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Certification of Standard Reference Material® 2261a:

Chlorinated Pesticides in Hexane
(Nominal Mass Concentration 2 µg/mL)

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Amy Cuthbertson
James H. Yen

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October 2026



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Abstract

Standard Reference Material (SRM) 2261a Chlorinated Pesticides in Hexane (Nominal Mass Concentration 2 µg/mL) is intended primarily for use in the calibration of gas chromatographic (GC) methodology used for the determination of chlorinated pesticides. This publication documents the production, analytical methods, density determination, and statistical results used to characterize the replacement material for SRM 2261.

Keywords

Chlorinated Pesticides; Gas Chromatography-Mass Spectrometry (GC-MS); Reference Material; Value Assignment.

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1. Introduction

Chlorinated pesticides are a class of compounds that have multiple chlorine atoms attached to the carbon structures, which make the molecules very stable and fat-soluble. Between the 1940s and 1970s, these pesticides saw widespread use due to their broad-spectrum efficacy, exceptional persistence, and low production costs. Soon after, it was observed that the persistent nature of the compounds caused widespread environmental contamination as well as animal and human health risks. After the U.S. Environmental Protection Agency was established in 1970, the use of most of these compounds in the USA was banned or heavily restricted, notably the ban on DDT in 1972, ban of Aldrin and Dieldrin in 1974, and phaseouts of Chlordane and Heptachlor between 1983 and 1988. In 2001 the Stockholm Convention on Persistent Organic Pollutants (POPs) was adopted, further establishing the global push to eliminate, restrict, and minimize the use of these chemicals.

While some of the pesticides were banned decades ago, they are still found in the environment. SRM 2261 Chlorinated Pesticides in Hexane (Nominal Mass Concentration 2 $\mu\text{g/mL}$) [1] was designed to enable accurate calibration of analytical techniques (primarily gas chromatographic (GC) methods) for the more common of these compounds. The SRM was issued in early 1992 as solution of 15 components in hexane, although the certified values for two of the components were later withdrawn. While the number of SRM 2261 units sold per year has steadily declined (Fig. 1), an accurate calibrator continues to be essential for trusted chemical monitoring in the U.S. and around the globe (Fig. 2).

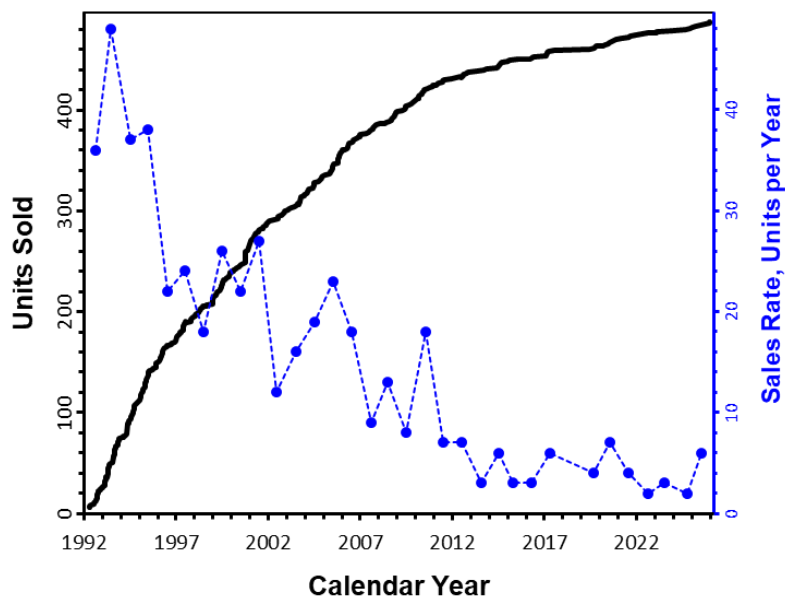


Fig. 1. Sales History of SRM 2261.

This panel displays the sales history of SRM 2261 as a function of time. The solid, black line depicts the cumulative distribution of sales as a function of the order date, plotted against using the "Units Sold" axis on the left. The dashed blue line depicts the units sold per year, plotted using "Sales Rate, Units per Year" axis on the right.

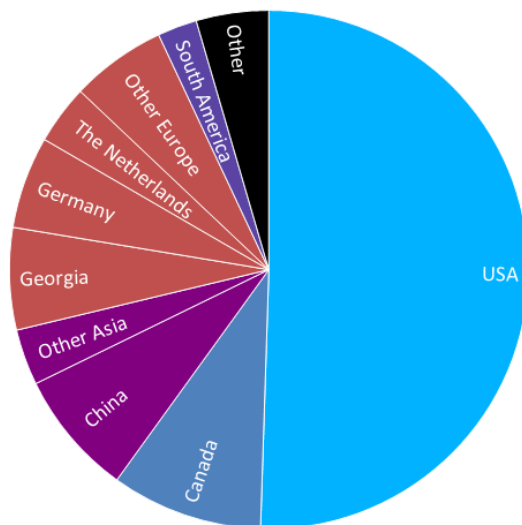


Fig. 2. Location of customers for SRM 2261, 1992 through 2025.

The chart displays the fraction of SRM 2261 sales to customers within countries or geographic regions that account for at least 2 % of the total number of units sold.

The last units of SRM 2261 were sold in December of 2025. SRM 2261a Chlorinated Pesticides in Hexane (Nominal Mass Concentration 2 µg/mL) has been prepared as very similar calibration solution to support the use of established analysis methods for the chlorinated pesticides. SRM 2261a is a hexane solution of the 13 chlorinated pesticides previously certified in SRM 2261. This publication details the gravimetric preparation, density determination, and gas chromatographic-mass spectrometry (GC-MS) measurement techniques used for value assignment of this replacement material.

2. Production

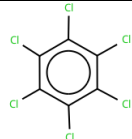
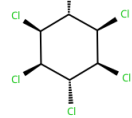
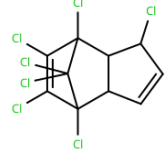
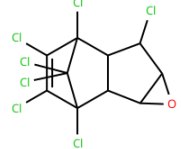
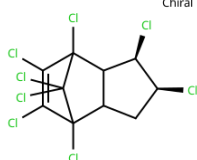
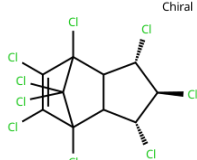
2.1. Safety Considerations

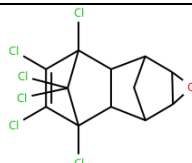
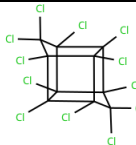
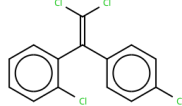
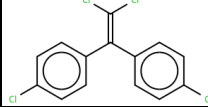
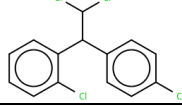
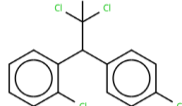
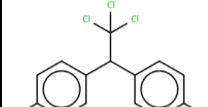
Chlorinated pesticides have been reported to have toxic, mutagenic, and/or carcinogenic properties. Hexane is highly flammable. All materials were handled in accord with established protocols.

2.2. Materials

Table 1 lists identifier(s), Chemical Abstracts Service (CAS) Registry numbers, molecular formulas, molar masses, and model structures for the chlorinated pesticides or breakdown products included in SRM 2261a. All 13 compounds were purchased from the same commercial source. Optima-grade hexane was used as solvent.

Table 1. Chlorinated Pesticides in SRM 2261a.

Compound Identifier(s)	CAS number	Molecular Formula	Molar Mass g/mol	Structure
HCB Hexachlorobenzene Perchlorobenzene	118-74-1	C ₆ Cl ₆	284.77	
γ-HCH γ-BHC Hexachlorocyclohexane Lindane	58-89-9	C ₆ H ₆ Cl ₆	290.82	
Heptachlor	76-44-8	C ₁₀ H ₅ Cl ₇	373.31	
Heptachlor epoxide	1024-57-3	C ₁₀ H ₅ Cl ₇ O	389.31	
<i>cis</i> -Chlordane α-chlordane	5103-71-9	C ₁₀ H ₆ Cl ₈	409.77	
<i>trans</i> -Nonachlor	39765-80-5	C ₁₀ H ₅ Cl ₉	444.21	

Compound Identifier(s)	CAS number	Molecular Formula	Molar Mass g/mol	Structure
Dieldrin HEOD	60-57-1	C ₁₂ H ₈ Cl ₆ O	380.90	
Mirex Dechlorane Perchlodecone Perchlorodihomocubane	2385-85-5	C ₁₀ Cl ₁₂	545.52	
2,4'-DDE o,p'-DDE	3424-82-6	C ₁₄ H ₈ Cl ₄	318.02	
4,4'-DDE p,p'-DDE	72-55-9	C ₁₄ H ₈ Cl ₄	318.02	
2,4'-DDD o,p'-DDD	53-19-0	C ₁₄ H ₁₀ Cl ₄	320.03	
2,4'-DDT o,p'-DDT	789-02-6	C ₁₄ H ₉ Cl ₅	354.48	
4,4'-DDT p,p'-DDT	50-29-3	C ₁₄ H ₉ Cl ₅	354.48	

2.3. Preparation and Ampouling

The chlorinated compounds were individually weighed using a calibrated microbalance, aiming for 12 mg of each pesticide. The compounds were gravimetrically dissolved in 6 L of hexane and held in a 10 L glass bottle that had been solvent rinsed and baked to dryness. The solution was stirred at room temperature for 20 h, then chilled in a -20 °C freezer for 2 h and transferred to the NIST Office of Reference Materials (ORM) for ampouling. A total of 3811 ampoules were filled with ≈1.2 mL of solution, flushed with argon, and heat sealed. The ampoules were stored sequentially in the order of production.

