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DETERMINATION OF V-10029 IN SOIL AND SOIL SEDIMENT METHOD RM-35S

DATE: MAY 13, 1997

INTRODUCTION

This method determines residues of V-10029 [KIH-2023, sodium 2,6-bis((4,6-dimethoxypyrimidin-2-yl)oxy) benzoate] in soil and soil sediment. This method is derived from the crop residue method developed by Kumiai Chemical Industry Co., Ltd[1].

Briefly, V-10029 residues are extracted from soil using a mixture of acetonitrile and water, then partitioned with dichloromethane/water. The aqueous phase, containing the V-10029 residues, is acidified, extracted with ethyl acetate, methylated with diazomethane and cleaned-up using a silica gel Sep-Pak. The V-10029 residues are quantified as the methyl ester of V-10029 (KIB-4662) by gas chromatography using a nitrogen-phosphorus specific flame-ionization detector (NPD). Confirmatory procedures are included in the method using an alternate GC column or a mass-selective detector.

REAGENTS

Acetone - pesticide quality or equivalent.

Acetonitrile - pesticide quality or equivalent.

Diazomethane - solution in diethyl ether. See Appendix I for preparation and safety considerations.

Dichloromethane - pesticide quality or equivalent. Must be alcohol free.

Ethyl acetate - pesticide quality or equivalent.

Hexane - pesticide quality or equivalent.

Hydrochloric acid - 36.5-38.0%, Baker-Analyzed, JT Baker Cat.# 9530-00; or equivalent.

Methanol - pesticide quality or equivalent.

1-Octanol - 99+%, Aldrich Cat # 29,324-1 or equivalent.

REAGENTS (CONTINUED)

Sodium chloride - reagent grade or equivalent.

Sodium dihydrogenphosphate, monohydrate ($NaH_2PO_4 \cdot H_2O$), reagent grade or equivalent.

Sodium hydrogenphosphate, heptahydrate ($Na_2HPO_4 \cdot 7H_2O$), reagent grade or equivalent.

Sodium sulfate - anhydrous, granular, AR grade or equivalent.

Water - deionized.

REAGENT SOLUTIONS

Acetonitrile:water, 9:1 (v/v) - Combine 9 parts acetonitrile with 1 part deionized water. For example, add 900 mL acetonitrile and 100 mL of deionized water sequentially to a reagent bottle. Store at room temperature.

Hexane:ethyl acetate, 4:1 (v/v) - Combine 4 parts of hexane with 1 part of ethyl acetate. For example, add 400 mL of hexane and 100 mL of ethyl acetate sequentially to a reagent bottle. Store at room temperature.

Hexane:ethyl acetate, 9:1 (v/v) - Combine 9 parts of hexane with 1 part of ethyl acetate. For example, add 900 mL of hexane and 100 mL of ethyl acetate sequentially to a reagent bottle. Store at room temperature.

Hydrochloric acid, 5.0 N - carefully add 416 mL of concentrated acid to a 1L flask, partially filled with deionized water. Dilute to volume with deionized water, stopper and shake. Store at room temperature.

Sodium dihydrogenphosphate, 0.05M - dissolve 6.90 grams of $NaH_2PO_4 \cdot H_2O$ in 1 liter of deionized water. Stopper and shake well until dissolved. Store at room temperature.

Sodium hydrogen phosphate, 0.05M - dissolve 13.4 grams of $Na_2HPO_4 \cdot 7H_2O$ in 1 liter of deionized water. Stopper and shake well until dissolved. Store at room temperature.

Buffer Solution, pH 7.4 - combine 800 mL of 0.05 M disodium hydrogen phosphate with 216 mL of 0.05 M sodium dihydrogenphosphate. Mix well. Store at room temperature.

Water saturated with sodium chloride - add 1000 grams of sodium chloride to 3 L of deionized water and stir or shake intermittently for at least one hour. Decant the supernatant solution into another vessel for use. Store at room temperature.

REFERENCE STANDARDS

V-10029 (KIH-2023) - analytical standard of known purity. Prepare a stock solution containing 1 mg/mL in methanol. Prepare a secondary stock solution containing 10 μg/mL in methanol by diluting the stock solution. Prepare a fortifying solution containing 1.0 μg/mL by diluting the secondary stock solution with acetone. All solutions should be kept refrigerated when not in use.

