imal gains in weight and in PER's with rats. No pancreatic hypertrophy occurred in rats fed soy flour in which 55–69% of the TIA had been destroyed.

Although Chan and de Lumen (1982b) have demonstrated by rat feeding studies that winged bean trypsin inhibitors caused pancreatic hypertrophy and growth inhibition, there was, however, uncertainty regarding the biological threshold level of TIA at which these biological effects occurred. In this connection, it is important to note that there is signifciant varietal differences in the effectiveness of autoclave inactivation of TIA and the level of TIA in the winged beans. Thus, even though 20-min autoclave treatment generally destroyed 80% of the TIA in the winged bean meals, the residual TIA in the six varieties of winged bean meals after this treatment differs significantly, ranging from 5000 to 1300 IU (g of seed)$^{-1}$.

It is also interesting to note that the residual TIA in the winged bean meals of varieties 046, 185, 100, and 095 after 5 min of autoclave treatment is higher than the TIA of untreated varieties 141 and 207. On the other hand, after 5 min of autoclave treatment, TIA in winged bean meals of varieties 141 and 207 was substantially reduced. Thus whereas 5-min autoclaving is almost certainly inadequate to abolish the deleterious effect of TIA in winged bean meals of varieties 046, 185, 100, and 095, the same treatment may be sufficient for varieties 141 and 207.

Even though prolonged heat treatment could destroy virtually all the winged bean meal TIA, excessive heat treatment could cause functional as well as nutritional damage to protein. The breeding of varieties of winged beans with thermolabile TIA should therefore offer a more satisfactory solution to eliminate the uncertainties entailed in the heat processing of the winged beans.

ACKNOWLEDGMENT

Technical assistance was provided by Ho Meng Yew, Cheah Cheng Im, and Tan Soon See.

LITERATURE CITED

Cerny, K.; Kordylas, M.; Pospisil, F.; Svabensky, O.; Zajic, B. *Br. J. Nutr.* **1971**, *26*, 293.
Chan, J.; de Lumen, B. O. *J. Agric. Food Chem.* **1982a**, *30*, 42.
Chan, J.; de Lumen, B. O. *J. Agric. Food Chem.* **1982b**, *30*, 46.
Chase, T., Jr.; Shaw, E. *Biochem. Biophys. Res. Commun.* **1967**, *29*, 508.
Claydon, A. *Sci. New Guinea* **1975**, *3*, 103.
Jaffe, W. G.; Korte, R. *Nutr. Rep. Int.* **1976**, *14*, 449.
Kakade, M. L.; Rackis, J. J.; McGhee, J. E.; Puski, G. *Cereal Chem.* **1974**, *51*, 376.
Khor, H. T.; Tan, N. H.; Wong, K. C. *Proc. Malays. Biochem. Soc. Conf.* **1980**, *6*, 157.
Kortt, A. A. *Biochim. Biophys. Acta* **1979**, *577*, 371.
Kortt, A. A. *Biochim. Biophys. Acta* **1980**, *624*, 237.
Liener, I. E.; Kakade, M. L. In "Toxic Constituents of Plant Foodstuffs", 2nd ed.; Liener, I. E., Ed.; Academic Press: New York, 1980; p 7.
Masfield, G. B. *Field Crop Abstr.* **1973**, *26*, 157.
National Academy of Sciences "The Winged Bean: A High Protein Crop for the Tropics"; National Academy of Sciences, U.S. Government Printing Office: Washington, DC, 1975.
Rackis, J. J.; McGhee, J. E.; Booth, A. N. *Cereal Chem.* **1975**, *52*, 85.
Richardson, M. *Food Chem.* **1981**, *6*, 235.
Sohonie, K.; Bhandarkar, A. P. *J. Sci. Ind. Res., Sect. B* **1954**, *13*, 500.
Tan, N. H.; Ho, M. Y.; Ramasamy, P.; Chin, K. O. *Proc. Symp. Fed. Asian Ocean Biochem., 2nd* **1979**, 86.
Wong, K. C. *Proc. Conf. Malays. Food Self-Sufficiency, UM-GAMA* **1975**, 103.

