Coumaphos Disposal by Combined Microbial and UV–Ozonation Reactions

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Coumaphos [*O,O*-diethyl *O*-(3-chloro-4-methyl-2-oxo-2*H*-1-benzopyran-7-yl) phosphorothioate] is a widely used insecticide for control of livestock insects. Large volumes of wastewater are generated annually from livestock dip operations. Microbial metabolism by a *Flavobacterium sp.* and fragmentation by UV–ozonation were examined alone and in combination as detoxification mechanisms for wastewater taken directly from a dip vat containing 1500 mg/L of coumaphos. Coumaphos was oxidized slowly when subjected to combined UV irradiation and ozonolysis. Microbial metabolism was rapid as measured by HPLC and resulted in cleavage of the phosphorothioate linkage to yield chlorferon (3-chloro-4-methyl-7-hydroxycoumarin) and diethyl thiophosphoric acid. The microbe did not fragment the benzene ring portion of the molecule as determined by loss of ^{14}C from [benzo ring U-^{14}C]coumaphos. Microbial metabolism followed by UV–ozonation or ozonation alone was most effective in cleaving the phosphorothioate linkage, ring fragmentation, and destroying the microorganism. A major product of chlorferon oxidation was 2,4-dihydroxyacetophenone.

Coumaphos [*O,O*-diethyl *O*-(3-chloro-4-methyl-2-oxo-2*H*-1-benzopyran-7-yl) phosphorothioate] is used to control ectoparasites on livestock and poultry, fly larvae in poultry fecal material, and gastrointestinal nematodes. It is widely used as an acaricide in cattle dip vat solutions for control of *Boophilus microplus* (Canestrini), the southern cattle tick, and *Boophilus annulatus* (Say), the cattle tick, in the cooperative USDA Animal and Plant Health Inspection Service (APHIS) Veterinary Science (VS) Tick Eradication Program with the Texas Animal Health Commission. One estimate (Schubert, 1985) is that between 75 000 and 125 000 U.S. cattle and about 300 000 Mexican cattle were dipped for tick control along the U.S.–Mexican border in 1984. The volume of these dip vats varies from 10 595 to 17 028 L (estimated average of 12 487 L), and about 42 vats are in operation year round. Maintaining a homogeneous solution of coumaphos in the dipping vats has been a major problem. Recent formulation research has shown that coumaphos-flowable liquid was easier to maintain in suspension than the wettable powder in a comparison study, thus overcoming the sediment problem (Ahrens et al., 1982).

Considerable research has been conducted on the environmental properties of coumaphos (Waggoner, 1985). The half-life of coumaphos was 23 days in buffered aqueous solution at pH 9.0 and 50 °C. In pond water at pH 5.5 and temperature in the range of 20–35 °C, the half-life was less than 7 days. Degradation of coumaphos was slow in a sandy loam soil under laboratory conditions, with a half-life of approximately 300 days. Volatility studies with ^{14}C-labeled coumaphos over 6 months in the same soil revealed that less than 1% loss occurred via volatile ^{14}C products. The major degradation products were chlorferon (3-chloro-4-methyl-7-hydroxycoumarin) and the oxygen analogue. In a field study when 300 ppm coumaphos was uniformly incorporated into the surface 15 cm of a silty loam soil, the half-life was approximately 200 days. The solubility of coumaphos in water at 25 °C is approximately 50 ppb.

Large volumes of waste liquid are generated from used dip vat operations that are difficult to degrade by soil disposal due to the long persistance of coumaphos. Experimentation with UV–ozonation of pesticide wastewater has proved to be an effective pretreatment step for rendering a number of compounds more amenable to subsequent soil metabolism (Kearney et al., 1983a,b; Kearney et al., 1984; Kearney et al., 1985).

Our objective was to examine the rates of decomposition of coumaphos from a dip vat solution used in a tick control program by UV–ozonation and by microbial metabolism and a combination step prior to addition to soils.

