Synthesis and Studies with 2-C^{14}-Labeled Bromacil and Terbacil

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The half life of 2-C^{14}-labeled bromacil and terbacil in soil was determined to be approximately 5 to 6 months when 4 pounds of active ingredient per acre was applied to the surface of Butlertown silt loam. About 90% of the residual activity recovered from the soil in these field tests was intact bromacil and terbacil. In laboratory studies, 25 to 32% of the total applied C^{14}-activity was lost as $C^{14}O_2$ from soil in 6- to 9-week exposures. In orange seedlings, bromacil was the principal residue in the plants. 5-Bromo-3-*sec*-butyl-6-hydroxymethyluracil and a few related minor metabolites have been identified by thin-layer chromatography as metabolites of bromacil in both soil and orange plants. No 5-bromouracil or 5-chlorouracil was detected as a metabolite of bromacil or terbacil, respectively, in these soil and plant studies.

Bromacil (Hyvar X, E. I. du Pont de Nemours and Co., 5-bromo-3-*sec*-butyl-6-methyluracil) is an effective herbicide for the control of deep-rooted perennial broadleaf and grass weeds. Terbacil (Sinbar, E. I. du Pont de Nemours and Co., 3-*tert*-butyl-5-chloro-6-methyluracil) is an effective herbicide for the control of annual and perennial weeds. Residue tolerances have been established covering the use of terbacil for weed control in sugar cane, citrus fruits, peaches, apples, and pears. Bromacil and terbacil residues can be determined by microcoulometric gas chromatography according to the procedures described by Pease (1966, 1968). Results of mammalian metabolism studies on bromacil in the rat and terbacil in the dog are reported by Gardiner *et al.* (1969) and Rhodes *et al.* (1969)

This paper reports the syntheses of 2-C^{14}-labeled bromacil and terbacil and the results of studies of the disappearance and metabolism of these C^{14}-labeled herbicides in soils and their uptake and distribution in plants. Both compounds were labeled in the 2-position, so that the C^{14}-label would be retained by all metabolites in which the uracil ring had not been degraded.

SYNTHESES AND ANALYSES

Bromacil and terbacil were synthesized by four-step reactions from potassium cyanate-C^{14} with over-all yields of 18.6 and 5.1%, respectively. The labeled starting material, potassium cyanate-C^{14}, was obtained from New England Nuclear Corp.

Bromacil-2-C^{14}. Bromacil was prepared by a four-step synthesis which proceeded through the intermediates, *sec*-butylurea-2-C^{14}, ethyl 3-(3-*sec*-butyl-2-C^{14}-ureido)crotonate, and 3-*sec*-butyl-6-methyluracil-2-C^{14}. The reaction sequence is shown in Figure 1.

STEP 1. *sec*-Butylamine (0.966 gram, 13.2 mmoles) was weighed into a 15-ml. test tube fitted with a ground glass jointed gas inlet-outlet head. The tube and contents were chilled in a dry ice–acetone bath for about 3 minutes. To this, while stirring with a Nichrome wire, was slowly added 0.873 ml. of 12.6*N* hydrochloric acid.

The vial containing the 0.595 gram (7.33 mmoles, 11 mc.) of potassium cyanate-C^{14} was opened and 0.296 gram (4.05 mmoles) of nonradiolabeled potassium cyanate was added. Water (2.0 ml.) was added and solution was effected with the aid of an eye dropper drawn to a capillary.

This solution was transferred to the reaction tube containing the *sec*-butylamine hydrochloride. The vial was washed twice with 0.5-ml. portions of water, which were also added to the reaction tube. The reaction mixture was stirred with the wire and warmed in a water bath to ca. 40° C. Solution occurred, and the wire was withdrawn and washed with 0.5 ml. of water. The tube and contents were then heated on a steam bath for 45 minutes. A slow stream of nitrogen was passed over the contents of the tube to sweep out any gas that might have formed during the reaction. The effluent gas gave a negative test for radioactivity. The solution was chilled for 46 minutes in an ice-acetone bath and then sucked damp dry with a filter stick. The solid was washed with 0.3 and then 0.2 ml. of cold water. The tube containing the damp solid was placed in a drying pistol overnight at room temperature over P_2O_5 under vacuum (ca. 20 mm.). A 75.5% yield (0.964 gram) of 1-*sec*-butylurea-2-C^{14} (m.p. 164.5–68° C.) was obtained.

STEPS 2 AND 3. The total quantity of *sec*-butylurea-2-C^{14} prepared above (0.964 gram, 8.30 mmoles) was transferred to a

