Radiotracer Distribution and Excretion Study of Chlorophenothane in Rats

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Abstract □ ^{14}C-Labeled chlorophenothane was acutely administered orally and intraperitoneally to rats to study the tissue distribution, rate of excretion, and effect of the route of administration. Five days after dosing, the fat was the tissue with the highest radioactivity irrespective of the route of administration. At the 0.05 significance level, the lungs, spleen, liver, kidneys, and testes of intraperitoneally dosed rats showed greater concentration than those orally dosed. The fecal excretion of the latter group was higher than those of the former group. The greatest percentage of radioactivity excreted in feces after oral administration occurred in the first 24 hr. Urinary excretion by the two routes was statistically the same. Negligible $^{14}CO_2$ was detected in the respired air. The results of this experiment support previously reported nonradiotracer studies.

Keyphrases □ Chlorophenothane (*p,p'*-DDT), radiolabeled—distribution, excretion, rats □ Radiolabeled chlorophenothane (*p,p'*-DDT)—distribution, excretion, rats □ Tissue distribution—radiolabeled chlorophenothane, rats □ Excretion studies—radiolabeled chlorophenothane, rats

Data on the accumulation of chlorophenothane[1] in animals following single and/or multiple doses have been reported in the literature. Distribution of chlorophenothane following a single dose (intravenous, oral, or intraperitoneal) in rat tissues was studied by Judah (1). Comparable data for repeated oral dosing of rats was reported by Deichmann *et al.* (2), Laug and Fitzhugh (3), and Ludewig and Chanutin (4). Similar data were also available for rabbits (1, 5–9), goats (10), dogs (11), cats (7), and turkeys (12). The amount of chlorophenothane stored in fat was always greater than in other organs of the same animal.

The methods used to determine chlorophenothane in the previous studies included the estimation of organically bound chlorine (7, 8) and color development with the xanthydrol–potassium hydroxide–pyridine procedure (13). Stiff and Castillo (9) failed to show chlorophenothane in tissues of animals using the latter method. Their findings were criticized by Judah (1) because they used the alcoholic saponification step, which was reported to destroy chlorophenothane to 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene (*p,p'*-DDE), a product that does not yield color with the xanthydrol–potassium hydroxide–pyridine method (14). Schechter and Haller (15) and Schechter *et al.* (16) reported a specific spectrophotometric method for the estimation of chlorophenothane and related compounds. The colors were developed by the interaction of the intensively nitrated compounds and methanolic sodium methoxide in benzene solution. Their method was applied to biological materials by Ofner and Calvery (5) and Judah (1). Laug (17) developed a biological assay method—depending on the toxic response of the housefly—to determine chlorophenothane content in animal tissues and excreta. Results of the bioassay method were in good agreement with those obtained using the chemical method of Schechter and Haller (15).

The development of highly sensitive detectors resulted in several GC methods for the estimation of chlorophenothane and related materials in biological tissues and fluids (18–27). Analysis of chlorinated insecticides in these biological samples by electron-capture GC requires several methods of extraction and cleanup which are time consuming (22–24), involve many pieces of glassware, suffer surface adsorption losses (18), and demand special solvent and equipment treatments before use (28). In some GC methods the procedures recommended were destructive to chlorophenothane (27). A high degree of technical skill, extreme care in preparation of apparatus (20), and pretreatment of samples before analysis (27) were among other requirements for the GC procedures.

Studies on the losses of pesticides during sample preparation were reported by Chiba and Morley (29). Filtration, partitioning, washing, concentration, and evaporation to low volume or dryness were examined separately. The losses of insecticide residues during filtration, washing, or partitioning were relatively small but cumulative and cannot be neglected. The most significant loss was caused by evaporation to dryness (29, 30). These methods were evaluated using ^{14}C-chlorophenothane (29–31) and conducting parallel GC and radiometric measurements (27). Application of ^{14}C-chlorophenothane demonstrated the failure of the colorimetric method of Schechter *et al.* (16) to extract 25% of chlorophenothane metabolites in bile and feces (32). Forty-four percent of the radioactivity in the bile was lost in the heating step for nitration, and an additional 10% of the radioactivity was retained by the sodium hydroxide wash step (33) of the same colorimetric method.

