

**COMPLETE SEPARATION OF ISOMERIC PENTA- AND
HEXACHLORONAPHTHALENES BY CAPILLARY GAS
CHROMATOGRAPHY**

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1. Introduction

Polychlorinated naphthalenes (PCNs) are a group of 75 compounds which have historically been used in similar applications as PCBs, namely as dielectrics for flame-resistance and insulating in capacitors, transformers, cable and wires. They were also used as lubricants, fungicides and insecticides, and as binding agents and plasticizers [1] and have been found in PCB formulations [2] and incinerator emissions [3]. PCN congeners are named according to IUPAC rules and have been systematically numbered by Weidmann and Ballschmiter [4].

PCNs are widespread environmental pollutants and are of particular concern due to their dioxin-like toxicity and their ability to bioaccumulate. Several penta-, hexa- and heptachloronaphthalene congeners (CN-54, 63, 66, 67, 68, 69, 70, 73) induce enzyme activity and have been assigned toxic equivalency factors relative to 2,3,7,8-tetrachlorodibenzo-p-dioxin (2,3,7,8-TCDD) [5-7]. Many of these congeners are bioaccumulative, a property that has been related to their structure. PCNs which have all adjacent carbon atoms substituted with chlorine, of which there are 15, tend to bioaccumulate [1]. These include: CN-42, 52, 58, 60, 61, 64, 66, 67, 68, 69, 71, 72, 73, 74, and 75.

Current methods employed for PCN analyses usually involve separation by gas chromatography (GC) and mass specific detection using individual standards or well-characterized commercial mixtures. Relative retention indices for PCNs have been determined for two of the more common capillary GC column stationary phases, (5%-phenyl)-methylpolysiloxane (DB-5) and (14%-cyanopropyl-phenyl)-methylpolysiloxane (TC-1701) [6]. However, several of the toxic and bioaccumulative PCNs coelute on these phases, namely CN-52/60, 66/67, 64/68 and 71/72, preventing a more detailed assessment of their toxic potential and bioaccumulation tendency.

Several capillary GC stationary phases have been investigated to maximize PCN separation, especially for the hexachloronaphthalenes (HxCN) and CN-66/67 in particular [8-10]. None of the columns tested was successful in resolving CN-66/67. However, Imagawa and Yamashita [10] found that CN-64/68 and CN-71/72 separated using α -cyclodextrin and β -cyclodextrin columns, respectively. Resolution of CN-66/67 has only been achieved by high performance liquid chromatography (HPLC) using 2-(1-pyrenyl)-ethyltrimethylsilylated silica gel stationary phase [9, 11].

This paper presents the first gas chromatographic separation of CN-66/67 (1,2,3,4,6,7- and 1,2,3,5,6,7-HxCN), and the resolution of all possible penta-, hexa-, and heptachloro-

naphthalenes. The implications of this enhanced separation method are discussed and the method is applied to PCNs in ambient air.

2. Experimental

Individual chloronaphthalene standards were purchased from Wellington Laboratories (Guelph, ON, Canada; CN-49, 50, 55, 69) and Promochem LLC (Laramie, WY, USA; CN-52, 53, 54, 66, 67, 68, 69, 70, 71, 72, 73, 74). Technical PCN mixtures Halowax 1014, 1013, 1099 and 1051 were from U.S. Environmental Protection Agency (Research Triangle Park, NC, USA).

Based on the limited success of some earlier work in resolving coeluting HxCN isomers [10] using cyclodextrin-based capillary GC columns, we selected four phases for investigation. The selected columns employed stationary phases consisting of derivatized cyclodextrins doped into 14% cyanopropyl/phenyl / 86% dimethylpolysiloxane (Rt-1701). We obtained four columns from Restek Corporation (Bellefonte, PA, USA) and these columns and phases were: Rt-bDEXm (permethylated β -cyclodextrin), Rt-bDEXse (2,3-di-O-ethyl-6-O-*tert*-butyldimethyl-silyl- β -cyclodextrin), Rt-bDEXsa (2,3-di-acetoxy-6-O-*tert*-butyldimethylsilyl- β -cyclodextrin), and Rt-bDEXcst (a proprietary cyclodextrin material). Column dimensions were 30 m x 0.25 mm i.d. and 0.25 mm film thickness.

