

US EPA ARCHIVE DOCUMENT

Method of Analysis for
Ethephon in Straw

From Report Titled
Detailed Method of Analysis
of Ethephon in Straw

Union Carbide Agricultural
Products Company, Inc.

June 1962

DETAILED METHOD OF ANALYSIS FOR RESIDUES
OF (2-CHLOROETHYL)PHOSPHONIC ACID (ETHEPHON*) IN WHEAT,
OAT AND BARLEY GRAIN, STRAW AND MILLING FRACTIONS
REGISTRATION ANALYTICAL CHEMISTRY
UNION CARBIDE AGRICULTURAL PRODUCTS CO., INC., JUNE 1982

Reagents

- Methanol - Mallinckrodt Nanograde, or equivalent
- Methanol - reagent grade (use only where specified)
- Diethyl ether - Mallinckrodt #0848 or redistilled in glass
- Hydrochloric acid solution - "10%", dilute 10 ml. conc. HCl to 100 ml. with methanol
- Citric acid solution - "1%", dissolve 10 gm C.P. citric acid in 1 L methanol
- Diazomethane solution - prepare from N-methyl-N-nitroso-p-toluenesulfonamide (Diazald, Aldrich Chemical Co. #D2800-0; or Eastman Kodak #7066) according to manufacturer's instructions. Observe all safety precautions. Do not store over KOH or other desiccants, but do store in tightly capped brown bottles in freezer. Use Teflon cap liner.
- Standard ethephon solutions - weigh 10.0 mg. ethephon into a 2 oz. polyethylene, screw-capped bottle. Add 10.0 ml. methanol and shake until dissolved. Transfer 1.0 ml. to a second 2 oz. polyethylene bottle, using a 1 ml. polyethylene pipet. Add 9.0 ml. methanol and mix well. Transfer 1.0 ml. from the second bottle to a third 2 oz. polyethylene bottle, again using a 1 ml. polyethylene pipet. Add 9.0 ml. methanol and mix well. This final solution contains 0.01 micrograms ethephon per microliter and may be used for fortification of crop samples for determination of recoveries. A polyethylene pipet must be used for dispensing this solution (See Note A).
- Standard dimethylethephon solutions - weigh 10.0 mg. ethephon into a 2 oz. polyethylene, screw-capped bottle (See Note A). Add 2 ml. methanol and shake until dissolved. Add diazomethane solution until a permanent yellow color is obtained. Cap tightly and let stand for 15 minutes. Remove excess diazomethane by using a gentle stream of dry nitrogen until solution is colorless. Quantitatively transfer solution to a 15 ml. graduated, screw-capped

*Ethephon is the accepted common name for the major active ingredient in formulations available as "ETHREL[®]" plant regulators. ETHREL[®] is a registered trademark of Union Carbide Agricultural Products Co., Inc.

centrifuge tube, using methanol to rinse. Adjust volume to 10.0 ml. with methanol. Make three serial 10:1 dilutions with methanol, resulting in a solution containing 1 nanogram (acid equivalent) dimethylethephon per microliter. This solution may be used for gas chromatographic calibration.

Special Equipment

- Polyethylene bottles, 2 oz., screw-capped with Teflon cap liners
- Polyethylene pipets, 1 ml. graduated
- Blender, one gallon, Waring Blendor or equivalent
- Soxhlet extractors, Pyrex, 30 x 110 mm., Corning #3740 or equivalent
- Standard Taper Teflon sleeves for all Soxhlet extractor joints
- Flasks, 300 ml. single-neck, Standard Taper, for use with Soxhlet extractors
- Extraction thimbles, single thickness, 25 x 80 or 25 x 100 mm., S & S #603 or equivalent, pre-extracted with reagent grade methanol for at least 8 hours. Methanol may be re-used several times.
- Vacuum pump or aspirator capable of maintaining a vacuum of 25 - 26 inches of water
- Rotary flash evaporator
- Centrifuge capable of maintaining 2000 rpm
- Gas chromatograph, or equivalent, equipped with flame photometric detector in phosphorus mode.

