Environmental Health Perspectives
Vol. 18, pp. 167-179, 1976

Model Ecosystem Evaluation of the Environmental Impacts of the Veterinary Drugs Phenothiazine, Sulfamethazine, Clopidol, and Diethylstilbestrol

by Joel R. Coats,* Robert L. Metcalf,* Po-Yung Lu,* Daniel D. Brown,* Janet F. Williams,* and Larry G. Hansen†

Four veterinary drugs of dissimilar chemical structure were evaluated for environmental stability and penchant for bioaccumulation. The techniques used were (1) a model aquatic ecosystem (3 days) and (2) a model feedlot ecosystem (33 days) in which the drugs were introduced via the excreta of chicks or mice. The model feedlot ecosystem was supported by metabolism cage studies to determine the amount and the form of the drug excreted by the chicks or mice. Considerable quantities of all the drugs were excreted intact or as environmentally short-lived conjugates. Diethylstilbestrol (DES) and Clopidol were the most persistent molecules, but only DES bioaccumulated to any appreciable degree. Phenothiazine was very biodegradable; sulfamethazine was relatively biodegradable and only accumulated in the organisms to very low levels.

Data from the aquatic model ecosystem demonstrated a good correlation between the partition coefficients of the drugs and their accumulation in the fish.

Introduction

Animal wastes are an important contribution to environmental pollution in the United States. The agricultural industry raises annually about 107 million cattle, 53 million hogs, 26 million sheep, 375 million chickens, 104 million turkeys, and 11 million ducks. These animals produce annually about 1.14×10^9 tons of solid wastes and 4.35×10^8 tons of liquid wastes (*1*). Such animal wastes which aggregate about 10 times the U.S. total of human excretory wastes have become a major source of pollution, especially in cattle feed lots and poultry farms where 100,000 or more animals may be confined in very limited areas.

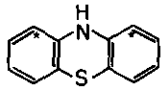
The less obvious pollution problems from animal wastes result from the widespread use of veterinary drugs—antibiotics, chemotherapeutants, parasiticides, nutritional additives, and growth-promoting additives. These are given as feed supplements or by direct administration to the animals. An indication of the extent to which chemical supplements are used in animal feeds is given by Huber (*2*), who records that in 1966 in the United States, $215 million were spent on animal feed additives and $115 million health pharmaceuticals. More than half of the antibiotics

*Department of Entomology, School of Life Sciences, University of Illinois, Urbana-Champaign, Illinois 61801.
† Department of Veterinary Physiology and Pharmacology, College of Veterinary Medicine, University of Illinois, Urbana-Champaign, Illinois 61801.

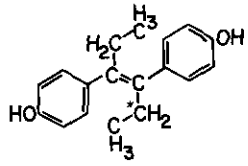
produced are used for agricultural purposes, primarily as feed additives, and two-thirds of the 60 million tons of feed produced commercially contain medication, with 75% of these requiring legal withdrawal times before the treated animals can be marketed.

In addition to the deliberate additives listed above there are accidental additives which contaminate feed such as polychlorinated biphenyls (PCBs), persistent organochlorine insecticides, plasticizers, and flame retardants (PBBs).

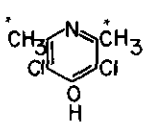
The environmental fates and degradative pathways for nearly all of these substances are little known as are the possible levels of toxic effects, bioconcentration factors, and food chain relationships of the parent compound and its metabolites on the living elements of the ecosystem. The model ecosystem studies discussed here were developed to model the environmental impact of a feed lot on a sewer, an adjacent pond, or other aquatic drainage. Four representative radiolabeled veterinary drugs, the anthelmintic phenothiazine, the coccidiostat Clopidol, the bacteriostat sulfamethazine, and the growth promoter diethylstilbestrol were chosen for evaluation; the asterisks (*) in the structure denote the radiolabels.



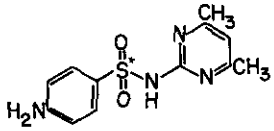
phenothiazine



diethyl stilbestrol



Clopidol



sulfamethazine

Materials and Methods

Radiolabeled compounds were purchased from commerical suppliers as follows: ^{14}C-labeled diethylstilbestrol (monoethyl-1^{14}C) or DES, specific activity 52 mCi/mole, radiopurity >98% from Amersham-Searle Corp.; ^{14}C-ring (uniform label) phenothiazine, specific activity 3.29 mCi/mole, radiopurity 98% from California Bionuclear Corp.; ^{35}S-labeled sulfamethazine (sulfanilamido-4,6-dimethylpyrimidine or sulfadimidine), specific activity 26.1 mCi/mole, radiopurity 98% from Amersham-Searle Corp. Dow Chemical Company generously supplied ^{14}C-Clopidol (2,6-^{14}C-label) or 3,5-dichloro-2,6-dimethyl-4-pyridinol, specific activity 0.943 mCi/mole, radiopurity 99%.

Radioassay

Liquid scintillation was used for analysis of the concentrations of radiolabeled compounds in samples of water, feces, urine, and tissues of organisms. The cocktail was composed of 100 g naphthalene, 5 g diphenyl oxazole (PPO), made up to 1 liter with 1,4-dioxane. Quench corrections were made by using the channels ratio method (*3*). Radioautographs of thin layer chromatography plates (0.25 mm thick, fluorescent silica gel GF-254 from E. Merck) were made by using Eastman no-screen x-ray film. The plates were evaluated quantitatively by scraping 1 cm × 2 cm sections or fluorescent spots into vials of scintillation fluid and counted.

Model Metabolites

Preparations of the model compounds were as follows: DES-mono-β-D-glucuronide was isolated from rabbit urine (*4*); the structure was confirmed by mass spectromety. Acetyl-DES was prepared by using pyridine and acetic anhydride per equivalent of DES. DES was obtained from Sigma Chemical Company.

Phenothiazine sulfoxide was prepared by adding one equivalent of H_2O_2 to phenothiazine in acetone solution and allowing the sulfoxide to crystallize slowly. The product decomposed at 240°C; the literature value is 250°C (*5*). Infrared spectroscopy revealed the sulfoxide bond stretching absorbance at 1078 cm^{-1}.

Phenothiazine sulfone was prepared by using peracetic acid as the oxidizing agent (*6*). The compound melted at 260°C (literature mp 258°C), and the compound absorbed in the infrared at 1157 and 1288 cm^{-1}.

