for OCDD. This variation is similar to that observed by Zitko (1975) for other organochlorine compounds in biological samples that were collected during a carefully controlled sampling program.

During our initial attempts, apparent contamination was observed in both reagent blanks and sample extracts. A previous author (Wilkinson, 1975) has also observed gas chromatographic interferences in the determination of OCDD. Interferences produced apparent positive results. For example, a response corresponding to 40 ppt of HCDD was observed in the extract of sample D-05654 subsample 3. After purification of the liquid chromatographic adsorbents as described in the Experimental Section, the interferences were significantly reduced and the apparent positive result could not be reproduced. All three subsamples were analyzed to insure the initial analysis was indeed a false positive result. Reagent blanks were analyzed concurrently with each set of samples to insure that this interference did not reoccur. Control milk samples were analyzed for PCP, HCDD, and OCDD. In each case the controls were identical to the reagent blanks.

By analyzing the milk for residues of pentachlorophenol, HCDD, and OCDD, we had hoped to provide data on the types of impurities present in PCP. Tetrachlorophenols would be expected to bioconcentrate less than pentachlorophenol; and since they are present in the starting material at lower concentrations, would not be expected in the milk if PCP is not observed. Chlorinated dibenzofurans have been observed in PCP at concentrations comparable to the dioxins (Buser and Bosshardt, 1976). Although chlorinated dibenzofurans and heptachlorodibenzodioxins were not quantitatively determined, their presence would have been observed in the EC–GC chromatograms if present at concentrations similar to HCDD and OCDD.

The clean-up procedure described, coupled with EC–GC, provides a suitable screening technique for the determination of PCP, HCDD, and OCDD in bovine milk. As shown in Figures 2 and 3, the electron-capture gas chromatograms are relatively free of interfering components. In the event that a positive HCDD or OCDD response or significant interference is observed, confirmation of identity by GC–MS with comparable detection limits is required.

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A Simple Apparatus and Quantitative Method for Determining the Persistence of Pesticides in Soil

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A simple, noncumbersome apparatus and full supporting methodologies have been developed for quantitative studies on the rate and extent of degradation of ^{14}C-labeled pesticides in soil under aerobic conditions. Evolved $^{14}CO_2$ is monitored periodically using interchangeable Drierite–Ascarite–polyurethane towers with analysis by liquid scintillation counting after release of $^{14}CO_2$ from Ascarite with acid and retrapping. Aerobic conditions are maintained by diffusion through the tower while volatile organics are trapped in the polyurethane plug. Using multiple incubation flasks with a given soil and ^{14}C pesticide, the rate of degradation can be determined by periodic analysis of a whole flask. The overall accountability of the radioactivity using this methodology is nearly quantitative as shown by control experiments and by the results of many aerobic metabolism studies.

Two basic types of incubation systems have been developed and utilized to measure the rate and extent of degradation of pesticides and other ^{14}C-labeled compounds. The merits of these systems, as discussed by Parr and Smith (1969) and Bartha and Pramer (1965), have been summarized below. The open system employing a manifold assembly has been described by Parr and Smith

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(1969). Although this system has advantages in terms of maintaining the desired atmospheric composition, multiple trapping capabilities, and precision, disadvantages include its space requirements, personal attention required, and its complexity when applied to large multiflask experiments. Variations on this basic approach have appeared recently (Kearney and Konston, 1976; Goswami and Koch, 1976). The second commonly utilized system is the closed system which utilizes static or intermittent aeration; this system was developed by Norman and Newman (1941) and modified by Bartha and Pramer (1965). Examples of the application of this latter system include studies by Bartha et al. (1967), Tiedje and Mason (1974), and Sprankle et al. (1975). This latter closed system requires less equipment, space, and personal attention; however, the oxygen content is not controlled and reduction can affect the metabolism (Goswami and Koch, 1976). A feature common to almost all the aforementioned systems is that unambiguous distinction between $^{14}CO_2$ and other volatile ^{14}C activity trapped in the base requires separate special analysis.

As a consequence of the limitations of existing aerobic incubation systems for degradation studies with ^{14}C-labeled compounds, we have developed a simple apparatus requiring minimal space, needing little personal attention, consisting of simple equipment, and permitting free exchange of air between the incubation flask and the atmosphere. This paper describes the incubation system in detail, validates the methodology with respect to air exchange, details the trapping, release, retrapping, and assay of evolved $^{14}CO_2$, the trapping of volatile organics using polyurethane foam, and summarizes the results of nearly 7 years of experience involving many aerobic studies with a variety of ^{14}C-labeled compounds. A preliminary account of this work has been presented (Marvel et al., 1976). Anderson (1975) has recently described a conceptually analogous aerobic incubation system.

