Glass-Catalyzed Decomposition of Oxycarboxin in Aqueous Solution

David T. Stanton[1]

Studies of the commercial fungicide oxycarboxin have shown a time-dependent decrease in the concentration of the pesticide in dilute aqueous solutions. Additional study showed the decomposition of oxycarboxin involved the container surface and was surface-type dependent. The rate of degradation was shown to follow first-order kinetics and was temperature and solvent related. A tentative structure has been assigned to the decomposition product, and a mechanism is suggested to account for its formation catalyzed by the container surface.

Compound loss during analysis has been documented in the chemical literature; in particular, interaction of a compound with the surface of the container in which it is stored has been reported. Interaction may be in the form of reaction with the container surface or adsorption to it; however, there are many more examples of the latter in the chemical literature. An example of a material reacting with the surface of a container was reported by Hanneman and Klimisch (1972). The study described the decomposition of organopolysiloxanes in acetone solution catalyzed by the surface of glass containers and how storage in polyethylene containers significantly reduced the rate of decomposition. Several examples of loss due to adsorption have been reported. The loss of dinitroanaline herbicide trifluralin was observed by Strachan and Hess (1982) when aqueous solutions of the pesticide were stored in glass containers. The study showed that the compound was adsorbed onto the surface of the container, making it difficult to maintain a constant concentration of the material in solution. A similar study by Sharom and Solomon (1981) involved the adsorption of permethrin, a pyrethroid insecticide, on glass and plastic surfaces. Later, Helmuth et al. (1983) demonstrated that pretreatment of glass containers with Carbowax 20M effectively reduced the adsorptive losses of synthetic pyrethroids and other lipophilic compounds from aqueous solutions.

During a soil residue study involving two commercial oxathiin fungicides, carboxin (Figure 1, I) and oxycarboxin (Figure 1, III), it was noted that the concentrations of aqueous solutions of carboxin remained stable over time while the concentrations of similar solutions of oxycarboxin decreased with time. Several possible causes for the apparent instability of oxycarboxin solutions were considered. It was shown by others that oxycarboxin is susceptible to acid-catalyzed hydrolysis (Corbeil et al., 1973) and base-catalyzed hydrolysis (Kulka, 1980). Although both carboxin and oxycarboxin would be susceptible to hydrolysis, the carboxin solutions appeared to be stable while the solutions of oxycarboxin were not. Thus, hydrolysis was not considered to be a major factor. Oxycarboxin has been shown to undergo photolysis (Buchenaure, 1975), which was considered as a potential cause of the loss but was later discounted as preliminary studies indicated that the presence or absence of light had little effect on the stability of oxycarboxin in solution. Apparent solution instability could also be attributed to adsorption or reaction of the compound with the surface of the glass container. This possibility was strongly suggested when preliminary studies showed that the loss of oxycarboxin could be inhibited if the solutions were stored in plastic containers. Furthermore, samples stored in soda lime glass containers exhibited a slower rate of loss of oxycarboxin as compared to samples stored in containers composed of borosilicate glass.

Such degradation of oxycarboxin in solution has not been previously reported in the literature; therefore, this study was conducted to characterize the factors responsible for the observed loss of oxycarboxin in solution and to determine the effect of specific variables on the rate of loss.

To effectively study the causes of the apparent instability of oxycarboxin in solution, it was necessary that the analytical procedure used be rapid, sensitive, selective, and facile. The approach offering the best combination of these qualities was a high-performance liquid chromatography (HPLC) method developed by Wolkoff et al. (1976). Adjustments were made to the original method to improve resolution and peak shape.

EXPERIMENTAL SECTION

Apparatus. Chromatography was performed using a high-performance liquid chromatograph consisting of a Model 6000A pump, a Model M-45 pump, a Model 450 variable-wavelength ultraviolet absorbance detector, a Model U6K injection valve (all Waters Associates, Milford, MA), a Model 308 computing integrator (Laboratory Data Control, Boca Raton, FL), and a Model 285 dual pen strip-chart recorder (Linear Instruments, Irvine, CA). Separation of the compounds of interest was achieved on a Spherisorb ODS, 5-μm, 250 mm × 4.6 mm HPLC column. Isocratic conditions were employed with a mobile phase composition of 40% (v/v) acetonitrile in water, with a flow of 1.5 mL/min. Detection of the components was carried out at 254 nm. Total time required for a single determination was 6.0 min.

