Analytical method for dichlobenil & BAM in water

Reports:	ECM: MRID 45321801. Carter, D.S. 2000. Analytical method for determining dichlobenil and its metabolite 2,6-dichlorobenzamide in water. Analytical Method No. AC-7005. Uniroyal Chemical Company Study No. 99055. Report prepared, sponsored and submitted by Uniroyal Chemical Company, Inc., Crop Protection Division, Middlebury, Connecticut; 74 pages. Final report issued May 10, 2000. ILV: MRID 49051904. Noon, P. 2001. Independent laboratory validation of Uniroyal Chemical Company analytical method "Analytical method for determining dichlobenil and its metabolite 2,6-dichlorobenzamide in water" (Analytical Method No. AC-7005, Uniroyal study number 99055). Sponsor Study No: 2001-089. NCL Study Number: 20.080. Report prepared by North Coast Laboratories, Ltd., Arcata, California; sponsored and submitted by Uniroyal Chemical Company, Inc., Middlebury, Connecticut; 94 pages. Final report issued July 23, 2001.
Document No.:	MRIDs 45321801 & 49051904
Guideline:	850.6100
Statements:	ECM: The study was conducted in compliance with FIFRA GLP standards, with the exception that nonvalidated computer systems were used and spreadsheet calculations were confirmed using a hand calculator (p. 3). Signed and dated No Data Confidentiality, GLP, Quality Assurance and Certification of Authenticity statements were provided (pp. 2-5). ILV: The study was conducted in accordance with the USEPA FIFRA Good Laboratory Practice (GLP) standards (40 CFR Part 160; p. 5). Signed and dated No Data Confidentiality, GLP, Quality Assurance and Certification of Authenticity statements were provided (pp. 2-5).
Classification:	This analytical method is classified as **acceptable**. However, mass spectra chromatograms were not included in the ILV report. Method reproducibility was hindered by the volatility of the analytes and distortion of the dichlobenil GC peak by the keeper solvent. The determination of the LOQ and LOD were not based on scientifically acceptable procedures.
PC Code:	027401
Reviewer:	Gregory Orrick USEPA
	Signature: Greg J Orrick **Date:** Jun. 12, 2014

All page citations refer to MRID 45321801 (ECM) unless otherwise noted.

Executive Summary

This analytical method, No. AC-7005, is designed for the quantitative determination of dichlobenil and 2,6-dichlorobenzamide (BAM) in water using GC/MS/SIM (selective ion monitoring mode). The method is quantitative for dichlobenil and its transformation product at the stated LOQ of 0.10 µg/L, which is less than the lowest toxicological level of concern in water (*i.e.*, the vascular aquatic plant NOAEC of 6 µg/L). No major issues were discovered by the independent laboratory.

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Table 1. Analytical Method Summary

Analyte(s) by Pesticide	MRID		EPA Review	Matrix	Method Date	Registrant	Analysis	Limit of Quantitation (LOQ)
	Environmental Chemistry Method	Independent Laboratory Validation						
Dichlobenil & 2,6-dichlorobenzamide	45321801	49051904	PDF	Water	5/10/00	Uniroyal Chemical Company, Inc.	GC/MS/SIM	0.10 µg/L

I. Principle of the Method

A Bakerbond divinylbenzene (DVB) Speedisk™ was cleaned using acetone, *n*-propanol and methanol (15 mL each) and then conditioned with 10 mL methanol and 25 mL Milli-Q™ water without drying (pp. 11, 15-17; Figure 1, p. 26). Samples (1 L) were extracted using the pre-conditioned Speedisk™ at *ca*. 80 mL/min under vacuum. The analytes were eluted with methylene chloride (3 x 5 mL) by gravity followed by slight vacuum. After 1-heptanol (160 µL) was added as a keeper, the sample was concentrated to *ca*. 1400 µL under very gentle nitrogen stream in a room temperature water bath. The internal standard 2,4,6-trichlorobenzonitrile (100 L) was added prior to GC/MS analysis using selected ion monitoring (SIM). The GC column was an RTX-200, 30 m x 0.25 mm x 0.5 µm (p. 13). Quantification of analytes was performed by applying the linear regression equation of the calibration curve.