Phenothiazone was obtained from Dr. G. D. Koritz, and was prepared by the oxidation of phenothiazine with $FeCl_3$ (*7*). Phenothiazine was obtained from Eastman Chemical Company.

Clopidol was provided by Dow Chemical Company.

N^4-acetyl sulfamethazine was prepared by using acetic anhydride in acetic acid (*8*). N^4-methyl sulfamethazine was synthesized by re-

fluxing sulfamethazine in methanol with KOH and excess methyl iodide. Sulfamethazine was furnished by Dr. R. F. Bevill. The compounds and their derivatives were separated on TLC plates by using the solvent systems listed in Table 1. Chromogenic detection methods were also employed as aids in locating model metabolites on TLC plates (Table 1).

Toxicity Methodology

The compounds and their model metabolites were each tested for lethal effects to the species of organisms to be used in the model ecosystem. Three-liter glass containers were each filled with two liters of standard reference water. Various predetermined concentrations of compound were added in small volumes of appropriate solvents (acetone, methanol, or water), and air was bubbled in for 8 hr. The organisms were added and observed for lethal or toxic effects after 24 and 48 hr.

Aquatic Model Ecosystem

A 3-day, 2-liter aquatic model ecosystem was used to determine relative uptake and degradation of each compound (*9*). The ecosystem contained 2 liters of standard reference water and the following organisms: *Oedogonium cardiacum*, *Daphnia magna*, *Culex pipiens quinquefasciatus*, *Physa* sp., and *Gambusia affinis*. Following equilibration, the compounds were added in minimum amounts of appropriate solvents. Two days later the system was dismantled, organisms were analyzed by grinding and extracting with appropriate solvents, theh combusting the residues to determine unextractable radioactivity. The water was extracted, then HCl was added until pH 2 was reached; refluxing and extraction produced a water-hydrolyzed fraction.

Dosing

Labeled compounds were administered orally in olive oil to Swiss white mice: ^{14}C-DES at 0.5 mg/kg, ^{14}C-phenothiazine at 2 mg/kg, ^{35}S-sulfamethazine at 100 mg/kg. One day old chicks were fed 0.0125% ^{14}C-Clopidol in their feed, and injected subcutaneously at 0.05 mg/kg ^{14}C-DES in propylene glycol.

Metabolism Cages

Mouse feces and urine were collected and separated within the "econo metabolism unit"

Table 1. Chromatographic and chromogenic properties of four veterinary drugs and metabolites.

Compound	R_f by silica gel TLC		Solvent system		Color	
	I	II	I	II	Ultraviolet irradiation	Spray reagent
DES	0.60	0.83	Benzene: acetone (7:3)	Hexane: chloroform: isopropanol: acetic acid (13:10:6:1)	Yellow	Brown[a]
DES acetate	0.63	0.91			Light orange	—
β-Glucuronide	0.00	0.08			Tan	Brown[a]
Clopidol	0.70	0.73	Chloroform: ethanol: acetic acid (16:4:1)	Butan-1-ol: acetic acid: water (2:1:1)	—	
α-Hydroxyclopidol	0.51[b]	0.69[a]			—	
Carboxylic acid derivative	0.05[b]	0.59[a]			—	
Phenothiazine	0.58	0.72	Hexane: toluene: acetone: methanol (10:7:2:1)	Chloroform: diethyl ether (1:1)	Green	Brown[c]
Phenothiazone	0.41	0.55			Red	Tan[c]
Phenothiazine sulfone	0.20	0.41			—	Brown[c]
Phenothiazine sulfoxide	0.12	0.13			Tan	Gray[c]
Sulfamethazine	0.73	0.47	Diethyl ether: isopropanol (4:1)	Ethyl acetate	Tan	Yellow[d]
N^4-Methyl sulfamethazine	0.79	0.52			Tan	Yellow[d]
N^4-Acetyl sulfamethazine	0.60	0.27			—	Yellow (4 hr)[d]
N^4-Sulfate conjugate	0.50	0.13			—	Yellow (0.5 hr)[d]

[a] Spray reagent: ceric ammonium nitrate in 2*N* nitric acid.
[b] Values from Cameron et al. (*17*)
[c] Spray reagent: cupric sulfate in water.
[d] Spray reagent: *p*-dimethylaminobenzaldehyde in 1*N* HCl.

cages. Dry feces weights were recorded. Feces were ground with mortar and pestle, and 15 mg samples were taken for assay by the Schoniger oxygen flask combustion technique (*10*).

The daily urine samples were made up to 25 ml with methanol and two 1 ml aliquots were taken for counting. Extraction of feces and urine followed the usual procedures (*11*), utilizing appropriate solvents.

Model Feedlot Ecosystem

A 33-day terestrial-aquatic model ecosystem was used to follow the qualitative and quantitative fate of the drugs being evaluated. The system was comprised of 15 kg of white quartz sand and 7 liters of standard reference water in a 10-gal aquarium. The biotic components of the system were: *Sorghum vulgare*, the alga *Oedogonium cardiacum*, the water flea *Daphnia magna*, the mosquito larva *Culex pipiens quinquefasciatus*, the snail *Physa* sp., the mosquito fish *Gambusia affinis*, and a complement of microbes and zooplankton. The detailed methodology for the model ecosystem has been described previously (*12*). The following modifications were made to facilitate the tracing of a veterinary drug through the model ecosystem: a 10 cm × 18 cm × 27 cm cage constructed from 0.25 in. wire mesh was supported from the top of the aquarium using glass rods, three mice or baby chicks, dosed as described for the metabolism cage study, were placed in the cage and supplied with food and water. The cage was positioned over the sand-water interface to allow optimal input of excretory products into the aqueous phase without inciting an extensive algal bloom. The excretion rate data from the metabolism cage experiment determined the duration of the animals' confinement over the model ecosystem. The most suitable location for the cage was where one-fourth of the excrement fell directly into the water, and three-fourths onto the terrestrial phase (Fig. 1). The maximum allowable excretory input was that of three 1-day chicks or three 20-g mice in the suspended cage for 3 days following treatment. The daphnia were quite sensitive to excess chick excrement in the water. The system was maintained at constant temperature (25 ± 1°C) and photoperiod (12 hr diurnal cycle of 5000 ft-candles) in a Percival environmental plant-growth chamber. The level of radioactivity was monitored by withdrawal of 1 ml aliquots of water for counting. On day 26, 300 *Culex* larvae were added; on day 30, 50 of them were removed, as well as 50 *Daphnia*, for quantitative and

