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Project Summary

EPA Method Study 22 Method 612-Chlorinated Hydrocarbons

Jack R. Hall, J. Richard Florance, Dennis L. Strother, and Marlene N. Wass

An interlaboratory study in which 20 laboratories participated was conducted to provide precision and accuracy statements for the proposed EPA Method 612-Chlorinated Hydrocarbons for measuring concentrations of the Category 3 chemicals hexachloroethane, hexachlorobutadiene, 2-chloronaphthalene, 1-2, dichlorobenzene, 1,3-dichlorobenzene, 1,4-dichlorobenzene, 1,2,4-trichlorobenzene, hexachlorobenzene, and hexachlorocyclopentadiene in municipal and industrial aqueous discharges. Hexachlorocyclopentadiene was eliminated from the study because of its instability in the solvent used to prepare sample concentrates.

The study design was based on Youden's plan for collaborative tests of analytical methods. Three Youden pair samples of the test compounds were spiked into six types of test waters and then analyzed. The test waters were distilled water, tap water, a surface water, and three different industrial wastewater effluents. The resulting data were statistically analyzed using the computer program entitled "Interlaboratory Method Validation Study" (IMVS). Using the mean recovery for each subject compound, the mean recoveries for the method were in the range of 64 to 90%. Overall precision was in the range of 26 to 41%, and single-analyst precision was in the range of 16 to 24%. In general, mean recoveries, overall standard deviation (S), and the single-analyst standard deviations (SR) were directly proportional to the true concentration levels. In all cases, there was no evidence of a statistically significant effect on accuracy or precision due to water type.

This Project Summary was developed by EPA's Environmental Monitoring and Support Laboratory, Cincinnati, Ohio, to announce key findings of the research project that is fully documented in a separate report of the same title (see Project Report ordering information at back).

Introduction

EPA first promulgated guidelines establishing test procedures for the analysis of pollutants in 1973, following the passage of the Federal Water Pollution Control Act in 1972 by Congress. Pursuant to the amendment and publication of these guidelines, EPA entered into a Settlement Agreement—the Consent Decree—which required the study and, if necessary, regulation of 65 "priority" pollutants and classes of pollutants of known or suspected toxicity to the biota. Subsequently, Congress passed the Clean Water Act of 1977, mandating the control of toxic pollutants discharged into ambient waters by industry.

In order to facilitate the implementation of the Clean Water Act, EPA selected for initial study 129 specific toxic pollutants, 113 organic and 16 inorganic. The organic pollutants were divided into 12 categories based on their chemical structure. Analytical methods were developed by EPA for these 12 categories through in-house and contracted research and may eventually be required for the monitoring of the 113 toxic pollutants in industrial wastewater effluents, as specified by the Clean Water Act of 1977.

This report describes the interlaboratory study of Method 612 Chlorinated Hydrocarbons, which is proposed for the Category 3 chemicals: hexachloroethane, hexachlorobutadiene, 2-chloronaphthalene, 1,2-dichlorobenzene, 1,3-dichlorobenzene, 1,4-dichlorobenzene, 1,2,4-trichlorobenzene, hexachlorobenzene, and hexachlorocyclopentadiene. Hexachlorocyclopentadiene, one of the category

compounds, was dropped from the method study because of its instability in the solvent used to prepare sample concentrates. The primary objective of the study was to characterize the behavior of Method 612 in terms of accuracy, overall precision, single-analyst precision, and effect of water type on accuracy and precision. The study was conducted with the cooperation of 20 participating laboratories under auspices of the Environmental Monitoring and Support Laboratory (EMSL)-Cincinnati.

The data were collected from the 20 laboratories according to Youden's collaborative testing design. Formal statistical techniques compatible with the Youden design were used to identify outliers, estimate the method's accuracy and precision, and test for the effect of water type. The formal statistical analyses were carried out using the IMVS computer program. The information obtained from the statistical analyses was summarized and reduced to a descriptive form for the purpose of interpretation and presentation.