Received for review April 7, 1982. Accepted July 1, 1982. This work was supported by Research Grants Vote F 114/80, from the University of Malaya, and ICDUP.

Evaluation of the Bleidner Technique for Analysis of Soil-Bound 3,4-Dichloroaniline Residues

In-Soon You and Richard Bartha*

By radiochemical and conventional analysis, the effectiveness of the Bleidner distillation process for recovery of the herbicide residue 3,4-dichloroaniline (DCA) from its humic complexes was evaluated. An increase of alkali concentration to 12.5 N and the extension of the distillation period to 23 h attained the quantitative recovery of DCA from its freshly formed humic complexes. However, during 99 days of incubation in soil, DCA recovery efficiency declined to less than 70%. From a field soil treated with 3-(3,4-dichlorophenyl)-1,1-dimethylurea (diuron) at the rate of 1.76 kg ha^{-1} year^{-1} for the past 10 consecutive years, Bleidner distillation recovered 1 ppm of DCA, virtually all from humic complexes. The mineralization rate of bound DCA and the decline kinetics of DCA recovery both lead to the conclusion that the total bound DCA accumulation in the analyzed soil did not exceed 2.5 ppm and that in soils of the examined type the accumulation of bound DCA residues does not constitute a problem.

3,4-Dichloroaniline (DCA) is the major biodegradation product of several phenylcarbamate (Herrett, 1969), phenylurea (Geissbühler, 1969), and acylanilide (Bartha and Pramer, 1970) herbicides. At recommended treatment levels, 80–90% of the DCA released from herbicides in soil becomes solvent inextractable (Chisaka and Kearney, 1970) primarily by covalent binding to soil organic matter (Bartha, 1971; Hsu and Bartha, 1974). Humus-bound DCA appears to be a source of low-level crop contamination (Still and Mansager, 1969; Still et al., 1980) and gives rise to concern that xenobiotic residues might accumulate in humus (Bartha, 1981). A direct analytical technique for humus-bound DCA residues is clearly needed to resolve the regulatory and environmental concerns asssociated with humus-bound residues. Unfortunately, alkaline or

Department of Biochemistry and Microbiology, Cook College, New Jersey Agricultural Experiment Station, Rutgers University, New Brunswick, New Jersey 08903.

0021-8561/82/1430-1143$01.25/0

acid hydrolysis (Hus and Bartha, 1974) releases only half or less of the bound DCA, and the proportion of the tightly bound "nonhydrolyzable" DCA increases with time (Hsu and Bartha, 1976). Recent attempts by Worobey and Webster (1982a,b) to release intact 4-chloroaniline from its humic complexes by various techniques resulted in a maximal recovery of 46%. Using the Bleidner distillation technique, Bollag et al. (1978) achieved somewhat higher solubilization of radioactivity from some soil-boound anilines. DCA was not included in this study and the solubilized radioactivity was not chemically identified as the parent aniline compound. Using high concentrations of aniline or of other substituted anilines, Parris (1980) displaced high percentages of DCA and other chloroanilines from their humic acid complexes that had been prepared in vitro. For obvious reasons of interference, this approach is not promising for field analysis of soil-bound pesticide residues.

The classical technique for recovery of phenylamide herbicide residues from plant and soil material is the Bleidner distilltion (Bleidner et al., 1954; Geissbühler et al., 1971), but it is not clear from the existing literature whether this technique or its modifications are capable of recoverying humus-bound DCA residues and do so in a quantitative and reproducible manner. Comparison of gas chromatographic (GC) analysis with recovery of radioactivity from humus-bound [^{14}C]DCA allowed the optimization and evaluation of the Bleidner distillation process for soil-bound DCA recovery. Subsequently, the optimized process was used in the analysis of humus-bound DCA residues in a field soil that had been treated for the past 10 years by the DCA-based herbicide diuron.

