Fate of Buturon-^{14}C in Soil during Seven Seasons of Exposure under Outdoor Conditions

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Buturon (ring ^{14}C) was applied to wheat and soil in two successive years (3 kg/ha each year) at outdoor conditions. Residual radioactive substances in the upper soil layer (0–10-cm depth) were measured 1.5, 2.5, 3.5, 6.0, and 6.5 years after the first treatment. The decline of total radiocarbon was a two-stage process. Mass balance studies were conducted 1.5, 2.5, and 6.5 years after the first treatment; total recoveries were 83, 47, and 27%, respectively, of the applied radioactivity. Extractability of residues with cold chloroform was 20–30% of total residues. Whereas the chloroform-soluble conversion products identified after the first growing period mostly resulted from alterations within the side chain of buturon, after seven seasons only hydroxylated and/or methoxylated derivatives of 4-chloroaniline could be detected.

Buturon [*N*-(*p*-chlorophenyl)-*N'*-methyl-*N'*-(1-methyl-2-propynyl)urea], a urea herbicide, is mainly used on winter wheat and has little use on other crops against various weeds. Therefore, the study of its residual behavior in soil is important with respect to the quality of agricultural soils and, thus, also to the quality of human food of vegetable origin. Laboratory as well as outdoor studies on its fate in microorganisms and soil have been reported. Studies with the fungus *Rhizopus japonicus* resulted in an elimination of the isobutinyl group from the molecule (Wallnöfer et al., 1973). In laboratory experiments with plant–soil systems and buturon-^{14}C (Schuphan, 1977; Ebing and Schuphan, 1979; Haque and Schuphan, 1977), several metabolites were identified, which all resulted from alterations of the side chain of buturon. Removal of the complete side chain, resulting in 4-chloroaniline, was not observed. Similarly, in outdoor studies conducted in this Institute for one growing period (Haque et al., 1977), various conversion products were isolated from soil and identified. Most of these, also, were only derivatives containing the side chain of buturon in an altered form; 4-chloroaniline was present only as a trace metabolite.

No information is available thus far on further degradation pathways of these products in soil within longer time periods. Isolation of conversion products from soil after an exposure of more than one growing period and their identification have not been reported. Similarly, mass balance studies have been reported only for a 3-year period after application (Haque et al., 1977; Haque and Schuphan, 1977). Although buturon is regarded as a nonpersistent pesticide, conversion products were formed in soil, which persisted for several years. Therefore, the outdoor experiments reported were continued for a total of 6.5 years. The mass balance of radioactivity was studied, and the radioactive substances were isolated and identified. The results are described in this paper.

MATERIALS AND METHODS

Apparatus. Radioactivity measurements were performed in liquid scintillation counters Tri-Carb 3375 or 3380, Packard. Unextractable radioactivity in plant or soil samples was determined by combustion in a Packard oxidizer, Tri-Carb 306.

Radioactive zones on thin-layer plates were located with a thin-layer scanner from Berthold/Frieseke, Federal Republic of Germany.

Mass spectra of isolated conversion products were taken with a GLC–MS combination LKB 9000 from LKB Producter, Bromma, Sweden. The conditions were as follows: carrier gas He, 40 mL/min; ionization voltage 70 eV; accelerating voltage 3.5 kV; ion source temperature 250 °C; column 2 m long and 4 mm in diameter, packed with 1% SE-30 on Chromosorb W-AW-DMCS; column temperatures 100–230 °C; injector temperature 220 °C; detector temperature 240 °C.

Reagents. Ring-labeled buturon-^{14}C was synthesized in this laboratory (Attar et al., 1973; specific activity 5 mCi/mmol; radiochemical purity 99.2%). Inactive 4-chloroaniline (puriss., purity >99%) was purchased from Fluka. The origin of reference compounds for the metabolites of the first year is given in former publications (Haque et al., 1976, 1977).

The analysis of the soil used was as follows: coarse sand (0.2–2.0 mm in diameter) 49.2%; fine sand (0.06–0.2 mm in diameter) 27.0%; silt (0.002–0.06 mm in diameter) 10.3%; clay (<0.002 mm in diameter) 13.5%; humus 1.2%; pH 7.0.

