

influenced differently by cooking method depending on whether the samples were spiked or nonspiked. Naturally occurring residues of DDT were reduced significantly ($P < 0.01$) by the steritort process and by broiling with a concomitant increase in DDD as a result of dechlorination of DDT. These results are in agreement with earlier reports by Thronburg (1963), Langlois et al. (1964), Ott and Gunther (1964), Richey et al. (1967, 1969), Elkins et al. (1972), and Lane et al. (1978). Spiked samples, on the other hand, decreased in DDT content during the steritort process, but not during broiling. Moreover, there was no increase of DDD levels in spiked samples (low and high) when subjected to heat treatment. These findings may be a result of a greater tendency in spiked samples for further degradation of DDD to compounds which are not detected by electron capture gas chromatography.

Results for total residues also differed between spiked and nonspiked samples with respect to the 104 °C steritort process and broiling. There was no difference in total residue content between the two heat treatments with the ground beef containing naturally occurring residues, but there was a significant difference ($P < 0.05$) in the material which contained the spiked DDT when subjected to the steritort method and broiling. These data point up the necessity for studying samples containing residues from natural contamination such as feeding, rather than from artificially contaminated materials.

LITERATURE CITED

- Britten, W. A., Fairing, J. D., *J. Assoc. Off. Agric. Chem.* **33**, 599 (1950).
 Carter, R. H., Hubanks, P. E., Mann, H. D., Alexander, L. M., Schopmeyer, G. E., *Science* **107**, 347 (1948).

- Castro, C. E., *J. Am. Chem. Soc.* **86**, 2310 (1964).
 Deloach, H. K., Hemphill, D. D., *J. Assoc. Off. Anal. Chem.* **54**, 1352 (1971).
 Ecobichon, D. J., Saschenbucker, P. W., *Science* **156**, 663 (1967).
 Elkins, E. R., Farrow, R. P., Kin, E. S., *J. Agric. Food Chem.* **20**, 286 (1972).
 Farrow, R. P., Elkins, E. J., Jr., Cook, R. W., *J. Agric. Food Chem.* **14**, 430 (1966).
 Kallman, B. J., Andrews, A. K., *Science* **141**, 1050 (1963).
 Lane, L. G., Ammerman, G. R., Lane, R. H., *J. Food Sci.* **43**, 172 (1978).
 Langlois, B. E., Liska, B. J., Hill, D. L., *J. Milk Food Technol.* **27**, 264 (1964).
 Liska, B. J., Stemp, A. R., Stadelman, W. J., *Food Technol.* **21**, 435 (1967).
 Martin, W. D., Ph.D. Dissertation, Mississippi State University, Mississippi State, MS, 1974.
 McCaskey, T. A., Liska, B. J., *J. Dairy Sci.* **50**, 1991 (1967).
 Plimmer, J. R., Kearney, P. C., *J. Agric. Food Chem.* **16**, 594 (1968).
 Ott, D. E., Gunther, F. A., *J. Agric. Food Chem.* **12**, 239 (1964).
 Ralls, J. W., Cortes, A., *J. Food Sci.* **37**, 760 (1972).
 Ritchey, S. J., Young, R. W., Essary, E. O., *J. Food Sci.* **32**, 238 (1967).
 Ritchey, S. J., Young, R. W., Essary, E. O., *J. Food Sci.* **34**, 569 (1969).
 Snedecor, G. W., Cochran, W. G., "Statistical Methods", 6th ed, Iowa State University Press, Ames, Iowa, 1967.
 Stemp, A. R., Liska, B. J., *J. Dairy Sci.* **49**, 1006 (1966).
 Thornburg, W. W., in "Analytical Methods for Pesticides, Plant Growth Regulators and Food Additives", Vol. 1, Academic Press, New York, 1963, p. 540.
 Tressler, C. J., Jr., *J. Assoc. Off. Agric. Chem.* **30**, 140 (1947).

Received for review July 5, 1978. Accepted August 2, 1979.

Fate of Selected Fungicides in a Terrestrial Laboratory Ecosystem

Jay D. Gile* and James W. Gillett

The disposition of ¹⁴C-labeled pentachloronitrobenzene (PCNB), two of its analogues pentachlorophenol (PCP) and hexachlorobenzene (HCB), and captan was examined as seed-protectant coatings in a terrestrial microcosm chamber (TMC) in comparison to a reference compound, dieldrin (HEOD). The TMC contained a synthetic soil medium, agricultural crops, numerous invertebrates, and a gravid gray-tailed vole (*Microtus canicaudus*). Captan and PCP degraded more rapidly in soil and plants than did PCNB, which was degraded somewhat more quickly than HCB and HEOD. By 45 days postplanting, total soil residues (parent + metabolites + bound residues) had declined from a nominal 3 ppm to about 1 ppm for all chemicals but captan (0.26 ppm), while parent residues became undetectable for PCP and captan. Residues in invertebrates and the vole were low for all chemicals, but HCB and HEOD showed ecological magnification indices (EMs) in the vole of 17.7 and 2.1, respectively, as compared to 1.2 for PCNB. None of the chemicals adversely affected vole survival, although the PCNB-exposed vole had no surviving pups. Only HEOD greatly decreased cricket survival.