The literature shows that the use of ^{14}C-chlorophenothane to study the distribution of the pesticide in rats would be more specific and sensitive since the radiotracer technique is free from the problems of GC and colorimetric methods. Radioanalysis usually can be achieved at nanogram levels of the labeled compound (34). Furthermore, since traces of chlorophenothane could be found in almost all living organisms (27), the use of ^{14}C-chlorophenothane eliminates interference from the naturally occurring residues in contrast to the other two methods. The objective of this study was to use a radiotracer technique to: (*a*) determine the amount of chlorophenothane residues accumulated in 13 different tissues of adult rats after 5 days of a single oral or

[1] 1,1,1-Trichloro-2,2-bis(*p*-chlorophenyl)ethane (*p,p'*-DDT).

intraperitoneal dose, (*b*) determine urinary and fecal excretion, (*c*) determine excretion *via* exhaled carbon dioxide, and (*d*) determine the effect of the route of administration on any of these parameters.

EXPERIMENTAL

Liquid Scintillation Counting—An internal liquid scintillation spectrometer[2], equipped with bialkali phototubes, was used to assay for the ^{14}C activity present in the various samples. The scintillation solution used consisted of 0.4% 2,5-diphenyloxazole in an equal volume of toluene and 2-ethoxyethanol. The percent counting efficiency of each sample was determined by adding ^{14}C-toluene internal standard and recounting. Whenever practical, the samples were counted with less than 5% counting error at the 95% confidence level.

Radiochemical—The radiochemical purity of ^{14}C-chlorophenothane, ring uniformly labeled[3] (specific activity 3.85 mc./mmole), was determined by TLC and autoradiography. Twenty-four micrograms of labeled chlorophenothane (0.26 μc.) and 10 mcg. of carrier chlorophenothane[4], each dissolved in a benzene solution, were spotted on four TLC plates[5]. Each plate was developed in one of four solvent systems: *n*-hexane, *n*-hexane–chloroform (80:20), *n*-hexane–ethyl ether–acetic acid (90:10:1), and *n*-pentane–acetone (95:5). Chlorophenothane was located by visualizing under UV light and spraying with a silver nitrate chromogenic reagent as described by Bishara *et al.* (35). An amount of the labeled chlorophenothane solution equal to 1% of that originally spotted was placed on the upper right corner of each developed plate as a sensitivity spot. The plates were then exposed to X-ray film[6], with an exposure time sufficient to allow for 10^7 disintegrations from the sensitivity spots. The radioactive spots on the TLC plates were marked after superimposing the radiochromatogram on the plate. For determination of the radioactivity in the marked spot, the adsorbent layer was scraped and quantitatively transferred into a counting vial, which was then filled with thixotropic gel powder[7] and 15 ml. scintillation solution. The vial was allowed to stand at room temperature for a few hours with shaking on a mixer at intervals before being counted. The radiochemical purity of ^{14}C-chlorophenothane was found to be greater than 98.7%.

Radiorespirometry—Roth-type glass metabolism chambers[8] were used to draw air free of moisture and carbon dioxide through the chamber. Anhydrous calcium sulfate[9] and asbestos coated with sodium hydroxide[10] were used to remove the moisture and carbon dioxide, respectively, from the laboratory air. A flow rate of 500 ml./min. was maintained by a cartesian manostat[11]. Air leaving the chamber was again passed through anhydrous calcium sulfate before being bubbled through two gas scrubbing towers containing 100 ml. of a mixture of 2-ethoxyethanol and 2-aminoethanol (2:1) to trap respired carbon dioxide. The drying of the chamber exhaust air was to prevent water quenching while counting the samples. The second trap was included to ensure quantitative collection of carbon dioxide. However, the efficiency of the first trap approached 100% (36).

