

## INTRODUCTION

This method provides a means of determining diazinon and its metabolites as residues in soil. Validation levels ranged from 0.02 ppm to 4.0 ppm for diazinon and O-diazinon, while G-27550, demethylated G-27550 and GS-31144 ranged from 0.01 ppm to 2.0 ppm.

## PRINCIPAL OF METHOD

Diazinon and metabolites are extracted from a fifty gram aliquot of homogenized soil using 100 ml of 90:10 acetonitrile:water. The extract is filtered into a separatory funnel using an additional 60 ml of extraction fluid to wash the filter cake and extraction vessel. The extraction fluid is then partitioned against 50 ml of saturated aqueous NaCl and 100 ml of methylene chloride. A second extraction of the aqueous layer is done using 50 ml of methylene chloride and 50 ml acetonitrile. The third extraction of the aqueous layer is done using 50 ml of methylene chloride. All organic layers are combined and dried through anhydrous sodium sulfate. This is then evaporated just to dryness using vacuum rotary evaporation and reconstituted to 10 ml using acetone. Diazinon and O-diazinon residues are then determined by direct injection of the extract into a capillary GC with N-P detection. The other metabolites are determined by HPLC with UV detection after removing the acetone from the aliquot using a N<sub>2</sub> sparge, then reconstituting with HPLC mobile phase.

## MATERIALS AND METHODS

Diazinon (DZN) Reference Standard  
O-Diazinon (ODN) Reference Standard  
G-27550 (OPG) Reference Standard  
Demethylated G-27550 (DMG) Reference Standard  
GS-31144 (DHG) Reference Standard  
Methanol - HPLC quality  
Water - Deionized  
Acetone - HPLC quality  
Acetonitrile - Pesticide grade  
Sodium sulfate, anhydrous  
Sodium chloride  
Ammonium phosphate dibasic  
Phosphoric acid  
Glass fiber filter paper, GF/A  
HPLC with a variable wavelength UV detector  
HPLC column - Supelco LC8DB Cat. #5-8347  
Gas chromatograph with NP detector and temperature programming  
15 M x 0.53 mm ID DB-1 at 5µ GC Column  
HP3392A Integrator  
HP1000 computer with Beckman CALS®

## REAGENT SOLUTIONS

Extraction solvent - 3600 ml of acetonitrile mixed with 400 ml deionized water.

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Saturated NaCl - An amount of NaCl is added to a quantity of water such that an excess of solid NaCl is noted after two hours of vigorous stirring at room temperature. As a guide in making this solution approximately 360 grams of salt will dissolve in 1 liter of water.

HPLC buffer - Prepared by dissolving 0.5 g  $\pm 0.005$  g  $(\text{NH}_4)_2\text{HPO}_4$  in 1 liter of deionized water. This is then adjusted to a pH of  $7.5 \pm 0.05$  using  $\text{H}_3\text{PO}_4$ .

#### STOCK STANDARD PREPARATION

The parent and each individual metabolite are weighed to give concentration of 1 mg/ml in methanol when corrected for purity. For example if 25 mg of diazinon is required with a purity of 87.8%, the actual amount weighed is calculated by:

$$\text{Amount Needed (mg)} = \frac{25 \text{ mg} \times 100\%}{87.8\% \text{ Purity}} = 28.5 \text{ mg}$$

This process is then repeated for each metabolite and all are diluted in acetone.

#### DILUTED STANDARDS

GC and Spiking Standard: Stock solutions of parent and metabolites at 1 mg/ml concentrations are used to prepare the combined diluted solutions. Into the same 50 ml volumetric;

| <u>Amount to Pipet</u> | <u>Compound</u> | <u>Concentration</u> |
|------------------------|-----------------|----------------------|
| 5 ml                   | DMG             | 100 $\mu\text{g/ml}$ |
| 5 ml                   | DHG             | 100 $\mu\text{g/ml}$ |
| 5 ml                   | OPG             | 100 $\mu\text{g/ml}$ |
| 10 ml                  | ODN             | 200 $\mu\text{g/ml}$ |
| 10 ml                  | DZN             | 200 $\mu\text{g/ml}$ |

then bring to volume with methanol.

This solution is acronymed 4.0,2.0 PPM SPK SOL and is used for spiking and preparation of the following solutions.