As part of a continuing effort to enhance man's knowledge of the fate and effects of chemicals introduced into the environment, the U.S. Environmental Protection Agency has embarked upon a program which uses simulated ecosystems as an intermediate test between bench and field studies. The data presented herein reflect the second in a series of studies in which representatives from broad categories of pesticides were examined in a system

developed at the U.S. EPA's Corvallis Environmental Research Laboratory (CERL). The system used in this study is almost identical with one described earlier (Gillett and Gile, 1975, 1976).

The chemicals examined were or are commonly used as fungicides: pentachloronitrobenzene (PCNB), two of its analogues [pentachlorophenol (PCP) and hexachlorobenzene (HCB)] and captan [*N*-(trichloromethylthio)-4-cyclohexene-1,2-dicarboximide]. These were compared with the insecticide dieldrin (HEOD; 1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4:5,8-endo,exo-dimethanonaphthalene) which serves as a reference compound in our system.

*Terrestrial Systems Division, Corvallis Environmental Research Laboratory, U.S. Environmental Protection Agency, Corvallis, Oregon 97330.

Originally the disposition of PCNB was of interest as a candidate substitute chemical (EPA, 1976) for ethylenebisthiourea fungicides, as was captan (EPA, 1975). Subsequently both PCNB and captan were placed on the EPA Rebuttable Presumption Against Registration List. PCP is one of the most widely used pesticides in the United States, as well as being a metabolite of both PCNB and HCB (Mehendale et al., 1975). While HCB is no longer registered for use, it is still a major industrial waste (Darr, 1978). Since either PCNB or captan may replace or be replaced by other fungicides, a comparison of the persistence and distribution of the two should prove valuable.

The major goal of this study was to evaluate the suitability of the microcosm chamber for examination of different classes of chemicals. The second objective was to determine the disposition of the various fungicides within the system and compare this information to the performance of our reference chemical (dieldrin). Finally a different method of application was used so that examination of the reference compound data from this and previous work may indicate the importance of route of exposure on chemical disposition.

METHODS

Microcosm. The terrestrial microcosm chamber (Gillett and Gile, 1976) utilized in this study consisted of glass box (1 m × 0.75 m × 0.61 m) with a Plexiglas lid. The chamber received 2500 foot-candles of light at the soil surface with a 16-h daily photoperiod. Temperatures ranged from 30.25 °C during the day to 18.25 °C at night. The air flow rate was increased from previous tests to 50 L/min, resulting in ten air changes per hour. The soil medium consisted of 10% (by weight) Jiffy Mix Plus, 45% 20-grit sea sand, and 45% illite clay.

Flora and Fauna. The plants consisted of alfalfa (*Medicago sativa*) and perennial ryegrass (*Lolium perenne*). Bacteriophagic nematodes (*Pristionchos inheriteiri*), earthworms (*Lumbricus* spp.), pillbugs (*Armadillarium* and *Porcellia* spp.), mealworm larvae (*Tenebrio molitor*), gray crickets (*Achetus domesticus*), and garden snails (*Helix pomata*) represented various invertebrate categories. A gravid gray-tailed vole (*Microtus canicaudus*) was added as the top level in the food web.

Radiolabeled Material. The [¹⁴C]dieldrin (labeled in positions 1, 2, 3, 4, and 10) was a technical grade (≥85% HEOD) supplied by Amersham. ¹⁴C-labeled PCNB, PCP, HCB, and captan were supplied by New England Nuclear (≥95% purity), with the first three having a ring-U label and captan being labeled in the -SCH₂Cl₃ group.

Experimental Procedure. The soil mixture was added to each chamber in three 6–8-cm layers with each layer being compacted overnight. The 75 earthworms and approximately 40 000 nematodes were added with the last layer. The air flow rate was established at 50 L/min and the 16-h light cycle initiated.

The labeled material was added to the terrarium as a seed coating in equal concentrations on 30 g of alfalfa and 20 g of ryegrass. The seeds were coated batchwise by tumbling with the chemical in a rotary flask using a carrier of 250 mg of starch/clay (1:1) and 1.0 mL of glycerine. An additional 2.0 g of starch was added to the alfalfa carrier to help binding on the seeds' waxy coating. In all, 50 μCi of ¹⁴C-labeled pesticide was diluted with reference standard grade chemical for a total of 50.0 mg of pesticide/TMC (0.5 lbs/acre or 0.64 kg/ha). TMC IV (control) was treated only with equivalent amount of carrier-coated seeds.