EXPERIMENTAL SECTION

Chemicals. Uniformly ^{14}C-labeled DCA (specific activity 61 μCi/mg) was obtained from Amersham/Searle (Des Plaines, IL). Its radiochemical purity, as determined by thin-layer chromatography (TLC), was 97%. Unlabeled DCA was purchased from Aldrich (Milwaukee, WI) and was purified by recyrstallization from ligroin to a melting point of 71 °C. Carbonyl-^{14}C-labeled 3-(3,4-dichlorophenyl)-1,1-dimethylurea (diuron) and unlabeled analytical diuron standard were gifts from E. I. du Pont de Nemours & Co., Wilimington, DE. As specified by the donor, the radiolabeled diuron had a specific activity of 4.22 μCi/mg and a radiochemical purity of 99%. The analytical standard was 98% pure. Pesticide-grade isooctane was glass distilled prior to use. All other chemicals and solvents were analytical and pesticide grade, respectively.

Soils. The soil used in laboratory incubations was Nixon sandy loam, freshly collected from the grounds of the New Jersey Agricultural Experiment Station, New Brunswick, NJ. The characteristics of this soil and its processing prior to incubation were described earlier (Hsu and Bartha, 1974). The soil used for analysis of DCA residues were collected in July 1981 from an asparagus farm near Crosswicks, NJ. This field was treated for the last 10 years with the diuron-based herbicide Karmex (80% a.i.) at the rate of 2.2 kg ha^{-1} year^{-1} (2 lb acre^{-1} year^{-1}). The field was sampled at nine locations in a grid pattern to a depth of 0–10 cm and a sieved composite sample was prepared. The soil was a loamy sand (80% sand, 12% silt, 8% clay, and 0.8% organic matter by ignition) and had a pH of 6.1. All soil quantities and treatment levels are based on oven-dry weight.

Recovery of Diuron from Soil. Fresh soil samples, 25 g by dry weight each, were treated with radiolabeled diuron (2.4×10^5 dpm/sample, 1 ppm of diuron applied in 15 μL of ethanol). Some of the treated soil samples were immediately extracted by using, consecutively, three 150-mL aliquots of cold actone with rotary shaking (200 rpm) for the time periods of 2, 5, and 16 h, respectively. The radioactivity of the combined acetone extracts was determined by liquid scintillation counting (LSC). Of the extracted soil, 2.5-g samples were subjected to wet combustion (Allison et al., 1965). The resulting $^{14}CO_2$ was trapped and counted.

Similary diuron-treated soil samples were poisoned by 1% $HgCl_2$ to suppress microbial activity and were incubated in sealed microfernbach flasks for 7 days. At this time, the soil was extracted as described above, and the proportion of solvent-extractable and bound radioactivity was determined.

Preparation of Bound DCA Residues. For soil incubations, radiolabeled DCA was dissolved in 10 μL of ethanol and was added to a sufficient amount of water to adjust the moisture level of fresh 10-g soil samples to 60% of their water-holding capacity. [^{14}C]DCA was applied at 0.25 μCi/sample and was sufficiently diluted with unlabeled DCA to give a 5-ppm concentration in soil. The treated soil samples were incubated at 28 °C in the dark in modified microfernbach flasks that were periodically flushed through traps to quantitate the $^{14}CO_2$ evolved (Marinucci and Bartha, 1979). In some flasks, biological activity was suppressed by addition of 1% (w/w) $HgCl_2$. Replicate flasks were analyzed for soil-bound DCA residues after 1, 7, and 99 days.

The humic acid–DCA complex was prepared in vitro as described by Hsu and Bartha (1974). The complex was exhaustively washed with cold acetone to remove absorbed DCA and was stored dry for later analysis. The complex had a specific radioactivity of 1.1×10^4 dpm/mg.

