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ORIGINAL PAPER

Separation of 26 toxaphene congeners and measurement in air particulate matter SRMs compared to technical toxaphene SRM 3067

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Abstract Toxaphene is a complex technical mixture that has been found ubiquitously in the environment but has caused issues for analysis, especially of individual congeners. This paper reports the elution order of 26 major toxaphene congeners on three gas chromatographic columns. The three different stationary phases generally had similar elution orders for the toxaphene congeners, but fewer co-elutions occurred on a low-bleed, low-polarity column. These congeners (except for two that co-eluted and were not added to the calibration mixture) were examined in air particulate matter standard reference materials (SRMs), 1648a, 1649a, and 1649b as well as SRM 3067 toxaphene in methanol for assignment of reference values. SRM 3067 had mass fractions an order of magnitude greater than the air particulate SRMs, which ranged from 0.568±0.018 ng g^{-1} dry mass (B9-2006 in SRM 1648a) to 12.9±0.20 ng g^{-1} dry mass (B9-715 (P 58) in SRM 1649a). The three air particulate SRMs all had different mass fractions and proportions of congeners relative to the sum of the toxaphene congeners. SRM 3067 may be useful as a technical mixture toxaphene congener calibrant. SRMs 1648a and 1649b will serve as reference materials for the analysis of 21 (three congeners were not included due to values below the detection limit or a potential polychlorinated biphenyl co-elution) toxaphene congeners in atmospheric particulate samples.

Keywords Reference material · Air particulate · Toxaphene · Column separation

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Introduction

Toxaphene, also known as camphechlor, chlorocamphene, polychlorocamphene, and chlorinated camphene as well as various trade names, is an insecticide that theoretically contains over 32,000 congeners if optical isomers are included [1]. In air samples, various congeners are reported as major and include (AV Code [2] with Parlar number in parentheses [3]) B9-1025 (P 62), B8-1413 (P 26), B8-2229 (P 44), B9-1679, (P 50), B8-810 (P 49a), and B8-1412 [4–7]. Toxaphene was used heavily in the USA, especially in the southern USA on cotton crops, until it was banned for most uses in 1982 (all uses were banned in 1990) [8]. At least 450,000 metric tons of toxaphene have been applied worldwide with about 80% of the application occurring in the USA [9]. Breathing, eating, or drinking high levels of toxaphene can damage the lungs, nervous system, and kidneys and can cause death [8]. Since toxaphene is no longer used in the USA, concentrations have declined in

some areas [7, 10, 11]; however, toxaphene persists in soils where it was formerly applied and can volatilize to the atmosphere resulting in releases long after application [12]. It is not known whether toxaphene can affect reproduction or cause birth defects in people. However, animal studies have reported that toxaphene affects the development of newborn animals when their mothers are exposed to it during pregnancy [8]. The Health Canada acceptable daily intake/tolerable daily intake (ADI/TDI) for toxaphene is 0.2 μg/kg body weight per day [13].

A recent interlaboratory study for toxaphene in air samples, which reported relative standard deviations among laboratories of over 30% [4], emphasized the need for standard reference materials (SRMs) for toxaphene congeners to assist with interlaboratory agreement. Schantz et al. [14] discussed several suitable SRMs for toxaphene measurements, including SRM 1649 urban dust, which was collected from Washington, DC in 1976 and 1977 and recertified as SRM 1649a. Bulk material remaining from the collection of this SRM, which was originally sieved to a nominal particle size of <123 μm, was re-sieved to produce SRM 1649b with a nominal particle size of <63 μm (90% of particles <43.2 μm) [15–17]. SRM 1648a urban particulate matter is a subset of the same bulk material used to prepare SRM 1648, which was collected from St. Louis, Missouri in the mid-1970s (90% of particles <30.1 μm) [18, 19].

The objectives of this study were to (1) determine the elution order of 26 commercially available toxaphene congeners on different gas chromatographic (GC) columns, (2) assign toxaphene congener reference values to a technical toxaphene SRM (SRM 3067) and the selected air particulate SRMs described above (SRMs 1648a, 1649a, and 1649b), and (3) evaluate the differences in toxaphene congener mass fractions and proportions to the sum of the toxaphene congeners in these SRMs.

Experimental

SRMs analyzed

SRM 3067 toxaphene in methanol, SRM 1648a urban particulate matter, SRM 1649a, urban dust, and SRM 1649b urban dust were obtained from the Standard Reference Materials Program at the National Institute of Standards and Technology (Gaithersburg, MD, USA). SRM 3067 is a technical mixture of toxaphene in methanol prepared in 2003 and certified for total toxaphene concentration [20]. SRM 1649a is no longer available and has been replaced with SRM 1649b, but data are provided to examine the effect of particle size on toxaphene in air particulate material.

Toxaphene congener preparation

Individual toxaphene congeners were purchased from LGC Promochem (Teddington, Middlesex, UK) and are listed in Table 1 with the various names applied to each. These congeners are those most commonly found in the environment. Following purity and retention time determination, 23 of the 25 toxaphene congener solutions (for a total of 24 congeners) were gravimetrically combined into three calibration solutions ("A–C"), which each had an aliquot removed that was diluted to create three more solutions ("D–F") with an overall mass fraction range of 0.6 to 330 ng g^{-1}. The two toxaphene congeners that were not added due to potential co-elutions with more environmentally relevant congeners were 2-*endo*,3-*exo*,6-*exo*,8,9,10,10-heptachlorobornane (B7-1059) and 2-*exo*,3-*endo*,6-*exo*,8,9,10,10-heptachlorobornane (B7-1474).

