Benomyl

Method no.:	PV2107
Target Concentration:	10 mg/m^3 (ACGIH TLV)
Procedure:	Samples are collected by drawing known volumes of air through OSHA versatile sampler (OVS-2) tubes, containing a glass fiber filter and two sections of XAD-2 adsorbent. Samples are extracted with acetonitrile (ACN) and analyzed by high performance liquid chromatography (HPLC) using an ultraviolet (UV) detector.
Recommended air volume and sampling rate:	60 minutes at 1.0 L/min (60 L)
Detection limit of the overall procedure:	0.05 mg/m^3 - Based on the recommended air volume.
Status of method:	Partially Validated method. This method has been only partially evaluated and is presented for information and trial use.

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David B. Armitage

Carcinogen and Pesticide Branch
OSHA Salt Lake Technical Center
Sandy-84070

1 General Discussion

1.1 Background

1.1.1 History of procedure

This evaluation was undertaken to determine the effectiveness of the OVS-2 tube as a sampling device for benomyl. It follows the procedure developed for carbaryl. (Ref. 5.1)

The literature indicates that benomyl decomposes in many organic solvents to give carbendazim and n-butyl isocyanate (BIC). It is possible to analyze for benomyl in organic solvents, but this calls for the addition of BIC at 100 times the level of benomyl encountered to force the equilibrium back to the undecomposed benomyl. Due to this decomposition, the analytical method developed in this evaluation is for carbendazim (however, the analyte is referred to as benomyl throughout this evaluation). It follows a method developed by Zweig and Gao. (Ref. 5.2)

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

The acute oral LD_{50} for rats is >10,000 mg/kg. The acute skin absorption LD_{50} for rabbits is >10,000 mg/kg. (Ref. 5.3)

A low order of chronic toxicity has been found in several studies. The results of these studies as well as the original references can be found in the ACGIH documentation volume. (Ref. 5.3)

Due to its low toxicity, the ACGIH has given benomyl a 10 mg/m^3 TLV.

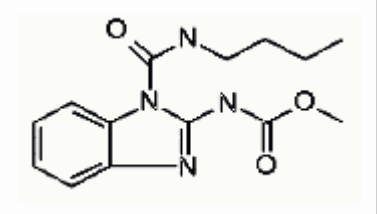
1.1.3 Potential workplace exposure

No estimate of worker exposure to benomyl could be found. Benomyl is used as a systemic fungicide. (Ref. 5.4)

1.1.4 Physical properties (Ref. 5.3 - 5.5)

CAS #:	17804-35-2
Molecular weight:	290.32
Molecular formula:	$C_{14}H_{18}N_4O_3$
Melting point:	decomposes
Vapor Pressure:	negligible
Appearance:	white crystalline solid
Solubility:	insoluble in water and oil, soluble in acetone, alcohol, dimethyl formamide, chloroform, and xylene.
Chemical name:	methyl 1-(butylcarbamoyl)-2-benzimidazole Carbamate
Synonyms:	Benex, Benlate, And Tersan 1991
UV maxima in ACN:	286 nm and 244 nm (i.e., values are for carbendazim)

Structure:



1.2 Limit defining parameters

The detection limit of the analytical procedure is 10 ng per injection. This is the amount of analyte which will give a peak whose height is approximately five times the baseline noise.

2 Sampling procedure

2.1 Apparatus

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

2.1.2 Samples are collected with OVS-2 tubes, which are specially made 13-mm o.d. glass tubes that are tapered to 6-mm o.d. These tubes are packed with a 140-mg backup section and a 270-mg sampling section of cleaned XAD-2. The backup section is retained by two foam plugs and the sampling section is between one foam plug and a 13-mm diameter glass fiber filter. The glass fiber filter is held next to the sampling section by a polytetrafluoroethylene (PTFE) retainer.

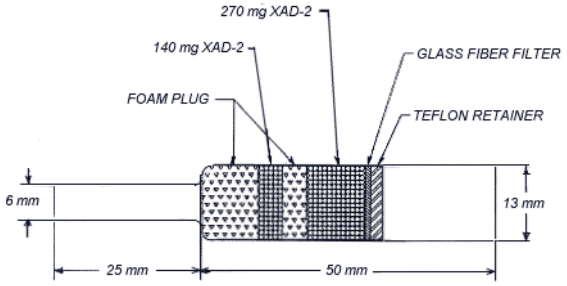


Figure 1. OVS-2 Sampling Device

2.2 Reagents

No sampling reagents are required.

2.3 Sampling technique

2.3.1 Attach the small end of the OVS-2 sampling tube to the sampling pump with flexible, plastic tubing such that the large, front section of the sampling tube is exposed directly to the atmosphere. Do not place any tubing in front of the sampler. Attach the sampler vertically (large end down) in the worker's breathing zone in such a manner that it does not impede work performance.