Recovery of Bound DCA from Soil and from Humic Acid Complexes. Laboratory-incubated soil samples were exhaustively solvent-extracted by using acetone in a Soxhlet apparatus for 8 h to remove solvent-soluble DCA. The acetone extract was counted for radioactivity. From the solvent-extracted soils, 3-g samples were either combusted for LSC of $^{14}CO_2$ or, alternatively, were subjected to Bleidner distillation (Bleidner et al., 1954) using the improved extraction head of Heizler (Geissbühler et al., 1971). Two 250-mL round-bottom flasks, one containing the soil sample plus 100 mL of 6.3–12.5 N NaOH and the other one 100 mL of isooctane, respectively, were connected to the apparatus. The flask containing the soil sample was magnetically stirred but antifoam was not used. The Bleidner distillation process was applied for various time periods up to 23 h. In initial optimization tests, the highest alkali concentration (12.5 N) and the longest distillation run (23 h) proved to be the most favorable for DCA recovery, and the latter conditions were subsequently used in the analysis of all samples. To remove free and absorbed DCA and/or diuron while minimizing potential losses of DCA by volatility, we exhaustively washed field soil samples with cold acetone but did not subject them to Soxhlet extraction. To the combined acetone extracts 1 mL of glacial acetic acid was added, and the solution was concentrated in a rotary evaporator to 5 mL. The concentrate was made alkaline by NaOH, and DCA and diuron residues were partitioned into isooctane and were measured by GC analysis. Subsequently, 25-g aliquots of the solvent-extracted soil were subjected to Bleidner distillation. From the humic acid–DCA preparation, 15–60-mg amounts were subjected to the same treatment.

In case of radiolabeled DCA residues, the isooctane fractions from the Bleidner distillations were subjected to LSC. Subsequently, DCA from these isooctane fractions

Table I. Recovery of Radioactivity[a] from Bound DCA Residues

sample	incubation, days	volatile + CO_2	Soxhlet extract	Bleidner isooctane	Bleidner NaOH	soil residue	recovery
soil	1	<0.1	48.5	38.4 (±6.1)		3.0 (±3.4)	90.0
soil	7	<0.3	33.2	46.8 (±1.6)	7.9 (±2.9)	3.4 (±2.3)	91.6
soil	99	6.9	8.5	52.3 (±0.4)	15.0 (±5.4)	11.4 (±0.7)	94.2
soil[b]	99	0.6	18.3	37.5 (±2.2)	11.2 (±2.1)	15.0 (±0.1)	82.6
HA-DCA complex	none			80.2 (±12.7)	17.1 (±3.0)		97.3

[a] All data are expressed as percentage of the originally applied radioactivity. The numbers represent the average of triplicate determinations with standard deviations shown in parentheses. [b] Poisoned by 1% $HgCl_2$.

was extracted into aqueous acid, and after the pH was raised, DCA was extracted back into ethyl acetate. The ethyl acetate solution was concentrated under a N_2 stream at room temperature, dried with Na_2SO_4, and analyzed by quantitative TLC. Radioactivity remaining in the soil residue and in the aqueous alkaline digest was quantitated after conversion to $^{14}CO_2$. Isooctane fractions from the field soil samples were concentrated 10-fold by distillation. The distillate was checked and found to be free of DCA. The residual isooctane solution was dried over Na_2SO_4 and analyzed by GC.

In case of some soil samples, the alkaline solution that resulted from the Bleidner distillation was further processed in order to recover additional DCA. After neutralization with concentrated HCl, the salt precipitate was removed by centrifugation, and the supernatant was extracted by ethyl acetate. DCA was analyzed in the solvent extract by quantitative TLC. The radioactivites of the solvent extract, the salt precipitate and the aqueous supernatant were also determined. In the latter two cases, this necessitated wet combustion of the samples prior to LSC.

Chromatographic and Radioassay Procedures. TLC separations were performed on 250-μm precoated silica gel G plates (Fisher, Pittsburgh, PA) developed with $CHCl_3$. Spots were visualized under UV light. For quantitative analysis of radioactive DCA on TLC plates, cold DCA carrier was added to the extracts. After development of the chromatogram, the DCA spot was scaped off and the cochromatographed radioactivity was determined by LSC.

CG analysis was performed by using a Model MT 200 Tracor instrument equipped with a ^{63}Ni electron capture detector, linearizer, and 180 cm long × 6.12 mm o.d. glass column packed with 5% UC-W98 on 60–80-mesh Chromosorb WAW (Hewlett-Packard, Avondale, PA). Operating conditions were as follows: CH_4–Ar (95:5); carrier flow, 90 mL/min; oven temperature, 200 °C; detector temperature 250 °C. Peaks were quantitated by area by using a Hewlett-Packard Model 3390A integrator. Under the above conditions, diuron and DCA had retention times of 0.92 and 1.46 min, respectively, and the detection limit for both was 1 ng/1-μL injection volume.