For liquid scintillation counting of extracts and water, a scintillation liquid based on dioxane was used. For counting $^{14}CO_2$ from samples combusted in the oxidizer, Carbosorb–Permafluor V (8:12) from Packard was used.

For column chromatography, silica gel 60 (0.063–0.200 mm; 70–230-mesh ASTM; Merck) was used. Thin- or thick-layer chromatography was carried out on plates self-coated with MN silica gel G (Fa. Macherey, Nagel & Co., Federal Republic of Germany), or ready-coated plates (PSC, silica gel without fluorescence indicator, 2 mm, Merck, Darmstadt, or aluminum oxide 60 E). All solvents used were analytical grade.

Application and Experimental Conditions. The experiments were carried out under outdoor conditions in a box 60 × 60 × 70 cm, constructed from water-resistant plywood, filled with about 160 kg of soil, and placed in a large pit so that the surface of the soil was at the same level as the surrounding ground. The base of the box contained holes to permit the drainage of excess water that was

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0021-8561/84/1432-0208$01.50/0

collected in a metal splash tray. Further details of the experimental setup have been reported previously (Haque et al., 1977).

In each of the first and second year of the experiment, 108.9 mg of buturon-^{14}C was sprayed on winter or summer wheat, respectively, as an aqueous formulation; for details, see Haque et al. (1977). The concentration of the applied buturon-^{14}C standard solution was ensured by counting its radioactivity in a liquid scintillation counter immediately before application. No further buturon treatment was carried out in the following years. In the third year, potatoes were grown in the same soil; in the next years, the soil was left fallow.

Samples of the upper soil layer (0–10-cm depth, about 1 kg each) were taken with an auger after the harvest of the first, second, and third growing season, as well as 6 and 6.5 years after the first buturon application. Three times (1.5, 2.5, and 6.5 years after the first application) complete mass balances of radioactive residues were established. Therefore, soil samples were taken at depths of 10–20, 20–40, and 40–50 cm; when crops had been grown, they were analyzed also to include their residues in the mass balance studies. The leaching water at the bottom of the box was collected during the entire experimental time and checked for radioactivity.

Extraction of Samples and Radioactivity Measurements. The moisture content of all soil samples was determined before extraction by air-drying of an aliquot to constant weight. All soil samples were extracted successively twice with chloroform by stirring with an electric stirrer for 4 days at room temperature, filtering, and washing with chloroform. Methanol, a solvent known for high extraction efficiency, was not used for the preparation of extracts intended for characterization of soluble residues because of potential reaction of methanol with buturon or related compounds to form methyl *N*-(*p*-chlorophenyl)-carbamate (Haque et al., 1976). However, for preparation of samples for characterization of bound residues, the soils after extraction with cold chloroform were reextracted with methanol in a Soxhlet for 48 h to ensure complete removal of all nonbound radioactive products from the soil.

The radioactivity in the extracts was assayed in a liquid scintillation counter with a dioxane-based liquid scintillator. The unextracted radioactivity was measured by combustion of about 200 mg of the sample to $^{14}CO_2$ in an automatic oxidizer, followed by liquid scintillation counting.

For the separate determination of unchanged buturon-^{14}C and its conversion products formed, the chloroform extracts were concentrated in a rotary evaporator and subjected to TLC analysis on self-coated silica gel plates in dichloromethane. The radioactive zones were located with a scanner. For their quantitative determination, TLC zones of 1 cm were scraped off and suspended in dioxane scintillation liquid for assaying radioactivity.

The sampling and analysis of plants has been reported elsewhere (Haque et al., 1976).