SRM 3067 toxaphene in methanol preparation

Two aliquots (0.5 mL) from three ampoules of SRM 3067 toxaphene in methanol were gravimetrically diluted in 25 mL of toluene. For use as a control material, three aliquots (0.5 mL) of calibration solution "B" (≈85 ng g^{-1}) were gravimetrically diluted in 12 mL of toluene. All SRM 3067 and calibration solution B dilutions were vortexed and an approximately 0.75-mL aliquot (exact mass known) was placed in a clean autosampler vial. A blank sample was also prepared by placing 0.75 mL of toluene in a clean autosampler vial. All autosampler vials were then spiked with approximately 0.25 mL (exact mass known) of a mixed polychlorinated biphenyl (PCB), PBDE, and pesticide internal standard solution (resulting in 98 to 125 ng of ^{13}C-*trans*-chlordane, the internal standard used to quantify the toxaphene congeners, added to the autosampler vial) and vortexed prior to analysis.

Air particulate matter SRMs preparation

Six replicates of approximately 0.5 g (gravimetrically measured) of each air particulate matter SRM (1648a, 1649a, and 1649b), a set of toxaphene calibration solutions, and three blanks were extracted using pressurized-fluid extraction (Dionex, Sunnyvale, CA, USA) as described previously [21]. The extracts were further cleaned using solid-phase extraction through both alumina and acidified silica columns and gel permeation chromatography with a 7.5 mm×300 mm 10 μm 100 Å PLGel column (Polymer Labs, Amherst, CA, USA).

Purity analysis

Purity of the individual toxaphene congeners (Table 1) was determined based on the response of GC with a flame ionization detector (GC-FID, Agilent 6890N, Palo Alto,

Table 1 Toxaphene congener names and relative retention times for various gas chromatographic (GC) columns (see text for details) with ions monitored by mass spectrometry (MS)

IUPAC name	AV code	Parlar no.	Western code	Other	LGC Promochem ID	Relative retention times			Monitored ions	
						DB-XLB	DB-17	DB-5MS	Quantitation	Confirmation
2-*Exo*,5-*endo*,9,10,10- pentachlorobornane			B[20010]-(012)[a]		DE-TOX 417	0.727	0.716	0.506	274	310
2-*Exo*,3-*endo*,6-*exo*,8,9,10-hexachlorobornane	B6-923		B[21002]-(111)[a]	hex-sed	DE-TOX 441	0.808	0.804	0.629	313	311
2-*Endo*,3-*exo*,5-*endo*,6-*exo*,8,9,10-heptachlorobornane	B7-1001		B[12012]-(111)[a]	hex-sed	DE-TOX 442	0.818	0.813	0.661	343	345
2-*Endo*,3-*exo*,5-*endo*,6-*exo*,8,8,10,10-octachlorobornane	B8-1413	26	B[12012]-(202)	Tox 8	DE-TOX 401	0.829	0.818	0.707	377	379
2,2,3-*Exo*,8,8,9,10-heptachlorocamphene		25	C[032001]-(21)		DE-TOX 448	0.840	0.854	0.697	341	343
2,2,3-*Exo*,8,8,9,9,10-octachlorocamphene		31	C[032001]-(22)		DE-TOX 449	0.860	0.859	0.748	375	377
2-*Exo*,3-*endo*,5-*exo*,8,9,10,10-heptachlorobornane	B7-1450		B[21020]-(112)[a]		DE-TOX 414	0.872	0.867	0.740	343	345
2-Exo,3-endo,6-exo,8,9,10,10-heptachlorobornane	*B7-1474*		*B[21002]-(112)[a]*		*DE-TOX 419*	*0.883*	*0.889*	*n.m.*	*n.m*	*n.m.*
2,2,5-*Endo*,6-*exo*,8,9,10-heptachlorobornane	B7-515	32	B[30012]-(111)	Tox B	DE-TOX 404	0.884	0.881	0.762	343	345
2,2,5,5,8,9,10-heptachlorobornane	B7-495		B[30030]-(111)[a]		DE-TOX 418	0.888	0.892	0.761	343	345
2,2,5,5,9,9,10,10-octachlorobornane	B8-789	38	B[30030]-(022)		DE-TOX 408	0.899	0.895	0.802	377	379
2,2,3-*Exo*,5-*endo*,6-*exo*,8,9,10-octachlorobornane	B8-531	39	B[32012]-(111)		DE-TOX 412	0.904	0.903	0.817	377	379
2-*Exo*,3-*endo*,5-*exo*,8,9,9,10,10-octachlorobornane	B8-1945	41	B[21020]-(122)		DE-TOX 454	0.908	0.905	0.823	377	379
2-Endo,3-exo,6-exo,8,9,10,10-heptachlorobornane	*B7-1059*		*B[12002]-(112)[a]*	*Last eluting hexane/SiO_2*	*DE-TOX 409*	*0.912*	*0.931*	*n.m.*	*n.m.*	*n.m.*
2-*Endo*,3-*exo*,5-*endo*,6-*exo*,8,9,10,10-octachlorobornane	B8-1414	40	B[12012]-(112)		DE-TOX 445	0.912	0.913	0.824	377	379
2,2,5-*Endo*,6-*exo*,8,8,9,10-octachlorobornane	B8-806	42a	B[30012]-(211)	Tox A	DE-TOX 439	0.920	0.926	0.839	377	379
2-*Exo*,5,5,8,9,9,10,10-octachlorobornane	B8-2229	44	B[20030]-(122)		DE-TOX 453	0.923	0.922	0.847	377	379
2-*Endo*,3-*exo*,5-*endo*,6-*exo*,8,8,9,10,10-nonachlorobornane	B9-1679	50	B[12012]-(212)[a]	Tox 9	DE-TOX 402	0.931	0.927	0.883	413	411
2,2,5-*Endo*,6-*exo*,8,9,10,10-octachlorobornane	B8-810	49a	B[30012]-(112)[a]		DE-TOX 444	0.949	0.956	0.892	377	379
2,2,3-*Exo*,5,5,9,9,10,10-nonachlorobornane	B9-718		B[32030]-(022)[a]		DE-TOX 410	0.950	0.945	0.912	413	411
2-*Endo*,3-*exo*,6-*exo*,8,8,9,10,10-octachlorobornane	B8-1471		B[12002]-(212)[a]		DE-TOX 455	0.958	0.971	0.910	377	379
2,2,3-*Exo*,5-*endo*,6-*exo*,8,9,10,10-nonachlorobornane (A) +	B9-743		B[32012]-(112)[a]		DE-TOX 422	0.961	0.963	0.936	413	411
2-*Exo*,3,3,5-*exo*,6-*endo*,8,9,10,10-nonachlorobornane (B)	B9-2006		B[23021]-(112)[a]			0.962	0.968	0.940	413	411
2,2,5-*Endo*,6-*exo*,8,8,9,10,10- nonachlorobornane	B9-1046	56	B[30012]-(212)		DE-TOX 423	0.966	0.971	0.946	413	411
2,2,3-*Exo*,5,5,8,9,10,10-nonachlorobornane	B9-715	58	B[32030]-(112)		DE-TOX 411	0.975	0.972	0.959	413	411
2,2,5,5,8,9,9,10,10-nonachlorobornane	B9-1025	62	B[30030]-(122)		DE-TOX 403	1.000	1.000	1.000	377	379