Spiking Standards: PPM concentrations are based on a 1 ml aliquot of solution into 50 g of soil. Spiking solutions are prepared as follows:

The acronym for this solution is 0.8,0.4 PPM SPK SOL - prepared by diluting the 4 and 2 ppm spiking solution using 5 ml and diluting to a volume of 25 ml in methanol. The effective concentration are then 40  $\mu\text{g/ml}$  for ODN and DZN while DHG, DMG and OPG concentrations are 20  $\mu\text{g/ml}$ .

The acronym for this solution is 0.1,0.05 PPM SPK SOL - prepared by diluting the 4 and 2 ppm spiking solution using 5 ml and diluting to a volume of 200 ml in methanol.

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The acronym for this solution is 0.02,0.01 PPM SPK SOL - prepared by diluting the 4 and 2 ppm spiking solution using 0.5 ml and diluting to a volume of 100 ml in methanol.

Individual spiking solutions are prepared in separate 100 ml volumetrics as follows diluted in methanol.

| <u>Acronym and Compound</u> | <u>Stock Concentration</u> | <u>Aliquot Volume</u> | <u>Final Concentration</u> | <u>PPM Equivalent</u> |
|-----------------------------|----------------------------|-----------------------|----------------------------|-----------------------|
| DHG-20                      | 1 mg/ml                    | 2 ml                  | 20 µg/ml                   | 0.4 ppm               |
| DMG-20                      | 1 mg/ml                    | 2 ml                  | 20 µg/ml                   | 0.4 ppm               |
| OPG-20                      | 1 mg/ml                    | 2 ml                  | 20 µg/ml                   | 0.4 ppm               |
| ODN-40                      | 1 mg/ml                    | 4 ml                  | 40 µg/ml                   | 0.8 ppm               |
| DZN-40                      | 1 mg/ml                    | 4 ml                  | 40 µg/ml                   | 0.8 ppm               |

GC STANDARDS

The following mixed standards are prepared and diluted with acetone:

| <u>Acronym</u> | <u>Parent Solution</u> | <u>Aliquot Volume</u> | <u>Dilution Volume</u> | <u>Concentration µg/ml</u> |                |
|----------------|------------------------|-----------------------|------------------------|----------------------------|----------------|
|                |                        |                       |                        | <u>ODN, DMG, DHG</u>       | <u>DZN OPG</u> |
| MX2,1          | 4,2 PPM SPK SOL        | 1                     | 100                    | 2                          | 1              |
| MX1,0.5        | MX2,1                  | 25                    | 50                     | 1                          | 0.5            |
| MX0.5,0.25     | MX2,1                  | 25                    | 100                    | 0.5                        | 0.25           |
| MX0.1,0.05     | MX1,0.5                | 5                     | 50                     | 0.1                        | 0.05           |
| MX0.05,0.025   | MX0.5,0.25             | 5                     | 50                     | 0.05                       | 0.025          |

The following individual standard solutions are prepared and diluted with acetone:

| <u>Acronym</u> | <u>Parent Concentration</u> | <u>Aliquot</u> | <u>Final Volume</u> | <u>Concentration</u> |
|----------------|-----------------------------|----------------|---------------------|----------------------|
| DMG-1          | 1 mg/ml                     | 0.05 ml        | 50 ml               | 1 µg/ml              |
| DHG-1          | 1 mg/ml                     | 0.05 ml        | 50 ml               | 1 µg/ml              |
| OPG-1          | 1 mg/ml                     | 0.05 ml        | 50 ml               | 1 µg/ml              |
| ODN-1          | 1 mg/ml                     | 0.05 ml        | 50 ml               | 1 µg/ml              |
| DZN-1          | 1 mg/ml                     | 0.05 ml        | 50 ml               | 1 µg/ml              |

HPLC STANDARDS

The mixed standards contain only DMG, DHG, and OPG.

The stock solution is made by diluting 1 ml of each DMG, DHG, and OPG at 1 mg/ml into a total volume of 100 ml of methanol. This solution is acronymed as MXD-10. The solution provides a 10 µg/ml solution for each compound.

The most concentrated solution for HPLC injection is acronymed MXD-1. This solution is prepared by diluting a 1 mg/ml aliquot of MXD-10.

to 10 ml with a solution of 0.5 g/l  $(\text{NH}_4)_2\text{HPO}_4$  adjusted to a pH of 7.5 with  $\text{H}_3\text{PO}_4$ . This gives a concentration of 1  $\mu\text{g}/\text{ml}$  for each compound.