The LOQ was the same in the ECM and ILV (0.10 µg/L; p. 22; p. 11 of MRID 49051904). In the ECM, the LOD was reported as 0.033 µg/L (one third of the LOQ). The LOD was not reported in the ILV.

II. Recovery Findings

ECM (MRID 45321801): Mean recoveries and relative standard deviations (RSD) were within guideline requirements (mean 70-120%; RSD ≤20%) for analysis of dichlobenil and 2,6-dichlorobenzamide (BAM) in pond water (pp. 20-21; Tables I-II, pp. 24-25). Confirmation of analyte and internal standard identities was performed by monitoring the retention times and the ratio of the $^{35}Cl/^{37}Cl$ isotope ions (pp. 16, 22).

ILV (MRID 49051904): Mean recoveries and RSDs were within guideline requirements for analysis of dichlobenil and BAM in pond water (p. 18; Table 2, p. 19). The method was validated with the "first" trial (pp. 7, 18; see Comment #5).

Table 2. Initial Validation Method Recoveries for Analytes in Pond Water

Analyte	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Pond Water (Middlebury, Connecticut)						
Dichlobenil	0.100 (LOQ)	10[1]	79.3-112.0	90.2	8.97	9.95
	1.00	10[2]	88.0-98.5	94.4	2.83	3.00
BAM*	0.100 (LOQ)	10[3]	87.8-111.6	100.7	8.57	8.50
	1.00	10[4]	83.2-101.1	93.0	5.52	5.94

Data were obtained from pp. 20-21; Tables I-II, pp. 24-25 in the study report.
* 2,6-Dichlorobenzamide.
1 Mean recoveries from the two subsets of five replicates were 94.3 ± 10.92% (RSD, 11.58%; range, 81.9-112.0%) and 86.0 ± 4.27% (RSD, 4.97%; range, 79.3-90.0%).
2 Mean recoveries from the two subsets of five replicates were 93.2 ± 3.12% (RSD, 3.35%; range, 88.0-95.5%) and 95.6 ± 2.15% (RSD, 2.25%; range, 92.9-98.5%).
3 Mean recoveries from the two subsets of five replicates were 95.4 ± 7.71% (RSD, 8.08%; range, 87.8-106.4%) and 106.1 ± 5.80% (RSD, 5.46%; range, 97.1-111.6%).
4 Mean recoveries from the two subsets of five replicates were 90.5 ± 6.07% (RSD, 6.71%; range, 83.2-98.2%) and 95.4 ± 4.03% (RSD, 4.23%; range, 90.8-101.1%).

Table 3. Independent Validation Method Recoveries for Analytes in Pond Water

Analyte	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Pond Water (Arcata, California)						
Dichlobenil	0.10 (LOQ)	5	78.0-90.9	83.3	5.05	6.07
	1.0	5	83.7-88.8	86.5	2.17	2.51
BAM*	0.10 (LOQ)	5	74.0-86.1	82.0	4.90	5.98
	1.0	5	77.9-88.0	84.2	4.11	4.88

Data were obtained from p. 18; Table 2, p. 19 of MRID 49051904.
* 2,6-Dichlorobenzamide.

III. Method Characteristics

The LOQ was the same in the ECM and ILV (0.10 µg/L). In the ECM, the LOQ was defined as the lowest fortification level which obtained average recoveries of 70-110% and a RSD <20% (p. 22). The LOD was set at one-third of the LOQ (0.033 µg/L). In the ILV, the LOQ was reported from the ECM, and no justification was provided (p. 11 of MRID 49051904). The LOD was not defined.