METHODS AND MATERIALS

Chemicals. Coumaphos standards and several suspected metabolite standards, including chlorferon, were gifts from Bayvet, Division of Miles Laboratories, Shawnee, KS 66201. [benzo ring U-^{14}C]Coumaphos (sp act. 21.1 mCi/mmol) was a gift from the Mobay Chemical Corp., Agricultural Chemicals Division, Kansas City, MO 64120. The [^{14}C]coumaphos was purified by thin-layer chromatography (TLC).

Twenty-three liters of dip vat solution were shipped in plastic containers from the USDA—APHIS, VS Tick Force Headquarters, P.O. Box 6299, Laredo, TX 78042. The commercial formulation used to prepare these solutions was Co-Ral (registered trademark name) Flowable Cattle Insecticide (42% coumaphos, 58% inert ingredients), EPA Reg. No. 11556-98. The dip vat samples were taken at several depths from formulated coumaphos used in a previous tick control program, and consequently the opaque solutions contained soil, cattle urine, and fecal material. Some solutions were passed through a 10-μm core cotton filter with a polypropylene core (Filterlite Model LMOIU-3/4, Filterlite Corp. Timonium, MD 21093) at a rate of 7.5 L/min in an attempt to remove some extraneous organic materials prior to UV–ozonation.

Analytical Methods. Two methods were employed for the analysis of coumaphos in solution. Unless stated otherwise, all experimentation was performed on the raw, unfiltered dip vat solutions. A gas chromatographic method involved removing a 5-mL sample and diluting it with 100 mL of methanol in a 250-mL polypropylene screw-cap bottle. The sample was shaken vigorously for 1 min. The bottle was centrifuged for 10 min at 1800*g*. Fifty milliliters of the supernatant were filtered through No. 2 Whatman filter paper and the filtrate plus rinses collected in a round-bottomed flask. The methanol extract was dried over anhydrous Na_2SO_4 and evaporated to near dryness in vacuo. The flask was rinsed three times with 5 mL of benzene; the benzene and rinses were passed through a Na_2SO_4 filter and collected in a 15-mL graduated test tube.

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The volume was adjusted to 10 mL for analysis on a Hewlett-Packard 5710A gas chromatograph fitted with a flame ionization detector. The column was a 5% DC-200 on 80/100 Chromosorb WAW DMCS 700. The column temperature was 240 °C, injector 250 °C, and detector 300 °C. Nitrogen carrier gas flow rate was 70 mL/min, air 200 mL/min, and hydrogen 45 mL/min. Based on ^{14}C-labeled coumaphos, the recovery was 99.4% in the final benzene extract. The limit of detection was 1.0 mg/L. No attempts were made to examine the sensitivity of the method below 1 mg/L.

A HPLC method was also used for the analysis of coumaphos and chlorferon. A 0.1-mL sample was diluted with 0.9 mL of methanol in a 1.5-mL polypropylene Eppendorf micro test tube. The tube was vortexed and then centrifuged at 11600*g* for 2 min. The supernatant was analyzed on a Waters C-18 Nova-Pak radially compressed cartridge column. The solvent system used was 75% methanol in 0.75 mM phosphoric acid (pH 2.0) at a flow rate of 2 mL/min. The pumps were Waters M 6000 A (Waters Associates, Inc., Milford, MA 01757). Coumaphos and chlorferon were detected by UV in a Perkin-Elmer LC-95 UV/visible spectrophotometric variable-wavelength detector at 320 nm. Chlorferon exhibited a major peak at 320 nm and coumaphos two broad peaks at 288 and 312 nm when scanned with a Beckman Model 25 spectrophotometer. Based on ^{14}C-labeled coumaphos, a recovery of 100% can be counted in the methanol supernatant injected into the HPLC. The limit of detection was 1.0 mg/L. As with the GLC method, no attempts were made to extend the method below concentrations of 1 mg/L.

For product studies, larger samples (10–30 mL) were periodically removed from the UV lamp system and acidified with 0.1 N HCl to pH 2.0. The samples were extracted three times with equal volumes of ethyl acetate, dried over Na_2SO_4, and carefully reduced to 0.5 mL under N_2. Pyridine (100 μL) was added to the ethyl acetate extract, reduced in volume to 0.1 mL, and derivatized with 100 μL of *N,O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA). A portion of the Me_3Si derivative was injected into a 30-m flexible silica capillary column coated with SE-30 and temperature programmed from 90 to 200 °C at 5 °C/min. The mass spectrometer is a Finnigan 4021 with an Incos data system, operated in an electron impact mode with an electron energy at 70 eV and a source temperature of 250 °C.