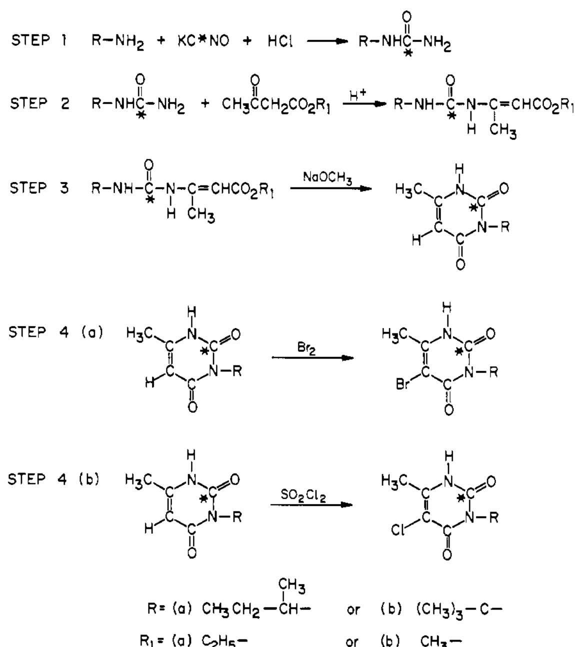

Figure 1. Synthesis of 2-C^{14}-labeled bromacil and terbacil

a. Bromacil
b. Terbacil

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200-ml. round-bottomed flask with the aid of 25 ml. of warm benzene. To this slurry were added 1.445 grams (11.1 mmoles) of ethyl acetoacetate and 21.6 mg. of *p*-toluenesulfonic acid. The vial used to weigh out the ethyl acetoacetate was washed with 5 ml. of benzene, which was added to the reaction vessel. The mixture was stirred and refluxed for 10 hours. The water given off by the reaction was removed by a modified condenser–water trap. A few particles of calcium chloride were present in the trap to help prevent small water droplets from passing through. Foaming was controlled with a foam breaker attached to the stirrer. At the outset and toward the end of this condensation reaction, a slow stream of nitrogen was passed through the reaction flask to sweep out any gas that might have formed. The effluent gas gave a negative test for radioactivity. The cooled reaction mixture which contained the ureido intermediate was allowed to stand overnight.

The condenser–water trap was removed and a solution of 1.0 gram (18.5 mmoles) of sodium methoxide, dissolved in a total of 6.0 ml. of absolute ethanol, was added. The condenser–water trap was cleaned and replaced. The mixture was stirred, refluxed for 45 minutes, then cooled. Thirty milliliters of water was added along with a little activated charcoal, and the mixture was stirred to allow the product to dissolve in the aqueous phase. The entire mixture was filtered through a sintered glass funnel and the aqueous phase separated. This contained the 3-*sec*-butyl-6-methyluracil-2-C^{14} in solution as the sodium salt.

STEP 4. The solution of 3-*sec*-butyl-6-methyluracil-2-C^{14} sodium salt was placed in a 150-ml. five-necked flask fitted with a magnetic bar stirrer, pH probe, condenser, thermometer, and 5-ml. buret with a needle valve. The volume was made up to 50 ml. with water. The pH was adjusted by dropwise addition of sulfuric acid with stirring to 4.60. The pH probe was removed, and washed by adding 10 ml. of acetic acid to the reaction solution. The redox probe was put in place, and the mixture was heated by a mantle to 85° C. When this operating temperature was reached, the buret containing bromine was put in place. Bromine was added in small increments until the reaction was complete as measured potentiometrically. A total of 0.575 ml. (1.80 grams, 11.25 mmoles) of bromine was used. The redox probe was removed and washed with water, and the reaction mixture allowed to cool slowly to 5° with stirring. The product crystallized during the cooling period and the reaction fluid was removed with a filter stick. The damp solid was washed with 10 ml. of water and then dissolved in 15 ml. of 1*N* sodium hydroxide. Impurities were extracted with 25 ml. of benzene, and the aqueous phase was treated with Darco and filtered. The basic aqueous phase was gradually acidified with 6*N* hydrochloric acid with stirring, precipitating 5-bromo-3-*sec*-butyl-6-methyluracil-2-C^{14}. The aqueous solution was removed with a filter stick and the solid was washed with 10 ml. of water. After drying, a sample of the 0.612 gram yield of crude product melted at 151–54° C. The total sample was dissolved in 12.5 ml. of warm acetonitrile, treated with activated carbon, and filtered. The filtrate was concentrated and dried under a slow stream of N_2. The yield was 0.578 gram (m.p. 152–57°). This was recrystallized with 1.5 ml. of absolute ethanol, sucked dry with a filter stick, and dried overnight in a drying pistol at room temperature under vacum (ca. 20 mm.). The yield was 0.425 gram (m.p. 156–58° C.). An additional crystallization from 0.8 ml. of ethanol gave 0.349 gram of 99.9% radiochemically pure bromacil-2-C^{14} with a specific activity of 1.13 mc. per mmole (sample I), 11.0% of the theoretical yield. The product (m.p. 157–58.5° C.) was identical to reference-grade bromacil.

Residual amounts of bromacil-2-C^{14} left in the combined mother liquors from the above recrystallizations were recovered by the addition of 1.0 gram of pure, nonradiolabeled bromacil, followed by recrystallization from nitromethane or ethanol. Sample II consisted of 0.939 gram of bromacil-2-C^{14} (m.p. 156.5–58° C.), specific activity 0.233 mc. per mmole, 99.9% radiochemical purity, 6.1% of theoretical yield. Sample III, obtained in a similar manner from the mother liquors from sample II, consisted of 1.024 grams of bromacil-2-C^{14} (m.p. 157–58.5° C.), specific activity 0.0555 mc. per mmole, 99.8% radiochemical purity, 1.5% of theoretical yield.

The total per cent of theoretical yield obtained, based on C^{14}-activity, was 18.6%. The reported values for radiochemical purity of all samples were determined by thin-layer chromatography, radioautography, and liquid scintillation counting.

Terbacil-2-C^{14}. Terbacil-2-C^{14} was prepared by a four-step synthesis which proceeded through the intermediates *tert*-butylurea-2-C^{14}, methyl 3-(3-*tert*-butyl-2-C^{14}-ureido)crotonate, and 3-*tert*-butyl-6-methyluracil-2-C^{14}. The reaction sequence is shown in Figure 1. This procedure in general is the same as that employed for making bromacil-2-C^{14}. The lower yield of terbacil-2-C^{14} obtained was probably due to the greater steric hinderance of the *tert*-butyl group in comparison with the *sec*-butyl group, especially during step 1 of the synthesis.

