

Analytical method for dodine in soil

- Reports:** ECM: EPA MRID No. 44985702. Yang, J. 1998. Dodine: Validation of Method of Analysis for Dodine in Soil using GC-MSD. Report prepared, sponsored, and submitted by Rhône-Poulenc Ag Company, Research Triangle Park, North Carolina; 138 pages. Rhône-Poulenc Study No.: EC-97-384. Final report dated March 18, 1998.
- ILV: EPA MRID No. 44985703. Petit, J.B. 1998. Independent Laboratory Validation of Analytical Method: "Dodine: Method of Analysis for Dodine in Soil using GC-MSD". Report prepared by Centre Analytical Laboratories, Inc. ("CAL"), State College, Pennsylvania, and sponsored and submitted by Rhône-Poulenc Ag Company, Research Triangle Park, North Carolina; 71 pages. Centre Analytical Study No.: 019-005; Rhône-Poulenc Study No.: EC-98-423. Final report dated July 16, 1998.
- Document No.:** MRIDs 44985702 & 44985703
- Guideline:** 850.6100
- Statements:** ECM: The study was conducted in accordance with USEPA FIFRA (40 CFR Part 160), except that 1) not all data were recorded and/or corrected according to GLPs, 2) routine maintenance of GC-MSD was not performed and documented as per the SOP, and 3) some training files were incomplete (p. 3 of MRID 44985702). Signed and dated No Data Confidentiality, GLP, Quality Assurance, and Authenticity statements were provided (pp. 2-5).
ILV: The study was conducted in accordance with USEPA FIFRA (40 CFR Part 160; p. 3 of MRID 44985703). Signed and dated No Data Confidentiality, GLP, Quality Assurance, and Authenticity statements were provided (pp. 2-5).
- Classification:** This analytical method is classified as unacceptable. Since the reported method LOQ for the ECM was not based on scientifically acceptable procedures defined in 40 CFR Part 136, the reported LOQ is the lowest level of method validation (LLMV) rather than LOQ. The ILV was not conducted independently of the ECM since technical issues were discussed between the ECM and ILV personnel. An insufficient number of samples (n = 2) were prepared for the ECM soil matrices at the LOQ. The reproducibility of the method could not be determined at 10×LOQ. An updated ECM report should include a significant ILV modification. The number of ILV trials required to validate the method was not reported. The ILV soil matrix was not characterized or described. ECM soil matrices were not characterized or described; however, the ECM soil matrices originated from a submitted dodine field dissipation study. The ECM calibration curve range was inappropriate for the analytical method. The specificity of the method was not supported by ECM chromatographic data for the California soil.

PC Code: 044301**EFED Final
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This Data Evaluation Record has been altered by the Environmental Fate and Effects Division subsequent to signing by CDM/CSS-Dynamac Joint Venture personnel. The CDM/CSS-Dynamac JV role does not include establishing Agency policies.

Executive Summary

This analytical method, Rhône-Poulenc Study No. EC-97-384, is designed for the quantitative determination of dodine in soil using GC-MSD at the LOQ of 10 ng/g (equivalent to 10 ug/kg) for soil. The lowest toxicity Level of Concern (LOC) for terrestrial plants is a NOAEC of 2.6 lb a.i./A (IC25 = 2.6 lb a.i./A) which is considered equivalent to a dodine concentration of 1300 ppb in soil using standard assumptions. Therefore, the LOQ (10 ppb) is much lower than the lowest toxicological LOC in soil matrices. Since the reported method LOQ was not based on scientifically acceptable procedures defined in 40 CFR Part 136, the reported LOQ is the lowest level of method validation (LLMV) rather than LOQ. The lowest concentration tested with sufficiently accurate and precise recoveries is the LLMV. Based on the performance data submitted by the ILV and ECM, the LLMV was equivalent to the ECM reported method LOQ for dodine in the tested soil matrices (10 ug/kg).