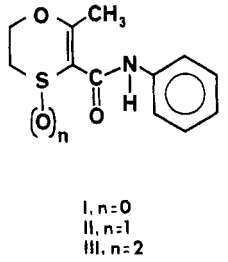
Reagents. Analytical-grade oxycarboxin (99.2%), carboxin (99.4%), and carboxin sulfoxide (Figure 1, II) (99%) were obtained from Uniroyal Chemical Inc. and were used without further purification. All solvents were HPLC grade and were filtered by 0.45-μm filters prior to use.

Procedure. The effects of light, temperature, solvent type, container type, and container surface treatment were studied in order to determine the influence of these variables on the stability of oxycarboxin in solution. Investigation of the effects of light and temperature was made by preparing three sets of five 20-mL, borosilicate glass vials, adding 5.0 mL of a 31.6 μg/mL aqueous oxycarboxin solution to each vial and capping tightly. The first set of five vials was immediately wrapped in aluminum foil to exclude light and allowed to stand at room temperature (22.2 °C). The second set was placed in storage at 5 °C while the third set was allowed to stand at room temperature and exposed to normal fluorescent room lighting at a distance of 1–3 m. The three sets were incubated under

Uniroyal Chemical Company Inc., Naugatuck, Connecticut 06770.

[1] Present address: 321 Toftrees Ave., Apt. 331, State College, PA 16803.

0021-8561/87/1435-0856$01.50/0



I, n=0
II, n=1
III, n=2

Figure 1. Structures of carboxin (I), carboxin sulfoxide (II), and oxycarboxin (III).

these conditions for a period of 96 h. After incubation, subsamples of each vial of the covered, room-temperature set were transfered to a set of five 20-mL polypropylene vials that were then stored at −7.0 °C for later analysis. Composite samples of each set were then prepared by transfering 1.0-mL aliquots from each sample in a set to one 20-mL, polypropylene vial that was then stored at −7.0 °C for later analysis.

Effects of plastic vials on the stability of oxycarboxin solutions were determined by incubating 5.0-mL aliquots of the 31.6 μg/mL solution used above in a set of five 20-mL, polypropylene vials under conditions indentical with those used for the exposed, room-temperature set above. A composite sample was then prepared and stored for later analysis. In order to study the effect of glass surface treatment, a set of clean 20-mL, borosilicate vials were treated with a 5% (v/v) solution of dimethyldichlorosilane in toluene. After treatment, aliquots of 5.0 mL of the 31.6 μg/mL aqueous oxycarboxin solution were incubated in the vials under the same conditions used for the plastic set. A composite sample was then prepared for later analysis.

Solvent effects on the loss of oxycarboxin were studied of 30 μg/mL solutions of oxycarboxin in acetonitrile, water, methanol, and methylene chloride. A set of five glass vials was prepared for each solvent by pipetting 5.0 mL of the respective solutions into the vials and capping tightly. The vials were then incubated under conditions identical with those used for the exposed-glass, room-temperature set above. Composite samples of each set were later analyzed as described above.

To determine the kinetics of the reactions involved in the observed loss of oxycarboxin, the effects of temperature and concentration on the reaction rate were measured at temperatures of 0 ± 2.9, 25 ± 0.8, and 39.6 ± 0.5 °C and at concentrations of 1.1, 10.5, and 21.1 μg/mL in water. A set of 14 20-mL borosilicate glass vials was prepared for each concentration and temperature studied, with the exception of the high-temperature sets containing 16 vials each. Aliquots of 5.0 mL of the appropriate solution were added to separate vials that were then tightly capped. The samples were incubated for a period of 52.9 h at the temperatures specified above. At different time points, two vials from each concentration and each temperature were removed for analysis. The contents of each pair of vials was combined in a clean 20-mL plastic vial and frozen until analyzed.

RESULTS AND DISCUSSION

The chromatographic conditions described above were found to give good precision and reproducibility. A calibration curve covering the expected working range of 0.3–31.7 μg/mL aqueous oxycarboxin gave a linear curve with a correlation coefficient of 0.999. The limit of detection (signal/noise ratio 2) for aqueous oxycarboxin was found to be 50 ng/mL with no sample preconcentration. A typical chromatogram is shown in Figure 2.