Method 612 was developed by IT Enviroscience under a contract with the Physical and Chemical Methods Branch, EMSL-Cincinnati. It requires extraction of the pollutants with methylene chloride, solvent exchange, concentration by Kuderna-Danish, extract cleanup on activated Florisil®, and subsequent gas chromatographic (GC) analysis using electron capture (EC) detection.

Procedure

The interlaboratory study design was based on Youden's plan for collaborative evaluation of precision and accuracy for analytical methods. According to Youden's design, samples are analyzed in pairs. A Youden pair consists of two samples of similar, but distinctly different concentrations. The analyst is requested to perform only a single analysis for each sample and report only one value as in routine use of the method.

Selection of Laboratories

Of the 20 participating laboratories, 19 were selected as the result of competitive bidding after their technical capabilities and experience in trace organic analyses of wastewater had been evaluated. The twentieth laboratory was a volunteer from within the EPA.

Preparation of Ampuls

All starting materials were reagent-grade quality or better. Distilled-in-glass methyl ethyl ketone was the solvent. Separate stock solutions for each of the eight chlorinated hydrocarbons were prepared by dissolving a precisely weighed amount of the compound into Class A volumetric glassware containing the solvent. Appropriate volumes of the stock solutions were mixed and diluted to volume in 2000-mL volumetric flasks. The flasks were refrigerated overnight at 4°C. The following day, approximately 3-mL of the refrigerated solution was transferred into 5-mL glass ampuls using an all-glass or Teflon® delivery system. After the ampuls were cooled in a freezer at -30°C for three hours, they were sealed by a professional glass blower using the pull-and-twist technique.

True Value and Stability of Concentrates

An important segment of this study was to verify the true values and stability of the chlorinated hydrocarbons in the methyl ethyl ketone concentrates before they were used in the study. To achieve this verification, three replicate ampuls were randomly selected from each concentrate level batch and analyzed by triplicate injection into a gas chromatograph equipped with a recording integrator. To check stability these analyses were carried out at 0, 45, and 90 days after the ampuls were sealed and before the study was started. All concentrate values determined experimentally were within instrumental error or the calculated true values.

Preliminary Study

Previous EPA method studies have shown that more realistic results can be obtained if all participants thoroughly understand the analytical and sample handling procedures before undertaking the full study. To familiarize the analyst with these procedures, each of the 20 laboratories was sent a low-level Youden pair of sample concentrates (different from those to be used in the actual study) for spiking distilled water, along with instructions, a copy of the method, and data report sheets.

The results of these analyses were collected, statistically analyzed, and discussed with the laboratories' representatives in a one-day conference meeting at EMSL-Cincinnati. The meeting also allowed discussion of analytical problems and clarification of any methodology procedures.

Actual Interlaboratory Study

A summary of the test design using Youden's nonreplicate technique based on pairs with slightly dissimilar analyte concentrations is given below:

- Twenty laboratories were sent three Youden pairs in sealed glass ampuls containing various levels of the eight chlorinated hydrocarbons in methyl ethyl ketone.
- When an analyst was ready to start the analyses, the ampuls were opened and aliquots were diluted to volume in the appropriate water types according to instructions.
- Each sample (ampul) was analyzed only once.
- The six water types were analyzed with and without spiking, and the added level of constituent was determined by difference and reported as $\mu g/L$ in each water sample.
- The three levels of chlorinated hydrocarbons used in the study were within the working range of the method and represented the range of levels one would normally expect to encounter in application of the method to actual samples.

Description and Distribution of Samples

The individual laboratories provided their own samples of laboratory-distilled water, tap water, and a local surface water. The source for each surface water is listed in the final report.

The wastewater effluent samples representative of the industries of concern were collected by the prime contractor as grab samples in 55-gallon stainless steel drums which had been precleaned with acetone, methylene chloride, and distilled water. The unfiltered wastewater samples were mixed and transferred into one-quart bottles using an all-Teflon system and stored in a refrigerator at 4°C until shipment to the laboratories. Each laboratory was sent 36 ampul concentrates (six sets of three Youden pairs), seven one-quart bottles each of the three industrial effluent types, instructions, and data report sheets. The wastewater samples were packed in ice in coolers and sent by air freight to minimize sample change and assure comparability of the wastewaters from laboratory to laboratory.

