Method no.:	PV2082
Matrix:	Air
Target concentration:	10 ppb (81.5 µg/m³)(OSHA Regulation 1910.1003, lowest feasible limit)
Procedure:	Samples are collected by drawing a known volume of air through an OSHA versatile sampler (OVS-2) tubes, containing a glass fiber filter and two sections of XAD-2 adsorbent. Samples are desorbed with ethyl acetate and analyzed by liquid chromatography (LC) using an ultra-violet detector (UV).
Recommended air volume and sampling rate:	240 L at 1.0 L/min
Reliable quantitation limit:	0.018 ppb (0.15 µg/m^3)
Special requirements:	Samples should be stored in a refrigerator when not in transit.
Status of method:	Partially Evaluated Method. This method has been subjected to established evaluation procedures, and is presented for information and trial use.

Date: September 1994 Chemist: Wayne Potter

Organic Service Branch I
OSHA Salt Lake Technical Center
Salt Lake City, UT 84115-1802

1. General Discussion

1.1 Background

1.1.1 History

Airborne 4-nitrodiphenyl has been determined by collection on a glass fiber filter and silica gel tube connected in series and analyzed by gas chromatograph with a flame ionization detector in NIOSH proposed method P & CAM 273. One of the potential disadvantages of this method is connecting the glass fiber filter and silica gel tube is series. Another disadvantage is that flame ionization detection is not very sensitive to 4-nitrodiphenyl.

In this method, airborne 4-nitrodiphenyl is collected on one OVS-2 sampler and analyzed on a liquid chromatograph with an ultra-violet (UV) detector. This detector is very sensitive to 4-nitrodiphenyl which allows a much lower detection limit. 4-Nitrodiphenyl under 29 CFR 1910.1003 is considered a potential human carcinogen. A target concentration of 10 ppb was picked as the lowest feasible amount to work with.

1.1.2 Toxic effects (This section is for information only and should not be taken as the basis of OSHA policy.) (Ref. 5.1, 5.2, and 5.3)

The International Agency for Research on Cancer Working Group on the Evaluation of the Carcinogenic Risk of Chemicals to Man found no reports on carcinogenicity of 4-Nitrodiphenyl to man. However, the group concluded that it is not possible to separate the exposures to 4-nitrodiphenyl from exposures to 4-aminobiphenyl because the former is converted to the latter by reduction. 4-Aminobiphenyl is a recognized human bladder carcinogen (Ref. 5.1).

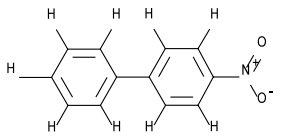
4-Nitrodiphenyl is a confirmed carcinogen with experimental carcinogenic, neoplastigenic and tumorigenic data. It is a poison by intraperitoneal route and moderately toxic by ingestion. When heated to decomposition it emits toxic fumes of NOx. (Ref. 5.2).

Exposure to 4-nitrodiphenyl can cause headaches, lethargy, dizziness, dyspnea, ataxia, weakness, methemoglobinemia; urinary burning and acute hemorrhagic cystitis. (Ref. 5.3).

1.1.3 Workplace exposure (Ref. 5.4)

4-Nitrodiphenyl is used as a dye intermediate, fungicide, plasticizer for cellulosics and a wood preservative. No data is available on the extent of work place exposure.

1.1.4 Physical properties and other descriptive information (Ref. 5.1 unless otherwise indicated).

Synonyms:	Nitrodiphenyl, 4-nitrobiphenyl, p-phenyl-nitrobenzene, 4-phenyl-nitrobenzene (Ref. 5.5)
CAS number:	92-93-3
IMIS:	1875
RTECS:	DV5600000; 20760 (Ref. 5.5)
Molecular weight:	199.22
Boiling point:	340°C @ 101.3 kPa (760 mmHg)
Melting point:	114°C
Odor:	Sweetish odor
Color:	Yellow to white needles
Solubility:	Insoluble in water, slightly soluble in cold alcohol, very soluble in ether.
Molecular formula:	$C_{12}H_9NO_2$
Structural formula:	

The analyte air concentrations throughout this method are based on the recommended sampling and analytical parameters. Air concentrations listed in ppm are referenced to 25°C and 101.3 kPa (760 mmHg).

1.2 Limit defining parameters

1.2.1 Detection limit of the overall procedure (DLOP)

The detection limit of the overall procedure is 0.011 µg per sample (0.006 ppb or 0.046 µg/m³). This is the amount of analyte spiked on the sampler that will give a response that is significantly different from the background response of a sampler blank.