2.3.2 After sampling for the appropriate time, remove the sampling device and seal the tube with plastic end caps.

2.3.3 Wrap each sample end-to-end with a Form OSHA-21 seal.

2.3.4 Submit at least one blank with each set of samples. Handle the blanks the same as the other samples except draw no air through them.

2.3.5 Submit any bulk samples in a separate container. Do not ship them with the air samples.

2.4 Extraction efficiency

2.4.1 Glass fiber filter

Five 13-mm glass fiber filters were each liquid spiked with 50 µL of an 838 µg/mL solution of benomyl in methanol. These five filters, along with an unspiked filter, were place in separate 4-mL vials, sealed with PTFE-lined septa, and allowed to sit overnight at room temperature. They were then extracted with 3.0 mL of acetonitrile and analyzed.

Table 2.4.1
Glass Fiber Filter Extraction Study

filter #	amount spiked (µg)	amount recovered	% recovered
1	41.90	42.08	100.4
2	41.90	40.60	96.9
3	41.90	43.35	103.5
4	41.90	41.71	99.6
5	41.90	41.64	99.4
6	0.00	0.00	blank

average recovery = 100.0%

2.4.2 XAD-2 adsorbent

An amount of XAD-2 adsorbent equal to the A section (270 mg) of an OVS-2 tube was placed in each of five 4-mL vials which were then sealed with PTFE lined septa.

The adsorbent in each vial was then liquid spiked with 50 µL of an 838 µL/mL solution of benomyl in methanol by injecting the solution onto the adsorbent through the septum. The vials were allowed to equilibrate overnight in a drawer at room temperature. They were then desorbed with 3.0 mL of acetonitrile and analyzed.

Table 2.4.2
XAD-2 Adsorbent Desorption Study

adsorbent #	(µg) spiked	(µg) recovered	% recovered
1	41.9	39.13	93.4
2	41.9	39.55	94.4
3	41.9	40.05	95.6
4	41.9	40.70	97.1
5	41.9	41.22	98.4
6	0.0	0.00	blank

average recovery = 95.8%

2.4.3 OVS-2 tubes

Nine OVS-2 tubes were each liquid spiked with 50 µL of an 838 µg/mL solution of benomyl in methanol by spiking the glass fiber filter. The tubes were sealed and stored overnight in a drawer at room temperature. A blank tube was also stored with the nine above. Three of the spiked tubes and the blank were then extracted and analyzed as in Section 3.4.

Table 2.4.3
OVS-2 Tube Extraction Study

tube #	(μg) spiked	(μg) recovered	% recovered
OVS-1	41.90	41.74	99.6
OVS-2	41.90	41.93	100.1
OVS-3	41.90	42.47	101.4
OVS-BL	0.00	0.00	blank

average recovery = 100.4%

2.5 Retention efficiency

The remaining six OVS-2 tubes each had 60 liters if humid air (65% relative humidity) drawn through them. Three of these tubes were extracted as in Section 3.4 and analyzed immediately. The remaining three tubes were returned to the drawer for the storage study.

Table 2.5
Retention Efficiency Study

tube #	(μg) spiked	(μg) recovered	% recovered
OVS-4	41.90	42.75	102.0
OVS-5	41.90	43.66	104.2
OVS-6	41.90	42.57	101.2

average recovery = 102.5%

2.6 Sample Storage

The remaining three spiked tubes from section 2.5 above were stored for a total of 7 days in a drawer at room temperature. They were then extracted and analyzed as in Section 3.4.

Table 2.6
Storage Study

tube #	(μg) spiked	(μg) recovered	% recovered
OVS-7	41.90	40.76	97.3
OVS-8	41.90	40.76	97.3
OVS-9	41.90	41.51	99.1

average recovery = 97.9%

2.7 Recommended air volume and sampling rate

2.7.1 The recommended air volume is 60 L.

2.7.2 The recommended flow rate is 1.0 L/min.

2.8 Interferences

It is not known if any compounds will interfere with the collection of benomyl. Suspected interferences should be reported to the laboratory with submitted samples.

2.9 Safety precautions

2.9.1 Attach the sampling equipment to the employee in such a manner that it will not interfere with work performance or employee safety.

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

3 Analytical procedure

3.1 Apparatus

3.1.1 A high performance liquid chromatograph equipped with a UV detector, and manual or automatic injector. A Waters 510 pump, Waters 710B autosampler, and Waters 490E UV detector were used in this evaluation.

3.1.2 An HPLC column capable of separating benomyl from any interference. A 25-cm × 4.6-mm i.d. Chromasil, 5 µm C18 column was used in this evaluation.

3.1.3 An electronic integrator or other suitable means of measuring detector response. A Hewlett-Packard 3357 data system was used in this evaluation.