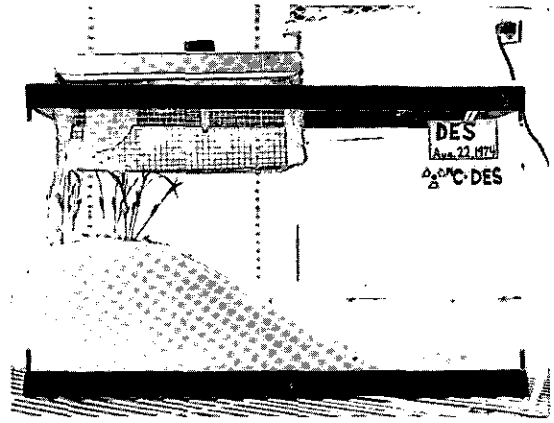


FIGURE 1. Model feedlot modification of the laboratory model ecosystem.

qualitative analysis of radiolabel in their bodies. Three *Gambusia* were added to feed on the remaining *Culex* larvae and *Daphnia* for 3 days. On day 33 *Gambusia*, algae, and snails were removed for analysis. Extraction procedures were as described above for the aquatic ecosystem. One liter of twice-filtered water was extracted with a solvent, taken to pH 2 with concentrated HCl, and refluxed, and extracted again, to yield fractions called "water-unhydrolyzed" and "water-hydrolyzed". The identification of metabolites using TLC was then completed for extracts of the water and all organisms in the model ecosystem.

Water Solubility and Partition Coefficients

The value for water solubility of phenothiazine and DES was determined by radioassay at 25°C; determinations for Clopidol and sulfamethazine utilized unlabeled drugs. Distilled water was used, and the pH of the water was not controlled. Partition coefficients were determined by using a system of 1-octanol and water (*13*). Table 2 gives these physicochemical parameters for the four drugs studied.

Results and Discussion

Toxicity Tests

The evaluation of the drugs and their primary metabolites for acute lethal effects on the biotic components of the model ecosystem produced Table 3. Phenothiazine and N^4-methyl sulfamethazine exhibited some degree of toxicity.

Table 2. Environmental properties of four veterinary drugs.

Drug	H_2O solubility, ppm	Octanol/H_2O partition	Aquatic model ecosystem			
			Fish		Snail	
			EM[a]	Unextractable	% EM	Unextractable, %[b]
Diethylstilbestrol	0.40	302	13.7	53.5	484	29.5
Phenothiazine	0.63	1589	356	88.2	37	92.1
Clopidol	ca. 10	3.19	5.71	93.3	62	30.8
Sulfamethazine	380	0.93	ca. 1	94.6	ca. 1	62.2

[a] EM = ecological magnification =concentration of parent drug in an organism/concentration of parent drug in the water.
[b] Unextractables =[(total radiolabel−radiolabel extracted by acetonitrile)/total radiolabel]×100.

Table 3. Acute toxicities of four veterinary drugs and some metabolites.

	LC_{50}, ppm				
	Oedogonium cardiacum	*Daphnia magna*	*Culex pipiens quinquefasciatus*	*Physa* sp.	*Gambusia affinis*
DES	>10	4	4	>10	> 1
DES acetate	>10	10	>10	>10	>10
Phenothiazine	> 1	0.1	0.8	> 1	> 0.5
Phenothiazone	> 1	1	>10	> 1	> 1
Phenothiazine sulfoxide	> 1	1	>10	> 1	> 1
Phenothiazine sulfone	> 1	> 1	>10	> 1	> 1
Clopidol	> 7	> 7	> 7	> 7	>10
Sulfamethazine	>10	>10	>10	>10	>10
N^4-Acetyl Sulfamethazine	>10	>10	>10	>10	>10
N^4-Methyl sulfamethazine	> 1	> 1	> 0.5	> 1	> 0.5

Metabolism Cage Studies

The comparison of excretion of DES by orally dosed mice and subcutaneously injected chicks is presented in Table 4. The dose injected into the chicks was eliminated considerably more slowly. The degree of metabolism by both animals is shown in Tables 5 and 6. A large proportion of the DES was excreted by the mouse in the feces, mostly as free DES and its metabolites with some as conjugates. All DES found in the urine was either conjugated or metabolized to more polar products. The chick excreted about 8% of the administered dose as free DES and 40% as hydrolyzable conjugates of the parent molecule. Other metabolism studies involving beef cattle, sheep, rabbits, cats, rats, and chickens have shown glucuronide and sulfate conjugates to be major metabolites. Our experience with these metabolites indicates they are quite short-lived in an aquatic environment and are easily hydrolyzed to release free DES.

Phenothiazine was rapidly eliminated by the mouse (Table 4), only 14% of the dose being excreted as intact phenothiazine. Table 7 gives the results of the metabolism study.

The predominant metabolite was the primary sulfur-oxidation product, phenothiazine sulfoxide. The sulfone also occurs, as well as two major unknown polar metabolites thought to be leucophenothiazone (R_f = 0.05) and thionol (at the origin). Earlier work by use of colorimetric assay techniques found ring-hydroxylation products (leucophenothiazone, phenothiazone, thionol) and their conjugates to be the major metabolites in dairy cows (*14*) and rabbits, dogs, pigs, sheep, and horses (*15*).

The total dose of Clopidol ingested by chick during 24 hr is excreted somewhat more slowly than was determined with rats (*16*). About 37% of radiolabel excreted during 3 days was in the form of free Clopidol, with significant amounts of the α-hydroxylated product (23%) and the car-

Table 4. Excretion rate and primary metabolites for four veterinary drugs.

Drug	Animal	Route of administration	Radiolabel excreted, %[a]	Intact drug excreted, %[a]	Primary metabolite
DES	Mouse	Oral	95	61	Polar conjugates (14%)
DES	Chick	Injected S.C.	66	48	Polar conjugates (6%)
Phenothiazine	Mouse	Oral	95	14	Sulfoxide (42%)
Clopidol	Chick	In feed	73	28	α-Hydroxyl (20%)
Sulfamethazine	Mouse	Oral	62	17	N^4-Acetyl (8%)

[a] Percent of administered dose, 72 hr.

Table 5. Metabolism of ^{14}C-diethylstilbestrol by male Swiss mice.