The italicized congeners were not added to the final mixture due to potential co-elutions on the low-bleed, low-polarity column and were not measured on the low-bleed, 5% phenyl phase column

[a] Code created from instructions in Wester et al. [30]

CA, USA) with both a 60 m × 0.25 mm × 0.25 μm low-bleed, low-polarity column (DB-XLB, Agilent) and a 60 m × 0.25 mm × 0.25 μm 50% phenyl phase column (DB-17, Agilent). Each had a 5 m×0.25 mm guard column (Restek Siltek, Bellefonte, PA, USA) connected. Single injections of 5.0 μL were made into the inlet heated at 200 °C with a pulse pressure of 345 kPa until 0.90 min and then reduced to 196 kPa. The carrier gas was He. The head pressure on the column was at 196 kPa with a constant flow rate of 1.3 mL/min. The FID was maintained at 300 °C, with a hydrogen flow of 30 mL/min, breathing grade air flow at 400 mL/min, and the makeup gas nitrogen at 24.8 mL/min. The oven was temperature-programmed from 90 °C with a 1 min hold to 150 °C at 10 °C/min, then to 280 °C at 4 °C/min, and finally to 300 °C at 10 °C/min with a 10 min hold (51.5 min total run time).

SRM 3067 analysis

Samples were analyzed using GC/mass spectrometry (GC/MS; Agilent 6890N/5973 inert) operated in negative chemical ionization (NCI) mode with selected ion monitoring (SIM). Monitored ions are shown in Table 1 for the toxaphene congeners. The internal standard, ^{13}C-*trans*-chlordane, quantification ion was 418, with the confirmation ion of 420. The dwell time for each ion was 50 ms. Cool on-column injections (2 μL) were performed (143 kPa, track oven mode) onto a 5 m×0.25 mm guard column (Restek) connected to a 60 m × 0.25 μm film thickness × 0.25 mm i.d. low-bleed, 5% phenyl phase column (DB-5MS, Agilent). The GC oven was held at 60 °C for 1 min, ramped at 25 °C/min to 170 °C, ramped to 270 °C at 2 °C/min, then ramped to 300 °C at 10 °C/min, and held isothermally for 10 min (64.4 min total run time). Helium was the carrier gas set at a constant flow rate of 1.2 mL/min. The quadrupole, source, and transfer line were maintained at 150, 150, and 300 °C, respectively. The samples were injected a second time onto a 5 m×0.25 mm guard column (Restek) connected to a 60 m × 0.25 μm film thickness × 0.25 mm i.d. low-bleed, low-polarity column (DB-XLB, Agilent). The GC oven was held at 60 °C for 1 min, ramped at 25 °C/min to 235 °C, and ramped at 4 °C/min to 300 °C and held isothermally for 4 min (28.3 min total run time). At 23 min, the helium flow was increased from 1.2 to 2.0 mL/min at 50 mL/min. Methane was used as the reaction gas. All other conditions were the same as the first injection. A representative chromatogram from the low-bleed, low-polarity column is shown in Fig. 1.

Air particulate matter SRMs analysis

New methodology was developed that allows for better sensitivity and faster sample analysis (see Fig. 1 for representative chromatograms compared to SRM 3067), so slightly different methods were used for the analysis of the air particulate SRMs. Samples were analyzed using GC/MS in NCI with SIM on a 30 m × 0.18 mm × 0.18 μm i.d. low-bleed, low-polarity column (DB-XLB, Agilent) with a 5 m× 0.25 mm guard column (Restek) added to the beginning of the column. As organochlorines pesticides were also analyzed with the toxaphene congeners, dwell times for the ions were between 15 and 40 ms (Electronic supplementary materials contain full GC/MS acquisition details). A programmed temperature vaporization injector (Agilent 6850) was used for sample introduction. The inlet was cooled with liquid nitrogen to 10 °C for 1.5 min during the injection of 20 μL (4×5 μL) of the sample, and the solvent vent flow rate was 65 mL/min of nitrogen. The inlet was then heated at 720 °C/min to the final transfer temperature of 250 °C with no hold time, and then ramped at 20 °C/min to 280 °C and held for 10 min to bake off any remaining compounds from the liner. The GC oven was held at 120 °C for 1.0 min, ramped at 30 °C/min to 230 °C, then ramped at 10 °C/min to 260 °C, ramped at 4.8 °C/min to 284 °C and finally at 9.6 °C/min to 300 °C, and held isothermally for 1.67 min (16 min total run time). Helium was the carrier gas with a flow rate of 1.5 mL/min held for 4.5 min, ramped at 50 to 0.7 mL/min held for 8.5 min, and finally ramped at 50 up to 1.4 mL/min and held for 3 min. All other conditions were as for the SRM 3067 analysis.

