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Sample Preparation Techniques for Evaluating Methanol and Formaldehyde Emissions from Methanol Fueled Vehicles and Engines

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1. Introduction

The recently released "Proposed Emission Standards and Test Procedures for Methanol-Fueled Vehicles and Engines"[1] requires the evaluation of methanol and formaldehyde levels, in addition to the usual analyses of currently regulated emissions. The analyzers used to measure the concentrations of the currently regulated gaseous emissions are capable of making their respective measurements directly from dilute exhaust samples that are collected during testing. In contrast, the methanol and formaldehyde samples must be specially prepared for chromatographic analysis. The Test and Evaluation Branch have designed methanol and formaldehyde sampling systems which use separate vacuum pumps to draw dilute exhaust through traps, which capture the compounds for analysis.[2] Water filled impingers are used to extract methanol, while silica gel cartridges coated with a complexing agent extract formaldehyde from the sample gas. Both methods require post-test processing and analysis to determine sample concentrations. The methanol and formaldehyde sample preparation procedures involve liquid solutions, and are called "wet chemistry" procedures.

This report describes the wet chemistry procedures used by the Emission Control Technology Division, Test and Evaluation Branch (TEB). It is divided into two sections, the first dealing with formaldehyde wet chemistry procedures and the second dealing with methanol wet chemistry procedures. The report assumes the reader is familiar with EPA vehicle test procedures for currently regulated emissions but is relatively unfamiliar with chemistry laboratory techniques.

The purpose of this report is to document the wet chemistry procedures used by TEB and to explain why they are used. The new formaldehyde and methanol wet chemistry procedures are detailed in Appendices 1 and 2 respectively.

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[1] "Proposed Emission Standards and Test Procedures for Methanol-Fueled Vehicles and Engines, Draft Regulations," U.S. Environmental Protection Agency, Office of Mobile Sources, Emission Control Technology Division, Summer 1986.

[2] Pidgeon, William M., "Formaldehyde Sampling From Automobile Exhaust: A Hardware Approach," U.S. EPA, Report No. EPA-AA-TEB-88-01, Office of Mobile Sources, Emission Control Technology Division, Test and Evaluation Branch, July 7, 1988.

2. Formaldehyde Sample Preparation Procedure

TEB collects formaldehyde samples using Sep-Pak silica gel cartridges that are coated by EPA with 2,4-dinitrophenylhydrazine (DNPH), according to procedures developed by Silvestre B. Tejada.[3] The cartridges require less preparation immediately before a vehicle test than the impingers previously used, since DNPH coating can be performed weeks before using the cartridges, and they are much smaller and easier to handle (1 cm outer diameter, 2.7 cm long, with 5 mm outer diameter tube ends at inlet and outlet having respective lengths of 1 and 2 cm).

When exhaust sample gas diluted by a constant volume sampler flows through the cartridge, the formaldehyde in the sample reacts with the DNPH, forming a formaldehyde-DNPH derivative. The wet chemistry procedure involves eluting (flushing) the cartridge with acetonitrile, producing a solution that can be evaluated by liquid chromatography for the mass concentration of the derivative. The mass concentration of formaldehyde is determined by the ratio of molecular weights, formaldehyde to formaldehyde-DNPH derivative.

Tejada's elution techniques form the basis of the TEB procedure, developed to meet the requirements of our chemistry laboratory environment. This procedure has been designed to minimize variability and sample contamination, while increasing productivity. In the following description of TEB's procedure, the underlined portions are those which differ from either Tejada's techniques or from previous TEB practice. Those changes are explained in the subsequent paragraphs.