Isolation and Identification of Buturon-^{14}C Metabolites. The combined chloroform extracts of soil samples (0–50-cm depth) of the last experimental year, after concentration, were applied on ready-coated preparative silica gel plates, and the plates were developed in dichloromethane. The radioactivity was resolved in four radioactive zones (R_f 0–0.2, 0.25–0.3, 0.5, and 0.8, respectively; see Table II). The two less polar zones (fractions 3 and 4 in Table II) contained only low amounts of radioactivity and, therefore, were not analyzed further. The two major fractions (1 and 2 in Table II) were scraped off from the plate and eluted with ethyl acetate. Each desorbed radioactive fraction was further purified by column chromatography [silica gel 100 from Merck, eluted successively with *n*-hexane, *n*-hexane/ethyl acetate (5:1), benzene/ethyl acetate (3:1), and ethyl acetate] and repeated TLC using various solvents on precoated silica gel [dichloromethane, hexane–acetone (17:1), benzene–acetone 95:5)] or aluminum oxide plates [hexane/ethyl acetate (1:1)]. After these purifications, the radioactive fractions were subjected to GC–MS analysis.

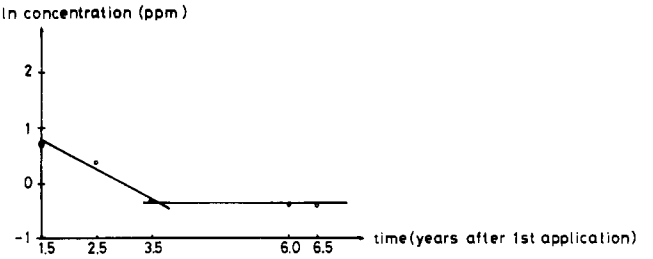

Figure 1. Decrease of radioactive residues in the upper soil layer (0–10-cm depth) with time after two applications of buturon-^{14}C (in years after the first application; the second application was 1 year after the first one).

Characterization of Unextractable Residues. The soil samples of the last experimental year (6.5 years after the first buturon application) were used for the characterization of soil-bound residues. After exhaustive extraction with chloroform and methanol, the soil was separated into humin, humic acid, and fulvic acid fractions according to the method of Kearney (1976). The radioactivity present in these fractions was determined by liquid scintillation counting, if necessary, after combustion to $^{14}CO_2$.

RESULTS AND DISCUSSION

Time Course of Residue Losses in the Upper Soil Layer. Although the half-life concept gives only an approximate description of losses of chemicals from soil, it is used in literature for this purpose, at least for limited and defined time spans. After the second application of buturon-^{14}C to soil under outdoor conditions, a two-stage decline of total ^{14}C residues was observed for the 5 years studied (Figure 1). In the second stage, beginning about 3.5 years after the first application, the residue decline is so slow that the residues remain nearly constant for the next 3 years studied. This behavior is similar to that of 4-chloroaniline, which, $^1/_2$ year after application to soil, shows only a very slow residue decrease (Freitag et al., 1984).

Mass Balance Studies. Mass balance studies including residues in deeper soil layers, leaching water, and plants give information on the distribution of residual radioactivity and, thus, partial information on the contribution of different factors to the residue decrease in the upper soil layer. Table I gives mass balance data for 1.5, 2.5, and 6.5 years after the first application of buturon. The table shows that the removal of radioactive residues from the soil by harvesting plants as well as by leaching water is of minor importance as compared to the loss to the atmosphere.

This loss of radioactivity to the atmosphere can be attributed to total degradation to carbon dioxide (mineralization) and/or conversion to volatile products of buturon by abiotic factors as well as by microorganisms (Ebing and Schuphan, 1979). These processes are more active during

Table I. Mass Balance of Radioactive Residues in Soil at Indicated Years[a] after Application of Buturon-^{14}C

sample, time after 1st application	% ^{14}C of total applied 1.5 years	2.5 years	6.5 years
soil, total	82.7	46.9	27.0
plants, 0.5 year (winter wheat)	1.0	1.0	1.0
plants, 1.5 years (summer wheat)	0.50	0.50	0.50
plants, 2.5 years (potatoes)		0.49	0.49
leaching water, 0-0.5 year	0.14	0.14	0.14
leaching water, 0.5-1.5 years	0.80	0.80	0.80
leaching water, 1.5-2.5 years		0.12	0.12
leaching water, 2.5-6.5 years			0.68
sum of recovered residues	85.14	49.95	30.73
loss to the atmosphere	14.86	50.05	69.27

[a] Years after first application.

