formation and could be isolated for future investigations.

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Supplementary Material Available: A listing of the observed and calculated structure factor amplitudes (7 pages). Ordering information is given on any current masthead page.

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Fate and Impact of Wood Preservatives in a Terrestrial Microcosm

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The transport and effects of ^{14}C-labeled wood preservatives [creosote with labeled phenanthrene or acenaphthene, pentachlorophenol, and bis(tri-*n*-butyltin) oxide] impregnated in wood posts were examined in a terrestrial microcosm chamber (TMC-II) in comparison to a reference compound, the insecticide dieldrin. The TMC-II contained a Willamette Valley topsoil, ryegrass, invertebrates, and a gravid gray-tailed vole (*Microtus canicaudus*). Approximately 2.5 months after introduction of the posts, 95% of the chemicals remained in the posts. Of the material released into the ecosystem, most remained in the upper soil layer immediately surrounding the posts. Concentrations in plants ranged from 0.7 ppm for dieldrin to 8.8 ppm for phenanthrene. Residue accumulation by the invertebrates was highly variable. Of the chemicals tested, creosote accumulated in the vole to the greatest extent (e.g., whole body concentrations of 7.2 and 37.0 ppm for phenanthrene and acenaphthene, respectively). Only dieldrin exhibited any acute toxic effects (e.g., cricket survival).

The preservation of wood from fungal and insect attack is a major function of pest control. Chemical preservatives can extend the useful life of wood by decades, thereby reducing the demand on wood production. Although wood is a renewable resource, the limitations on resource renewal make it necessary to protect our timber supply. However, there are problems associated with each of the wood preservatives. Creosote is a potential carcinogen included in a Rebuttable Presumption Against Reregistration (*Fed. Regist.*, 1978). Practically nothing is known about the fate of creosote in the environment resulting from treated telephone poles, bridge structures, railroad ties, and other wooden materials. Aquatic studies on pentachlorophenol indicate there may be environmental damage caused by the parent material and congeners in the technical product. Bis(tri-*n*-butyltin) oxide, which is not registered as a wood preservative in the United States, is used as such mostly in Europe on above-ground structures. It is quite toxic to fish, but little is known of its terrestrial fate and effects. Chlordane and heptachlor (cyclodiene insecticides) have been banned except for structural uses, but even this limited usage is under dispute.

Wood preservation represents the extreme of controlled release of a chemical. Barring deterioration by pests, accident, or storm, a wood structure is expected to have utility for decades. Wood preservative agents act as toxicants, feeding inhibitors, or growth inhibitors for boring animals (i.e., termites, carpenter ants, woodpeckers and wood-rotting fungi). Chemical release is not desirable in this type of application. Adverse effects can be anticipated from exposure to these chemicals if they are released into the environment. However, the characteristically slow release rate of wood preservatives makes the evaluation of that release rate and potential impacts extremely difficult. The terrestrial microcosm chamber (TMC) is a

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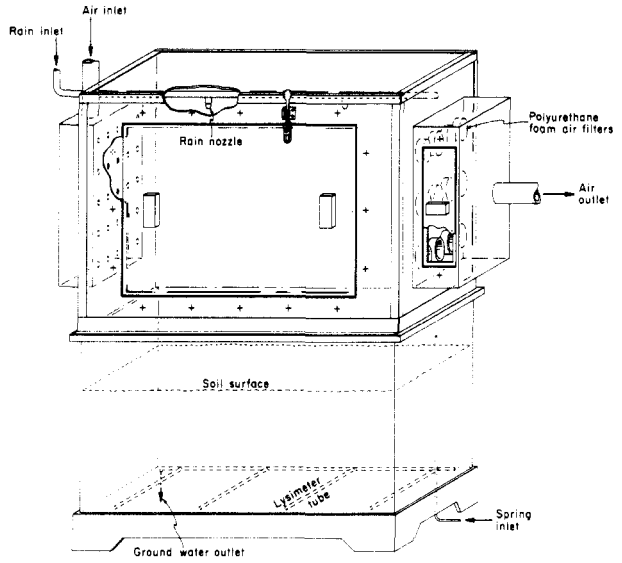

Figure 1. Schematic of terrestrial microcosm chamber (TMC-II). The bottom section is welded high-density linear polyethylene fitted with porous clay lysimeter tubes, under vacuum to a collection reservoir. The top part of the chamber (which may be removed for cleaning, etc.) has Pyrex glass end panels drilled with 20 2.5-cm holes (inlet) or 12 7.5-cm holes (outlet) with Lucite air plenums cemented to them, a UV-transparent Pyrex top, and Pyrex side doors held in place by clamps mounted on Lucite side panels, all held in an aluminum L-channel frame. Doors are sealed with a neoprene gasket; the weight of the top is self-sealing between the top and bottom chambers. The overhead water system (individually controlled) is constructed of acrylic tubing fitted with stainless steel spray nozzles.

system which may permit evaluation of that release over a shorter time span.