KIB-4662 (V-10029- methyl ester) - analytical standard of known purity. Prepare a stock solution containing 1 mg/mL in acetone. Prepare a minimum of four linearity standards by diluting this stock solution with acetone to concentrations ranging from 0.05 to 1.0 μg/mL. (See Note 1). Prepare a calibrating solution containing 1.0 μg/mL by diluting the stock solution with acetone. (The calibrating solution may be used as one of the four required linearity standards). All solutions should be kept refrigerated when not in use.

EQUIPMENT

Baker SPE-12G Column Processor (12-port vacuum manifold) - J.T. Baker Product # 7018-00 or equivalent system.

Beakers - 400 mL

Büchner funnels - 9 cm diameter.

Centrifuge tubes - 15 mL, calibrated, with stoppers.

Filter flasks - 500 mL.

Filter funnels - approximately 10 cm diameter.

Filter paper - Whatman GF/A glass fiber or equivalent, 9 cm diameter.

Gas Chromatograph - Hewlett-Packard Model 5890, equipped with a packed column glass insert for splitless injection (HP Part No. 5080-8732, packed with approximately 5 mm of silanized glass wool), an NP detector, automatic sampler, and HP ChemStation or equivalent system.

Glass wool - Pyrex® or equivalent.

Mason jars - 1 pint with plastic screw cap lids or equivalent.

N-Evap - Organomation Model 111 or equivalent. Install in an efficient fume hood.

Omni-Mixer with adaptor for use with 1-pint Mason jars.

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EQUIPMENT (CONTINUED)

Pasteur pipets - 5¾" and 9".

pH Meter - Corning Model 240 or equivalent.

Rotary evaporator - Büchi or equivalent, equipped with a temperature controlled water bath.

Round-bottom flasks - 50 mL, 500 mL, and 1000 mL.

Separatory funnels - 250, 500 mL

Sep-Pak Plus Silica Gel Cartridges - Waters Part # 20520 or equivalent. See Note 2.

Syringe - Yale hypodermic, 10 mL, glass Luer Tip.

Ultrasonic cleaner - Branson 3200 or equivalent.

Vials - glass, 6-dram with polyethylene lined screw cap.

Vortexer - Vortex Genie 2™, VWR or equivalent.

ANALYTICAL PROCEDURES

1. Extraction

Weigh 100 grams (± 0.1 grams) of soil into a one pint Mason jar. At this point, if required by the testing facility, control samples for method recovery should be fortified with V-10029 (See Note 3). Add 30 mL of deionized water and stir to thoroughly mix. Add 150 mL of acetonitrile:water (9:1, v/v) to the sample and blend on the Omni-Mixer for 10 minutes.

Filter the sample into a 500 mL filter flask using a Büchner funnel and Whatman GF/A glass fiber filter paper (use 2 pieces of filter paper to minimize clogging). Rinse the Mason jar with three 30 mL portions of acetonitrile:water (9:1, v/v) and add each portion to the Büchner funnel.

Transfer the combined filtrates to a 1000 mL round-bottom flask. Rinse the filter flask with two 20 mL portions of acetonitrile:water (9:1, v/v) and add to the round-bottom flask. Evaporate the acetonitrile using a rotary-evaporator and water bath set to <35°C. Approximately 60 mL of water will remain.

2. Dichloromethane/Water Partitioning

Transfer the aqueous extract to a 500 mL separatory funnel. Add 50 mL of pH 7.4 buffer solution to the round-bottom flask, briefly rotate the flask in an ultrasonic water bath to assist removal of residues adhering to the walls of the flask, then transfer to the separatory funnel. Add 5 mL of saturated sodium chloride and 50 mL of dichloromethane to the separatory funnel and shake vigorously for approximately one minute. Allow the phases to separate, then drain the lower dichloromethane layer into a clean 250 mL separatory funnel and transfer the aqueous phase to a 400 mL beaker. **Do not discard the aqueous phase.**

Add 50 mL of pH 7.4 buffer and 5 mL of saturated sodium chloride to the dichloromethane in the 250 mL separatory funnel and shake for one minute. Allow the phases to separate, then discard the lower dichloromethane layer. Combine the aqueous phase in the 250 mL separatory funnel with the aqueous phase from the first extract in the 400 mL beaker.