Table 2 lists the masses and nominal mass fractions of the solution components, adjusted by nominal purity claimed by the manufacturer. These mass fraction values are not definitive since the manufacturer's purity values were not confirmed nor does the composition of the total solution necessarily represent the post-production composition of the solution in the ampoules.

Table 2. Gravimetric composition of the solution used to prepare SRM 2261a.

Compound	Mass, mg	Nominal Purity, %	Mass Fraction, μg/g
HCB	12.465	99	3.08
γ-HCH	11.610	99.7	2.89
Heptachlor	12.680	99.9	3.16
Heptachlor epoxide	9.730	97	2.35
<i>cis</i> -Chlordane	11.762	99.7	2.92
<i>trans</i> -Nonachlor	11.913	100	2.97
Dieldrin	12.070	98.8	2.97
Mirex	11.834	100	2.95
2,4'-DDE	11.955	99.9	2.98
4,4'-DDE	12.169	99.9	3.03
2,4'-DDD	11.610	99.9	2.89
2,4'-DDT	12.970	97.3	3.15
4,4'-DDT	12.215	100	3.04
Hexane	4011460		

3. Chlorinated Pesticide Measurements

3.1. Materials

In accordance with ISO 33405:2024 guidance on homogeneity testing [2], the SRM 2261a production run was stratified into 16 blocks of ≈ 238 ampoules each. The first and last ampoules were selected for analysis along with one randomly selected ampoule from each of the remaining 14 blocks. Three additional ampoules were used in a later analysis, one each from the 2nd, 6th, and 15th blocks.

Optima-grade hexane was the solvent for all solutions and as the procedural blank.

Two ampoules of SRM 2261 were used to prepare check samples.

Three ampoules of SRM 2261 were used to prepare six calibration solutions. Calibrants A, B, and C were gravimetrically prepared into 30-mL amber glass bottles. These calibrants were then used to gravimetrically prepare calibrants D, E, and F.

A multicomponent internal standard (IS) solution was previously prepared at NIST for a related project. The IS contained ^{13}C -labeled HCB, *trans*-chlordane, *trans*-nonachlor, oxychlordane, 4,4'-DDE, 4,4'-DDD, and 4,4'-DDT. The solution also contained ^{13}C -labeled polychlorinated biphenyl (PCB) congeners 28, 52, 118, 153, 180, 194, and 206; polybrominated diphenyl ether (PBDE) congener 104; and fluorinated PBDE congeners 47, 160, 208, and 209.

3.2. Sample Preparation

Two sample sets were prepared. Sample set 1 used sixteen SRM 2261a ampoules, evaluating homogeneity across the ampoule fill sequence and other potential sources of between- and within-ampoule analysis variability. Sample set 2 was prepared about a month later, evaluating three SRM 2261a ampoules. Table 3 lists the identifiers and the SRM 2261a ampoule fill-orders in both sets.

Table 3. Sample Set 1 and 2 Test Samples.

Set 1 Day 1		Set 1 Day 2		Set 1 Day 3		Set 2	
Sample	Ampoule	Sample	Ampoule	Sample	Ampoule	Sample	Ampoule
S01a	1	S02	307	S03a	644	S17a	366
S01b	1	S05a	964	S03b	644	S17b	366
S04	796	S05b	964	S06	1206	Cal A2	
S07a	1440	S08	1689	S09a	1959	Blank 4	
S07b	1440	S11a	2404	S09b	1959	Cal B2	
S10	2156	S11b	2404	S12	2685	S18a	1257
S13a	2875	S14	3123	S15a	3359	S18b	1257
S13b	2875	S16	3811	S15b	3359	Check 4	
Blank 1		Blank 1		Blank 3		Cal C2	
Check 1		Check 2		Check 3		Blank 5	
Cal A1		Cal B1		Cal C1		S19a	3406
Cal E1		Cal F1		Cal D1		S19b	3406
						Cal D2	
						Blank 6	
						Cal E2	
						Check 5	
						Cal F2	

3.2.1. Sample Set 1

The sixteen ampoule homogeneity samples were prepared over three consecutive days. Two test samples were prepared from eight of the ampoules, only one test sample was prepared from the other eight ampoules. Twelve test samples were prepared on each of the three days: eight preparations from five or six SRM 2261a ampoules, one hexane blank, one SRM 2261 check sample, and two calibrants. Samples were gravimetrically transferred into amber autosampler ampoules using a gastight syringe followed by addition of the internal standard solution, also gravimetrically delivered using a gastight syringe. All samples were diluted with hexane to approximately 1.5 mL in the autosampler ampoules and stored at -20 °C until analyzed together as a group of 24 test materials.

3.2.2. Sample Set 2

Seventeen test samples were prepared on the same day. The set consisted of two preparations from each of three SRM 2261a ampoules, three hexane blanks, two SRM 2261 check samples, and six calibrants.

3.3. GC-MS Instrumental Methods

Two gas chromatography mass spectrometry (GC-MS) methods were used to accurately determine the mass fraction concentrations of chlorinated pesticides in SRM 2261a. GC-MS Method 1 was run on Set 1 one day after finishing their preparation. Method 2 was run the conjoined Set 1 and Set 2 test samples several weeks after finishing preparation of Set 2.

Both methods injected 2 μ L of each test sample into an Agilent 7890A GC System equipped with an Agilent 7693 Autosampler coupled to an Agilent 7000 GC/MS Triple Quad. The GC oven was held at 60 $^{\circ}$ C for 1.0 min, ramped to 170 $^{\circ}$ C at 25 $^{\circ}$ C/min, ramped to 270 $^{\circ}$ C at 2 $^{\circ}$ C/min, ramped to 325 $^{\circ}$ C at 25 $^{\circ}$ C/min, then held isothermally for 10 min. Total run time for each sample was 67.6 min. The carrier gas (helium) was set at a constant flow rate of 0.8 mL/min.

The MS source was operated in electron impact (EI) ionization mode with selected ion monitoring (SIM). Table 4 lists the monitored quantifier and qualifier ions for the 13 pesticide measurands and associated internal standard compounds. Time segments were used to limit the number of ions monitored and increase dwell times. (Quantifier ions are used to estimate measurand concentration; qualifier ions are used to confirm measurand identity.) Table 4 also lists the retention times (RT) for both methods.

Table 4. Monitored ions and retention times.

Peak	Monitored Ions, <i>m/z</i>		Retention Time, min			
	Quantifier	Qualifier	Method 1	Time Segment	Method 2	Time Segment
HCB	284	286	12.15	1	13.08	1
¹³ C ₆ -HCB	290	292	12.15	1	13.07	1
γ -HCH	219	217	13.36	1	14.40	1
Heptachlor	272	274	17.03	1	17.71	1
¹³ C ₁₂ -PCB 52	304	302	18.21	1	19.32	2
¹³ C ₁₀ -oxychlordane	397	399	21.61	1	22.77	2
Heptachlor epoxide	353	355	21.62	1	22.97	2
¹³ C ₁₀ - <i>trans</i> -chlordane	383	385	23.21	2	-	
2,4'-DDE	246	248	23.58	2	24.86	3
<i>cis</i> -chlordane	373	375	24.15	2	25.79	3
¹³ C ₁₀ - <i>trans</i> -nonachlor	419	421	24.36	2	26.31	3
<i>trans</i> -nonachlor	409	411	24.38	2	26.34	3
¹³ C ₁₂ -4,4'-DDE	258	260	25.97	2	27.87	4
4,4'-DDE	246	248	25.98	2	27.90	4
Dieldrin	263	279	26.08	2	27.90	4
2,4'-DDD	235	237	26.41	2	28.55	4
¹³ C ₁₂ -PCB 118	338	340	28.40	3	31.13	5
¹³ C ₁₂ -4,4'-DDD	247	249	29.14	3	32.20	5
2,4'-DDT	235	237	29.31	3	30.87	5
¹³ C ₁₂ -PCB 153	372	374	30.09	3	32.37	5
¹³ C ₁₂ -4,4'-DDT	247	249	32.12	3	34.61	5
4,4'-DDT	235	237	32.13	3	34.63	5
6-F-PBDE 47	504	344	35.16	3	-	
¹³ C ₁₂ -PCB 180	406	408	37.52	3	40.26	5
Mirex	274	272	40.11	3	42.26	5

3.3.1. GC-MS Method 1

Method 1 was used to analyze the 24 test samples of sample Set 1. The method used cool-on-column injection onto a 5 m × 0.25 mm Restek Siltek guard column (Bellefonte, PA) connected to a 30 m × 0.18 mm × 0.18 μm film thickness DB5MS capillary column (Agilent, Palo Alto, CA). The column was set on oven track mode.