The TMC (Gillett and Gile, 1976) was used to study the fate and effects of a variety of pesticides (Gile and Gillett, 1979a,b; Gile et al., 1980). Following a critical review of current terrestrial microcosm technology (Gillett and Witt, 1979), the TMC was redesigned to be more realistic by providing increased airflows, greater photolytic capability, and increased soil depth. Preliminary testing of the TMC-II system indicated improved performance (e.g., greater plant growth, invertebrate survival, etc.). Therefore, we conducted an experiment to evaluate the effects and fate of the following selected chemicals used in wood preservation: (1) dieldrin (HEOD; 1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-*endo*,*exo*-dimethanonaphthalene), used as the positive control in previous TMC studies (Gile and Gillett, 1979a,b) and representing the closely related cyclodiene insecticides chlordane and heptachlor for the purposes of these tests; (2) creosote coal tar distillate [phenanthrene (21%) and acenaphthene (9%)]; (3) pentachlorophenol (PCP), a common household and commercial wood preservative; (4) bis(tri-*n*-butyltin) oxide (TBTO).

EXPERIMENTAL SECTION

Microcosm. The TMC-II (Figure 1) has been modified from the original (Gillett and Gile, 1976) by increasing the soil depth to 40 cm, increasing the headspace to 0.85 m, using UV-transmitting glass for the top, and utilizing the polyurethane from filter system of Beall et al. (1976) for trapping volatiles in the air. Each TMC-II receives about 38 000 lm/m^2 (3500 ft-c) at the soil surface during a 16-h-day/8-h-night cycle from a single 1000-W Sylvania Metalarc lamp. The airflow through the chamber was approximately 300 L/min, resulting in 35 air changes/h. Air temperatures ranged from a high of 21 ± 1 °C (day) to 15 ± 1 °C (night) with soil temperatures between 15 and 18 °C, depending upon depth.

The soil used in this experiment is a typical Willamette Valley topsoil (2.2% organic carbon; pH 6.3; cation-exchange capacity 17%). Perennial ryegrass (*Lolium perenne* L.) was planted as the primary vegetation. Invertebrates were represented by indigenous soil microorganisms, earthworms (*Lumbricus* spp.), pill bugs (*Armadillarium* and *Porcellia* spp.), mealworm larvae (*Tenebrio molitar*), gray crickets (*Acheta domesticus*), and garden snails (*Helix pomata* L.). A gravid female gray-tailed vole (*Microtus canicaudus*) was the only vertebrate included.

Chemicals. The [^{14}C]dieldrin was a technical grade (≥85%) purchased from Amersham/Searle (Arlington Heights, IL), ^{14}C-labeled PCP (≥95% purity) was purchased from New England Nuclear (Boston, MA), and ^{14}C-labeled TBTO, phenanthrene, and acenaphthene (≥95% purity) were purchased from Dynapol (Palo Alto, CA). Commercially available creosote was supplied by Koppers, and the commercial Penta (5% active ingredient) was a Weldwood product purchased locally. Scintillation-grade toluene was purchased from Mallinckrodt.

Preparation of Wood Posts. Ponderosa pine blocks supplied by the Forest Research Laboratory of Oregon State University were selected for treatment, since they were readily available, of known quality (heart wood), and frequently used by Oregon State University in wood preservative experiments. (Initially the pine blocks were treated by using heat and pressure to simulate in a very general manner the treatment practice normally used by industry. Such an application using these blocks resulted in a chemical loading far in excess of the desired 120–160 mg/cm^3. Although the treatment process can certainly impact chemical dispersal, we felt that achieving a more realistic loading of the test chemical was of greater importance. Therefore, the treatment process was modified as described to achieve the desired chemical concentration). Seven blocks (3.3 × 2.6 × 14 cm) were treated with each chemical to saturate them to a level of about 120–160 mg/cm^3 (8–10 lb/ft^3), determined gravimetrically, containing approximately 5 × 10^7 dpm of ^{14}C. Each chamber received a designated chemical on four blocks, driven halfway into the center of each quadrant, containing one of the following. (1) [^{14}C]Phenanthrene in Commercial Creosote (Koppers). One hundred microcuries of [^{14}C]-phenanthrene (chemical purity 99%) was mixed with 1 L of coal tar creosote at 100 °C for 1 h and then placed in a beaker with the blocks and heated for 1 h. The blocks were removed and dried overnight. (2) [^{14}C]Acenaphthene in Commercial Creosote. One hundred microcuries of [^{14}C]acenaphthene (chemical purity 99%) was mixed with 1 L of coal tar creosote and handled the same as phenanthrene. (3) [^{14}C]Pentachlorophenol in Commercial Penta (PCP, Weldwood). One hundred fifty-four microcuries of [^{14}C]pentachlorophenol was purified to 90% (by TLC) and added to 1 L of commercial 5% Penta solution. The blocks were placed in a 1500-mL beaker in a desiccator under modest vacuum (about 4 mmHg). After 2 h, the treatment solution was poured over the blocks which were left at atmospheric pressure for 1 h. If the blocks showed excess loading after drying, they were returned to the desiccator until the loading was reduced to 170–220 mg/cm^3. (4) Bis(tri-*n*-butyltin) Oxide (TBTO; Alpha Products). One hundred thirteen microcuries of [^{14}C]-bis(tri-*n*-butyltin) oxide (chemical purity 99%) was combined with 50 g of bis(tri-*n*-butyltin)oxide (chemical purity 97%) and toluene to make 1 L of solution. The blocks were treated in the same manner as pentachlorophenol.