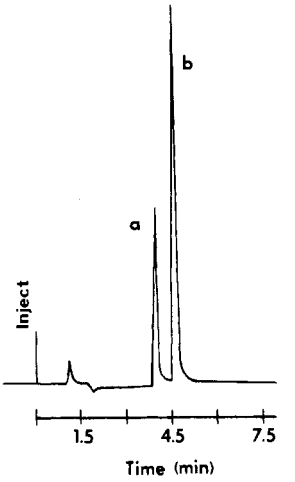


Figure 2. HPLC chromatogram of an aged-in-glass solution of oxycarboxin (b) showing the presence of the degradation product (a).

Table I. Summary of the Effects of Storage for 96 h, at Different Storage Conditions, on the Stability of Oxycarboxin Solutions

container type	temp, °C	solvent	% degradation[a]
glass, exposed	22.2	H_2O	84.7
glass, covered	22.2	H_2O	91.4
plastic	22.2	H_2O	6.2
glass, exposed	5.0	H_2O	50.7
glass, treated	22.2	H_2O	13.0
glass, exposed	22.2	CH_3OH	19.5
glass, exposed	22.2	CH_3CN	0.6
glass, exposed	22.2	CH_2Cl_2	3.8

[a] Degradation expressed as percent of a zero-time sample.

The effects of the sample treatments are described below. In all cases where a loss of oxycarboxin was observed there was a corresponding increase in a new compound that could not be traced to sample contamination. It was noted that individual vials in a given set would exhibit different degrees of oxycarboxin loss when all other variables were held constant. The samples in the covered, room-temperature set above exhibited losses in the range of 81.5–94.8% with a mean of 90.8%. Due to the dissimilarity between samples, it was necessary to prepare multiple vials for each variation of storage conditions studied. However, the task of analyzing each replicate individually was impractical. The analysis of a composite sample consisting of equal aliquots from each vial from the covered, room-temperature set gave a value of 91.4% loss, which falls well within the 95% confidence limit range of 90.8 ± 6.8% obtained for the individually analyzed set. The technique of composite sampling was therefore shown to be a valid approach to reducing the number of individual analyses while still providing useful data.

Table I summarizes the effects of different storage conditions and solvents on the stability of oxycarboxin solutions. Comparison of the uncovered glass vials to the foil-covered glass vials indicates that exposure to normal fluorescent room lighting had little effect on the stability of oxycarboxin in aqueous solution. The rate of degradation was found to be temperature dependent, since the cold storage sample exhibited a slower rate of loss compared to the room-temperature set. Use of silanized vials demonstrated that a surface interaction in the untreated vials accelerated the loss of oxycarboxin. The small loss suggests that some available active sites on the vial surface remained, even after treatment, to produce a loss. The loss of oxycarboxin in solution was also shown to be solvent related. It was apparent that protic solvents as well as the

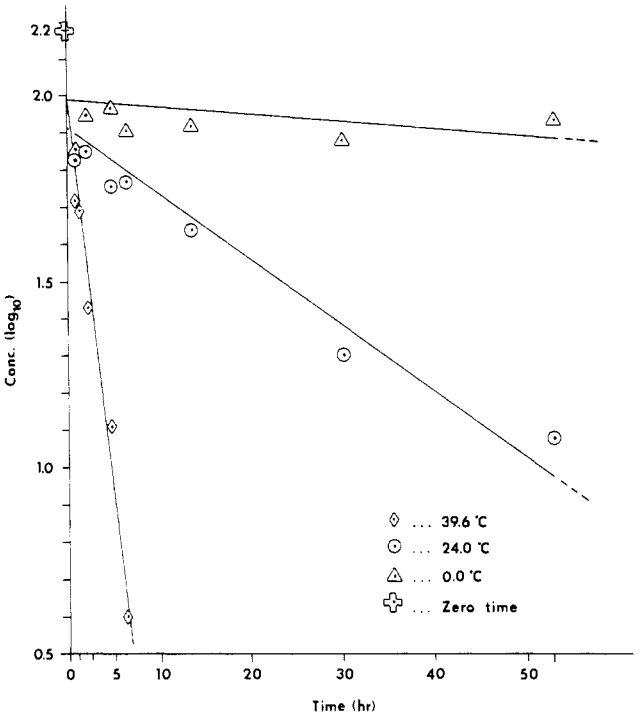


Figure 3. Correlation of experimental data to a first-order decomposition for 1.1 ppm aqueous oxycarboxin solutions at 0, 24, and 39.6 °C.