The SRM 2261a and IS peaks are identified in the extracted ion chromatogram (EIC) shown in Fig. 3. An EIC is a chromatogram filtered to plot specific mass-to-charge ratios (m/z), or a narrow m/z range, to isolate the signals of interest from background noise and co-eluting species. This enables high-sensitivity detection and accurate quantification of individual compounds within complex mixtures. Total ion chromatograms (TIC) for a representative blank, calibrant, SRM 2261a, and SRM 2261 check sample are shown in Fig. 4. A TIC plots the combined intensity of all detected ions across the full mass range as a function of retention time, and provides a comprehensive, broad-overview snapshot of the sample's overall composition and general chromatographic separation.

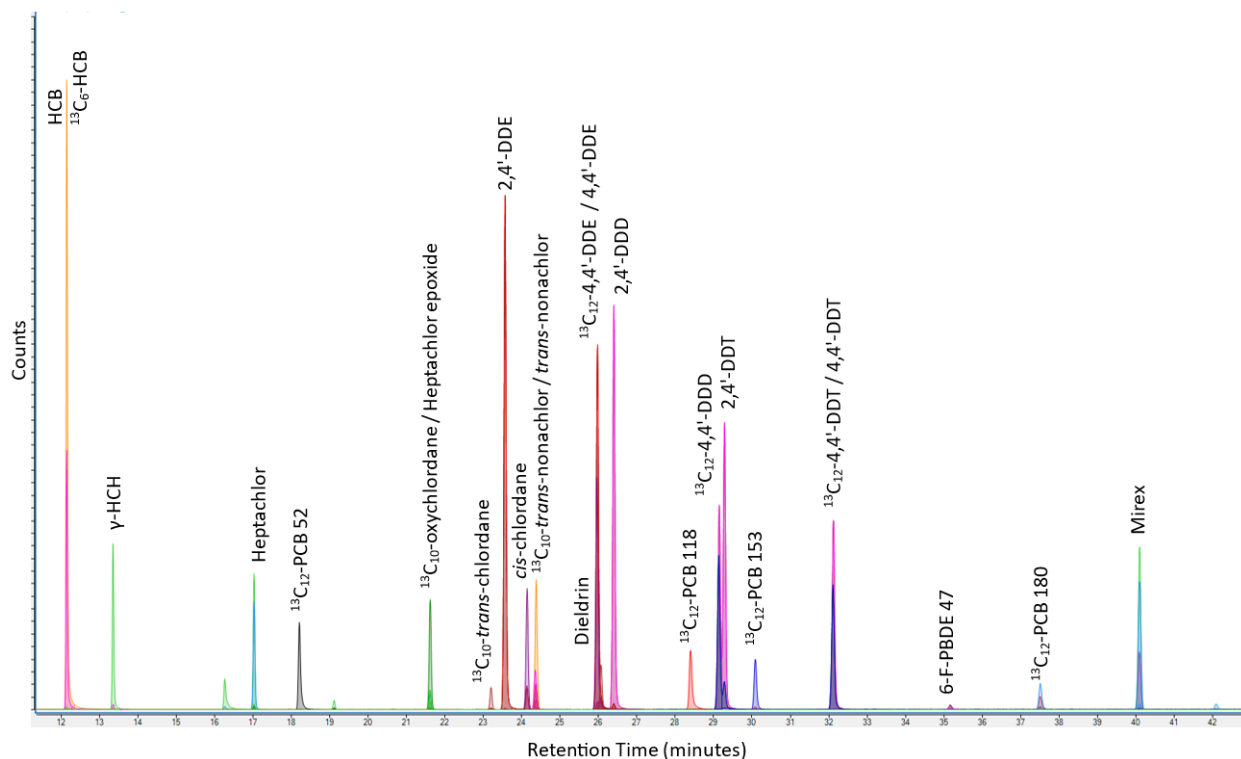


Fig. 3. Method 1 extracted ion chromatogram (EIC) of a SRM 2261a test sample.

Example Method 1 GC-MS EIC for a calibrant. The pesticide and relevant internal standard peaks are labeled. Different colors are associated with specific ions, e.g. lime green at 13.3 min = 219 m/z .

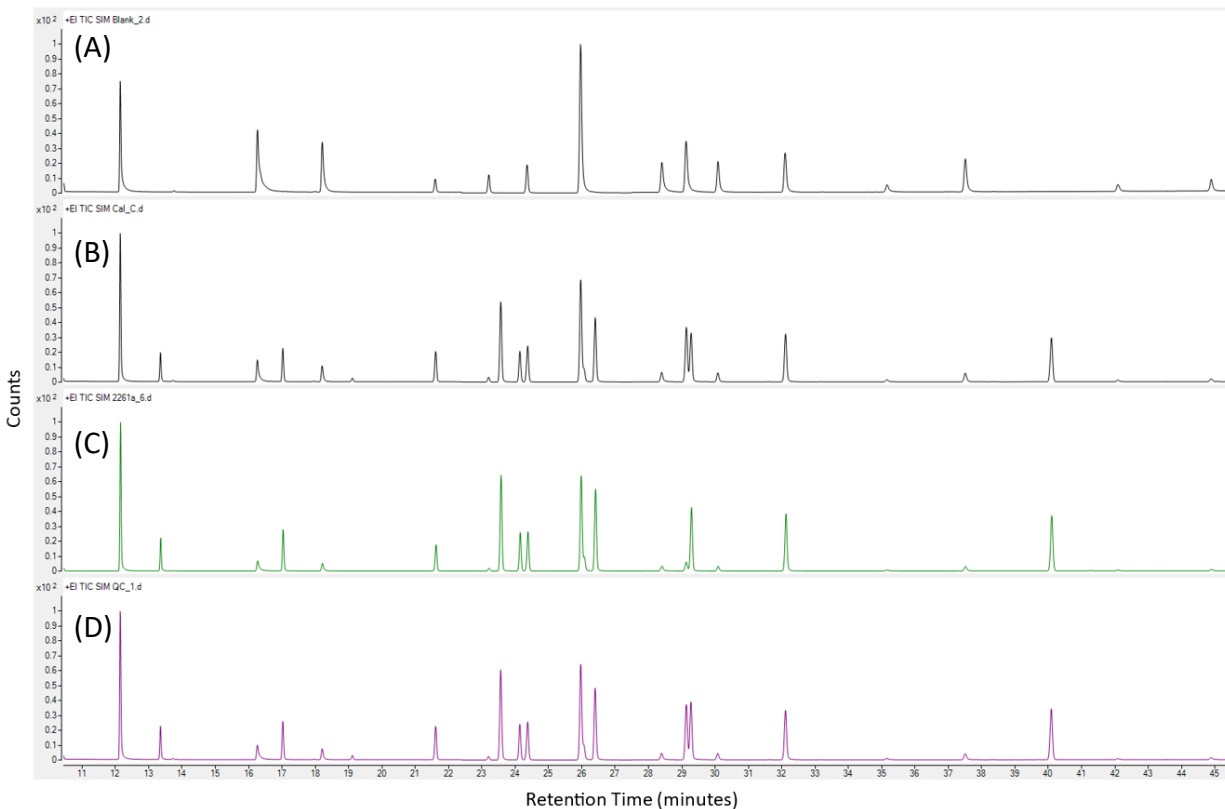


Fig. 4. Method 1 total ion chromatograms (TIC) of Set 1 test samples.

Example Method 1 GC-MS TICs for injections of a (A) system blank, (B) calibration solution, (C) SRM 2261a, and (D) SRM 2261. Note that a system blank contains the internal standard compounds and is used to determine limits of detection (LODs).

3.3.2. GC-MS Method 2

Method 2 was used to analyze the 24 test samples of Set 1 and 17 test samples of Set 2. The method used cool-on-column injection onto a 5 m × 0.25 mm Restek Siltek guard column connected to a 30 m × 0.18 mm × 0.18 μm film thickness DB-XLB capillary column (Agilent, Palo Alto, CA). The column was set on oven track mode.

The SRM 2261a and IS peaks are identified in the EIC shown in Fig. 5. TICs for a representative blank, calibrant, SRM 2261a, and SRM 2261 check sample are shown in Fig. 6.