The solution MXD-1 is then diluted further using HPLC mobile phase:

| Acronym  | Parent Solution | Aliquot Solution | Dilution Volume | $\mu\text{g}/\text{ml}$ of Each |
|----------|-----------------|------------------|-----------------|---------------------------------|
| MXD-0.5  | MXD-1           | 5                | 10              | 0.5                             |
| MXD-0.25 | MXD-0.5         | 5                | 10              | 0.25                            |
| MXD-0.05 | MXD-0.5         | 1                | 10              | 0.05                            |

Individual HPLC standards for each component are prepared by dilution as indicated in HPLC mobile phase.

| Acronym | Parent Concentration | Aliquot Volume | Final Volume | Final Concentration         |
|---------|----------------------|----------------|--------------|-----------------------------|
| DMG-0.5 | 1 mg/ml              | 0.025 ml       | 50 ml        | 0.5 $\mu\text{g}/\text{ml}$ |
| DHG-0.5 | 1 mg/ml              | 0.025 ml       | 50 ml        | 0.5 $\mu\text{g}/\text{ml}$ |
| OPG-0.5 | 1 mg/ml              | 0.025 ml       | 50 ml        | 0.5 $\mu\text{g}/\text{ml}$ |
| ODN-0.5 | 1 mg/ml              | 0.025 ml       | 50 ml        | 0.5 $\mu\text{g}/\text{ml}$ |
| DZN-0.5 | 1 mg/ml              | 0.025 ml       | 50 ml        | 0.5 $\mu\text{g}/\text{ml}$ |

#### EXTRACTION AND CLEAN UP

Step by step directions for this procedure are:

- 1) Fifty grams ( $\pm 0.5$  g) of ground homogenized soil is weighed into a 250 ml HDLPE bottles.
- 2) Any of the samples which require the addition of spiking solution are spiked at this time.
- 3) One hundred ml of 90:10  $\text{CH}_3\text{CN}:\text{H}_2\text{O}$  extraction solution is added.
- 4) The samples are then capped with a polypropylene linerless cap and shaken for 30 minutes on an oscillating shaker at high speed (280 excursions/minutes).
- 5) The solutions are then filtered into a 500 ml separatory funnel through glass fiber filter paper wet with 10 ml extraction solvent.
- 6) The extraction vessel and the filter cake are washed 2 times with 20 ml of extraction solvent, then finally with 10 ml extraction solvent.
- 7) Fifty ml of saturated NaCl is added to the separators and they shaken for 30 seconds.
- 8) One hundred ml of methylene chloride is then added to the separators and this is then shaken for 1 minute.

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- 9) The lower aqueous layer is then drained into a beaker and reserved for further extraction.
- 10) A 500 ml flat bottom flask is fitted to receive the organic layer by placing 1-1½" of anhydrous Na<sub>2</sub>SO<sub>4</sub> into a powder funnel plugged with glass wool.
- 11) The organic layer is passed through the drying medium into the 500 ml flat bottom flask.
- 12) The aqueous portion of the extract is returned to the original 500 ml separatory funnel and 50 ml of methylene chloride and 50 ml of acetonitrile are added to the aqueous extract.
- 13) The separator is then shaken for 1 minute and the phases are allowed to separate.
- 14) The lower aqueous phase is returned to the beaker and the upper organic layer is passed through the drying funnel into the same 500 ml flat bottom.
- 15) The aqueous layer is again returned to the original separators and 50 ml of methylene chloride is added.
- 16) The separator is shaken for 1 minute and the layers are allowed to separate.
- 17) The lower organic layer is passed through the drying funnel, and the upper aqueous layer is discarded.
- 18) The 500 ml flat bottom containing the cleaned up extract is then evaporated to dryness using a rotary vacuum evaporator with the aid of a water bath. The water bath temperature must not exceed 30°C or losses of DMG and DHG are likely to occur.
- 19) Evaporation flasks are to be removed immediately from vacuum and heat as soon as they come to dryness.
- 20) The contents of the evaporation flask are then rinsed quantitatively, using 4 or 5 individual washing of the evaporator at 2-2.5 ml of acetone to a total volume of 10 ml.
- 21) Individual volumetric aliquots of acetone extracts are then blown to dryness using a gentle stream of nitrogen and a room temperature water bath.
- 22) To achieve the low level detection limit for HPLC analysis the aliquot must be twice the reconstitution volume. For example a 2 ml aliquot of acetone is blown to dryness (step 21) and reconstituted with 1 ml of HPLC mobile phase. Smaller aliquots may be taken as needed for dilution as well as increasing the dilution volume. Solutions to be injected onto the HPLC must be diluted with HPLC mobile phase.