After scattering the seeds on the soil surface, they were covered with 1–2 cm of soil mixture and a fine layer of

Table I. Mass Balance ¹⁴C (10⁶ dpm)

	PCNB	PCP	HCB	cap- tan	HEOD
amount applied	48.9	49.0	48.1	48.6	48.9
amount recov.	37.6	37.1	39.5	16.4	48.7
% recov.	77	76	61	34	99.6
unaccounted dpm ^a	11.3	11.9	18.6	32.2	0.3

^a May reflect the volatile nature of the compounds and the conversion of the ¹⁴C-labeled material to ¹⁴CO₂, which was not detectable by the filtering apparatus.

washed sea sand. On day 19 after planting, 100 each of crickets, pillbugs, and *Tenebrio* as well as six snails were added to each terrarium. An additional 50 crickets and *Tenebrio* were added with the gravid vole on day 30. On days 34, 36, and 45 crickets and/or *Tenebrio* were added as a food supplement for the vole.

Daily censuses of visible animal species were conducted with weekly soil samples for nematode enumeration. Weekly samples of plants and crickets were taken for ¹⁴C analysis. Air filters were sampled on a daily basis, as were the air scrubber contents and condensate for the first week. Thereafter the scrubber contents and condensates were sampled on days 9, 12, 13, 20, 27, 34 and at termination of a TMC. Weekly ¹⁴C samples were included in ¹⁴C mass balance determinations.

At termination the macrobiota were removed from the TMC, counted, and frozen until analysis. A grid of fifty 10 × 15 cm units was created on the soil surface. From this grid 26 designated units were removed from the first 6-cm layer for residue analysis. This same pattern was applied to the second 6-cm layer. Sampling in soil levels 3 and 4 involved pooling of the units so that 13 and 6 samples were taken, respectively, from levels 3 and 4. After the soil samples were removed, all interior surfaces were cleaned with acetone and ethanol until free of radioactivity.

Analysis. All analysis for ¹⁴C was conducted by the fractionation procedures outlined earlier (Gile and Gillett, 1979), using a Packard Model 3385 Tri-Carb liquid scintillation spectrometer. When an organic extract of soil, plant, or animal tissue contained in excess of 10⁴ dpm, the sample was further analyzed by TLC on silica gel G plates (500 μm) developed by hexane/ether (1:1). Materials on the TLC plates were located by radioautography, removed manually, and counted. Due to low ¹⁴C content, none of the aqueous samples were analyzed by TLC.

RESULTS

Mass Balance. As shown in Table I the ability to recover ¹⁴C varied dramatically with the chemical under consideration. Values ranged from 34% for captan to 99% for dieldrin. Figure 1 illustrates the comparative distribution within each TMC. Figures 1e and 1f represent the variation in HEOD distribution with method of application; Figure 1e represents seed coating and Figure 1f foliar treatment from previous tests (Gile and Gillett, 1979). Recovery of HEOD from seed treatment (99%) greatly exceeded the average recovery from foliar application (83.8%).

Residue Distribution. Concentrations of ¹⁴C-labeled materials in the upper 6 cm of soil were approximately 0.9 ppm equivalents for all chemicals (PCNB, 0.87; PCP, 0.78; HCB, 0.93; HEOD, 1.03) except captan which was 0.26 ppm (Figure 2). Of the PCNB in the upper layer, 44% was as extractable parent. In excess of 50% of HCB and HEOD were present as extractable parent, while PCP and captan were present only as metabolite or bound residue. Vertical distribution of all chemicals showed some movement within the soil column. PCP, the most water-soluble chemical examined (2 mg/L), exhibited the

Table II. Total^a Pesticide Residues in Macroinvertebrates (ppm Fresh Weight)

species ^g	sample day ^b	chemical				
		PCNB	PCP	HCB	captan	HEOD ^d
cricket	28	0.20	3.87	0.20	0.52	0.35
	29					0.09
snail	termination ^c	0.61			0.91	
	adult					
adult	29	0.39	4.30	0.25	0.27	0.97
	36	0.47	1.50	0.30	0.46	3.62
	37				0.55	
	41				0.60	
juvenile	termination	0.30	1.16	0.43	0.47	2.94
	termination	1.02 ^e	<i>f</i>	0.12	0.06	
<i>Tenebrio</i>	termination	0.38	<i>f</i>	235.15 ^d	0.56	
pillbug	29					8.8
earthworm	termination	0.60	6.17	4.30	0.28	71.4
	termination				0.05	

^a Total residue includes extractable parent, metabolites, and bound residue as ¹⁴C equivalent of parent ($\mu\text{g/g}$ if fresh wt).