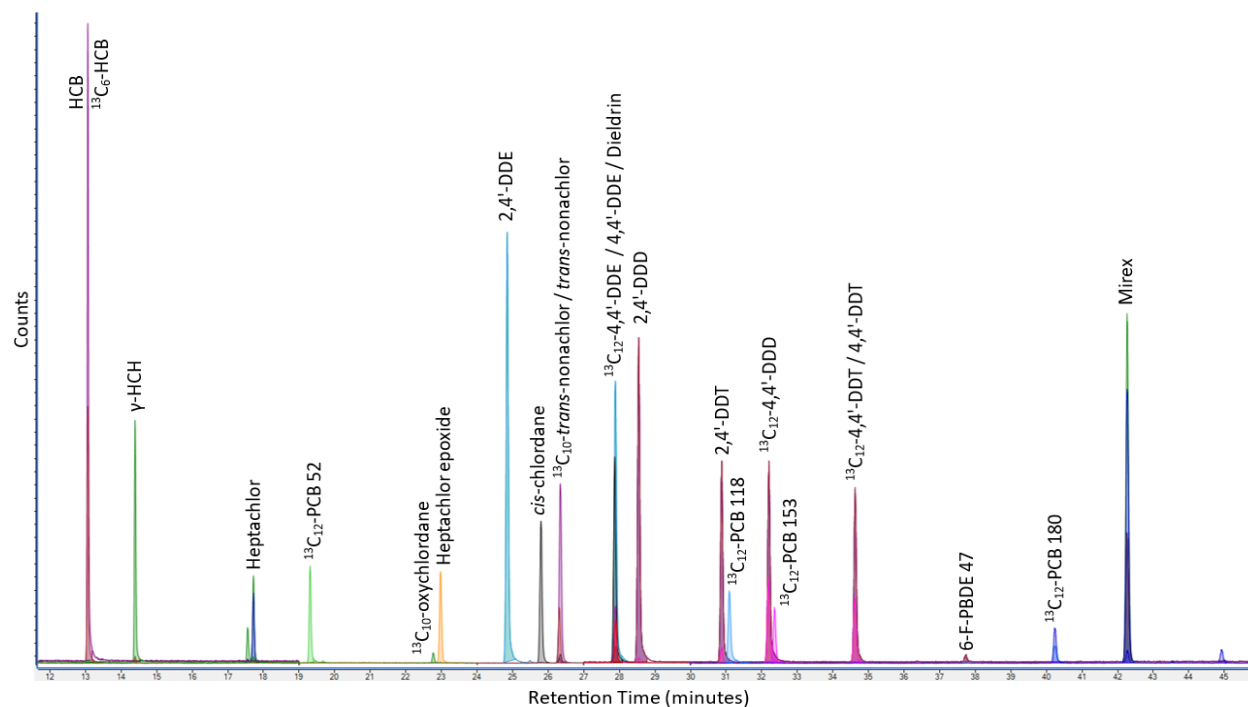


Fig. 5. Method 2 extracted ion chromatogram (EIC) of calibrant.

Example Method 2 GC-MS EIC for a calibrant. The pesticide and relevant internal standard peaks are labeled. Different colors are associated with specific ions, e.g. green at 14.4 min = 219 m/z .

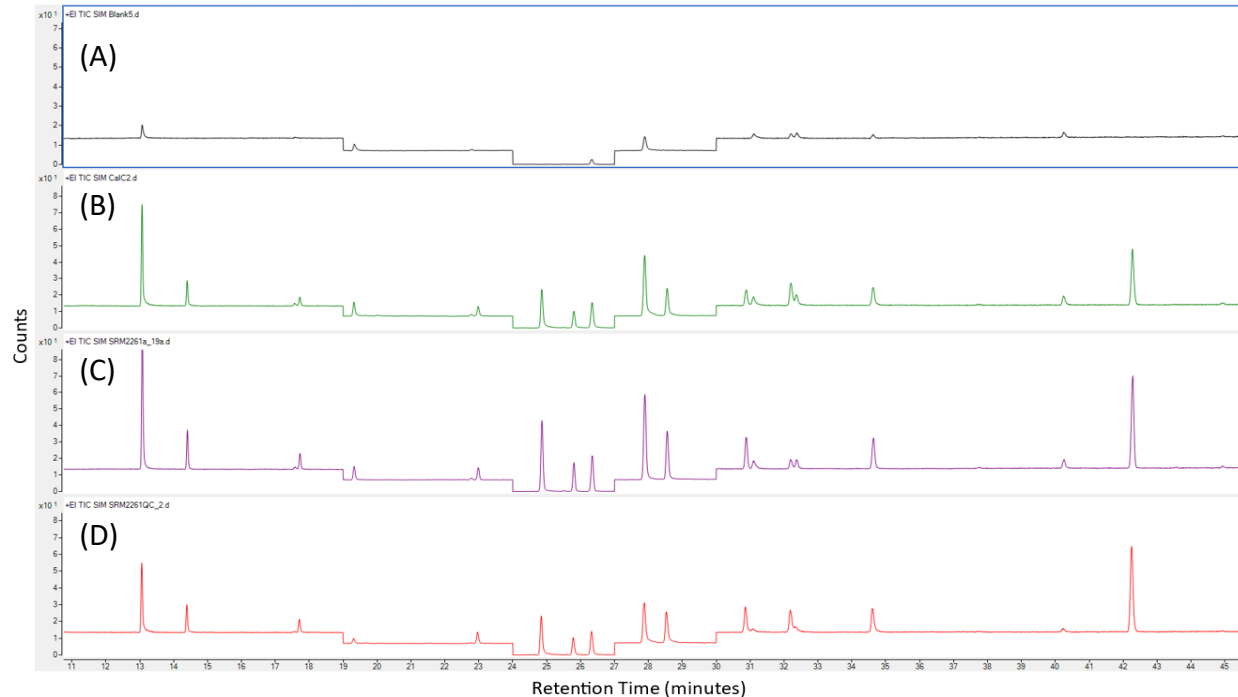


Fig. 6. Method 2 total ion chromatograms (TIC) of Set 1 test samples.

Example Method 2 GC-MS TICs for injections of a (A) system blank, (B) calibration solution, (C) SRM 2261a, and (D) SRM 2261. Baseline shifts are due to different time segments.

3.4. Quantitation

Using NIST's Environmental Metrology Measurement Assistant (EMMA) [3], masses of each measurand were calculated using equally-weighted linear regression with five or six-calibration points that bracketed the observed peak area ratios. Mass fractions were determined by dividing the calculated mass of each measurand by the sample mass. The IS used for each of the measurands, the number of calibration points used in the regression, the R^2 goodness-of-fit statistic, and the limit of detection (LOD) are listed in Table 5 for the Method 1 analysis and in Table 6 for the Method 2 analyses. The pesticide measurands are listed in elution order. The LODs were estimated using 3 blanks and the least concentrated calibrant.

Table 5. Method 1 internal standard, goodness-of-fit, and limit of detection.

Measurand	Corresponding IS	# Point	R^2	LOD, $\mu\text{g/g}$
HCB	$^{13}\text{C}_6\text{-HCB}$	6	0.989	0.27
$\gamma\text{-HCH}$	$^{13}\text{C}_6\text{-HCB}$	6	0.991	0.27
Heptachlor	$^{13}\text{C}_6\text{-HCB}$	6	0.989	0.27
Heptachlor epoxide	$^{13}\text{C}_{10}\text{-oxychlordanes}$	6	1.000	0.27
2,4'-DDE	$^{13}\text{C}_{12}\text{-4,4'-DDE}$	6	0.998	0.27
<i>cis</i> -chlordanes	$^{13}\text{C}_{10}\text{-trans-nonachlor}$	6	1.000	0.27
<i>trans</i> -nonachlor	$^{13}\text{C}_{10}\text{-trans-nonachlor}$	6	0.999	0.27
4,4'-DDE	$^{13}\text{C}_{12}\text{-4,4'-DDE}$	6	0.998	0.27
Dieldrin	$^{13}\text{C}_{12}\text{-4,4'-DDE}$	6	0.999	0.27
2,4'-DDD	$^{13}\text{C}_{12}\text{-4,4'-DDE}$	6	0.999	0.39
2,4'-DDT	$^{13}\text{C}_{12}\text{-4,4'-DDE}$	6	0.996	0.45
4,4'-DDT	$^{13}\text{C}_{12}\text{-4,4'-DDT}$	6	0.996	0.27
Mirex	$^{13}\text{C}_{12}\text{-4,4'-DDT}$	6	0.990	0.28

Table 6. Method 2 internal standard, goodness-of-fit, and limit of detection.