Radioactivity was counted on a Beckman LS 230 instrument. Soil, humus, and opaque solutions were subjected to wet ashing (Allison et al., 1965), and $^{14}CO_2$ was trapped and counted in Oxifluor (New England Nuclear, Boston, MA). The radioactivity of clear solvent solutions was counted in Aquasol (New England Nuclear). Counts were corrected for background and for quenching by using the external standard ratio method. All analytical determinations were made at least in triplicate.

RESULTS

Recovery of DCA from Laboratory-Incubated Soil. The results of this experiment are presented in Table I. $^{14}CO_2$ evolution was insignificant in poisoned controls and during brief incubation times (1–7 days); it amounted to a modest percentage during the 99-day incubation period. The proportion of solvent-extracted radioactivity, consisting of free and physically absorbed DCA and of DCA polymerization products (Bartha, 1971), decreased with incubation time but remained relatively high in poisoned controls. An explanation for the latter finding may be the lack of mineralization activity in the poisoned samples that removes preferentially free or absorbed DCA. The sum of mineralized and solvent-extractable radioactivity (15.4%) is comparable to the sum of that of the same two fractions in the poisoned samples (18.8%).

Table II. Distribution of Radioactivity Resistant to Solvent Extraction[a]

sample	incubation, days	DCA, %	NaOH solution, %	soil residue, %
soil	1	92.7		7.3
soil	7	80.6	13.5	5.8
soil	99	66.5	19.1	14.4
soil[b]	99	58.9	17.5	23.5
HA-DCA complex	none	82.4	17.6	

[a] Calculated from analytical data presented in Table I. DCA in isoctane was determined by quantitative cochromatography. [b] Poisoned by 1% $HgCl_2$.

Table II shows the recovery of radioactivity that remained in the soil after exhaustive solvent extraction, i.e., of the chemically bound DCA. It is obvious that the Bleidner distillation process is much more efficient than the alkaline or acid hydrolysis that recovered approximately half of the bound DCA (Hsu and Bartha, 1974). However, recovery even by the more efficient Bleidner technique declined with increasing incubation times. On TLC, 90–95% of the radioactivity in isooctane moved with the added DCA standard. Except for some radioactivity at the origin, no other radioactive spots were detected on the chromatogram. Therefore, the lack of 100% recovery of radioactivity in the DCA spot was attributed to volatility and adorption losses inherent to the TLC technique, and all radioactivity in the isooctane fractions was listed as unchanged DCA. The results give experimental support to an earlier contention (Hsu and Bartha, 1976) to the effect that all or most of the radioactivity not released by ordinary acid or alkaline hydrolysis consists of tightly bound DCA rather than of unrecognizable ^{14}C-labeled compounds.

Recovery of DCA from Field Soil. The solvent extract of a field soil, treated for the past 10 consecutive years with diuron at 1.76 kg $acre^{-1}$ $year^{-1}$ and sampled 100 days after the last application, revealed that the extract contained the barely measurable amount of 0.005 ppm of diuron and no detectable DCA. Bleidner distillation recovered from the same soil 1.0 ± 0.07 ppm of DCA. The immediate recovery by Bleidner distillation of a 1-ppm DCA spike was virtually quantitative (0.98 ± 0.07 ppm).

The immediate (zero-time) solvent extraction of soil spiked by 1 ppm of radiolabeled diuron was similarly efficient, yielding a 0.96 ± 0.01 ppm recovery. From 1 ppm of radiolabeled diuron incubated in poisoned soil for 7 days, solvent extraction recovered 0.90 ± 0.04 ppm of diuron, showing that, without microbial transformation, diuron does not become solvent inextractable even on prolonged contact with soil.