Table II. Rate Constants (k) Calculated for the Degradation of Oxycarboxin in Aqueous Solution at Three Different Temperatures and Concentrations

concn, ppm	temp, °C	k	log k
1.1	0.0	4.08×10^{-3}	-2.32
1.1	24.0	3.98×10^{-2}	-1.40
1.1	39.6	5.01×10^{-1}	-0.30
10.5	0.0	9.21×10^{-3}	-2.04
10.5	24.0	3.86×10^{-2}	-1.41
10.5	39.6	4.26×10^{-1}	-0.37
21.1	0.0	6.91×10^{-4}	-3.13
21.1	24.0	1.88×10^{-2}	-1.72
21.1	39.6	2.46×10^{-1}	-0.61

glass surface played a major role in the degradation of oxycarboxin.

It was suspected that any active sites on the container surface would be in great excess relative to the concentrations of oxycarboxin studied and thus the loss of oxycarboxin would follow pseudo-first-order kinetics. Examination of the data obtained from studies of three concentrations of oxycarboxin at different temperatures over time support this hypothesis. If indeed first-order kinetics was being obeyed, then a plot of the log of the concentration vs. time would result in a linear curve with a slope of $-k/2.303$ (Frost and Pearson, 1961). Figure 3 shows the correlation of the curves obtained from least-squares regression analysis of the data obtained for aqueous solutions of oxycarboxin containing 1.1, 10.5, and 21.1 ppm. The data conform to linear curves with coefficients of correlation averaging 0.950. Table II lists the rate constants derived from these curves. Deviations noted in the early sampling of the 0 °C set were due to difficulties encountered in cooling the samples rapidly enough to avoid reaction at warmer temperatures. The data obtained strongly indicate a temperature-dependent decomposition process that is a pseudo-first-order reaction giving only one detectable product.

In an effort to characterize the unknown product, a sample from the above studies was partially purified by the collection of fractions of the HPLC effluent as the peak

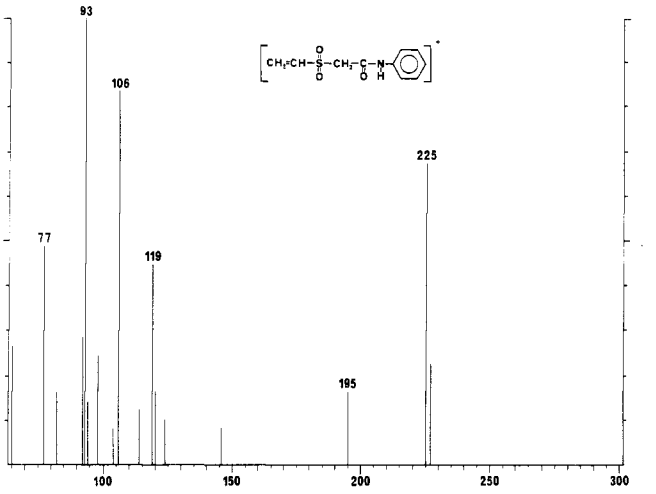


Figure 4. Electron impact (EI) mass spectrum of the decomposition product of oxycarboxin.