A thin-layer chromatographic method was used to purify [^{14}C]coumaphos and [^{14}C]chlorferon. The [^{14}C]coumaphos received from Mobay was dissolved in 1 mL of benzene, and the entire volume was spotted on an acetone-washed precoated silica gel 60 F254 20 × 20 cm TLC plate (E. Merck, Darmstadt, Germany). The plates were developed in 9:1 benzene–ethyl acetate. Air-dried plates were radioautographed on NS-5T "no screen" X-ray film (Eastman Kodak Co., Rochester, NY 14650) for 24 h. Areas corresponding to coumaphos (R_f 0.42–0.56) were scraped off the plate, and the silica gel was extracted with 50 mL of benzene and reduced to 10 mL under N_2. The original [^{14}C]coumaphos was 94.2% pure. A product of >99% purity was achieved after TLC.

For the purification of [^{14}C]chlorferon, the benzene extracts generated from the microbial metabolism studies were spotted on acetone-washed TLC plates. The plates were also spotted with authentic coumaphos and chlorferon standards. All plates were developed with either 9:1 benzene–ethyl acetate or 5:4:1 hexane–chloroform–methanol. Air-dried plates were placed on X-ray film, and standards were visualized under UV light. After the plates were examined, all of the benzene extracts were pooled, and radioactivity corresponding to chlorferon was scraped off the plates and rechromatographed until a single labeled band was obtained in two solvent systems. The ^{14}C-labeled chlorferon was chromatographically identical with standard chlorferon.

UV–Ozonation. The apparatus for laboratory-scale UV irradiation and in situ ozone generation has been described previously (Kearney et al., 1985). Briefly, 250 mL of the dip vat solution or a metabolized solution was irradiated with a 450-W (254-nm) quartz mercury lamp (Hanovia Catalog No. 679-A36) housed in a water-cooled quartz-walled immersion well and a 300-mL reaction vessel fitted with a gas inlet tube. Ozone was generated by slowly bubbling oxygen into the reaction vessel during the irradiation period at a rate of 60 mL/min. In some studies ozone was generated from a Model GTC-1B ozone generator (Griffin Technics Corp., 178 Route 46, Lodi, NJ 07644) at a rate of 32 g/h from O_2 feed gas (manufacturer's estimate).

The loss of coumaphos from a raw and filtered dip vat solution containing 1500 mg/L coumaphos was examined via UV–ozonation or ozonation alone as a function of time.

Degradation of chlorferon with 0.1 μCi of [benzo U-^{14}C]chloroferon was examined in the same lamp system in distilled water at concentrations of 60 and 250 mg/L. To achieve these concentrations of chlorferon, it was necessary to maintain the pH at or above 8 and mix the solution for several hours on a magnetic stirrer. Two-milliliter samples were removed every 2 min for the 60 mg/L solution and every 5 min for the 250 mg/L solution. Loss of chlorferon was measured by HPLC.

UV–ozonation of a dip vat solution previously subjected to microbial metabolism was examined at 15-min intervals over a $2^1/_2$-h period.

Microbial Metabolism. *Flavobacterium sp.* ATCC 27551, previously reported to degrade diazinon and parathion (Sethunathan and Yoshida, 1973), was obtained from the American Type Culture Collection, 12301 Parklawn Drive, Rockville, MD 20852-1776. A streptomycin-resistant variant designated 27551S2 selected by growth on nutrient agar plates containing streptomycin was used in this study. The organism was grown in nutrient broth (Difco, Detroit, MI) to late log phase at 30 °C in a gyrorotary shaker. The bacteria were collected by centrifugation (6000*g*, 10 min, 20 °C), and the pelleted cells were suspended by vortexing in a minimum volume of 50 mM potassium phosphate buffer (pH 7.0) containing 1 mM $MgSO_4$. Cell suspensions were tested for their ability to degrade diethyl thiophosphorate compounds by diluting 10 μL of suspension into 100 μL of potassium phosphate buffer containing approximately 20 μg of parathion and noting the rapid appearance of a yellow color due to the accumulation of *p*-nitrophenol formed by enzymatic hydrolysis of parathion. For degradation of dip vat coumaphos, the cell suspension was diluted 1:10 directly into dip vat material and mixed. The coumaphos–cell suspension mixture was placed in a shaker and incubated at 30 °C.