The ECM and ILV soil matrices were not characterized or described in the studies; however, the ECM reported that the California, New Jersey, Washington, and Georgia soil matrices originated from the submitted dodine field dissipation study Rhône-Poulenc Study No. US96X10342 (MRID 44985701). It could not be determined if the ILV was provided with the most difficult matrices with which to validate the method or if the ILV soil matrix covered the range of soils used in the terrestrial field dissipation studies. The ILV validated the method with the significant modification of increasing the amount of 1,1,1,5,5,5-hexafluoro-2,4-pentanedione for the success of the derivatization reaction, as well as some minor modifications of the analytical method. An updated ECM report should be submitted with the ILV modification of 100 µL of 1,1,1,5,5,5-hexafluoro-2,4-pentanedione for the derivatization reaction since the ILV laboratory could not validate the method without this modification. The number of ILV trials required to validate the method was not reported. The ILV was not conducted independently of the ECM since the ECM study author was the ILV Sponsor Study Monitor and technical issues were discussed in

communications between the ILV study personnel and the ILV Sponsor Study Monitor.

The reproducibility of the method was demonstrated for the LOQ (10 ug/kg); however, an insufficient number of samples (n = 2) were prepared for the ECM soil matrices. The reproducibility of the method could not be determined at 50, 100, 500, and 5000 ug/kg since only one set of performance data was submitted for each fortification. All ILV data regarding linearity and specificity were satisfactory for dodine in the soil matrix. ECM linearity was not acceptable since the ECM calibration curve range was 20-150 ug/kg which did not include the LOQ concentration of 10 ug/kg. ECM specificity was acceptable for all soil matrices, except the California soil, based on quantified residues in the untreated controls. The LOD was not reported in the ILV.

Table 1. Analytical Method Summary

Analyte(s) by Pesticide	MRID		EPA Review	Matrix	Method Date (dd/mm/yyyy)	Registrant	Analysis	Limit of Quantitation (LOQ)
	Environmental Chemistry Method	Independent Laboratory Validation						
Dodine	44985702 ¹	44985703 ²	Taime Harris	Soil	18/03/1998	Rhône- Poulenc Ag Company	GC- MSD	10 µg/kg

¹ In the ECM, the soil matrices collected from the dodine field dissipation study Rhône-Poulenc Study No.

US96X10342 (MRID 44985701; sites in California, New Jersey, Washington, and Georgia) were used in the study (pp. 11-12 of MRID 44985702). The soil descriptions and characterizations were not included in the ECM study report (see Reviewer's Comment #6).

² In the ILV, the soil matrix was an untreated California soil sample, from a depth of 0-6 inches, received from the Sponsor (assigned CAL ID 981898; p. 11 of MRID 44985703). The soil descriptions and characterizations were not included in the ILV study report (see Reviewer's Comment #6).

I. Principle of the Method

Soil samples (*ca.* 50 g) were fortified at 10, 50, 500, and 5000 ug/kg fortification solutions, as necessary, in a 250-mL nalgene bottle and allowed to sit for *ca.* 10 minutes (Tables 2-5, pp. 20-23; Appendix A, p. 31; Appendix A, pp. 41, 47-49 of MRID 44985702). The samples were extracted twice with *ca.* 70 mL of 0.05M KOH in methanol:water (90:10, v:v) via shaking on a horizontal shaker for *ca.* 15 minutes then centrifuging at *ca.* 2500 rpm for *ca.* 5 minutes. The solvents were removed with filtration using a 9-cm Buchner funnel (GF/A filter paper) with slow vacuum. The soil was extracted again using *ca.* 70 mL of 1% HCl in methanol via shaking for *ca.* 15 minutes then filtered using Buchner funnel. The Nalgene bottle and cap and Buchner funnel tip and adapter were rinsed with methanol, which was added to the soil extract (Extract A). The extract volume was adjusted to 250 mL with methanol. A 50-mL aliquot of Extract A was evaporated to *ca.* 10 mL at 40°C via rotary evaporation. Salt (7.90-7.94 g) was added to a 125-mL separatory funnel. The evaporated extract was added to the funnel using a 25-mL rinse with distilled water. The separatory funnel was shaken until all salt dissolved (saturated salt solution) then *ca.* 45 mL of dichloromethane was added. After shaking vigorously for *ca.* 1 minute, the dichloromethane was drained though *ca.* 10 g of sodium sulfate. The