Measurand	Corresponding IS	# Points	Sample Set 1		Sample Set 2	
			R^2	LOD, $\mu\text{g/g}$	R^2	LOD, $\mu\text{g/g}$
HCB	$^{13}\text{C}_6\text{-HCB}$	4	0.994	0.089	0.999	0.076
$\gamma\text{-HCH}$	$^{13}\text{C}_6\text{-HCB}$	4	0.993	0.089	1.000	0.076
Heptachlor	$^{13}\text{C}_{12}\text{-PCB 52}$	4	0.997	0.090	0.999	0.198
Heptachlor Epoxide	$^{13}\text{C}_{10}\text{-Oxychlordanes}$	4	0.997	0.090	0.991	0.076
2,4'-DDE	$^{13}\text{C}_{10}\text{-trans-Nonachlor}$	5	0.995	0.090	0.993	0.076
<i>cis</i> -Chlordanes	$^{13}\text{C}_{10}\text{-trans-Nonachlor}$	5	0.998	0.089	0.999	0.076
<i>trans</i> -Nonachlor	$^{13}\text{C}_{10}\text{-trans-Nonachlor}$	5	0.998	0.090	0.997	0.077
4,4'-DDE	$^{13}\text{C}_{13}\text{-4,4'-DDE}$	5	0.996	0.090	0.999	0.076
Dieldrin	$^{13}\text{C}_{13}\text{-4,4'-DDE}$	5	0.995	0.089	0.998	0.076
2,4'-DDD	$^{13}\text{C}_{13}\text{-4,4'-DDE}$	5	0.989	0.089	0.998	0.076
2,4'-DDT	$^{13}\text{C}_{12}\text{-4,4'-DDD}$	4	0.985	0.089	1.000	0.076
4,4'-DDT	$^{13}\text{C}_{12}\text{-4,4'-DDT}$	5	0.998	0.089	0.998	0.076
Mirex	$^{13}\text{C}_{12}\text{-4,4'-DDT}$	5	0.994	0.090	0.993	0.077

Because the IS solution contained PCB and PDBE labeled compounds, multiple IS choices were available. IS selected on 1) chemical structure similarities and on 2) RT matching provided very similar results as the IS shown above for 2,4'-DDE, 2,4'-DDT, and Mirex.

3.5. Method Performance Check

SRM 2261 samples were included in each set of analyses as a check on method performance. The differences between the estimated mass fractions for the 13 pesticide measurands and their certified values [1] are displayed in Fig. 7.

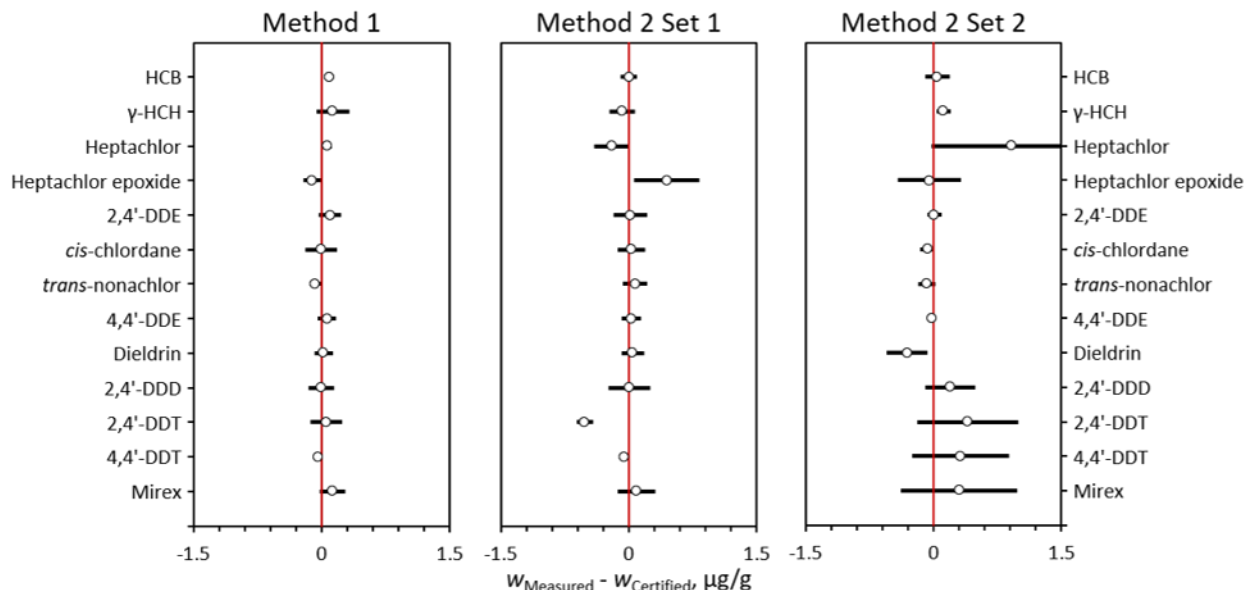


Fig. 7. SRM 2261 check sample results compared with certified values.

Symbols represent the difference between the mean mass fractions for the SRM 2291 components and their certified values. Error bars represent $\approx 95\%$ confidence limits on the mean differences. The red vertical line in each panel represents zero difference.

Nearly all of the results agree with the certified values. The only significant differences are the 2,4'-DDT results from Method 2 Set 1 and the heptachlor results from Method 2 Set 2.

3.6. SRM 2261a Results

The EMMA-calculated results for the 24 Set 1 SRM 2261a samples analyzed using Method 1 are presented in Table 7. Results for the 23 Set 1 SRM 2261a samples analyzed using Method 2 are presented in Table 8; no Method 2 results were obtained for sample S09b due to a failed injection. The Method 2 results for the six SRM 2261a Set 2 samples are presented in Table 9.

Table 7. SRM 2261a Method 1 Results, µg/g.

Sample	HCB	γ-HCH	Heptachlor	Heptachlor epoxide	2,4'-DDE	cis-chlordane	trans-nonachlor	4,4'-DDE	Dieldrin	2,4'-DDD	2,4'-DDT	4,4'-DDT	Mirex
S01a	3.06	2.70	3.04	2.22	3.00	2.90	2.91	3.03	2.74	2.80	2.56	2.94	3.11
S01b	3.13	3.09	3.03	2.11	2.97	2.72	2.79	3.04	2.77	2.85	2.72	3.02	3.01
S02	3.10	2.70	2.99	2.20	3.01	2.90	2.82	3.00	2.68	2.73	2.56	2.97	3.19
S03a	2.76	2.58	2.61	2.10	3.10	2.76	2.76	3.04	2.79	2.75	2.59	2.51	2.94
S03b	2.80	2.48	2.71	2.14	3.11	2.85	2.76	3.05	2.77	2.77	2.59	2.58	2.86
S04	2.98	2.41	2.51	2.12	2.98	2.87	2.82	2.86	2.62	2.42	2.00	2.35	3.45
S05a	3.25	2.95	3.18	2.14	3.08	2.78	2.77	3.06	2.76	2.80	2.69	3.10	3.09
S05b	3.06	3.20	3.34	2.17	2.91	2.83	2.79	3.07	2.76	3.16	3.16	3.38	2.48
S06	2.98	2.73	3.14	2.25	3.00	2.99	2.94	3.12	2.77	2.97	2.72	2.98	2.95
S07a	3.11	2.74	3.06	2.22	3.05	2.90	2.86	3.05	2.76	2.82	2.59	3.01	3.23
S07b	3.16	2.95	3.04	2.13	3.03	2.82	2.79	3.06	2.76	2.80	2.69	2.99	3.06
S08	3.21	2.98	3.07	2.12	2.98	2.81	2.78	3.03	2.74	2.77	2.62	2.99	3.10
S09a	2.89	2.51	2.68	2.12	2.94	2.88	2.82	2.94	2.63	2.72	2.34	2.63	3.11
S09b	2.69	2.73	3.03	2.27	2.94	2.92	2.90	3.04	2.70	3.15	3.18	3.13	2.32
S10	3.09	2.71	3.04	2.22	2.78	2.83	2.85	3.00	2.64	2.81	2.59	3.05	3.00
S11a	3.13	2.86	3.09	2.17	3.06	2.84	2.78	3.08	2.77	2.83	2.73	3.04	2.94
S11b	3.16	3.06	3.01	2.07	3.00	2.71	2.81	3.03	2.73	2.80	2.69	3.00	2.90
S12	2.88	2.58	2.76	2.19	3.05	2.87	2.82	3.07	2.77	2.86	2.64	2.79	2.96
S13a	3.04	2.71	2.93	2.16	2.97	2.86	2.84	2.96	2.63	2.73	2.43	2.89	3.00
S13b	3.03	2.70	2.94	2.17	3.00	2.87	2.85	2.99	2.68	2.81	2.53	2.94	3.11
S14	3.07	2.77	3.13	2.18	3.12	2.89	2.81	3.07	2.78	2.84	2.70	2.98	2.96
S15a	3.15	2.93	3.18	2.18	3.03	2.85	2.80	3.07	2.75	2.89	2.76	3.12	2.92
S15b	2.99	2.63	2.83	2.16	2.99	2.89	2.85	2.98	2.67	2.73	2.40	2.78	3.12
S16	3.07	2.81	2.94	2.17	2.92	2.90	2.86	2.94	2.60	2.79	2.45	2.96	3.05
n:	24	24	24	24	24	24	24	24	24	24	24	24	24
Mean:	3.03	2.77	2.97	2.166	3.001	2.852	2.824	3.024	2.720	2.81	2.62	2.92	2.99
SD:	0.14	0.19	0.19	0.049	0.074	0.063	0.047	0.057	0.060	0.14	0.23	0.22	0.22

Table 8. SRM 2261a Method 2 Set 1 Results, µg/g.