STEP 1. To dilute the potassium cyanate-C^{14} (0.770 gram, 33 mc., >97% radiochemical purity) to approximately 1 mc. per mmole, 1.907 grams of nonradiolabeled potassium cyanate was added. These materials were dissolved in 5.2 ml. of warm water.

The *tert*-butylurea-C^{14} was prepared in a jacketed, fritted, filter funnel. With a positive air pressure on the underside of the frit, 4.3 ml. of water and 3.20 grams of sulfamic acid were placed in the funnel. The funnel was swirled for a few seconds to accomplish solution. An ice water flow was started through the jacket of the funnel, and 3.86 ml. of *tert*-butylamine was added dropwise to the solution on top of the frit. A steam flow was started through the jacket of the funnel, and the entire mixture was stirred with a thermometer until the temperature reached 80° C. The steam flow through the funnel jacket was stopped and the warm solution of potassium cyanate-C^{14} was added quickly to the funnel, followed by 2-ml. portions of wash water to complete the transfer. To complete the reaction, the mixture was slowly stirred and steam-heated for 3 minutes. At this point the reaction mixture was cooled to 0° C. with ice water. The white solid precipitate which had formed was filtered off and washed with two 5-ml. portions of ice water. The remaining crude *tert*-butylurea-2-C^{14} was dried further by a brief passage of steam through the funnel jacket, then dissolved on the frit by methanol, and finally filtered through the frit into a filter flask. The filtrate was transferred to a sublimation apparatus and the methanol removed under a nitrogen stream while the apparatus was warmed by the vapor of boiling methanol. The resulting crude *tert*-butylurea-2-C^{14} was purified by sublimation in vacuo during an 8-hour period with heating by a 120–25° C. oil bath and cooling of the condenser by tap water (ca. 15° C.). The sublimate was removed from the condenser by methanol and the methanol solution was evaporated to dryness under a nitrogen stream in a tared 50-ml. Soxhlet flask. The flask and contents were dried for 15 hours to afford 1.08 grams of *tert*-butylurea-2-C^{14}.

STEPS 2 AND 3. These steps were performed in a micro-Soxhlet extraction apparatus. To the Soxhlet flask containing the 1.08 grams of *tert*-butylurea-2-C^{14} were added 20 ml. of xylene, 1.00 ml. of methyl acetoacetate, 2 drops of concentrated sulfuric acid, and a Teflon-coated magnetic stirring bar. The Soxhlet extraction tube was filled with Drierite. Rapid stirring was started. A 70-mm. vacuum was applied and maintained by a manostat. The mixture was heated and allowed to boil rapidly under reflux for 3 hours. During the reaction, the Drierite in the extraction tube removed the water produced by the reaction. The vacuum was then released and the reaction mixture was treated with a solution of 0.61 gram of sodium methoxide in 3.7 ml. of methanol, followed by an additional 1.8 ml. of wash methanol. The methanol was removed by distillation during a 30-minute period. The remaining xylene mixture was cooled to room temperature, treated with 5.9 ml. of water, and stirred to obtain two clear layers. The aqueous layer was drawn off in a separatory funnel, washed with ether, cooled to 0° C., and acidified with concentrated hydrochloric acid to precipitate the 2-C^{14}-uracil as a white solid. This product was collected by filtration at 0° C. on a tared funnel, washed with ice water, sucked dry, and then dried overnight to give 0.63 gram (37%) of 3-*tert*-butyl-6-methyluracil-2-C^{14}.

STEP 4. The 0.63 gram of 3-*tert*-butyl-6-methyluracil-2-C^{14} obtained in step 3 was dissolved on the funnel in a minimum of hot chloroform and filtered into a 100-ml. round-bottomed flask, fitted with aTeflon-coated magnetic stirring bar. A total of 0.32 ml. of sulfuryl chloride was added and the solution was stirred for 30 minutes at 40° C. Evaporation of the chloroform solvent under a stream of nitrogen gave a solid, which was dried for 3 hours.

The solid was treated with 6.3 ml. of ether and 6.3 ml. of water; then, with stirring and cooling, 27 drops of 50% sodium hydroxide solution was added. This mixture was transferred to a 125-ml. separatory funnel with the aid of ether and water. Shaking the funnel gave two clear layers. The aqueous layer was withdrawn, washed with ether, cooled to 0° C., and acidified with concentrated hydrochloric acid to precipitate a white solid which was collected by filtration, washed with water, sucked dry, and dried overnight to afford 0.47 gram (62.5%) of the chlorinated, radiolabeled uracil. This material was dissolved in 60 ml. of boiling ether, the solution filtered, and the filtrate diluted with 50 ml. of hexane. Slow evaporation of the solution to a volume of 30 ml. by gentle warming caused white crystals to deposit. The mixture was cooled for a few minutes (to 0° C.) and the white crystals were filtered off, washed with cold hexane, and dried. The 0.42 gram of radiolabeled uracil thus obtained was recrystallized from ether-hexane to afford 0.301 gram (4.1% of theoretical yield) of terbacil-2-C^{14} (sample A). All filtrates were retained.

To the combined filtrates, 1.30 grams of pure, nonradiolabeled terbacil was added, and the mixture was recrystallized from ether-hexane to produce 1.268 grams (1.0% of theoretical yield) of terbacil-2-C^{14} (sample B). Thus, the total per cent of theoretical yield obtained in this synthesis, based on C^{14}-activity, was 5.1%.