Preliminary Experiment—The purposes of this preliminary experiment were: (*a*) to obtain samples containing ^{14}C-chlorophenothane for investigating the application of three methods of tissue solubilization for liquid scintillation counting, (*b*) to have an idea about the effect of the dose chosen, and (*c*) to learn about the distribution of the activity among the different tissues and excreta in a short period of 24 hr. A male Sprague-Dawley strain descendent white laboratory rat[12] weighing 249 g. was dosed orally with 0.5 ml. ^{14}C-chlorophenothane in peanut oil equivalent to 10 mg. chlorophenothane and 5.3 μc. The animal was placed in a 20 × 11.5-cm. stainless steel metabolism cage[13] with food and water *ad libitum*. The rat was sacrificed 24 hr. after dosing by decapitation with a small guillotine[14], and the following organs were removed: heart, lungs, brain, spleen, testes, liver, muscle (left rear leg), epididymal and perirenal fat, adrenals, kidneys, stomach, intestine, and blood which was collected over 2 ml. of 4% sodium citrate. Feces and urine were collected every 6 hr. The excreta and all organs were kept in a freezer until analyzed.

Table I—Comparison of Tissue Solubilization Methods

Method	Percent Counting Efficiency	d.p.m./mg.	Tissue
Perchloric acid	55.2	42.76	Liver
Soluene	71.5	41.68	
Hyamine hydroxide	42.1	41.41	
Perchloric acid	55.6	28.17	Kidney
Soluene	73.3	22.96	
Hyamine hydroxide	40.4	26.10	
Perchloric acid	69.5	286.51	Epididymal fat
Soluene	68.9	296.28	
Hyamine hydroxide	48.9	323.43	
Perchloric acid	68.7	1.96	Blood
Soluene	57.4	3.15	
Hyamine hydroxide	36.0	1.94	

Replicates of 50-mg. portions of minced wet tissue samples were prepared for counting by three different methods, and the results of each replicate were averaged. The first procedure was a slight modification of the Mahin and Lofberg acid digestion method (37) using perchloric acid and hydrogen peroxide. To about 50 mg. of the tissue in a counting vial, 0.2 ml. of perchloric acid (70%) was added and mixed well to ensure thorough wetting of the tissue. Two-tenths milliliter of hydrogen peroxide (30%) was then added and the contents were mixed. The vials were put in the oven for 15 min. at 70–75° and then were cooled to room temperature. Another 0.5 ml. of hydrogen peroxide solution was added, and the samples were bleached for 30 min. in the oven. The vials were allowed to cool to room temperature and 15 ml. of the scintillation solution was added, mixed, and counted. In the second method, hyamine hydroxide[15] was used to solubilize the tissues as described by Rowles (38). The third procedure was performed as reported by Larsen (39) who used Soluene[16] to digest tissues. Blank samples for each of the vials from the three methods were prepared and used in determining the corresponding background.

Triplicates of 50 mg./organ, 100–300 mg. urine, and 20 mg. feces were digested and counted. The carcass was frozen in liquid nitrogen and then transferred to a blender[17]. Five hundred milliliters of water was added, and the carcass was blended for about 10 min. Ten aliquots, each weighing 250–300 mg., were digested. Similar amounts of ^{14}C activity were found in all replicates of the four (liver, kidney, epididymal fat, and blood) tissue samples used for comparison of the solubilizing methods. Table I summarizes the data obtained. Assumption of the stability of ^{14}C-chlorophenothane under the conditions of the perchloric acid–hydrogen peroxide method was made because of similar activity data obtained using the other two methods. The Soluene procedure required a longer digestion time than the previous method. Furthermore, a feathery white precipitate was collected on the bottom of the vial when left to stand. The hyamine hydroxide technique was also slow and had the lowest counting efficiency. For expediency, simplicity, and economy, the perchloric acid–hydrogen peroxide method was adopted for solubilizing the tissues in all animal studies. All organs and blood collected showed ^{14}C activity. The urine and the feces showed activity after 6 hr. The carcass samples indicated an average body retention of 83.5% of the administered dose. The dose administered did not elicit any toxic signs, and the solution in peanut oil did not cause diarrhea.