^b Number of days after planting. ^c Termination day for PCNB = 47, PCP = 41, HCB = 39, captan = 44, HEOD = 41.

^d Except for *Tenebrio* in HCB treatment, extractable parent material from any added pesticide was found only with HEOD.

^e Sample taken on day 36 instead of at termination. ^f No sample available. ^g Crickets, *Tenebrio* and pillbugs initially added on day 19 with subsequent additions of crickets and *Tenebrio* as food source for vole on an as-needed basis. Snails were added on day 20.

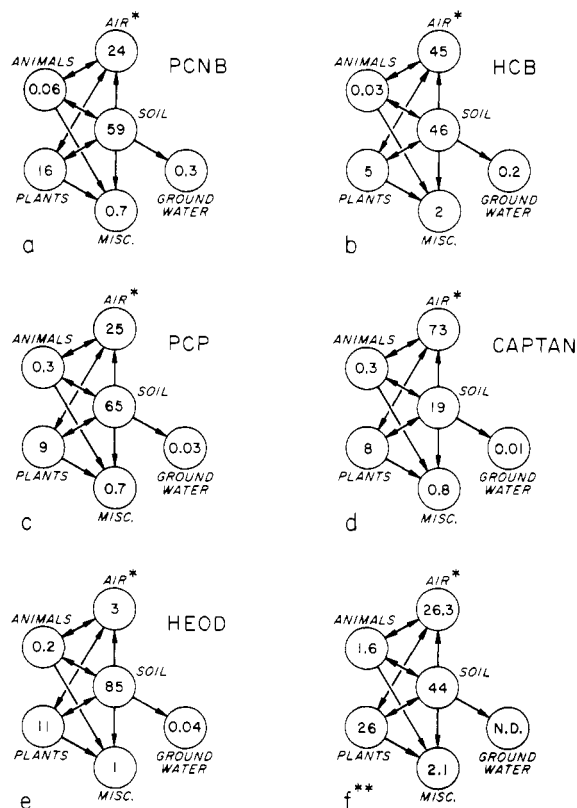


Figure 1. ¹⁴C mass balance (% of applied) in TMC (seed protectant coatings). (a) PCNB, (b) HCB, (c) PCP, (d) captan, (e) HEOD, (f) HEOD (foliar spray). Compartmental mass, volumes: soil, 100 kg; groundwater, 10–50 L. (*) Amount measured plus estimated loss as ¹⁴CO₂. (**) Average of five runs; Gile and Gillett (1978).

greatest movement followed by PCNB and captan (solubilities of ≤ 0.5 mg/L); the least soluble HCB and HEOD were relatively immobile. HEOD residue patterns from seed treatment were comparable to foliar application, although metabolites were more abundant from seed treatment.

Plants. Dieldrin accumulated in the plants to a much greater extent than any of the fungicides (Figure 3). Both HEOD and HCB were present mainly as extractable parent, whereas virtually all of the PCP or captan were

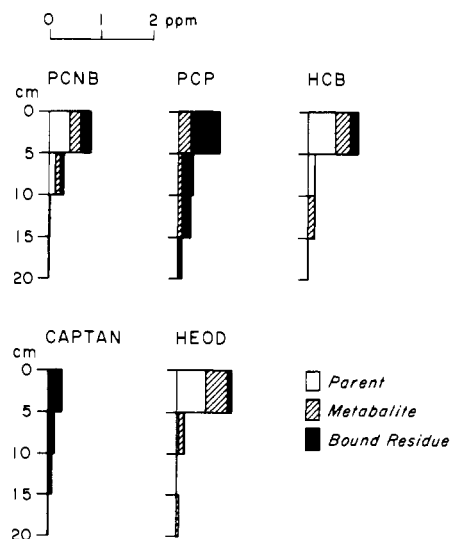


Figure 2. Vertical profile of terminal ¹⁴C distribution in soil from TMC treatment with seeds coated with ¹⁴C-labeled pesticides.

bound or as metabolites. Some extractable PCNB was present but the majority was either metabolite or bound. Temporal changes in residue levels in rye and alfalfa were erratic and generally reflected a decline with time (Figure 3). PCNB in rye was an exception which may be a result of a lower rate of uptake by the foliage. In general initial uptake rates for all chemicals were faster in alfalfa (Figure 3).