EXPERIMENTAL SECTION

Chemicals. 2-Chloro-2′,6′-diethyl-*N*-butoxymethylacetanilide-*carbonyl*-^{14}C (butachlor-*1*-^{14}C; 10.27 mCi/mmol), *S*-(2,3-dichloroallyl-2-^{14}C)-diisopropylthiocarbamate (diallate-*2*-^{14}C; 9.62 mCi/mmol), and *N,N*-diallyl-2-chloroacetamide-*2*-^{14}C (CDAA-*2*-^{14}C; 10.40 mCi/mmol) were synthesized by Freeman (1975). $Na_2{}^{14}CO_3$, $NaH^{14}CO_3$, and sucrose-^{14}C were obtained from New England Nuclear. Each of the carbon-14 labeled compounds possessed a chemical and radiochemical purity of greater than 96% based on thin-layer chromatography with Baird Atomic beta camera detection, nuclear magnetic resonance, and gas chromatography using internal standardization and/or radioactive gas proportional counter.

Soils. Soil metabolism studies have been carried out using two different soils. The soils with their respective percentages of organic matter, sand, silt, clay, and water holding capacity (percent by weight), cation-exchange capacity (mequiv/100 g), and pH were as follows: Ray silt loam (1.2, 4.6, 84.2, 10.0, 23.9, 10.4, and 8.1); Drummer silty clay loam (3.4, 2.4, 68.8, 25.3, 28.8, 24.6, and 6.2).

Aerobic Incubation Apparatus. The aerobic soil metabolism apparatus is shown in Figure 1. The apparatus consists of a 250-mL Erlenmeyer flask and a two-piece trapping tower. All joints in the apparatus are 34/45 standard taper sealed by means of Teflon sleeves (Fisher No. 14-320). The trapping tower consists of two parts. The lower part (12 cm) fits into the standard taper joint of the flask; this portion is plugged with a porous polyurethane plug (2 × 3.3 cm; Fisher Scientific) and filled with indicator

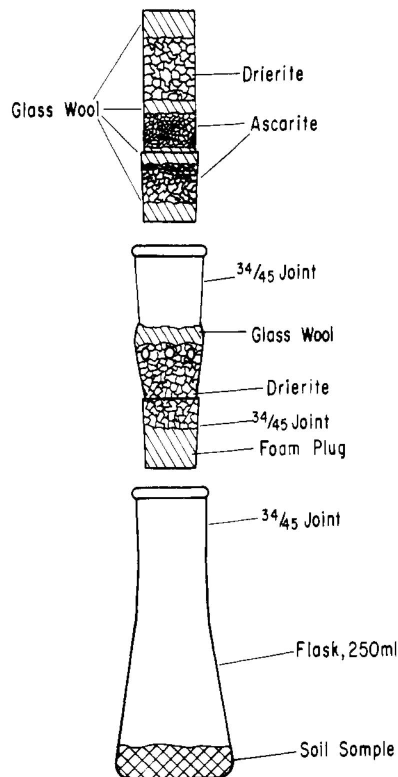

Figure 1. Aerobic soil metabolism incubation system.

Drierite (4 cm layer). The upper part (10 cm) of the trapping tower was plugged with glass wool and layered sequentially from the bottom with 15 g of Ascarite (1:1 mixture of 8–20 and 20–40 mesh), glass wool, Ascarite (15 g), glass wool, Drierite (3 cm layer), and glass wool.

Release and Assay of $^{14}CO_2$ from Ascarite. Two quantitative methods have been developed to assay the $^{14}CO_2$ trapped on the Ascarite. The first method involves simple, routinely available glassware while the second makes use of a spinning band column and offers a tenfold increase in sensitivity.

In release method 1, the bottom layer of Ascarite from the aerobic apparatus is placed in a 500-mL Erlenmeyer flask having a 34/45 standard taper outer joint. This flask was fitted with an apparatus consisting of an inner 34/45 standard taper joint, a glass inlet tube, and an outlet tube. The inlet was closed with a rubber septum. The outlet was connected by polyethylene tubing to a gas diffusion stone which was placed into a cylinder (3.2 × 28 cm) containing 50 mL of 0.25 N NaOH. Excess concentrated HCl was introduced through the rubber septum on the inlet. It was not necessary to measure the HCl accurately since Ascarite contains an indicator that turns yellow upon acidification. The release flask was then flushed through the inlet septum with nitrogen at 2.3 psi for 20 min. Five milliliters of the 0.25 N NaOH solution were assayed for radioactivity by liquid scintillation counting in Insta-Gel (Packard Instrument Co.).