3. Ethyl Acetate/Water Partitioning (See Note 4)

Carefully adjust the pH of the combined aqueous phases to 3.0 by adding 5.0 N HCl (dropwise, approximately 1 mL) while monitoring the sample with a pH meter. Return the acidified aqueous phase to the 500 mL separatory funnel, add 5 mL of saturated sodium chloride solution, and 100 mL of ethyl acetate. Shake for approximately 1 minute then allow the phases to separate. Drain the lower aqueous phase into the 400 mL beaker and pour the upper ethyl acetate phase through a 10 cm filter funnel containing approximately 50 grams of sodium sulfate (suspended on a plug of glass wool) and collect in a 500 mL round-bottom flask.

Return the aqueous phase to the separatory funnel and re-extract with an additional 50 mL portion of ethyl acetate as described above. Drain the lower aqueous phase into the 400 mL beaker and discard. Pour the upper ethyl acetate phase through the sodium sulfate into the 500 mL round-bottom flask containing the first extract. Rinse the sodium sulfate with two 10 mL portions of ethyl acetate.

Evaporate the combined ethyl acetate layers to approximately 2 mL using a rotary-evaporator and water bath set to <35°C. Transfer the extract to a 6 dram screw cap vial using three 2 mL portions of ethyl acetate to rinse the round-bottom flask. Sonicate each rinse for approximately 15 seconds. Evaporate the ethyl acetate to approximately 2 mL under a gentle stream of nitrogen using the N-Evap and a water bath set to <35°C. Proceed immediately to Step 4. Methylation. See Note 4.

4. Methylation (See Appendix I for Preparation of Diazomethane)

Caution: Diazomethane is not only exceedingly toxic, but its solutions have been known to explode unaccountably. All work with diazomethane should be carried out behind safety shields in efficient hoods. Never allow diazomethane solutions to contact ground-glass joints or sharp glassware edges. See Appendix I for further safety considerations.

Add 3.0 mL of diazomethane solution to the vial containing the sample extract and securely cap. Allow to stand at room temperature for approximately 15 minutes. Add 100 µL of 1-octanol and evaporate **until only the octanol remains** using a gentle stream of nitrogen in the N-evap. Do not immerse the samples in the water bath. Add 1 mL of hexane to the vial and evaporate again **until only the octanol remains** using a gentle stream of nitrogen in the N-evap. Do not immerse the samples in the water bath.

5. Sep-Pak Silica Gel Cartridge Cleanup (See Note 2)

Attach a Sep-Pak silica gel cartridge to the Baker-SPE vacuum manifold. Attach a 10 mL glass syringe (plunger removed) to the column and pre-condition the column with 10 mL of hexane. **Do not exceed a flow rate of 5 mL/minute. Do not allow the column to dry before the sample is applied.**

Add 6 mL of hexane to the vial containing the methylated sample extract and sonicate the extract for 15 seconds. Transfer the extract to the column. Apply gentle vacuum to the sample until the solvent reaches the top of the column packing. **Do not allow the column to dry.** Repeat this procedure with another 6 mL of hexane followed by 6 mL of hexane:ethyl acetate (9:1, v/v). Rinse the sample vial with each solvent before adding to the column. Discard these eluants.

Place a 6 dram screw cap vial under the column and elute the KIB-4662 from the column as follows: add 10 mL of hexane:ethyl acetate (4:1, v/v) to the original sample vial, sonicate for approximately 15 seconds, then transfer to the column. Apply gentle vacuum to the sample until all the solvent has been eluted from the column.

Transfer the eluate to a 50 mL round-bottom flask using three 1 mL portions of acetone to rinse the vial. Evaporate the eluate **just to dryness** using a rotary-evaporator and water bath set to <35°C. Transfer the extract to a 15 mL calibrated centrifuge tube using three 1 mL portions of acetone to rinse the round-bottom flask (sonicate each rinse for 15 seconds). Evaporate the extract to slightly less than 1.0 mL using a gentle stream of nitrogen in the N-evap. Do not immerse the samples in the water bath. After allowing the sample to equilibrate to room temperature, adjust to exactly 1.0 mL with acetone. Sonicate each sample for approximately 15 seconds, then transfer the extract to an autosampler vial and store at ≤0°C until GC analysis.