PRODUCT PURIFICATION BY COLUMN CHROMATOGRAPHY. Because of the presence of a very small amount of an unidentified radiochemical impurity (of high R_f value relative to the uracil product), it was necessary to purify the product further.

A slurry of 50 grams of silica gel (Will Scientific Co. No. WI85893) in 100 ml. of chloroform was poured into a column (30-mm. diameter) and packed to give a 15-cm. length of silica gel, which was topped with 1 cm. of sand. The column was washed by elution of 500 ml. of chloroform.

Sample A of terbacil-2-C^{14} was dissolved in a small amount of chloroform and placed on the column and the pure sample was eluted from the column (following the impurity) by successive use of chloroform, acetone, and methanol. Sample B was chromatographed as above on the same size and type of column as used for sample A. The purified sample A was 99.8% radiochemically pure and had a specific activity of 0.894 mc. per mmole. Purified sample B was 98.2% radiochemically pure and had a specific activity of 0.0532 mc. per mmole.

LABORATORY EXPOSURE OF 2-C^{14}-LABELED BROMACIL AND 2-C^{14}-LABELED TERBACIL TO MOISTURE, LIGHT, AND NONSTERILE SOIL

A two-piece glass apparatus was constructed as follows. The lower section consisted of a 4-inch length of tubing, having a diameter of 3 inches. It was closed at the bottom with a disk containing 16 0.5-cm.-diameter holes. The top section, containing a quartz thimble for an ultraviolet-source and inlet and exit leads for the passage gases, was closed at the top. The open end of each section was flat-ground to permit joining and sealing with an O-ring.

The bottom section of the vessel was packed with tillable Keyport silt loam to a depth of 5 inches and was forced into an external bed of Keyport soil to a depth of about 1 inch. The top and bottom sections were joined, the soil inside and outside the vessel was thoroughly wetted, and the whole apparatus was equilibrated for 7 days to allow the soil to settle. After the equilibration period, 10.80 mg. of 2-C^{14}-labeled bromacil having a specific activity of 0.900 μc. per mg. was placed on the soil surface. The total radioactivity applied was 9.72 μc., and the application rate corresponded to about 20 pounds of bromacil per acre. An ultraviolet source in the form of a mercury vapor tube was inserted into the quartz thimble directly over the soil surface to get low intensity ultraviolet radiation into the vessel. The top and bottom sections of the apparatus were rejoined, and external air was drawn through the vessel and over the soil surface using an aspirator. A small gas bubbling tower filled with water was used to saturate the inlet air with moisture before it entered the vessel. Two small traps containing 5 ml. of 2.5*N* sodium hydroxide were inserted in series in the exit line.

During the 9-week exposure of the experiment, the outside soil was wetted every 2 days to keep the soil column inside the vessel moist. In addition, the apparatus was opened periodically to apply water directly to the soil surface. Over the 9-week period, the equivalent of 1.6 surface inches of rain was applied directly to the soil. The ultraviolet light was operated for 8 hours a day, 5 days a week. Aliquots of the sodium hydroxide in the gas traps were analyzed periodically for C^{14} by liquid scintillation counting.

The procedure described above was repeated with 2.30 mg. of 2-C^{14}-labeled terbacil having a specific activity of 4.13 μc. per mg. However, 30 ml. of 1.0*N* sodium hydroxide was used in the gas traps and a third and final trap was added, preceded by a tube packed with cupric oxide. This tube was heated to 750° C. to oxidize any possible C^{14}-containing fragments. The total activity applied was 9.50 μc. and the application rate corresponded to 4.5 pounds of terbacil per acre. The exposure period for the terbacil experiment was 6.5 weeks.

Analysis of Gas Traps. For the periodic analysis of the gas traps, 1 ml. of the base from each trap was added to a 20-ml. scintillation vial filled with Cab-O-Sil and 15 ml. of

Table I. Per Cent Applied C^{14} Present in Various Fractions

	CO_2 Traps	Soil	% C^{14} in Soil Extracts as Intact Herbicide
Bromacil-2-C^{14}	25.3	47.5	>94
Terbacil-2-C^{14}	32.0	57.8	78

scintillator solution. Results of these tests are given in Table I. For each compound, the contents of the gas traps were shaken with ethyl acetate and were treated with Ba^{2+}. These results indiate that $C^{14}O_3{}^{2-}$ was the only C^{14}-containing material in traps and that, therefore, $C^{14}O_2$ was the gas evolved from the systems. Nothing was found in the third trap after the oxidizing furnace in the terbacil experiment.

Extraction of Treated Soils. The soil inside the vessel and the soil outside were wet ball-milled for 2 hours, spread on large trays to air dry, then passed through a 10-mesh screen. Duplicate 3-gram aliquots of each soil sample were analyzed for total C^{14} by combustion techniques according to the procedure of Smith *et al.* (1964). These data are also given in Table I. The total over-all recovery for bromacil is believed to be lower than that for terbacil because of the relatively inefficient gas-trapping system used in the bromacil experiment.

Fifty-gram samples of the soil were extracted by shaking with 150 ml. of 80% ethanol in a wrist-action shaker for 48 hours. The soils were rinsed with a second 150-ml. portion of 80% ethanol. The combined extracts from each sample were taken to dryness, then made to 2.0 ml. with 80% ethanol for TLC analysis.