Analysis and Reporting

A water spiking technique involving a water-soluble solvent concentrate (methyl ethyl ketone is approximately 28% soluble in water) was used in this study. Each analyst was instructed to add separate 2.0-mL aliquots of each concentrate to the bottle containing approximately

1 L of water or wastewater. The spiked sample was stirred for 15 minutes and then handled as a routine sample in the method. The results of the method's measurement for each constituent (in micrograms per liter of water) were then corrected by subtracting any blank sample reading and reported on data sheets by ampul and water type. Method 612 was utilized by the participating laboratories with no reported deviations.

The data were reviewed for completeness and abnormal results, and were then entered into the computer for statistical treatment. Any laboratory reporting unusually high or low data was requested to review them for errors in calculations, but was not told how its reported data varied from the true values.

Treatment of Data

The objective of this interlaboratory study was to obtain information about the accuracy and precision associated with measurements generated by Method 612. This objective was met through the use of statistical analysis techniques designed to extract and summarize the relevant information about accuracy and precision from the data reported by the participating laboratories. The statistical techniques were similar to the techniques recommended in the ASTM Standard Practice D2777-77.

The algorithms required to perform the statistical analyses were integrated into the IMVS system of computer programs. The analyses performed by IMVS included several tests for the rejection of outliers (laboratories and individual data points); summary statistics by concentration level for mean recovery (accuracy); overall and single-analyst standard deviation (precision); determination of the linear relationship between mean recovery and concentration level; determination of the linear relationship between the precision statistics and mean recovery; and a test for the effect of water type on accuracy and precision.

Results and Discussion

The IMVS computer program was designed to output the raw data in tabular form and compile summary statistics including: number of data points; true value; mean recovery; accuracy as percent relative error; overall standard deviation; overall percent relative standard deviation; single-analyst standard deviation; and single-analyst percent relative standard deviation. The statistical analyses performed by the IMVS program included the determination of the linear relationship between both the overall (S) and single-analyst (SR) precision statistics and mean recovery along with accuracy statements based on the determination of the linear relationship between mean recovery (X) and concentration level. The results of the regression analyses indicate apparent linear relationships for each of the above cases.

For all data for the eight compounds, 25% of the raw data were rejected as determined by laboratory ranking and individual outlier tests. The data rejection was found to be nonuniform among laboratories. More than 82% of the rejected data were generated by eight of the 20 participating laboratories, and for one, 98% of its total raw data were rejected as outliers. Nine laboratories' raw data were rejected for between one and four of the compounds studied in all water types.

Regression equations for single-analyst precision, overall precision, and accuracy are presented in Table 1. Mean recoveries of the subject compounds were in the range of 64 to 90%. Overall precision was in the range of 26 to 41%, and single-analyst precision was in the range of 16 to 24%.

Based on the IMVS test for the effect of water type on precision and accuracy, there was no statistical significance between distilled water and the corresponding wastewater for any of the associated parameters during EPA Method Study 22.

Conclusions and Recommendations

Based on the results of EPA Method Study 22, EPA Method 612 is a viable analytical method for measuring trace concentrations of the eight Category 3 chemicals used. As a result of the collaborative study conducted in the IMVS data analysis, the following conclusions and recommendations can be made concerning Method 612.