Table 4. Method Characteristics

	Dichlobenil	BAM
Limit of Quantitation (LOQ)	0.10 µg/L	0.10 µg/L
Limit of Detection (LOD)	0.033 µg/L	0.033 µg/L
Linearity (calibration curve r^2 and concentration range)	$r^2 = 0.9983$[1] (0.050-0.150 µg/mL) $r^2 = 0.9975$[1] (0.50-1.50 µg/mL)	$r^2 = 0.9974$[1] (0.050-0.150 µg/mL) $r^2 = 0.9985$[1] (0.50-1.50 µg/mL)
Repeatable	Yes	Yes
Reproducible	Yes	Yes
Specific	Yes	Yes

Data were obtained from pp. 20, 22; Figures 5-6, pp. 30-31; Figures 8-9, pp. 33-34.
1 ILV calibration curves yielded similar linearity, $r^2 = 1.000$, for dichlobenil and BAM in the concentration ranges of 0.050-0.150 µg/mL and 0.50-1.50 µg/mL (see p. 18 and Appendix B, Figures B17-B18, pp. 42-43; Appendix B, Figures B32-B33, pp. 57-58 of MRID 49051904).

IV. Method Deficiencies and Reviewer's Comments

1. The determination of the LOD and LOQ were not based on scientifically acceptable procedures. The LOQ was defined as the lowest fortification level with average recoveries of 70-110% and a RSD ≤20% and the LOD was not determined experimentally (the LOD was reported as one-third of the LOQ; p. 22). Detection limits should not be based on the arbitrarily selected lowest concentration in the spiked samples.

2. Mass spectra chromatograms accompanying GC chromatograms were not included in the ILV report. The ILV reported a shift of the retention time of dichlobenil between the reference standards (10.95 min.) and the samples (11.19 min.), indicating that the cyan group may have been involved (Appendix A, p. 24 of MRID 49051904). The retention time of the internal standard, which also contains a cyan group, shifted as well (12.20 min. to 12.31 min.). No further investigation was performed to explain the shift, and chromatograms were not included to verify the identity of the analytes, especially dichlobenil and the internal standard. No retention time shift or retention time labels were reported in the ECM although the reviewer believed that a retention time shift may have occurred based on the chromatograms; however, this was not an issue since the MS chromatograms were included (Figure 10, p. 35; Figures 14-15, pp. 39-40**)**.

3. In the ECM, the study author noted two general potential difficulties with the analytical method: 1) dichlobenil is volatile and volatilizes easily from the sample extracts during concentration; and 2) BAM quantification is subject to fluctuations from instrumental drift (p. 18). To minimize volatility of dichlobenil, the method uses 1-heptanol as a keeper and the study author states that care should be taken to avoid conditions that favour volatilization (elevated temperatures, sample agitation from nitrogen stream, concentration to very low volumes). The keeper should be added conservatively since large amounts can overload the GC column. Regarding the problem of BAM quantification, the study author prescribed the analysis of calibration solutions at the beginning and end of each sample set. Also, the fortifications of BAM at low concentrations were most likely to be affected by minor instrumental fluctuations.

4. In the ILV, the study author reported that the methylene chloride would not elute by gravity and a substantial vacuum was required (Appendix A, p. 24 of MRID 49051904). When the vacuum was employed, a small amount of water was collected concurrently. Therefore, the water was removed via Pasteur pipette after the sample was centrifuged (1000 rpm for 3 minutes). In the ECM, the method states that the methylene chloride is to elute by gravity, and a slight vacuum may be applied to elute any remaining analyte (p. 16). The use of substantial vacuum and centrifugation in the ILV was a minor modification of the ECM. The main problem the modification could cause is the volatilization of the analytes and subsequent lower recovery. Since the ILV recoveries met guideline requirements, the minor modification did not significantly affect results.