TEB now performs the elution by connecting the inlet end of the DNPH coated cartridge to the outlet end of a syringe body at the luer tip where the needle would normally be connected. The plunger is removed from the syringe body before attaching the cartridge. The "syringe body - cartridge" assembly is placed in a holding rack with a 5 milliliter (ml) volumetric flask below the outlet end of the cartridge. Either a pipet or a

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[3] Tejada, Silvestre B. "Evaluation of Silica Gel Cartridges Coated *In Situ* with Acidified 2,4-Dinitrophenylhydrazine for Sampling Aldehydes and Ketones in Air," U.S. EPA, International Journal of Environmental Analytical Chemistry, Vol. 26, pp. 167-185, 1986.

glass syringe is used to measure 5.0 ml[4] of acetonitrile and to dispense the acetonitrile into the syringe body. The acetonitrile is pushed through the cartridge and into the volumetric flask with the syringe plunger. Then the eluate (the resulting solution) in the volumetric flask is brought to 5.00 ml with pure acetonitrile. This make-up step is made necessary by hangup of acetonitrile within the cartridge. The sample solution is then transferred to vials for analysis by liquid chromatography (LC).

Before discussing the revisions, clarification of certain terminology may be in order. A luer tip is a standardized male/female fitting, utilizing a friction fit between the syringe body, which has the male component, and the needle (or a cartridge), which has the female component. Since the cartridges are unmarked, the inlet and outlet ends of the cartridges are determined by the convention used in the DNPH coating process. EPA's convention is that the short tube (1 cm) is the inlet and the long tube (2 cm) is the outlet. The DNPH is added from the "inlet end."

2.1 Elution Changes

The first substantive change in TEB's revised procedure from Tejada's technique is that the cartridge is eluted in the direction of loading flow. Typically an elution would be performed in the reverse direction, but considerations unique to vehicle exhaust sampling justify inverting the cartridge during elution. Specifically, dilute vehicle exhaust will contain some quantity of particulate, which, if drawn through the formaldehyde sample probe, can be deposited on the inlet frit of the primary sampling cartridge. Eluting in the reverse direction of loading could cause any particulate on the frit to be washed into the eluate and be transferred into the sample vial. This particulate could interfere with the function of the liquid chromatograph, compromising not only the data from the contaminated sample, but all subsequent data as well.

The revised procedure differs further from Tejada's in that while he allowed the elution to take place by gravity feed, TEB forces the acetonitrile through the cartridge with a syringe plunger. The extended time requirements of the gravity technique were prohibitive; eluting by gravity feed requires approximately 5 minutes whereas

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[4] The number of significant figures will reflect the expected precision of the measurement. Precision is further discussed on pages 7 and 9.

forced elution takes less than 30 seconds. Both Tejada and Marcus Haubenstricker, PhD, a chemist at EPA's Motor Vehicle Emission Laboratory (MVEL), stated that the forced elution would not adversely affect DNPH recovery, if not performed too rapidly. Five milliliters of acetonitrile forced into the cartridge in 15 seconds (20 ml/min) appears to be sufficiently slow to avoid problems, as determined through preliminary qualification tests. Twenty milliliters per minute is recommended as the maximum elution rate, unless faster rates demonstrate equivalent results.

Another change from Tejada's procedures is a decrease in the quantity of acetonitrile used in the elution. While Tejada used 6 ml, his gravity feed technique resulted in greater hangup of acetonitrile in the cartridge than is the case with forced elution. If 6 ml are forced into the cartridge, the target volume of 5.00 ml of eluate is exceeded. Tejada and Haubenstricker also concurred with this modification of the elution procedure.

2.2 Volume Measurement Change

Another more important change from previous TEB practice is the use of volumetric flasks rather than centrifuge tubes for a critical volume measurement. The eluate is collected in a volumetric flask calibrated to contain 5.00 ml. This volume represents the dilution level of the sample, and must be known accurately if the mass emissions are to be determined from the concentration reported by the LC.

Previously, graduated centrifuge tubes were used to collect the eluate and the centrifuge tube's markings were used to measure 5.0 ml of solution. The volumetric flasks improve the accuracy and the repeatability of this measurement.