Samples were taken by removing 100 μL of well-shaken material and diluting directly into 900 μL of methanol. The mixture was vortexed vigorously and centrifuged at 12000*g* for 2 min. The supernatant fluid was analyzed by HPLC.

In a separate study, 30 μCi of [^{14}C]coumaphos was added to two flasks containing 100 mL of dip vat solution and incubated with a 1:10 cell suspension dilution for 4 and 68 h. At the termination of the experiment, loss of

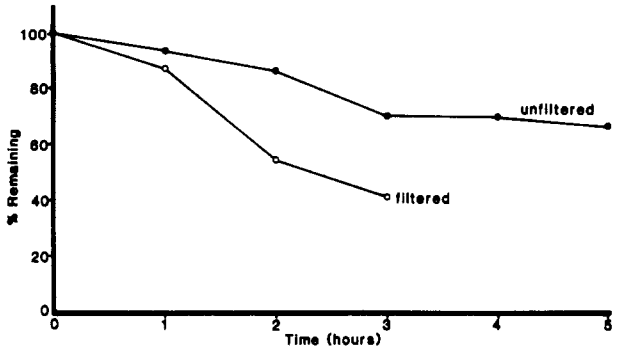


Figure 1. Degradation of 1500 mg/L of coumaphos in raw and filtered cattle dip vat solution by UV–ozonation as a function of time. The filtered solution was passed through a 10-μm pore size filter. Coumaphos loss was measured by GLC.

[^{14}C]coumaphos was measured by liquid scintillation analysis and loss of coumaphos by HPLC. All of the labeled solutions were extracted with benzene, and the benzene extracts were dried with anhydrous Na_2SO_4 and reduced to 1 mL. [^{14}C]Chlorferon was purified by TLC.

Cell Destruction. The numbers of 27551 S2 cells present in the dip vat material before, during, and after the UV–ozonation process were determined by diluting the material in sterile 50 mM potassium phosphate buffer (pH 7.0) containing 1 mM $MgSO_4$ and plating on nutrient agar plates supplemented with 200 μg/mL of streptomycin (Sigma Chemical Co., St. Louis, MO). After 48-h growth at 30 °C the cell numbers were determined by counting the number of bacterial colonies on the plates.

Soil Metabolism. Ten milliliters of cattle dip vat solution containing 0.1 μCi of [benzo U-^{14}C]coumaphos and 15 mg of unlabeled coumaphos were added to 50 g of Drummer silty clay loam in duplicate biometer flasks (Bartha and Pramer, 1965), and metabolism was determined by measuring the evolution of $^{14}CO_2$ at 30 °C. The Drummer soil had the following properties: field capacity 33.5%, pH 6.9, 5.5% organic carbon content. Duplicate flasks were also amended with 10 mL of dip vat labeled coumaphos solution previously subjected to UV–ozonation for 5 h and 10 mL of the same solution subjected to metabolism by the streptomycin-resistant variant of *Flavobacterium sp.* 27551 S2, followed by UV–ozonation of 200 mL of the metabolized solution. Two 1-mL samples of 0.1 N NaOH solution in the side arm of the flask were added to 10 mL of Beckman Ready-Solv HP (Beckman Instruments, Inc., Fullerton, CA 92634), and ^{14}C was determined by liquid scintillation analysis.