Thin-Layer Chromatography. Thin-layer plates of kieselgel (250-micron thickness) were spotted with 10-μl. aliquots of each extract and with 10 μl. of a reference solution of the appropriate herbicide. The plates were developed to 10 cm. in chloroform and then to 10 cm. in ethyl acetate. The R_f values for bromacil and terbacil under these conditions were 0.53 and 0.44, respectively. The adsorbent was removed from each plate in 1-cm. increments starting 0.5 cm. below the origin. Each increment was assayed for C^{14} by liquid scintillation counting methods, the silica gel being added directly to a dioxane-based scintillation solution in a counting vial. Samples were counted in a Nuclear-Chicago Model 6801 liquid scintillation system. The per cent herbicide in the extract was determined by dividing the activity in the increment which corresponded in R_f to the reference material by the total activity applied to the plate. The results are shown in Table I.

EXPOSURE OF 2-C^{14}-LABELED BROMACIL AND 2-C^{14}-LABELED TERBACIL TO FIELD CONDITIONS

Soil Treatment and Sampling. During the spring of the year, six 12-inch sections of 4-inch-diameter stainless steel tubing were driven into the ground (Butlertown silt loam) to isolate undisturbed columns of soil. About $^1/_2$ inch of each cylinder was left protruding above the ground surface. The area within the cylinders was cleared of vegetation and debris, and 5-ml. aliquots of a 50% methanol solution containing radiolabeled bromacil were added to three of the cylinders and solutions of radiolabeled terbacil were added to the other three cylinders. The active material was watered into the soil with five separate rinses, which in total were equivalent to about 0.25 inch of rain. A stainless steel, 8-mesh screen was suspended above the samples to break up large raindrops and prevent splash. The level of herbicide treatment was 3.69 mg. (8.10 μc.) of bromacil and 3.45 mg. (14.25 μc.) of terbacil. These correspond to an application level of about 4 pounds per acre.

Table II. Per Cent of Original C^{14} Activity in Various Soil Fractions

Soil Depth, Inches	Bromacil 5-week exposure	Bromacil 14-week exposure	Bromacil 1-year exposure	Terbacil 5-week exposure	Terbacil 14-week exposure	Terbacil 1-year exposure
0–1	34.2	25.2	4.3	17.4	46.2	4.5
1–3	24.0	17.7	7.1	24.8	17.5	3.2
3–5	9.6	12.5	5.9	11.8	5.6	2.7
5–8	0.7	5.8	4.6	12.1	1.3	3.3
8–12	0.3	1.8	1.6	6.3	0.2	0.8
Total	68.8	63.0	23.5	72.4	70.8	14.5

One cylinder each was dug up after 5 weeks' exposure to field conditions, while another was left in the field for 14 weeks. A third cylinder of each treatment remained in the field for one year. The soil in each cylinder was removed for analysis in the following increments as measured from the surface: 0 to 1, 1 to 3, 3 to 5, 5 to 8, and 8 to 12 inches. All increments were wet-ball-milled for 2 hours, spread on large trays to dry, then passed through a 10-mesh screen.

Analysis and Results. Duplicate 3-gram aliquots of each soil increment were analyzed for total C^{14} by the wet combustion method (Table II).

In a separate experiment using unlabeled bromacil and terbacil, field plots were treated at the rate of 4 pounds per acre. The soil (0- to 4-inch depth) from these plots was analyzed for bromacil and terbacil residues, one year after treatment, by the gas chromatographic methods of Pease (1966, 1968). These analyses showed that 0.44 p.p.m. of bromacil and 0.21 p.p.m. of terbacil remained in the soil after one year. These results compare favorably with values calculated from the radiochemical data in Table II, 0- to 5-inch depth, which indicated residues of 0.49 and 0.30 p.p.m., respectively, after one year. These latter values were calculated by assuming that the entire C^{14}-residue was either bromacil or terbacil.

The soil increments from each cylinder were extracted and analyzed for the intact herbicides as described for the laboratory soils. About 90% of the total activity in the extracts was bromacil and terbacil, even after field exposures of up to 1 year.

The soil extract from the 0- to 1-inch layer of the 5-week bromacil sample was also analyzed for metabolic breakdown products of bromacil. The soil extract was cochromatographed with metabolites isolated from rat urine (Gardiner *et al.*, 1969). Five microliters of the soil extract and 1 to 10 μg. of each of the rat urine metabolites were spotted as one spot in the corner of a TLC plate. The plate was developed for 10 cm. in ethyl acetate, rotated 90°, and developed in 0.15N sodium chloride for 10 cm. The locations of nonradioactive reference spots of bromacil and its metabolites were detected under short wavelength ultraviolet light and marked on the plate. The TLC plate was then placed next to Ansco Non-screen x-ray film for 3 weeks to allow the C^{14} activity to expose the film (Figure 2). The dotted circles indicate the location of the reference compounds; with but one exception the dark, radioactive areas can be identified as falling with one of the reference spots. The areas of the plate, which showed exposure on the radioautogram, were removed from the plate