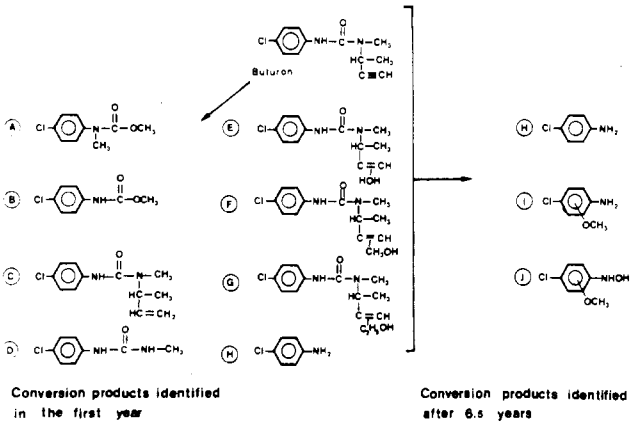

Figure 2. Conversion products of buturon isolated from soil.

the first years and slow down with the lapse of time, probably as a consequence of the formation of stable complexes with humic acids and/or clay, which are nonaccessible to biotic or abiotic degradation.

Extractability of Radioactive Products and Characterization of Extractable Residues. The extractability of radioactive residues by cold chloroform averaged 20–40% of the total residues present in the soil samples taken 1.5 years after starting the experiment or later; extracting with methanol in a Soxhlet for 48 h solubilized an additional portion of about 4–6%.

Whereas, in the first two experimental years with application of buturon, more than half of the chloroform-extractable radioactive residues were unchanged buturon, its portion decreased rapidly in the next years. Two and a half years after the first treatment, only about 7% of the soluble residues showed the R_f value of buturon; however, its identity could no longer be confirmed by mass spectrometry. The concentrations of radioactive substance fractions 6.5 years after the first application, calculated for the molecular weight of buturon, are presented in Table II.

Isolation and Identification of Individual Conversion Products. A multitude of conversion products was formed from buturon in soil during the experimental time. One group being more polar than buturon on TLC, and amounting to 23% of the chloroform-extractable radioactivity in the first year, was shown to contain at least six conversion products (Figure 2, formulas C–H). These result from elimination of the isobutinyl group, addition of H_2, H_2O, CH_3OH, or C_2H_5OH to the triple bond, or elimination of the entire side chain. The latter product (4-chloroaniline) was present only in trace amounts (Haque et al., 1977). Several metabolite groups less polar than buturon were observed at all sampling times. In the first year, two carbamates (Figure 2, formulas A and B) were identified (Haque et al., 1977).

The soil samples taken at the end of the experimental time (6.5 years) were used for the identification of long-lived residues derived from buturon in soil. From the chloroform extract, only the two major radioactive fractions (1 and 2 in Table II) were sufficient for isolation and identification of individual components. The major, more polar group (fraction 1 in Table II; about 50% of the extracted activity, R_f 0.61 in the solvent 5% acetone in benzene), was subjected to GC–MS analysis. A molecular ion at 173 ($C_7H_8O_2NCl$) and the occurrence of masses at 158 ($M^+ - CH_3$), 155 ($M^+ - H_2O$), and 146 ($M^+ - CNH$) suggest the presence of (chloromethoxyphenyl)hydroxylamine in this fraction (Figure 2, formula J); the presence of further radioactive compounds could not be excluded. The other radioactive TLC fraction (1 in Table II; about 30%, R_f 0.21 in acetone–hexane, 3:17) on GC–MS analysis was found to contain a mixture of 4-chloroaniline (m/e 127, 100) and methoxychloroaniline (m/e 157, 142, 114) (Figure 2, formulas H and I). The mass spectrum of metabolite H was identical with that of an authentic reference compound. Each of the structures proposed for metabolites I and J implies two possible isomers for which no authentic reference compounds were available. Thus, the positions of the substituents within the molecule were not established. However, mass spectrometric data are sufficient to demonstrate that oxygenated derivatives of 4-chloroaniline occurred in soil. It is worth mentioning that except for 4-chloroaniline no other metabolite of buturon previously reported was isolated or identified after 6 years of exposure. The only relatively stable metabolite of buturon seems to be 4-chloroaniline, which is metabolized further to oxygenated products. The other previously reported degradation products of buturon are probably converted to 4-chloroaniline by complete elimination of the side chains, or bound to soil, or mineralized to CO_2 by enzymes of microorganisms or by abiotic reactions. 4-Chloroaniline has been reported also to be incorporated into soil constituents (Hsu and Bartha, 1974; Bollag et al., 1978; Freitag et al., 1984) and to be mineralized to carbon dioxide, as demonstrated by laboratory experiments (Kloskowski et al., 1981; Bollag et al., 1978).