Table I. Chemical and ^{14}C Loading in Posts

chemical	chemical loading, mg/cm^3 [a]	^{14}C loading, 10^6 dpm[a]	TMC-II net loss, %		% ^{14}C recovered from TMC-II ecosystem
			gravimetric[b]	radiometric[c]	
HEOD	160 [12][d]	4.8 [4]	1.1 [9.6]	31 [30]	5.7
phenanthrene	135 [12]	3.6 [5]	(1.3)[e] [9.8]	18 [100]	5.0
acenaphthene	151 [5]	3.9 [5]	4.8 [7.2]	45 [18]	6.5
PCP	214 [12]	9.2 [11]	1.5 [14.5]	17 [54]	6.3
TBTO	167 [2]	6.0 [12]	(1.6) [4.1]	4 [100]	4.0

[a] Values represent $\bar{x}$ of four posts from TMC-II. [b] Loss determined gravimetrically and corrected for storage loss. [c] Loss as determined by ^{14}C analysis of three posts from each TMC-II. [d] Coefficient of variation in brackets. [e] Net gain in parentheses.

(5) Dieldrin (HEOD). Ninety-two microcuries of [^{14}C]-dieldrin (94% HEOD) was combined with 50 g of technical-grade dieldrin (85% HEOD; Shell Development Co.) and toluene in 1 L of solution, and the blocks were treated with this solution in the same manner as Penta (above). (6) Control. The blocks were subjected to solvent (toluene) and desiccation.

Of the three blocks per chemical remaining after treatment, one was analyzed immediately to verify chemical concentration, while the remaining two were frozen until completion of the study to check for chemical loss not due to exposure within the system. The method of analysis for all posts was the same. Each post was individually pulverized and extracted in a Soxhlet extraction apparatus with hexane for 24 h. A sample of the residue was then combusted in a sample oxidizer to collect $^{14}CO_2$.

Experimental Procedures. The soil was added to each of the six chambers in two equal portions to a total depth of 40 cm, with 6 L of water and a 24-h compaction period following each addition. At this point, the airflow was started and adjusted to 300 L/min followed by initiation of the 16-h light cycle and air temperature profile. After the systems had acclimated for 72 h, air and soil temperature and moisture were monitored on a daily basis throughout the remainder of the study. Then 40 g of ryegrass seed was scattered over the soil surface and covered with approximately 1 cm of soil. Water was added to each TMC-II via an overhead sprinkler system 3 times a week (12 L total/week). When the ryegrass was approximately 10–15 cm high, 50 earthworms were added to each TMC. After 1 week, 50 each of pill bugs, *Tenebrio* larvae, and crickets plus six snails were added to each TMC. Subsequently crickets were added as numbers declined to less than 10/chamber as a test of bioactivity (Gillett et al., 1980). Twenty-five days after planting the ryegrass, four treated posts were added to each TMC as previously described. Air filters were sampled daily for the first 10 days after treatment and at 48–72 h intervals thereafter. An animal census was conducted daily. Samples of grass and snails were collected biweekly; groundwater was sampled weakly. Cricket samples were collected when available. The vole was not added until day 54 after treatment to increase the longevity of the systems. All chambers were terminated 19–26 days later.

At termination, the macrofauna were enumerated, weighed, and frozen for analysis, and plant material was removed, weighed, separated into roots and shoots, and frozen. The vole nest, tunnels, and paths were diagramed, and the adult females were dissected into brain, gastrointestinal tract (including viscera except heart and lungs), and carcass. Pups were frozen whole.