	Excreted label, %			
	Urine (8.9%)		Feces (91.1%)	
	Unhydrolyzed (4.2%)	Hydrolyzed (4.7%)	Unhydrolyzed (81.4%)	Hydrolyzed (9.7%)
DES	—	2.9	57	3.6
Unknown IV (Rf=0.58)[a]	—	—	1.6	0.6
Unknown V (R_f= 0.48)	—	—	0.6	0.8
Unknown VII (R_f= 0.35)	0.5	—	2.3	0.7
Unknown VIII (R_f= 0.30)	—	—	1.2	0.9
Unknown X (R_f= 0.18)	2.9	—	1.1	1.1
Unknown XI (R_f= 0.10)	—	—	6.1	1.1
Unknown XII (R_f= 0.05)	0.3	—	11.4	0.6
Polar (R_f= 0.00)	0.5	1.8	—	0.3

[a] Solvent system: benzene: acetone (7:3).

Table 6. Metabolism of ^{14}C-diethylstilbestrol by baby chicks.

	Excreted label, %	
	Excrement (13.4%)	Hydrolyzed excrement (86.6%)
DES	10.9	61
Unknown IV (R_f= 0.58)[a]	—	8
Unknown V (R_f= 0.48)	0.8	5
Unknown VI (R_f= 0.43)	0.4	—
Unknown VII (R_f= 0.35)	0.7	2
Unknown VIII (R_f= 0.30)	0.3	1
Unknown IX (R_f= 0.25)	—	1
Unknown XII (R_f= 0.05)	0.2	1
Polar (R_f= 0.00)	0.1	8

[a] Solvent system: benzene: acetone (7:3).

boxylic acid derivative (16%) (see Table 8). Acid hydrolysis revealed small amounts of conjugates of Clopidol and the α-hydroxyl product present. The metabolism agrees well with previous work (*17*), which identified the same major degradation products in rabbits.

Sulfamethazine was eliminated by the mouse according to the data shown in Table 4, somewhat slower than observed in sheep (*18*). The metabolism was primarily to the N^4-acetylated product as shown by Table 9. This parallels the results of other studies with cows and sheep (*8, 18*).

Model Aquatic Ecosystem

The fate of the four compounds is expressed as concentrations (ppm) of the parent molecule and detectable metabolites in each component of the small aquatic ecosystem (Table 10). The crucial

Table 7. Metabolism of ^{14}C-phenothiazine by female Swiss mice.

	Excreted label, %			
	Urine (72%)		Feces (28%)	
	Unhydrolyzed (10%)	Hydrolyzed (62%)	Unhydrolyzed (15%)	Hydrolyzed (13%)
Unknown I (R_f= 0.79)[a]	—	—	0.5	1.4
Unknown II (R_f= 0.69)	—	—	0.8	0.4
Phenothiazine	0.1	11	2.3	1.2
Unknown III (R_f= 0.55)	0.1 tr.	— 0.9	— 1.6	— 0.2
Phenothiazone				
Unknown IV (R_f= 0.35)	—	1.5	1.3	0.6
Unknown V (R_f= 0.27)	0.4 0.2	— 2.2	0.7 0.6	0.4 1.7
Phenothiazine sulfone				
Unknown VI (R = 0.17)	—	—	0.8	—
Phenothiazine sulfoxide	3.6	36	1.8	2.6
Unknown VII (R_f= 0.09)	—	—	1.8	0.9
Unknown VIII (R_f= 0.05)	1.9	4.0	2.1	1.4
Polar (R_f= 0.00)	3.7	6.6	0.8	2.2

[a] Solvent system I: hexane:toluene:acetone:methanol (10:7:2:1).

Table 8. Metabolism of ^{14}C-Clopidol by baby chicks.

	Excreted label, %	
	Excrement (94.2%)	Hydrolyzed excrement (5.8%)
Clopidol	37	1.4
α-Hydroxyclopidol	23	4.1
Unknown I (R_f = 0.37) [a]	0.8	–
Unknown II (R_f = 0.26)	–	0.3
Unknown III (R_f = 0.17)	2	–
Unknown IV (R_f = 0.12)	8	–
Carboxylic acid derivative	16	–
Polar (R_f = 0.00)	8	–

[a]Solvent system: chloroform: ethanol: acetic acid (16:4:1).

values extracted from the concentrations are the ecological magnification (EM) for each organism and the biodegradability index (BI) for each organism. These values are determined as follows:

$$EM = \frac{\text{concentration of parent in organism}}{\text{concentration of parent in water}}$$

$$BI = \frac{\text{Concentration of metabolites more polar than parent}}{\text{concentration of parent plus less polar metabolites}}$$

These indices were originally devised to reflect degree of bioconcentration and ease of biodegradation for a series of DDT analogs (*19*). They have since been utilized to assess the comparative environmental fate of many classes of insecticides, herbicides, fungicides, industrial chemicals, and heavy metals. The EM and BI values can be determined for the 33-day model feedlot ecosystem, as well as the 3-day aquatic model.

Diethylstilbestrol concentrated to a considerable degree in the alga and snail and to a lesser extent in the fish. BI values ranged from 0.42 in the snail to 1.2 in the fish and 1.4 in the daphnia.

Phenothiazine concentrated less than DES in the snail but more in all the other organisms. However, the BI values were higher for phenothiazine, ranging from 0.6 to 9.4, indicating that it was more easily metabolized by the organisms. By comparison phenothiazine was concentrated in the body (due to high lipophilicity) more than DES, but was also a better substrate for enzymatic oxidation reactions, especially sulfoxidation.

Clopidol concentrated to fairly low levels and was metabolized very slowly as demonstrated by the absence of metabolites in the body extracts. The appearance of trace amounts of the primary degradation products in the water was the only evidence of metabolism. The polar derivatives in the water indicated that Clopidol was probably excreted by most organisms via a conjugation process.

Sulfamethazine failed to concentrate to levels high enough to analyze the organisms for metabolites. However, if all ^{35}S label in the organisms were considered parent molecule, the EM values would all be less than 1.6. Sulfamethazine essentially did not bioconcentrate. Equal concentrations of polar and nonpolar products were found in the water after the 2-day exposure to the organism complex (Table 10).

Analysis of Aquatic Model Ecosystem Results

A comparison of EM values for the fish *Gambusia* from the 3-day model aquatic system with octanol/water partition coefficients revealed an excellent correlation (r = 0.987). The log EM values were plotted vs. log octanol/water par-

Table 9. Metabolism of ^{35}S-sulfamethazine by female Swiss mice.