Procedure

- (1) Weigh about 200 gm of hard-frozen sample plus about 200 gm dry ice into a blender and grind until no particles are larger than 1/8".
- (2) Allow dry ice to sublime in freezer overnight.
- (3) Weigh 10 gm grain or mill fraction sample into a pre-washed extraction thimble (use 5 gm for straw samples). Fortification of samples for determination of recoveries may be done at this point. (See Note D).
- (4) Cover sample with a wad of glass wool.
- (5) Extract grain or mill fraction samples with 100 ml methanol for 4 hours. Extract straw samples by attaching thimble to top (inside) of extractor with a paper clip or small clamp and adding four 25 ml portions of 1% methanolic citric acid solution to top of thimble, waiting 5 minutes between portions.

Procedure (cont'd)

- (6) Add 0.5 ml "10%" methanolic HCl to methanol extract; discard thimble and contents.
- (7) Quantitatively transfer extract to a glass-stoppered 100 ml graduated cylinder, using methanol to rinse.
- (8) Adjust to a convenient volume with methanol.
- (9) Pipet 1/10 of the extract obtained in step 10 into a 15 ml screw-capped, graduated centrifuge tube.
- (10) Concentrate to 1.5 ml, using a gentle stream of dry nitrogen and a 30-35°C water bath.
- (11) Add 0.5 ml "10%" methanolic HCl.
- (12) Add 8 ml diethyl ether, mix well, let stand for 10 minutes, mix once again, then centrifuge at 2000 rpm for 10 minutes (See Note B).
- (13) Decant supernatant to a clean screw-capped, graduated centrifuge tube.
- (14) Rinse residue with 2 x 1 ml diethyl ether and add rinsings to supernatant.
- (15) Concentrate to 1-1.2 ml using a gentle stream of dry nitrogen and a 30-35°C water bath.
- (16) In a good fume hood, add diazomethane solution until a permanent yellow color is obtained (See Note C.)
- (17) Cap tightly and let stand for 15 minutes.
- (18) Concentrate to 1-1.5 ml using a gentle stream of dry nitrogen and a 30-35°C water bath.
- (19) Centrifuge at 2000 rpm for 10 minutes.
- (20) Adjust volume to 0.8-0.9 ml using a gentle stream of dry nitrogen and a 30-35°C water bath. To avoid losses of dimethylethephon, DO NOT TAKE BELOW 0.8 ML!! Adjust to 1.0 ml with diethyl ether.
- (21) Add one ml ethyl acetate and shake well. Add two ml diethyl ether and shake well. Add one ml hexane and again shake well. Allow to stand overnight. A very small quantity of viscous material may settle out. Pipet the clear supernatant into a 15 ml centrifuge tube and concentrate to 1.0 ml using a gentle stream of dry nitrogen and a 45-50°C water bath. DO NOT TAKE BELOW 0.8 ml!
- (22) Grain or mill fraction samples, representing 1 gm/ml, are now ready for gas chromatographic analysis. Straw samples require a further clean-up, as follows:

Materials and Reagents

Disposable Pasteur pipets-9 inch
Glass wool
Sodium sulfate, anhydrous
Florisil, 60-100 mesh; activate at 130°C for at least 24 hours;
add 5 ml water to 95 gm Florisil and mix well; allow to
stand 24 hours and again mix well. Deactivated Florisil
should be used the day it is ready.
Acetone, Baker analyzed
Ethyl ether, Baker analyzed
Ethyl acetate, Baker analyzed
Hexane, Baker analyzed

Preparation of Column

Push a small wad of glass wool into the bottom of a nine inch disposable pipet column. Add one inch of deactivated Florisil to the column and then add about $\frac{1}{2}$ inch of anhydrous sodium sulfate. Prewet column with 1 ml acetone and discard acetone.

Sample Clean-up

Transfer sample extract to column and rinse sample tube with one ml acetone. As soon as the last of the sample extract reaches the top of the bed, add the rinse solvent from the sample tube. When the last of this reaches the top of the bed, add an additional two x 2 ml of acetone in a similar manner. Collect all eluate from the column (~5 ml) in a 15 ml centrifuge tube and concentrate to 1.0 ml. This solution, representing 0.5 gm straw/ml, is now ready for gas chromatographic analysis.

- (23) Analyze a 2-8 μ l aliquot of the prepared extract. The operating parameters used at Morse Laboratories to generate wheat and barley residue data are as follows:

Cas chromatograph: Microtek 220 equipped with a flame photometric detector in the phosphorus mode

Column: 6' x $\frac{1}{8}$ " O.D. glass packed with 20% OV-11 on 100/120 mesh Chromosorb W HP

Temperatures:

Column: 182°C

Inlet: 230°C

Detector: 200°C

Carrier gas: Nitrogen at 95 ml/min.