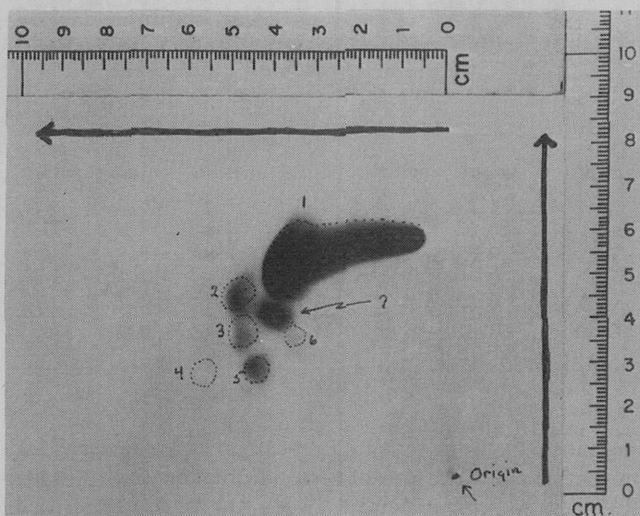

Figure 2. TLC radioautogram of bromacil soil extract

Spot 1. Bromacil
Spot 2. 5-Bromo-3-*sec*-butyl-6-hydroxymethyluracil
Spot 3. 5-Bromo-3-(2-hydroxy-1-methylpropyl)-6-methyluracil
Spot 4. 5 - Bromo - 3 - (2 - hydroxy - 1 - methylpropyl) - 6 - hydroxymethyluracil
Spot 5. 5-Bromo-3-(3-hydroxy-1-methylpropyl)-6-methyluracil
Spot 6. 3-*sec*-Butyl-6-methyluracil
Spot 7. Unidentified

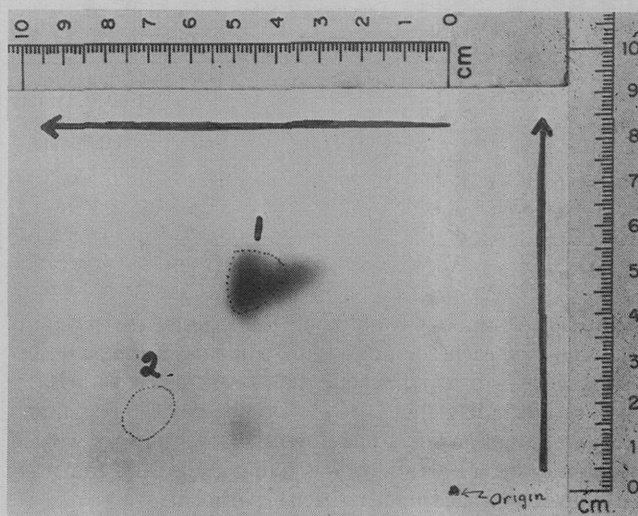

Figure 3. TLC-radioautogram of extract of terbacil-treated soil

(0-1 inch depth, 5-week exposure in field)
Spot 1. Terbacil
Spot 2. 5-Chlorouracil

and analyzed by liquid scintillation counting for total C^{14} (Table III).

The principal soil residue was tentatively identified as bromacil by TLC procedures as shown. To confirm this identification, the isolated material was eluted from the TLC plate with acetone which was evaporated. The infrared spectrum of the isolated material was identical to the spectrum of reference-grade bromacil.

The extracts of the 0- to 1-inch and the 8- to 12-inch increments from the 5-week soil cylinders from both the bromacil and the terbacil test were analyzed for the possible presence of C^{14}-labeled 5-bromouracil and 5-chlorouracil, respectively. Ten-microliter aliquots of each extract and 5 μl. (ca. 10 μg.) of the appropriate nonradiolabeled reference compounds were spotted as a single spot in a corner of the TLC plate for cochromatography. Each plate was developed in the first dimension for 10 cm. in chloroform, for 5 cm. in 3 to 1 ethyl acetate–methanol, and finally for 10 cm. in 10:10:1 ethyl acetate–chloroform–methanol. The R_f values for 5-bromouracil and 5-chlorouracil were approximately 0.3 with this combination of solvents. The TLC plates were then rotated 90° and developed in H_2O for 10 cm.; the R_f values for 5-bromouracil and 5-chlorouracil were 0.75 and 0.70, respectively. The silica containing the 5-bromouracil (or 5-chlorouracil) spots, as detected under ultraviolet light, was scraped from the plates and analyzed for total C^{14} by liquid scintillation counting. For the 5-bromouracil analysis, a counting rate of 28 c.p.m. was obtained for the 0- to 1-inch soil increment and 28 c.p.m. for the 8- to 12-inch soil increment. For the 5-chlorouracil analysis, 32 c.p.m. was obtained for the 0- to 1-inch increment and 29 c.p.m. for the 8- to 12-inch increment. Background counting levels in the presence of some added silica from the TLC plates were 28 and 29 c.p.m., respectively.

These results show that the extracts contain no C^{14}-labeled 5-bromouracil or 5-chlorouracil, since essentially background counting rates were observed in the treated samples. The sensitivity of the analyses was estimated at 0.01 p.p.m., and this sensitivity was demonstrated by recovery studies on soil fortified with either 2-C^{14}-labeled 5-bromouracil or 5-chlorouracil. Recovery data are given in Table IV and a typical radioautogram is presented in Figure 3. Thus, C^{14}-labeled 5-bromo- and 5-chlorouracil are not produced as a result of the metabolism of C^{14}-labeled bromacil and terbacil in soil.

Table III. Relative Amounts of Bromacil and Its Metabolites in 0- to 1-Inch Soil Layer after 5-Week Exposure

Area No.	Tentative Identification	Estimated P.P.M. in 0–1 Inch Soil Layer[a]	% of Total Activity Found on TLC Plate
1	Bromacil	4.9	89.5
2	5-Bromo-3-*sec*-butyl-6-hydroxylmethyluracil	0.14	2.5
3	5-Bromo-3-(2-hydroxy-1-methylpropyl)-6-methyluracil	0.06	1.1
5	5-Bromo-3-(3-hydroxy-1-methylpropyl)-6-methyluracil	0.09	1.7
7	Unidentified	0.28	5.5

[a] Estimated by assuming all compounds have same specific activity as bromacil-2-C^{14}.