RESULTS AND DISCUSSION

UV–O_3 Studies. The oxidative decomposition of coumaphos in dip vat solutions via UV–ozonation is shown in Figure 1. After 3 h the rate of degradation appeared to level off with no further loss of coumaphos. Inspection of the solutions suggested that the presence of extraneous materials may have hindered the process by binding the parent coumpound and thereby slowing the reaction. Prior filtration and then subjecting this solution to UV–ozonation did enhance the rate of coumaphos decomposition (Figure 1). The filtering step did lower the coumaphos concentration in solution by about 13.0% after five passes through the pump. In a separate study ozone produced by the Griffin generator was fed directly into a dip vat solution (750 mL) contained in a 1-L graduated cylinder. After 6 h of ozonation, 61.4% of the original unfiltered coumaphos was recovered.

At this point it was concluded that UV–ozonation or ozonation alone was not a viable option for pretreatment

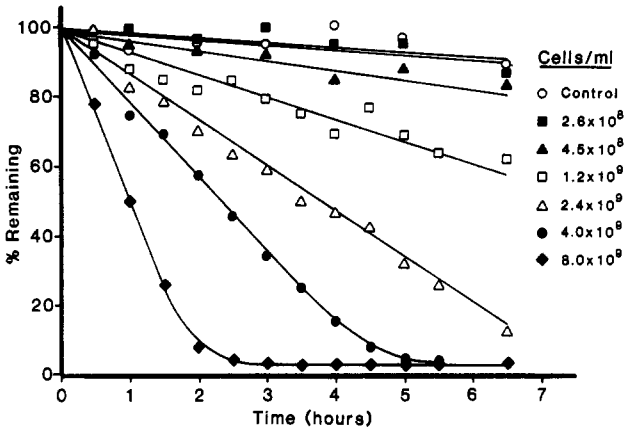


Figure 2. Metabolism of 1500 mg/L of coumaphos in cattle dip vat solution by a *Flavobacterium sp.* using six cell densities. Coumaphos loss was measured by HPLC.

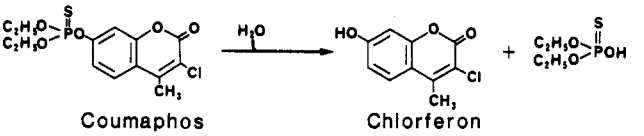


Figure 3. Hydrolysis reaction of coumaphos to chlorferon catalyzed by *Flavobacterium sp.* as measured by TLC analysis of [benzo ring U-^{14}C]coumaphos reaction products.

of coumaphos in dip vat solutions prior to soil disposal due to the slow and incomplete rate of reaction. It is difficult to compare rates of decomposition of coumaphos in these solutions to formulated pesticides in previous UV–ozonation studies. In previous studies (Kearney et al., 1984) aqueous solutions of 2,4-D (1500 mg/L) and atrazine (4480 mg/L) were degraded more than 80% in 2–3 h.

Microbial Metabolism. Preliminary studies on the microbial metabolism of coumaphos in dip vat solutions suggested the rates were more rapid and less influenced by interfering substances than UV–ozonation. The effect of cell density on coumaphos metabolism with time is shown in Figure 2. The *Flavobacterium sp.* isolated by Sethunathan and Yoshida (1973) hydrolyzed both diazinon and parathion by a constitutive enzyme. With diazinon, the heterocyclic group, i.e. the 2-isopropyl-6-methyl-4-hydroxypyrimidine, was used by the bacterium as its sole source of carbon, whereas it did not metabolize the resultant *p*-nitrophenol from parathion. Subsequent research by Brown (1980) described the isolation and partial purification of phosphotriesterase from the same *Flavobacterium.* The substrate specificity of the partially purified enzyme was restricted to compounds with electron-withdrawing aromatic or heterocyclic leaving groups such as parathion, paraoxon, diazinon, and their analogues. Substrate analogues having the weakly electrophilic 4-aminophenyl group were not hydrolyzed and in some cases acted as competitive inhibitors.