The DLOP is defined as the concentration of analyte that gives a response (Y_{DLOP}) that is significantly different (three standard deviations (SD_{BR})) from the background response (Y_{BR}).

$$Y_{DLOP} - Y_{BR} = 3(SD_{BR})$$

The direct measurement of Y_{BR} and SD_{BR} in chromatographic methods is typically inconvenient, and difficult because Y_{BR} is usually extremely low. Estimates of these parameters can be made with data obtained from the analysis of a series of samples whose responses are in the vicinity of the background response. The regression curve obtained for a plot of instrument response versus concentration of analyte will usually be linear. Assuming SD_{BR} and the precision of data about the curve are similar, the standard error of estimate (SEE) for the regression curve can be substituted for SD_{BR} in the above equation. The following calculations derive a formula for the DLOP:

$$SEE = \sqrt{\frac{\sum (Y_{obs} - Y_{est})^2}{n - k}}$$

Y_{obs} = observed response
Y_{est} = estimated response from regression curve
n = total no. of data points
k = 2 for a linear regression curve

At point Y_{DLOP} on the regression curve

$$Y_{DLOP} = A(DLOP) + Y_{BR}$$

A = analytical sensitivity (slope)

therefore

$$DLOP = \frac{(Y_{DLOP} - Y_{BR})}{A}$$

Substituting 3(SEE) + Y_{BR} for Y_{DLOP} gives

$$DLOP = \frac{3(SEE)}{A}$$

The DLOP is measured as mass per sample and expressed as equivalent air concentrations, based on the recommended sampling parameters. Ten samplers were spiked with equal descending increments of analyte, such that the highest sampler loading was 0.2 µg/sample. This is the amount, when spiked on a sampler, that would produce a peak approximately 10 times the background response for the sample blank. These spiked samplers, and the sample blank were analyzed with the recommended analytical parameters, and the data obtained used to calculate the required parameters (A and SEE) for the calculation of the DLOP. Values of 52245.1 and 187.194 were obtained for A and SEE respectively. DLOP was calculated to be 0.011 µg/sample (0.006 ppb or 0.046 µg/m^3).

Table 1.2.1
Detection Limit of the Overall Procedure

mass per sample (µg)	area counts (µV-s)
0	0
.02	1.570
.04	2.642
.06	4.090
.08	4.971
.10	6.065
.12	6.953
.14	8.325
.16	8.988
.18	9.915
.20	11.132

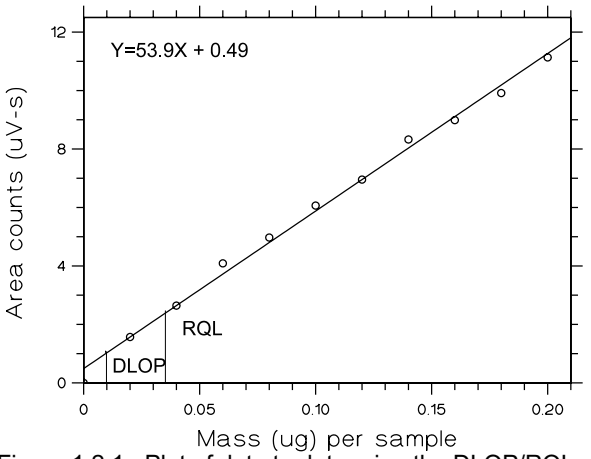


Figure 1.2.1. Plot of data to determine the DLOP/RQL.

1.2.2 Reliable quantitation limit (RQL)

The reliable quantitation limit is 0.036 µg per sample (0.018 ppb or 0.15 µg/m^3). This is the amount of analyte spiked on a sampler that will give a signal that is considered the lower limit for precise quantitative measurements.