[2] Tri-carb model 2002, Packard Instrument Co., Downers Grove, Ill.
[3] Mallinckrodt-Nuclear, St. Louis, Mo.
[4] Aldrich Chemical Co., Milwaukee, Wis.
[5] Precoated 250-μ TLC plates, aluminum oxide (type E) F_{254}, Brinkmann Instruments, Inc., Westbury, N. Y.
[6] Kodax No-Screen Medical X-ray film, Eastman Kodak Co., Rochester, N. Y.
[7] Cab-O-Sil, Packard Instrument Co., Downers Grove, Ill.
[8] Delmar Scientific Laboratories, Maywood, Ill.
[9] Drierite, Hammond Drierite Co., Xenia, Ohio.
[10] Ascarite, Arthur H. Thomas Co., Philadelphia, Pa.
[11] Manostat Division of Greiner Scientific Corp., New York, N. Y.
[12] Laboratory Supply Co., Indianapolis, Ind.
[13] Acme Metal Products, Chicago, Ill.
[14] Harvard Apparatus Co., Dover, Mass.
[15] Hydroxide of Hyamine, 10-x, Packard Instrument, Downers Grove, Ill.
[16] Soluene, 100; Packard Instrument, Downers Grove, Ill.
[17] Waring Products Co., Winsted, Conn.

Tissue Distribution Study—A solution of ^{14}C-chlorophenothane was prepared in peanut oil so that each 0.5 ml. was equivalent to 12.9 μc. and 10 mg. chlorophenothane. Six male Sprague-Dawley strain descendent rats, weighing between 176 and 186 g., were used for this study. The rats were acclimatized to laboratory conditions and handling for a period of two days. Food[18] and water were allowed *ad libitum* until 24 hr. before dosing when the food was removed. Three rats were dosed orally, and the remaining three rats were dosed intraperitoneally. The administered dose of chlorophenothane represented 0.2 of the LD_{50} (40) to prevent any toxic manifestation and was calculated individually depending on the rat's body weight. The activity administered ranged from 11.15 to 11.66 μc. The rats were immediately housed in the glass Roth-type metabolism cages. Laboratory air was drawn through the system as previously described under radiorespirometry.

To ensure rapid absorption, the rats were denied food for 6 hr. following the dosing but water was allowed *ad libitum*. The traps were changed every 6 hr. during the 24-hr. carbon dioxide collection period. Triplicate aliquots of 3 ml. each from all collected traps were pipeted into a counting vial, 15 ml. of the scintillation solution was added, and the samples were counted. After the first 24 hr. the top of the glass metabolism chamber was removed and replaced by a 5-mm. mesh screen to keep the rats from leaving the metabolism chamber. Five days after dosing, the animals were sacrificed by decapitation with a small guillotine. The blood was collected into a beaker containing 2 ml. of 4% sodium citrate. The same 13 organs removed from the rat of the preliminary experiment were removed from all six rats of this experiment. The organs were rinsed with saline to remove excess blood and were then dried, weighed, and frozen until analyzed. The stomach and intestines were cut open and washed with saline to remove any contents. The washings were added to the carcass to allow for the material balance determination. Triplicates of 50 mg. of each tissue were solubilized using the perchloric acid–hydrogen peroxide method. The adrenals were assayed in duplicates (left and right adrenals). The carcass was homogenized as previously mentioned (preliminary experiment), and 10 aliquots of the homogenate were digested and counted. The glass metabolism cages were rinsed with 5 × 10 ml. water, and the wash was added to the carcass before homogenization.

Excretion Study—The excretion study was conducted simultaneously with the tissue distribution study. The same rats were used for the two studies, and data from both experiments were added together to calculate the material balance. Feces and urine samples were collected for each animal in tared vials at 6, 12, 18, 24, 30, 36, 42, 48, 60, 72, 84, 96, 108, and 120 hr. from dosing. The excreta samples were accurately weighed and frozen until analysis. The feces pellets were ground in a glass mortar, and triplicates were digested and counted. Urine samples were transferred to a counting vial by Pasteur disposable pipets[19].

RESULTS AND DISCUSSION

Tissue Distribution Study—Two of the three rats dosed orally showed tremors and some convulsions 2 hr. after ^{14}C-chlorophenothane administration. These symptoms continued for 6 hr. Similar observations were reported by Judah (1). The amount of ^{14}C activity detected in the carbon dioxide trapping solutions was very small. The mean cumulative expired $^{14}CO_2$ at the end of the 24 hr. following oral or intraperitoneal administration of ^{14}C-chlorophenothane was 0.010 or 0.007% of the administered dose, respectively. This was in agreement with Hayes' (41) conclusion that the degradation of chlorophenothane to carbon dioxide by roaches represented the most radical *in vivo* change of a residual organochlorine pesticide. On the other hand, Robbins and Dahma (42) found that less than 1% (0.5–0.6%) of the topically applied or injected ^{14}C-chlorophenothane was expired by roaches at $^{14}CO_2$.