Incubation of ring-labeled coumaphos in the dip vat solution with the *Flavobacterium sp.* revealed no loss of radioactivity from the solution after 4 or 68 h. A 90% loss of coumaphos occurred after 4 h as measured by HPLC. Two labeled spots were detected on the X-ray film of the TLC plates from the 4- and 68-h incubation study. These spots cochromatographed with coumaphos (R_f 0.50, and 0.65) and chlorferon (R_f 0.11, 0.30) in the 9:1 benzene–ethyl acetate and 5:4:1 hexane–chloroform–methanol solvent systems, respectively. The reaction is shown in Figure 3. In contrast to the hydroxypyrimidine group from diazinon, chlorferon was not used as a carbon source by the *Flavobacterium sp.* From an environmental standpoint, it could

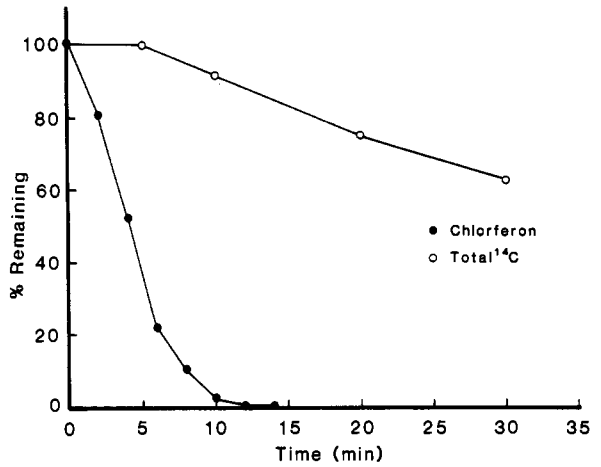


Figure 4. Rate of degradation of 60 mg/L of [benzo ring U-^{14}C]chlorferon in aqueous solution via UV–ozonation. The loss of total ^{14}C from solution (○) and loss of purified chlorferon (●) are plotted as a function of time (min). The rate constant was $k = 0.289$, $t_{1/2} = 4.25$ min, $r^2 = 0.95$.

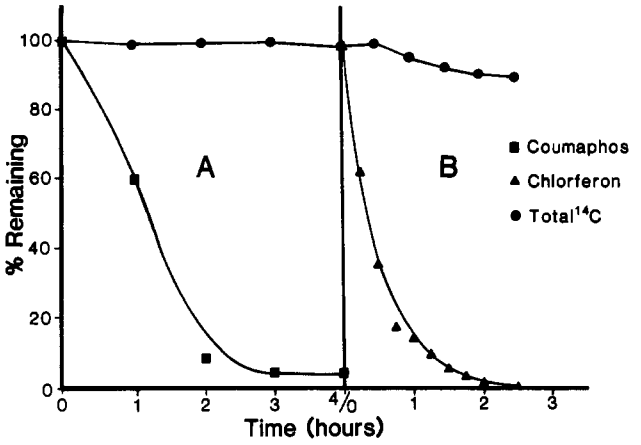


Figure 5. Combined effects of microbial metabolism of 1500 mg/L of coumaphos in cattle dip vat solution by *Flavobacterium sp* (A) and subsequent UV–ozonation of the resultant chlorferon (B) on a cattle dip vat solution containing 1500 mg/L of coumaphos. The pH of the solution after microbial metabolism B was adjusted to 10.0 to bring the chlorferon into solution.

be argued that a chlorinated phenolic product of microbial metabolism may be undesirable, i.e. it may elicit some untoward, although undocumented, biological effects. Chlorferon is a chlorinated, methylated derivative of the umbelliferones (7-hydroxycoumarins), the latter compounds being widely distributed in nature (Dean, 1963). The 4-methylumbelliferone has been the subject of several photochemical studies, due to its pH-dependent absorption and fluorescence spectra (Wolfbeis et al., 1980). Introduction of an electron-accepting substituent into the 3-position of umbelliferone causes the molecule not to undergo phototautomerizations (Wolfbeis, 1977). Photochemical ring-opening reactions are well documented with simple α-pyrones (Chapman, 1974), and the resultant ketene intermediates are most often trapped by their reaction with water or methanol (Chapman et al., 1973; McIntosh and Chapman, 1973; Pong and Shirk, 1973). UV–ozonation has been demonstrated to be particularly effective in destroying phenolic compounds in water, and therefore the rate of oxidative cleavage of chlorferon in pure and dip vat solutions was examined.

