o-TOLUIDINE
m-TOLUIDINE
p-TOLUIDINE

Related Information: Chemical Sampling - o-Toluidine, m-Toluidine, p-Toluidine

---

Method no.:	73
Matrix:	Air
Procedure:	Samples are collected closed-face by drawing known volumes of air through sampling devices consisting of three-piece cassettes, each containing two sulfuric acid-treated glass fiber filters separated by a spacer. Analysis is performed by quantitating the heptaflurobutyric acid anhydride derivatives of the amines by gas chromatography using an electron capture detector.
Recommended air volume and sampling rate:	100 L at 1 L/min

	o-toluidine	m-toluidine	p-toluidine
Target conc.: ppm (mg/m^3)	2 (8.8)	2 (8.8)	2 (8.8)
Reliable quantitation limits: ppb (μg/m^3) (based on a 100-L air volume)	0.22 (097)	0.18 (0.79)	0.13 (0.55)
Standard errors of estimate at the target concentration: (Section 4.7.)	5.6%	5.6%	5.7%

Status of method:	Evaluated method. This method has been subjected to the established evaluation procedures of the Organic Methods Evaluation Branch.

Date: August 1988 Chemist: Carl J. Elskamp

Organic Methods Evaluation Branch
OSHA Analytical Laboratory
Salt Lake City, Utah -84115

1. General Discussion

1.1. Background

1.1.1 History

The previous OSHA-recommended procedure (Ref. 5.1.) for collection of air samples for o- and p-toluidine using silica gel tubes was based on NIOSH Method S168 (Ref. 5.2.). The analysis was done by GC after desorbing the samples with 95% ethanol. This method was fully validated by NIOSH for o-toluidine at the 5-ppm level. There was no OSHA-recommended sampling or analytical scheme for m-toluidine. The purpose of this evaluation was to validate a sampling and analytical procedure at the 2-ppm level for all three isomers based on methods validated for other aromatic amines.

Methodology exists which has previously been validated by the OSHA Analytical Laboratory for a number of other aromatic amines. (Ref. 5.3. - 5.5.) The collection of air samples had involved closed-face sampling using a sampling device consisting of a sulfuric acid-treated glass fiber filter

and a support pad contained in a two-piece cassette. Thus, the collected free amines are converted to the more stable and less volatile corresponding amine salts on the filter surface. To further enhance the stability of samples, the filters are transferred to small vials containing 2 mL of deionized water after sampling. The analysis involves converting the amine salts to free amines by addition of sodium hydroxide, extracting the amines into toluene, removing a portion of the toluene extract, and derivatizing the free amines in the extract with heptaflurobutyric acid anhydride (HFAA) according to the reaction

$RNH_2 + (C_3F_7CO,)_2O > RNHCOC_3F_7 + C_3F_7COOH$

The derivatives are determined by capillary gas chromatography using an electron capture detector.

Based on two modifications of this methodology, a sampling and analytical procedure for o-, m-, and p-toluidine was validated. The first modification was to utilize a sampling device consisting of a cassette containing two sulfuric acid-treated filters separated by a spacer instead of a cassette containing a single acid-treated filter and, a support pad with no spacer. (This new sampling device is also recommended in the updated methods of Refs. 5.3. -5.5.) This was done because it was found that when using the latter sampling device, a significant amount of toluidine was found on the support pad after sampling from a generation apparatus as described in Section 2.4.1. There was no analyte found on the back filter when sampling the same concentration of toluidine with the new sampling device. The front filter contained essentially 100% of the analyte which had been injected into the generation apparatus. This new design has an added advantage because the back filter can be analyzed to determine if there was any breakthrough from the front filter. The closed-face sampling technique was retained because open-face sampling produced no improvement in collection efficiency. The second modification was to eliminate transferring the filters to vials containing deionized water after sampling. It was found that toluidine samples were stable when the filters were left in the cassettes, even after storage at room temperature.

1.1.2. Toxic effects (This section is for information only and should not be taken as the basis of OSHA policy.)

All three isomers of toluidine can cause anoxia (due to formation of methemoglobin) and hematuria in man. Exposure can occur from inhalation of the vapors or by skin absorption. In numerous epidemiological studies, it was found that there was an increased incidence of bladder cancer in workers exposed to o-toluidine, but the workers were simultaneously exposed to other possible carcinogens as well. Based on these studies, the IARC Working Group judged that although o-toluidine cannot specifically be identified as the responsible agent, for practical purposes it should be regarded as a carcinogenic risk to humans. Neither m- nor p-toluidine have been tested as thoroughly as o-toluidine, but they do show the same toxicity profile and the dose ranges which produce the same toxic effects are comparable. o-Toluidine produced a variety of cancerous tumors in rats and mice after oral administration. p-Toluidine produced malignant liver tumors in mice after oral administration, but was not carcinogenic to male rats at the same dose level. It appears that p-toluidine is a weaker carcinogen than o-toluidine. m-Toluidine was not carcinogenic to male rats and female mice. There were inconclusive results for male mice. In comparison to the other two isomers, m-toluidine appears to have, no carcinogenic or mutagenic activity. (Ref. 5.6.)

All three isomers have been assigned a TLV of 2 ppm with a "skin" notation. Both o- and p-toluidine also have an A2 designation as suspected human carcinogens. (Ref. 5.7.) o-Toluidine has been assigned a PEL of 5 ppm with a "skin" notation. (Ref. 5.8.) There are no assigned PELs for m- and p-toluidine.

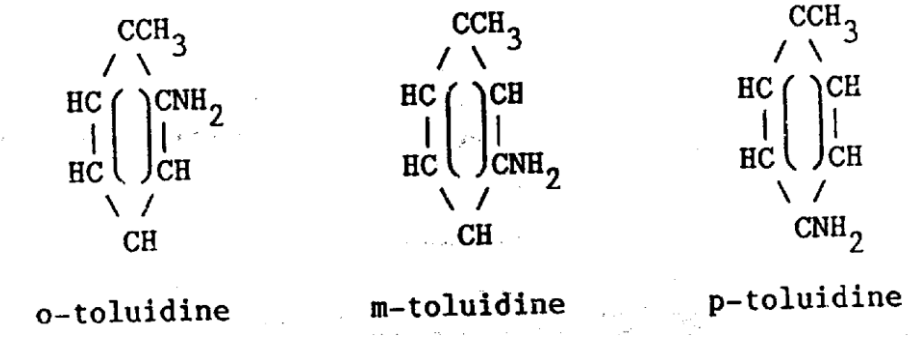
1.1.3. Potential workplace exposure

The major uses of toluidine are as intermediates in the synthesis of dyestuffs, rubber chemicals, pharmaceuticals, and pesticides. (Ref. 5.6.)

1.1.4. Physical properties and other descriptive information (Ref. 5.9.)

	o-toluidine	m-toluidine	p-toluidine
CAS no.:	95-53-4	108-44-1	106-49-0
molecular weight:	107.2	107.2	107.2
boiling point:	199.7 ˚C	203.3˚ C	200.4˚ C
melting point:	-16.3˚ C -	50.5˚ C	44.5˚ C
description:	colorless liquid	colorless liquid	colorless leaflets
specific gravity:	1.004 @ 20 ˚C	0.989 @ 20 ˚C	1.046 @ 20 ˚C
vapor pressure:	133 Pa @ 44 ˚C	133 Pa @ 41 ˚C	133 Pa @ 42 ˚C
vapor density:	3.69 (air=1)	3.90 (air=1)	3.90 (air=1)
flash point:	185˚F (CC)		188˚F (CC)
autoignition temperature:	900 ˚F		900 ˚F
synonyms:	o-methyl-aniline; 2-methyl-aniline; o-amino-toluene; 2-amino-toluene	m-methyl-aniline; 3-methyl-aniline; m-amino-toluene; 3-amino-toluene	p-methyl-aniline; 4-methyl-aniline; p-amino-toluene; 4-amino-toluene

structural formula:



The analyte air concentrations throughout this method are based on the recommended sampling and analytical parameters. Air concentrations listed in ppm and ppb are referenced to 25˚C and 760 mm Hg. Although the derivatives of the amines are analyzed, the equivalent masses of the amines are listed throughout the method.