There are two problems with making the 5.00 ml measurement with the centrifuge tubes. The first problem is the relatively large diameter of the centrifuge tubes. The large diameter causes small changes in meniscus level to result in relatively large volume changes. Since several technicians perform the procedure, it is reasonable to assume that each differs slightly in interpreting the proper level of the meniscus relative to the 5 ml mark. Although these interpretation differences will also occur with volumetric flasks, the variability in volume will be diminished since the diameter of the volumetric flask is appreciably less than that of the centrifuge tube. As an example, if the true meniscus level is 1 mm lower than the 5 ml mark, then the flask's

observed volume will be 0.039 ml less than 5 ml or 0.78% low, whereas the centrifuge tube's volume will be 0.179 ml less or 3.59% low. The area of the flask neck is typically only 0.390 cm^2 as compared to the centrifuge tube's area of 1.794 cm^2. So the volume measurement error is over 4 times greater with the centrifuge tube for the same 1 mm error in meniscus reading.

Also, the large diameter of the centrifuge tube increases the likelihood of errors in reading the meniscus level. The volumetric flask's narrow neck makes it significantly easier to determine the relative position of the meniscus and the 5 ml mark, allowing an individual technician to improve the repeatability of his or her measurements, in addition to improving correlation among technicians.

The second problem with the centrifuge tube is the variability of the markings themselves. The centrifuge tubes previously used are marked from 0.1 ml to 15 ml in increments of 0.1 ml. Class A volumetric flasks, in contrast, are only marked to contain a single volume and are calibrated at this volume. As an example to illustrate the difference in marking accuracy, Ace Glass, Inc. lists the tolerance of their Class A 5 ml volumetric flask at ±0.02 ml or a 0.4% error band whereas their 15 ml centrifuge tubes, previously used, have a tolerance of ±0.20 ml at volumes above 3 ml or a 10 times larger error band at 5 ml of 4.0%.

In conclusion, the sample preparation accuracy and repeatability will be improved by switching from centrifuge tubes to volumetric flasks for collecting the formaldehyde that is eluted from the DNPH coated silica cartridges.

2.3 Sample Contamination Issue

Most of the emission tests run by TEB are two or three mode tests utilizing a primary and a secondary cartridge for each mode. Analyses suggested that the secondary samples may occasionally be contaminated with eluate from the primary samples. This contamination causes significant errors in the test results. The new wet chemistry procedure (Appendix 1) includes measures that should reduce the risk of sample contamination. These include using disposable pipets, a new glassware washing procedure, and other precautions justified by experience.

2.4 Formaldehyde Sample Preparation Procedure Summary

The new formaldehyde wet chemistry procedure is expected to increase productivity, accuracy, and repeatability and reduce errors due to sample contamination. These improvements were made by changing the elution procedure, using class A volumetric flasks in place of centrifuge tubes for volume measurements, and by revising written procedures to include methods to aid in avoiding sample contamination.

3. Methanol Sample Preparation Procedure

The procedure developed for methanol sampling and presently in use employs water-filled impingers through which are pumped a sample of the dilute exhaust or evaporative emissions. The methanol in the sample gas dissolves in the water. After the sampling period is complete, the solution in the impingers is prepared for gas chromatograph (GC) analysis.

The wet chemistry procedure by which TEB prepares methanol samples for the GC is derived from a procedure used by the Southwest Research Institute under contract with the EPA.[5] In qualifying TEB methanol sampling systems and procedures for testing, portions of the post-test wet chemistry procedures currently in place were identified as areas in which test accuracy and repeatability could be further improved.

The original procedure called for direct GC analysis of the solution in the impingers at the end of the test. Vial samples for the GC would be drawn directly from the impinger. However, MVEL's GC is programmed to accept methanol samples containing a known concentration of an internal standard, added prior to analysis. The internal standard allows the GC to compensate for errors in the automatic sample injection system.

During the operation of the GC, an automatic syringe injects a very small quantity (on the order of one microliter) of sample into the analysis column. Because the response of

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[5] Smith, Lawrence R, and Urban, Charles M., "Characterization of Exhaust Emissions from Methanol- and Gasoline-Fueled Automobiles," Southwest Research Institute (EPA Contract), Report No. EPA-AA-TEB-88-01, August 7, 1982.

the instrument is affected by the quantity of sample injected, variability in the automatic syringe can introduce error. The internal standard allows the instrument to correct for this variation. A compound, in this case isopropyl alcohol (isopropanol), is introduced into every sample at identical concentrations. The instrument is programmed to scale its response to this known concentration. For instance, if the injected quantity was below the nominal volume, the detected quantity of the internal standard and all other compounds would be low. The instrument would then scale up the reported concentrations proportionally to compensate for the low injected volume, delivering a more accurate analysis.

