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# [$^{14}$]C Acrolein Accumulation and Metabolism in Leaf Lettuce

A. J. Nordone,[1] M. F. Kovacs,[1] R. Doane[2]

[1]TAS Inc., 1000 Potomac Street NW, Washington, DC 20007, USA
[2]Baker Performance Chemicals, Inc., 3920 Essex Lane, Houston, Texas 77027, USA

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The increasing use of chemicals in modem agriculture has resulted in widespread concern regarding pesticide residues entering the food supply (NRC 1993). MAGNACIDE® H Herbicide (active ingredient: 92% minimum acrolein, inhibited) is used in agricultural canals as an injectable aquatic herbicide to control aquatic weeds. Acrolein is highly toxic to both plants and animals, producing a variety of adverse effects (Beauchamp et al. 1985).

Acrolein has been detected in a number of foods, including white bread (Mulders and Dhont 1972), cooked potatoes (Talima et al., 1967), and ripe tomatoes (Hayase et al. 1984), and in heated and aged bone grease at average levels of 4.2 ppm (Maslowska and Bazylak 1985). Little information exists regarding the potential of acrolein to accumulate in food crops that have been irrigated with MAGNACIDE® H treated water. During the summer irrigation months, MAGNACIDE® H Herbicide is used routinely in the irrigation canals of the western United States. The aim of this study was to determine whether acrolein would accumulate in leaf lettuce that had been irrigated with MAGNACIDE® H treated water. The effects of both single and repeated applications were examined using concentrations of acrolein in water of 75 ppm, which is five times the recommended maximum application rate, and the highest concentration that did not result in visible damage to the plants.

## MATERIALS AND METHODS

[2,3-$^{14}$C]-labeled acrolein was obtained from Sigma Chemical Company (St. Louis, MO). The specific activity of the compound was 8.9 mCi/mmole (0.159 mCI/mg). The radiochemical purity of the compound was determined before each application by HPLC and was found to be 94.7% ± 1.7% pure. Unlabeled acrolein (96.05% pure) received from Baker Performance Chemicals, Inc., Houston, TX, (BPCI) was used for dilution of the radioactive material and as an anlytical standard. All other reagents were of analytical grade or better. The field phase of this study was

*Correspondence to:* A. J. Nordone

carried out at Plant Sciences Inc. (Watsonville, CA). Wooden beds (flats) were prepared approximately 2 wk prior to the study initiation by using boxes (90 cm x 75 cm and 60 cm in depth), lined with plastic and filled with a sandy loam, typical of that used in lettuce agriculture, to approximately 5 cm from the top. A total of six flats were used for the study: three controls and three treatments. During the entire study, flats were maintained outside in separate fenced areas. Flats were manually sprinkle irrigated as required for crop growth. On application days, irrigation (if required) was performed early in the day to prevent dilution of the application solution with irrigation water of the leaf surface.

Leaf lettuce, variety Gene Corp Green (Salinas, CA), was used for the study. Prior to the main study, a tolerance test indicated that acrolein applied at 75 ppm did not show any phytotoxic symptoms. Plants received either a single irrigation application containing 75 ppm $^{14}$C-acrolein on day 0 (three-leaf stage) or four applications on days 0, 17, 34 and 50. Plants receiving the single application were randomly sampled on days 0, 29 and 53 (final harvest), whereas those receiving multiple applications were randomly sampled on days 0, 17, 34, 51, 52 and 53 (final harvest). Plants were harvested by gently pulling the entire plant out of the soil. Roots were cut off, and leaves and roots were placed in separate Zip Lok® bags and immediately frozen on dry ice. Roots were rinsed with water prior to processing to remove adhering soil particles. On study days 50 (before and after application), 51, 52 and 53, approximately 100 g of leaves from each lettuce plant were rinsed with 100 mL of water to determine if any of the radiocarbon residues could be removed by rinsing. These rinses were radioassayed by liquid scintillation (Beckman, model 500CE, Fullerton, CA).