1.2 Limit defining parameters

1.2.1. Detection limit of the analytical procedure

The detection limits of the analytical procedure are 15.2, 12.3, and 8.6 fg per injection for o-, m-, and p-toluidine respectively. These are the amounts of analytes - which produce peaks with heights that are approximately 5 times the baseline noise. (Section 4.1.)

1.2.2. Detection limit of the overall procedure

The detection limits of the overall procedure are 97.0, 79.0, and 55.3 ng per sample for o-, m-, and p-toluidine respectively. These are the amounts of analytes spiked on sample filters which allow recoveries of amounts of analytes equivalent to the detection limits of the analytical procedure. These detection limits correspond to air concentrations of 0.22 ppb (0.97 □g/m^3), 0.18 ppb (0.79 □g/m^3), and 0.13 ppb (0.55 □g/m^3) for o-, m-, and p-toluidine respectively. (Section 4.2.)

1.2.3. Reliable quantitation limit

The reliable quantitation limits are 97.0, 79.0, and 55.3 ng per sample for o-, m-, and p-toluidine respectively. These are the smallest amounts of analytes which can be quantitated within the requirements of a recovery of at least 75% and a precision (±1.96 SD) of ±25% or better. These reliable quantitation limits correspond to air concentrations of 0.22 ppb (0.97 □g/m^3), 0.18 ppb (0.79 □g/m^3), and 0.13 ppb (0.55 □g/m^3) for o-, m-, and p-toluidine respectively. (Section 4.3.)

The reliable quantitation limits and detection limits reported in this method are based upon optimization of the instrument for the smallest possible amount of analyte. When the target concentration of an analyte is exceptionally higher than these limits, they may not be attainable at the routine operating parameters.

1.2.4. Instrument response to the analyte

The instrument response over concentration ranges representing 0.5 to 2 times the target concentrations is linear for all three analytes. (Section 4.4.)

1.2.5. Recovery

The recoveries of o-, m-, and p-toluidine from samples used in a 15-day storage test remained above 91, 92, and 93% respectively. The sample filters were stored in cassettes in a closed drawer at approximately 21˚C. (Section 4.5.). The recovery of analyte from the collection medium during storage must be 75% or greater.

1.2.6. Precision (analytical method only)

The pooled coefficient of variation obtained from replicate injections of analytical standards at 0.5, 1, and 2 times the target concentrations is 0.014 for all three isomers. (Section 4.6.)

1.2.7. Precision (overall procedure)

The precisions at the 95% confidence level for the 15-day storage test are ±10.9, ±10.9, and ±11.2% for o-, m-, and p-toluidine respectively. These include an additional ±5% for sampling error. The sample filters were stored in cassettes in a closed drawer at approximately 21˚C. (Section 4.7.) The overall procedure must provide-results at the target concentration that are ±25% or better at the 95% confidence level.

1.2.8. Reproducibility

Six samples, spiked by liquid injection, and a draft copy of this procedure were given to a chemist unassociated with this evaluation. The samples were analyzed after 17 days of storage at approximately 21˚C. No individual sample result deviated from its theoretical value by more than the precision of the overall procedure as reported in Section 1.2.7.(Section 4.8.)

1.3. Advantages

1.3.1. The acid-treated filter provides a convenient method of sampling for a number of aromatic amines.

1.3.2. The analysis is rapid, sensitive, and precise.

1.4. Disadvantages

None

2. Sampling Procedure

2.1. Apparatus

2.1.1. Samples are collected using a personal sampling pump that can be calibrated within ±5% of the recommended flow rate with the sampling device attached.

2.1.2. Samples are collected closed-face using a sampling device consisting of two sulfuric acid-treated 37-mm Gelman Type A/E glass fiber filters contained in a three-piece cassette. The filters are prepared by soaking each filter with 0.5 mL of 0.26 N sulfuric acid. (0.26 N sulfuric acid can be prepared by diluting 1.5 mL of 36 N sulfuric acid to 200 mL with deionized water.) The filters are dried in an oven at 100˚C for 1 h and then assembled into three-piece 37-mm polystyrene cassettes without support pads. The front filter is separated from the back filter by a polystyrene spacer. The cassettes are sealed-with shrink bands and the ends are plugged with plastic plugs.

2.2. Reagents

None required

2.3. Sampling technique

2.3.1. Remove the plastic plugs from the sampling device immediately before sampling,

2.3.2. Attach the sampling device to the sampling pump with flexible tubing and place the device in the employee's breathing zone.

2.3.3. Seal the sampling device with the plastic plugs immediately after sampling.

2.3.4. Seal and identify each sampling device with an OSHA Form 21.

2.3.5. Submit at least one blank sampling device with each sample set. Handle the blanks in the same manner as the air samples, but draw no air through them.

2.3.6. Record the volume of air sampled (in liters) for each sample, along with any potential interferences.

2.4. Collection efficiency

2.4.1. Generation apparatus

Collection efficiency studies were conducted by drawing air through sampling devices that were attached to empty impingers. Microliter amounts of toluidine standards (in toluene) were injected into the impingers before sampling commenced. The inlets of the impingers were attached to a humid air generator so air at approximately 80% relative humidity could be drawn through the generation apparatus. The sample generations were done at room temperature. It was found that a majority of the toluidine was flushed from the impinger and collected in the first 30 to 40 min of, sampling at 1 L/min, with 70-80% flushed in the first 10 min. (Section 4.9.)

2.4.2. Collection efficiency at 2 times the target concentration

Three individual collection efficiencies were determined at 2 times the target concentration for each analyte. This was done by adding an amount of the toluidine isomer of interest, which was equivalent to 4 ppm for a 100-L air sample (approximately 1750 μg), to each impinger before sampling at 1 L/min for 100 min. The average collection efficiency was 99.4 (SD = 2.1), 100.6 (SD = 3.3), and 95.7% (SD = 1.5) for o-, m-, and p-toluidine respectively. There was no analyte found on the back filter of any of the samples.

2.4.3. Collection efficiency at 10 times the target concentration

A collection efficiency determination was also made in a similar manner at 10 times the target concentration (20 ppm for 100 L or approximately 8760 μg) for each analyte. The combined collection efficiency of the front and back filter was 97.8, 103.8, and 97.6% for o-, m-, and p-toluidine respectively. The collection efficiency of the front filter was 80.9, 81.4, and 79.7% for o-, m-, and p-toluidine respectively, while the remainder of the analytes were collected on the back filters.

2.5. Extraction efficiency

2.5.1. The average extraction efficiencies from six filters for each amine spiked at the target concentration were 99.3, 99.0, and 98.4% for o-, m-, and p-toluidine respectively. (Section 4.10.)

2.5.2. The stability of extracted and derivatized samples was verified by reanalyzing the above samples 24 h later using fresh standards. The average extraction efficiencies for the reanalyzed samples were 101.2, 100.8, and 100.2% for o-, m-, and p-toluidine respectively. (Section 4.10.)

2.6. Recommended air volume and sampling rate

2.6.1. The recommended air volume is 100 L.

2.6.2. The recommended sampling rate is 1 L/min.

2.6.3. If a smaller air volume is desired, the reliable quantitation limits will be larger. For example, the reliable quantitation limit for o-toluidine for a 15-L air sample would be 1.5 ppb.

2.7. Interferences (sampling)

2.7.1. Any compound in the sampled air that will react with the sulfuric acid on the treated filters or with the collected analyte is a potential sampling interference.

2.7.2. Suspected interferences should be reported to the laboratory with submitted samples.

2.8. Safety precautions (sampling)

2.8.1. Attach the sampling equipment to the employees so that it will not interfere with work performance or safety.

2.8.2. Follow all safety procedures that apply to the work area being sampled.

3. Analytical Procedure

3.1. Apparatus

3.1.1. A GC equipped with an electron capture detector. For this evaluation, a Hewlett-Packard 5890A Gas Chromatograph equipped with a Nickel 63 electron capture detector and a 7673A Automatic Sampler was used.