The RQL is considered the lower limit for precise quantitative measurements. It is determined from the regression line data obtained for the calculation of the DLOP (Section 1.2.1), providing at least 75% of the analyte is recovered. The RQL is defined as the concentration of analyte that gives a response (Y_{RQL}) such that

$$Y_{RQL} - Y_{BR} = 10(SD_{BR})$$

therefore

$$RQL = \frac{10(SEE)}{A}$$

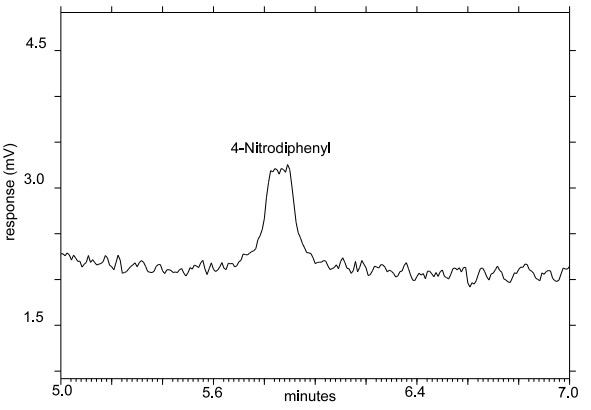


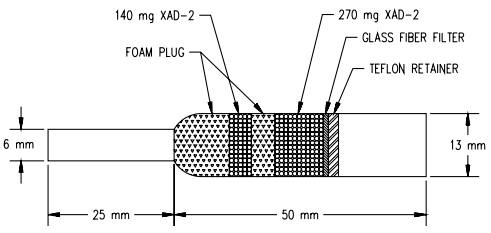
Figure 1.2.3. Chromatogram of the RQL.

2. Sampling Procedure

2.1 Apparatus

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

2.1.2 Samples are collected on OVS-2 tubes, which are specially made 11-mm i.d. × 13-mm o.d. x 5.0 cm long glass tubes that taper to 6-mm o.d. × 2.5 cm. Each tube is packed with a 140-mg back section and a 270-mg front section of XAD-2 and a 13-mm diameter glass fiber filter. The back section is retained by two foam plugs and the sampling section is between one foam plug and the glass fiber filter. The glass fiber filter is held next to the sampling section by a polytetrafluoroethylene (PTFE) retainer. These tubes are commercially available from SKC Inc. and Forest Biomedical.



2.2 Technique

2.2.1 Immediately before sampling, remove the caps. All tubes should be from the same lot.

2.2.2 Attach small end of the sampling tube to the pump with flexible tubing. Position the tube so that sampled air passes through the front section of the tube first.

2.2.3 Air being sampled should not pass through any hose or tubing before entering the sampling tube.

2.2.4 Attach the sampler vertically with the open end pointing downward, in the worker's breathing zone, and positioned so it does not impede work performance or safety.

2.2.5 After sampling for the appropriate time, remove the sample and seal the tube with plastic end caps. Wrap each sample end-to-end with a Form OSHA-21 seal.

2.2.6 Submit at least one blank sample with each set of samples. Handle the blank sampler in the same manner as the other samples except draw no air through it.

2.2.7 Record sample volumes (in liters of air) for each sample, along with any potential interferences.

2.2.8 Ship any bulk samples in separate containers from the air samples.

2.2.9 Submit the samples to the laboratory for analysis as soon as possible after sampling. If delay is unavoidable, store the samples in a refrigerator.

2.3 Desorption efficiency

The desorption efficiencies of 4-nitrodiphenyl were determined by liquid-spiking the 13-mm glass fiber filters and also an amount of XAD-2 adsorbent equal to the adsorbing section (270mg) of an OVS-2 tube with the analyte at 0.1 to 2 times the target concentration. The loadings on the tubes were 2.01, 10.05, 20.1, and 40.2µg of 4-nitrodiphenyl. These samples were stored overnight at ambient temperature and then desorbed and analyzed by LC-UV. The average desorption efficiency over the studied range was 99.3%.

Table 2.3.1
Desorption Efficiency of 4-Nitrodiphenyl
From GFF

Tube #	% Recovered			
	0.1 × 2.01µg	0.5 × 10.05ug	1.0 × 20.1µg	2.0 × 40.2µg
1	97.2	101.8	103.9	101.6
2	92.5	103.1	100.9	100.6
3	93.3	101.4	100.2	100.7
4	95.6	104.9	101.4	100.8
5	87.6	102.5	101.9	101.3
6	92.4	100.0	100.8	100.9
average	93.1	102.3	101.5	101.0
overall average	99.5			
standard deviation	±4.28			

Table 2.3.2
Desorption Efficiency of 4-Nitrodiphenyl
From XAD-2

Tube #	% Recovered			
	0.1 × 2.01µg	0.5 × 10.05ug	1.0 × 20.1µg	2.0 × 40.2µg
1	100.3	102.0	100.1	100.4
2	94.8	102.2	100.1	100.4
3	97.1	94.0	97.6	100.9
4	101.2	94.6	100.0	100.1
5	97.1	95.5	101.4	100.6
6	96.8	98.7	100.2	99.4
average	97.9	97.8	99.9	100.3
overall average	99.0			
standard deviation	±1.31			