The data concerning the tissue distribution of ^{14}C-chlorophenothane and its radioactive metabolites in rats 5 days after administration are presented in Table II. The data revealed that the tissue means of the rats dosed intraperitoneally were greater than the means of animals dosed orally. However, statistically significant differences (43) between the means of the two routes of administration at the 5% level were found only for lungs, spleen, liver, kidneys, and testes. The highest activity was found in the fatty tissue irrespective of the route of dosing. These findings are in complete agreement with previously reported work on the storage of chlorophenothane in the fat of the rat (11, 32, 44–49). McCully *et al.* (49) reported a greater concentration of total chlorophenothane residues in rat's fat and liver following intraperitoneal injection than by oral or intramuscular administration. The authors indicated that their results were preliminary observations and were not presented on a statistical basis. No data about the accumulation of chlorophenothane in the rat's adrenal gland were given by Judah (1). However, he reported that the rabbit adrenal showed a high concentration of chlorophenothane residue. The high level of ^{14}C activity in the rat's adrenal of this study was in complete support of the data of Ludewig and Chanutin (4). The average carcass activity retained by the three rats dosed intraperitoneally was 52.32% of the administered dose as compared to 41.60% for orally dosed animals. The standard errors of the means were 3.48 and 3.25, respectively.

Table II—Tissue Distribution of ^{14}C-Chlorophenothane and/or Its Radioactive Metabolites 5 Days after Administration to Rats

Tissue	Percent of Administered —Radioactivity per Gram of Tissue[a]— Oral Administration	Intraperitoneal Administration
Adrenal	0.37 ± 0.03	0.91 ± 0.48
Lung[b]	0.05 ± 0.00	0.07 ± 0.00
Heart	0.04 ± 0.01	0.05 ± 0.01
Spleen[b]	0.01 ± 0.00	0.10 ± 0.02
Liver[b]	0.11 ± 0.01	0.25 ± 0.03
Kidney[b]	0.03 ± 0.00	0.06 ± 0.00
Testes[b]	0.01 ± 0.00	0.03 ± 0.00
Muscle (left rear leg)	0.06 ± 0.01	0.08 ± 0.01
Stomach	0.45 ± 0.16	1.93 ± 0.88
Brain	0.02 ± 0.00	0.03 ± 0.00
Intestine	0.06 ± 0.00	0.17 ± 0.05
Epididymal fat	2.28 ± 0.33	2.26 ± 0.25
Perirenal fat	2.39 ± 0.29	3.86 ± 0.77
Blood	0.01 ± 0.00	0.02 ± 0.01

[a] Mean ± *SE* for three rats. [b] Significant difference between the means of the two routes of administration at the 5% confidence level.

Excretion Study—The urinary and fecal ^{14}C activity excreted by both groups of rats is presented in Table III. Elevated urinary excretion of orally dosed rats was noticed in the collected samples at 42 and 72 hr. after dosing with ^{14}C-chlorophenothane. This finding agreed with Smith and Stohlman (7), who reported the peak urinary excretion following a single oral dose of chlorophenothane to rabbits in 2–3 days. The total urinary elimination reported by the same authors ranged from 1.8 to 5.1% of the administered dose. The data from this study showed 2.63 and 1.86% of the administered dose eliminated by orally and intraperitoneally dosed animals, respectively. The difference between the two percentages was statistically nonsignificant (43).

The excretion of ^{14}C-chlorophenothane and/or its radiolabeled metabolites in feces clearly indicated a rapid elimination of about

Table III—Urinary and Fecal Excretion[a] of ^{14}C by Rats Dosed with ^{14}C-Chlorophenothane