3.1.2. A GC column capable of separating the amine derivatives from the solvent and interferences. A 15-m x 0.32-mm i.d. (1.0-μm film) SPB-5 fused silica capillary column purchased from Supelco, Inc. was used in this evaluation.

3.1.3. An electronic integrator or some other suitable means of measuring peak areas or heights. A Hewlett-Packard 18652A A/D converter interfaced to a Hewlett-Packard 3357 Lab Automation Data System was used in this evaluation.

3.1.4. Small resealable glass vials with Teflon-lined caps capable of holding at least 5 mL. Glass, 7-mL scintillation vials are recommended.

3.1.5. Small resealable glass vials with Teflon-lined caps capable of holding 4 mL. WISP autosampler vials are recommended.

3.1.6. A dispenser or pipet for toluene capable of delivering 2.0 mL.

3.1.7. Pipets (or repetitive pipets with plastic or Teflon tips) for dispensing the sodium hydroxide and buffer solutions, capable of delivering 3 mL and 1 mL, respectively.

3.1.8. Repetitive pipets, one to deliver 25 μL of HFAA and one to transfer 25 μL aliquots of standards and samples.

3.2. Reagents

3.2.1. Saturated and 0.17 N Na0H solutions, prepared from reagent grade sodium hydroxide.

3.2.2. Toluene. American Burdick and Jackson "High Purity Solvent" brand toluene was used.

3.2.3. Heptafluorobutyric acid anhydride (HFAA). HFAA from Pierce Chemical Company was used.

3.2.4. Phosphate buffer, prepared from 136 g of reagent-grade potassium dihydrogen phosphate and deionized water. The pH is adjusted to 7.0 with the saturated sodium hydroxide solution. The final volume is adjusted to 1.0 L with deionized water.

3.2.5. Toluidine, reagent grade. The three isomers used in this evaluation were purchased from Aldrich Chemical Company, Inc., Milwaukee WI.

3.3. Standard preparation

3.3.1. CAUTION. FOR SAFE LABORATORY PRACTICE, THESE AROMATIC AMINES SHOULD BE CONSIDERED CARCINOGENIC TO HUMANS. Restrict the use of pure compounds and concentrated standards to regulated areas. Prepare concentrated stock standards by diluting the pure amines with toluene. Stock standards appear to be stable for at least three months when refrigerated.

3.3.2. Prepare analytical standards by injecting microliter amounts of diluted stock standards into 4-mL vials containing 2.0 mL of toluene.

3.3.3. Transfer 25-μL aliquots of the analytical standards to 4-mL vials containing 2.0 mL of toluene.

3.3.4. Add 25 μL of HFAA to each vial. Recap and shake the vials for 10 s.

3.3.5. After allowing 10 min for the derivatives to form, add 1 mL of the phosphate buffer to each vial to destroy the excess HFAA and to extract the heptaflurobutyric acid that is formed.

3.3.6. Recap and shake the vials for 10 s.

3.3.7. After allowing the two layers to separate, analyze the toluene (upper) layer of each standard by GC.

3.3.8. Bracket sample concentrations with analytical standard concentrations. If sample concentrations are higher than the upper range of prepared standards, prepare additional standards to ascertain detector response or derivatize a smaller aliquot of the toluene extract of the high samples.

3.4. Sample preparation

3.4.1. Transfer the sample filters to individual 7-mL scintillation vials.

3.4.2. Add 3 mL of 0.17 N Na0H and 2.0 mL of toluene to each vial.

3.4.3. Recap and shake the vials end-to-end for 10 min.

3.4.4. Allow the layers to separate and transfer a 25-μL aliquot of the toluene layer of each sample to separate 4-mL vials, each containing 2.0 mL of toluene.

3.4.5. Add 25 μL of HFAA to each vial. Recap and shake the vials for 10 s.

3.4.6. After allowing 10 min for the derivatives to form, add 1 mL of the phosphate buffer to each vial to destroy the excess HFAA and to extract the heptaflurobutyric acid that is formed.

3.4.7. Recap and shake the vials for 10 s.

3.4.8. After allowing the two layers to separate, analyze the toluene (upper) layer of each sample by GC.

3.5. Analysis

3.5.1. GC conditions and information

zone temperatures:	column, 110˚C injector, 200˚C detector, 300˚C	
gas flows:	column, 3.7 mL/min hydrogen (35 kPa head pressure) make up, 80 mL/min nitrogen	
injection volume:	1.0 μL	
split ratio:	40:1	
column:	SPB-5, 1.0-μm film, 15 m x 0.32-mm i.d. fused silica (Supelco, Inc.)	
retention times of derivatives:	o-toluidine,	4.0 min
	m-toluidine,	4.8 min
	p-toluidine,	5.2 min
chromatogram:	Section 4.11.	

3.5.2. Measure peak areas or heights by use of an integrator or by other suitable means.

3.5.3. Construct a calibration curve by plotting response (peak areas or heights) of standard injections versus micrograms of analyte per sample. Bracket sample concentrations with standards.

3.6. Interferences (analytical)

3.6.1. Any compound that elutes in the same general time as the HFAA derivative of the amine of interest is a potential interference. Suspected interferences reported to the laboratory with submitted samples by the industrial hygienist must be considered before samples are derivatized.

3.6.2. GC parameters may be changed to possibly circumvent interferences.

3.6.3. Retention time on a single column is not considered proof of chemical identity. Analyte identity should be confirmed by GC/MS if possible.

3.7. Calculations

The analyte concentration for samples is obtained from the calibration curve in micrograms of analyte per sample. If any analyte is found on any back filter, that amount is added to the amount found on the corresponding front filter. If any analyte is found on the blank filters, the combined

amount is subtracted from the sample amounts. The air concentrations are calculated using the following formulae.

$$\mu g/m^3 = \frac{\text{(micrograms of analyte per sample)(1000)}}{\text{(liters of air sampled)(extraction efficiency)}}$$

where extraction efficiencies are: o-toluidine, 99.3%
m-toluidine, 99.0%
p-toluidine, 98.4%

$$ppb = (\mu g/m^3)(24.46)/(107.2) = (\mu g/m3)(0.2282)$$

where, 24.46 is the molar volume (liters) at 25˚C and" 760 mm Hg 107.2 is the molecular weight of toluidine

3.8. Safety precautions (analytical)

3.8.1. CAUTION. FOR SAFE LABORATORY PRACTICE, THESE AROMATIC AMINES SHOULD BE CONSIDERED CARCINOGENIC TO HUMANS. Restrict the use of pure compounds and concentrated standards to regulated areas. Avoid skin contact and inhalation of all chemicals.

3.8.2. Use all chemicals to a fume hood if possible.

3.8.3. Wear safety glasses and a lab coat at all times while in the lab area.

4. Backup Data

4.1. Detection limit of the analytical procedure

The injection volume listed in Section 3.5.1., 1.0 μL with a 40:1 split, was used in the determination of the detection limits of the analytical procedure. The detection limits of 15.2 fg of o-toluidine, 12.3 fg of m-toluidine, and 8.6 fg of p-toluidine were determined by analyzing dilute standards equivalent to 97.0 ng of o-toluidine, 79.0 ng of m-toluidine, and 55.3 ng of p-toluidine per sample. (The samples are extracted into 2.0 mL of toluene and further diluted by transferring 25-μL aliquots to 2.0 mL of toluene before the derivatization step.) These amounts were judged to give peaks with heights approximately 5 times the baseline noise. A chromatogram is shown in Figure 4.1.