Invertebrates. The total pesticide residue levels in all invertebrate species remained relatively low (Table II). Exceptions to this are the *Tenebrio* in the HCB treatment and pillbugs in the dieldrin treatment. The high value in the *Tenebrio* is due solely to the presence of a questionably large amount (234.93 ppm) of extractable parent material. Extractable parent material was not present from any of the other fungicides, while free HEOD was largely responsible for residue levels in all invertebrates except crickets. The fungicide residues were comprised mainly of bound residues with some aqueous material. In general PCP metabolites and bound residues accumulated to a greater extent in all invertebrates except for the two previously mentioned instances. Poor recovery of crickets, juvenile snails, *Tenebrio*, and earthworms prevented

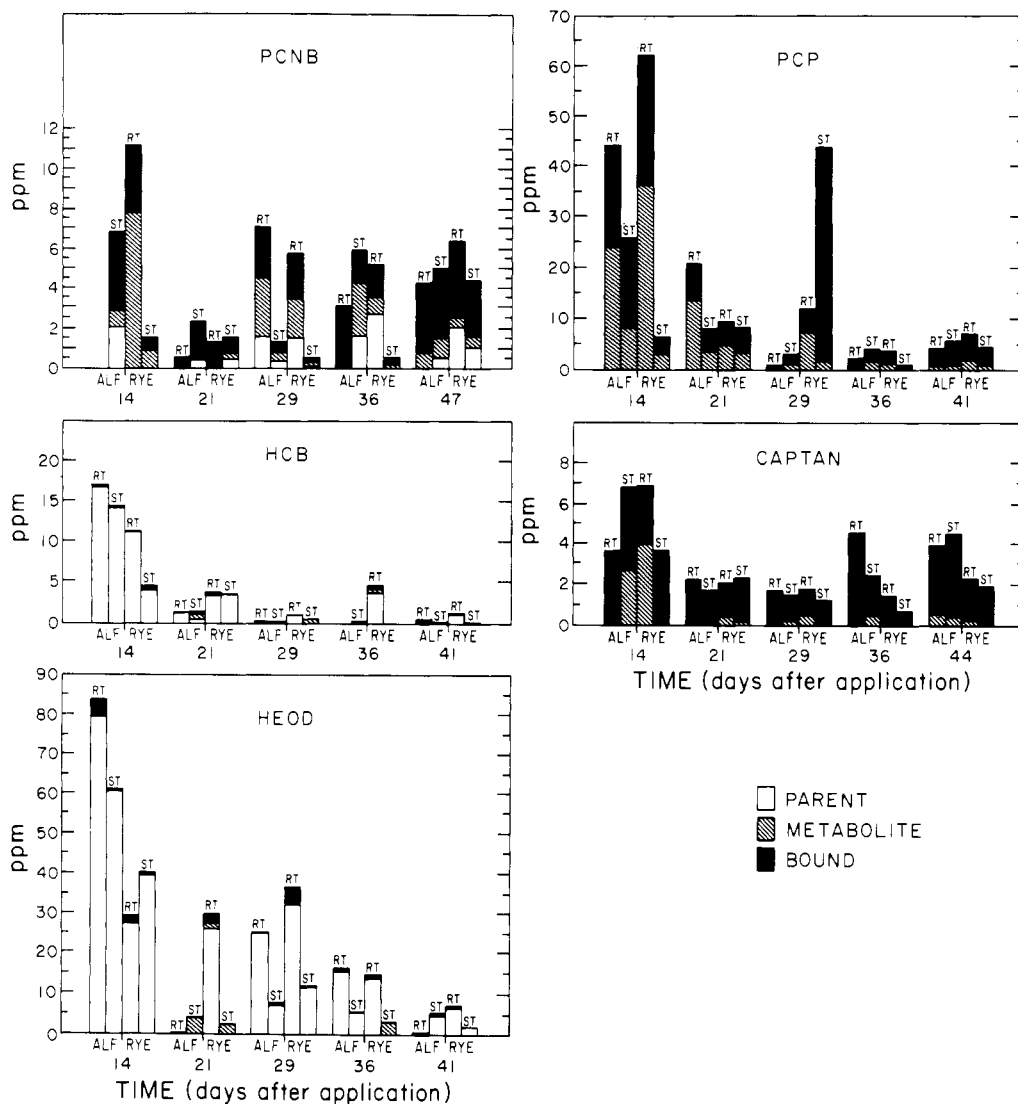


Figure 3. Residue patterns in alfalfa and ryegrass shoots and roots from TMC treatment with seeds coated with ^{14}C -labeled pesticides.

determination of the residue levels in these invertebrates for some chemicals.