Time[b]	—Oral Administration— Urine	Feces	Intraperitoneal Administration Urine	Feces
6	0.03 ± 0.00	—[c]	0.03 ± 0.00[d]	—[c]
12	0.06 ± 0.02	26.27 ± 13.08[d]	0.03 ± 0.01	0.07 ± 0.06
18	0.15 ± 0.04	11.89 ± 4.18	0.07 ± 0.02	0.40 ± 0.11
24	0.13 ± 0.03	2.48 ± 0.74	0.16 ± 0.04	0.44 ± 0.04
30	0.13 ± 0.07	0.99 ± 0.15[d]	0.06 ± 0.02	0.20 ± 0.10
36	0.13 ± 0.05	1.12 ± 0.57	0.04 ± 0.01	0.48 ± 0.05
42	0.42 ± 0.08	2.06 ± 0.58	0.11 ± 0.01	0.92 ± 0.09
48	0.17 ± 0.03	1.88 ± 0.34	0.08 ± 0.00	0.57 ± 0.02
60	0.21 ± 0.04	1.93 ± 0.50	0.16 ± 0.03	1.03 ± 0.15
72	0.50 ± 0.27	3.04 ± 0.53	0.17 ± 0.03	1.90 ± 0.34
84	0.13 ± 0.02	1.85 ± 0.24	0.45 ± 0.31	1.26 ± 0.17
96	0.24 ± 0.02	2.76 ± 0.74	0.14 ± 0.09	2.02 ± 0.37
108	0.10 ± 0.03	1.33 ± 0.20	0.10 ± 0.04	1.05 ± 0.18
120	0.22 ± 0.06	1.85 ± 0.12	0.26 ± 0.12	2.09 ± 0.42

[a] Mean ± *SE* for three rats. Values are percent of administered activity. [b] Hours after administration of ^{14}C-chlorophenothane. [c] Negligible activity (<0.0003). [d] One rat did not defecate or urinate.

[18] Wayne Lab-Blox, Allied Mills, Chicago, Ill.
[19] Fisher Scientific Co., Pittsburgh, Pa.

Table IV—Total Tissue Radioactivity 5 Days after Administration of ^{14}C-Chlorophenothane to Rats[a]

Tissue	Oral Administration	Intraperitoneal Administration
Adrenal	0.01 ± 0.00	0.02 ± 0.01
Lung	0.05 ± 0.01	0.07 ± 0.00
Heart	0.03 ± 0.01	0.04 ± 0.01
Spleen[b]	0.01 ± 0.00	0.10 ± 0.03
Liver[b]	1.27 ± 0.10	2.55 ± 0.34
Kidney[b]	0.05 ± 0.01	0.12 ± 0.02
Testes[b]	0.03 ± 0.00	0.09 ± 0.01
Muscle (left rear leg)	0.10 ± 0.02	0.24 ± 0.09
Stomach	0.85 ± 0.35	3.13 ± 1.64
Brain	0.03 ± 0.00	0.05 ± 0.01
Intestine	0.56 ± 0.13	1.13 ± 0.55
Epididymal fat	3.95 ± 0.39	5.18 ± 0.55
Perirenal fat	1.96 ± 0.28	2.25 ± 0.38
Blood	0.05 ± 0.00	0.15 ± 0.09
Total[b]	8.95 ± 1.13	15.11 ± 1.82

[a] Mean percent of administered dose ± *SE* for three rats. [b] Significant difference between the means of the two routes of administration at the 5% confidence level.

40% of the administered dose in orally dosed rats by the end of the first 24 hr. This amount was assumed to be excreted without absorption. The validity of this assumption was favored by the very low 24-hr. fecal excretion level (0.9%) of the intraperitoneally dosed rats. Slow absorption of chlorophenothane administered to the rat by stomach tube was reported by Judah (1). The author recovered 72-89% of the dose from the gut in 3 hr. Irregular and incomplete chlorophenothane absorption from the gut was reported by Smith and Stohlman (7), who recovered 50% of a single dose in feces. In this study, 59.44% of the administered dose was recovered from orally dosed animals at the end of 5 days after dosing. The activity recovered in the feces from intraperitoneally dosed rats for the same period was 12.44%. This recovery was higher than the value of 2.5% reported by Judah (1).

Since 40.6% of the orally administered dose was excreted in the feces in 24 hr., the amount available for absorption was in the proportion of 60:100 for orally and intraperitoneally dosed rats. The total tissue radioactivities reported in Table IV show 8.95 and 15.11% of dosed activity for oral and intraperitoneal routes, respectively. The relation between these two concentrations was 59.2:100, which accounted for the 40.6% excretion of activity discussed above. Despite the high tissue activity levels of the intraperitoneally dosed rats (Table II), presumably due to more complete absorption, the blood levels in the two groups were the same. This accounted for the similar urinary elimination (Table III) for for the two routes at the end of 5 days after dosing. The data of Table II show similar concentrations of the pesticide in the fatty tissue for both routes. This could be explained by the high affinity of chlorophenothane to be stored in fat. It would appear, therefore, that the fat levels of the orally dosed animals reached the same value of the intraperitoneally dosed rats at the expense of the other tissues.