The top (10-cm) layer of soil was removed by quadrant around each post in an annular square as follows: 0–5 cm from post, 5–10, and ≥10. The balance of the soil was removed in three 10-cm layers. Each sample was homogenized and (five) 100-g subsamples removed for analysis. Following removal of all soil samples, the interior surfaces of each TMC were cleaned with acetone and ethanol to remove residual radioactivity.

Analysis. Water samples were extracted in a separatory funnel with acetone; soil samples were extracted by blending with hexane–2-propanol (3:2). Plant tissue was freeze-dried (−20 °C), pulverized in a ball mill, and then combusted on a Packard sample oxidizer. Invertebrates were extracted by blending with acetone, and animal tissue was extracted by blending with hexane–2-propanol and then acetone. Air filters were extracted in hexane in a Soxhlet extractor for 24 h. Extract was separated from sample residue by using a Büchner funnel under vacuum. Following extraction, all sample residues (except water) were combusted on a Packard sample oxidizer. All extracts and residues were analyzed for ^{14}C with a Packard Tri-Carb liquid scintillation spectrometer by using commercially available counting cocktails. The ^{14}C activity of any sample was assumed to be the sum of the activities for all extracts plus combusted residue.

If an organic extract of soil, plant, or animal tissue contained in excess of 10^3 dpm, the sample was further analyzed by thin-layer chromatography (TLC) using silica gel G plates and an ether–hexane (1:1) solvent. Autoradiography was used to ascertain the presence of metabolites and quantify residual parent material. In addition, creosote samples were analyzed for phenanthrene and acenaphthene by high-performance liquid chromatography (HPLC) on a Varian 5000 with a Vari-Chrom detector operating at 290 nm. A Micro-Pak CN-10 column was used with organic extracts, whereas a Micro-Pak MCH-10 was used with aqueous solutions. Dieldrin was quantified by gas chromatography using a Varian 2600 (^3H/scandium electron capture detector) with a 6-ft glass column of Chromosorb W coated with 6% SP2401 and 4% SP30 operating at 200 °C. In some instances, metabolites could be tentatively identified by cochromatography with analytical standards, but resource limitations prevented more extensive studies.

RESULTS

Residue Distribution. Estimation of the mass of material proved difficult, since the wood preservatives were distributed nonhomogeneously over the length of the wood blocks and there were definite variations between aboveground and below-ground portions, depending upon the chemical. In addition, the low specific activity of the treatment solutions (average 4×10^3 dpm/mg) further complicated analysis. Table I compares the estimated residues and ^{14}C in the treated blocks at the start of the experiment, with losses at the end of the experiment determined both gravimetrically and radiometrically. Estimated export (Table I) from the blocks (except TBTO and HEOD) is within the experimental error of determi-

Table II. Residue Profile in Upper Soil Layer (ppm)[a,b]

chemical	distance from post, cm[c] 0-5	5-10	≥10	wt-av concn for top layer[d]
HEOD	1.46 [1.79]	0.32 [0.24]	0.13 [0.07]	0.40
phenanthrene	0.73 [0.74]	ND[e]	0.69 [0.83]	0.60
acenaphthene	10.40 [9.50]	1.33 [2.0]	0.58 [0.81]	1.19
PCP	5.36 [3.14]	1.23 [0.79]	0.93 [0.79]	1.22
TBTO	4.35 [2.74]	0.08 [0.08]	0.61 [0.95]	0.74

[a] HEOD analysis via GC; all others are total ^{14}C equivalents as determined via liquid scintillation. [b] Average of four quadrants; standard deviation is in brackets. [c] A 10-cm square (10 cm deep) was removed after removal of the post. The second segment was 20 cm on a side and the third represents the rest of the quadrant (37 × 50 cm). [d] Top layer = 10 cm in depth. None was detected at 10 cm. [e] Detection limit = 0.02 ppm equiv.

Table III. ^{14}C-Labeled Residues in Macroinvertebrates (ppm)

species[b,c]	sample[a] day	chemical HEOD	phenanthrene	acenaphthene	PCP	TBTO
cricket	2	0.63	–[d]	–	–	–
	35	11.79 (2)	0.74 (5)	–	0.78 (1)	1.28 (1)
	72-75	–	–	5.92 (5)	1.16 (9)	4.04 (2)
	84	2.02 (5)	1.22 (7)	–	–	–
snail	35	7.53	–	–	–	–
	37	0.79	5.65	11.2	1.56	4.94
	72-75	–	–	–	2.71	4.01
	82	–	3.27	–	–	–
pill bugs	64	–	–	–	2.14	–
	72-75	–	–	0.99	–	5.14
	82-84	1.05	1.72	–	–	–
Tenebrio	72-75	–	–	9.01	ND[e]	ND
	82-84	30.5	–	–	–	–
worm	72-75	–	–	71.90	7.67	7.87
	82-84	3.70	18.30	–	–	–

[a] Posttreatment exposure time except for crickets (in parentheses). [b] Snails, pill bugs, *Tenebrio*, and earthworms were added prior to treatment; crickets were added prior to treatment and thereafter as part of a bioassay experiment. [c] Residue values reflect a composite sample of all invertebrates collected for any given chemical on a particular sample day. [d] No sample available. [e] Detection limit = 0.25 ppm equiv.