dichloromethane extraction was repeated twice, then the sodium sulfate was rinsed with *ca.* 10 mL of dichloromethane. This combined dichloromethane extract (Extract B) was evaporated to dryness using a rotary evaporator at 30°C and then a gentle stream of nitrogen. The residue of Extract B was dissolved in 3 mL of 1-chlorobutane, then transferred to the reaction vial with an additional 3 mL of 1-chlorobutane. Methanol (100 µL) and 1,1,1,5,5,5-hexafluoro-2,4-pentanedione (30 µL) were added to the reaction vial, and the mixture was stirred on a heating block at 100°C for 1 hour. After cooling, the product was evaporated to dryness at 30-35°C with a gentle stream of nitrogen with a moisture trap (all moisture must be removed for good recoveries of derivatized product). Methanol was added to the desired volume (up to 9 mL), and the solution was mixed with sonication. The sample was filtered (Gelman nylon filter) prior to GC-MSD analysis.

Samples were analyzed using a Hewlett-Packard 5890A GC coupled with a Hewlett-Packard Model 5972A Mass Selective Detector (p. 13; Appendix A, Appendix A, pp. 49-50 of MRID 44985702). The following GC-MSD conditions were used: J & W Scientific DB-5 (30 m x 0.25 mm column; 0.25 µm film thickness), helium carrier gas (15 PSI with 1.5 minutes 45 PSI pulse at injection), injector temperature 250°C, detector temperature 325°C, oven temperature program (100°C and hold for 1 min., ramp 20°C/min. to 195°C and hold 0 min., ramp 5°C/min. to 275°C and hold for 3 min., ramp 30°C/min. to 300°C and hold for 5 min.), and injection volume of 1.0 µL. Derivatized dodine was identified using the following ions: *m/z* 244.00 (quantitation) and *m/z* 245.00 and *m/z* 399.20 (qualifier ions). Expected retention time was 8.5 minutes (Appendix A, Appendix A, p. 53).

The independent laboratory performed the ECM as written, with the significant modification of increasing the amount of 1,1,1,5,5,5-hexafluoro-2,4-pentanedione from 30 µL to 100 µL which was necessary for the success of the derivatization reaction (pp. 13-15, 18-19 of MRID 44985703). A Hewlett-Packard 5890 Series GC coupled with a Hewlett-Packard Model 5973 Mass Selective Detector was used. Several minor modifications of the GC-MSD parameters were noted. The following GC-MSD conditions were used: Alltech SE-54 (30 m x 0.25 mm column; 0.25 µm film thickness), helium carrier gas (45 PSI for 1.0 min. at injection), injector temperature 250°C, detector temperature 300°C, oven temperature program (120°C for 1 min., ramp 20°C/min. to 180°C and hold 0 min., ramp 5°C/min. to 220°C and hold 0 min., ramp 20°C/min. to 300°C and hold for 5 min.), and injection volume of 2 µL. Derivatized dodine was identified using the following ions: *m/z* 244.0 (quantitation) and *m/z* 245.0 and *m/z* 399.2 (qualifier ions). Expected retention time was *ca.* 9 minutes. An updated ECM report should be submitted with the ILV modification of 100 µL of 1,1,1,5,5,5-hexafluoro-2,4-pentanedione for the derivatization reaction since the ILV laboratory could not validate the method without this modification.

The Limit of Quantification (LOQ) for dodine in soil was reported as 10 ug/kg in the ECM and the ILV (p. 16; Table 1, p. 19 of MRID 44985702; pp. 10-11 of MRID 44985703). The Limit of Detection (LOD) for dodine in soil was reported in the ECM as the Method Detection Limit (MDL), which was reported as 3 ug/kg. The LOD was not reported in the ILV. Since the LOQ was not based on scientifically acceptable procedures defined in 40 CFR Part 136, the reported LOQ is the lowest level of method validation (LLMV) rather than LOQ.