The percent recovered activities (material balance) were 103.57 ± 6.73 and 81.74 ± 3.69 for the orally and intraperitoneally dosed animals, respectively. These data represent the mean ± standard error for the three rats per route of administration. The low recovery of activity in the intraperitoneally dosed rats may be due to the difficulty in obtaining true representative samples for counting because of the large volume of the homogenized carcass (500 ml.) with respect to the small counted aliquots (0.25 ml.). Such effect is not as drastic in the orally dosed carcass since more activity is excreted.

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Dissolution of Alkyl Vinyl Ether–Maleic Anhydride Copolymers and Ester Derivatives

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Abstract □ The dissolution properties of several alkyl vinyl ether–maleic anhydride copolymers and their ethyl, *n*-propyl, and *n*-butyl half- and quarter-ester derivatives were studied. An immersion refractometric method was employed to determine the polymer dissolution properties. Rates of polymer dissolution and lag times prior to the initiation of measurable dissolution rates were shown to be dependent upon several variables. The polymer variables studied included polymer molecular weight, degree of esterification, ester chain length, and ether substituent. Other factors studied were pH of the dissolution medium, type of sample (cast films or compressed disks), and inclusion of a plasticizing agent. Each factor was shown to influence the resulting polymer dissolution properties.

Keyphrases □ Dissolution rates, alkyl vinyl ethers–maleic anhydride copolymers and partial esters—determination, related to use as timed-release coatings □ Alkyl vinyl ether–maleic anhydride copolymers and partial esters—dissolution rates, related to use as timed-release coatings □ Maleic anhydride–alkyl vinyl ether copolymers and partial esters—dissolution rates, related to use as timed-release coatings □ Refractometry, immersion—determination of dissolution rates, alkyl vinyl ether–maleic anhydride copolymers

The existence of many commercial drug products containing similar amounts of identical active ingredients, but produced by different manufacturers and often having widely varying prices, has raised the question of generic equivalence of such products. Timed-release formulations command an expanding portion of the solid dosage form medications, but recent clinical evaluations have demonstrated vast differences in drug availability from competing products of the timed-release categories (1–5).

Recent experiments with copolymers containing maleic anhydride or maleic acid have indicated that polymers containing this chemical moiety may possess timed-release properties when used as coating materials. Nessel *et al.* (6, 7) demonstrated that the *n*-butyl half-ester of poly(methyl vinyl ether–maleic anhydride) copolymer was capable of producing a timed-release dosage form when coated on granules that were subsequently compressed into tablets. Wagner *et al.* (8) produced enteric disintegration properties from tablets coated with styrene–maleic acid copolymers. Lappas and McKeehan (9) found that partial esters of poly(methyl vinyl ether–maleic anhydride) might be suitable to control the release of drugs upon reaching a specific intestinal pH. Further studies (10) showed that some control of *in vivo* initial drug release could be obtained from such partial esters when applied as coatings to solid dosage forms.

Heyd *et al.* (11) recently reported fundamental studies concerning the surface phenomena associated with the dissolution of a model polymer series, ethylene–maleic acid, and reported in detail the use of immersion refractometry as a method of determining polymer dissolution parameters (12).

The investigations herein described were undertaken to elucidate the dissolution properties of the poly(alkyl vinyl ether–maleic anhydride) class of copolymers and their partial ester derivatives, with the ultimate objective of establishing the relationship between such properties and the usefulness of the various polymers as timed-release coating materials. The dissolution properties reported here were subsequently related to the *in vitro* and *in vivo* drug release characteristics from tablets coated with these polymers.

EXPERIMENTAL

Materials Studied—The polymers investigated were poly(alkyl vinyl ether–maleic anhydride) copolymers and selected partial ester derivatives synthesized in this laboratory. The general chemical structure of the anhydride copolymers is reproduced here, where R_1 was either a methyl or an isobutyl substituent. The copolymers were