In release method 2, which is now used exclusively in our laboratory, the lower layer of Ascarite from the aerobic trapping towers is transferred to a 250-mL round-botton flask and dissolved in 50 mL of distilled water. The flask is attached to the CO_2 release apparatus and immersed in an ice bath. The release apparatus was a gas inlet adapter (Ace Glass Co. No. 5190) equipped with a 25-mL Teflon stopcock dropping funnel. The dropping funnel was equipped with a gas bypass to the flask so that no pressure buildup occurred in the funnel when the stopcock was closed and nitrogen gas was applied at the top of the

dropping funnel. Nitrogen was flushed through the assembled apparatus at 300 mL/min and passed into the bottom of the spinning band column accessory of the Peterson Automatic Combustion Apparatus (Peterson, 1969). The flow of phenethylamine trapping solution (Peterson, 1969) was started through the top of the spinning band column, and immediately thereafter concentrated sulfuric acid (25 mL) was added to the cooled, vigorously stirred Ascarite solution. The addition of the sulfuric acid was completed within 1 min while the flow of 20 mL of the phenethylamine trapping and counting solution through the spinning band column required 2 min; the trapping solution was collected in a counting vial. Two additional 20-mL phenethylamine solution rinses were collected in two separate counting vials under nitrogen flow for each $^{14}CO_2$ release. The three vials containing collected $^{14}CO_2$ were then measured by liquid scintillation counting using the Nuclear Chicago Mark I scintillation counters utilized in all these studies. Normally, less than 5% of the released $^{14}CO_2$ is collected in the latter two counting vials containing phenethylamine solution rinses. Routinely, recoveries with standard $Na_2{}^{14}CO_3$ in the presence of Ascarite are run with each set of analyses as described in the standardization section of this method.

Standardization of Methods for Releasing $^{14}CO_2$ from Ascarite. Using $^{14}CO_2$ release method 1, $NaH^{14}CO_3$ (65 210 dpm), $NaHCO_3$ (200 mg), 100 mL of water, and 20 g of Ascarite were placed in the release flask. Triplicate samples were flushed for 20 min at 2.3 psi of nitrogen gas as described in detail previously. Liquid scintillation counting of 5-mL aliquots of the basic trapping solution in Insta-Gel was used to measure the ^{14}C content.

Using $^{14}CO_2$ release method 2 (i.e., the spinning band column method), 15 g of Ascarite was dissolved in 50 mL of H_2O, added to a 250-mL round-bottom flask, and fortified with $Na_2{}^{14}CO_3$ (202 000 to 269 000 dpm) in 30 different check recoveries. Release, trapping, and analysis for $^{14}CO_2$ recovery were carried out as described previously.

Standardization of Aerobic $^{14}CO_2$ Collection Methods. Triplicate aerobic incubation flasks containing a side arm sealed with a septum were set up to contain 3300 dpm of $NaH^{14}CO_3$, 300 mg of $NaHCO_3$, and 100 mL of H_2O. With the trapping tower in place, the solution was acidified through the septum with concentrated hydrochloric acid (5 mL) and the flask flushed with air for 1.25 at 1000 mL/min. The lower Ascarite layer was analyzed for trapped $^{14}CO_2$ as described previously using release method 1.

Establishment that flushing of the aerobic incubation flasks was not required to collect $^{14}CO_2$ is based on the experiments described below. In the first study, four aerobic shake flasks containing 1.0 mg (2.54×10^6 dpm) of sucrose-^{14}C, 5 g of Ray silt loam soil, and 100 mL of water were set up. After 7 days, two of the flasks were flushed with air for 1.25 h at 1000 mL/min while the other two were not flushed prior to assaying the lower Ascarite layers for $^{14}CO_2$ content using release method 2. In the second experiment, the study was carried out in triplicate analogous to the nonflushed flasks described above; however, after 3 days incubation, the trapping towers were removed and immediately replaced with new trapping towers, and then the flasks were flushed with air. The $^{14}CO_2$ content on the Ascarite layers was measured in each of the nonflushed traps using release method 2. In a final study, $NaH^{14}CO_3$ (156 000 dpm), 3 mg of $NaHCO_3$, and 50 mL of H_2O were placed in the aerobic incubation flask, the trapping tower attached, and 5 mL of concentrated HCl added through a septumed side arm using a syringe. After standing for 3 days, analysis of the lower Ascarite layers from triplicate flasks was carried out with release method 2.