	Excreted label, %				
	Urine (84%)			Feces (16%)	
	Unextractable (18%)	Unhydrolyzed (59%)	Hydrolyzed (7%)	Unhydrolyzed (2%)	Hydrolyzed (14%)
Unknown I (R_f = 0.57)[a]	–	–	0.1	0.40	0.5
N^4-Methyl sulfamethazine	–	0.1	0.2	0.14	1.2
Sulfamethazine	–	22	2.9	0.40	1.7
Unknown III (R_f = 0.39)	–	1.0	0.3	0.08	1.2
N^4-Acetyl derivative	–	6.5	1.6	0.68	3.8
Unknown IV (R_f = 0.20)	–	3.6	0.3	0.04	1.7
Polar (R_f =0.00)	–	3.3	0.4	0.04	0.8
Unextractable	18	22	1.1	0.21	2.9

[a] Solvent system: diethyl ether: isopropanol (4:1).

Table 10. Environmental fate of ^{14}C-diethylstilbestrol (DES), ^{14}C-phenothiazine, ^{14}C-Clopidol, and ^{35}S-sulfamethazine in a model aquatic ecosystem.

	Parent molecule equivalent, ppm					
	H_2O	*Oedogonium* (alga)	*Daphnia* (daphnia)	*Culex* (mosquito)	*Physa* (snail)	*Gambusia* (fish)
DES						
Total extractable ^{14}C	0.000740	0.0458	0.0353	0.0245	0.1506	0.0129
Unknown III (R_f = 0.63)[a]	0.000014	0.0074	0.0145	0.0076	0.0215	0.0033
DES	0.000174	0.0172	—	—	0.0843	0.0024
Unknown IV (R_f = 0.58)[a]	0.000145	—	—	—	0.0092	—
Unknown V (R_f = 0.48)	0.000090	—	—	—	0.0056	—
Unknown VI (R_f = 0.43)	0.000054	—	—	—	0.0068	—
Unknown VII (R_f = 0.35)	0.000014	—	—	—	0.0040	—
Unknown VIII (R_f = 0.30)	0.000010	—	—	—	—	—
Unknown IX (R_f = 0.25)	0.000007	—	—	—	—	—
Unknown X (R_f = 0.18)	0.000006	0.0027	—	—	0.0028	—
Unknown XI (R_f = 0.10)	0.000033	0.0040	—	—	0.0020	—
Polar (R_f = 0.0)	0.000133	0.0145	0.0208	0.0169	0.0144	0.0072
Unextractable ^{14}C	*0.000060*	*0.0006*	*0.0301*	*0.0412*	*0.0629*	*0.0149*
EM	1	99	—	—	484	14
BI		0.86	1.4	2.2	0.42	1.2
Phenothiazine						
Total extractable ^{14}C	0.0212	7.590	1.470	1.110	1.161	1.690
Unknown I (R_f = 0.74)[c]	0.0008	—	—	—	—	—
Unknown II (R_f = 0.69)	0.00017	—	—	—	—	—
Phenothiazine	0.00303	0.7920	0.610	0.259	0.112	1.080
Phenothiazone	0.00323	0.726	—	0.108	—	—
Unknown IV (R_f = 0.35)	*0.00058*	*0.462*	—	—	—	—
Phenothiazine sulfone	0.00123	1.122	—	—	—	—
Phenothiazine sulfoxide	0.02027	1.452	0.391	0.297	0.649	0.460
Unknown VIII (R_f = 0.05)	0.00091	1.386	—	0.160	—	—
Polar (R_f = 0.0)	0.00133	1.650	0.469	0.286	0.400	0.150
Unextractable ^{14}C	0.0129	85.7	7.57	5.24	13.6	14.3
EM	1	261	201	85	37	356
BI		9.4	1.4	3.3	9.5	0.6
Clopidol						
Total extractable ^{14}C	0.01914	0.0218	0.0403	0.0150	0.0616	0.0056
Clopidol	0.00098	0.0218	0.0403	0.0150	0.0616	0.0056
α-Hydroxyclopidol	trace	—	—	—	—	—
Carboxylic acid derivative	trace	—	—	—	—	—
Polar	0.0014	—	—	—	—	—
Unextractable ^{14}C	0.01667	0.1524	0.0244	0.0741	0.0274	0.0784
EM	1	22	41	15	15	
EM	1	22	41	15	62	5
BI		—	—	—	—	—
Sulfamethazine						
Total extractable ^{35}S	0.03064	0.0171[b]	0.0115[b]	0.0028**		0.0008*
N^4-Methyl sulfamethazine	0.00320					
Sulfamethazine	0.01102					
N^4-Acetyl sulfamethazine	0.00375					
Unknown II (R_f = 0.33)[d]	0.00277					
Unknown III (R_f = 0.10)	0.00153					
Unknown IV (R_f = 0.05)	0.00192					
Polar	0.00403					
Unextractable ^{35}S	0.00242	0.0791	0.0214	0.0148	0.0205	0.0141

[a]Solvent system: benzene: acetone (7:3).
[b]Too low to analyze.
[c]Solvent system: hexane: toluene: acetone: methanol (10:7:2:1).
[d]Solvent system:

tition coefficient as shown in Figure 2. These values conform well to the predicted relationship (*9*). The correlation coefficient $r = 0.9207$ and the F value = 11.13 indicated a high degree of significance. Clearly, the bioconcentration of the chemicals by the fish is closely related to the lipid-partitioning properties of the chemicals.

The unextractable radioactivity of the organisms of the model ecosystem is a measure of the extent to which xenobiotic compounds are totally degraded *in vivo* and the radiolabeled atoms are reconstituted into tissue components. It has been shown that there is a high degree of negative correlation between ecological magnification of pesticides in model ecosystem biota and per cent unextractable radioactivity. DDE had the lowest value determined, 0.25%, and is well known to be virtually nondegradable in living organisms (*20*).

The values for the unextractable radioactivity for the drugs studied here are recorded in Table 2. Sulfamethazine, phenothiazine, and Clopidol had very high values and DES was intermediate.

Model Feedlot Ecosystem

We compared two modes of introducing DES into the ecosystem; oral dosing of mice (using olive oil), and subcutaneous injection of baby chicks (in propylene glycol).

At the conclusion of the 33-day experiment, in the mouse ecosystem DES constituted 17% of extractable radioactivity while in the chicken ecosystem DEA accounted for 25% of extractable radioactivity.