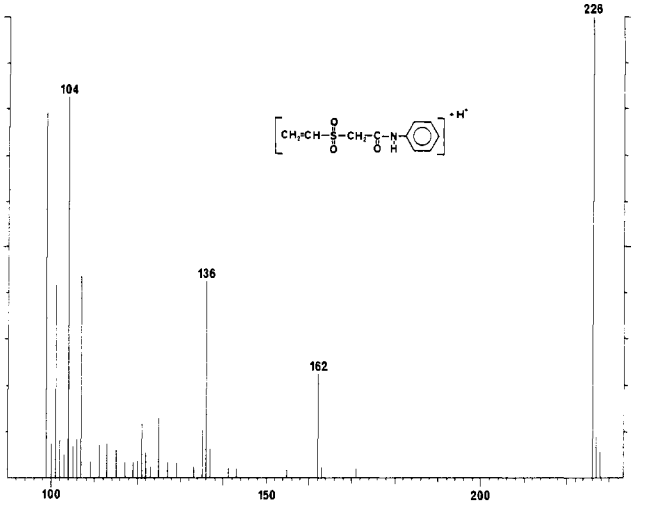


Figure 5. Chemical ionization (CI) mass spectrum of the decomposition product of oxycarboxin.

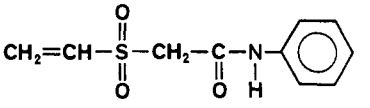


Figure 6. Structure of the decomposition product 2-(vinylsulfonyl)acetanilide.

of interest eluted. This sample was submitted for mass spectral analysis. Electron impact data gave a value for the molecular ion of m/e 225 (Figure 4), which was confirmed by a molecular ion of m/e 226 obtained from a methane-CI analysis of the sample (Figure 5). Fragmentation in the EI spectrum indicated a loss of 106 mass units, corresponding to a loss of $C_3H_6SO_2$, which was also detected as a separate fragment ion. A peak at m/e 93 indicated the aniline ring was still intact. A compound that can reasonably be derived from the degradation of oxycarboxin and is also supported by the mass spectral data is shown in Figure 6. This compound has been previously reported as a degradation product of oxycarboxin formed by refluxing a methanol solution of oxycarboxin for a period of 13 days (Ross et al., 1972). The reported mass spectral data agree with that obtained in this study.

As discussed previously, the oxathiin ring can undergo base-catalyzed opening. Once the ring is opened, it is a simple step to hydrolytically remove an acetyl group to give 2-(vinylsulfonyl)acetanilide. The proposed mechanism is depicted in Figure 7. The deacetylation step is supported

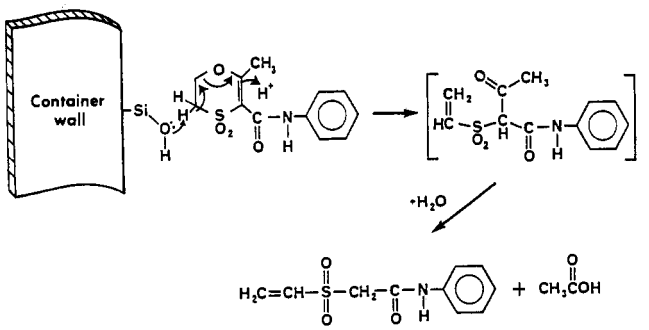


Figure 7. Proposed mechanism for the conversion of oxycarboxin to 2-(vinylsulfonyl)acetanilide involving the surface of a glass container.

by a similar reaction reported for the ring-opened form of carboxin (Corbeil et al., 1973). It is proposed that the silanol groups available at the glass surface act as a base to catalyze the ring-opening step.

The results of this study provide significant impact on analysis of oxycarboxin in general. It is apparent that the glass-catalyzed decomposition of oxycarboxin is rapid enough to cause great variation in recovery levels in metabolism and environmental fate studies. It is possible, for example, that one could misinterpret the absence of oxycarboxin residues as losses occurring in the sample matrix rather than attributing the loss to storage conditions used during isolation or extraction steps, while the presence of 2-(vinylsulfonyl)acetanilide could be misinterpreted as a real product of biological or environmental decomposition. At this point, there are no data available to suggest that the losses noted here have a significant effect on studies involving much more concentrated solutions of oxycarboxin such as would be encountered in standard assay procedures. However, it is clear that the storage of calibrated standard solutions of oxycarboxin in glass containers for extended periods of time is a questionable practice that should be accompanied by periodic analysis to confirm solution integrity.

Registry No. III, 5259-88-1; CH_2=$CHSO_2CH_2CONHC_6H_5$, 75983-69-6; H_2O, 7732-18-5; CH_3OH, 67-56-1; CH_3CN, 75-05-8; CH_2Cl_2, 75-09-2.