Statistical analysis

SRM reference value assignment depended on the number of analytical techniques available. The means of results from different methods and expanded uncertainties were determined with the freely available NIST Dataplot (http://www.itl.nist.gov/div898/software/dataplot.html/) using one of two methods. If two analytical techniques were used, an unweighted mean was used and an expanded uncertainty about the mean, with coverage factor 2 (approximately 95% confidence), was calculated by combining a between-method variance [22] with a pooled, within-method variance following the ISO Guide to the Expression of Uncertainty in Measurement [23, 24]. If only one analytical technique was used, the mean of the replicates was used. The expanded uncertainty, U, was calculated as $U = ku_c$, where u_c is one standard deviation of the analyte mean, and the coverage factor, k, was determined from the Student's t distribution corresponding to the associated degrees of freedom and a 95% confidence level for each analyte. Limits of detection were determined by the maximum of either the lowest observable calibration solution divided by the mass of the sample extracted or the mean of the blanks plus three times the standard deviation of the blanks divided by the mass of the sample extracted.

The mass fractions of toxaphene congeners in air particulate SRMs were compared using individual

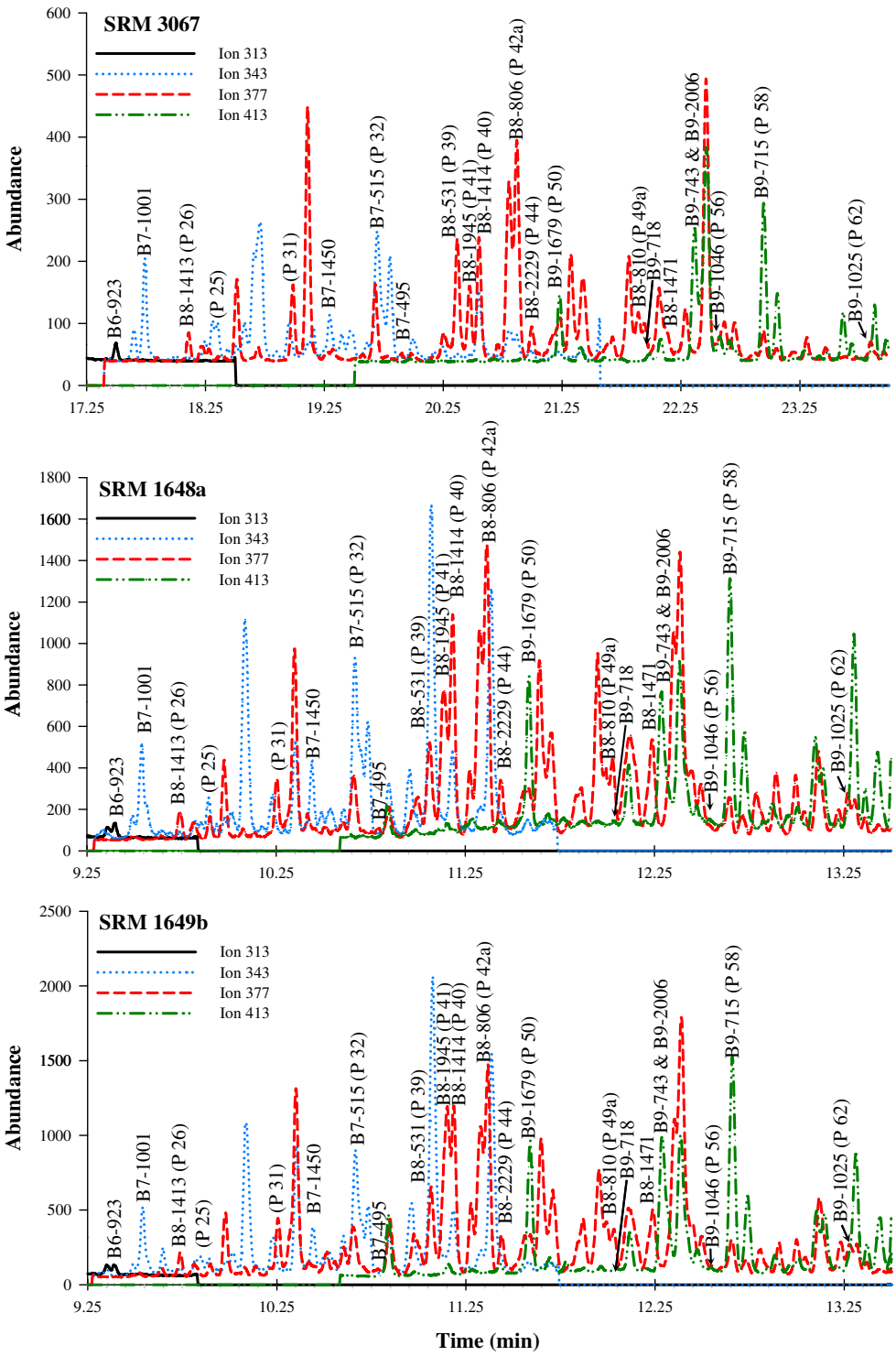

Fig. 1 Representative chromatograms from gas chromatography/mass spectrometry (GC/MS) analysis of air particulate matter SRMs on 30 m × 0.18 mm × 0.18 μm i.d. low-bleed, low-polarity column and of SRM 3067 toxaphene in methanol on 60 m × 0.25 mm × 0.25 μm i.d. low-bleed, low-polarity column

analyses of variances and Tukey–Kramer multiple comparisons with commercially available software (JMP 7.0.2, SAS Institute, Cary, NC, USA). The null hypothesis of no difference between/among mass fractions was rejected if the probability (P) of obtaining the result by chance alone was less than 0.05 (5% significance, 95% confidence).