UPTAKE AND METABOLISM OF 2-C^{14}-LABELED BROMACIL BY YOUNG ORANGE TREES

Two-year-old Hamlin orange trees, grafted on sour orange stock, were transplanted to 4-liter stainless steel beakers with

Table IV. Recoveries of 2-C^{14}-Labeled 5-Bromouracil and 5-Chlorouracil from Fortified Soil Samples

P.P.M. Added to Soil: 5-Bromo-	P.P.M. Added to Soil: 5-Chloro-	Background C.P.M.	C.P.M. in TLC Spot	% Recovery
0.27	—	28	9763	53.9
0.027	—	30	713	39.8
0.013	—	28	250	27.8
—	0.12	29	5045	45.5
—	0.012	29	465	46.3

1-inch holes punched in the bottoms. The bottoms of the beakers had been covered with glass wool and gravel and the beakers had been filled with washed quartz sand. The plants were maintained for several weeks with Nutri-leaf nutrient solution containing iron chelate.

Battery jars were modified, so that gas inlets and outlets were attached. The equipment was designed to isolate not only the plant but also the aerial and root sections of the plant from each other. A Lucite plate with a hole in it to pass the stem of the plant separated the upper and lower jars. Mortite caulking compound was used to seal the gaps between the sections and the section around the stem. A small stand supported the plant above the level of the nutrient solution. A monitoring device was inserted into one of the upper battery jars, which turned on an external water spray whenever the temperature exceeded 90° F. The lower jars were wrapped with aluminum foil and black polyethylene sheets to minimize growth of algae in the jars.

Tygon tubing connected each upper jar to a series of three traps. Each trap was fritted and contained 100 ml. of 1*N* NaOH. A hot tube furnace between the second and third trap in each line contained a 12-inch section of 1-inch-diameter Vycor tubing filled with cupric oxide which was maintained at 500° C. The flow rates were monitored with Brooks flowmeters, tube size R 215-A. Flows averaged 0.5 cu. foot per hour. A volume equivalent to the volume of the battery jars passed through the upper sections every 3 hours.

The nutrient solution was recirculated through the sand every 3 days by using a peristaltic pump to transfer the solution through external Tygon tubing.

Duplicate experiments were performed where 10.3 mg. of bromacil-2-C^{14} (21.7 μc.) was added to 1000 ml. of the nutrient solution. The specific activity of the bromacil used in this work was 4677 d.p.m. per μg. The plants were placed in the lower battery jars, the C^{14}-containing nutrient solution was added, and the system was assembled and sealed. Run 1 ran for 27 days, while run 2 ran for 28 days.

The sodium hydroxide traps were analyzed for C^{14}-content at the conclusion of each run. No activity was found in any of the traps indicating that bromacil is not metabolized to CO_2 in the foliar portion of the plants.

After the termination of the experiment, the leaves were pulled off the orange tree and examined separately. The stem was divided into an upper and lower portion, where the lower portion consisted of everything from the sand surface to just under the first branch. The root was pulled out of the sand and rinsed with water, which was allowed to drain back into the sand.

The leaves from each plant were dried on paper towels, weighed, and placed in a blender with 100 ml. of 80% ethanol for 15 minutes. The extract was decanted and the residue was blended with a second portion of 80% ethanol. The residue from this step was air-dried and analyzed by wet combustion techniques for unextracted C^{14}-activity. The 80% ethanol extract was evaporated to dryness in the hood and the residue was dissolved in a known volume of methanol. The extract was analyzed for C^{14} content.

The upper and lower stems and the roots from each plant were chopped into very small pieces no more than 0.75 sq. cm. in size and were extracted and analyzed according to the procedure described for the leaves. The total C^{14} found in each plant part is given in Table V.

The extracts were spotted on TLC plates and cochromatographed with nonradioactive reference materials as previously described. The developed plates were then exposed to Ansco Nonscreen x-ray film for 3 weeks. The developed x-ray film showed the presence of three C^{14}-labeled compounds, which were shown by TLC to be bromacil, 5-bromo-3-*sec*-butyl-6-hydroxymethyluracil, and an unknown metabolite in the approximate ratio of 10:5:1. The film showed no exposure in the 5-bromouracil area. An illustrative radioautogram for the root extract is shown in Figure 4. The area of the TLC plate containing the 5-bromouracil reference spot was scraped from the plate and counted as previously described. The 5-bromouracil spot contained 31 counts per minute and a background contained 29 counts per minute.

Table V. C^{14}-Residue in Orange Plant

Tissue	Total Activity Detected, μc.		C^{14} Residue, P.P.M.[a]		
	Run 1	Run 2	Run 1	Run 2	Av.
Root	0.870	0.570	10.6	6.6	8.5
Lower stem	0.100	0.041	1.3	0.6	1.0
Upper stem	0.022	0.005	1.9	0.3	1.1
Leaves	0.046	0.012	1.8	0.7	1.2

[a] Expressed as p.p.m. bromacil.

DISTRIBUTION OF 2-C^{14}-LABELED TERBACIL IN SUGAR CANE FOLLOWING INJECTION

Three series of sugar cane plants were grown in the greenhouse to a height of about 3 feet for use in this study. Each series consisted of five plants. One series was used as an untreated control, a second was treated at 100 μg. of 2-C^{14}-labeled terbacil per plant, and the third was treated at 210 μg. per plant.

