



Project Summary

Development of Preservation Techniques and Establishment of Maximum Holding Times: Requirements of the National Pollutant Discharge Elimination System and Safe Drinking Water Act

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This project was designed to statistically determine the length of time a sample can be stored for analysis of ten National Pollutant Discharge Elimination System (NPDES) compliance parameters and two Safe Drinking Water Act (SDWA) parameters. Accepted preservation and storage techniques were used on spiked representative real-world samples. Experimentally determined maximum holding times (MHT) were calculated for each parameter in three different water matrices and under several different preservation conditions for certain parameters.

Fifteen experiments were conducted for the ten NPDES compliance parameters and three experiments for the two SDWA compliance parameters. The ten NPDES parameters were phenols, cyanide, mercury, ammonia, nitrate plus nitrite, fluoride, total Kjeldahl nitrogen, total phosphorus, total organic carbon, and sulfide. Multiple experiments were performed to evaluate MHTs for different preservation techniques for phenols and mercury. The two SDWA parameters were nitrate and fluoride, with two experiments performed to evaluate MHTs for different preservation techniques for nitrate.

This Project Summary was developed by EPA's Environmental Monitoring and Support Laboratory, Cincinnati, OH, 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).

Experimental Design and Approach

The experiments performed during this study are summarized in Tables 1 and 2. For each of the 15 wastewater experiments, three different wastewater samples were collected. For the three drinking water experiments, three finished drinking waters were collected from potable water treatment plants treating different raw waters using different methods. For wastewater or drinking water parameters with multiple experiments (e.g., phenols and mercury in wastewater and nitrate in drinking water), sample matrices were collected at the same site, and mixing and analyses were started on the same day. The protocol to prepare samples for a time-sequenced analysis of one parameter in a matrix was performed 54 times during the project (three matrices

Table 1. Summary of Experimentally Determined MHTs for Wastewater Parameter Experiments

Experiment number	Parameter	Preservation technique	Container [plastic (P) glass (G)]	Proposed MHTs† (days)	Experimentally determined MHT*		
					M1	M2	M3
1	Phenols	Cool 4°C H ₂ SO ₄ pH<2	P	28	32	32	32
2	Phenols	Cool 4°C 1 g/L CuSO ₄ H ₃ PO ₄ pH<4	G	1**	16	5	3
3	Phenols	Cool 4°C H ₂ SO ₄ pH<2	G	28	32	32	32
4	Cyanide	Cool 4°C NaOH pH>12	P	14	32	32	—
5	Mercury	HNO ₃ pH<2 0.05% K ₂ Cr ₂ O ₇	P	28	6.8	4.5	10.5
6	Mercury	HNO ₃ pH<2 0.05% K ₂ Cr ₂ O ₇	G	28	32	11.1	32
7	Mercury	HNO ₃ pH<2	G	38**	5.3	NV	32
8	Mercury	HNO ₃ pH<2	P	13**	2.7	NV	16
9	Ammonia	Cool 4°C Conc. H ₂ SO ₄ pH<2	P	28	32	32	32
10	Fluoride	None	P	28	32	32	32
11	TKN	Cool 4°C Conc. H ₂ SO ₄ pH<2	P	28	32	32	32
12	Nitrite plus nitrate	Cool 4°C Conc. H ₂ SO ₄ pH<2	P	28	32	32	32
13	Organic carbon	Cool 4°C Conc. H ₂ SO ₄ pH<2	P	28	32	32	32
14	Total phosphorus	Cool 4°C Conc. H ₂ SO ₄ pH<2	P	28	32	32	32
15	Sulfide	Cool 4°C 2 ml/L of 2N zinc acetate	P	28	32	32	32

*Experimentally determined MHTs are either 90 percent LCL of calculated MHT or maximum time of experiment.

†MHTs are those proposed by EPA.

**MHTs for these preservation techniques were recommended by EPA.

NV: Calculated MHTs and LCL were not considered valid because the lack-of-fit variability from the statistical test was large.

Table 2. Summary of Experimentally Determined MHTs for Drinking Water Parameter Experiments

Experiment number	Parameter	Preservation technique	Container [plastic (P) glass (G)]	Proposed MHTs† (days)	Experimentally determined MHT*		
					M1	M2	M3
1	Nitrate**	Conc. H ₂ SO ₄ pH<2	P	14	16	16	16
2	Nitrate**	Cool 4°C	P	2	16	16	16
3	Fluoride	None	P	30	32	32	32

*Experimentally determined MHTs are all the maximum time of experiment.