- The accuracy of the method could be expressed as a linear function of the true concentration. In the majority of equations, the slope represents the percent recovery attributable to the method.
- The precision of the method could be expressed as a linear function of the mean recovery. In the majority of equations, the slope represents the relative standard deviation attributable to the method, both as single-analyst and overall standard deviations.
- The average mean recovery of the six concentrations in six water types for each compound compared well with data generated on distilled water industrial effluents during the development of this method.
- No significant difference in method performance was attributable to the water type from which the analysis was performed.
- One laboratory had trouble with the extract concentration step using the Kuderna-Danish apparatus and some laboratories had to use peak height measurements for quantitation because occasional interference peaks in wastewater created faulty integration when using recording integrators.
- When using the method, the analyst should develop recovery and precision data for the wastewater being analyzed. These data should be based on the concentration levels determined or expected.
- In future interlaboratory studies, very detailed instruction should be given to the participating laboratories to ensure labeling of each chromatogram. In this study it was very difficult to interpret much of the raw chromatographic data because of inadequate labeling. Other points to be emphasized in future studies are that (a) blanks and spiked samples must be analyzed at the same sensitivity and (b) calculations and record keeping should be uniform or consistent to aid in data interpretation.

Table 1. *EPA Method Validation Study*

Regression Equations for Accuracy and Precision for Compounds 1 - 4

Water Type	Hexachloroethane	Hexachlorobutadiene	2-Chloronaphthalene	1,2-Dichlorobenzene
Applicable Conc. Range	(1.02 - 14.80)	(3.12 - 36.80)	(19.10 - 268.00)	(29.80 - 356.00)
Distilled Water				
Single-Analyst Precision	SR = 0.23X + 0.07	SR = 0.18X + 0.08	SR = 0.28X - 1.17	SR = 0.22X - 2.95
Overall Precision	S = 0.36X - 0.00	S = 0.53X - 0.12	S = 0.38X - 1.39	S = 0.41X - 3.92
Accuracy	X = 0.74C - 0.02	X = 0.61C + 0.03	X = 0.75C + 3.21	X = 0.85C + 0.70
Tap Water				
Single-Analyst Precision	SR = 0.33X - 0.10	SR = 0.29X - 0.23	SR = 0.16X + 0.25	SR = 0.17X + 10.94
Overall Precision	S = 0.34X - 0.04	S = 0.42X + 0.14	S = 0.26X - 0.71	S = 0.31X + 5.35
Accuracy	X = 0.78C - 0.09	X = 0.64C + 0.18	X = 0.74C + 3.41	X = 0.87C + 6.70
Surface Water				
Single-Analyst Precision	SR = 0.17X + 0.51	SR = 0.30X + 0.05	SR = 0.13X + 0.92	SR = 0.30X - 4.00
Overall Precision	S = 0.46X + 0.07	S = 0.39X - 0.06	S = 0.18X + 1.20	S = 0.32X - 2.18
Accuracy	X = 0.83C + 0.06	X = 0.62C + 0.12	X = 0.77C + 3.67	X = 0.81C + 0.44
Waste Water 1				
Single-Analyst Precision	SR = 0.24X - 0.05	SR = 0.25X + 0.21	SR = 0.12X + 14.77	SR = 0.13X + 4.87
Overall Precision	S = 0.26X + 0.07	S = 0.37X + 0.02	S = 0.24X + 8.18	S = 0.26X + 4.26
Accuracy	X = 0.80C + 0.05	X = 0.66C + 0.08	X = 0.74C + 11.64	X = 0.85C - 0.52
Waste Water 2				
Single-Analyst Precision	SR = 0.25X + 0.03	SR = 0.25X + 0.44	SR = 0.12X + 8.51	SR = 0.22X + 1.60
Overall Precision	S = 0.45X - 0.09	S = 0.39X + 0.39	S = 0.27X + 2.91	S = 0.34X - 0.63
Accuracy	X = 0.73C - 0.03	X = 0.63C + 0.39	X = 0.72C + 7.64	X = 0.83C + 3.41
Waste Water 3				
Single-Analyst Precision	SR = 0.20X - 0.05	SR = 0.18X + 0.14	SR = 0.14X + 2.92	SR = 0.21X + 2.78
Overall Precision	S = 0.27X - 0.03	S = 0.38X - 0.06	S = 0.26X + 2.79	S = 0.32X + 0.77
Accuracy	X = 0.81C - 0.13	X = 0.71C + 0.31	X = 0.74C + 2.55	X = 0.87C + 5.53