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6. Gas Chromatography Measurement

Analyze the samples, along with calibrating standard solutions, using the following operating conditions:

Column: DB-5 (30 M x 530 μm) wide bore capillary (1.5 μm film thickness, J&W Cat # 125-5032 or equivalent).
Column Oven Temperature: 270°C. Hold Time: 10 minutes.
Column Temperature Program: 30°C/minute.
Final Column Temperature: 320°C. Hold Time: 5 minutes.
Detector Temperature: 300°C
Injector Temperature: 250°C
Carrier Gas: Helium at 10 mL/min
Make-Up Gas: Helium at 20 mL/min
Air: 102 mL/min
Hydrogen: 3.6 mL/min
Injection Size: 1.0 μl

The GC parameters shown above are given only as a guide. They may be modified as needed to optimize the chromatography or to resolve matrix interferences. Each set of chromatograms must be clearly labelled with the GC parameters used. See Note 5 for alternative GC parameters.

The recommended sequence of samples and standards for analysis is: calibrating standard, sample, sample, sample, calibrating standard, etc. (The calibrating standard vials contain 1.0 μg/mL of KIB-4662 in acetone). This sequence may, however, be modified if the reproducibility requirement is met. (See Note 6). Each sequence must begin and end with a calibration standard.

7. Calculations

The amount of V-10029 in each sample is calculated using the following formula:

$$ppm\ V\text{-}10029 = \frac{B \times C \times V \times MW \times DF}{A \times W}$$

where:

B = integration counts for KIB-4662 in the sample.
C = concentration of KIB-4662 in the calibrating standard (1.0 μg/mL).
V = final volume of the sample extract (1.0 mL).
MW = molecular weight factor (452 ÷ 444 = 1.02) to convert KIB-4662 to V-10029.
DF = dilution factor, used if the sample extract is diluted prior to analysis.
A = mean integration counts for KIB-4662 in the calibrating standards.
W = sample weight (100 grams).

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LIMITS OF DETECTION AND QUANTITATION

The limit of detection (LOD) of V-10029 in soil and soil sediment analyzed by this method is 0.0005 ppm (0.5 ppb). The validated limit of quantitation (LOQ) is 0.001 ppm (1.0 ppb).

ANALYSIS TIME

A trained analyst can complete the analysis of a set of eight samples for V-10029 in approximately 8 hours. The results are available within 24 hours of initiating the analysis.

NOTES

1. At Valent, linearity of the gas chromatograph must be determined each day that samples are analyzed (Valent SOP #VR-007). Linearity is determined by analyzing a series of linearity standards containing 0.05 to 1.0 μg/mL of KIB-4662. The response for each standard is normalized to response per 1.0 μg/mL by dividing the response of each standard by its concentration. The coefficient of variation (CV) of these responses must be 10% or less. Sample extracts must be diluted to bring the concentration of KIB-4662 within the range of linearity established.

2. Each batch of Sep-Pak disposable cartridges must be checked for recovery of KIB-4662 as follows: Transfer 5 mL of the 1.0 μg/mL KIB-4662 standard solution to a 6 dram screw cap vial, add 100 μL of 1-octanol, and evaporate **until only the octanol remains** using a gentle stream of nitrogen in the N evap. Do not immerse the vial in the water bath. Re-dissolve in 6 mL of hexane and transfer to a Sep-Pak silica gel disposable cartridge and elute the KIB-4662 as described under Step 5. **Sep-Pak Silica Gel Cartridge Cleanup**.

   Evaporate the eluate **just to dryness** using a rotary-evaporator and water bath set to <35°C. Re-dissolve the eluate in 5.0 ml of acetone and analyze with the 1.0μg/mL calibrating standard as described under Step 6. **Gas Chromatography Measurement**. If the KIB-4662 peak for the eluant is less than 90% of the calibrating standard, then the elution profile of KIB-4662 must be determined.

3. At Valent, a standard operating procedure (SOP# VR-002) requires that a fortified control sample be analyzed with each set of samples. If the testing facility does not require concurrent analysis of fortified control samples, or if a UTC sample is not available, this method requirement may be waived.

   The level of fortification is generally 0.001 ppm (the LOQ of the method) and/or 0.005 ppm. These fortifications are made by adding 0.10 mL and 0.5 mL, respectively, of the 1.0 μg/mL fortifying solution to a 100 gram sample. Method recoveries must be 70% to 120% to be acceptable unless approved by the chemist responsible for the analysis.

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NOTES (CONTINUED)

4. The free acid of V-10029 is an unstable compound. It is therefore important that the ethyl acetate extraction and methylation steps be performed as quickly as possible.