A confirmatory gas chromatographic system, utilizing a somewhat more polar column and alternative means of detection, is given below:

Gas chromatograph: Hewlett-Packard 402-B equipped
with an alkali thermionic detector

Column: 6' x $\frac{1}{8}$ " O.D. glass packed with
6% FFAP on 60/80 mesh Chromosorb G,
AW, DMCS

Temperatures:

Column: 175°C

Inlet: 190°C

Detector: 345°C

Gas flows: Carrier gas (He) 40-45 ml/min; Hydrogen
45-50 ml/min; Air 220-240 ml/min.

Either of the above systems may be used as the "primary" means of analysis, the other being available for confirmation of over-tolerance residues.

- (24) Analyze a standard solution with each set of samples. Determine peak height or peak area. During the development of the preceding method of analysis, it was found that the dimethylethephon peak from "grown-in" or fortified samples was sometimes slightly sharper and slightly more symmetrical than standard dimethylethephon peaks. This phenomenon is thought to be due to a transient blockage of "active sites" by crop extractives. As a result of this short-lived increase in column efficiency, the recovery of ethephon from fortified samples may appear to be somewhat greater than 100%, when peak height rather than peak area is measured. The use of peak areas seems to eliminate this problem. It should be noted that, in either case, correction for spike recovery of the amount of ethephon found in treated samples is valid, since the increased column efficiency applies to both treated and fortified samples. Since both flame photometric (phosphorus) and alkali thermionic detector responses are linear throughout the range of ethephon content found in grain, mill fraction or straw samples, or can be made linear by dilution of samples, the amount of ethephon in a given sample may be determined by simple proportion.