Results and discussion

Quality assurance

The individual toxaphene congeners were determined to be >98% pure (a few congeners had small peaks at different retention times). However, two congeners, 2-

endo,3-*exo*,6-*exo*,8,9,10,10-heptachlorobornane (B7-1059) and 2-*exo*,3-*endo*,6-*exo*,8,9,10,10-heptachlorobornane (B7-1474), were not included in the final calibration solution mixture due to potential co-elutions with more environmentally relevant congeners (Table 1).

The determined mass fractions for the calibration solution dilutions used as a control material were all within 10% of the expected values, with most congeners within 5%. Likewise, the individual SRMs all had relative uncertainties below 10% with most congeners below 5% (Tables 2 and 3). 2-*Exo*,5-*endo*,9,10,10- pentachlorobornane was not detected (LOD≤0.567 ng g^{-1}) in any of the SRMs; however, the instrumental response for this congener is very low. B6-923 was not detected (LOD≤ 0.325 ng g^{-1}) in the air particulate matter SRMs. Due to their non-detection, these two congeners are not listed. Co-elution of B8-789 (P 38) with PCB congeners on a low-bleed, 5% phenyl phase column has previously been reported [25]. The peak for this congener in SRM 3067 on the low-bleed, 5% phenyl phase column was not integratable, but there was a time-shifted peak on the low-bleed, low-polarity column compared to the calibration solutions (Electronic supplementary material Fig. S1). Considering the large number of toxaphene congeners, this time-shifted peak is probably a different congener. In addition, B8-789 (P 38) was not detected by Vetter et al. [26] in the technical toxaphene mixture produced in the USA. Although toxaphene technical mixtures did vary in congeneric compositions [11], care should be taken with any report of this congener.

Table 2 Results for toxaphene congeners in SRM 3067 toxaphene in methanol (ng g^{-1}; n=6) on two gas chromatographic (GC) columns compared to reference values calculated from these data along with the percent each congener contributes to the certified total toxaphene mass fraction (26.1±1.0 μg g^{-1})

Toxpahene congener	Maximum limit of detection	Low bleed, low polarity			Low bleed, 5% phenyl phase			Percent difference	Reference values	Percent of total toxaphene
		Mean	95% CI	Relative uncertainty[a]	Mean	95% CI	Relative uncertainty[a]			
B6-923	0.695	53.9	2.1	4.0	22.2	0.70	3.2	−140	22.2±0.70[b]	0.085
B7-495	0.521	122	6.1	5.0		Co-elution			122±6.1[b]	0.47
B7-515 (P 32)	0.636	491	7.8	1.6		Co-elution			491±7.8[b]	1.9
B7-1001	0.653	108	1.1	1.0	99.8	2.0	2.0	−8.4	104±4.9[c]	0.40
B7-1450	0.867	106	3.3	3.1	109	4.9	4.5	2.3	108±3.5[c]	0.41
B8-531 (P 39)	0.534	131	2.4	1.8	142	5.3	3.8	8.0	137±7.0[c]	0.52
B8-806 (P 42a)	0.781	398	5.5	1.4	404	7.1	1.8	1.3	401±5.8[c]	1.5
B8-810 (P 49a)	0.730	221	3.2	1.4	417	1.8	0.42	47	221±3.3[b]	0.85
B8-1413 (P 26)	0.620	123	2.4	2.0	133	4.0	3.0	7.8	128±6.2[c]	0.49
B8-1414 (P 40)	0.622	287	4.6	1.6		Co-elution			287±4.5[b]	1.1
B8-1471	0.723	18.6	1.0	5.3	86.9	2.8	3.3	79	18.6±0.96[b]	0.071
B8-1945 (P 41)	0.687	116	1.0	0.83		Co-elution			116±1.1[b]	0.44
B8-2229 (P 44)	0.687	146	4.2	2.9	148	4.7	3.2	1.1	147±3.4[c]	0.56
B9-715 (P 58)	0.570	251	1.1	0.42	271	5.0	1.8	7.3	261±12[c]	1.0
B9-718	0.608	86.7	2.8	3.2		Not detected			86.7±2.8[b]	0.33
B9-743	0.280	111	2.1	1.9	58.7	2.3	3.9	−89	58.7±2.3[b]	0.22
B9-1025 (P 62)	0.637	399	9.8	2.5	416	11	2.7	4.1	408±12[c]	1.6
B9-1046 (P 56)	0.711	99.5	1.6	1.6	96.2	3.4	3.5	−3.4	97.9±2.7[b]	0.38
B9-1679 (P 50)	0.724	163	1.8	1.1	170	6.9	4.1	3.8	167±5.4[c]	0.64
B9-2006	0.280	52.5	2.7	5.2	57.4	2.3	4.1	8.5	55.0±3.4[c]	0.21
(P 25)	0.686	165	3.6	2.2	145	5.2	3.6	−14	155±12[c]	0.59
(P 31)	0.713	135	2.4	1.8	146	6.3	4.3	7.6	141±7.2[c]	0.54

