

Table II. Taste Evaluation of Commercial Lemon, Persian Lime, and Lem'n Lime Juice Ades at a ° Brix:Acid Ratio of 14.4

Juice ade	Statistics of panel rankings		Flavor characteristics
	\bar{X}	S	
Commercial lemon	1.0	0	Good
Lem'n lime	2.9 ^a	1.2	Mild
Persian lime	5.0	0	Good

^a Significantly different at the 99.9% confidence level from either commercial lemon or Persian lime.

as the best means of obtaining ades of approximately equal strength. The three ades were used to evaluate the lemon-lime character of lem'n lime juice (see Table II). All panelists selected the lemon and lime ades correctly, and both are judged good flavors. Seven of 10 panelists judged lem'n lime to have combined lemon and lime flavor characteristics. This judgement is significantly different from each of the lemon and lime samples at the 99.9% confidence level (Miller and Freund, 1965).

Lem'n lime juice was markedly milder in flavor than either lemon or lime juices (see Table II) and some panelists detected an off-flavor that detracted from the observed lemon and lime flavor. The juice shows promise only as a mild lemon-lime flavoring, with perhaps more lemon-like than lime-like flavor characteristics. Essential oil from lem'n lime fruit does not show promise as an acceptable alternative to lemon-lime oil mixtures in flavoring food products. The presence of thymol and thymol methyl ether and the absence of neral and geranial in the essential oil cause a strong, non-citrus-type aroma that suggests its use in products other than those for which

strong lemon-lime aromas are desired such as pharmaceutical or cosmetic preparations.

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COMMUNICATIONS

Chemical Combinations of 2,4-Dichlorophenoxyacetic Acid (2,4-D) and Polystyrene. Preparation and Application for the Control of Duckweed

By the reactions of 2,4-D with functionalized polystyrene, the herbicide was bound to the polymer via the ester and anhydride bonds. The release of 2,4-D from these preparations was estimated by the effect on the growth of duckweed (*Lemna minor*) under laboratory conditions. The anhydride released the herbicide at a faster rate than the ester. The release from both these preparations was much slower than from a mechanical mixture of 2,4-D and polystyrene.

Combinations of biologically active compounds and polymers which provide controlled release of the active compounds over an extended period have received considerable attention in recent years (*Chem. Eng. News*, 1975; Allen et al., 1971). Such preparations are especially attractive when the active agent is an insecticide or a herbicide. An ideal preparation would reduce environmental hazards by preventing the flow of large amounts of the free agent in the soil or water. As the predetermined amount is released over a long period, frequent applications are unnecessary thus saving time and labor.

Two approaches have generally been used to prepare such combinations (Allen et al., 1971). One provides the release of the active agent by diffusion through polymers in which the agent is dissolved or encapsulated. The second approach provides the release by the breakdown

of a polymer (usually cellulose) containing the agent as a side chain. In this report, we wish to present our investigations where the agent is chemically bound to a non-degradable synthetic polymer by two different linkages. The purpose is to demonstrate the feasibility of obtaining control over the release by changing the nature of the covalent bond between the active agent and the inert polymer.

In our study, 2,4-dichlorophenoxyacetic acid (2,4-D) was chosen as the active agent due to its wide use as a herbicide. The carboxylic acid function provided a suitable means for its covalent bonding to the polymer matrix, which was cross-linked polystyrene. This polymer satisfied the main criterion, namely the ease of introducing different chemical functions in its backbone. It is known to readily undergo the usual nucleophilic aromatic substitution

reactions and for this reason has been the polymeric matrix of choice in related applications such as in solid phase peptide synthesis (Merrifield, 1963), ion-exchange resins (Wheaton and Bauman, 1951), and reagents in organic synthesis (Leznoff, 1974). By allowing the preformed polystyrene to react, in the solid state, polymers with different chemical functions and similar physical properties can be obtained. "Popcorn" polystyrene (Letsinger and Hamilton, 1958), a white, brittle, insoluble, and highly porous polymer with irregular shape, was used in the present work. The fundamentals of the controlled-release systems were investigated *in vitro* primarily for the control of aquatic weeds by using duckweed (*Lemna minor*) as an example. This weed is known to clog stagnant water bodies, such as ponds, and 2,4-D is commonly used to control this weed (Stovell, 1960; Breth, 1969).

EXPERIMENTAL SECTION

Preparation of 2,4-D-Polystyrene Resins. "Popcorn" Polystyrene. The copolymerization of styrene with 0.2% divinylbenzene was carried out according to the procedure described by Letsinger and Hamilton (1959). The polymer was washed repeatedly with chloroform, dried at 60°C *in vacuo*, and reduced to 40–100 mesh in a Waring blender.