Sample	HCB	γ-HCH	Heptachlor	Heptachlor epoxide	2,4'-DDE	cis-chlordane	trans-nonachlor	4,4'-DDE	Dieldrin	2,4'-DDD	2,4'-DDT ^b	4,4'-DDT	Mirex
S01a	3.04	2.44	2.42	2.12	3.31	3.05	3.03	3.03	2.55	2.84	2.56	2.87	3.18
S01b	3.13	2.99	3.55	1.99	2.88	2.84	2.91	3.19	2.91	3.25	2.78	3.25	3.30
S02	3.00	2.93	2.98	2.26	2.79	2.89	2.97	3.11	2.82	2.84	2.78	3.43	3.25
S03a	2.56	2.16	1.75	3.29	3.25	3.18	2.88	3.15	3.05	2.92	2.49	2.93	3.27
S03b	2.69	2.27	2.16	1.84	3.09	2.90	2.94	2.79	2.58	2.55	2.27	2.45	2.97
S04	2.99	2.73	2.92	2.25	2.76	2.75	2.88	2.98	2.81	2.99	2.44	3.07	2.91
S05a	3.02	2.91	3.03	1.86	2.86	2.65	2.53	3.00	2.60	2.64	2.58	2.66	3.08
S05b	2.97	2.62	2.55	1.93	2.91	2.92	2.92	3.01	2.93	2.84	2.35	2.78	2.83
S06	2.95	2.78	2.80	2.51	3.31	3.28	3.31	3.20	2.96	2.99	2.63	2.94	3.22
S07a	3.32	2.92	3.20	2.52	2.82	2.98	2.98	3.18	2.86	3.00	2.71	3.32	3.33
S07b	3.11	2.50	2.34	2.84	3.61	3.30	3.07	3.00	2.32	2.71	2.67	2.97	3.15
S08	3.25	2.85	3.26	2.22	2.67	2.74	2.76	3.06	2.58	2.89	2.78	3.19	3.21
S09a	2.97	2.31	2.39	3.00	2.64	2.73	2.59	3.06	2.64	2.78	2.52	2.97	3.49
S09b ^a													
S10	2.99	2.82	2.80	2.40	3.04	3.01	3.01	3.11	2.86	2.80	2.84	3.10	3.43
S11a	3.15	3.04	3.38	2.17	2.76	2.77	2.88	3.20	2.84	3.08	2.40	2.94	2.78
S11b	3.13	2.85	2.91	2.42	2.84	2.91	2.89	3.00	2.80	2.82	2.48	2.89	2.84
S12	2.72	2.66	2.56	2.23	2.92	2.96	3.00	3.07	2.82	2.96	2.19	2.69	2.72
S13a	3.12	2.82	3.17	2.00	2.78	2.80	2.89	3.05	2.75	3.04	2.60	3.06	3.02
S13b	3.11	2.84	3.06	2.09	2.91	2.91	3.01	3.17	2.81	3.08	2.58	3.07	2.91
S14	3.08	2.98	3.04	2.16	2.90	2.80	2.88	3.19	2.82	2.98	2.76	3.16	3.20
S15a	3.32	2.65	2.43	2.45	3.08	2.93	2.81	2.91	2.74	2.64	2.55	2.83	2.78
S15b	2.94	2.46	2.98	1.97	3.11	2.99	3.10	3.04	2.74	2.94	2.44	2.94	3.08
S16	3.04	2.76	2.67	2.21	2.80	2.83	2.72	3.10	2.84	2.73	2.49	2.76	3.09
n:	23	23	23	23	23	23	23	23	23	23	23	23	23
Mean:	3.03	2.71	2.80	2.29	2.96	2.92	2.91	3.07	2.77	2.88	2.56	2.97	3.09
SD:	0.18	0.25	0.42	0.36	0.24	0.17	0.16	0.10	0.16	0.17	0.17	0.22	0.22

^a Failed injection, no results.

^b Results uncertain based upon SRM 2261 check sample analysis (Fig. 7).

Table 9. SRM 2261a Method 2 Set 2 Results, µg/g.

Sample	HCB	γ-HCH	Heptachlor ^a	Heptachlor epoxide	2,4'-DDE	cis-chlordane	trans-nonachlor	4,4'-DDE	Dieldrin	2,4'-DDD	2,4'-DDT	4,4'-DDT	Mirex
S17a	3.01	3.00	3.97	2.67	3.17	2.94	3.04	3.10	2.45	3.24	3.24	3.04	2.80
S17b	3.19	3.07	5.19	2.35	3.34	2.83	2.89	3.26	2.52	3.34	3.29	3.32	3.23
S18a	3.16	3.05	4.99	2.36	3.28	2.91	2.92	3.23	2.44	3.32	3.49	3.29	3.20
S18b	3.04	3.18	4.69	2.09	3.30	2.91	2.94	3.26	2.38	3.44	3.08	3.34	3.08
S19a	3.03	2.94	3.62	2.20	3.06	2.86	2.91	3.18	2.53	3.09	3.05	3.12	2.75
S19b	2.98	2.82	3.23	2.15	3.01	2.84	2.85	3.03	2.73	2.91	3.22	3.01	3.00
<i>n</i> :	6	6	6	6	6	6	6	6	6	6	6	6	6
Mean:	3.07	3.01	4.28	2.30	3.19	2.88	2.93	3.18	2.51	3.22	3.23	3.19	3.01
SD:	0.08	0.12	0.79	0.21	0.14	0.05	0.06	0.09	0.12	0.19	0.16	0.15	0.20

^a Results uncertain based upon SRM 2261 check sample analysis (Fig. 7).

3.7. Ampoule Fill Order Analysis

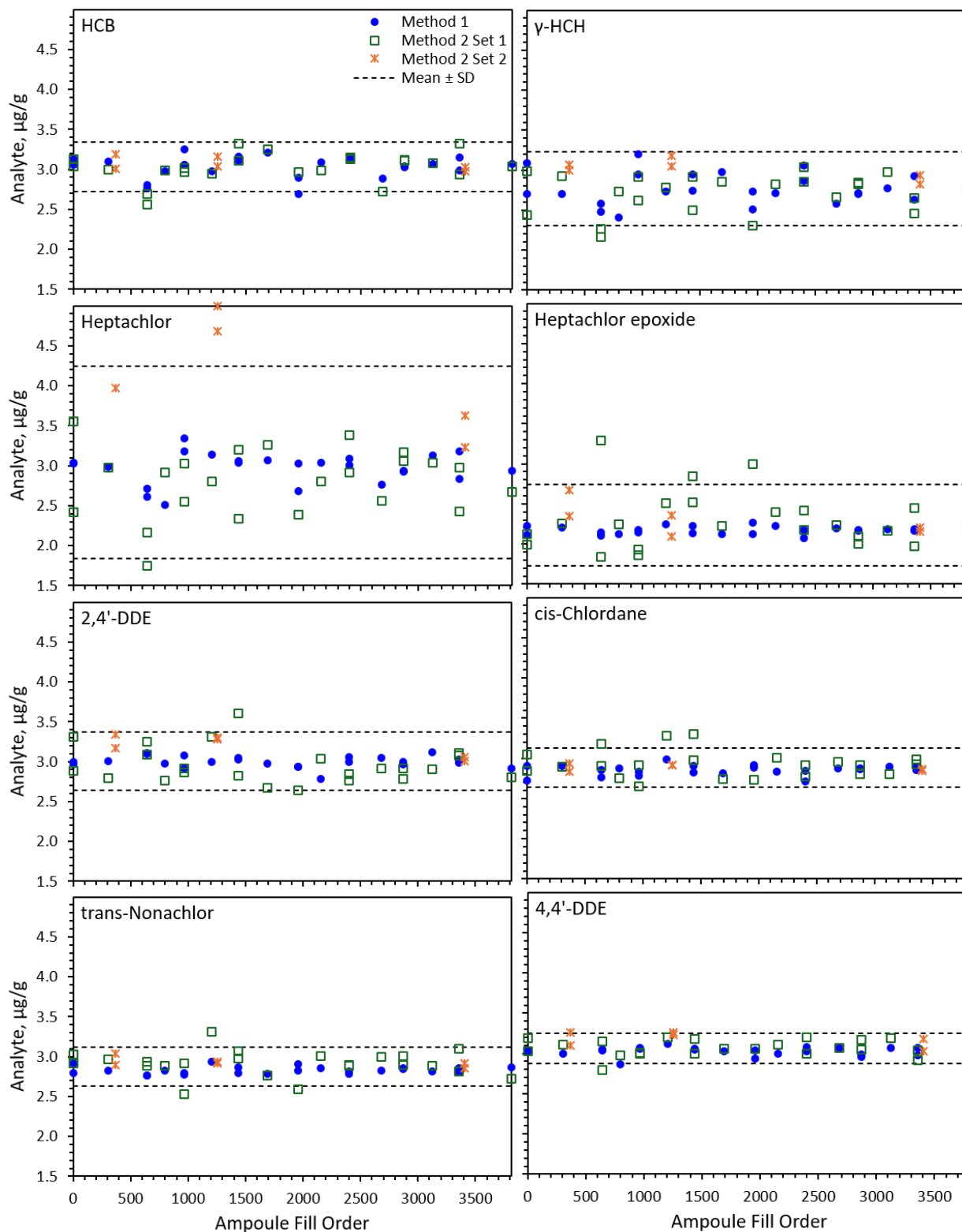
3.7.1. Homogeneity

Material homogeneity was assessed by plotting mass fractions of the 13 pesticide measurands as a function of ampoule fill order. As displayed in Fig. 8, there are no significant fill-order trends for any of the 13 measurands.