The degradation of labeled chlorferon in distilled water is shown in Figure 4. The reaction is rapid and obeys first-order kinetics ($k = 0.289$, $r^2 = 0.95$) with a half-life of 4.25 min. There was loss of ^{14}C from the solution, in-

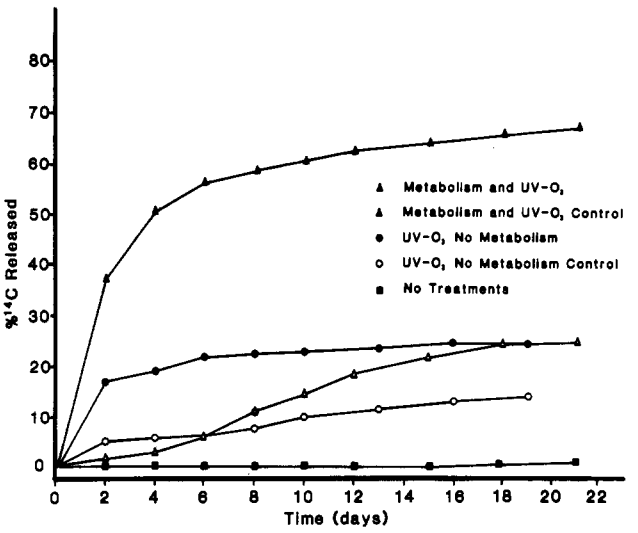
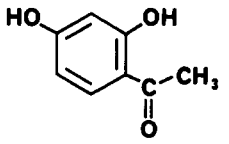


Figure 6. Soil degradation of 1500 mg/L of [benzo ring U-^{14}C]coumaphos previously subjected to no treatment (■), UV–ozonation (○), and microbial metabolism by a *Flavobacterium sp.*, followed by UV–ozonation of the same solution (▲). $^{14}CO_2$ evolution was measured in soil biometer flasks containing 50 g of Drummer soil and 10 mL of cattle dip vat solution (300 mg/L of coumaphos in soil based on initial concentration). Control flasks contained 50 g of autoclaved soil and 10 mL of dip vat solution subjected to UV–ozonation or metabolism followed by UV–ozonation.

dicating cleavage of the benzene portion of the substituted coumarin. Thin-layer chromatography of the solution revealed two compounds, chlorferon and an unknown, at R_f 0.10 and 0.16, respectively, in the 9:1 benzene–ethyl acetate solvent system.

Products from the UV–ozonation of the 250 mg/L chlorferon solutions were examied by GLC–mass spectrometry. The Me_3Si derivative of a single major ring-containing product was detected in the ion chromatogram of the 10-min sample. The important ions and their relative intensities were m/z 296 (1.9%), a base peak at 281 (100%), 209 (11.7%), 147 (2.3%), 133 (5.3%), 75 (14.7%), 73 (41.6%), and 45 (6.2%). Only peaks above 1% are reported. The molecular ion was at m/z 296. This spectrum and retention time (18.8 min) are in excellent agreement with the $(Me_3Si)_2$ derivative of 2,4-dihydroxyacetophenone (I), m/z 296 (0.6%), 281 (100%), 209 (7.5%),



I

147 (1.2%), 133 (2.2%), 75 (6.5%), 73 (38.8%), and 45 (3.5%). The HPLC retention times for I and an authentic standard were identical. The $(Me_3Si)_2$ derivative of glycolic acid was identified by comparing the retention time and fragmentation pattern with a standard, and other derivatives of ring fragment products are under study. A minor product was tentatively identified as a hydroxylated chlorferon.