Because the internal standard must be present in each sample at a precise concentration, the amount of sample solution to which the isopropanol is added must be accurately determined. The TEB procedure previously involved pouring the solution from the impinger into a graduated centrifuge tube, and then bringing the volume to the 15 ml mark with additional reagent (water). Errors were introduced: sample remained unaccounted for on the sides of the impinger; the solution in the centrifuge tube was diluted when additional water was added; the rated tolerance of the centrifuge tube graduations was poor; a wide meniscus increased the likelihood of technician error.

To reduce or eliminate errors from these sources, the procedure has been revised further. After mixing the solution in the impinger to ensure uniform concentration, a 10 ml glass pipet nominally accurate to $\pm$ 0.2% at 20^{o}C is used to remove sample to the centrifuge tube, which is no longer used as a volumetric measure. The rated tolerance of the graduated centrifuge tubes is $\pm$ 1.3% at the volume in question, or six times larger than that of the pipets. The consistency with which the technician can bring the meniscus to the calibration line is also enhanced with the use of pipets. The pipet is narrow at the calibration mark, producing a well defined meniscus which can be adjusted precisely. The liquid surface area in the centrifuge tubes, by contrast, is more than eight times that of the pipets at the point where the measurement is made, and a well defined meniscus is difficult to maintain and read.

An appropriate quantity of isopropanol is then added to the sample solution in the centrifuge tube. An electronic pipet with a rated accuracy of 0.8% and precision of 0.15% is employed to add 400 microliters of a prepared solution of isopropanol in water. The dilution ratio of 200 µl added to each 5 ml of solution produces the concentration of internal standard for which the GC is programmed. The resulting solution is mixed and placed in vials for GC analysis.

placed in vials for GC analysis.

This revised chemistry procedure should increase the accuracy and repeatability of methanol emission evaluation. The essential elements have been employed by TEB technicians over a development period of several months; their input went into the development of the procedure, particularly with regard to contamination control and efficiency. Certain substitutions could be made without compromising the procedure; for instance, a cheaper test tube could be used in place of the centrifuge tube, or a calibrated manual pipet could be used for the internal standard dilution, provided accuracy and precision levels were maintained.

4. Appendices

Following are the formaldehyde and methanol wet chemistry procedures, as currently employed by TEB. These instructional step-by-step listings are given to the technicians during their training on the procedure. They come about from the application of our experience with methanol vehicle testing, while drawing on the work of others, such as Silvestre Tejada.

Some of the specifics of these procedures could be altered without compromising the data. For instance, while any method could be used to transfer formaldehyde sample to the vials, we chose long-stemmed disposable pipets, which not only can reach the sample through the narrow necks of the volumetric flasks, but are single-use, to reduce the chances of cross contamination between samples. The electronic and automatic pipets are used for convenience and to improve repeatability. A calibrated syringe or some other method might prove as effective in another lab, or for a smaller scale operation. Any changes should be evaluated with regard to the issues of accuracy, repeatability and contamination.

Appendix 1: Revised Formaldehyde Wet Chemistry Procedure - TEB

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1. **Select the required number of cartridges**: one primary and one secondary for each bag or SHED sample taken.

2. **Mount the cartridges securely on the sampling system,** with the shorter end as the inlet. Handle cartridges only by the center section, not the tips, to avoid contamination.

PERFORM THE EMISSIONS TEST.

Note: Do not replace cartridges in their original glass tubes after the test. The tubes will be reused for fresh cartridges and should not be contaminated. Specially designated tubes may be used to carry the cartridges to the Chemistry Lab.

Use disposable gloves and eye protection. Wash hands before touching gloves to help avoid sample contamination.