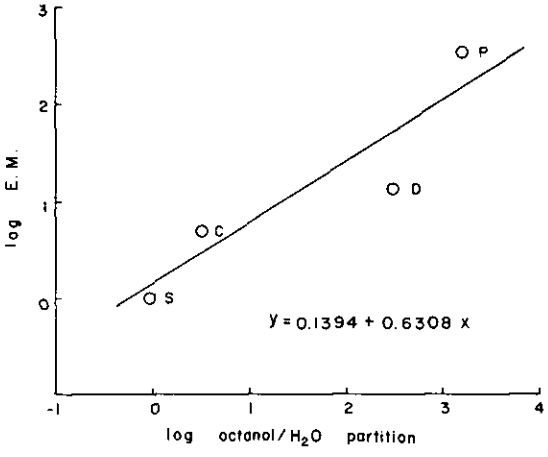


FIGURE 2. Relationship of log (EM) of fish in model aquatic ecosystem to log (octanol/water partition coefficient) for (S) sulfamethazine, (C) Clopidol, (D) diethylstilbestrol, and (P) phenothiazine. The correlation coefficient $r = 0.9207$, and $F = 11.13$.

The snails and fish in both systems accumulated DES, as well as more lipophilic metabolites corresponding in R_f value with acetylated DES and methylated DES. These data are presented in Tables 11 and 12. The other organisms in the ecosystem also contained some DES.

Phenothiazine was considerably more biodegradable as only 4% of the extractable ^{14}C was in the form of the parent molecule. Table 13 shows the amounts of phenothiazine and its metabolites (mostly sulfoxide and polar compounds) in the water. None of the organisms contained detectable levels of radioactivity on day 33 of the experiment further proving the ease of degradation of phenothiazine to polar nonaccumulating compounds.

Analysis of the water showed 16% of the extractable radioactivity was sulfamethazine; however it did not accumulate to a very large degree in any of the organisms. This may be attributed to sulfamethazine's moderately high water solubility and very low partition coefficient (see Table 2) which allow rapid elimination and minimal storage in lipoid tissues. The primary metabolite in the water is the N^4-acetyl sulfamethazine, while the organisms each contained some sulfamethazine, its acetylated and methylated derivatives as well as polar products (Table 14).

The Clopidol molecule is environmentally more stable than phenothiazine or sulfamethazine; in the model feedlot ecosystem there was nearly as much parent compound as polar metabolites present in the water. Table 15 shows the distribution of metabolites in the water of the Clopidol ecosystems; the α-hydroxylation product is the primary metabolite. The organisms concentrated the ^{14}C label in their tissues to the levels shown in Table 16. Bioconcentration to this degree is rather insignificant, inasmuch as autoradiography confirmed all the activity to be in the form of very polar metabolites; the one exception is that the snail contained an appreciable quantity of the carboxylic acid derivative of Clopidol, which is also quite polar. Apparently the Clopidol molecule is easily excreted by the organisms exposed to it in the model ecosystems; the moderately high water solubility and low partition coefficient are consistent with the observations that Clopidol is not accumulated in the body because it can be readily eliminated.

Reproducibility

The model feedlot ecosystem experiments with ^{14}C-Clopidol were performed independently in

Table 11. Distribution of ^{14}C-DES and its metabolites in a model feedlot ecosystem after oral dosing of male Swiss white mice.

	DES equivalents, ppb						
	Unhydrolyzed water	Hydrolyzed water	*Oedogonium* (alga)	*Daphnia* (daphnia)	*Culex* (mosquito)	*Physa* (snail)	*Gambusia* (fish)
Total extractable ^{14}C	0.037	0.078	9.2	13.8	20.8	11.5	12.6
Unknown I (R_f = 0.09)[a]	—	—	—	—	2.2	0.5	1.1
Unknown II (R_f = 0.73)	—	—	1.8	2.5	1.6	1.4	2.4
Unknown III (R_f = 0.63)	—	—	1.9	—	—	1.3	2.9
DES	0.0117	0.0078	3.2	2.2	—	0.7	0.7
Unknown VI (R_f = 0.43)	0.0124	—	—	1.9	5.7	1.8	0.9
Unknown VII (R_f = 0.35)	0.0041	—	—	—	1.9	1.3	2.2
Unknown VIII (R_f = 0.030)	0.0030	—	2.3	2.5	5.0	1.4	
Unknown XI (R_f = 0.10)	0.0024	0.025	—	—	1.7	0.8	1.1
Unknown XII (R_f = 0.05)	0.0034	0.023	—	2.5	—	1.0	—
Polar (R_f 0.00)	0.0005	0.022	—	2.2	2.7	1.4	1.2
Unextractable	—	0.298	—	—	—	—	—
EM	—	—	164	113	—	36	36
BI	—	—	0.33	1.9	4.5	2.0	0.76

[a] Solvent system: benzene: acetone (7:3).

Table 12. Distribution of ^{14}C-DES and its metabolites in a model feedlot ecosystem after subcutaneous injection of baby chicks.

	DES equivalents, ppb						
	Unhydrolyzed water	Hydrolyzed water	*Oedogonium* (alga)	*Daphnia* (daphnia)	*Culex* (mosquito)	*Physa* (snail)	*Gambusia* (fish)
Total extractable ^{14}C	0.05	0.14	22.3	31.1	23.1	70.7	5.3
Unknown I (R_f = 0.90)[a]	—	—	—	4.7	9.2	—	1.1
Unknown II (R_f = 0.73)	—	—	0.67	14	6.7	29.3	3.7
Unknown III (R_f = 0.63)	—	—	0.99	5.6	—	—	0.3
DES	0.010	0.039	8.76	1.71	1.16	32.3	0.09
Unknown V (R_f = 0.48)	0.0075	—	3.19	0.44	—	—	0.02
Unknown VI (R_f = 0.43)	0.0075	—	2.84	—	—	—	0.05
Unknown VII (R_f = 0.35)	0.0070	—	1.56	—	—	—	—
Unknown VIII (R_f = 0.30)	0.0115	0.018	1.40	—	—	—	—
Unknown IX (R_f = 0.25)	0.0019	—	0.65	—	—	—	—
Unknown XI (R_f = 0.10)	0.0017	0.034	2.25	4.4	—	—	—
Unknown XII (R_f = 0.05)	0.0012	0.021	—	0.6	2.61	—	—
Polar	0.0017	0.028	—	—	3.35	9.1	0.03
Unextractable ^{14}C	—	0.469	—	—	—	—	—
EM	—	—	179	35	24	659	1.8
BI	—	—	1.14	0.21	0.35	0.15	0.02

[a] Solvent system: benzene: acetone (7:3).

triplicate using three model ecosystems, each with three chicks fed 10 g of feed contaminated with ^{14}C-Clopidol at 0.0125% for 3 days. The three systems were assayed independently to measure the degree of replicatability of results. As shown in Tables 15 and 16, the replicates were in very good agreement, in fact beyond our expectations, especially since the degree to which the ^{14}C-contaminated chicken feed was spilled directly into the three systems was somewhat random and uncontrollable, despite every precaution to make feeding complete.