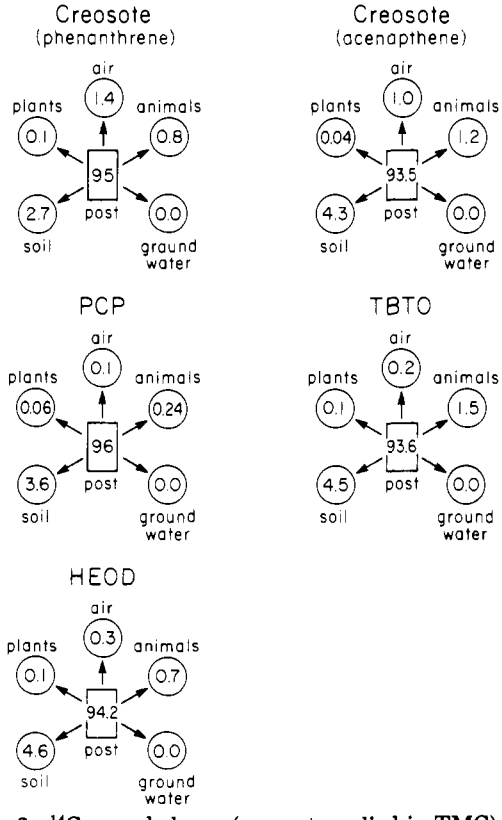

Figure 2. ^{14}C mass balance (percent applied in TMC).

nation of loadings, but use of mean values permits adequate accounting of the overall distribution (shown in Figure 2). The substantial difference in the initial and final loading with HEOD and TBTO reflects a loss of the toluene solvent and not the test chemical, as is evident from the ^{14}C analysis of the posts (Table I). Even under refrigeration, the HEOD and TBTO blocks lost most of the toluene. Of the materials leaving the blocks, the majority was retained in the soil. Crickets were eliminated from mass balance considerations, since some were added to the system after treatment.

Soil. The creosote treatment labeled with [^{14}C]-acenaphthene exhibited the highest concentration in area immediately surrounding the posts, followed by PCP, dieldrin, TBTO, and the ^{14}C-labeled phenanthrene/creosote treatment (Table II). All of the chemicals were detected in the ≥10-cm zone from the post, although the dieldrin concentration was substantially lower. No ^{14}C-labeled materials were detected below the first 10-cm layer. While creosote materials ([^{14}C]acenaphthene) exhibited the highest single concentration, PCP materials produced the highest overall average soil concentration.

Plants. Very little of any of the chemicals was detected in plant material (Figure 2). Terminal residue concentrations in plant tissue were estimated at 0.7 ppm for dieldrin, 8.8 ppm for phenanthrene, 2.7 ppm for acenaphthene, 3.5 ppm for PCP, and 4.2 ppm for TBTO.

Animals. The most striking feature of chemical distribution among the invertebrate species is the relationship to habitat. The crickets and snails residing above ground and relying heavily on vegetation as a food source generally exhibited lower levels of all chemicals than the soil-dwelling and litter-feeding species (pill bugs, *Tenebrio*, and earthworms). Of the three wood preservatives, labeled materials

Table IV. Residue Concentrations in Vole[a] as ^{14}C Equivalents (ppm)

sample	chemical				
	HEOD	phenanthrene	acenaphthene	PCP	TBTO[e]
vole whole body[b]	3.68	7.20	37.00	3.20	1.94
vole brain	2.23	7.79	ND[f]	1.23	0.45
vole GI tract	13.60	8.24	55.40	6.60	2.03
vole feces	c	87.40	123.80	46.30	4.61
vole pups	d	d	10.32	4.53	3.00

[a] Data represent one vole per chemical, except for pups, which were a composite sample of all individuals per chemical. [b] Carcass plus brain and GI tract. [c] No feces sample available for collection. [d] No pups—female not pregnant. [e] ^{14}C from labeled TBTO was present entirely as bound residue. [f] Detection limits = 0.05 ppm equiv.

from creosote were accumulated to the greatest degree by all the invertebrates except pill bugs (Table III). Dieldrin showed the highest accumulation in crickets and *Tenebrio*.