4.2. Detection limit of the overall procedure

The detection limits of the overall procedure were determined by analyzing filters spiked with loadings equivalent to the detection limits of the analytical procedure. Samples were prepared by injecting 97.0 ng of o-toluidine, 79.0 ng of m-toluidine, and 55.3 ng of p-toluidine onto acid-treated filters. These amounts are equivalent to 0.22 ppb (0.97 $\mu g/m^3$), 0.18 ppb (0.79. $\mu g/m^3$), and 0.13 ppb (0.55 $\mu g/m^3$) for o-, m-, and p-toluidine respectively. The results are given in Tables 4.2.1. - 4.2.3.

Table 4.2.1.
Detection Limit of the Overall Procedure for o-Toluidine

sample no.	ng spiked	ng recovered
1	97.0	91.9
2	97.0	99.7
3	97.0	112.4
4	97.0	110.4
5	97.0	93.0
6	97.0	99.4

Table 4.2.2.
Limit of the Overall Procedure for m-Toluidine

sample no.	ng spiked	ng recovered
1	79.0	73.9
2	79.0	82.5
3	79.0	95.0
4	79.0	92.9
5	79.0	76.3
6	79.0	82.7

Table 4.2.3.
Detection Limit of the Overall Procedure for p-Toluidine

sample no.	ng spiked	ng recovered
1	55.3	52.4
2	55.3	60.0
3	55.3	66.0
4	55.3	68.0
5	55.3	55.0
6	55.3	59.4

4.3. Reliable quantitation limit

The reliable quantitation limits were determined by analyzing filters spiked with loadings equivalent to the detection limits of the analytical procedure. Samples were prepared by injecting 97.0 ng of o-toluidine, 79.0 ng of m-toluidine, band 55.3 ng of p-toluidine onto acid-treated filters. These amounts are equivalent to 0.22 ppb (0.97 $\mu g/m^3$), 0.18 ppb (0.79 $\mu g/m^3$), and 0.13 ppb (0.55 $\mu g/m^3$) for o-, m-, and p-toluidine respectively. The results are given in Tables 4.3.1. - 4.3.3.

Table 4.3.1.
Reliable Quantitation Limit for o-Toluidine
(Based on samples and data of Table 4.2.1.)

sample no	%recovered	statistics
1	94.7	X = 104.3
2	102.8	
3	115.9	
4	113.8	SD = 8.9
5	95.9	precision = (1.96)(*8.9)
6	102.5	= ±17.4

Table 4.3.2.
Reliable Quantitation Limit for m-Toluidine
(Based on samples and data of Table 42.2.)

sample no	% recovered	statistics
1	93.5	X = 106.2
2	104.4	
3	120.2	
4	117.6	SD = 10.8
5	96.6	precision = (1.96)(*10.8)
6	104.7	= ±21.2

Table 4.3.3.
Reliable Quantitation Limit for p-Toluidine
(Based on samples and data of Table 4.2.3.)

sample no	% recovered	statistics	
1	94.8	X = 108.8	
2	108.5		
3	119.3		
4	123.0	SD =	10.9
5	99.5	precision =	(1.96)(±10.9)
6	107.4	=	±21.4

4.4. Instrument response to the analyte

The instrument response to the analytes over the range of 0.5 to 2 times the target concentrations was determined from multiple injections of analytical standards. These data are given in Tables 4.4.1. - 4.4.3. and Figure 4.4. The response is linear for each of the three analytes with slopes (in area counts per micrograms of analyte per sample) of 36,600 for o-toluidine, 45,200 for m-toluidine, and 51,000 for p-toluidine.

Table 4.4.1.
Instrument Response to o-Toluidine

x target conc.	0.5x	1x	2x
μg/sample	449.7	899.3	1799
ppm	1.03	2.05	4.11
area counts	103602	180031	311905
	103921	183269	319433
	104083	182732	315745
	107357	179883	325345
	105446	180043	324259
	105706	183823	314638
$\bar{X}$	105019	181630	318554

Table 4.4.2.
Instrument Response to m-Toluidine

x target conc.	0.5x	1x	2x
μg/sample	433.3	866.6	1733
ppm	0.99	1.98	3.96
area counts	131144	228739	398220
	131594	233098	407556
	131707	232368	402949
	136031	228917	415059
	133624	228910	414073
	133868	233818	401662
$\bar{X}$	132995	230975	406586

Table 4.4.3.
Instrument Response to p-Toluidine

x target conc.	0.5x	1x	2x
μg/sample	438.0	875.9	1752
ppm	1.00	2.00	4.00
	157379	277113	486818
	157905	282392	498306
	157971	281618	492569
	163198	277168	507306
	160389	277185	506414
	160661	283227	490875
$\overline{X}$	159584	279784	497048

4.5. Storage test

Storage samples were generated by spiking sulfuric acid-treated glass fiber filters with amounts of analyte equal to the target concentrations (899.3 μg of o-toluidine, 866.6 μg of m-toluidine, 875.9 μg of p-toluidine). The filters were then assembled in three-piece cassettes with back filters. Thirty-six samples were prepared. One hundred liters of air at 79% relative humidity and 23.5˚C were then drawn through each sampling device. Six samples were analyzed immediately, fifteen were stored in a refrigerator at 0˚C, and fifteen were stored in a closed drawer at approximately 21˚C. Six samples, three from refrigerated and three from ambient storage, were analyzed at intervals over a period of fifteen days. The results are given in Tables 4.5.1. - 4.5.3. and shown graphically in Figures 4.5.1.1., 4.5.1.2., 4.5.2.1., 4.5.2.2., 4.5.3.1. and 4.5.3.2. The standard errors of estimate are 5.6, 5., and 5.7% and the 95% confidence limits (±1.96 SD) are ±10.9, ±10.9, and ±11.2% for o-, m, and p-toluidine respectively.

These values were obtained from Figures 4.5.1.2., 4.5.2.2. and 4.5.3.2.

Table 4.5.1.
Storage Test for o-Toluidine

days of storage	% recovery refrigerated			ambient		
0	95.9	93.6	92.5	95.9	93.6	92.5
0	91.5	93.8	95.7	91.5	93.8	95.7
3	99.9	98.5	95.2	99.0	96.2	97.4
6	86.8	91.3	92.9	91.3	92.8	93.8
9	95.4	94.5	95.5	96.2	95.5	93.1
12	91.8	94.3	89.7	93.8	93.7	91.7
15	89.6	90.3	89.7	89.6	88.5	87.1

Table 4.5.2.
Storage Test for m-Toluidine

days of storage	% recovery refrigerated			ambient		
0	96.0	93.8	92.7	96.0	93.8	92.7
0	91.6	93.7	95.2	91.6	93.7	95.2
3	99.6	97.8	94.6	98.5	95.7	97.0
6	87.0	91.5	93.2	92.0	93.4	94.4
9	95.5	94.4	95.7	96.5	96.2	93.4
12	92.7	95.0	90.6	94.5	94.2	92.2
15	89.9	90.4	89.8	89.8	88.9	87.3

Table 4.5.3.
Storage Test for p-Toluidine

days of storage	% recovery refrigerated			ambient		
0	95.1	94.0	92.2	95.1	94.0	92.2
0	91.3	91.9	93.5	91.3	91.9	93.5
3	98.2	96.1	92.7	96.4	93.5	94.5
6	87.4	92.4	94.3	93.3	95.2	96.0
9	96.8	94.4	97.7	97.8	98.1	94.3
12	95.1	96.0	92.2	95.7	95.7	92.8
15	90.8	90.9	90.6	90.0	89.2	87.3

4.6. Precision (analytical method only)

The precision of the analytical method for each analyte is the pooled coefficient of variation determined from replicate injections of standards. The precision of the analytical method for each analyte is given in Tables 4.6.1. - 4.6.3. These tables are based on the data presented in Section 4.4.