Total radioactivity in lettuce samples was determined by oxidation of triplicate 250 to 500 mg aliquots (R. J. Harvey, Model OX400 and OX600). Lettuce leaves and roots were extracted in preparation for chromatographic analysis by first being homogenized using a commercial food processor. Five-to ten-gram samples of the homogenates were added to 50 mL centrifuge tubes with 10 to 20 mL deionized water. The mixture was thoroughly stirred by vortexing for 1 min and then placed in a sonicator for 5 min. The mixture was then centrifuged at 0°C and 2500 rpm for 10 min. The supernatant was removed and analyzed by Liquid Scintillation Counter (LSC). The remaining solid residue was extracted with 20 mL lN aqueous HCL solution in the same manner as above and the supernatant analyzed by LSC. The solid residue was heated in 10 mL 6N NaOH solution at approximately 70°C for 60 min and, after cooling, was centrifuged as above. The supernatant was analyzed by LSC. The final solid residue was combusted as previously described. Total recovered radiocarbon ranged from 82.5 to 100.8% throughout the study.

HPLC analysis utilized a Perkin Elmer Model 410 LC pump and a Supelco LC-18 column (25 cm x 4.6 mm i.d., 5 µm particle size). Eluting compounds were detected with a Perkin Elmer Model LC235 Diode Array Detector and associated

recorder. Radiolabeled compounds were detected with a Beckman radioactive flow detector (Beckman Instruments, Fullerton, CA) or by the collection of fractions as described below. Column eluent was collected, for each individual analysis, as 0.5 mL (0.5 min.) fractions with an ISCO fraction collector. These were subsequently assayed by LSC. These fractions were collected from the time of sample injection through 50 min after injection. Radiochromatograms were reconstructed from individual fraction DPM utilizing software developed at PTRL West, Inc. (Richmond, CA).

Extracts were analyzed by injecting sufficient quantity of sample radiocarbon to detect 0.01 ppm $^{14}$C-acrolein equivalents as a single peak (100 to 250 µl). Each sample was fortified with sufficient unlabeled acrolein for the ultraviolet detection of acrolein such that identification of the radiolabel could follow from comparison of the observed retention time of eluting $^{14}$C products and the observed retention time of unlabeled acrolein. The HPLC flow rate was 1 mL/min., and the gradients for 0 to 40, 40 to 50, and 50 to 65 min were acetonitrile:water, 0: 100%, 100:0%, and 0: 100%, respectively.

Thin layer chromatography (TLC) analysis was performed on silica gel F254 plates (0.25 mm) developed in one dimension, utilizing either (TLC system 1) n-butanol:acetic acid: water (6:1:1 v/v/v) or (TLC system 2) toluene:ethylformate: formic acid (5:7: 1 v/v/v) prepared immediately prior to development. After the solvents evaporated, each plate was placed on an Ambis radiographic scanner, for quantitation of radiocarbon.

Acid hydrolysis was attempted on selected whole concentrated extracts and chromatographically isolated fractions of extracts. Samples were concentrated by nitrogen evaporation or roto-evaporation as necessary to remove organic solvents and excess water. To 0.5 mL of concentrated aqueous samples was added to 2.0 mL of 3.75 M aqueous HCL (final HCL concentration of 3.0 M) in a teflon-lined, screw-capped vial. The sealed vial was heated in a water bath (60 to 80°C) for 3 to 5 hr. Samples were analyzed by HPLC and TLC after appropriate concentration of partitioning with ethyl acetate.

## RESULTS AND DISCUSSION

Acrolein was only detected in samples taken immediately after application following either single or multiple applications in irrigation water at a concentration of 75 ppm. With a single application, total radioactive residues (TRR) in leaves fell from 0.79 ppm (79% acrolein) 1 hr after application to 0.003 ppm (0.0% acrolein) 53 d post application. Corresponding values for the roots were 0.012 and 0.006 ppm, respectively (Table 1). Multiple applications of acrolein at 75 ppm resulted in TRR in leaves of approximately 1.10 ppm (91% acrolein) and 1.04 ppm (49% acrolein) 1 hr after the day 0 and day 50 treatments, respectively. This fell to 0.77 ppm on day 53 (0.0 % acrolein). Corresponding

values for the roots were 0.142, 0.05 and 0.09 ppm, respectively (Table 1). In all samples, the only other product detected was observed eluting at the solvent front by HPLC. Identification of these polar constituents was attempted using TLC, and a total of four products were observed. Further attempts to characterize these products were not successful.