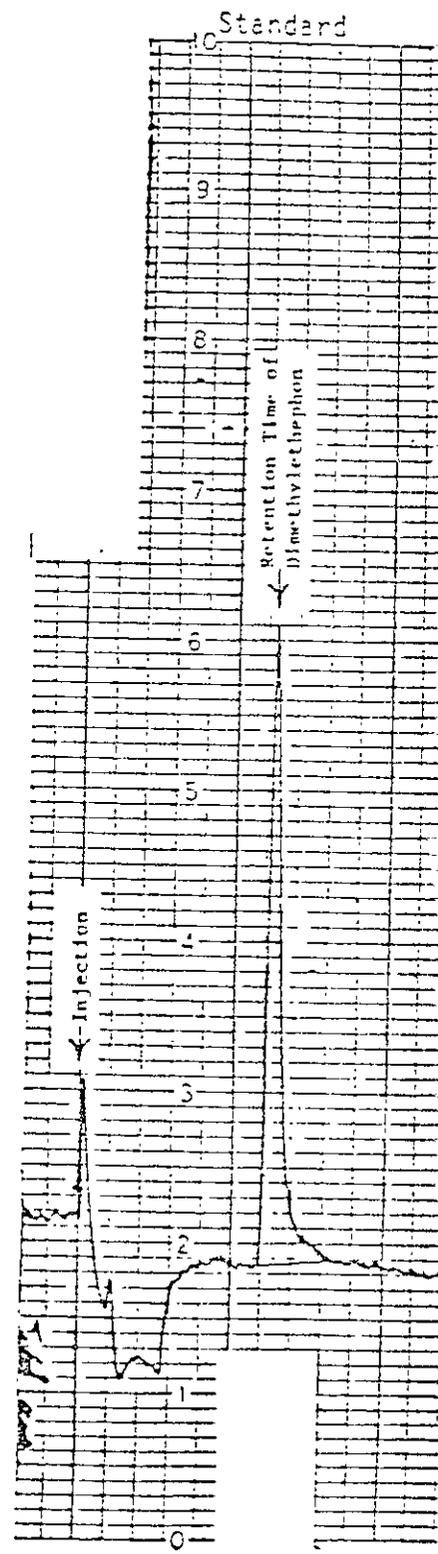
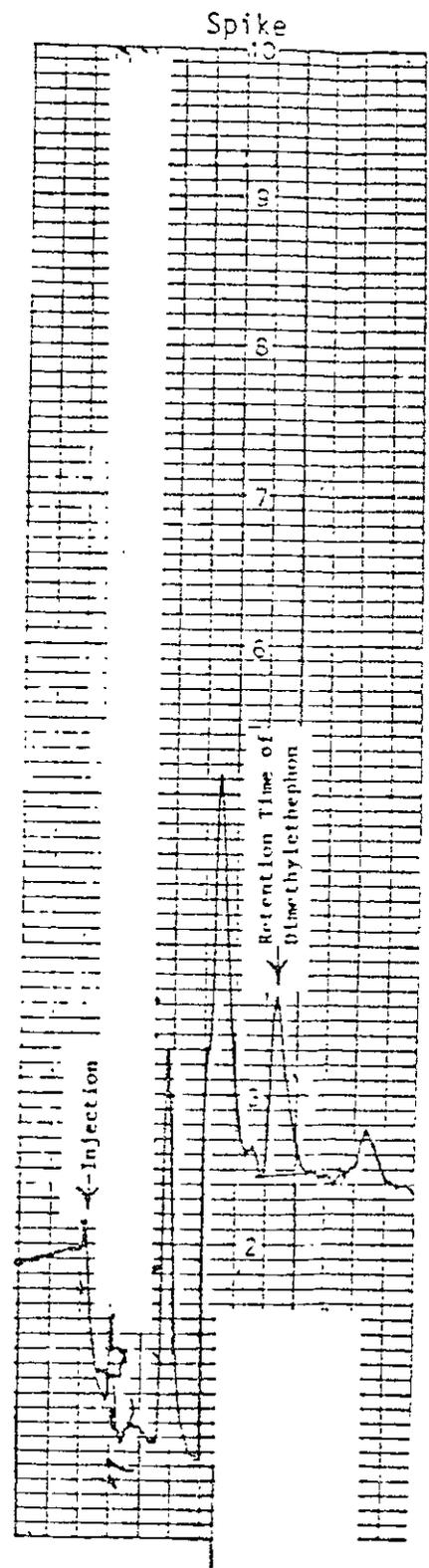
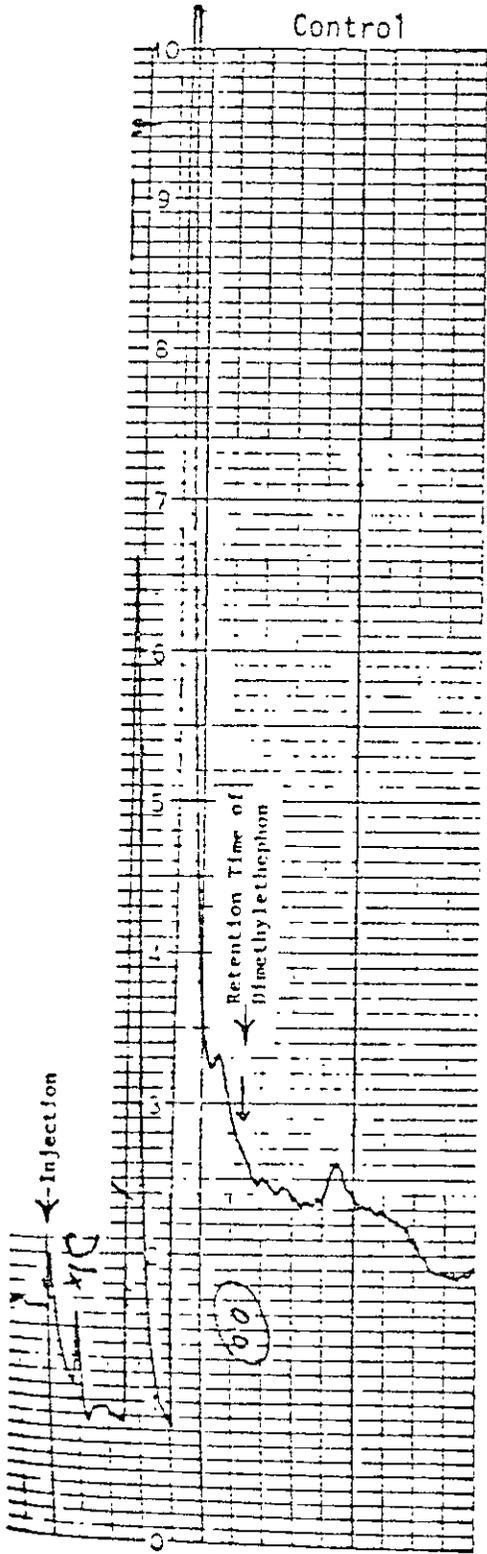
NOTES:

