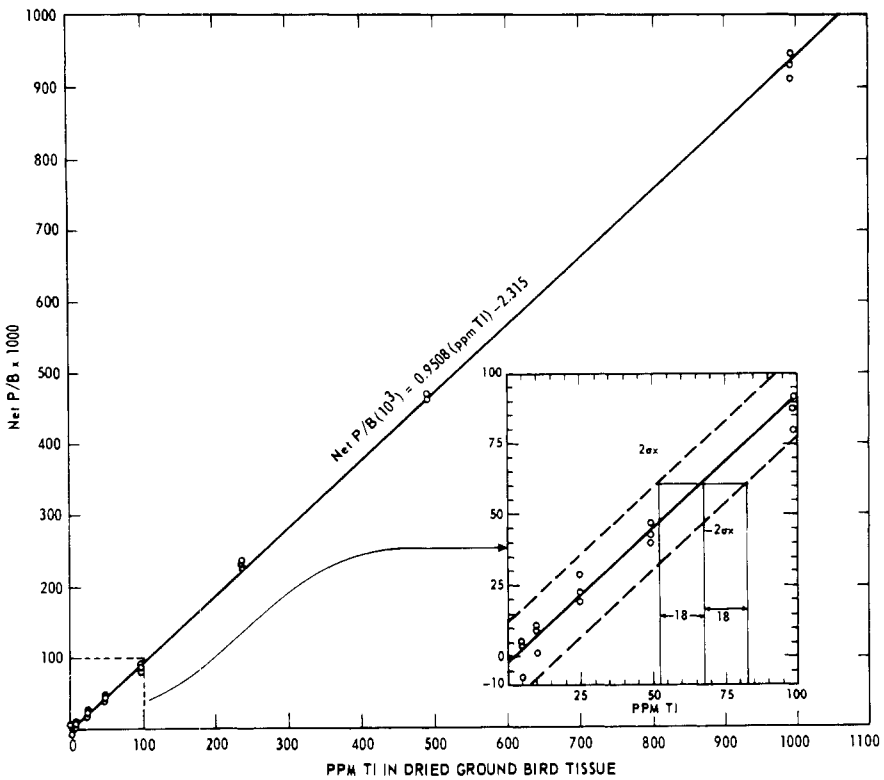

Figure 2. Thallium Lβ net peak to background ratio in dried ground bird tissue containing known additives of thallium

Linearity of response (net P/B × 1000) to increasing concentration of Tl (5 to 987 p.p.m.) is fit by the relation $y = a + bx$ where y = net P/B(1000), $a = -2.315$ P/B(1000), $b = 0.9508$ net P/B(1000)/p.p.m. Tl, x = p.p.m. Tl. The expanded inset replots the 0 to 100 p.p.m. data including the 95% confidence interval (2σ) on the P/B (1000) measurements which corresponds to ± 18 p.p.m.

weight basis would be computed using the appropriate desiccation value. Whereas the 3- to 5-gram samples of dry tissue indicated a detection limit of about 18 μg. Tl per gram, and were derived from 10 to 17 grams of fresh tissue, a lower detection limit of 5 μg. Tl per gram of fresh tissue would be expected from a 10-gram sample, dried and measured once. This suggests that at least 5 p.p.m. of Tl in a 10-gram sample of fresh tissue is required in order to prepare a dried sample with adequate Tl for the determination.

As the effective atomic number of most biologic materials does not vary markedly, it is probable that this method can be extended to other elements present in trace concentrations in biologic media if one uses the appropriate corrections for scattering within media of diverse composition in the region of the characteristic wave length of interest. For thallium determinations, a preliminary spectral scan in the region of the L-emission lines verified the absence of interfering elements. The proximity of the WLβ lines of the tube target to the thallium Lα line necessitated utilization of the less intense Tl Lβ emission. However, Tl sensitivity for the range of concentrations studied was sufficiently adequate and appears to equal or exceed most of the published wet-chemical methods (*1*, *2*).

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INSECTICIDE DETERMINATIONS

Ultraviolet Spectrophotometric Method for Fenthion

F. B. IBRAHIM and J. C. CAVAGNOL
Research Department, Chemagro Corp., Kansas City, Mo.

An improved analytical method has been developed for fenthion based on the UV absorption at 252 mμ after separating impurities on a Florisil column with heptane.

Fenthion, *O,O* - dimethyl *O* - [4-(methylthio)-*m*-tolyl] phosphorothioate, is also designated as BAY 29493, Baytex, Entex, or Tiguvon. Baytex formulations are used for mosquito control, Entex formulations for fly and roach eradication, and Tiguvon formulations for parasite control in animals.

A method for the determination of technical fenthion was developed by Farbenfabriken Bayer (*3*). This utilized a colorimetric measurement of the diazo coupling product of 4-amino-2-nitrobenzenesulfonic acid in alkaline buffer solution with 4-methylthio-*m*-cresol, an hydrolysis product of fenthion.

Hirano and Tamura (*5*) described a colorimetric method in which fenthion was hydrolyzed in a caustic alcohol solution, and the phenol produced was condensed with 4-aminoantipyrene to give an orange-colored pigment. This was extracted into chloroform and determined spectrophotometrically at 458 mμ.