2.4 Retention efficiency

The sampling tubes were spiked with 40.2 µg (20 ppb or 160 µg/m³) 4-nitrodiphenyl, allowed to equilibrate overnight at room temperature, and then had 240L humid air (80% RH at 25°C) drawn through them at 1.0 Lpm. The sampling tubes were opened and the GFF, the front section and the back section were each put in separate vials. The samples were desorbed and analyzed by LC-UV. The retention efficiency averaged 98.8%. There was no 4-nitrodiphenyl found on the back sections of the tubes.

Table 2.4
Retention Efficiency of 4-Nitrodiphenyl

Tube #	% Recovered			
	GFF	Front Section	Back Section	Total
1	90.5	8.1	0	98.6
2	77.0	18.6	0	95.6
3	85.7	14.4	0	100.1
4	78.9	18.8	0	97.7
5	84.0	16.0	0	100.0
6	89.8	10.7	0	100.5
		average		98.8

2.5 Sample storage

The glass fiber filter of twelve sampling tubes were each spiked with 20.1 µg (80 µg/m³) of 4-nitrodiphenyl. They were sealed and stored at room temperature. The next day 240 L of humid air (80% RH at 25°C) was drawn through each tube at 1.0 L/min. Half of the tubes were stored in a drawer at ambient temperature and the other half were stored in a refrigerator at 0°C. After 7 days of storage three samples from the tubes stored under refrigeration and three samples from ambient storage were analyzed. The remaining samples were analyzed after 14 days of storage. The amounts recovered, which are not corrected for desorption efficiency, indicate that the samples should be refrigerated.

Table 2.5
Storage Test for 4-Nitrodiphenyl

Ambient Storage		Refrigerator Storage	
Time (days)	% Recovered	Time (days)	% Recovered
7	93.8	7	98.6
7	91.4	7	102.0
7	91.4	7	100.1
14	85.6	14	99.1
14	83.1	14	99.7
14	77.4	14	99.5
average	87.1	average	99.8

2.6 Recommended air volume and sampling rate.

Based on the data collected in this evaluation, 240 L air samples should be collected at a sampling rate of 1.0 L/min.

2.7 Interferences (sampling)

2.7.1 It is not known if any compounds will severely interfere with the collection of 4-nitrodiphenyl on OVS-2 sampling tubes. In general, the presence of other contaminant vapors in the air will reduce the capacity of the sampling tube to collect 4-nitrodiphenyl.

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 worker in such a manner that it will not interfere with work performance or safety.

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

2.8.3 Wear eye protection at all times while in the work areas.

3. Analytical Procedure

3.1 Apparatus

3.1.1 The instrument used in this study was a liquid chromatograph equipped with an ultra-violet detector, specifically a Waters model 600E system controller, a Waters 490E detector and a Waters 717 autosampler.

3.1.2 An LC column capable of separating the analyte from any interferences. The column used in this study was a Supelco LC-8-DB, 5 µm (4.6×250 mm).

3.1.3 An electronic integrator or some suitable method of measuring peak areas.

3.1.4 Four milliliter vials with Teflon-lined caps.

3.1.5 A 10 µL syringe or other convenient size for sample injection.

3.1.6 Pipets for dispensing the desorbing solution. A 2 mL dispenser was used in this study.

3.1.7 Volumetric flasks - 10 mL and other convenient sizes for preparing standards.

3.2 Reagents

3.2.1 4-Nitrodiphenyl, Reagent grade.

3.2.2 Ethyl Acetate, HPLC grade.

3.2.3 Acetonitrile, HPLC grade.

3.2.4 Water, HPLC grade.

3.3 Standard preparation

3.3.1 At least two separate stock standards are prepared by diluting a known quantity of 4-nitrodiphenyl with the desorbing solution of ethyl acetate. The concentration of these stock standards was 2010 µg/mL.

3.3.2 Dilutions of these stock standards were prepared to bracket the samples. The range of the standards used in this study was from 2.01 to 40.2 µg/mL.

3.4 Sample preparation

3.4.1 Sample tubes are opened and the front section (GFF and 270 mg adsorbent), and back section of each tube are placed in separate 4 mL vials.