3.7.2. Between Set and Method Differences

There are, however, significant differences between Methods and/or Sets for a few of the measurands, and specific values were excluded prior to final value assignment.

- Heptachlor results for the Set 2 samples are on average larger than they are for the Set 1 samples. This is concordant with the significantly larger than expected results for the SRM 2261 check samples.
- The Method 2 results are variable for 2,4'-DDT compared to the Method 1 results. This may be related to an unresolved peak at 29.28 min that follows the ¹³C₁₂-4,4'-DDD peak in samples and calibrants. While SRM 2261a does not contain 4,4'-DDD, SRM 2261 and the calibrants do. This could cause peak area selection to be different in the different types of materials and the subsequent calibration to be inaccurate for 2,4'-DDT in SRM 2261a.



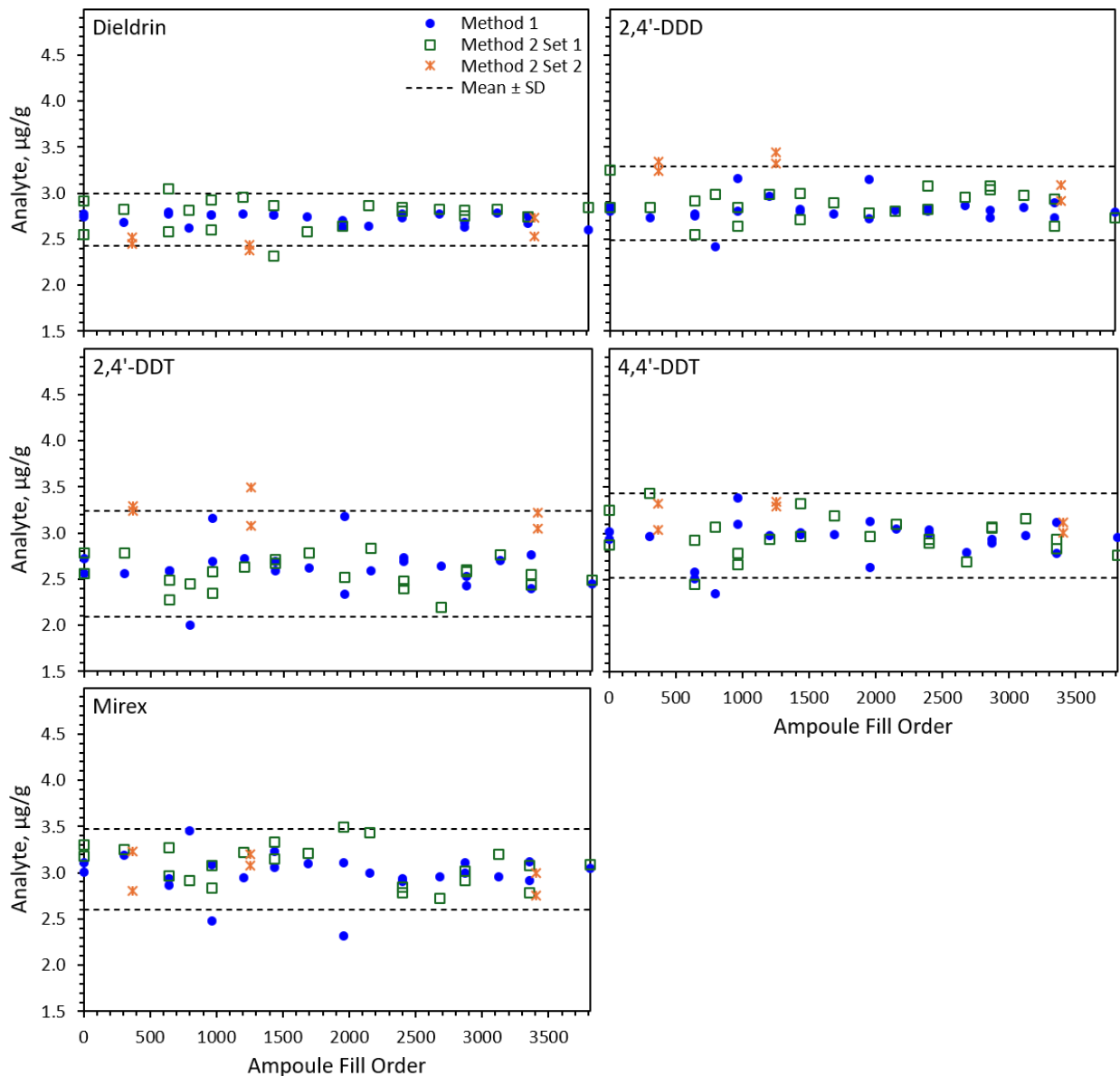


Fig. 8. SRM 2261a results for individual measurands as functions of ampoule fill order.

Each panel presents SRM 2261a results for one measurand, identified in the upper left corner, as a function of the ampoule fill order. Blue, solid circles denote results for the Set 1 test sample obtained using Method 1. Green, open squares denote results for the Set 1 samples obtained using Method 2. The orange “*” symbols denote results for the Set 2 test samples using Method 2. The dashed horizontal lines bound an approximate 95 % confidence region on the combined results.

3.7.3. Sample Preparation and GC-MS Injection Order Trend Analysis

The potential contributions to Set 1 sample measurement variability from sample preparation and GC-MS sample injection sequences are displayed in Fig. 9. To minimize between Method differences, the results for each sample with each Method were centered on the Method's mean for that measurand. To minimize between measurand differences, the 13 mean-centered values for each sample for each Method were averaged.

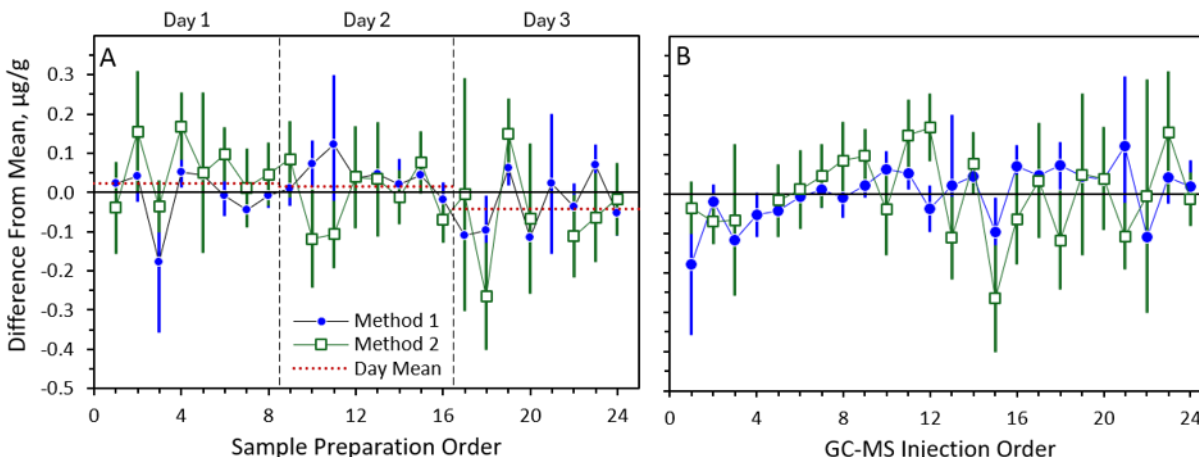


Fig. 9. SRM 2261a composite results for Set 1 samples as functions of preparation and injection order.

Panel A summarizes Set 1 results using Methods 1 and 2 as a function of sample preparation order. Panel B summarizes the same values as a function of GC-MS injection order. Blue, solid circles denote 13-measurand composite mean-differences derived from the Method 1 results for each sample. Open squares denote 13-measurand mean-differences derived from the Method 2 results. Error bars represent $\approx 95\%$ confidence intervals on the mean value. The dashed vertical lines in panel A indicate the three preparation sessions; the horizontal dotted lines denote the average mean-difference for the eight samples prepared during each of the three days.

There is weak evidence for a small difference ($\approx 0.1 \mu\text{g/g}$) in the results for samples prepared on Day 3 relative to those prepared on Day 1 and Day 2.

There appears to be a small ($\approx 0.2 \mu\text{g/g}$) injection order effect in the Method 1 results, increasing from the first injection to a plateau by the 10th SRM 2261a injection. The variability among Method 2 results masks any injection-order trend that may be present.