Verificiation of Aerobic Incubation Conditions. In order to check the efficiency of aeration, duplicate aerobic incubation systems were set up with 50 g of Ray silt loam soil with moisture at 75% of field capacity and kept at 25 °C. At 3 and 6 days after initiation, triplicate 0.2-mL samples were taken with a gas syringe through side-arm septa and analyzed by gas chromatography. Gas chromatographic analysis for nitrogen and oxygen content was carried out at 62 °C on a 6 ft × 0.125 in. i.d. stainless steel column of molecular sieve 5A (60/80 mesh) using thermal conductivity detection. At the same time, ambient air samples were also analyzed for comparison of peak areas of oxygen and nitrogen.

Soil Degradation Studies. Fifty grams of air-dried soil which has been screened through a 4-mesh sieve was placed in each flask. For CDAA-*2*-^{14}C studies, 0.50 mg of labeled material in 0.5 mL of water was applied to Drummer soil in duplicate flasks to give a 10 ppm treatment rate. For diallate-*2*-^{14}C, 0.10 mg of labeled material in 0.5 mL of ethanol was added to Ray soil in duplicate flasks. Similarly, 0.150 mg of butachlor-*1*-^{14}C in 0.5 mL of ethanol was added to Ray soil in each of ten flasks. The moisture content of the soil was adjusted to 75% of field capacity. Each week as the trapping towers were replaced, the tared flasks were weighed, and the water (ca. 1 g) lost by evaporation was replenished. The flasks were all incubated at 25 °C in the dark. The flasks were analyzed as described below. Trapping towers were replaced weekly with fresh units. The evolved trapped $^{14}CO_2$ was determined as described above using release method 2. The foam plugs were extracted with methylene chloride and the extracted ^{14}C determined by liquid scintillation counting. At 1 and 2 weeks for all treatments and 4, 6, and 10 weeks in addition for butachlor-*1*-^{14}C flasks, the soil from a flask was removed and extracted exhaustively, and the extracts were analyzed for radioactivity using liquid scintillation counting. The extracts were further examined for parent using both thin-layer chromatography with beta camera detection–quantitation and gas chromatography with radioactive detection. The exhaustively extracted soils were analyzed for residual ^{14}C content by combustion (Peterson et al., 1969; Peterson, 1969) using weighed aliquots of the lyophilized soil.

RESULTS AND DISCUSSION

The design of the aerobic incubation system (Figure 1) readily maintains the desired aerobic state and permits quantitative trapping of both the evolved $^{14}CO_2$ and volatile organics. The necessary movement of air by diffusion through the trapping tower has been established by gas chromatographic analysis of the oxygen content of the flasks as a function of time. No detectable change (<1%) occurred in 6 days with the trapping towers in place. The aerobic microflora are clearly exposed to ambient air levels with this apparatus. Control experiments with ^{14}C-labeled carbonate and sucrose-^{14}C have established that the trapping of evolved $^{14}CO_2$ can be carried out nearly quantitatively by simple diffusion without flushing. This would be expected if rapid interchange of air was occurring. Acidification of three aerobic incubation flasks containing ^{14}C-labeled carbonate and equipped with a trapping tower gave an average recovery of $^{14}CO_2$ on the Ascarite of 93.6 ± 0.8% after standing for 3 days vs. 101.9 ± 8.3% by flushing. Experiments with sucrose-^{14}C have established that the amount of $^{14}CO_2$ collected on Ascarite was independent

Table I. Metabolism of CDAA and Diallate on Soil for 2 Weeks[a]

	^{14}C as % of total applied					
Compound	CO_2	Plug	Extracted	Parent herbicide remaining	Soil bound	Accountability
CDAA	8.3	1.1	40.3	6.7	48.6	98.3
Diallate	7.4	26.5	42.9	40.3	20.0	96.8

[a] Results are an average of duplicate flasks for each soil.