**Table 1.** Total radioactive residues (ppm acrolein equivalents) in lettuce after $^{14}$C-acrolein treatments (Trt).

| | | Single Treatment | | Multiple Treatment | |
|---|---|---|---|---|---|
| Day | Sample | Leaves | Roots | Leaves | Roots |
| 0 | After Trt 1 | 0.794 | 0.012 | 10.97 | 0.142 |
| 17 | Before Trt 2 | NA | NA | 0.115 | 0.035 |
| 29 | | 0.015 | 0.013 | NA | NA |
| 34 | Before Trt 3 | NA | NA | 0.180 | 0.031 |
| 50 | Before Trt 4 | NA | NA | 0.213 | 0.038 |
| | After Trt 4 | NA | NA | 1.040 | 0.047 |
| 51 | | NA | NA | 0.825 | 0.074 |
| 52 | | NA | NA | 0.753 | 0.076 |
| 53 | Final Harvest | 0.003 | 0.006 | 0.766 | 0.094 |

NA: not applicable

The TRR acrolein equivalents observed in the rinses, expressed as ppm in lettuce as well as the total ppm acrolein equivalents in the unrinsed lettuce, are described in Table 2.

At 53 d following the single application, no radiocarbon is observed in the rinse. In the multiple application study, immediately prior to the day 50 fourth application and 16 d after the day 34 third application, 1.9% of the radiocarbon was washable from the lettuce leaves. However, immediately after the fourth application, 14.4 % of the radiocarbon was rinseable from the leaves. This percentage declined to 9.0%, 3.1% and 5.1% at 1, 2 and 3 d, respectively, after the fourth application.

We have demonstrated that the use of MAGNACIDE® H Herbicide, when applied in irrigation water at a concentration of 75 ppm, either as single or multiple applications, does not lead to the accumulation of acrolein within lettuce leaves or roots or on the surface of the plant. Field experiments have demonstrated that the presence of acrolein in agricultural canals is transient (Nordone et al. 1996a; Nordone et al. 1996b) with a half-life ranging from 2 to 10 hr. The degradation of acrolein in water is due largely to its rapid hydrolysis to 3-hydroxy-propanal and biotransformation to acrylic acid and allyl alcohol (Smith et al. 1995). Thus, under normal circumstances, irrigated crops would be exposed to minimal levels of acrolein and are more likely to come in contact with acrolein metabolizes that

**Table 2.** Total radiocarbon residues (ppm acrolein equivalents) in lettuce rinses after $^{14}$C-acrolein treatments.

| Day | Sample | Total Radioactive Residues Total PPM | Rinse PPM | Percent of Total in Rinse |
|---|---|---|---|---|
| Single Application | | | | |
| 53 | Leaves Final Harvest | 0.003 | 0.000 | 0.000 |
| Multiple Application | | | | |
| 50 | Leaves Before 4th Application | 0.213 | 0.004 | 1.88 |
| | Leaves After 4th Application | 1.040 | 0.150 | 14.42 |
| 51 | | 0.825 | 0.074 | 8.97 |
| 52 | | 0.753 | 0.023 | 3.05 |
| 53 | | 0.766 | 0.039 | 5.09 |

Note: Total PPM refers to the TRR detected by combustion of unrinsed samples. Rinse PPM refers to the TRR detected in rinsates of subsamples. Percent of total is calculated as (Rinse TRR ppm x 100)/(Total TRR ppm).

are metabolized to carbon dioxide in both microbial and mammalian systems (Smith et al. 1995; Ghanayem et al. 1987).

These results indicate that acrolein degrades very rapidly in lettuce. Samples taken 1 d after the final application of the radiolabeled compound were shown to contain no $^{14}$C-acrolein. Although not identified, four metabolic products were observed using TLC analysis. These were hydrolyzable by HCL into many products, suggesting that they consist of multiple, conjugated polar metabolizes of $^{14}$C-acrolein or biological components which have incorporated $^{14}$C-fragments resulting from further degradation of $^{14}$C-acrolein metabolizes.

This study indicates that, under normal use scenarios, irrigation of crops with MAGNACIDE® H Herbicide treated water is highly unlikely to result in the accumulation of biologically significant levels of acrolein either on or within the crop.

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