5. If matrix interferences are encountered during the analysis of V-10029, sample extracts may be re-injected using the following alternate GC parameters:

   Column: DB-17 (30 M x 530 μm) wide bore capillary (1.0 μm film thickness, J & W Scientific Cat # 125-1732 or equivalent).
   Column Oven Temperature: 260°C. Hold Time: 15 minutes.
   Detector Temperature: 300°C
   Injector Temperature: 250°C
   Carrier Gas: Helium at 30 mL/min
   Air: 110 mL/min
   Hydrogen: 3.6 mL/min
   Injection Size: 1.0 μl

   If the alternate GC parameters are unsuccessful at resolving matrix interferences or if confirmation of residues is required, sample extracts may be analyzed by GC/MS using the following parameters:

   Instrument - Hewlett-Packard Model 5890 gas chromatograph equipped with a 2 mm I.D. glass insert for splitless injection (Restek Part No. 20713, packed with approximately 5 mm of silanized glass wool), an HP 5970A Mass Selective Detector with a direct capillary interface, automatic sampler, and HP ChemStation or equivalent system.

   Column: DB-5MS (15 M x 250 μm, 0.25 μm film thickness)
   Injector Temperature: 280°C. Splitless Injection: 1.0 min purge
   Initial Column Oven Temperature: 150°C. Hold time: 1.0 minutes.
   Column Temperature Program Rate: 25°C/minute
   Final Column Temperature: 300°C. Hold time: 3 minutes
   Transfer Line Temperature: 280°C
   Carrier Gas: Helium at approximately 1 mL/min (5 psig column head pressure)
   Injection Size: 2.0 μl
   Acquisition Mode: SIM, 1 ion (385.2 m/z)*
   Solvent Delay: 2 minutes
   Dwell Time per ion: 1000 msec.

   *All qualifying ions are present at <5% of the base peak and therefore are unsuitable for quantitation (See Figure 7).

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6. At Valent, reproducibility of an analytical run is determined by calculating the CV from the peak units obtained for the calibrating standards analyzed during the run. For a run to be acceptable, these CV's must be 10% or less unless approved by the chemist responsible for the analysis (Valent SOP #VR-013).

REFERENCE

1. Matsushita, H., Tadaaki, U., *Analytical Method of KIH-2023 in Rice Straw and Hulled Grain by Gas Chromatography with a Nitrogen-phosphorus Detector*, File No. LEC-11-P-02, Kumiai Chemical Ind. Co., Ltd., December 6, 1990.

METHOD APPROVAL

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June 16, 1997

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APPENDIX I

PREPARATION OF DIAZOMETHANE

METHOD 1 (1-50 mmoles)

Caution: Diazomethane is not only exceedingly toxic, but its solutions have been known to explode unaccountably. All work with diazomethane should be carried out behind safety shields in efficient hoods. Never allow diazomethane solutions to contact ground-glass joints or sharp glassware edges. See below for further safety considerations.

EQUIPMENT

Mini-Diazald Apparatus - available from Aldrich Chemical Co. (Cat # Z10,889-8). See Figure 1.

Round-bottom flask - 100 mL, with Clear-Seal joint.

Separatory funnel - 125 mL, with Teflon stopcock and Clear-Seal joints.

Heated Water bath.

REAGENTS

Acetone - reagent grade.

Deionized water.

Diazald® - Cat. #D2800-0, Aldrich Chemical Co.

Diethyl Ether - reagent grade or equivalent.

Ethanol - denatured, OMNISOLV, VRW Cat # EM-EX0278-1 or equivalent. Prepare 95% (v/v) ethanol by adding 5 parts of deionized water to 95 parts of absolute (100%) ethanol.

Potassium Hydroxide, reagent grade or equivalent.

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PROCEDURE

1. Fill the condenser with dry-ice, then add acetone slowly until the cold-finger is about one-third full.

2. Add ethanol (95%, 10 mL) to a solution of potassium hydroxide (5 g) in water (8 mL) in the reaction vessel.

3. Attach a 100 mL receiving flask (with Clear-Seal joint) to the condenser and cool the receiver in an ice bath. Provide an ice-cooled ether trap at the sidearm.

4. Place a separatory funnel (with Clear-Seal joint) over the reaction vessel and charge funnel with a solution of Diazald (5.0 g, 23 mmol) in ether (45 mL).