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23) For GC analysis the dilution ratio is 1-1 to achieve the low level detection limit. For example 1 ml of the extracts is blown to dryness (step 21) and reconstituted with 1 ml of acetone. Note: This step is needed to discharge any residual acetonitrile from the extraction solution. When evaporating acetone extracts, when a water bath is used, that water temperature should not exceed 30°C.

#### HPLC PARAMETERS

Column - Supelco LC 8 DB, 15 cm x 4.6 mm; 5  $\mu$  packing  
Cat #5-8347

Detection - UV at 225 nm

Injection Volume - 200  $\mu$ l

Flow rate - 2 ml/minute

Mobile Phase - 900 ml of 0.5 g  $(\text{NH}_4)_2\text{HPO}_4$  1 liter in HPLC quality water adjusted to a pH of 7.5  $\pm$ 0.05 using  $\text{H}_3\text{PO}_4$  mixed with 100 ml of methanol. Mobile phase  $\text{H}_2\text{O}/\text{MeOH}$  ratios may require adjustment for new columns or aged columns.

Autosampler rinse - 10% methanol in HPLC quality water

Note: Subsequent dilutions of samples must be made in mobile phase after removal of acetone.

#### GC PARAMETERS

Injector - 250°C using a disposable glass insert in the splitless mode with an injection volume of 2  $\mu$ l.

Column - DB-1 15 M x 0.53 mm ID at 5  $\mu$  film thickness (J & W Cat #125-1015 using an initial temperature of 150°C for 1 minute and then ramping at 10°C/minute to 230°C and holding for 2 minutes. The column flow rate is set at 7-9 ml/minute. This results in a head pressure of 9-11 psi.

Detector - A N-P detector at 320°C is used with gas flows set at, He make up at 18-20 ml/minute,  $\text{H}_2$  fuel at 3.3-3.5 ml/minute, and air flow set at 100-120 ml/minute.

Note: Dilutions of samples above the curve should be made using acetone.

#### QUANTITATION OF SAMPLES

Sample concentrations are interpolated from the standard curve response for each component. The found ppm is then calculated by the equation.

$$\text{Found ppm} = \frac{\mu\text{g/ml found} \times \text{extract volume (ml)} \times \text{dilution volume (ml)}}{\text{sample weight (g)} \times \text{aliquot volume (ml)}}$$

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Recovery of fortified control samples is calculated by:

$$\% \text{ Rec.} = \frac{\text{Found ppm (fortified)} - \text{Found ppm *(control)} \times 100}{\text{ppm added to fortified samples}}$$

\*This value is calculated from the standard curve even if the level is below the lowest standard curve point. Values less than zero are not used in this calculation.

Moisture in analytical samples is determined as in ABC S.O.P. 7.28. The calculations are also given in that SOP. Briefly the wet soil and containers are weighed and then dried to a constant weight at 105-130°C. The dry soil and containers are weighed after attaining a constant weight, usually overnight. Calculations given are:

$$\% \text{ Moisture} = \frac{B - C}{B - A} \times 100$$

A = weight of container (g)

B = weight of wet soil and container (g)

C = weight of dry soil and container (g)

The final correction would convert the metabolites to equivalent amount of the parent compound and this is:

$$\text{Molecular Ratio} = \frac{\text{Molecular weight of parent (g/mole)}}{\text{Molecular weight of metabolites (g/mole)}}$$

The final step is to convert the ppm found to the corrected ppm.

$$\text{corrected ppm (as parent)} = \frac{\text{Found ppm} \times \text{molar ratio} \times 100\% \times 100\%}{\% \text{ Recovery} \times (100 - \text{soil moisture } \% )}$$

Attachment 1 is a typical HPLC chromatogram with the peaks of interest labeled.

Attachment 2 is a typical GC chromatogram with the peaks of interest labeled.

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