Creosote was accumulated to a much greater extent in the vole than any of the other test chemicals. The high concentration from the [^{14}C]acenaphthene/creosote treatment is a result of the high level of bound residues detected in the GI tract (Table IV). While this may be considered as material not accumulated, the high concentration of ^{14}C in the vole brain from the phenanthrene/creosote treatment does indicate the possibility of substantial accumulation of creosote into the animal's system. Dieldrin also exhibited higher concentrations than PCP or TBTO. None of the treatments caused any mortality of the voles or produced any gross visible behavioral change.

Air. Cumulative loss (Figure 3) of ^{14}C-labeled materials sorbed on the polyurethane foam filters accounts for only 0.1–1.4% of the chemical applied to the wood blocks (Figure 2). In preliminary trials, about 99% of the HEOD introduced into the filter material is trapped, retained for at least 48 h by the filter plugs, and recovered by Soxhlet extraction. Efficiencies are not known for the remaining materials, but general experience (Beall et al., 1976; Nash et al., 1977) has shown most nonpolar chemicals, such as these wood preservatives, to be readily trapped in high efficiency. Unfortunately, CO_2 cannot be trapped with sufficient accuracy and efficiency at the airflow rates employed in the TMC-II to provide that measure of ultimate degradation of the labeled chemicals. Moreover, the inaccuracies in assessing net (and therefore unaccounted) losses from loading measurements do not permit estimation of CO_2 by difference in this experiment.

Miscellaneous. Miscellaneous components of the system included the groundwater or leachate and the various solvent and aqueous rinses acquired during the cleanup of the systems. Any ^{14}C that might be contained in these components was below instrument detection limits.

Residue Characterization. Only soil samples immediately surrounding the treatment posts contained sufficient extractable ^{14}C-labeled materials to permit analysis by gas chromatography or high-performance liquid chromatography. Analysis of the residues from both creosote treatments verified that the ^{14}C detected via liquid scintillation was associated with the original labeled component, either phenanthrene or acenaphthene. The HPLC analysis of the PCP treatment indicated that pentachloroaniline was the major material detected in the soil, followed by the parent material (PCP) and tetrachlorohydroquinone. The GC analysis of soil from the dieldrin

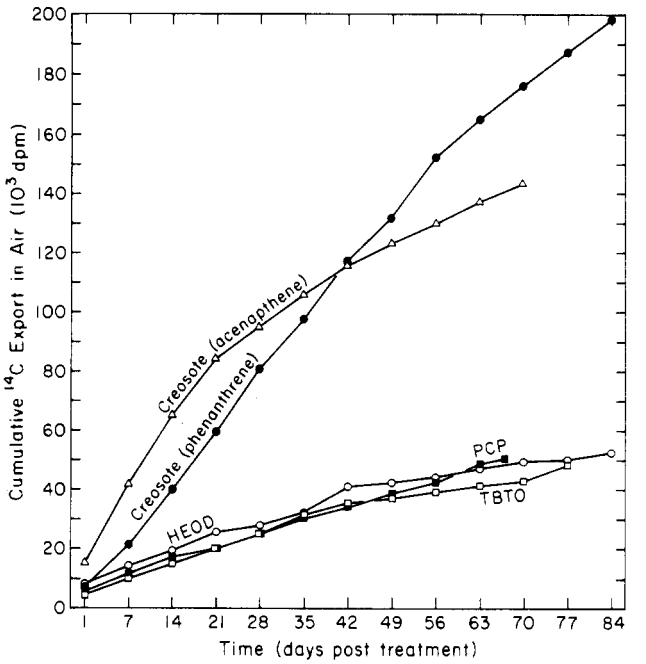

Figure 3. Cumulative ^{14}C export in air (does not include $^{14}CO_2$).

treatment revealed that virtually all of the extractable material was present as parent (HEOD). No intact TBTO was detected in any extract examined by chromatographic methods.

Effects. The main measures of effect employed in this study were of species survival and interaction. Posttreatment mortality of crickets was significant only for the HEOD chamber (Gillett et al., 1980), prior to addition of the vole. Adventitious predation on crickets by the vole led to high rates of cricket loss, noted especially in the control, PCP, and (to a lesser extent) TBTO treatments. However, HEOD or creosote exposure led to significant reduction in predation by the vole.

Survival of other invertebrates varied considerably between TMC-IIs. Adventitious insects occurred in all chambers except the HEOD treatment; *Collembola* spp. were especially evident from the unsterilized soil. No effect on adult vole survival, fecundity, or pup survival was evident with any of the treatments. All pregnant voles produced litters ranging in size from four to six pups with 100% survival to weaning at normal weight. Additional details on effects assessment are presented elsewhere (Gillett et al., 1980).