5. Warm the reaction vessel to 65°C with a water bath and add the Diazald solution over a period of 20 minutes. The rate of distillation should approximate the rate of addition. Replenish the cold finger with dry ice as necessary.

6. When all the Diazald has been used up, slowly add 10 mL of ether and continue the distillation until the distillate is colorless. The ethereal distillate will contain about 700 mg (16.6 mmol) of diazomethane.

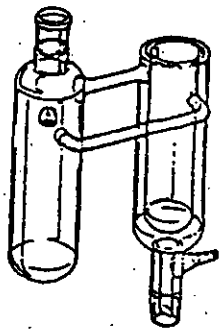

Figure 1. Mini-Diazald Apparatus

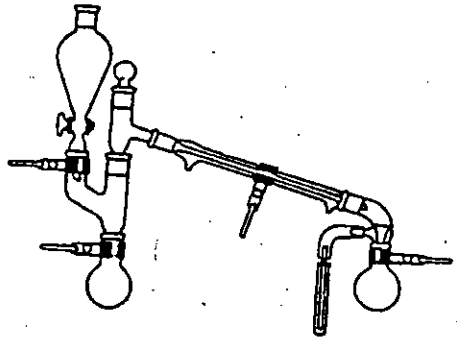

Figure 2. Diazald Kit

METHOD 2 (5-100 mmoles)

EQUIPMENT

Diazald Kit - available from Aldrich Chemical Co. (Cat # Z10,025-0). See Figure 2.

Round-bottom flasks - 500 mL, with Clear-Seal joint.

Separatory funnel - 125 mL, with Teflon stopcock and Clear-Seal joints.

Heated Water bath.

REAGENTS

Deionized water.

Diazald® - Cat. #D2800-0, Aldrich Chemical Co.

Diethyl Ether - reagent grade or equivalent.

Ethanol - denatured, OMNISOLV, VRW Cat # EM-EX0278-1 or equivalent. Prepare 95% (v/v) ethanol by adding 5 parts of deionized water to 95 parts of absolute (100%) ethanol.

Potassium Hydroxide, reagent grade or equivalent.

PROCEDURE

1. Assemble the Diazald Kit as shown in Figure 2. Place the reaction vessel (500 mL round-bottom flask) in a water bath. Place the receiving flask in an ice bath.

2. Add ethanol (95%, 50 mL) to a solution of potassium hydroxide (10 g) in water (16 mL) to the reaction vessel.

4. Charge the dropping funnel with a solution of Diazald (21.4 g, 0.1 mole) in ether (200 mL).

5. Warm the reaction vessel to 65°C and add the Diazald solution over a period of 30 minutes. The rate of distillation should approximate the rate of addition. Replenish the ice bath with ice as necessary.

6. When all the Diazald has been used up, slowly add 10 mL of ether and continue the distillation until the distillate is colorless. The ethereal distillate will contain approximately 3 g (70 mmol) of diazomethane.

SAFETY CONSIDERATIONS

"Although quite safe when handled as a dilute solution in an inert solvent, diazomethane presents several safety hazards of which all users of the reagent should be aware. It is both extremely toxic and highly irritating, causing pulmonary edema when inhaled in high concentration. Long-term, low-level exposure may lead to sensitization, resulting in asthma-like symptoms. Also, diazomethane and several of its chemical precursors have been cited as carcinogens.

"Diazomethane has been known to explode quite unaccountably, both as a gas and as a liquid, although rough surfaces are proven initiators of detonations. Thus, ground-glass joints and any glassware which have not been carefully fire polished must never be allowed to come in contact with diazomethane or its solutions. In addition, contact with alkali metals or drying agents such as calcium sulfate can result in an explosion. If moisture must be removed from a solution containing diazomethane, the recommended drying agent is potassium hydroxide pellets. Finally, solutions should not be exposed to strong light, which has been reported to initiate detonations.

"Fortunately, if the reagent is generated using the proper equipment and is handled only as a dilute solution at low temperature (ca. 0°C), the risks cited above are minimized. Of course, all reactions involving diazomethane should be carried out in an efficient fume hood and behind a sturdy safety shield. Finally, it is recommended that solutions of diazomethane be used immediately and not stored, even at low temperature."[1]

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[1] Black, T. Howard, "The Preparation and Reactions of Diazomethane", *Aldrichimica Acta* **1983**, 16(1),3.