3. In the Chemistry Lab, **insert a 10 ml plastic syringe body** into the inlet end of each cartridge. **Place a plastic pipet tip** over the outlet to facilitate elution.

4. Place an appropriate number of 5 ml volumetric flasks into the rack provided, and place a cartridge-syringe body assembly into each.

For each cartridge:

5. Using a glass pipet or syringe, **measure 5 ml of acetonitrile** into the syringe body. Pour the acetonitrile from the reagent bottle into a small, clean beaker and draw from there as needed. Do not introduce a pipet or syringe into the reagent bottle, to avoid contaminating the supply.

6. With the syringe plunger, **push the acetonitrile through the cartridge** into the flask. This step should take no less than 15 seconds. Eluting too rapidly will

result in incomplete recovery of the DNPH derivative. Dispose of the cartridge and pipet tip.

7. Using the automatic pipet, **fill the volumetric flask** to the calibration line with acetonitrile; do not allow the dispenser tip to contact the flask. **Agitate the flask** vigorously for ten or more seconds to mix the sample. (The Vortex Genie is a preferred method, provided no sample is lost.)

8. Using a disposable pipet, **transfer the solution into chromatograph sample vials**, approximately 1.5 ml per vial, or up to the shoulder of the vial. Care must be taken to avoid contamination of the sample. This means always using a new disposable pipet for each cartridge sample. Throw away the disposable pipets immediately after use. Prepare one vial from each cartridge, unless otherwise directed in the test plan. Label each vial with an indelible **black** pen, including test number, date, bag or sample number, primary or secondary, and whatever additional information may be required to properly identify the sample.

9. **Cap the vials** with the tool provided. The caps should be secure enough that they will not slip when twisted by hand. Use the **red** caps (double Teflon lined silicon) for formaldehyde samples.

10. **Repeat steps 5 - 9 for each cartridge.** Samples may be contaminated by glassware which has not been thoroughly cleaned and dried, by accidental contact with other sample solutions (e.g., a disposable pipet used for a primary cartridge will seriously contaminate the secondary if used in place of a new pipet), or by contact with the skin.

11. Place the sample vials in the rack provided. Place any data which may be needed by the Chemistry Lab to process the samples under the rack.

12. Dispose of excess sample in the waste DNPH bottle. Clean all glassware thoroughly after use, disposables excepted. Rinse several times with acetonitrile (until yellow color is gone), then tap water, followed by deionized water, prior to drying. Dispose of all contaminated disposable pipets, pipet tips or other items, to prevent their accidental reuse.

13. At the conclusion of the day's testing, the waste DNPH bottle should be emptied

into the receptacle provided. The waste bottle is not meant for storage. After cleaning, the glassware should be placed in the oven overnight to dry. This should include any beakers or pipets used, as well as the volumetric flasks.

Additional Notes:

- If multiple cartridge samples are being prepared simultaneously, it is preferable to perform a step on the secondaries before moving to the primaries. This can help reduce the impact of accidental contamination.

- The glass syringes used to measure and dispense acetonitrile in this procedure are manufactured as a matched syringe body/plunger set. Before reassembling the syringe after drying, ensure that the control numbers on the syringe body and plunger match. Attempted assembly of unmatched parts can result in breakage.

- All supplies should be readily available in the Chemistry Lab. Both TEB technicians and certain Chemistry Lab personnel familiar with these procedures should be able to locate all equipment and supplies mentioned in this procedure. Comparable equipment may be substituted, provided accuracy and sample integrity are not compromised. For instance, sample may be poured into a disposable test tube, and then transferred to the sample vials with a dropper, if the disposable pipets are unavailable. Any substituted glassware should be cleaned with acetonitrile prior to use, disposables excepted. If the equipment or supplies are unavailable for proper performance of the wet chemistry, the end plugs of the cartridges should be replaced, and the cartridge placed in a labeled glass tube in the freezer, until the proper equipment can be located. Be certain to note any unusual handling of the sample in the test documentation.

Appendix 2: Revised Methanol Wet Chemistry Procedure - TEB

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1. **Select required number of impinger sets**: one primary and one secondary for each bag or SHED sample taken.