†MHTs for these preservation techniques were required by EPA.

**Additional analyses for nitrate experiments support longer experimentally determined MHT's of at least 32 days.

for 18 experiments). The protocol for preparing samples for time-sequenced analysis included:

1. Collecting grab samples and shipping to the laboratory within 24 hours;
2. Compositing and homogenizing the grab samples in a large-volume mixing container;
3. Collecting three aliquots for analysis from the composite prior to addition of preservative;
4. Collecting three aliquots for analysis from the composite containing the preservative;
5. Adding the study analyte to the preserved composite and mixing;
6. Collecting 100 aliquots (dispensed to ensure a large enough population for representative random sampling) from the spiked, preserved composite; and
7. Randomly selecting 18 fixed aliquots of the 100 aliquots chosen for time-sequences analysis.

The time sequence was either 0, 1, 2, 4, 8, and 16 days or 0, 2, 4, 8, 16, and 32 days.

The time-sequenced experimental data were used to calculate experimentally determined maximum holding times (MHTs) according to a mathematically acceptable definition. The experimentally determined or calculated MHT was defined as the 90 percent lower confidence limit of a specified critical time (CT) (see Figure 1). The CT was defined as the time at which a 10 percent change in the analyte concentration (from the intercept at Day 0) occurred and when the precision of the method allowed that 10 percent change to be a statistically significant difference at the 90 percent confidence level. When the precision of the method was not sufficient to discern a 10 percent change in the analyte concentration, the CT was that time at which the percent change (greater than 10 percent) in the analyte concentration represented a statistically significant difference at the 90 percent confidence level. An upper limit of a 15 percent change in the analyte concentration was set for determining a valid CT. The experimentally determined MHTs were specified as either the 90 percent lower confidence limit (LCL) of the CT or the maximum time of the designed experiment when the LCL was longer.

A statistical protocol was developed to provide information to support the use of a zero or first order kinetic model to calculate a CT and its 90 percent LCL (or

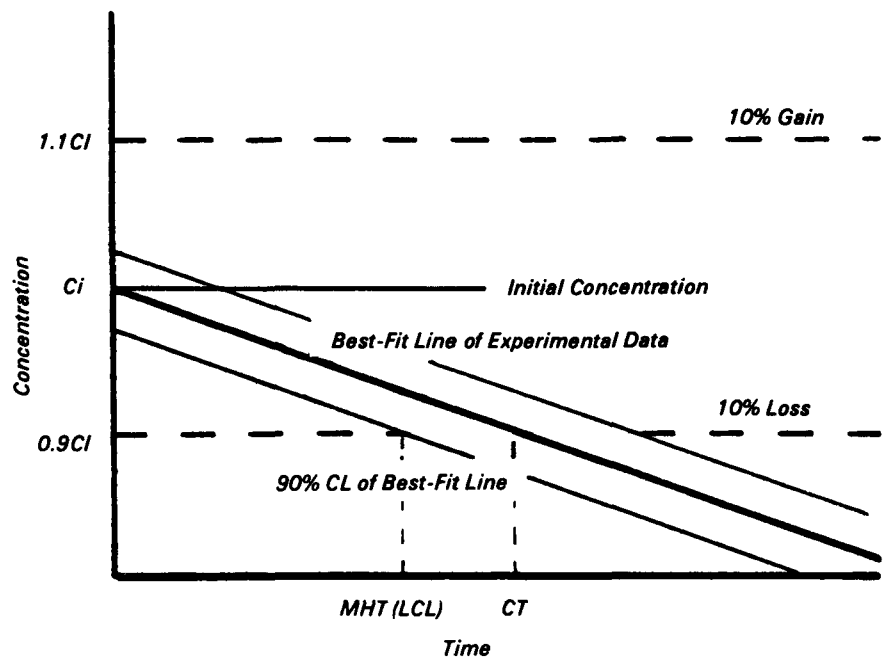


Figure 1. Graphic representation of MHT definition.

MHT). Only the raw data zero-order model (concentration versus time) and the transformed on first-order model (natural log of concentration versus time) were evaluated since loss or gain of the constituent was expected to follow zero-or-first-order kinetics.