3.4.2 Each section is desorbed with 2 mL of ethyl acetate.

3.4.3 The vials are sealed immediately and allowed to desorb for one hour on a mechanical shaker.

3.5 Analysis

3.5.1 Liquid chromatograph conditions.

Injection size:	5 µL (an injection size greater than 5 µL will cause peak splitting).
Column:	Supelco LC-8-DB, 5 µm, 25cm × 4.6mm i.d.
Mobile phase:	55% Acetonitrile in water (v/v)
Flow rate:	1 mL/min
UV detector:	305 nm
Retention time:	6.0 min

Chromatogram:

3.5.2 Peak areas are measured by an integrator or other suitable means.

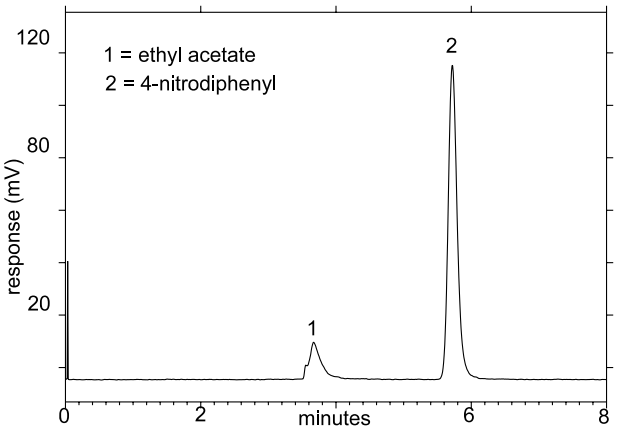


Figure 3.5.1. Chromatogram at the target concentration.

3.6 Interferences (analytical)

3.6.1 Any compound that produces a response to UV at 305 nm, and has a similar retention time as the analyte is a potential interference. If any potential interferences were reported, they should be considered before samples are desorbed. Generally, chromatographic conditions can be altered to separate an interference from the analyte.

3.6.2 When necessary, the identity or purity of an analyte peak may be confirmed by a GC-mass spectrometer or by another analytical procedure.

3.7 Calculations

3.7.1 The calibration curve was made from at least four standards at different concentrations bracketing the samples.

3.7.2 The values for the samples are obtained from the calibration curve.

3.7.3 To calculate the concentration of analyte in the air sample the following formulas are used:

$$\text{mass of analyte in sample} = \frac{(\text{mg/mL})(\text{desorption volume})}{\text{desorption efficiency}}$$

$$\text{number of moles of analyte} = \frac{\text{mass of analyte in sample}}{\text{molecular weigth}}$$

Volume the analyte will occupy at 25 °C and 760 mmHg is number of moles of analyte times the molar volume at 25 °C and 760 mmHg.

$$\text{ppm} = \frac{(\text{volume analyte occupies})(10^6)}{\text{air volume}}$$

3.7.4 The above equations can be consolidated to the following formula.

$$\text{ppm} = \frac{(\text{mg/mL})(\text{DV})(24.46)(10^6)(\text{g})(\text{mg})}{(10\ \text{L})(\text{DE})(\text{MW})(1000\ \text{mg})(1000\ \text{mg})}$$

µg/mL = concentration of analyte in sample or standard
24.46 = molar volume (liters/mole) at 25 °C and 760 mmHg
MW = molecular weight (g/mole)
DV = desorption volume
10 L = 10 liter air sample
DE = desorption efficiency
* All units must cancel.

3.7.5 This calculation is done for each section of the sampling tube and the results added together after a blank correction is performed, if necessary.

3.8 Safety precautions

3.8.1 Avoid skin contact and inhalation of all chemicals.

3.8.2 Wear safety glasses, gloves and a lab coat at all times while in the laboratory areas.

4. Recommendations for Further Study

Collection studies should be performed.

5. References

5.1 American Conference of Governmental Industrial Hygienists, Inc. "Documentation of the Threshold Limit Values", 5th ed., 1986. Cincinnati, Ohio, p.433.

5.2 Sax, N., "Dangerous Properties of Industrial Materials", 8th edition, Van Nostrand Reinhold Co., New York, 1992, p. 2524.

5.3 Cameo Database, 1992, NOAA.

5.4 Lewis, R., "Hawley's Condensed Chemical Dictionary", twelfth edition, Van Nostrand Reinhold Co., New York, 1993, p.826.

5.5 Sweet, D., "Registry of Toxic Effects of Chemical Substances", 1986-86 Edition, U.S. Department of Health and Human Services, Public Health Service, Center for Disease Control, NIOSH, 1987, Vol. 2, p.1186.