PCN analyses were conducted by gas chromatography - negative ion mass spectrometry (GC-NIMS) using a Hewlett-Packard 5890 Series II Plus GC with a 5989B MS engine operating with methane at a nominal pressure of 1.3 Torr. Standards solutions in isoctane (2 mL) were injected in splitless mode using a 0.5 min splitless hold time. The carrier gas was helium at 40 cm s⁻¹ and the temperature programs were 110°C for 0.5 min, 20°C min⁻¹ to 160°C, 0.5°C min⁻¹ or 1.0°C min⁻¹ to 225°C then held for 20 min. The inlet and MS transfer line temperatures were 225°C, while the ion source and quadrupole temperatures were 150 and 100°C, respectively. Ions 232, 266, 300, 334, 368 and 404 were monitored in the selective ion mode.

The mass percent composition of PCNs in Halowax 1014 was determined by GC-flame ionization detection (FID) on a 60 m DB-5 column (J&W Scientific; 0.25 mm i.d., 0.25 mm film thickness) and the Rt-bDEXcst column with hydrogen carrier gas at 40 cm s⁻¹. Samples (2 mL) were injected in splitless mode using a 0.5 min splitless hold time. The GC temperature program was 110°C for 0.5 min, 10°C min⁻¹ to 160°C, 2.0°C min⁻¹ to 250°C, 10°C min⁻¹ to 270°C and held for 15 min for the DB-5 column while GC conditions listed above were used for the Rt-bDEXcst column. A series of C16-C28 *n*-alkanes was injected to calibrate detector response and normalize PCN congener response to the response of an alkane having similar retention time. This was done to account for possible injector discrimination. The procedures and mass percent composition calculations have been described previously [12]. Mass percent compositions determined by FID were confirmed using available single PCN congeners by GC-MS.

The selected air sample was collected over Lake Ontario aboard the CCGS Limnos in July 1998 using a high volume air sampler and polyurethane foam (PUF) plugs. The extraction, clean-up, and fractionation procedures used for PCN analysis are described elsewhere [12].

3. Results and Discussion

3.1 Separation of Halowax Mixtures

The four columns were initially screened for separation of CN-66/67 using a mixture of the two individual CNs and a 0.5°C min⁻¹ temperature ramp. No separation of the two congeners was found using the Rt-bDEXm or Rt-bDEXse columns. The Rt-bDEXm column contains permethylated β -cyclodextrin, similar to the β -cyclodextrin phase evaluated by Imagawa and Yamashita [10]. On the Rt-bDEXsa column, the two isomers began to separate but no valley was observed. Using the Rt-bDEXcst column, separation of CN-66/67 was near baseline.

The Rt-bDEXcst was then evaluated for separation of PCNs in the commercial mixture Halowax 1014. Chromatograms for the tetra- (ion 266), penta- (ion 300), hexa- (ion 334) and

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heptachloronaphthalenes (ion 368) (TeCNs, PeCNs, HxCNs and HpCNs) were obtained. PeCN, HxCN and HpCN congeners were identified using available individual commercial standards and by mass percent composition compared to that determined on the DB-5 column. Mass percent compositions are compared as determined on the 60 m DB-5 (FID) and 30 m Rt-bDEXcst (FID and single congeners) columns in this study and using a 30 m DB-5MS column in a previous study [12].

All 14 PeCNs were separated on the Rt-bDEXcst column using the $0.5^{\circ}\text{C min}^{-1}$ temperature ramp. Using a temperature ramp of $1.0^{\circ}\text{C min}^{-1}$, CN-49 and 54 coeluted, although the dominating PeCNs remained separated. CN-52/60 and CN-53/55, which typically coelute [6], were baseline resolved on this column. The presence of CN-55 in Halowax mixtures has been questioned [1] but the lack of a peak at the retention time of the individual standard confirmed its absence from Halowax 1014. CN-56 eluted within the elution range of the HxCNs but the secondary HxCN ions did not interfere. This was not the case for the TeCNs for which secondary PeCN ions sometimes interfered with TeCN primary ions, rendering identification difficult without a complete set of standards.