[a] The relative uncertainty is calculated as the 95% confidence interval divided by the corresponding mean and multiplied by 100

[b] The reference values are the means of results obtained using one analytical technique. The expanded uncertainty, U, is calculated as $U = ku_c$, where u_c is one standard deviation of the analyte mean, and the coverage factor, k, is determined from the Student's t distribution corresponding to the associated degrees of freedom and a 95% confidence level for each analyte

[c] The reference value is an unweighted mean of the results from two analytical methods. The uncertainty listed with each value is an expanded uncertainty about the mean, with coverage factor 2 (approximately 95% confidence), calculated by combining a between-method variance [22] with a pooled, within-method variance following the ISO Guide to the Expression of Uncertainty in Measurement ([23] see also [24]).

Table 3 Results for toxaphene congeners for air particulate matter SRMs 1648a, 1649a, and 1649b (ng g^{-1} dry mass; n=6 for each SRM) on a low-bleed, low-polarity column

Toxpahene congener	Maximum limit of detection	SRM 1648a		SRM 1649a		SRM 1649a	
		Reference values[a]	Relative uncertainty[b]	Reference values[a]	Relative uncertainty[b]	Reference values[a]	Relative uncertainty[b]
	0.325						
B7-495	*0.959*	*1.77±0.066*	*3.7*	*1.75±0.10*	*5.8*	*1.67±0.11*	*6.7*
B7-515 (P 32)	0.796	10.3±0.22	2.1	10.8±0.20	1.9	8.42±0.12	1.4
B7-1001	0.305	1.33±0.026	2.0	1.24±0.030	2.5	1.06±0.024	2.3
B7-1450	0.691	2.91±0.072	2.5	2.62±0.058	2.2	2.09±0.039	1.9
B8-531 (P 39)	0.284	2.33±0.055	2.3	**3.03±0.076**	**2.5**	2.40±0.036	1.5
B8-806 (P 42a)	0.519	9.43±0.21	2.2	10.7±0.28	2.6	7.97±0.17	2.1
B8-810 (P 49a)	0.477	5.20±0.10	1.9	**7.54±0.24**	**3.2**	5.52±0.20	3.5
B8-1413 (P 26)	0.290	1.80±0.047	2.6	**2.09±0.048**	**2.3**	1.78±0.033	1.8
B8-1414 (P 40)	0.291	11.2±0.22	2.0	10.6±0.11	1.1	8.84±0.15	1.7
B8-1471	*0.516*	*1.83±0.071*	*3.9*	*1.72±0.12*	*7.0*	*1.90±0.14*	*7.2*
B8-1945 (P 41)	0.751	4.27±0.070	1.6	6.48±0.18	2.7	6.13±0.23	3.7
B8-2229 (P 44)	0.857	5.00±0.10	2.1	5.86±0.12	2.0	3.91±0.037	0.9
B9-715 (P 58)	0.308	9.37±0.22	2.3	12.9±0.20	1.5	8.69±0.27	3.1
B9-718	0.496	1.40±0.025	1.8	1.20±0.054	4.5	1.32±0.020	1.5
B9-743	0.131	2.53±0.045	1.8	4.26±0.17	3.9	3.07±0.08	2.6
B9-1025 (P 62)	0.713	8.78±0.14	1.6	10.9±0.29	2.7	7.44±0.19	2.6
B9-1046 (P 56)	1.067	**4.11±0.17**	**4.1**	2.62±0.10	3.9	2.81±0.068	2.4
B9-1679 (P 50)	0.580	7.74±0.26	3.3	**11.0±0.42**	**3.8**	7.86±0.29	3.7
B9-2006	0.131	0.568±0.018	3.2	1.19±0.082	6.9	1.09±0.021	2.0
(P 25)	0.385	2.61±0.058	2.2	1.48±0.033	2.3	1.19±0.023	1.9
(P 31)	0.333	1.11±0.020	1.8	1.88±0.036	1.9	1.47±0.023	1.6

Percent moisture for SRM 1648a, 1649a, and 1649b was 1.02%, 1.23%, and 0.97%, respectively. Congeners in italics were not significantly different (P>0.05), while the remaining congeners were generally significantly different (P<0.0001) among all three SRMs (except as indicated with bold emphasis where that one SRM was significantly higher than the others for that congener)

[a] The reference values are the means of results obtained using one analytical technique. The expanded uncertainty, U, is calculated as $U = ku_c$, where u_c is one standard deviation of the analyte mean, and the coverage factor, k, is determined from the Student's t distribution corresponding to the associated degrees of freedom, and a 95% confidence level for each analyte

[b] The relative uncertainty is calculated as the expanded uncertainty divided by the corresponding reference value and multiplied by 100

Separation of toxaphene congeners

The three different GC column stationary phases generally had similar elution orders for the toxaphene congeners, but fewer co-elutions occurred on the low-bleed, low-polarity column (Table 1). The later elution of congeners B7-1059 and B8-1471 on the 50% phenyl phase column compared to low-bleed, low-polarity column (and low-bleed, 5% phenyl phase column for B8-1471) was the only major difference in elution order (a few congeners swapped elution orders on the 50% phenyl phase and low-bleed, 5% phenyl phase compared to the other columns; Table 1). The elution orders matched those reported for similar congeners on the low-bleed, 5% phenyl phase column [5, 7, 27–29], but we are unaware of a report of the separation of all 26 congeners examined here. The same elution order on the low-bleed, low-polarity column for 16 of the 26 congeners examined here was reported by Smalling and Maruya [28]. To our knowledge, this is the first report of the separation of these congeners on a 50% phenyl phase column.