DISCUSSION

The first application of the Bleidner distillation with the specific aim of releasing substituted aniline residues resistant to solvent extraction (Bollag et al., 1978) clearly showed the advantages of this technique for solubilization of the bound residues. In this earlier report, however, only about one-third of the solubilized radioactivity was present in isooctane, with two-thirds remaining in the aqueous alkali. In isooctane, the only radiolabeled compounds were the respective anilines. The labeled compounds in aqueous alkali solution were not identified, but we assumed that these were anilines still attached to humic and polyphenolic molecules. To make the Bleidner distillation more suitable for analysis of bound anilines, it was necessary to improve the yield of the free parent compound. In case of DCA, this was achieved by using a higher alkali concentration and longer treatment period. An additional 3–4% increase in DCA recovery was achieved by neutralization, salt removal, and reextraction of the aqueous solution by ethyl acetate, but on a routine basis the modest improvement in recovery probably does not justify this added effort. Our results confirm the view of Bollag et al. (1978) that "hydrolyzable" and "nonhydrolyzable" aniline residues are relative rather than absolute terms. We seem to deal with a continuous range of bond strengths, and increasingly severe treatments liberate increasing portions of the bound DCA. A great advantage of the Bleidner distillation process is that the liberated DCA is removed from prolonged contact with the humic hydrolysate, thus lessening the chance of any reattachment.

The modified Bleidner process recovers solvent-inextractable DCA from freshly formed humic complexes in an essentially quantitative manner. However, the aging of the complexes during incubation in soil leads to decreased DCA recovery. This fact was reported earlier (Hsu and Bartha, 1976), and in spite of the overall better performance of the Bleidner distillation as compared to simple hydrolysis, the same trend is apparent in Table I of this publication. Microbial activity does not appear to be essential for the above shift, being evident also in $HgCl_2$-poisoned soil samples.

In consideration of the declining recovery of bound DCA with time, the analytical data obtained from the field soil that had been treated for the past 10 consecutive years by diuron need to be interpreted with caution. The amount of DCA determined (1.0 ppm) is probably substantially less than the amount actually present in the soil, and the shifting recovery efficiency prevents recovery corrections by a constant factor. Nevertheless, the kinetic consideration of our experimental data allows us to define the range of recovery within fairly narrow limits and thus allows the resolution of the question whether or not a significant accumulation of bound DCA residues has occurred.

From Figure 1 it is evident that the decline of DCA recovery is asymptotic. By projection, the decrease of DCA recovery beyond the 99-day incubation period will be very slow, and even in years, it is unlikely to become lower than 50%. If this minimal recovery value of 50% is assumed, the 1 ppm of DCA found may correspond to a maximum of 2 ppm of soil-bound DCA accumulation.

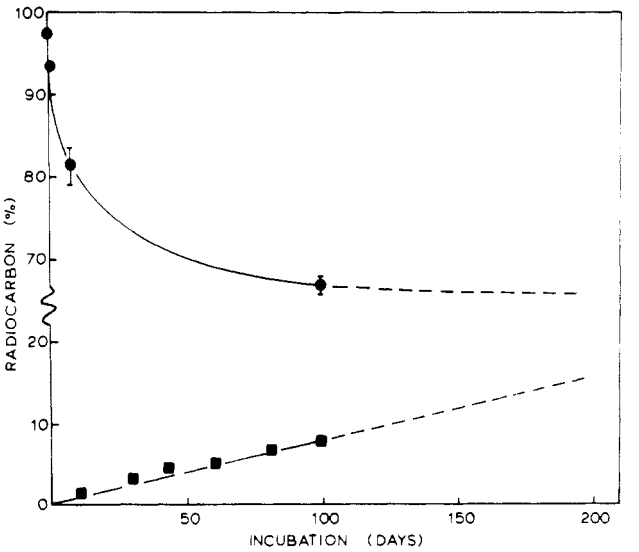

Figure 1. Decline in the recovery of bound DCA and $^{14}CO_2$ evolution from the same bound residues as plotted against incubation time. Decline in DCA recovery (●) appears to be asymptotic; $^{14}CO_2$ evolution (■) is linear. The dashed portions of the lines represent projections.