DISCUSSION

Lack of chemical dispersal of wood preservatives into the surrounding ecosystem during the relatively short exposure period is expected, because of the way wood posts are impregnated and the inherent nature of traditional wood perservative chemicals. The few data that exist in the literature suggest creosote in terrestrial systems is relatively immobile (Stasse, 1964). In a literature review, Leach and Weinert (1976) concluded that creosote losses from pressure-treated wood were minimal. While PCP has been extensively studied, data on losses from point sources (impregnated wood) are not available. A laboratory study (Gile and Gillett, 1979b) indicated that 65% of the PCP applied as a seed coating remained in the upper 5 cm of soil, primarily as bound residue. In the United States, TBTO is registered for aquatic uses only; loss rates of distribution data for terrestrial systems are not readily available.

Because the amount of materials released from the posts was small, the slight differences between chemical dis-

Table V. Vapor Pressures of Test Materials

chemical	vapor pressure, mmHg
dieldrin[a]	7.8×10^{-7} (25 °C)
PCP[b]	0.12 (100 °C)
acenaphthene[b]	10.0 (131 °C)
phenanthrene[b]	1.0 (118 °C)
TBTO[c]	2.0 (180 °C)

[a] Spencer (1973). [b] Sax (1979). [c] Wilkinson and Hilgard (1977).

tribution for various treatments in the TMC ecosystem may be caused by sampling errors, sample preparation, or analysis. Even if the differences between chemicals were not significant, the relative distributions within the ecosystem are consistent for all chemicals. With a point source of application, such as the wood posts, one would expect to find the majority of the material in the soil immediately surrounding the posts (Table I). Movement away from the posts is probably a function of volatility, absorptivity, solubility, and biologic disturbance (e.g., vole tunneling). On the basis of solubility alone, PCP (2 mg/L) would be expected to move downward to a greater extent (Table II) than the other chemicals, all of which are quite insoluble. The lack of detectable material below 10 cm even with PCP can be attributed to the minimal release from the posts, biodegradation, relatively strong binding in the upper soil layer, and instrument detection limits.

The observed accumulation of chemicals by soil invertebrates demonstrates the importance of soil as a chemical repository. An Ecological Magnification (EM) Index calculated for the vole based on total ^{14}C-labeled materials (ppm of ^{14}C equivalents in vole/ppm of ^{14}C equivalents in soil), rather than only extractable parent (Metcalf et al., 1971), results in values of 9.2 for HEOD, 12 for creosote/phenanthrene, 31 for creosote/acenaphthene, 2.6 for PCP, and 2.6 for TBTO. The values for HEOD and the two creosote treatments reflect the maximum case, since the parent material was measured both directly (by chromatographic methods) and indirectly by liquid scintillation. In contrast, the values for PCP and TBTO reflect an indeterminant situation, since parent residue levels could not be determined in either vole or soil. The calculated EM values together with the residues detected in the brain suggest that the accumulation of creosote may not be transitory. In addition, the actual concentration of creosote may be substantially higher, since the ^{14}C-labeled components of creosote comprised approximately 30% of the total. For example, based on the whole body concentrations for phenanthrene and acenaphthene in voles (Table IV), whole body concentrations of creosote could be approximately 36 and 411 ppm. Observation of the voles in TMC-II indicate that plant material comprises approximately 70% of their diet with crickets accounting for about 30%. Although plant residues were low, the volume of material consumed could contribute substantially to the residue levels of all chemicals.

From known vapor pressures (Table V) we would predict higher vaporization rates for phenanthrene and acenaphthene in comparison to those for dieldrin, PCP, and TBTO. This is confirmed by the data presented in Figure 3. Because volatility limits release from wood, the preservatives are likewise limited in environmental distribution.

The estimated total loss of HEOD from the posts amounted to about 5.6 kg/ha, of which 25% was released in the first few hours. The acute effects on the vole and extent of bioaccumulation were much less severe than when the TMC was treated by foliar spray with 1.12 kg/ha (about 1 lb/acre) (Gile and Gillett, 1979a). For example, the latter treatment resulted in vole mortalities, within 2–5 days after the animals were placed in the chamber up to 20 days posttreatment, and high brain (14.5 ppm) and whole body (EM = 60) residues. These contrast with the brain residues (2.2 ppm) and relatively low bioaccumulation (EM = 9.2) under chronic exposure from the posts. These results continue to emphasize the role of temporality and route of exposure in evaluation of the fate and effects for exposure and hazard assessment. Although the results are not yet predictable by mathematical model or simplistic monotonic treatment of process characteristics (e.g., vapor pressure), the microcosm system provides an integrated look at how these several processes are related and what the outcome may be for a given chemical and exposure set.