Chloromethylated Polystyrene (I). To a stirred suspension of the resin beads (70.0 g) in chloroform (500 ml) at 0°C, a solution of anhydrous tin chloride (25 ml) in chloromethyl methyl ether (75 ml) was added over 30 min. The mixture was stirred in an ice bath for 1 h. The resin was separated by filtration, washed exhaustively with chloroform, dioxane, dioxane-water, water, dioxane, and chloroform, and dried at 60°C *in vacuo* (75 g; 1.5 mequiv of chlorine per g).

2,4-D Ester on Polystyrene (II). To a hot solution of 2,4-D (8.84 g, 40 mmol) and potassium hydroxide (2.24 g, 40 mmol) in methyl Cellosolve (250 ml) and water (25 ml), the chloromethylated resin beads (I, 25 g) were added along with triethylamine (2 ml). The mixture was stirred under reflux for 10 h. The beads were separated by filtration and washed completely with hot methyl Cellosolve, water, dioxane, and chloroform, then dried at 60°C *in vacuo* (27.5 g; 10% 2,4-D as evidenced by base hydrolysis with 2 N KOH in dioxane-water and recovery by chloroform extraction).

Succinylated Polystyrene (III). Polystyrene beads (20.0 g) were suspended in tetrachloroethane (40 ml). To the stirred suspension, powdered succinic anhydride (20 g) was added followed by a solution of anhydrous aluminum chloride in nitrobenzene and tetrachloroethane (100 ml each). More solvent (200 ml each of tetrachloroethane and nitrobenzene) was added and the mixture stirred at 85°C for 2 h. After cooling, ice water (300 ml) was added. The beads were separated by filtration, washed completely with dioxane-water, dioxane, and chloroform, and then dried at 60°C *in vacuo* (22 g, 2.0 mequiv/g of acid as evidenced by base titration and oxygen content).

2,4-D Anhydride on Polystyrene (IV). The succinylated resin (5.0 g) was suspended in benzene (50 ml) and oxalyl chloride (4 ml) was added dropwise at reflux. After 30 min, the acid chloride resin (ir, strong band at 1760 cm^{-1}) was washed with benzene and suspended in ethyl acetate (50 ml) and a solution of 2,4-D (1.77 g, 8 mmol) and triethylamine (0.8 g, 8 mmol) in ethyl acetate (50 ml) was added. After 1 h, the resin was removed by filtration and washed with ethyl acetate, dioxane, dioxane-water, and chloroform (5.5 g, 10% 2,4-D as evidenced by base hydrolysis).

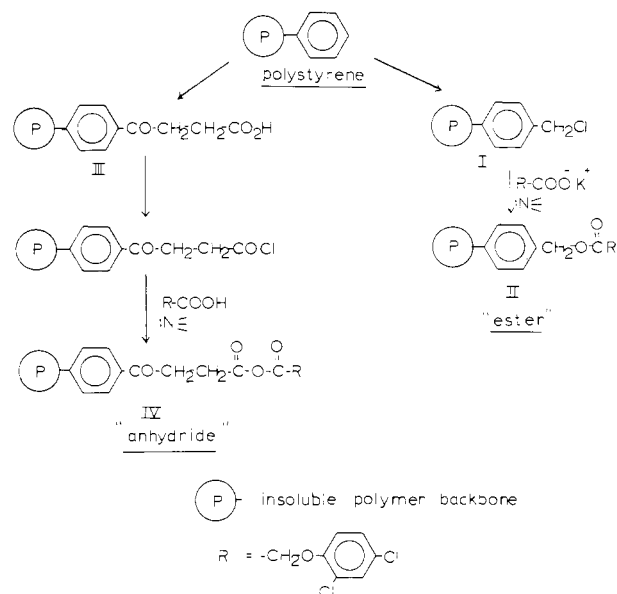


Figure 1. Covalent bonding of 2,4-D to polystyrene.

Growth Studies. The *Lemna* cultures were grown in 50 ml of sterile Hillman M medium (Hillman, 1961), adjusted to pH 4.8 with NaOH, in 125-ml Erlenmeyer flasks. These cultures were maintained at $25 \pm 2^\circ\text{C}$ under continuous illumination consisting of cool white fluorescent light. Various 2,4-D preparations were aseptically added to the culture medium. The culture medium was not replenished during the course of the experiment. Each culture was inoculated with 4 fronds from 10-day-old stock cultures.

After 7 and 14 days of incubation, eight cultures were harvested for each treatment and the number of fronds counted. Any degree of frond development was scored as a whole frond. The frond number for each culture was calculated as a percentage of the control value prior to determining the SE of the mean. Control cultures, containing unmodified medium, were processed as above.

RESULTS

"Popcorn" polystyrene was prepared by copolymerization of styrene with small amounts of divinylbenzene (0.2%) according to the procedure described by Letsinger and Hamilton (1959). Three 2,4-D-polystyrene preparations were used in the study. The reaction scheme is shown in Figure 1.