3.8. Comparison with Gravimetric Estimates

The differences between the estimated mass fractions for the 13 pesticide measurands in SRM 2261a samples and the gravimetric preparation results using the manufacturer's purity estimates are displayed in Fig. 10.

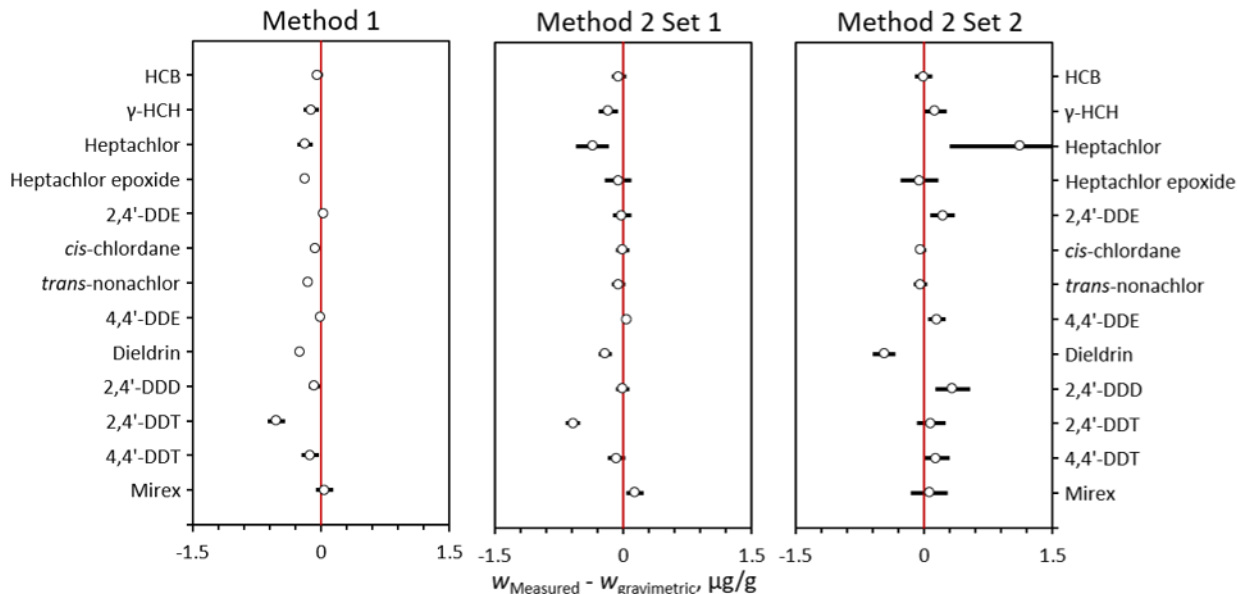


Fig. 10. SRM 2261a results compared with gravimetric preparation estimates.

Open circles represent the difference between the mean mass fractions for the SRM 2261a components and gravimetric preparation values using the manufacturer's estimated purities. In the absence of independent purity assessments, gravimetric values are assigned $\approx 95\%$ uncertainties of 2% . Error bars represent $\approx 95\%$ confidence limits on the mean differences. The red vertical line in each panel represents zero difference.

Most of the SRM 2261a results either agree with the gravimetric values or the differences are inconsistent across Methods or Sets. The only consistently negative differences are for dieldrin, plausibly due to too-high manufacturer's purity estimates. There are no consistently positive differences.

3.9. Metrological Traceability

The mass fractions of the measurands are metrologically traceable to the International System of Units (SI) of mass through the use of SRM 2261 as the primary calibrant. SRM 2261 was prepared in a similar manner to SRM 2261a, with comprehensive purity characterization performed for each component compound.

4. Density

Accurate density values are required to convert gravimetric mass fractions into mass-per-volume units. Due to the volatility of hexane, an alternative approach using 1 mL volumetric flasks was employed.

4.1. Materials, Equipment and Instrumentation

Optima-grade hexanes was used to test the density approach. Milli-Q (18.2 MΩ·cm resistivity) water was used to calibrate Class A 1 mL volumetric flasks. All weighings were performed on a calibrated analytical balance. Six ampoules of SRM 2261a were weighed for density determination.

4.2. Method Procedure

Flask volume was determined each day using the measured weight of water and the 0.99756 g/mL density of water at 23 °C [4]. An empty, capped 1 mL volumetric flask was weighed. The flask was then filled to the line with test solution (hexane or SRM 2261a), capped, wiped with lint-free cloth, and weighed. The flask was emptied, rinsed with Milli-Q water, and dried. The flask was then filled to the line with Milli-Q water, capped, wiped with a lint-free cloth, and weighed. Finally, the flask was emptied and set aside to dry overnight.

The temperature, approximate relative humidity, and time were recorded at the beginning and end of the weighing. Corrections for observed temperature were made by the volumetric expansion formula [4]

$$V_{23} = V_{\text{obsd}} (1 - (T_{\text{obsd}} - 23)) \times 0.000214$$

where V_{23} is the volume at 23 °C, V_{obsd} is the observed volume, and T_{obsd} is the observed temperature in °C.

4.3. Method Validation

The method was validated by determining the density of hexane using two flasks, labeled A and B. The densities calculated from both flasks, (0.677 and 0.680) g/mL, are consistent with the expected density of this lot of hexanes (0.671 g/mL at 25 °C, specifications between 0.653 g/mL and 0.673 g/mL)[5] with less than 1.5 % positive bias.

4.4. Results for SRM 2261a

The density determinations for SRM 2261a were conducted over 3 days, using 2 ampoules on each day. The room temperature slightly between measurement days, so each density determination was completed using its own water calibration measurement. The results for each measurement day as well as the average calculated density are presented in Table 10.

Table 10. SRM 2261a density measurements.

Day	Flask	Ampoule	$T_{\text{obsd}}, ^\circ\text{C}$	V_{23}, mL	m_{SRM}, g	$\rho_{\text{SRM}}, \text{g/mL}$
1	A	32	20.7	0.99217	0.67450	0.6798
	B	145	20.8	0.99558	0.66650	0.6695
2	A	208	21.1	0.99399	0.67148	0.6768
	B	3601	21.0	0.99188	0.67576	0.6788
3	A	3650	20.8	1.00113	0.66914	0.6744
	B	3745	20.8	1.00049	0.66885	0.6718
$n:$						6
Mean:						0.6752
SD:						0.0042

4.5. Metrological Traceability

Density values are metrologically traceable to the SI unit of mass.

5. Value Assignment Calculations

Statistical analysis was provided by the NIST Statistical Engineering Division.

5.1. Chlorinated Pesticides

For each chlorinated pesticide, results were obtained from two CGC-MS methods run at NIST. For each method, the method mean is the mean of the measurements available for that measurand, with the uncertainty of each such mean being the standard uncertainty of that mean. For each measurand, the value is the mean of the two method estimates. The uncertainty of this combined mean is estimated using a bootstrap procedure based on a Gaussian random effects model for the between-method effects [6,7,8,9]. As the results from SRM 2261 form the basis of these measurements, the uncertainty also incorporates the measurand value's relative (unexpanded) uncertainty from the SRM 2261 Certificate of Analysis.

5.2. Density

The density result is the mean of density measurements made at NIST, with the uncertainty being the standard uncertainty of that mean, also incorporating an uncertainty component related to mass measurement of flasks.

6. Certificate of Analysis

The official results for this SRM are presented in the Certificate of Analysis (COA) for SRM 2261a Chlorinated Pesticides in Hexane (Nominal Mass Concentration 2 µg/mL). A NIST COA is defined: [10]

“In accordance with ISO 33401:2024, a NIST SRM certificate is a document containing the name, description, and intended purpose of the material, the NIST logo, the name of NIST as a certifying body, instructions for proper use and storage of the material, certified property value(s) with associated uncertainty(ies), method(s) used to obtain property values, the period of validity, if appropriate, and any other technical information deemed necessary for its proper use. A Certificate is issued for an SRM certified for one or more specific physical or engineering performance properties and may contain non-certified in addition to certified values. A Certificate of Analysis is issued for an SRM certified for one or more specific chemical properties.”

For the most current version of the COA for NIST SRM 2261a, please visit:
https://www-s.nist.gov/srmors/view_detail.cfm?srm=2261a.

References

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- [9] Searle S, Casella G, McCulloch C. (1992) Variance Components. John Wiley, Hoboken, NJ. <https://doi.org/10.1002/9780470316856>.
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Appendix A. List of Acronyms

CAS	Chemical Abstracts Service
COA	Certificate of Analysis
EIC	extracted ion chromatogram
EMMA	Environmental Metrology Measurement Assistant
GC	gas chromatography
GC-MS	gas chromatography with mass spectrometric detection
IS	internal standard
LOD	limit of detection
MS	mass spectrometry
NIST	National Institute of Standards and Technology
PBDE	polybrominated diphenyl ether
PCB	polychlorinated biphenyl
SI	International System of Units
SRM	Standard Reference Material
TIC	total ion chromatogram