More congeners co-eluted on the 50% phenyl phase and low-bleed, 5% phenyl phase columns than the low bleed, low polarity. Congener pairs B7-495 and B7-515 (P 32) and B8-1945 (P 41) and B8-1414 (P 40) co-eluted on the low-bleed, 5% phenyl phase column, while B8-531 (P 39) and B8-1945 (P 41) and B9-1046 (P 56) and B9-715 (P 58) co-eluted on the 50% phenyl phase column. The only potential co-elutions on the low-bleed, low-polarity column were pairs B9-743 and B9-2006, which are sold in the same racemic mixture, and while not baseline- resolved, do have

two distinct peaks, and B8-810 (P 49a) and B9-718, which have different quantitation ions. Smalling and Maruya [28] had similar results when comparing column separations. Thus, only the low-bleed, low-polarity column was used for analysis of the air particulate SRMs.

Toxaphene congeners in SRM 3067

The major congeners found in SRM 3067 were B7-515 (P 32), B9-1025 (P 62), and B8-806 (P 42a; Table 2 and Fig. 2). Compared to technical toxaphene mixtures Melipax and Toxaphene, SRM 3067 had higher percentages of congeners B7-495/B7-515 (P 32), B8-1413 (P 26), and B9-718 and lower percentages of B7-1450, B8-1471, B8-531 (P39), B8-810 (P 49a), B9-1025 (P62), B9-1046 (P 56), and B9-743 (Fig. 3 [26]). The differentials could be due to differences in technical formulations (see [11]) or, as pointed out by Vetter et al. [26], differential degradation due to exposure to light and varying temperatures. Unfortunately, the origins and storage conditions of SRM 3067 were not documented prior to its use as a SRM, so these questions may never be answered.

The sum of the 22 toxaphene congeners (3.73± 0.12 μg g^{-1}) was only 14% of the certified total toxaphene mass fraction (26.1±1.0 μg g^{-1}; Table 2). The toxaphene congeners chosen for examination are those most often reported in the literature as recalcitrant in the environment and are thus thought to be most relevant for a wide variety of environmental SRMs. However, these obviously do not reflect the majority of toxaphene congeners comprising the technical mixture. As evidenced by the large unlabeled peaks shown in Fig. 1, several major congeners remain unidentified in SRM 3067. Further examination of other toxaphene congeners would be beneficial.

The low-bleed, 5% phenyl phase and low-bleed, low-polarity columns generally provided similar results (<10% difference by mass) for the determination of toxaphene congeners in SRM 3067 (Table 2). Only four congeners, B6-923, B8-810 (P 49a), B8-1471, and B9-743, had percent differences ranging from 49% to 140% (Table 2). In these cases, the lower mass fraction was used as the large difference was believed to be due to co-elutions. In addition, the low-bleed, 5% phenyl phase column did not have quantifiable values for five other individual toxaphene congeners (B7-495, B7-515 (P 32), B8-141 (P 40), B8-1945 (P 41), and B9-718), so only one analytical method was available for certification of nine of the 22 toxaphene congeners (Table 2). As the toxaphene congeners were only available in solution form, these 22 toxaphene congener mass fractions will all be listed on the certificate of analysis for SRM 3067 as reference values. This material may also serve as a technical mixture calibrant for these congeners.

Toxaphene congeners in air particulate matter SRMs

Mass fractions of the toxaphene congeners in the air particulate matter SRMs ranged from 0.568±0.018 ng g^{-1} dry mass (B9-2006 in SRM 1648a) to 12.9±0.20 ng g^{-1} dry mass (B9-715 (P 58) in SRM 1649a; Table 3). The major congeners (mean among all three SRMs) were B9-715 (P 58), B8-1414 (P 40), B7-515 (P 32), B8-806 (P 42a), B9-1679 (P 50), and B9-1025 (P62; Table 3 and Fig. 2). At 1.78 to 7.54 ng g^{-1} dry mass, the congeners B8-1413 (P 26), B8-2229 (P 44), and B8-810 (P 49a) reported by others as major toxaphene congeners in air [4–7] were still strong contributors, although not the most significant (Table 3, Fig. 2).

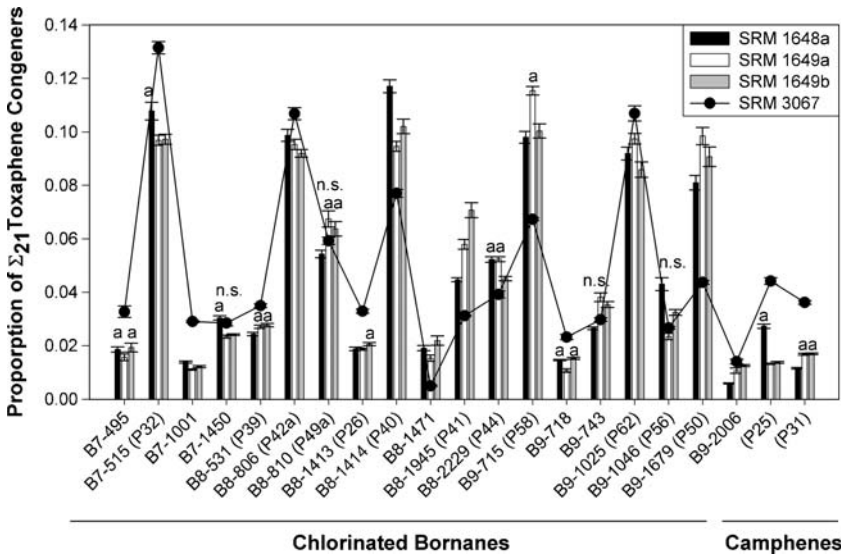

Fig. 2 Comparison (mean±SD) of proportions of toxaphene congeners to the sum of the congeners in air particulate matter SRMs 1649a, 1649b, and 1648a and compared to SRM 3067 toxaphene in methanol. Among the air particulate matter SRMs, all congeners were significantly different ($P \leq 0.0006$) with most significantly different among all three SRMs (*a* indicates an SRM with greater proportion than the others for that congener). The comparisons between the air particulate matter SRMs and SRM 3067 were significant ($P < 0.002$) except as indicated by *n.s.*