Unbound Herbicide or "Mixture". This preparation, which consists of 2,4-D uniformly dispersed in the polymer beads (40–100 mesh), was obtained by allowing the resin to swell in chloroform solution of the herbicide and then slowly removing the solvent under reduced pressure in a flash evaporator. A mixture consisting of 10% unbound herbicide was obtained.

2,4-D Ester on Polystyrene (II). The ester bond was formed by the treatment of chloromethylated polystyrene (I) with the potassium salt of 2,4-D in the presence of a tertiary amine. The product was completely washed with organic solvents in a soxhlet to remove any traces of the free acid. The ir spectrum was typical of esters (strong absorption at 1735 and 1175 cm^{-1}) and the preparation contained 9–10% of the active compound.

2,4-D Anhydride on Polystyrene (IV). The preparation was carried out according to the procedure described by Shambhu and Digenis (1973) for benzoic acid. The only modification was the use of ethyl acetate as the solvent. The ir spectrum of the product exhibited the anhydride

Table I. Effect of 2,4-D-Polystyrene Combinations on the Growth of *Lemna minor* under Laboratory Conditions

Concn of 2,4-D in the medium, ^a ppm	Observed growth of <i>Lemna minor</i> ^{b,c}					
	After 7 days			After 14 days		
	Ester bound 2,4-D	Anhydride bound 2,4-D	2,4-D and polystyrene mixture	Ester bound 2,4-D	Anhydride bound 2,4-D	2,4-D and polystyrene mixture
10	92	65	44	98	49	26
30	96	53	24	94	20	10
60	84	50	0	82	18	0
90	80	40	0	75	13	0
150	75	36	0	63	10	0
300	73	21	0	60	0	0

^a Ppm of 2,4-D in the medium obtained by adding the required amount of the resin beads. ^b Expressed as percent of control = (no. of fronds in flasks with resin beads)/(no. of fronds in flasks (no 2,4-D)) × 100. ^c Results within ± 3.

carbonyls at 1750 and 1715 cm⁻¹. The final product contained between 8 and 11% herbicide.

Growth Regulator Studies. Control cultures were grown for 14 days to select the appropriate growth periods to assess the activity of polymer preparations. Eight cultures were harvested each day and frond production was determined. Such data revealed that significant growth of minimal variability occurred after 7 days; hence this period was selected to evaluate the immediate release of herbicide from various 2,4-D preparations. A second growth assay was taken after 14 days. This interval permitted an assessment of longer term growth. Beyond the second assay period, growth in the control cultures was extensive and could not be measured accurately under the experimental conditions.

Comparative weedicidal action of the three 2,4-D polymers estimated under laboratory conditions in *L. minor* is illustrated in Table I. Growth in *Lemna* could be observed only at concentrations below 10 ppm of free 2,4-D in the medium. Unbound herbicide or "mixture" incorporated into the culture medium produced growth effects similar to those observed with free 2,4-D.

Little retardation of *Lemna* growth resulted by adding 60 ppm of the ester-bonded 2,4-D to the culture medium, as judged by frond production. Increasing the concentration up to 300 ppm did not significantly enhance growth inhibition. *Lemna* frond production after 7 and 14 days of growth was inhibited only 73 and 60%, respectively (Table I). The results of exposing *Lemna* to anhydride-bonded 2,4-D do not parallel any of the above preparations. Addition of 10 ppm of this sample to the growth medium caused approximately 40% growth inhibition. *Lemna* was adversely affected at higher concentrations.

DISCUSSION

The 2,4-D-polystyrene preparation, where the acid is not chemically bound ("mixture"), shows little, if any,

sustained release properties as the results are parallel to those obtained with free 2,4-D. Ester preparation II shows little release when up to 60 ppm of this resin is present in the medium in the chemically bound form. With an excess of the ester-bonded 2,4-D (300 ppm), the little retardation of growth could be due to release of only a small amount of the herbicide. The activity exhibited by anhydride preparation IV lies between the above two extremes, the release being faster than that for ester and considerably slower than that for the mixture. Growth inhibition values for the second assay period are significantly higher than those for the first assay period in all anhydride-bonded 2,4-D resins, indicating continuous release of the herbicide. The half-lives of carboxylic anhydrides in aqueous media are known to be on the order of a few hours (Bunton and Perry, 1960). The effectiveness of the 2,4-D anhydride resin even after 7 days indicates that the release of the herbicide into the medium is controlled not only by the rate of hydrolysis but also by other slow processes such as diffusion through the polymer matrix.

The preliminary results presented here indicate that chemical bonding to an insoluble undegradable support might be a potentially useful technique of synthesizing sustained release preparations. Admittedly, the method is limited to active compounds with suitable chemical functions such as a carboxylic acid. A number of herbicides and drugs (including antibiotics) do possess this function (Beasley and Collins, 1970). Polystyrene is a nontoxic material and is not known to have any undesirable effect on the environment when present in small amounts.

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