Table II. Metabolism of Butachlor on Ray Silt Loam Soil

	^{14}C as % of total applied						
Time, weeks	CO_2	Plug	Acetonitrile extract	Ammoniacal methanol extract	Soil bound	Total accountability	Butachlor remaining
0			100.7		0.8	101.5	101
1	1.0	0.2	93.5		11.9	106.6	87
2	2.8	0.1	88.4		17.4	108.7	76
4	8.5	0.1	41.8	28.4	26.6	105.3	22
6	14.8	0.1	27.9	32.6	25.3	100.6	3
10	24.6		21.8	23.3	29.7	99.4	0.3

of flushing the incubation system and that subsequent flushing of a nonflushed flask with air resulted in a negligible additional recovery of $^{14}CO_2$. Flasks flushed had 29.9 ± 3.4% of the applied ^{14}C activity as $^{14}CO_2$ after 7 days while nonflushed flasks had 31.4 ± 0.1% as $^{14}CO_2$. Similarly, 20.1 ± 1.0% was collected as $^{14}CO_2$ in different experiments and only an additional 0.08% by subsequent flushing.

An integral part of this incubation system methodology is the method for releasing and retrapping of $^{14}CO_2$ trapped by the Ascarite of the trapping towers. Two quantitative methods have been developed to assay $^{14}CO_2$ trapped on Ascarite. The first method described in the Experimental Section in detail requires only simple, inexpensive glassware. Control experiments gave essentially a quantitative recovery of 96.8 ± 7.2% with this method. The second method utilizes the spinning band accessory of the Peterson Automatic Combustion Apparatus (Peterson, 1969) and a phenethylamine containing counting cocktail to retrap quantitatively $^{14}CO_2$ liberated analogously from Ascarite. Thirty control experiments with release method 2 have given a recovery of 102.8 ± 3.5%. Although the second method is now utilized exclusively by us, the choice between these two methods depends upon the equipment, resources, and personnel available in a given situation. In addition to permitting the analysis of $^{14}CO_2$, both of these release methods also distinguish between carbon dioxide and other possible volatile carbon-14 products present. This latter feature can be very important for certain types of compounds.

Overall validation of the aerobic incubation system has been obtained through actual metabolic studies with ^{14}C-labeled pesticides. The results of these studies have been summarized in Tables I and II. Considering the radioactivity in evolved CO_2, trapped organic volatiles, extracts, and bound to soil, an essentially quantitative total recovery of ^{14}C activity was obtained. This excellent total accountability is consistent with the quantitative nature of the overall aerobic incubation system. The indicated rapid steady degradation of all three compounds has been observed in unpublished data (Rueppel et al., 1977a) and as illustrated in detail in Table II for butachlor. The rates of degradation observed for CDAA and butachlor using the aerobic incubation system, for example, are comparable to results obtained using the well-established, standard method of incorporation of the pesticide at comparable rates into large pans of the same moist soil. These latter unpublished results (Lauer and Arras, 1976; Daniels and Pounds, 1971) indicated half-lives of 0.2 and 1–2 weeks for CDAA and butachlor, respectively; the data in Tables I and II indicate comparable half-lives of 0.2 and 1–2 weeks, respectively. Butachlor was 97% dissipated in 6 weeks using the aerobic incubation system and 92 and 98% dissipated at 4 and 8 weeks in the other study. Previously published soil degradation studies with glyphosate and glyphosine have also found a good correspondence with similar comparisons (Rueppel et al., 1977b; Marvel et al., 1975). The aerobic incubation flask unquestionably provides representative, reasonable data.

Additional validation of the system results from an examination of the many other actual applications of the aerobic incubation system. Using this system, the average total accountability from 72 aerobic experiments with 11 different ^{14}C-labeled compounds was 95.7% with a standard deviation of 9.1% (Marvel et al., 1976; Marvel et al., 1975; Rueppel et al., 1977b). These experiments represented significant differences in terms of $^{14}CO_2$ evolution, chemical structure, polarity, solubility, volatility, and experimental duration.

In summary, the aerobic incubation system and the supporting methodology in this paper circumvents many of the limitations of previous systems while maintaining most of the advantages. The goal of developing a simple, compact, quantitative apparatus and precise, accurate supporting methodology in order to determine the rate, extent, and mechanism of the degradation of pesticides in soil has been accomplished. The apparatus and techniques should be generally applicable to the study of the rate, extent, and pathway of degradation of ^{14}C-labeled pesticides and other compounds; the effects of climate, ^{14}C-label position, soil, and environment can be examined without great expenditures of time, space, labor, and radioactive material. Applications to metabolism studies in natural waters and sediments as well as flooded soils can be readily anticipated.