II. Recovery Findings

ECM (MRID 44985702): Mean recoveries and relative standard deviations (RSDs) met requirements (mean 70-120%; RSD \leq 20%) for analysis of dodine in one soil matrix (California) at the 5 \times LOQ (50 ug/kg) and 50 \times LOQ (500 ug/kg); no samples of the CA soil were fortified at the LOQ (10 ug/kg; Table 2, p. 20). Recoveries were acceptable (77.9-105.4%) for analysis of dodine in two soil matrices (Washington and Georgia) at the LOQ (10 ug/kg); however, only two samples were prepared at each fortification, precluding the calculation of the means and RSDs (Tables 3-5, pp. 21-23). Recoveries were also acceptable (71.9-88.9%) for analysis of dodine in one soil matrix (Washington) at 50 \times LOQ (500 ug/kg); however, only two samples were prepared at each fortification, precluding the calculation of the means and RSDs. Recoveries were not acceptable (<70%) for one or both replicates of the New Jersey soil at the LOQ (10 ug/kg), 50 \times LOQ (500 ug/kg), and 500 \times LOQ (5000 ug/kg); and Washington and Georgia soils at 500 \times LOQ (5000 ug/kg). No samples were prepared at 10 \times LOQ (100 ug/kg). One ion was quantified; a confirmatory method is not usually required when GC-MSD or LC/MS is used as the primary method to generate study data. Recovery results, except for those of the Georgia soil, were corrected for residues quantified in the untreated controls (1.79-4.55 ug/kg; pp. 14-15; Tables 2-5, pp. 20-23). The soil matrices collected from the dodine field dissipation study Rhône-Poulenc Study No. US96X10342 (MRID 44985701; sites in California, New Jersey, Washington, and Georgia) were used in the ECM study (pp. 11-12). The soil descriptions and characterizations were not included in the ECM study report (see Reviewer's Comment #6).

ILV (MRID 44985703): Mean recoveries and relative standard deviations (RSDs) met requirements (mean 70-120%; RSD \leq 20%) for analysis of dodine in one soil matrix (California) at the LOQ (10 ug/kg) and 10 \times LOQ (100 ug/kg; Table 1, p. 21). One ion was quantified; a confirmatory method is not usually required when GC-MSD or LC/MS is used as the primary method to generate study data. The soil matrix was an untreated California soil sample, from a depth of 0-6", received from the Sponsor (assigned CAL ID 981898; p. 11). The soil descriptions and characterizations were not included in the ILV study report (see Reviewer's Comment #6). The method was validated by the ILV in one soil matrix with the significant modification of increasing the amount of 1,1,1,5,5,5-hexafluoro-2,4-pentanedione from 30 μ L to 100 μ L which was necessary for the success of the derivatization reaction, as well as some minor modifications of the analytical method (pp. 18-19). An updated ECM report should include the ILV modification of 100 μ L of 1,1,1,5,5,5-hexafluoro-2,4-pentanedione for the derivatization reaction since the ILV laboratory could not validate the method without this modification. The number of ILV trials required to validate the method was not reported.

Table 2. Initial Validation Method Recoveries for Dodine in Soil^{1,2}

Analyte	Fortification Level (ug/kg)	Number of Tests	Recovery Range (%)	Mean Recovery ⁴ (%)	Standard Deviation ⁴ (%)	Relative Standard Deviation ⁴ (%)
California Soil						
Dodine	50	5 ³	79.5-114.7	96.8	13.3	13.7
	500	5 ³	65.3 -94.2	77.7	12.6	16.2
New Jersey Soil						
Dodine	10 (LOQ)	2	61.7 , 76.8 ⁵	--	--	--
	500	2	66.7 , 68.0 ⁵	--	--	--
	5000	2	62.7 , 65.6 ⁵	--	--	--
Washington Soil						
Dodine	10 (LOQ)	2	77.9, 85.1 ⁵	--	--	--
	500	2	71.9, 88.9 ⁵	--	--	--
	5000	2	58.4 , 72.0 ⁵	--	--	--
Georgia Soil						
Dodine	10 (LOQ)	2	83.2, 105.4 ⁵	--	--	--
	500	2	65.5 , 72.2 ⁵	--	--	--
	5000	2	58.3 , 62.9 ⁵	--	--	--

Data (recovery results, except for those of the Georgia soil, were corrected for residues quantified in the untreated controls; pp. 14-15; Tables 2-5, pp. 20-23) were obtained from Tables 2-5, pp. 20-23 of MRID 44985702 and DER Excel Attachment. Since the LOQ was not based on scientifically acceptable procedures defined in 40 CFR Part 136, the reported LOQ is the lowest level of method validation (LLMV) rather than an LOQ. The reported LOQ of the ECM differed from that of the ILV.