Table 4.6.1.
Precision of the Analytical Method for o-Toluidine

x target conc.	0.5x	1x	2x
μg/sample	449.7	899.3	1799
ppm	1.03	2.05	4.1
SD (area counts)	1429	1835	5420
CV	0.014	0.010	0.017

$\overline{CV}$ = 0.014

Table 4.6.2.
Precision of the Analytical Method for m-Toluidine

x target conc.	0.5x	1x	2x
μg/sample	433.3	866.6	1733
ppm	0.99	1.98	3.96
SD (area counts)	1867	2368	6874
CV	0.014	0.010	0.017

$\overline{CV}$ = 0.014

Table 4.6.3.
Precision of the Analytical Method for p-Toluidine

x target conc.	0.5x	1x	2x
μg/sample	438.0	875.9	1752
ppm	1.00	2.00	4.00
SD (area counts)	2243	2924	8454
CV	0.014	0.010	0.017

$\overline{CV} = 0.014$

4.7.Precision (overall procedure)

The precision of the overall procedure is determined from the storage data. The determination of the standard error of estimate (SEE) for a regression line plotted through the graphed storage data allows the inclusion of storage time as one of the factors affecting overall precision. The SEE is similar to the standard deviation, except it is a measure

$$SEE = \left[\frac{\Sigma(Y_{obs} - Y_{est})^2}{n - k}\right]^{1/2}$$

where:

n = total no. of data points
k = 2 for linear regression
k = 3 for quadratic regression
Y_{obs} = observed % recovery at a given time
Y_{est} = estimated % recovery from the regression line at the same given time

of dispersion of data about a regression line instead of about a mean. It is determined with the following equation:

An additional 5% for pump error is added to the SEE by the addition of variances; The precision at the 95% confidence level is obtained by multiplying the SEE (with pump error included) by 1.96 (the z-statistic from the standard normal distribution at the 95% confidence level). The 95% confidence intervals are drawn about their respective regression lines in the storage graphs as shown in Figures 4.5.1.1., 4.5.1.2., 4.5.2.1., 4.5.2.2., 4.5.3.1. and 4.5.3.2. The precisions of the overall procedure of ±10.9 for o-toluidine, ±10.9 for m-toluidine, and ±11.2% for p-toluidine were obtained from Figures 4.5.1.2., 4.5.2.2. and 4.5.3.2. respectively.

4.8. Reproducibility

Six samples were prepared by injecting microliter quantities of standards onto acid-treated filters. The samples, were stored at approximately 21˚C for 17 days. The samples were analyzed by a chemist unassociated with this evaluation. The results are given in Tables 4.8.1. - 4.8.3. All of the amounts found were corrected for extraction efficiency. The extraction efficiency is 99.3% for o-toluidine, 99.0% for m-toluidine, and 98.4% for p-toluidine. The precision of the overall procedure is ±10.9% for o-toluidine, ±10.9 for m-toluidine, and ±11.2% for p-toluidine.

Table 4.8.1.
Reproducibility for o-Toluidine

sample no.	μg found	μg expected	% found	% deviation
1	1013	1066	95.0	-5.0
2	1962	2084	94.1	-5.9
3	1167	1187	98.3	-1.7
4	1942	2084	93.2	-6.8
5	1142	1187	96.2	-3.8
6	1068	1066	100.2	+0.2

Table 4.8.2.
Reproducibility for m-Toluidine

sample no.	μg found	μg expected	% found	% deviation
1	912.7	960.5	95.0	-5.0
2	1772	1877	94.4	-5.6
3	1052	1070	98.3	-1.7
4	1754	1877	93.4	-6.6
5	1029	1070	96.2	-3.8
6	961.5	960.5	100.1	+0.1

Table 4.8.3.
Reproducibility for p-Toluidine

sample no.	μg found	μg expected	% found	% deviation
1	718.2	756.8	94.9	-5.1
2	1402	1479	94.8	-5.2
3	829.0	842.8	98.4	-1.6
4	1386	1479	93.7	-6.3
5	811.0	842.8	96.2	-3.8
6	756.7	756.8	100.0	0.0

4.9.Generation apparatus for collection efficiency studies

Vapor generation rates were determined for each isomer using the generation apparatus as described in Section 2.4.1. The tests were conducted by injecting known amounts of toluidine standards (in toluene) into an empty impinger which were equivalent to approximately 4 ppm for a 100-L air sample. The actual amounts were 1749 μg of o-toluidine, 1757 μg of m-toluidine, and 1750 μg of p-toluidine. Samplers were changed every 10 min for the first 50 min and a final sample was taken from 50 to 100 min. The samples were taken at 1.0 L/min at room temperature. The relative humidity of the air drawn through the impingers was approximately 80%. The results are given in Tables 4.9.1. - 4.9.3. and shown graphically in Figure 4.9. The percent vaporized values in Figure 4.9. were normalized to 109% for each analyte.

Table 4.9.1.
Generation Rate for 1749 μg of o-Toluidine

time (min)	μg found	cumulative μg	cumulative %
0 to 10	1255	1255	71.8
10 to 20	427.4	1682	96.2
20 to 30	48.4	1731	99.0
30 to 40	--	1731	99.0
40 to 50	--	1731	99.0
50 to 100	--	1731	99.0

Table 4.9.2.
Generation Rate for 1757 μg of m-Toluidine

time (min)	μg found	cumulative μg	cumulative %
0 to 10	1423	1423	81.0
10 to 20	253.6	1677	95.4
20 to 30	55.1	1732	98.5
30 to 40	20.9	1753	99.8
40 to 50	5.7	1758	100.1
50 to 100	--	1758	100.1

Table 4.9.3.
Generation Rate for 1750 μg of p-Toluidine

time (min)	μg found	cumulative μg	cumulative %
0 to 10	1229	1229	70.2
10 to 20	316.1	1545	88.3
20 to 30	103.6	1649	94.2
30 to 40	35.8	1684	96.2
40 to 50	12.6	1697	97.0
50 to 100	6.9	1704	97.4

4.10. Extraction efficiency

Six sample filters for each amine were spiked with the target concentration amounts by liquid injection (899.3 μg of o-toluidine,866.6 μg of m-toluidine, and 875.9 μg of p-toluidine). These samples were analyzed to determine the extraction efficiencies. To determine the stability of extracted and derivatized samples, these same samples were reanalyzed after setting at room temperature for 24 h using fresh standards. The results are given in Tables 4.10.1. - 4.10.3.

Table 4.10.1.
Extraction Efficiency for o-Toluidine

sample no.	% extracted	after 24 h
1	98.3	99.7
2	97.3	99.6
3	99.5	103.1
4	98.7	99.4
5	101.5	103.2
6	100.4	101.9
$\bar{X}$	99.3	101.2

Table 4.10.2.
Extraction Efficiency for m-Toluidine

sample no.	% extracted	after 24 h
1	98.2	99.4
2	97.0	99.3
3	99.2	102.8
4	98.6	99.1
5	101.2	102.9
6	100.1	101.3
$\bar{X}$	99.0	100.8

Table 4.10.3.
Extraction Efficiency for p-Toluidine

sample no.	% extracted	after 24 h
1	97.5	98.8
2	96.4	98.7
3	98.6	102.2
4	97.9	98.5
5	100.5	102.2
6	99.4	100.8
$\bar{X}$	98.4	100.2

4.11. Chromatogram

A chromatogram of an analytical standard is shown in Figure 4.11. The chromatogram is from a 1.0-μL injection of a standard approximately equal to the target concentration for each analyte (899.3, 866.6, and 875.9 μg of o-, m-, and p-toluidine per sample respectively) for a 100-L sample.