5. In the ILV, the report stated that "the method was validated in the first trial" (p. 7 of MRID 49051904); however, an initial validation was reportedly performed without success (pp. 7, 18; Appendix F, pp. 69-72; Appendix G, pp. 73-91 of MRID 49051904). The results of this initial trial were not included in the study report because 1-octanol was used as the keeper rather than 1-heptanol which is specified in the ECM. 1-Octanol was not an appropriate keeper because it "caused significant fronting on the analyte peaks, which made quantification somewhat arbitrary" (p. 7 of MRID 49051904). Also, the samples were fortified at 1xLOQ and 5xLOQ instead of 1xLOQ and 10xLOQ.

6. The ILV reported two minor modifications to the GC/MS portion of the method: the GC column was a HP-5MS (30 m, 0.25 mm i.d., 0.25 µm film) and the GC/MS operating conditions were altered (p. 11; Appendix A, p. 23 of MRID 49051904).

7. Matrix characterization of the pond water was reported in the ECM (Appendix B, pp. 45-51) and in the ILV (p. 11; Appendix E, pp. 63-68 of MRID 49051904).

8. The linear regression equations of the reviewer-generated calibration curves did not exactly match those reported in the ECM and ILV; however, the equations and r^2 values were similar, in general.

9. It was reported for the ILV that a single analyst completed a sample set consisting of 13 samples in 8 hours or 1 calendar day (p. 24 of MRID 49051904).

V. References

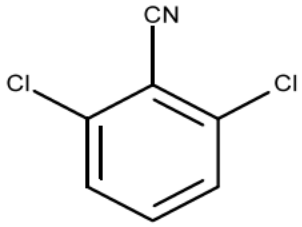
U.S. Environmental Protection Agency. 2012. Ecological Effects Test Guidelines, OCSPP 850.6100, Environmental Chemistry Methods and Associated Independent Laboratory Validation. Office of Chemical Safety and Pollution Prevention, Washington, DC. EPA 712-C-001.

40 CFR Part 136. Appendix B. Definition and Procedure for the Determination of the Method Detection Limit-Revision 1.11, pp. 317-319.

Attachment 1: Chemical Names and Structures

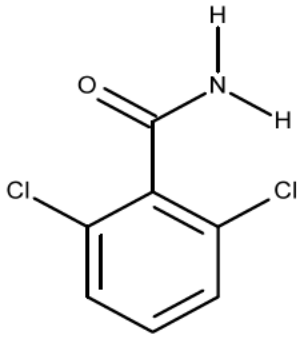
Dichlobenil; Casoron

IUPAC Name: 2,6-Dichlorobenzonitrile.
CAS Name: 2,6-Dichlorobenzonitrile
CAS Number: 1194-65-6.
SMILES String: c1cc(c(c(c1)Cl)C#N)Cl



2,6-Dichlorobenzamide; BAM; C-03

IUPAC Name: 2,6-Dichlorobenzamide.
CAS Name: --
CAS Number: 2008-58-4.
SMILES String:



Attachment 2: Excel Calculations

027401_45321801+_
DER-Fate_850.6100_

Test Material: Dichlobenil

MRID: 45321801

Title: Analytical method for determining dichlobenil and its metabolite 2,6-dichlorobenzamide in water.

MRID: 49051904

Title: Independent laboratory validation of Uniroyal Chemical Company analytical method "Analytical method for determining dichlobenil and its metabolite 2,6-dichlorobenzamide in water" (Analytical Method No. AC-7005, Uniroyal study number 99055).

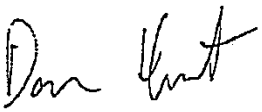
EPA PC Code: 027401

OCSPP Guideline: 850.6100

For CDM Smith

Primary Reviewer: Lisa Muto **Signature:** 

Date: 3/10/14

Secondary Reviewer: Dan Hunt **Signature:** 

Date: 3/10/14

QC/QA Manager: Joan Gaidos **Signature:** 

Date: 3/10/14