Another line of reasoning as to the maximal level of DCA accumulation can be developed from the conversion of bound DCA to $^{14}CO_2$ (mineralization). This process was found to be linear during the 99-day laboratory experiment and indicated a projected 25%/year mineralization rate. It is recognized that, under field conditions, mineralization will be less even and may stagnate during winter and drought periods, but stimulatory effects of plant rhizospheres during the growth season (Hsu and Bartha, 1979) probably more than compensate for such temporary slowdowns. Assuming a mineralization rate for bound DCA in the field that is equal to the measured laboratory rate of 25%/year, and assuming that the 1.76 kg/ha diuron treatment results in 0.9 ppm of diuron in the plough layer of the field, we found that the theorethical accumulation of bound DCA residues from 10 years of treatment, according to the calculation scheme of Hill et al. (1955), should be between 2 and 3 ppm at the time of sampling.

The two independent lines of reasoning lead to an essentially identical result, and therefore, we feel confident to conclude that the level of bound DCA accumulation during a fairly typical 10-year field treatment regime did not exceed 2–2.5 ppm, a level that seems in no way to be alarming. This conclusion should be qualified by the fact that the Nixon sandy loam and the loamy sand from the Crosswicks field were both light mineral soils with low humus contents. Before the accumulation question of bound aniline residues can be put to rest, it will be necessary to ascertain that other soil types, especially organic ones, behave similarly.

Our findings on the behavior of bound DCA residues affect the interpretation of phenylamide herbicide residue data. The Bleidner distillation, commonly used in analysis of phenylureas and occasionally of other phenylamides, results in hydrolysis of the parent herbicide. Subsequently, the substituted aniline is measured spectrophotometrically following diazotization (Bleidner et al., 1954; Hill et al., 1955; Geissbühler et al., 1971) or by GC (Kirkland, 1962; Webley and McKone, 1964). The above approaches do not distinguish between parent herbicides and their metabolites including soil-bound aniline residues. They all elicite the same reponse as long as the aromatic amine is liberated on hydrolysis. Therefore, it is certain that such analytical data include anilines liberated from humic complexes al-

though their quantitative recovery was not assured in these measurements.

ACKNOWLEDGMENT

The gift of radiolabeled and analytical-grade diuron from E. I. du Pont de Nemours & Co. is gratefully acknowledged.

LITERATURE CITED

Allison, L. E.; Bollen, W. B.; Moodie, C. D. In "Methods of Soil Analysis"; Black, C. A., Ed.; American Society of Agronomists, Inc.: Madison, WI, 1965; Chapter 89, p 1346.
Bartha, R. *J. Agric. Food Chem.* **1971**, *19*, 385.
Bartha, R. *ASM News* **1981**, *46*, 356.
Bartha, R.; Pramer, D. *Adv. Appl. Microbiol.* **1970**, *13*, 317.
Bleidner, W. E.; Baker, H. M.; Levitsky, M.; Lowen, W. K. *J. Agric. Food Chem.* **1954**, *2*, 476.
Bollag, J.-M.; Blattmann, P.; Laanio, T. *J. Agric. Food Chem.* **1978**, *26*, 1302.
Chisaka, H.; Kearney, P. C. *J. Agric. Food Chem.* **1970**, *18*, 854.
Geissbühler, H. In "Degradation of Herbicides"; Kearney, P. C.; Kaufman, D. D., Eds.; Marcel Dekker: New York, 1969; pp 79–111.
Geissbühler, H.; Kossmann, K.; Baunok, I.; Boyd, V. F. *J. Agric. Food Chem.* **1971**, *19*, 365.
Herrett, R. A. In "Degradation of Herbicides"; Kearney, P. C.; Kaufman, D. D., Eds.; Marcel Dekker, New York, 1969; pp 113–145.
Hill, G. D.; McGahen, J. W.; Baker, H. M.; Finnerty, D. W.; Bingeman, C. W. *Agron. J.* **1955**, *47*, 93.
Hsu, T.-S.; Bartha, R. *Soil Sci.* **1974**, *116*, 444.
Hsu, T.-S.; Bartha, R. *J. Agric. Food Chem.* **1976**, *24*, 121.
Hsu, T.-S.; Bartha, R. *Appl. Environ. Microbiol.* **1979**, *37*, 36.
Kirkland, J. J. *Anal. Chem.* **1962**, *34*, 428.
Marinucci, A. M.; Bartha, R. *Appl. Environ. Microbiol.* **1979**, *38*, 1020.
Parris, G. E. *Environ. Sci. Technol.* **1980**, *14*, 1105.
Still, C. C.; Hsu, T.-S.; Bartha, R. *Bull. Environ. Contam. Toxicol.* **1980**, *24*, 550.
Still, G. G.; Mansager, E. R. *Weed Res.* **1969**, *9*, 218.
Webley, D. J.; McKone, C. E. *Misc. Rep. Trop. Pestic. Res. Inst.* **1964**, *No. 441*.
Worobey, B. L.; Webster, G. R. B. *J. Agric. Food Chem.* **1982a**, *30*, 161.
Worobey, B. L.; Webster, G. R. B. *J. Agric. Food Chem.* **1982b**, *30*, 164.