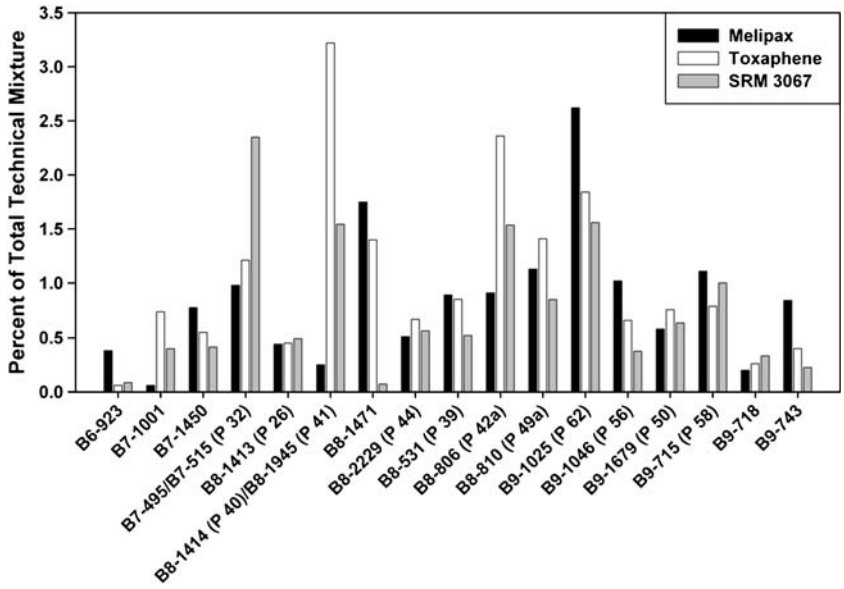

Fig. 3 Comparison of congeners in SRM 3067 toxaphene in methanol to percent of total technical mixture in relation to two other technical mixtures, melipax and toxaphene, as reported in Vetter et al. [26]

With the exception of toxaphene congeners B7-495 and B8-1471 ($P > 0.05$), the SRMs contained significantly different ($P < 0.0001$) mass fractions of individual congeners. In general, all three SRMs were different from each other, but four congeners (indicated with bold text in Table 3) were significantly higher in SRM 1649a than in the other SRMs, and B9-1046 (P 56) was higher in SRM 1648a than in the 1649 series SRMs (Table 3). Interestingly the finely sieved air particulate SRM, SRM1649b, had lower overall toxaphene congener values than SRM 1649a (only congener B9-718 was significantly higher in SRM 1649b compared to SRM 1649a). The PCBs and polycyclic aromatic hydrocarbons also tended to have lower mass fractions in the finer SRM 1649b with a few more exceptions than observed for the toxaphene congeners. Differential distribution of contaminants in coarse and fine air particulate may be due to differences in carbon content and affect volatilization and distribution of contaminants and may be of interest for further research. There are also major differences in the air particulate matter from St. Louis, Missouri (SRM 1648a) and Washington, DC (SRM 1649a), both with respect to mass fraction values (Table 3) and proportions of the congeners relative to the total toxaphene congeners (Fig. 2). All toxaphene congeners comprised significantly different proportions of total toxaphene ($P \leq 0.0006$) with most congeners different among all three SRMs, while only a few congeners had one or two of the SRMs that were higher than the other(s) (Fig. 2).

Air particulate matter SRMs compared to SRM 3067

The toxaphene congener mass fractions in SRM 3067 were an order of magnitude greater than those in the air particulate matter SRMs (Tables 2 and 3). The proportions of toxaphene congeners to the sum of the congeners were similar ($P > 0.05$) between SRM 3067 and the air particulate matter SRMs for congeners B7-1450, B8-810 (P 49a), B9-743, and B9-1046 (P 56), while toxaphene congeners B8-1414 (P 40), B8-1471, B8-1945 (P 41), B8-2229 (P 44), B9-715 (P 58), and B9-1679 (P 50) were significantly ($P < 0.002$) enhanced in the air particulate matter SRMs, and the remaining 11 congeners were significantly depleted (Fig. 2).

Conclusions

The 26 individual toxaphene congeners were analyzed on three GC columns, with a low-bleed, low-polarity column resulting in the fewest co-elutions. A technical toxaphene SRM, SRM 3067 toxaphene in methanol, and air particulate SRMs have been examined for 24 toxaphene congeners. SRM 3067 has mass fractions an order of a magnitude greater than the air particulate SRMs. The air particulate matter SRMs have different mass fractions and proportions of congeners to the sum of the toxaphene congeners. SRM 3067 may be useful as a technical mixture toxaphene congener calibrant, and SRMs 1648a and 1649b will serve as reference materials for 21 toxaphene congeners in particulate samples.

Disclaimer Certain commercial equipment or instruments are identified in this paper to adequately specify the experimental procedures. Such identification does not imply recommendations or endorsement by the National Institute of Standards and Technology nor does it imply that the equipment or instruments are the best available for the purpose.

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