The total loss of coumaphos by microbial metabolism (A) and subsequent UV–ozonation of chlorferon (B) in dip vat solutions is shown in Figure 5. Initially, we experienced considerable difficulty in getting rapid rates of chlorferon oxidation. Close examination of the culture flasks revealed a white material adhering to the flask walls. This was identified as undissolved chlorferon. After the culture solution was adjusted to pH 10, all of the chlorferon

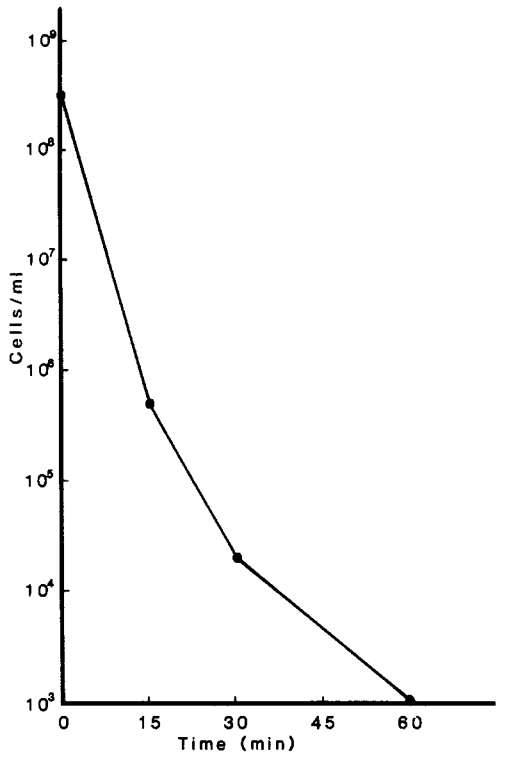


Figure 7. Cell-killing curve for a streptomycin-resistant *Flavobacterium sp.* in cattle dip vat solution containing 1500 mg/L of coumaphos subjected to UV–ozonation. Bacterial cells were enumerated by serial dilution on nutrient agar plates containing 200 μg/mL of streptomycin. After 48-h growth at 30 °C the cell numbers were determined by counting the number of colonies on the plates.

went into solution and yielded the rate data shown in Figure 5B. Ozonation in the absence of UV radiation was also very effective in destroying the chlorferon in the dip vat solutions. No detectable residues of chlorferon were measured after 30 min from ozone supplied by the Griffin generator. The mechanism appears to involve the direct attack of ozone on chlorferon.

Soil Metabolism. Soil metabolic rates for coumaphos, coumaphos subjected to prior UV–ozonation (5 h), and coumaphos subjected to prior microbial metabolism and then UV–ozonation are shown in Figure 6. No aromatic ring cleavage of unaltered coumaphos in the dip vat solution was observed during the 22-day soil study, which would seem to support the long persistence times observed by Waggoner (1985). The Drummer soil used in the biometer flask is microbially very active and contained large numbers of bacteria in a previous study (Burge, 1972). The rapid metabolism of the two-stage pretreatment dip vat solution suggests extensive fragmentation of the coumaphos molecule to yield some very degradable compounds, similar to rates observed in previous studies (Kearney, 1983a,b). No attempts were made to keep the control soils sterile, and consequently some breakdown occurred.

Cell Destruction. In a separate study the *Flavobacterium* cells were killed during the second-stage UV–O_3 step without pH adjustment (Figure 7). Cell destruction of coumaphos-degrading organisms as a consequence of ozonation may have important implications in preventing the escape of live cells from treatment tanks directly into the vats. Accidental release of selected or engineered organisms would destroy coumaphos' effectiveness as a tick control agent. Destruction of the viable cells was not unanticipated since water treatment by ozonation and ultraviolet radiation are used to sterilize water for drinking purposes (Block, 1982). Although not studied independently, the chlorferon in the presence of UV may have also affected the killing process. Oginsky et al. (1959) have shown that exposure of bacteria to long-wavelength UV in the presence of a psoralen derivative, 8-MOP (8-methoxypsoralen), resulted in cell killing that depended on both 8-MOP concentration and the influence of UV. It is not known whether the chlorine in the 3-position on the pyrone nucleus of chlorferon would affect the photocycloaddition products of psoralen type molecules to DNA via the 3,4-monoadducts as discussed by Ben-Hur and Song (1984).

Registry No. 2,4-$(OH)_2C_6H_4Ac$, 89-84-9; $(EtO)_2PSOH$, 2465-65-8; coumaphos, 56-72-4; chlorferon, 6174-86-3.

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