Radiolabeled terbacil was introduced through a small core which was removed from each plant at the fifth node. The labeled terbacil, in 10 μl. of methanol, was injected into the hole and the opening was covered with lanolin. After treatment, the plants were allowed to grow in the greenhouse for 3 weeks, then harvested by cutting the stalk at ground level. The leaves were removed from each plant and the stalks were cut into 2-inch pieces. The corresponding parts from each plant in a series were combined to form one sample for analysis. The stalk samples were pressed to remove the juice from the

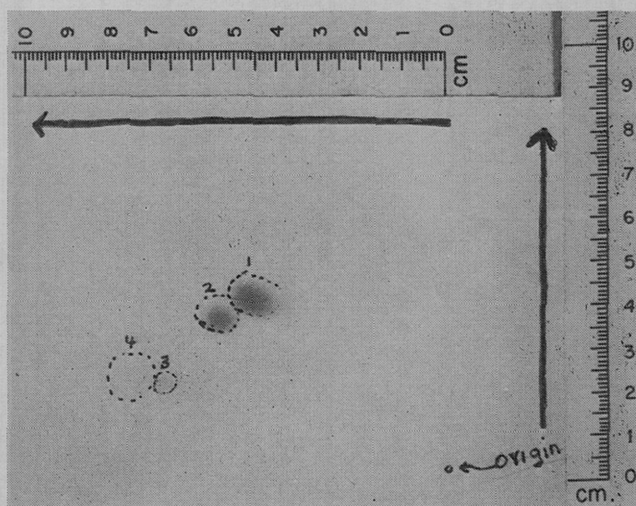

Figure 4. TLC radioautogram of orange root extract

Spot 1. Bromacil
Spot 2. 5-Bromo-3-*sec*-butyl-6-hydroxymethyluracil
Spot 3. Unknown metabolite
Spot 4. 5-Bromouracil

Table VI. Per Cent of Applied C^{14} Found in Sugar Cane Parts

Treatment	Juice	Pulp	Leaves	Recovery
100 μg.	4.0	7.7	88.3	87.9
210 μg.	1.9	7.3	90.8	93.3

pulp. Aliquots of the juice, pulp, and leaves from each series were analyzed for total C^{14} by combustion-liquid scintillation counting techniques (Table VI).

One hundred grams of the leaves were extracted with methanol in a Soxhlet extractor for 24 hours. The extract was taken to dryness under a hood and the residue was picked up in 10 ml. of methanol. Aliquots of the extract and residue were analyzed for total C^{14}; 88.3% of the activity in the leaves was extracted. The extract was analyzed for 5-chlorouracil by the procedure previously described; no 5-chlorouracil was detected in the extract. The background counting rate was 39 c.p.m. and the 5-chlorouracil test spot gave a counting rate of 41 c.p.m.

DISCUSSION

These studies have shown that 25.3% of the bromacil and 32.0% of the terbacil on soil samples treated at 20 and 4.5 pounds per acre, respectively, were decomposed to $C^{14}O_2$ under laboratory conditions in 9 and 6.5 weeks, respectively, and that the activity remaining on the soil was essentially all unaltered herbicide. Bromacil and terbacil had a half life of approximately 5 to 6 months on soil samples in field plots treated with the labeled herbicides at a rate of 4 pounds per acre. Analyses on the treated soils showed that about 90% of the residual activity recovered from the soil was bromacil and terbacil even after one-year exposure. Traces of four metabolites of bromacil were detected in the soil. Three have been identified by TLC cochromatography as 5-bromo-3-*sec*-butyl-6-hydroxymethyluracil, 5-bromo-3-(2-hydroxy-1-methylpropyl)-6-methyluracil, and 5-bromo-3-(3-hydroxy-1-methylpropyl)-6-methyluracil. No 5-bromouracil or 5-chlorouracil was formed as a metabolite of these herbicides.

These data indicate that one mode of degradation of these compounds proceeds through hydroxylation of the side chain alkyl groups, presumably followed by ring opening and then complete metabolism to carbon dioxide, ammonia, and hydrobromic or hydrochloric acid. The relatively short half life of these compounds on soil and the low concentrations of metabolites present suggest that the rate of ring opening and subsequent degradation is faster than the rate of side chain hydroxylation or other alteration on the ring structure.

Orange plants maintained for 4 weeks in sand on a nutrient solution containing 10 p.p.m. of bromacil-2-C^{14} took up less than 5% of the applied activity. Approximately 83% (8.5 p.p.m.) of this activity was found in the roots and 17% (1.1 p.p.m.) in the stem and leaves of the plant. Three C^{14}-labeled compounds were found in the plant extracts in a relative ratio of 10:5:1. The major component was identified by TLC cochromatography as bromacil and the major metabolite as 5-bromo-3-*sec*-butyl-6-hydroxymethyluracil. The minor metabolite has not been identified, but it was definitely shown that it was not 5-bromouracil.

Three weeks after injection of 100 or 210 μg. of terbacil-2-C^{14} into young sugar cane plants, about 90% of the recovered activity in the plants was in the leaves. The juice and pulp of the plant contained 3 and 7%, respectively. These data indicate that terbacil is translocated upward into the leaves by the plant, and that even if the herbicide is injected directly into the stalks, virtually all of the terbacil (or its possible metabolites) is found in the foliar portions of the plant after a short growth period. Analyses of plant extracts show that 5-chlorouracil is not formed as a metabolite of terbacil in sugar cane.

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