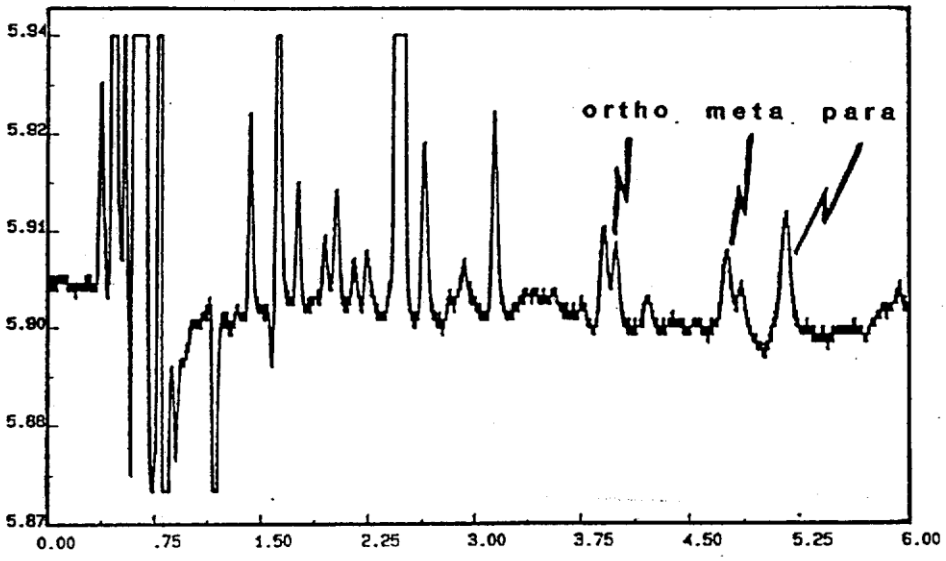


Figure 4.1. Detection limit chromatogram.

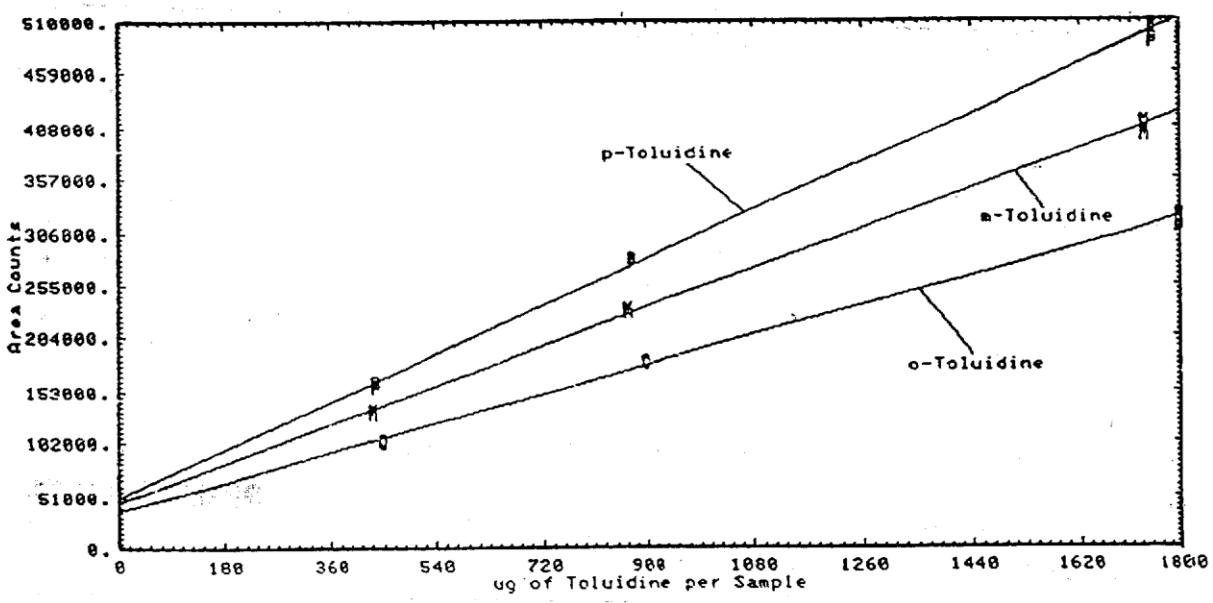


Figure 4.4. Instrument response.

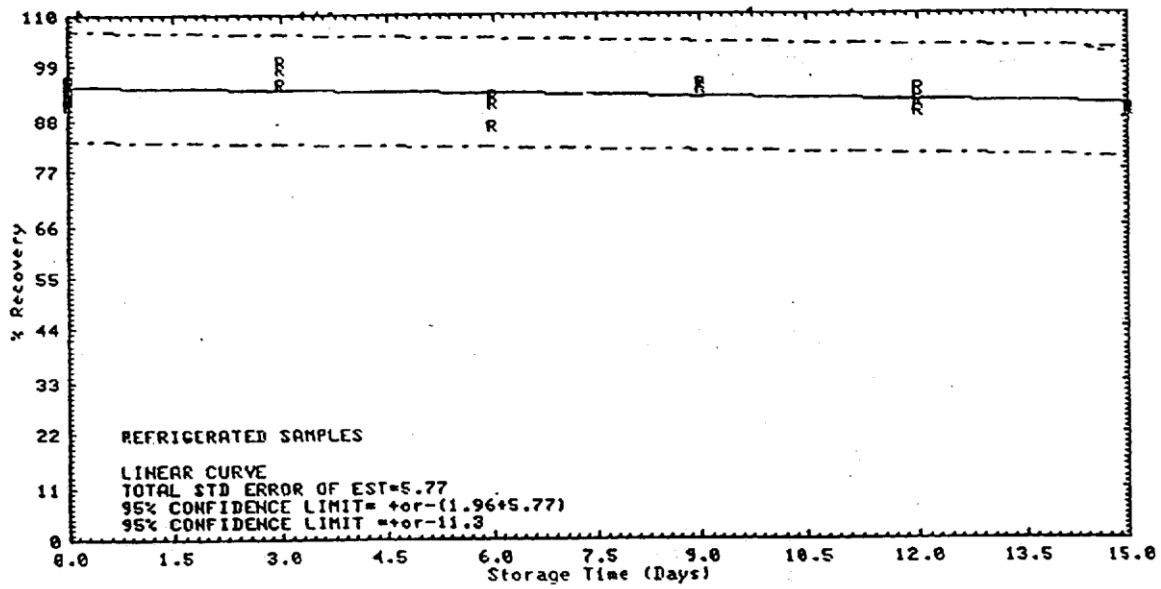


Figure 4.5.1.1. o-Toluidine refrigerated storage samples.

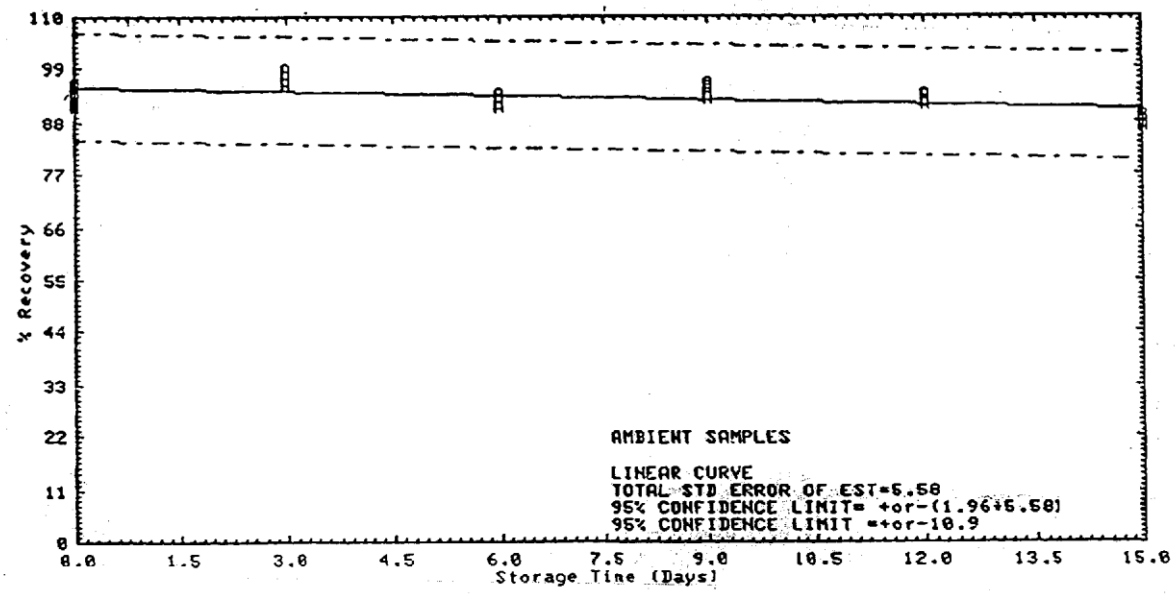


Figure 4.5.1.2. o-Toluidine ambient storage samples.