Vole. The vole in the captan treatment died 8 days after introduction and was replaced with another vole which survived until termination. Total ^{14}C -labeled pesticide equivalent residue levels ranged from 0.87 ppm with the captan treatment to 23.7 ppm with HCB. Except for the PCP kidney sample, the liver accounted for all extractable parent material present in the vole in the fungicide treatments. Extractable HEOD was present in 50% of the tissues analyzed from the dieltrin TMC. Except for the HEOD treatment few tissues contained extractable metabolites. Bound residues accounted for a majority of the ^{14}C present except for HCB, where a high level of extractable parent in the liver overshadowed the other components. Of the tissues analyzed, the liver contained the highest residue levels for all chemicals. Overall HEOD levels were substantially lower than those in previous studies using a foliar application. Residue levels in the pups were substantially higher in the HEOD terrarium when compared to the fungicides or when compared to foliar application of HEOD.

Air. Figure 4 represents ^{14}C trapped on particulate air filters and gas scrubbers. Captan showed a rapid initial loss, while the loss rates for the other chemicals remain low and constant through day 34. At termination, 7×10^3 dpm of ^{14}C was still being trapped daily in the dieltrin

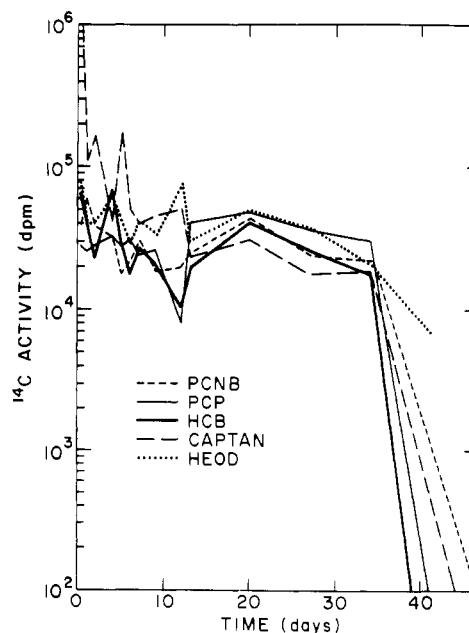


Figure 4. ^{14}C concentrations in air.

treatment while all other treatments exhibited 100 dpm on the daily air filter.

Table III. Physical Properties of Test Materials

chemical	solubility, mg/L	vapor pressure ^a	partition coeff. ^b
PCNB	0.44 ^c	5×10^{-5f} 11.3×10^{-5f}	16059
PCP	2.0 ^c	1.7×10^{-5e}	6400
HCB	0.02 ^d	1.09×10^{-5c}	13560
captan	<0.5 ^c	6×10^{-5g}	11
HEOD	0.05 ^e	7.78×10^{-7c}	4930

^a Vapor pressure for P, PCP and HCB reported at 20.25 °C; HEOD and captan at 25.25 °C; PCNB at 20.25 and 25.25 °C. ^b Metcalf (1978). ^c Spencer (1973). ^d Darr (1978). ^e Melnikov (1971). ^f EPA (1976). ^g EPA (1975).

Miscellaneous. Miscellaneous components of the system included the ground water or leachate and the various solvent and aqueous rinses acquired during cleanup of a terrarium. As might be anticipated with compounds of low solubility, very little ¹⁴C (0.01–0.3% of amount applied) was collected in the leachate. Cleanup rinses also exhibited very low ¹⁴C levels.

TLC. Examination of the TLC plates indicated the presence of one metabolite at 60 mm for PCNB and two for PCP at 115 mm and 60 mm in the soil. The *R_f* of the parent material for PCNB and PCP was 140 and 35 mm, respectively. No other metabolites were detected for the other chemicals in any sample. If present, metabolites could have been detected down to the 2% level of the amount applied on the plate.

DISCUSSION

Mass Balance. The recovery of [¹⁴C]HEOD was substantially greater with seed coating than with previous foliar treatments (Gile and Gillett, 1979). Presumably this is due to the insoluble nature of dieldrin and its low vapor pressure, 7.78×10^{-7} mm, at 25.25 °C (Spencer, 1973). While recovery of PCNB and PCP was within an acceptable range, recoveries of HCB and captan were unexpectedly low. Recovery of HCB should have been comparable to dieldrin based on low solubility, high octanol/H₂O partition coefficient and relatively low vapor pressure (Table III). While Figure 4 reflects only ¹⁴C actually detected, the authors believe that there may have been substantial losses of all chemicals except HEOD, as a result of chromatography through the air filters.

Recovery of captan was complicated by placing the ¹⁴C label in the -SCH₂Cl₃ group of captan which is broken down to tetrahydrophthalimide and CO₂ very rapidly (Pack, 1974). An alternate labeling site, the tetrahydrophthalimide ring, would be required to demonstrate the fate of the remainder of the molecule.

Distribution. Except for captan the majority of the pesticide residues was recovered from the soil as expected with a seed coating or soil treatment, given the propensity of soil to absorb nonpolar organic materials.