Received for review February 5, 1982. Accepted August 16, 1982. New Jersey Agricultural Experiment Station Publication No. D-01200-2-82, supported by State funds and a grant by American Cyanamid Co.

Formation of Two Thermal Degradation Products of β-Carotene

Philip N. Onyewu, Henryk Daun,* and Chi-Tang Ho

β-Carotene was heated at 210 °C for 4 h, and the thermal degradation products were separated by liquid column and thin-layer chromatography. Two compounds were tentatively identified as 3,7,10-trimethyl-1-1,12-bis(2,6,6-trimethylcyclohex-1-enyl)dodeca-1,3,5,7,9,11-hexaene and 3,6-dimethyl-1,8-bis(2,6,6-trimethylcyclohex-1-enyl)octa-1,3,5,7-tetraene. A mechanism for their formation is proposed.

Several studies have reported the formation of volatile compounds, mainly toluene, xylene, ionene, and 2,6-dimethylnaphthalene, as thermal degradation products of carotene. The summary of these studies is shown in Table I. The mechanism for the formation of toluene, xylene, and dimethylcyclodecapentaene from β-carotene has been proposed by Edmunds and Johnstone (1965) and advanced by Schwieter et al. (1969). It is said to involve the formation of a four-membered ring intermediate. This mechanism explains the formation or expulsion of toluene, xylene, and dimethylcyclodecapentaene from β-carotene but fails to show what is the remaining part of the carotene molecule after the expulsion.

Most of the work done on thermal degradation of β-carotene (though not in food) has emphasized almost exclusively the volatile degradation products. Only very few works were done on the nonvolatiles. Rost (1976) reported the isolation of polycyclic aromatic hydrocarbons upon heat treatment of crude edible oils at both the neutralizing temperature of 260 °C and bleaching plus deodorizing temperature at 270 °C.

At very high temperatures (400 and 700 °C), small amounts of polycyclic aromatic hydrocarbons (PAH) are formed as pyrolysis products of β-carotene (Halaby and Fagerson, 1971). Ouyang et al. (1980) studied the nonvolatile compounds generated under conditions simulating palm oil deodorization at 210 °C. Conventional deodorization is carried out at 360–425 °F (182–218 °C) (Schwitzer, 1959; Swern, 1964). Apocarotene and apocarotenals were reported as being formed by Ouyang and co-workers (see Table I).

In light of the above-mentioned studies, it becomes important to investigate the nonpolar nonvolatile thermal degradation products (TDP) of β-carotene. The use of these deodorization conditions has both theoretical value and practical value. The fact is that in most developed countries (especially Europe and the United States) in order for edible oils to be acceptable to consumers, the oils must be refined and deodorized. Moreover, refined oils undergo further heat treatment during cooking and frying. In the course of thermal treatments, carotenoids are degraded and chemicals such as PAH could be formed. The nonpolar PAH, if formed, would be in the nonpolar nonvolatile fraction of the TDP of β-carotene. The previously developed model system (Onyewu et al., 1981) was used to study the formation of the nonvolatile thermal degradation products of β-carotene.

EXPERIMENTAL SECTION

Previously developed model system by Onyewu et al. (1981) was employed. Two grams of β-carotene (lot 181029,

Department of Food Science, Cook College, New Jersey Agricultural Experiment Station, Rutgers University, New Brunswick, New Jersey 08903.

0021-8561/82/1430-1147$01.25/0