- A. It has been demonstrated that used glassware (presumably because it has been etched by repeated cleaning in an alkaline detergent) adsorbs ethephon very strongly in the absence of crop material. It has also been demonstrated that new glassware, which has not been so etched, and polyethylene labware, do not, exhibit this adsorption. Therefore, it is necessary that either new glassware or polyethylene labware (the latter is recommended) be used to prepare standard solutions. It should be noted that surface adsorption is not a problem once the ethephon has been methylated.
- B. When diethyl ether is added, precipitation may occur. If ether addition at this point is omitted, a similar precipitation may occur during methylation, with a serious loss of ethephon. It should also be noted that if acidification prior to ether addition is omitted, a serious loss of ethephon may occur.
- C. If the extract has too much color to show the yellow color of diaxomethane, add diaxomethane solution until no further bubbling occurs. then add an additional 2 ml.
- D. A modification of the method was occasionally found to be necessary for grain samples which showed unacceptably low recoveries of added ethephon. The modified method consists of grinding, multiple extractions with methanolic tartaric acid-HCl, pH adjustment, concentration, precipitation of interfering materials with methyl-t-butyl ether instead of diethyl ether and gas chromatographic analysis using flame photometric detection (phosphorus mode).

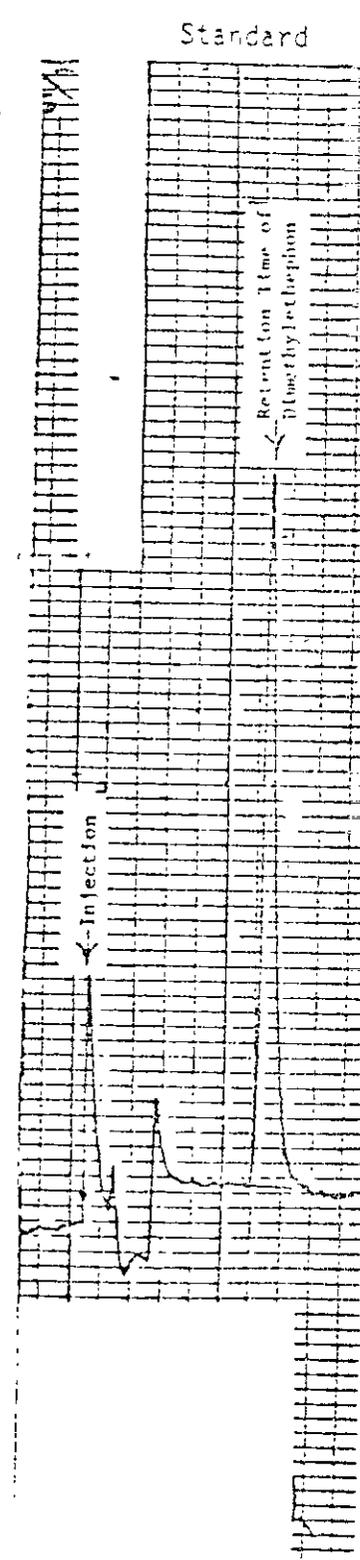
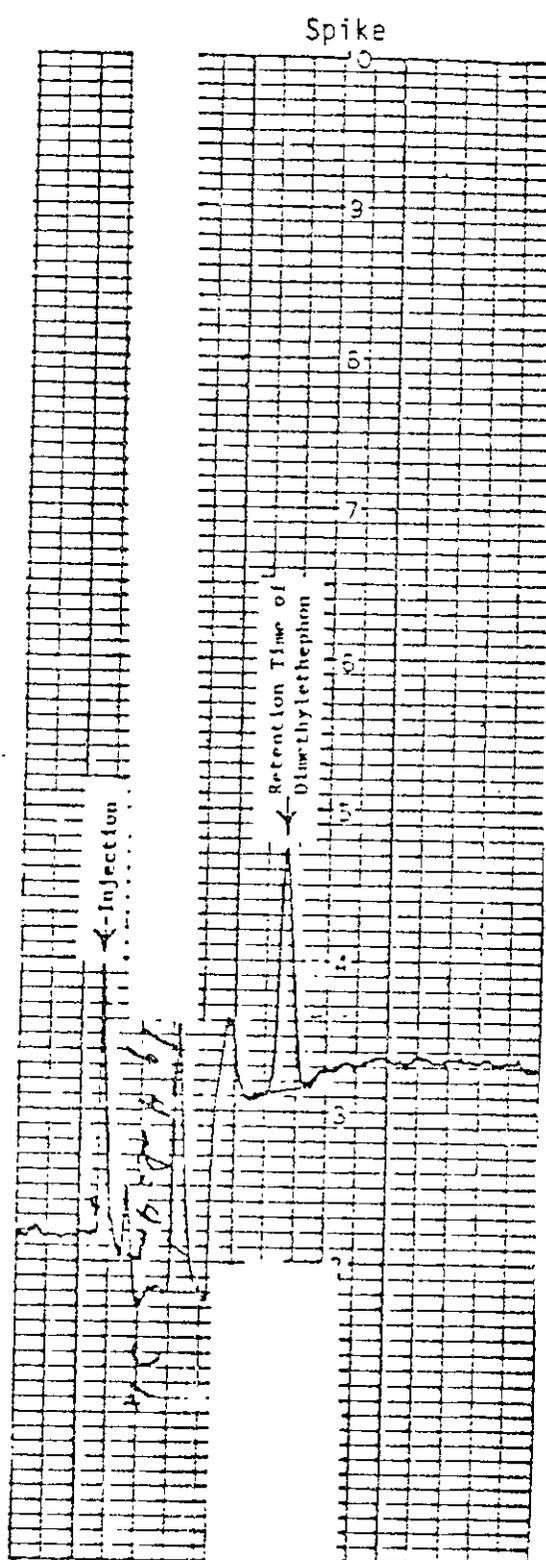
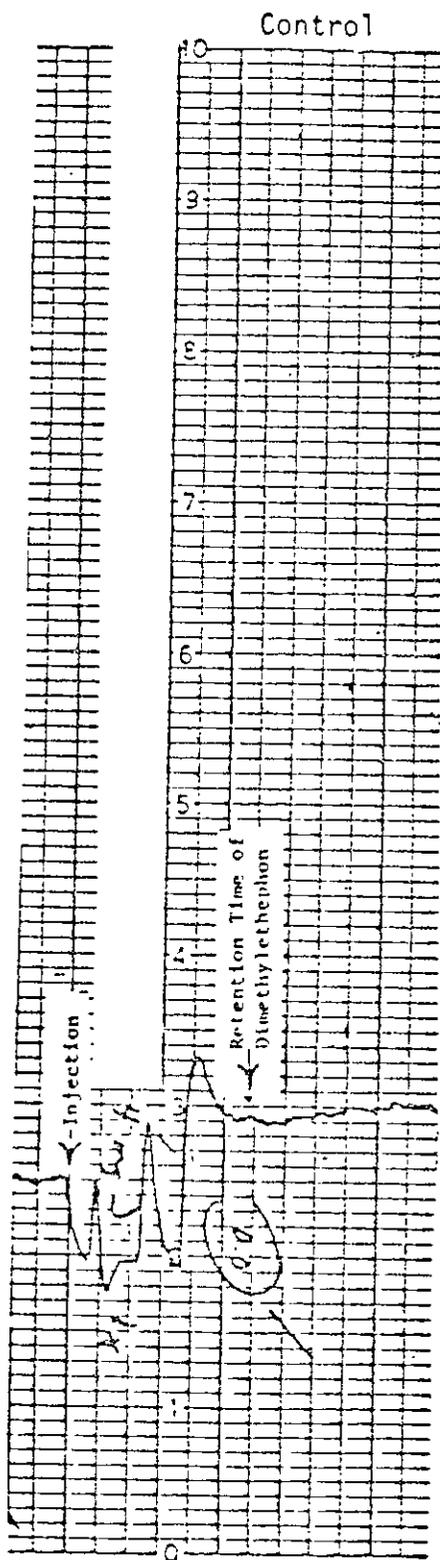
REPRESENTATIVE GAS CHROMATOGRAMS
BARLEY GRAIN



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BARLEY STRAW



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