Results

A summary of the results for the wastewater parameter experiments is presented in Table 1. The experimentally determined MHTs resulting from this study were longer than proposed MHT values published in the *Federal Register* (44/244: 75-28-75052, Tuesday, December 18, 1979) and recommended by EPA in *Methods for Chemical Analysis of Water and Wastes*, EPA-600/4-79-020, for the following parameters in wastewater:

Phenols	Nitrate plus nitrite
Ammonia	Total organic carbon
Fluoride	Total phosphorus
Total Kjeldahl	Sulfide
Nitrogen (TKN)	

A complete set of mercury and cyanide experimental results were unobtainable because of difficulties with the analytical procedures. The experimentally determined MHTs for fluoride and nitrate in drinking water were longer than the EPA required values and are summarized in

Table 2. Additional analyses for the nitrate in drinking water experiments supported experimentally determined MHTs of 32 days.

Conclusions

From the results obtained under the set of conditions used for this study, the following conclusions are drawn:

1. The experimentally determined MHTs were longer than the proposed holding times specified in the *Federal Register* and the recommended values from EPA for the analysis of the parameters in wastewater samples listed below.

Phenols	Nitrate plus nitrite
Ammonia	Total organic carbon
Fluoride	Total phosphorus
TKN	Sulfide

For mercury and cyanide, the experimentally determined MHTs were not longer than the proposed or recommended values.

2. The experimentally determined MHTs were longer than the EPA required holding time values for the analysis of nitrate and fluoride in samples from finished drinking water.
3. Cyanide concentrations in wastewater samples containing sulfur

compounds, which are converted to sulfide during the distillation procedure, cannot accurately be determined using the currently approved analytical method for cyanide.

4. The major reasons that some experimentally determined MHTs were lower than the proposed and recommended values were: the known variability for mercury analyses, especially at sample concentrations less than 2.0 micrograms per liter ($\mu\text{g/L}$), and the possible effectiveness of 0.05 percent potassium dichromate ($\text{K}_2\text{Cr}_2\text{O}_7$) in samples acidified with nitric acid (HNO_3) to extract mercury from the walls of new, HNO_3 -rinsed polyethylene Hedwin Cubitainers®. The slopes of the best-fit regression lines were not statistically similar for the two experiments using polyethylene Cubitainers®. Decreasing concentrations with the time were observed when samples were preserved with HNO_3 , except at the highest mercury concentration where no statistical change with time was observed. Increasing concentrations with time were observed when 0.05 percent $\text{K}_2\text{Cr}_2\text{O}_7$ and HNO_3 were used as preservative.
5. Different sample preservation techniques were studied for nitrate, phenols, and mercury analyses. The two preservation techniques for finished drinking water for nitrate analysis yielded the same experimentally determined MHTs. Of the three preservation experiments for phenols in wastewater, the two in which H_2SO_4 was added to samples stored in plastic or glass provided the longest experimentally determined MHTs. Of the four mercury-in-wastewater preservation experiments, HNO_3 and $\text{K}_2\text{Cr}_2\text{O}_7$ added to samples stored in glass provided the best experimentally determined MHTs.
6. The phenols experiments that used H_2SO_4 as the preservative consistently exhibited higher coefficients of variation for the unexplained variability from regression diagnostics compared to the copper sulfate/phosphoric acid preservation experiments. The highest CVs were from Sample Matrix 3, which also had the highest suspended solids content of the sample matrices collected for the phenols experiments. Since the higher variability is due to the high suspended solids content, the

higher CVs were accepted as reasonable for analytical variability, therefore, allowing the calculated MHT to be considered valid for that matrix.

7. A statistically significant lack of linear fit was encountered for the experimental data due primarily to larger day-to-day analytical variability compared to within-day analytical variability. This phenomenon is not entirely unexpected, and it has caused some concern from a purely statistical standpoint. Specifically, the concern arises from deriving a precise procedure to evaluate when the experimental data exhibits a true lack of linear fit. A true lack of linear fit for the experimental data implies that an MHT cannot be validly calculated. The procedure

used for this study represents a reasonable approach for the determination of MHTs. Further holding time studies should recognize the problems encountered during this study and possibly incorporate the following two recommendations. First, studies should provide a mechanism to precisely define project-specific analytical variability for comparison to lack-of-fit variability. Second, studies should use an analysis of the residuals from the linear regression line to detect outliers and determine the influence from extreme observations.

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Daniel F. Bender is the EPA Project Officer (see below).

The complete report, entitled "Development of Preservation Techniques and Establishment of Maximum Holding Times: Inorganic Constituents of the National Pollutant Discharge Elimination System and Safe Drinking Water Act," (Order No. PB 87-132 833/AS; Cost: \$18.95, subject to change) will be available only from:

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