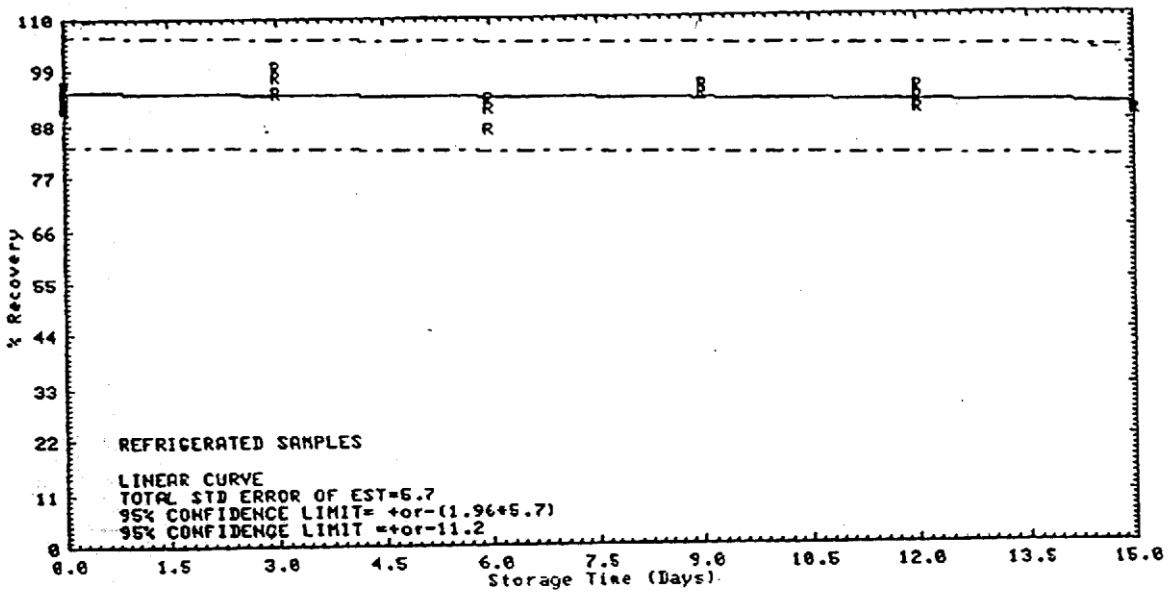


Figure 4.5.2.1. m-Toluidine refrigerated storage samples.

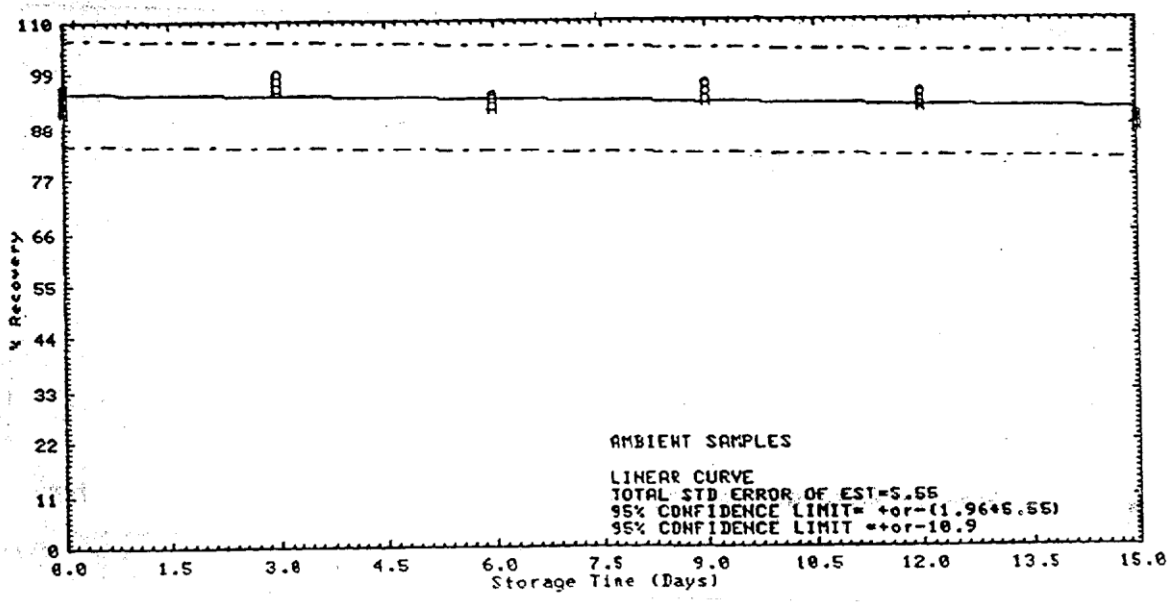


Figure 4.5.2.2. m-Toluidine ambient storage samples.

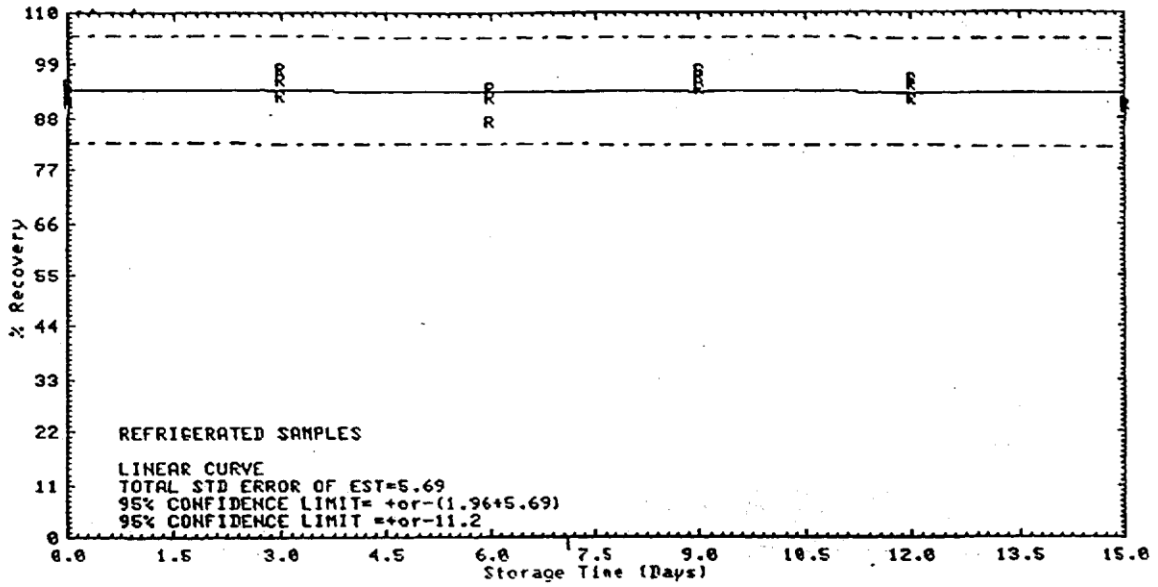


Figure 4.5.3.1. p-Toluidine refrigerated storage samples.