The maximum amounts of ^{14}C entering the water phase after feeding Clopidol for 3 days ranged from 0.16 to 0.20 ppm on day 26 (average 0.19 ppm) and declined to 0.13 to 0.18 ppm after 33 days. There was good consistency in the amounts of intact Clopidol and its α-hydroxy and carboxylic acid derivatives found in the water phase (Table 15). The agreement between the three replicated systems seems extraordinary considering the extremely small quantities detected. We conclude that the environmental parameters measured are basically functions of

Table 13. Environmental fate of ^{14}C-phenothiazine in the water of a model feedlot ecosystem introduced via mouse (oral dose) excrement.

	Phenothiazine equivalents, ppb
Total extractable ^{14}C	0.867
Phenothiazine	0.034
Unknown V (R_f = 0.27)[a]	0.060
Phenothiazine sulfone	0.130
Phenothiazine sulfoxide	0.251
Unknown VIII (R_f = 0.05)	0.101
Polar (R_f = 0.00)	0.288
Unextractable ^{14}C	5.33

[a]Solvent system: hexane: toluene: acetone: methanol (10:7:2:1).

Table 15. Environmental fate of ^{14}C-Clopidol in the water of a model feedlot ecosystem, introduced via baby chick excrement.

	Clopidol equivalents (3 replicates), ppm			
	I	II	III	Average (S.E.)
Total ^{14}C	160	180	130	157 ± 15
Clopidol	51	24	57	44 ± 10
α-Hydroxy Clopidol	30	16	16	21 ± 5
Unknown IV (R_f = 0.12)[a]	27	11	11	16 ± 5
Carboxylic acid derivative	23	12	15	17 ± 3
Polar (R_f = 0.00)	3.7	2.5	10	5.4 ±2.3
Unextractable ^{14}C	27	115	20	54 ± 31

[a] Solvent system: chloroform:ethanol:acetic acid (16:4:1).

Table 14. Environmental fate of ^{35}S-sulfamethazine in a model feedlot ecosystem, introduced via mouse (oral dose) excrement.

	Sulfamethazine equivalents, ppm						
	Unhydrolyzed water	Hydrolyzed water	*Oedogonium* (alga)	*Daphnia* (daphnia)	*Culex* mosquito	*Physa* (snail)	*Gambusia* (fish)
Total extractable ^{35}S	0.075	0.052	0.65	0.43	0.38	0.36	0.070
Unknown I (R_f = 0.57)[a]	0.0003	0.0002	—	—	—	—	—
N^4-Methyl sulfamethazine	0.0006	0.0005	0.078	0.170	0.075	0.057	0.0340
Sulfamethazine	0.016	0.0048	0.106	0.023	0.075	0.035	0.0158
Unknown II (R_f = 0.39)	0.003	0.0018	0.084	0.008	0.023	0.024	0.0031
N^4-Acetyl sulfamethazine	0.015	0.021	0.096	0.018	0.062	0.036	0.0063
Unknown III (R_f = 0.20)	0.0038	0.0016	0.094	0.002	0.028	0.028	0.0042
Unknown IV (R_f = 0.13)	0.0022	0.0042	0.048	0.011	0.031	0.020	0.0029
Unknown V (R_f = 0.02)	—	—	0.079	0.129	0.022	0.080	0.0047
Polar (R_f = 0.0)	0.034	0.018	0.065	0.070	0.068	0.085	0.0063
Unextractable ^{35}S	—	0.103	—	—	—	—	—
EM	—	—	5.1	1.1	3.6	1.7	0.76
BI	—	—	2.5	1.2	1.5	2.9	0.41

[a] Solvent system: diethyl ether:isopropanol (4:1).

intrinsic physical-chemical properties of the test compound (Fig. 2) and are relatively constant for a given compound.

Analysis of Model Feedlot Ecosystem Results

It can be concluded that chemicals of relatively high water solubilities and low partition coefficients do not accumulate to any great extent in the organisms of the 33-day terrestrial-aquatic model ecosystem. Such compounds tend not to be sequestered in fatty tissues and can be excreted rather easily by animals. Comparisions with data for industrial chemicals (*21*) and pesticides (*19,22*) indicate that compounds of lower water solubility and higher partition coefficients accumulate in organisms and biomagnify through food chains more than the chemicals evaluated here.

A second factor to consider is that of susceptibility to enzymatic degradation. The most lipophilic and least water-soluble compound we examined was phenothiazine. Despite its physical properties that could allow the compound to bioconcentrate and despite its having the highest EM in the short-term aquatic model ecosystem, phenothiazine failed to accumulate in the

Table 16. Levels of ^{14}C-label in organisms of a model feedlot ecosystem treated with ^{14}C-Clopidol.

	Clopidol equivalents (3 replicates), ppm			
	I	II	III	Average (S.E.)
Alga	2.58	1.07	0.88	1.51 ± 0.54
Daphnia	2.26	1.59	3.25	2.37 ± 0.48
Snail	1.57	1.74	1.91	1.74 ± 0.10
Mosquito	4.50	2.75	2.10	3.12 ± 0.72
Fish	0.38	0.66	0.31	0.45 ± 0.11
Water	0.16	0.18	0.13	0.16 ± 0.015

organisms in the 33-day model feedlot ecosystem. This was due to its oxidation to readily-excretable polar compounds by the mixed function oxidases of the organisms.

Clearly two parameters must be considered in any meaningful environmental evaluation of a chemical: (1) the water solubility/partitioning properties of the molecule and (2) functional groups present that will permit attack by degradative enzyme systems.