All 10 HxCNs were resolved on the Rt-bDEXcst column using either the 0.5 or $1.0^{\circ}\text{C min}^{-1}$ temperature ramp. Each of CN-66/67, CN-64/68 and CN-71/72 was separated. These congeners typically coelute [6], although CN-64/68 and CN-71/72 were resolved on two different cyclodextrin columns [10]. This is the first column reported to resolve CN-66/67 and each of the 3 coeluting HxCN pairs. The consistency of this stationary phase was confirmed by testing a second column from a separate batch.

Congener mass percentages determined on the Rt-bDEXcst column compared favorably to those determined on the DB-5 column and previously on the DB-5MS [12]. For previously coeluting congeners, their summed percentages were compared. The exception was PeCN-61 which coelutes with a TeCN congener(s) on the Rt-bDEXcst by GC-FID but is isolated by mass spectrometry. Mass percentages of single congeners in Halowax 1014 were determined with available standards on the Rt-bDEXcst column, confirming values measured by FID.

The ability to resolve coeluting pairs is significant, especially for CN-66 and 67 which are the most toxic PCN congeners [5-7]. In addition, CN-66 may be nearly four times as toxic as CN-67 [7]. Thus, resolution of these two congeners is essential for toxic exposure estimates from environmental samples. CN-66/67 and CN-52/60 are among the more bioaccumulative PCNs. Separating these isomers will determine if one accumulates to a greater extent and may improve our understanding of bioaccumulation mechanisms.

Resolution of the coeluting PeCN and HxCN pairs provides an opportunity to utilize isomer ratios as a tool in allocating sources of environmental PCN contamination. Isomer ratios and parent-metabolite compound ratios are often used to as indicators of local versus long range atmospheric transport of pesticides [e.g. 13]. CNs-52/60, 66/67, 64/68, and 71/72 are good candidates for ratio analysis since the coeluting pairs are considered to have identical vapor pressures [14]. Various commercial mixtures and combustion sources may contain these isomers in differing ratios. For example, the area ratio of CN-66 to CN-67 determined by NIMS differs greatly in Halowax 1014 (1.87 ± 0.01 , $n = 4$) and Halowax 1051 (5.13, single analysis). The ratios of CN-66/67 in Halowaxes 1013 and 1099 were 1.43 and 1.36, respectively. Ratios for CN-52/60 were 0.262 ± 0.001 ($n = 3$) for Halowax 1014, and 0.61, 0.98 and 0.87 for Halowaxes 1013, 1051 and 1099 (single analysis). It should be noted that only one set of Halowax standards (from EPA) was examined and it is unknown whether these ratios vary significantly among batches.

3.2 Application

Comparative chromatograms of PeCNs and HxCNs in Halowax 1014 and the Lake Ontario air sample collected between Burlington and Cape Vincent in July 1998 were compared. The CN-66/67 area ratio (NIMS) in the air was 1.3, lower than found in Halowax 1014 (1.87) and closer to Halowax 1013 and 1099 (1.36-1.43). Halowax 1013 and 1099 had significant

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market share as capacitor impregnants and in electroplating and carbon electrodes in the early 1970's [15]. The ratio of CN-52/60 also differs between Halowax 1014 (0.26) and the L. Ontario air sample (0.43), possibly from the influence of the other commercial mixtures.

In addition to commercial mixtures, other sources such as combustion may contribute to the PCN pattern over the lake. This is suggested by the enrichment of PeCN-54 relative to PeCN-49 in air compared to the Halowax 1014 standard. CN-54 was identified as a combustion product of municipal waste incinerators along with CN-66/67 [16]. Obtaining representative PCN compositions with full resolution of the PeCNs and HxCNs from a range of commercial mixtures and combustion-related sources will improve the ability to identify sources contributing to environmental PCN contamination.

4. Conclusion

Resolution of all 14 pentachloronaphthalenes and all 10 hexachloronaphthalenes was accomplished by capillary GC for the first time using a Rt-bDEXcst column. This capability will provide opportunities to improve understanding of environmental fate and transport processes of polychlorinated naphthalenes and to more fully characterize the isomeric composition of PCNs in environmental matrices and commercial mixtures. Better estimates of toxic potential can now be obtained, as in analyses to date many of the more toxic PCN congeners have coeluted with other, possibly less toxic, isomers.

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