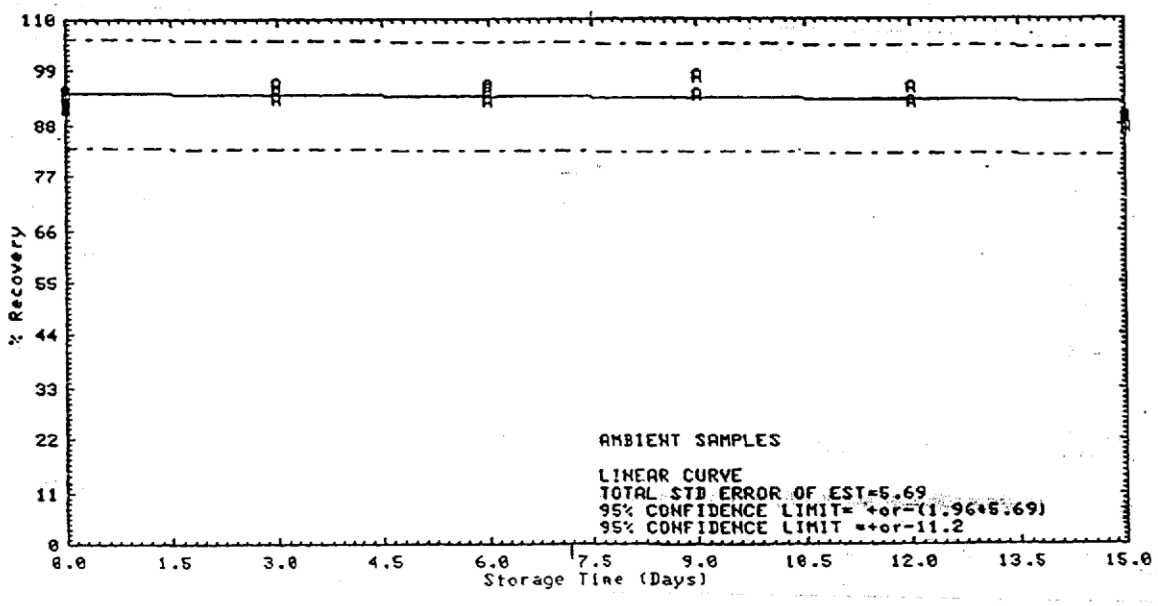


Figure 4.5.3.2. p-Toluidine ambient storage samples.

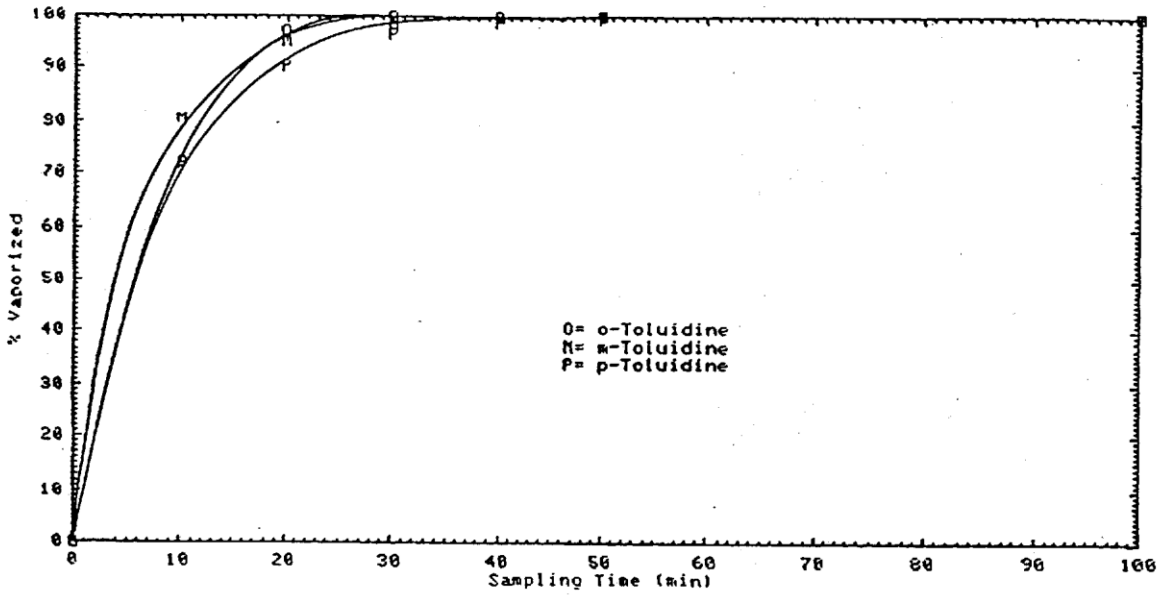


Figure 4.9. Toluidine vapor generation rates.

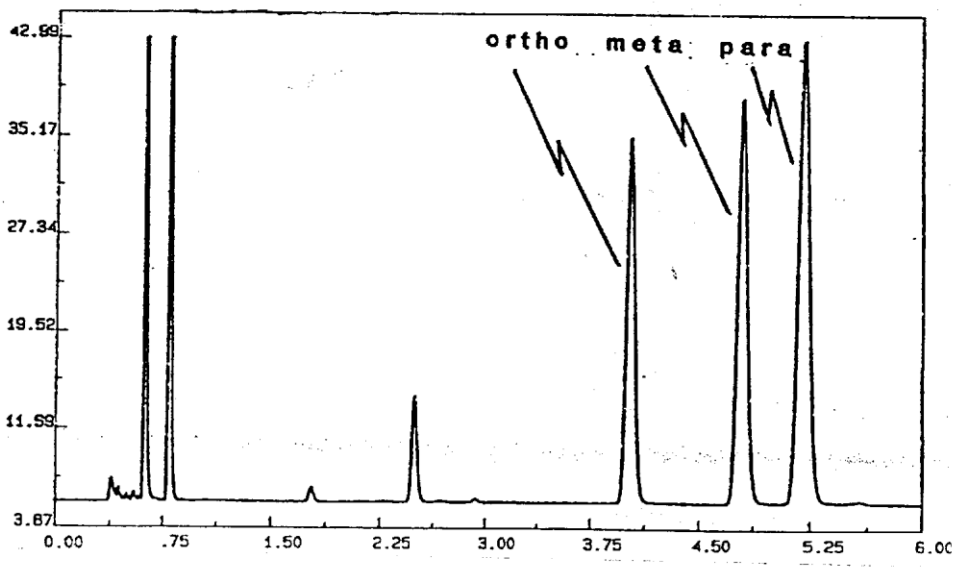


Figure 4.11. Target concentration chromatogram.

5. References

5.1."Chemical Information Manual", U.S Department of Labor, Occupational Safety and Health Administration, OSHA Instruction CPL 2-2.43, Directorate of Technical Support; National Technical Information Service: Springfield, VA 22161, October 20, 1987.

5.2."NIOSH Manual of Analytical Methods", 2nd ed.; U.S Department of Health, Education, and Welfare, National Institute for Occupational Safety and Health: Cincinnati, OH, 1977; Vol. 3, Method No. S168; DHEW(NIOSH) Publ.(U.S.), No. 77-157-C.

5.3.Elskamp, C. J. "OSHA Method No. 71; o-Dianisidine, 4,4'-Methylenebis(2-chloroaniline) o-Toluidine", OSHA Analytical Laboratory, unpublished, Salt Lake City, UT 84165, April 1988.

5.4.Elskamp, C. J. "OSHA Method No. 65; Benzidine, 3,3'-Dichlorobenzidine, 2,4-Toluenediamine, 2,6-Toluenediamine", OSHA analytical Laboratory, unpublished, Salt Lake City, UT 84165, August 1987.

5.5.Elskamp, C. J. "OSHA Method No. 57; 4,4-Methylenedianilin" OSHA Analytical Laboratory, unpublished, Salt Lake City, UT 84165, January 1986.

5.6."Documentation of the Threshold Limits Values and Biological Exposure Indices", 5th ed.; American Conference of Governmental Industrial Hygienists Inc.: Cincinnati, OH, 1986; pp 586-591.

5.7."Threshold Limit Values and Biological Exposure Indices for 1987-1988"; American Conference of Governmental Industrial Hygienists Inc.: Cincinnati, OH, 1987.

5.8."Code of Federal Regulations"; Office of the Federal Register National Archives and Records Service; U.S Government Printing Office: Washington, DC, 1985, 29 CFR Ch. XVII (7-1-85 Edition) 1910.1000, Table Z-1.

5.9.Sax, N. I. "Dangerous Properties of Industrial Materials"; Van Nostrand Reinhold: New York, 1979; p 1036.