Table III presents selected physical properties of these chemicals which could affect their distribution. From these physical properties one might surmise that PCP would be the most widely distributed, followed by captan and PCNB, with HCB and HEOD being equivalent. PCNB and PCP were practically identical in both distribution and mass balance (Table I); again the distribution of captan was confounded by the labeling site. Based on solubility HCB should have remained in the soil as did HEOD. HEOD behaved as anticipated; it should also be noted that the method of application dramatically affected the distribution of HEOD (Figure 1).

It is evident from Figure 1 that the chemicals did not behave in the TMC exactly as one might predict from

Table IV. Indices of Pesticide Disposition in the Vole

item	chemical				
	PCNB	PCP ^f	HCB ^g	captan ^e	HEOD
EM ^a	1.2	0	17.7	0 ^f	2.1
BI ^b	0.6	0	0.6	19.6	2.0
BRI ^c	0.8	0	0.4	8.35	1.4
TDI ^d	1.4	0	1.0	28.0	3.4

^aEM = (HEOD) whole body/(HEOD) soil at termination. ^bBI = (metabolites) whole body/(HEOD) whole body. ^cBRI = ¹⁴C combusted in residue/¹⁴C equivalent of HEOD, whole body. ^dTDI = total (metabolized and bound residue)/(HEOD) whole body. ^eVole a only; no extractable parent evident in vole b. ^fNo parent material present in soil or vole. ^gHigher EM due to high residue levels in liver.

physical data. Distribution is not a function of one chemical or physical property but the result of the interaction of all the chemical's physical and chemical attributes.

Indices of Performance. The indices of performance examined were ecological magnification (EM) index, biodegradation index (BI), bound residue index (BRI), and total degradability index (TDI). Due to lack of parent material in the invertebrates, as well as poor recovery of the organisms, these indices could be calculated for the vole only (Table IV). Because the soil is the major repository, whether treatment is as a seed coating or foliar application (Gile and Gillett, 1979), the concentration in soil is used as the basis for the EM calculations. Except for HCB all compounds demonstrated little or no ecological magnification. The relatively higher EM for HCB is due to a higher level of extractable parent material in the liver than with the other chemicals. The authors detected higher EM's (av 59.5) for HEOD with foliar application in previous studies (Gile and Gillett, 1979), suggesting that the method of application affects availability up through the food chain. From the calculated BI's and TDI's it appears that captan was more readily degraded; however, the indices for PCP could not be calculated due to a lack of parent material which by itself suggests an even higher degree of degradation. Captan exhibited the highest BRI value in the vole; this may be directly related to the preponderance of bound residues of captan in both soil and plant material.

It is important to note that while these EM values appear low when compared to those determined in aquatic systems, if the binding capacity of soil is considered then the values for HEOD, PCNB, and especially HCB represent considerable ecological magnification.

Metabolism. The metabolite of PCNB found in the soil is probably pentachloroaniline (PCA). The metabolites of PCP are presently unidentified.

CONCLUSIONS

The following conclusions may be derived from the data presented. (1) Method of application affects both mass balance of ¹⁴C and distribution of the material which can be generally well accounted for within the TMC. (2) Preliminary determinations of chemical fate based on physical properties may not coincide with actual distribution of the TMC for reasons not yet determined. (3) Under the described test conditions, none of the chemicals appeared to adversely affect the simulated ecosystem. The greatest observed impact was that of HEOD, an insecticide, on cricket mortality.

ACKNOWLEDGMENT

The authors express their appreciation to James Collins, William Albrecht, Wesley Lashbrook, Wayne Clark, Loren

Russel, and Howard Mercier for their valuable assistance in the operation of the TMC's and sample and data analysis. The voles were provided by L. G. Forslund, of the General Science Department of Oregon State University.

LITERATURE CITED

- Darr, J., "Hexachlorobenzene", Draft Phase I Report, Assessment Division, Office of Testing and Evaluation, Office of Toxic Substances, U.S. Environmental Protection Agency, Washington, DC, Sept 15, 1978.
- Gile, J. D., Gillett, J. W., *Arch. Environ. Contam. Toxicol.* 8, 107-124 (1979).
- Gillett, J. W., Gile, J. D., "Proceedings of Substitute Chemicals Program—The First Year of Progress", Vol. 3, Fredricksburg, VA, July 30, 1975, pp 5-28.
- Gillett, J. W., Gile, J. D., *Int. J. Environ. Stud.* 10, 15-22 (1976).
- Mehendale, H., Fields, M., Mathews, H., *J. Agric. Food Chem.* 23(2), 261-265 (1975).
- Melnikov, N. N., "Chemistry of Pesticides", Springer-Verlag, New York, 1971.
- Metcalf, R. L., University of Illinois, personal communication of unpublished data, 1978.
- Pack, D. E., "The Soil Metabolism of Carbonyl ¹⁴C-Captan", Chevron Chemical Co., San Francisco, CA, 1974, File No. 773.21 (Cited in U.S. EPA, 1975).
- Spencer, E. Y., "Guide to Chemicals Used in Crop Protection", Agriculture Canada, Publ. 1093, 1973.
- U.S. Environmental Protection Agency, "Initial Scientific and Minieconomic Review of Captan", EPA-540/1-75-012, Office of Pesticide Programs, Washington, DC, 1975.
- U.S. Environmental Protection Agency, "Initial Scientific and Minieconomic Review of PCNB", EPA-540/1-75-016, Office of Pesticide Programs, Washington, DC, 1976.