1 Dodine, as derivatized dodine, was identified using one ion: *m/z* 244.00.

2 The soil matrices collected from the dodine field dissipation study Rhône-Poulenc Study No. US96X10342 (MRID 44985701; sites in California, New Jersey, Washington, and Georgia) were used in the study (pp. 11-12). The soil descriptions and characterizations were not included in the ECM study report (see Reviewer's Comment #6).

3 Means, standard deviations, and relative standard deviations (RSDs) were not reported in the study but were reviewer-calculated based on recovery results (DER Excel Attachment). Rules of significant figures were followed.

4 Means, standard deviations, and RSDs could not be calculated since *n* = 2.

5 Ranges are not applicable since *n* = 2.

Table 3. Independent Validation Method Recoveries for Dodine in Soil^{1,2}

Analyte	Fortification Level (ug/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%) ³	Relative Standard Deviation (%)
Dodine	10 (LOQ*)	5	80-105	93	9.5	10.2
	100	5	77-90	85	5.8	6.8

*Data (uncorrected recovery results; pp. 16-18; Table 1, p. 21) were obtained from Table 1, p. 21 of MRID 44985703. Since the LOQ was not based on scientifically acceptable procedures defined in 40 CFR Part 136, the reported LOQ is the lowest level of method validation (LLMV) rather than an LOQ.

1 Dodine, as derivatized dodine, was identified using one ion: *m/z* 244.0. The quantitation ion was the same as that of the ECM.

2 The soil matrix was an untreated California soil sample, from a depth of 0-6 inches, received from the Sponsor (assigned CAL ID 981898; p. 11). The soil descriptions and characterizations were not reported in the study (see Reviewer's Comment #6).

III. Method Characteristics

The LOQ for dodine in soil was reported as 10 ug/kg in the ECM and 10 ug/kg in the ILV (p. 16; Table 1, p. 19 of MRID 44985702; pp. 10-11 of MRID 44985703). The LOD for dodine in soil was reported in the ECM as the Method Detection Limit (MDL), which was reported as 3 ug/kg. The LOD was not reported in the ILV. In the ECM, the LOD and LOQ were calculated from the standard deviation of seven samples fortified “at or near” the LOQ of the method and the average dodine concentration in five untreated control samples using the following equations:

$$\text{LOD (MDL)} = (\text{average UTC}) + (3 \times \text{standard deviation of fortified samples})$$

$$\text{LOQ} = (\text{average UTC}) + (10 \times \text{standard deviation of fortified samples}).$$

In the ECM, the LOQ was defined as the level of analyte in a substrate above which quantitative results may be obtained with a specified degree of confidence. No justification of the LOQ was reported in the ILV. No calculations to support the LOQ or LOD were reported in the ILV.

Since the LOQ was not based on scientifically acceptable procedures defined in 40 CFR Part 136, the reported LOQ is the lowest level of method validation (LLMV) rather than a valid LOQ.

Table 4. Method Characteristics – Soil

		Dodine
Limit of Quantitation (LOQ)*	ECM	10 ug/kg
	ILV	
Limit of Detection (LOD)	ECM	3 ug/kg
	ILV	Not reported
Linearity (calibration curve r and concentration range) ¹	ECM	r = 0.9970 ²
		20-150 ug/kg
	ILV	r = 0.9965
		5-100 ug/kg
Repeatable		All soil matrices were not characterized in study reports.
	ECM ³	Yes for 50 ug/kg and 500 ug/kg in one soil matrix. Yes for 10 ug/kg (LLMV) in two soil matrices, but n = 2. ⁵ Yes for 500 ug/kg in one soil matrix, but n = 2. ⁵ No samples prepared at 100 ug/kg.
	ILV ^{4,5}	Yes for 10 ug/kg (LLMV) and 100 ug/kg in one soil matrix.
Reproducible		Yes for 10 ug/kg (LLMV), but n = 2 in ECM . Only one set of performance data submitted for 50, 100, 500, and 5000 ug/kg.
Specific	ECM	Yes for three of four soils, matrix interferences were <28% of the LOQ (based on quantified residues). No for California soil, matrix interferences were 26-46% of the LOQ (based on quantified residues).
	ILV	Yes, no matrix interferences were observed.