LITERATURE CITED

Buchenaure, H. *Pestic. Sci.* **1975**, *6*, 525–535.
Corbeil, M. A.; Curcumelli-Rodostamo, M.; Fanning, R. J.; Graham, B. A.; Kulka, M.; Pierce, J. B. *Can. J. Chem.* **1973**, *51*, 2650–2658.
Frost, A. A.; Pearson, R. G. *Kinetics and Mechanism*; Wiley: New York, 1961.
Hanneman, L. F.; Klimisch, H. M. *J. Chromatogr.* **1972**, *70*, 81–86.
Helmuth, D. W.; Ghiasuddin, S. M.; Soderlund, D. M. *J. Agric. Food Chem.* **1983**, *31*, 1127–1129.
Kulka, M. *Can. J. Chem.* **1980**, *58*, 2044–2048.
Ross, J. A.; Tweedy, B. G.; Newby, L. C.; Bates, J. J., University of Missouri, Columbia, unpublished data, 1972.
Sharom, M. S.; Solomon, K. R. *Can. J. Fish. Aquat. Sci.* **1981**, *33*, 199–204.
Strachan, S. D.; Hess, F. D. *J. Agric. Food Chem.* **1982**, *30*, 389–391.
Tafuri, F.; Patumi, M. B.; Marucchini, C. *J. Agric. Food Chem.* **1978**, *26*, 1344–1346.
Wolkoff, A. W.; Onuska, F. I.; Comba, M. E.; Larose, R. H. *Biomed. Mass Spectrom.* **1976**, *3*, 248–254.

Received for review November 10, 1986. Accepted April 27, 1987.

Residues of Avermectin B_1a in Rotational Crops and Soils following Soil Treatment with [^{14}C]Avermectin B_1a

H. Anson Moye,* Marjorie H. Malagodi, Jau Yoh, Gary L. Leibee, Chia C. Ku,[1] and Peter G. Wislocki

[^{14}C]Avermectin B_1a was applied twelve times to muck and sandy loam soils and three times to sandy soil at 0.025–0.030 lb/acre per application. These applications simulated the intended use of avermectin B_1a on celery, vegetables, and cotton, respectively. Following three aging periods in each soil type, sorghum, lettuce, and carrot or turnip seeds were planted and harvested at one-fourth, half, and full size. Analysis of these crops by oxidative combustion demonstrated that crops grown in muck, sandy loam, and sandy soils contained radiolabeled residues ranging from below the limit of quantitation (BLQ) to 7.4 μg/kg of avermectin B_1a equivalents, BLQ to 11.6 μg/kg, and BLQ to 3.54 μg/kg, respectively. There was a general trend of decreasing residue concentrations with increasing preharvest intervals in crops grown in all soils. The radioactivity present in muck and sandy loam soils disappeared with half-lives ranging from 103 to 267 days and from 102 to 132 days, respectively.

Avermectins are a class of macrocyclic lactone pesticides that have been under investigation as acaricides/insecticides in citrus, orchard, and field crops (Price, 1983; Schuster and Everett, 1983; Wright, 1984; Reed et al., 1985; Burts, 1985). Abamectin (MK-0936) is currently being developed as a miticide/insecticide to control red imported fire ants and several phytophagus pests on horticultural and agronomic crops.

Since the use of a pesticide on a crop may lead to the presence of the pesticide or its degradation products in the soil, the potential exists that crops planted in that soil at a later date (rotational crops) might take up some of these residues. The present study was designed to determine the uptake and accumulation of [^{14}C]avermectin B_1a (the major component of abamectin) and all radiolabeled residues in rotational crops following soil treatment with the

Pesticide Research Laboratory, Department of Food Science and Human Nutrition, University of Florida, Gainesville, Florida 32611 (H.A.M., M.H.M., J.Y.), Central Florida Research and Education Center, Institute of Food and Agricultural Science, University of Florida, Sanford, Florida 32771 (G.L.L.), and Department of Animal Drug Metabolism, Merck Sharp & Dohme Research Laboratories, Three Bridges, New Jersey 08887 (C.C.K., P.G.W.).

[1]Present address: American Cyanamid Co., Princeton, NJ 08540.

0021-8561/87/1435-0859$01.50/0