2. Ensure that the glassware is clean and dry.

3. Using a glass pipet, **measure 15 ml deionized water** into each impinger body.

4. **Insert impinger tops securely** with a twisting motion and **clamp impinger set into place** on sampling system.

PERFORM THE EMISSIONS TEST.

Use disposable gloves and eye protection. Wash hands before touching gloves to help avoid sample contamination.

For each impinger:

5. In the Chemistry Lab, **remove the top** from the impinger, taking care to remove as little liquid as possible.

6. **Cover the top** of the impinger body with paraffin film and seal to prevent loss of sample. Ensure that only the protected side of the paraffin comes into contact with the sample solution.

7. Shake the impinger body thoroughly to **mix the sample** to a uniform concentration. Be sure to mix in the drops clinging to the sides.

8. Remove the paraffin, and, using a glass pipet, **transfer 10 ml of sample** into a clean, dry centrifuge tube. Use a clean, dry pipet for each impinger to avoid contamination. (This is extremely important. If a pipet used for a primary solution is subsequently used on a secondary, the test results will be erroneous.)

The centrifuge tube may not show 10 ml; however, the pipet is the more accurate of the two.

9. Using the electronic pipet, **add 400 µl isopropanol internal standard solution** to the centrifuge tube.

10. Cover the tube securely with paraffin film and **shake thoroughly.**

11. Remove the paraffin, and, using a disposable pipet, **transfer the sample solution to chromatograph vials,** approximately 1.5 ml per vial, or up to the shoulder of the vial. Care must be taken to avoid contamination of the sample. This means always using a new disposable pipet for each impinger sample. Throw away the disposable pipets immediately after use. Prepare one vial from each cartridge, unless otherwise directed in the test plan. Label each vial with an indelible **red** pen, including test number, date, bag or sample number, primary or secondary, and whatever additional information may be required to properly identify the sample.

12. **Cap the vials** with the tool provided. The caps should be secure enough that they will not slip when twisted by hand. Use the **brown** caps (Teflon lined butyl) for methanol samples.

13. **Repeat steps 5 - 12 for each impinger.** Samples may be contaminated by glassware which has not been thoroughly cleaned and dried, by accidental contact with other sample solutions (e.g., a pipet used for a primary impinger will seriously contaminate the secondary if used in place of a clean pipet), or by contact with the skin.

14. Place the vials in the lab tray provided for methanol samples. Place any data which may be required by the Chemistry Lab to process the samples under the rack.

15. Rinse all glassware thoroughly with deionized water prior to drying. Pipets should be placed in the oven to dry, while impingers may air dry on the racks provided. Dispose of all contaminated disposable pipets or pipet tips, to prevent their accidental reuse.

Additional notes:

- If multiple impinger samples are being prepared simultaneously, it is preferable to perform a step on the secondaries before moving to the primaries. This can help reduce the impact of accidental contamination.

- All supplies should be readily available in the Chemistry Lab. Both TEB technicians and certain Chemistry Lab personnel familiar with these procedures should be able to locate all equipment and supplies mentioned in this procedure. Comparable equipment may be substituted, provided accuracy and sample integrity are not compromised. For instance, sample may be pipeted into a test tube for spiking with isopropanol if centrifuge tubes are unavailable, or transferred to the sample vials with a dropper, if the disposable pipets cannot be located. Any substituted glassware should be cleaned with deionized water prior to use, disposables excepted. If the equipment or supplies are unavailable for proper performance of the wet chemistry, the sample should be poured from the impingers into a clean vial with a volume of approximately 20 ml (ask Chemistry Lab personnel for assistance locating a vial), sealed, and refrigerated until the proper supplies become available. Be certain to note any unusual handling of the sample in the test documentation.

- If glass pipets used to remove sample from the impingers contact no other solution, merely rinse the outside of the pipet with deionized water and place the pipet in the oven. Both the methanol and water will evaporate leaving a clean pipet. Never use a pipet to which drops of liquid are clinging. Use only clean, dry glassware.