These trials in the TMC-II represent only one of a number of scenarios that might be relevant for wood preservatives. Given the paucity of data available about creosote, PCP, and TBTO, situations such as the TMC-II reveal overall patterns of movement and relative distribution. These data are superior to that from the laboratory (directed at single, noninteracting processes) in terms of their comprehensive nature. However, we can give no assurance that they represent exactly even one situation in the field. If dieldrin, chlordane, or heptachlor were used to treat fence posts in a sheep pasture, we would expect adverse residues in the stock from grazing in the vicinity of the fence. If creosote were used in this manner, we similarly would anticipate potentially higher residues of chemicals that are suspect carcinogens. The magnitude of these concerns is moderated by the knowledge, provided by the TMC-II, that these residues would decline markedly with distance from the fence. Exposure assessment might therefore minimize the likelihood of adverse impact and confirm that by monitoring of grazing time/intake regarding distance from the fence. Hazard assessment of the situation is assisted by the TMC-II studies in demonstrating that release is nonzero (therefore worth performing) and that local effects might be present. Whether or not the chemical has greater impact on the system than the fence line itself is a matter of the risk assessment, which would take into account the exposure and hazard in relation to all impacts.

To extrapolate TMC-II data directly to the field we would require a validated and verified mathematical model. Several are currently under study as candidates for modeling the TMC system. If successful, a candidate would then be validated against site-specific and generic field experiments performed specifically for that purpose. Almost all of the systems being explored take into account seasonal variations in weather, local soil conditions, and related factors that make explicit the analysis of the fate of a chemical in that environment. Where compounds are retained in a point source and slowly distributed to the soil, which also acts as a moderating factor in distribution, the rate of transport, transformation, and bioaccumulation may be adequately represented by relatively simple sets of equations dominated by the stable (nondynamic) portions. Thus compounds with low mobility, such as the wood preservatives, may be more readily modeled than materials for which media movement (leaching, hydrodynamic flux, and air shed movement) are expected to be great, due to high volatility, low partitioning or sorption, and high solubility. While the TMC-II system does not necessarily represent any particular situation, we anticipate that it serves especially well for these wood preservatives.

CONCLUSIONS

The following conclusions on the transport and fate of the test chemicals are based solely on data collected from

the TMC experiment. (1) The mass of material (95%) remains at the site of application, with a sharp gradient existing in the soil for the chemical leaving the posts. (2) Steady, but small amounts of material are contributed to the ecosystem for distribution in the air and through the biota living in close proximity to the treated wood. (3) Of the chemicals tested, creosote components were dispersed to a greater extent than others. (4) The rate of release was too low to evidence acute effects on the vole, but insecticidal activity was pronounced and continuous for HEOD, and both creosote and HEOD had a depressing effect on vole predation of crickets. (5) Because of the high degree of mass accountability and because of adaptability of the test system to temporal and media-specific studies, the TMC-II system appears to be a useful means of studying toxic chemical fate and effects.

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Isolation and Identification of Two New [11]Cytochalasins from *Phomopsis sojae*

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Selected isolates of the soybean pathogen *Phomopsis sojae* produced two new [11]cytochalasin mycotoxins related to cytochalasin H and deacetylcytochalasin H. The mycotoxins were identified spectroscopically as 21-acetoxy-6,7-epoxy-18-hydroxy-10-phenyl-5,6,16,18-tetramethyl[11]cytochalasa-13,19-dien-1-one (epoxycytochalasin H) and 18,21-dihydroxy-6,7-epoxy-10-phenyl-5,6,16,18-tetramethyl[11]cytochalasa-13,19-dien-1-one (epoxydeacetylcytochalasin H). Both metabolites were toxic to day-old chickens and both showed plant growth inhibition in wheat coleoptile bioassay.

The soybean (*Glycine max* L. Merrill) is an important crop used worldwide for oil and protein for human consumption and feedstock for domestic animals. Few toxigenic fungi are known to invade soybeans, and little is known of potential hazards from consumption of molded soybeans. *Diaporthe phaseolorum* (CKe. Å ELL.) var. *sojae* Wehm. (imperfect state *Phomopsis sojae* Leh.) produces a pod and stem blight of soybean that is recognized worldwide. Infection occurs after pod formation and may be severe when harvest is delayed, especially when environmental conditions are warm and moist. Fungal invasion is often severe in fields with high plant populations and extensive lodging (Sinclair and Shurtleff, 1975). Environmental conditions in south Georgia during harvest are frequently favorable for colonization by *P. sojae*.

We recently reported the isolation, chemical characterization, and biological properties of two [11]cytochalasin metabolites, cytochalasin H (I) and deacetylcytochalasin H (II), from culture extracts of *Phomopsis* sp. (Wells et al., 1976; Beno et al., 1977; Cole et al., 1981). Biological assay of culture extracts of eleven selected isolates of *P. sojae* parasitic on soybean seeds produced two isolates that were toxigenic. We now report the chemical structures of

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