Data were obtained from p. 16; Table 1, p. 19 (LOQ/LOD); Tables 2-5, pp. 20-23 (recovery data); Appendix C, pp. 68-100 (calibration curves & chromatograms) of MRID 44985702; pp. 10-11 (LOQ/LOD); Table 1, p. 21 (recovery data); Figures 1-5, pp. 24-28 (calibration curves); Figures 6-9, pp. 29-32 (chromatograms) of MRID 44985703; DER Excel Attachment.

* Since the LOQ was not based on scientifically acceptable procedures defined in 40 CFR Part 136, the reported LOQ is the lowest level of method validation (LLMV) rather than LOQ. The lowest concentration tested with sufficiently accurate and precise recoveries is the LLMV.

- 1 The reported r values were reviewer-calculated from reported r² values (Appendix C, pp. 68-100 of MRID 44985702; Figure 5, p. 28 of MRID 44985703).
- 2 Value in ECM study report was only identified as “Corr Coef” (Appendix C, pp. 68-100 of MRID 44985702). The reviewer assumed that the value, 0.994, was the r² value and calculated and reported the r value (see DER Excel Attachment). If “Corr Coef” was the r value, then the linearity is not acceptable since acceptable linearity is r ≥ 0.995.
- 3 In the ECM, the soil matrices collected from the dodine field dissipation study Rhône-Poulenc Study No. US96X10342 (MRID 44985701; sites in California, New Jersey, Washington, and Georgia) were used in the study (pp. 11-12 of MRID 44985702). The soil descriptions and characterizations were not reported in the study (see Reviewer’s Comment #6).
- 4 In the ILV, the soil matrix was an untreated California soil sample, from a depth of 0-6 inches, received from the Sponsor (assigned CAL ID 981898; p. 11 of MRID 44985703). The soil descriptions and characterizations were not reported in the study (see Reviewer’s Comment #6).
- 5 The ILV validated the method in one soil matrix with the significant modification of increasing the amount of 1,1,1,5,5,5-hexafluoro-2,4-pentanedione from 30 µL to 100 µL which was necessary for the success of the derivatization reaction, as well as some minor modifications of the analytical method (pp. 18-19 of MRID 44985703). An updated ECM report should be submitted with the ILV modification of 100 µL of 1,1,1,5,5,5-hexafluoro-2,4-pentanedione for the derivatization reaction since the ILV laboratory could not validate the method without this modification. The number of ILV trials required to validate the method was not reported.

IV. Method Deficiencies and Reviewer's Comments

1. Since the reported method LOQ was not based on scientifically acceptable procedures defined in 40 CFR Part 136, the reported LOQ is the lowest level of method validation (LLMV) rather than a valid LOQ (p. 16; Table 1, p. 19 of MRID 44985702; pp. 10-11 of MRID 44985703). The LLMV is the lowest concentration tested with sufficiently accurate and precise recoveries. Based on the performance data submitted in the ILV and ECM, the LLMV was equivalent to the ECM-reported method LOQ for dodine in the tested soil matrices (10 ug/kg).
2. The ILV was not conducted independently of the ECM since the ECM study author (Ju Yang) was the ILV Sponsor Study Monitor and technical issues were discussed in communications between the ILV study personnel and the ILV Sponsor Study Monitor (pp. 1, 3, 5, 9 of MRID 44985702; pp. 1, 3, 5-6, 11, 19 of MRID 44985703). The communications included clarifications of the importance of moisture removal prior to derivatization, the fact that standard solutions should not be combined, and the numbering of samples and study number. ILV communications were summarized by date of communication in the study report and occurred prior to experiment start date. OCSPP guidelines state that the performing laboratories and personnel must differ between the ECM and ILV in order to ensure the independence of the validation of the external laboratory.
3. An insufficient number of samples ($n = 2$) were prepared/analyzed for the ECM soil matrices at the LOQ (10 ug/kg), and no samples were prepared/analyzed at $10 \times \text{LOQ}$ (100 ug/kg) in the ECM. OCSPP guidelines state that a minimum of five spiked replicates should be analyzed at each concentration (*i.e.*, minimally, the LOQ and $10 \times \text{LOQ}$) for each analyte. Means and relative standard deviations (RSDs) could not be calculated since $n = 2$.
4. The reproducibility of the ECM method could not be determined at 50 ug/kg, 100 ug/kg, 500 ug/kg, and 5000 ug/kg since only one set of performance data was submitted for each fortification. OCSPP guidelines state that two sets of performance data should be submitted, one for the initial or other internal validation and one for the ILV.
5. The ILV validated the method in one soil matrix with the significant modification of increasing the amount of 1,1,1,5,5,5-hexafluoro-2,4-pentanedione from 30 μL to 100 μL , which was necessary for the success of the derivatization reaction, as well as some minor modifications of the analytical method (pp. 18-19 of MRID 44985703). An updated ECM report should be submitted with the ILV modification of 100 μL of 1,1,1,5,5,5-hexafluoro-2,4-pentanedione for the derivatization reaction since the ILV laboratory could not validate the method without this modification.
6. It could not be determined if the ILV was provided with the most difficult matrices with which to validate the method or if the ILV soil matrix covered the range of soils used in the terrestrial field dissipation studies. The ILV soil matrix was not characterized or described in the study; however, it was reported as an untreated California soil sample,