Conclusions

The model feedlot ecosystem is an adaptation of the terrestrial- aquatic model ecosystem (*12*) and has been designed to screen veterinary drugs and feed additives for persistence in the environment and for food chain biomagnification. The method is quite reproducible with respect to rate of metabolic breakdown and degree of bioaccumulation, as demonstrated by the three-replicate experiment utilizing Clopidol. In combination with the aquatic model ecosystem and metabolism cage studies, the model feedlot ecosystem provides a precise quantitative and qualitative evaluation of the environmental fate of the compounds tested.

Examination of four synthetic veterinary drugs with considerable differences in biological, chemical, and physical properties provided valuable information about the environmental properties of the compounds.

Diethylstilbestrol is more resistant to degradation by the mouse or the chick than the other three drugs, despite the lower doses of DES administered. A significant portion of the DES excreted persisted in the water and organisms as the parent molecule. In view of its potency as a feminizing hormone and its known human carcinogenicity, DES may present a significant degree of environmental hazard.

Clopidol is fairly stable environmentally but the parent molecule did not accumulate in any of the organisms in the model feedlot ecosystem. Therefore it is not likely to cause deleterious effects to nontarget organisms.

Sulfamethazine has the highest water solubility and lowest octanol/water partition coefficient of the four durgs studied. It is readily excreted rather than stored in the body and is also susceptible to metabolism by the organisms.

Phenothiazine is quite lipophilic but is extremely susceptible to sulfoxidation and ring hydroxylation by both enzymatic and light-catalyzed reactions. The resultant oxidation products are more water soluble and easily excreted. Because of its biodegradability, it poses little threat to the environment except for toxicity to some aquatic organisms (Table 3).

None of the drugs evaluated was as recalcitrant as an organochlorine pesticide, but there was considerable variation in accumulation and biodegradation of the four compounds. The partition coefficient seems to be a good parameter to predict the fact of an organic molecule in a model ecosystem. The model feedlot ecosystem appears to be a useful tool for screening new veterinary drugs and feed additives for potential persistence and biomagnification.

This research was supported by the U.S. Food and Drug Administration through Contract FDA-223-74-8251 "Evaluation of the utility of the model ecosystem for determining the ecological fate of substances subject to FDA regulatory authority".

The authors are grateful to Dr. Buzz Hoffman, contract adminstrator, and Dr. John C. Matheson for assistance in planning this project. We also thank Dr. R. F. Bevill and Dr. G. D. Koritz for advice on the metabolism of sulfamethazine and phenothiazine.

REFERENCES

1. Lunin, J. Agricultural wastes and environmental contaminants. In: Advances in Environmental Science and Technology, Vol. 2, J. N. Pitts and R. L. Metcalf, Eds., Wiley, New York, 1971.
2. Huber, W. G. Antibacterial drugs as environmental contaminants. In: Advances in Environmental Science and Technology, Vol. 2, J. N. Pitts and R. L. Metcalf, Eds., Wiley, New York, 1971.
3. Bush, E. T. General applicability of the channels ratio method of measuring liquid scintillation counting efficiencies. Anal. Chem. 35: 1024 1963.
4. Dodgson, K. S., et al. Studies in detoxication. 15. On the glucuronides of stilbestrol, hexostrol, and dienostrol. Biochem. J. 42: 357 1948.
5. Barnett, E. B., and Smiles, S. The intramolecular rearrangement of diphenylamine ortho-sulphoxides. J. Chem. Soc. 95: 1253 1909.
6. Mital, R. L., and Jain, S. K. Phenothiazine sulphones-synthesis and IR spectra. Indian J. Chem. 9: 539 1971.
7. Houston, D. F., Kester, E. B., and DeEds, F. Phenothiazine derivatives: mono-oxygenated compounds. J. Amer. Chem. Soc. 71: 3816 1949.
8. Nielsen, P. The metabolism of four sulphonamides in cows. Biochem. J. 136: 1039 1973.
9. Lu, P. Y., and Metcalf, R. L. Environmental fate and biodegradability of benzene derivatives as studied in a model aquatic ecosystem. Environ. Health Perspect. 10: 269 1975.
10. Kelly, R. G., et al. Determination of ^{14}C and ^{3}H in biological samples by Schoniger combustion and liquid scintillation techniques. Anal. Biochem. 2: 267 1961.
11. Kapoor, I. P., et al. Comparative metabolism of methoxychlor, methiochlor, and DDT in mouse, insects, and in a model ecosystem. J. Agr. Food Chem. 18: 1145 1970.
12. Metcalf, R. L., Sangha, G. K., and Kapoor, I. P. Model ecosystem for the evaluation of pesticide biodegradability

and ecological magnification. Environ. Sci. Technol. 5: 709 1971.
13. Fujita, T., Iwasa, J., and Hansch, C. A new substituent constant, π, derived from partition coefficients. J. Amer. Chem. Soc. 86: 5175 1964.
14. Richardson, T., and Todd, A. C. Elimination of phenothiazine by lactating dairy cows. Amer. J. Vet. Res. 19: 610 1958.
15. Collier, H. B., Allen, D. E., and Swakes, W. E. Observations on the fate of phenothiazine in domestic animals. Can. J. Res., D, 21: 151 1943.
16. Smith, G. N., and Watson, B. L. The metabolism of ^{36}Cl-Clopidol (3,5-dichloro-2,6-dimethyl-4-pyridinol) in rats. Poultry Sci. 48: 437 1969.
17. Cameron, B. D., Chasseaud, L. F., and Hawkins, D. R. Methabolic fate of Clopidol after repeated oral administration to rabbits. J. Agr. Food Chem. 23: 269 1975.
18. Bevill, R. F., et al. Disposition of sulfonamides in food-producing animals. I. Concentration of sulfamethazine and its metabolites in plasma, urine, and tissues of lambs following intravenous administration. Amer. J. Vet. Res., in press.
19. Kapoor, I. P., et al. Structure activity correlations of biodegradability of DDT analogs. J. Agr. Food Chem. 21: 310 1973.
20. Metcalf, R. L., and Sanborn, J. R. Pesticides and environmental quality in Illinois. Bull. Ill. Nat. Hist. Surv. 31(9): 381 1975.
21. Metcalf, R. L., et al. Uptake and fate of di-2-ethylhexyl phthalate in aquatic organisms and in a model ecosystem. Environ. Health Perspect. No. 4: 27 1973.
22. Metcalf, R. L., et al. Model ecosystem studies of the environmental fate of six organochlorine pesticides. Environ. Health Perspect. No. 4: 35 1973.