Table II. Concentration of Radioactive Residues in Soil 6.5 Years after the First Application of Buturon-^{14}C (a Second Application Was Made after 1 Year; Residues in ppm = mg of Radioactive Products Equivalent to Buturon per kg of Air-Dried Soil)

sample, cm of depth	chloroform extract, TLC fractions[a] 1, R_f 0–0.2	2, R_f 0.25–0.3	3, R_f 0.5	4, R_f 0.8	methanol extract	bound residues, fractions humic acid	fulvic acid	humin + minerals	total	total residues
0-10	0.052	0.032	0.016	0.009	0.030	0.085	0.089	0.367	0.541	0.680
10-20	0.032	0.018	0.007	0.008	0.018	0.041	0.040	0.126	0.207	0.290
20-40	0.027	0.008	0.005	0.005	0.012	0.025	0.027	0.095	0.147	0.204
40-50	0.011	0.002	0.001	0.002	0.005	0.014	0.015	0.044	0.073	0.094

[a] Solvent: dichloromethane.

It is remarkable that the oxidation products of 4-chloroaniline identified in this study have not been detected after application of 4-chloroaniline-^{14}C to soil under outdoor conditions after one to two growing periods (Freitag et al., 1984). In laboratory experiments with microorganisms or enzymes, (4-chlorophenyl)hydroxylamine (Bordeleau et al., 1972; Kaufman et al., 1973) and ring-hydroxylated products (Fletcher and Kaufman, 1979; Kaufman et al., 1973) were formed, but according to our knowledge, the formation of methoxylated products similar to those found in this study has not been reported. The reason for this is not known. Possibly, these products result from long-term degradation of buturon without the intermediacy of 4-chloroaniline, or they are formed from 4-chloroaniline only after longer exposure times from its soil-bound residues during long-term turnover and degradation of humic substances in soil.

Characterization of Bound Residues. The distribution of soil-bound residues in the samples of the last experimental year (6.5 years after the first application) between various soil fractions is included in Table II. The soil was fractioned according to the behavior of its organic matter against alkali, as proposed by a classical fractionation method reported (Kearney, 1974). It can be seen from the table that the major part of bound residues is in the humin fraction. Since this fraction includes also the bulk of soil minerals, binding to the clay mineral fraction probably is an important pathway of fixation of buturon-derived residues in soil. The portions bound in humic and fulvic acids are comparable for all soil samples.

CONCLUSION

It may be concluded that, although buturon in its unchanged form is known as a nonpersistent pesticide, some of its conversion products do persist in soil for more than 6 years, mostly as soil-bound residues that probably release slowly 4-chloroaniline and oxygenated derivatives thereof for years. The concentration of these residues in soil shows a two-stage decline, which slows down with time. Since the experimental conditions of these outdoor experiments have been shown to give residue data close to those of real field conditions (Scheunert et al., 1977), the occurrence of buturon-derived xenobiotic residues must be expected for several years after application also for agricultural fields.

Registry No. Buturon, 3766-60-7; 4-chloroaniline, 106-47-8.

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Received for review December 20, 1982. Accepted October 18, 1983. Dedicated to Prof. Dr. F. Korte in celebration of his 60th birthday.