from a depth of 0-6", received from the Sponsor (assigned CAL ID 981898; p. 11 of MRID 44985703). It was not specified whether the soil sample was from the same source as the soil used in the ECM. OCSPP 850.6100 guidance does not address the use of the same matrices in the ECM and ILV validations.

ECM soil matrices were not characterized or described in the study. However, the ECM reported that the California, New Jersey, Washington, and Georgia soil matrices originated from the dodine field dissipation study Rhône-Poulenc Study No. US96X10342 (MRID 44985701; pp. 11-12 of MRID 44985702). Based on MRID 44985701, the soil textures are as follows: California [loamy sand 0-24 inches & 30-36 inches; 85-87% sand, 6-10% silt, 5-7% clay; 0.1-0.5% organic matter; sand 24-30 inches; 89% sand, 4% silt, 7% clay; 0.1% organic matter], New Jersey [loam 0-30 inches; 29-47% sand, 31-47% silt, 20-26% clay; 0.2-2.3% organic matter; sandy clay loam 30-36 inches; 55% sand, 23% silt, 22% clay, 0.2% organic matter], Washington [sand 0-36 inches; 89-93% sand, 4-8% silt, 3% clay; 0.2-1.2% organic matter], and Georgia [sand 0-6 inches; 87% sand, 10% silt, 3% clay; 1.0-1.5% organic matter; loamy sand/sandy loam/sandy clay loam 6-36 inches; 65-85% sand, 7-12% silt, 5-26% clay; 0.1-0.9% organic matter; Appendix B, pp. 141-144 of MRID 44985701]. In the ILV, the soil matrix was an untreated California soil sample, from a depth of 0-6 inches, received from the Sponsor (assigned CAL ID 981898; p. 11 of MRID 44985703).

OCSPP 850.6100 guidance suggests for a given sample matrix, the registrant should select the most difficult analytical sample condition from the study (*e.g.*, high organic content versus low organic content in a soil matrix) to analyze in order to demonstrate how well the method performs.

7. ECM linearity was not acceptable since the ECM calibration curve range was inappropriate for the analytical method. The calibration range was 20-150 ug/kg which did not include the LOQ concentration of 10 ug/kg (Appendix C, pp. 68-10 of MRID 44985702). The calibration range should span at least two data points above the highest concentration tested and at least two data points below the LOQ in order to have accurate quantification.

The reviewer noted that the ECM linearity coefficient was only identified as "Corr Coef" in ECM study report (Appendix C, pp. 68-100 of MRID 44985702). The reviewer assumed that the value, 0.994, was the r^2 value and calculated and reported the r value. If "Corr Coef" was the r value, then the linearity is not acceptable since acceptable linearity is $r \geq 0.995$.

8. The specificity of the method was not supported by ECM chromatographic data for the California soil since matrix interferences were 26-46% of the LOQ (based on quantified residues) in the untreated controls (Tables 2-5, pp. 20-23 of MRID 44985702). ECM representative chromatograms were truncated and only showed the analyte peak (Appendix C, pp. 68-100).

ECM recovery results, except for those of the Georgia soil, were corrected for residues quantified in the untreated controls (1.79-4.55 ug/kg; pp. 14-15; Tables 2-5, pp. 20-23 of MRID 44985702).