3.1.4 Vials, 4-mL glass with PTFE-lined septa.

3.1.5 Volumetric flasks, pipettes, and syringes for preparing standards, making dilutions, and performing injections.

3.2 Reagents

3.2.1 HPLC grade Acetonitrile (ACN).

3.2.2 HPLC grade water. A Millipore Milli-Q system was used to prepare the water in this evaluation.

3.2.3 Benomyl, 99+% (EPA).

3.3 Standard preparation

Prepare stock standard solutions by adding acetonitrile to pre-weighed amounts of benomyl. Prepare working range standard solutions by diluting stock solutions with acetonitrile. Store stock and dilute standards in a freezer.

Place aliquots of the working range standards in 4-mL vials and handle them along with the samples.

Note: A benomyl in methanol standard was used for spiking in the extraction, retention, and storage tests because benomyl is more soluble in methanol than acetonitrile. Methanol was not used for sample extraction because the literature indicates quicker and more complete decomposition of benomyl in acetonitrile (Ref. 5.2).

3.4 Sample preparation

3.4.1 Transfer the 13-mm glass fiber filter and the 270-mg section of the sampling tube to a 4-mL vial. Place the first foam plug and the 140-mg section in a separate vial. A small glass funnel can be used to facilitate the transfer of the adsorbent. Discard the rear foam plug. No not discard the glass sampling tube; it can be reused after it has been cleaned with surfactant or suitable solvent.

3.4.2 Add 3.0 mL of ACN to each vial.

3.4.3 Seal the vials the PTFE-lined septa and allow them to extract for one half hour. Shake the vials by hand periodically during this extraction time. Place the vials in a 45ºC water bath for an additional half hour to ensure complete decomposition of benomyl to carbendazim.

3.5 Analysis

3.5.1 Instrument conditions

Column:	25-cm × 4.6-mm i.d. Chromasil, 5 µm, C18
Mobile Phase:	70% acetonitrile/30% water
Flow rate:	1 mL/min
UV detector:	244 and 286 nm
Retention time:	3.5 minutes (carbendazim)
Injection volume:	10 µL

3.5.2 Chromatogram

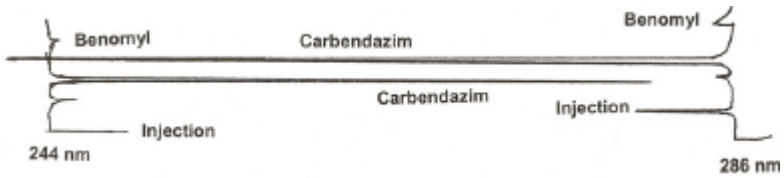


Figure 2. Chromatogram of Carbendazim (note the small Benomyl peak, benomyl not completely decomposed)

3.6 Interferences

3.6.1 Any compound having a similar retention time to the analyte is a potential interference. Generally, chromatographic conditions can be altered to separate interference from the analyte.

3.6.2 Retention time on a single column is not proof of chemical identity. Analysis by an alternative HPLC column, detection at another wavelength, comparison of absorbance response ratios, and confirmation by mass spectrometry are additional means of identification.

3.7 Calculations

3.7.1 A calibration curve is constructed by plotting detector response versus standard concentration.

3.7.2 The concentration of benomyl in a sample (µg/mL) is determined from the calibration curve. If benomyl is found on the backup section, it is added to the amount found on the front section. Blank corrections for each section should be performed before adding the results together.

3.7.3 The air concentration is then determined by the following formula.

$$mg/m^3 = \frac{(\mu g/mL\ in\ sample)(desorption\ vol, mL)}{(air\ vol, L)(desorption\ efficiency, decimal)}$$

3.8 Safety Precautions

3.8.1 Avoid exposure to all standards.

3.8.2 Avoid exposure to all solvents.

3.8.3 Wear safety glasses at all times.

4 Recommendations for further study

An HPLC column which gives better chromatographic results should be used for the analysis of benomyl in the future. One such column is a 25-cm × 4.6-mm i.d. Chromegabond, 5 µm TMS (see the n-butyl isocyanate in the presence of benomyl stopgap, Ref. 5.6). This method should be fully validated.

5 References

5.1 Burright, D., Method #63, "Carbaryl (Sevin)", OSHA Analytical Laboratory, unpublished, 1987.

5.2 Zweig, G., and R. Gao. Anal. Chem. 55:1448-51 (1983).

5.3 "Documentation of the Threshold Limit Values and Biological Exposure Indices, American Conference of Governmental Industrial Hygienists Inc., fifth edition, 1986.

5.4 "Farm Chemicals Handbook," Meister Publishing Co., 1985.

5.5 Windholz, M., Ed. "Merck Index," 10th ed.; Merck and Co., Rahway, NJ, 1983.

5.6 Armitage, D.B., Stopgap, "Benomyl with n-Butyl Isocyanate," OSHA Analytical Laboratory, unpublished, 1988.