Received for review May 14, 1979. Accepted August 13, 1979. The use of trade names of materials is intended solely for the reader's benefit and does not impute endorsement of that product to the exclusion of others serving equally well. The Environmental Protection Agency makes no such endorsement or warranty of performance.

Identification of Sensitized Photooxidation Products of Bromacil in Water

Aureliu J. Acher* and Ezra Dunkelblum

The dye sensitized photolysis of aerated aqueous solutions of bromacil (5-bromo-3-*sec*-butyl-6-methyluracil) exposed to solar irradiation has been investigated. The major product, formed in 83% yield, has been isolated and identified as a mixture of diastereoisomers of 3-*sec*-butyl-5-acetyl-5-hydroxyhydantoin. The techniques used were a combination of gas chromatography-mass spectrometry, proton and carbon-13 nuclear magnetic resonance, and infrared spectroscopy. In addition, silylation, reduction, and oxidation of the photooxidation product were carried out for further confirmation of the proposed structure.

The fate of agricultural chemicals in the environment is of great interest and importance. The extensive use of pesticides is a serious ecological problem. Bromacil (5-bromo-3-*sec*-butyl-6-methyluracil; I) is one of the most important herbicides used to control a wide range of grasses and broadleaf weeds (Martin, 1972). The photodecomposition of I has been studied in order to evaluate its fate in the environment. The UV-light photodecomposition of I was investigated in aqueous solutions (Kearney et al., 1969) and in solid films (Jordan et al., 1965), however, without the identification of the photoreaction products. Experiments done in simulated natural conditions with solar radiation (Moilanen and Crosby, 1974) yielded only 2.2% of a single dealkylated photoproduct (5-bromo-6-methyluracil). Addition of dye photosensitizers to aerated aqueous solutions of I leads to a quantitative and fast sunlight photochemical reaction (Acher and Saltzman, 1979).

Identification of the photoreaction products of I is important not only from the ecological point of view, but is also of photobiological interest since uracil derivatives are included in DNA and RNA. This accounts for some aspects of the photobiology of viruses and bacteria which

contain these genetic materials (Wang, 1976).

This paper describes the formation and the identification of the sensitized photooxidation products of I in water.

EXPERIMENTAL SECTION

Materials. Bromacil (5-bromo-3-*sec*-butyl-6-methyluracil; I), provided by Agan Ltd., Israel, was chromatographically pure, mp 158-159 °C. Methylene Blue (MB) (BDH, No. 26132 Q) was used as a 0.5% water solution.

Instrumentation. Combined gas chromatography-mass spectrometry (GC-MS) was performed on a Varian 2740 GC, equipped with a flame ionization detector, 2 m × 3 mm glass column with 3% OV-17 on Gas-Chrom Q 80-100 mesh coupled to a DuPont 490 B low-resolution (EI) mass spectrometer. The elution started at 100 °C; the program rate was 6 °C/min up to 220 °C; the carrier gas was helium.

The chemical ionization mass spectrum (CI) was recorded on a similar spectrometer with isobutane as reagent gas. The sample was introduced through the direct probe. High-resolution mass spectra were recorded on a Varian MAT-731. The IR spectra were run on a Perkin Elmer 257 instrument in KBr pellets or dichloromethane 1% solutions. The ¹H-NMR spectra were carried out on a Varian A60 or on a Bruker WH-270 MHz spectrometer, and the ¹³C-NMR spectra were run on a Varian FT-80A (20 MHz) spectrometer. All spectra are reported in δ from Me₄Si (internal standard). A Varian vis-UV spectrophotometer, Techtron, Model 635, was used for I deter-

Division of Soil Residues Chemistry, Institute of Soils and Water, ARO, The Volcani Center, Bet Dagan, Israel (A.J.A.), and the Division of Organic Agricultural Chemistry, Institute of Plant Protection, ARO, The Volcani Center, Bet Dagan, Israel (E.D.).