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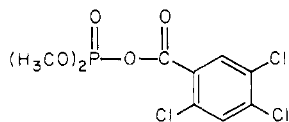
Crystal and Molecular Structure of Organophosphorus Insecticides. 9. Stirofos

Wayne J. Rohrbaugh and Robert A. Jacobson*

The crystal and molecular structure of stirofos (2-chloro-1-(2,4,5-trichlorophenyl)vinyl dimethyl phosphate), $(H_3CO)_2PO_2C_8H_3Cl_4$, has been determined by three-dimensional X-ray analysis. The compound crystallizes in the triclinic space group $P\bar{1}$: $a = 14.023$ (4), $b = 15.088$ (5), $c = 6.930$ (2) Å, $\alpha = 93.94$ (2), $\beta = 90.29$ (4), and $\gamma = 98.97$ (4)°. The structure was solved via direct methods and refined to a final residual of $R = 0.123$. The two molecules per asymmetric unit assume nearly identical configurations with the orientation of the phosphate group relative to the planar organic moiety differing markedly from previous studies of organophosphorus insecticides. The average angle between the P=O bond and the normal to the plane of the ring system was 85.8° compared to 23.7° for ronnel, 38.4° for coroxon, 23.9° for azinphos-methyl, and 23.5° for amidithion. In addition, the partial charge on phosphorus (via a CNDO II molecular orbital calculation) differed considerably from previous studies, i.e., stirofos, +1.385 e; amidithion, +1.063 e; and azinphos-methyl, +1.041 e.

Previous crystal structure analyses of organophosphorus insecticides have attempted to outline possible structural and electronic features of insecticide molecules which may be important factors when considering their toxicity (Baughman and Jacobson, 1975; Gifkins and Jacobson, 1976; Rohrbaugh et al., 1976; Rohrbaugh and Jacobson, 1977). As has been discussed, the ubiquitous or specific effectiveness of a particular insecticide may depend on its ability to accommodate a range of esteratic-anionic site separations in various acetylcholinesterase (AChE) enzymes. In addition, the effectiveness may be related to the relative charge densities on the corresponding sites of the insecticide that could be involved in enzyme binding (Rohrbaugh and Jacobson, 1977). Finally, gross topographical features of the insecticide molecules may also be important in regulating toxicity.

It is of interest to compare such molecular features among insecticides exhibiting varying degrees of toxic effectiveness. Since our previous studies have included azinphos-methyl (LD_{50} = 16 mg/kg) and amidithion (LD_{50} = 420 mg/kg), we decided to carry out a crystal structural investigation of stirofos, 2-chloro-1-(2,4,5-trichlorophenyl)vinyl dimethyl phosphate. It has an LD_{50} of 4000



mg/kg (Pesticides and Toxic Substances Effects Laboratory, 1973) and its structure determination should provide parameters useful for comparison to its more toxic counterparts.

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EXPERIMENTAL SECTION

Crystal Data. A sample of stirofos was kindly supplied by the Pesticides and Toxic Substances Effects Laboratory, U.S. EPA, Research Triangle Park, N.C. A nearly spherical crystal of radius 0.18 mm was mounted on a glass fiber with Duco cement and subsequently attached to a standard goniometer head. From five preliminary ω-oscillation photographs taken on an automated four-circle X-ray diffractometer at various χ and ϕ settings, only seven reflections of significant intensity were observed and their coordinates were input to the automatic indexing program ALICE (Jacobson, 1976). In spite of the crystal's poor diffraction characteristics, these seven reflections yielded a good cell, and intensity data were subsequently collected using this crystal.

The resulting reduced cell and reduced cell scalars indicated a triclinic crystal system. Inspection of the axial ω-oscillation photographs verified, within experimental error, the layer line spacings predicted for this cell by the automatic indexing program. A least-squares refinement of the lattice constants (Takusagawa, 1975) based on the $\pm 2\theta$ measurements of 13 moderately strong independent reflections on a previously aligned four-circle diffractometer (Mo Kα graphite monochromated X-radiation, λ = 0.70954 Å) yielded $a = 14.023$ (4), $b = 15.088$ (5), $c = 6.930$ (2) Å, $\alpha = 93.94$ (2), $\beta = 90.29$ (4), and $\gamma = 98.97$ (4)°.

Collection and Reduction of X-Ray Intensity Data. Data were collected at room temperature on an automated four-circle diffractometer designed and built in this lab-

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