9. The number of ILV trials required to validate the method was not reported.
10. The dodine field dissipation study Rhône-Poulenc Study No. US96X10342 (MRID 44985701) was provided to CDM/CSS-Dynamac Joint Venture personnel by EFED. CDM/CSS-Dynamac Joint Venture personnel used soil characterization data from MRID 44985701 to comment on the soil matrices used by the ECM. No performance data was taken from MRID 44985701; however, the reviewer noted that data from the verification recoveries portion of the analytical report for MRID 44985701 also had an insufficient number ($n = 2$) of samples fortified at the LOQ, $5 \times \text{LOQ}$, and $500 \times \text{LOQ}$ (p. 23; Appendix C, Table XII, pp. 385-394 of MRID 44985701).
11. No reagent blank was included in the ECM (Appendix A, p. 31 of MRID 44985702).
12. In the ECM, the study protocol contained example chromatograms for California, New Jersey, Washington, and Georgia soil which were not truncated (Appendix A, pp. 53-57 of MRID 44985702). Minor baseline noise was observed at the LOQ (10 ug/kg) fortification for all soils; however, the Washington soil showed multiple significant contaminants (30-50% of the LOQ peak height) near the analyte peak.
13. The determinations of the LOD and LOQ in the ECM and ILV were not based on scientifically acceptable procedures as defined in 40 CFR Part 136 (p. 16; Table 1, p. 19 of MRID 44985702; pp. 10-11 of MRID 44985703). The LOD for dodine in soil was reported in the ECM as the Method Detection Limit (MDL), which was reported as 3 ug/kg. The LOD was not reported in the ILV. In the ECM, the LOD and LOQ were calculated from the standard deviation of seven samples fortified "at or near" the LOQ of the method and the average dodine concentration in five untreated control samples using the following equations: 1) $\text{LOD (MDL)} = (\text{average UTC}) + (3 \times \text{standard deviation of fortified samples})$; and 2) $\text{LOQ} = (\text{average UTC}) + (10 \times \text{standard deviation of fortified samples})$. These equations are similar to scientifically acceptable procedures as defined in 40 CFR Part 136, but the addition of the average UTC is not acceptable. In the ECM, the LOQ was defined as the level of analyte in a substrate above which quantitative results may be obtained with a specified degree of confidence. No justification of the LOQ was reported in the ILV. No calculations to support the LOQ or LOD were reported in the ILV.
14. No matrix effects were observed in the ILV (p. 18 of MRID 44985703).

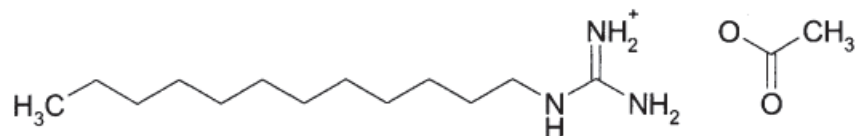
15. The total time required to complete one sample set of 13 samples was reported in the ILV study report as *ca.* 8 hours of work (p. 19 of MRID 44985703). GC/MS analysis required *ca.* 10 hours, with each injection requiring *ca.* 21 minutes. The total time required to complete one set of 13 samples was reported as 2 calendar days. The ECM study report stated that an analytical set can be completed within an 8-hour workday (p. 18 of MRID 44985702).

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.

USEPA. 2012. *Environmental Chemistry Method Guidance*. Memorandum From D. Brady to Environmental Fate and Effects Division. December 20, 2012. Environmental Fate and Effects Division. Office of Pesticide Programs. Office of Chemical Safety and Pollution Prevention. U.S. Environmental Protection Agency. Available at: <https://www.epa.gov/pesticide-science-and-assessing-pesticide-risks/environmental-chemistry-methods-guidance-pesticides>.

40 CFR Part 136. Appendix B. Definition and Procedure for the Determination of the Method Detection Limit-Revision 1.11, pp. 344-347, and Revision 2; 2015 and 2016.

Attachment 1: Chemical Names and Structures**Dodine****IUPAC Name:** 1-Dodecylguanidinium acetate**CAS Name:** Not reported**CAS Number:** 2439-10-3 (acetate)
112-65-2 (free base)**SMILES String:** Not found**Derivatized Dodine****IUPAC Name:** 2-Dodecylamino-4,6-bis(trifluoromethyl)pyrimidine**CAS Name:** Not reported**CAS Number:** Not applicable**SMILES String:** Not found