Regression Equations for Accuracy and Precision for Compounds 5 - 8

Water Type	1,3-Dichlorobenzene	1,4-Dichlorobenzene	1,2,4-Trichlorobenzene	Hexachlorobenzene
Applicable Conc. Range	(20.40 - 238.00)	(23.00 - 324.00)	(15.10 - 216.00)	(1.29 - 14.90)
Distilled Water				
Single-Analyst Precision	SR = 0.21X - 1.03	SR = 0.16X - 0.48	SR = 0.23X - 0.44	SR = 0.14X + 0.07
Overall Precision	S = 0.49X - 3.98	S = 0.35X - 0.57	S = 0.40X - 1.37	S = 0.36X - 0.19
Accuracy	X = 0.72C + 0.87	X = 0.72C + 2.80	X = 0.76C + 0.98	X = 0.87C - 0.02
Tap Water				
Single-Analyst Precision	SR = 0.23X + 2.91	SR = 0.20X + 2.80	SR = 0.15X + 0.60	SR = 0.22X - 0.08
Overall Precision	S = 0.35X + 2.65	S = 0.26X + 5.88	S = 0.30X + 0.72	S = 0.32X - 0.14
Accuracy	X = 0.79C + 4.02	X = 0.75C + 4.15	X = 0.68C + 1.97	X = 0.92C - 0.08
Surface Water				
Single-Analyst Precision	SR = 0.17X - 0.16	SR = 0.17X + 5.13	SR = 0.16X + 0.75	SR = 0.08X + 0.13
Overall Precision	S = 0.26X - 1.04	S = 0.42X + 0.22	S = 0.43X + 0.42	S = 0.22X - 0.01
Accuracy	X = 0.75C + 0.55	X = 0.73C + 5.56	X = 0.74C + 2.40	X = 0.97C - 0.06
Waste Water 1				
Single-Analyst Precision	SR = 0.16X - 0.47	SR = 0.24X - 0.81	SR = 0.39X - 1.97	SR = 0.11X + 0.19
Overall Precision	S = 0.34X + 0.03	S = 0.25X + 4.30	S = 0.42X - 1.05	S = 0.18X + 0.16
Accuracy	X = 0.78C + 4.47	X = 0.77C + 2.06	X = 0.75C + 0.70	X = 0.87C + 0.17
Waste Water 2				
Single-Analyst Precision	SR = 0.19X + 2.61	SR = 0.19X + 1.11	SR = 0.26X - 0.81	SR = 0.25X - 0.18
Overall Precision	S = 0.39X + 0.22	S = 0.34X + 1.80	S = 0.31X + 0.33	S = 0.39X + 0.16
Accuracy	X = 0.75C + 1.34	X = 0.78C + 2.25	X = 0.80C - 0.21	X = 0.99C + 0.09
Waste Water 3				
Single-Analyst Precision	SR = 0.15X + 0.58	SR = 0.23X - 1.94	SR = 0.15X + 1.52	SR = 0.24X - 0.04
Overall Precision	S = 0.32X - 0.13	S = 0.34X - 3.58	S = 0.34X - 0.71	S = 0.38X + 0.03
Accuracy	X = 0.71C + 2.52	X = 0.78C + 1.72	X = 0.78C + 2.11	X = 0.83C + 0.07

X = Mean Recovery
C = True Value for the Concentration

Jack R. Hall, J. Richard Florance, Dennis L. Strother, and Marlene N. Wass are with IT Enviroscience, Knoxville, TN 37919.
***Edward L. Berg** is the EPA Project Officer (see below).*
The complete report, entitled "EPA Method Study 22, Method 612—Chlorinated Hydrocarbons," (Order No. PB 84-187 772; Cost: $13.00, subject to change) will be available only from:
National Technical Information Service
5285 Port Royal Road
Springfield, VA 22161
Telephone: 703-487-4650
The EPA Project Officer can be contacted at:
Environmental Monitoring and Support Laboratory
U.S. Environmental Protection Agency
Cincinnati, OH 45268