Department of Chemistry, University of Jyväskylä

STRUCTURAL ANALYSIS OF SELECTED POLYCHLORINATED PERSISTENT ORGANIC POLLUTANTS (POPs) AND RELATED COMPOUNDS

Jari Koivisto

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II E. Kolehmainen, J. Koivisto, M. Nissinen, K. Rissanen, K. Laihia, Novel pentafulvalene derivatives: synthesis, crystal structures, ^{1}H and ^{13}C chemical shift assignments of *trans*- and *cis*-isomers of 2,2'-(4,5,6,7-tetrachloro-8,8-dimethoxy-3a,4,7,7a-tetrahydro-4,7-methanoindan-1,3a-dienylidene), *New J. Chem.* **1999**, *23*, 691.
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III E. Kolehmainen, J. Koivisto, V. Nikiforov, M. Peräkylä, K. Tuppurainen, K. Laihia, R. Kauppinen, S. A. Miltsov, V. S. Karavan, NMR spectroscopy in environmental chemistry: ^{1}H and ^{13}C NMR chemical shift assignments of chlorinated dibenzothiophenes based on two-dimensional NMR techniques and *ab initio* MO and DFT/GIAO calculations, *Magn. Reson. Chem.* **1999**, *37*, 743.
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IV J. Koivisto, E. Kolehmainen, V. Nikiforov, M. Nissinen, J. Linnanto, M. Lahtiperä, S. A. Miltsov, V. S. Karavan, A new potential toxaphene congener: synthesis, GC/EI-MS study, crystal structure, NMR analysis, and *ab initio* calculations of 3-*endo*,5-*endo*-dichloro-7,7-bis-chloromethyl-4-dichloromethyl-tricyclo[2.2.1.$0^{2,6}$]heptane, *Chemosphere* **2001**, *44*, 671.
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V J. Koivisto, E. Kolehmainen, V. Nikiforov, M. Nissinen, K. Tuppurainen, M. Peräkylä, S. A. Miltsov, V. S. Karavan, Syntheses, structures and spectroscopy of polychlorinated dihydrocamphenes. An experimental and theoretical study, accepted for publication in *ARKIVOC*.
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PREFACE

The research presented in this thesis has been carried out at the Department of Chemistry, University of Jyväskylä from December 1997 to spring 2001.

I am deeply grateful to my supervisor Professor Erkki Kolehmainen for introducing me into the field of NMR spectroscopy and for his valuable advice, encouragement and support during this work. I also wish to express my gratitude to Docent Katri Laihia and especially Professor Emeritus Jaakko Paasivirta for giving me the opportunity to participate in the project POP-REP.

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I dedicate this thesis to my late grandmother, Hanna.

Jyväskylä, June 2001

Jari Koivisto

ABSTRACT

Detailed structural data of persistent organic pollutants (POPs) are necessary for their toxicological studies and for a correct assessment or prediction of their degradation or accumulation in biota and environment.

In the present investigation the structures of 25 POPs, including eight chlordane derivatives, seven polychlorinated dibenzothiophenes (PCDTs), and ten potential toxaphene congeners, were studied by means of one- and two-dimensional NMR spectroscopy. The ^{1}H NMR spectral parameters were precisely evaluated by computer aided spectral analysis. In the case of PCDTs and toxaphene congeners, the structural analysis was supported by methods of quantum chemistry. In addition, two toxaphene structures were determined by single crystal X-ray analysis. Finally, two POP-related pentafulvalene derivatives were synthesized and their structures were elucidated by NMR and X-ray crystallography.

In the literature section of the thesis, a review of the application of NMR to structural analysis of selected POPs (technical chlordane, PCDTs and toxaphene) is presented. In some cases quantum chemical and X-ray crystallographic studies are included. Finally, synthetic, NMR spectroscopic, and X-ray crystallographic investigations of pentafulvalene and its derivatives are briefly discussed.

CONTENTS

ABBREVIATIONS

AM1	Austin method 1
BPW91	Becke's exchange functional with the Perdew-Wang 1991 correlation functional
B3LYP	Becke's three-parameter hybrid exchange functional with the Lee-Yang-Parr correlation functional
COSY	correlation spectroscopy
CW	continuous wave
δ	chemical shift (ppm)
DBT	dibenzothiophene
DDT	p,p'-dichlorodiphenyltrichloroethane
DEPT	distortionless enhancement by polarization transfer
DFT	density functional theory
ΔH_f	heat of formation energy
DMSO	dimethylsulphoxide
DQF	double-quantum filtered
ECD	electron capture detector
GC	gas chromatography
GIAO	gauge-including atomic orbital
HF	Hartree-Fock
HMBC	heteronuclear multiple bond correlation
HMQC	heteronuclear multiple-quantum coherence
HPLC	high-performance liquid chromatography
Hz	Hertz
INADEQUATE	incredible natural abundance double quantum transfer experiment ($^{13}C,^{13}C$ COSY)
IR	infrared
IUPAC	International Union of Pure and Applied Chemistry
J	scalar coupling
K_H	Henry's Law constant
LC/FTIR	liquid chromatography/Fourier transform IR spectroscopy

MS	mass spectrometry
NMR	nuclear magnetic resonance
m/z	mass-to-charge ratio
NOE	nuclear Overhauser effect
NOESY	NOE spectroscopy
NQR	nuclear quadrupole resonance
PCB	polychlorinated biphenyl
PCDD	polychlorinated dibenzo-*p*-dioxin
PCDF	polychlorinated dibenzofuran
PCDT	polychlorinated dibenzothiophene
PeCDT	pentachlorinated dibenzothiophene
PFG	pulsed field gradient
POP	persistent organic pollutant
ppm	parts per million
r^2	squared correlation coefficient
RDA	retro Diels-Alder fragmentation
RF	radio frequency
RMS	root-mean-square
ROE	NOE in a rotating frame
ROESY	rotating frame NOE spectroscopy
σ	isotropic shielding constant
	unimolecular nucleophilic substitution reaction
	bimolecular nucleophilic substitution reaction
σ_p^+	Hammett substituent constant
T	tesla
TeCDT	tetrachlorinated dibenzothiophene
THF	tetrahydrofuran
TMS	tetramethylsilane
UV	ultraviolet
Å	ångström, 10^{-10} m
2D	two-dimensional

1 INTRODUCTION

Persistent organic pollutants (POPs) are industrial chemicals or their by-products that, to a varying degree, resist photolytic, biological and chemical degradation.[1] POPs are often halogenated and characterized by low water solubility and high lipid solubility, leading to their bioaccumulation in fatty tissues.[2] They also have high air-water distribution ratios (Henry's Law constant K_H)[3], enabling them to travel long distances in the atmosphere before decomposition occurs.[4] Particularly injurious to the environment are those POPs which can biomagnify to a very high extent causing a toxic threat to humans and wildlife.[5] POPs enter the natural environment in accidents, but more commonly from industrial discharges, pesticide and preservative uses, urban wastes, and especially from chlorination and combustion processes.[6] The well-known organochlorine pesticides aldrin, chlordane, DDT, dieldrin, and toxaphene are important POPs.[5,6] Furthermore, the industrial or combustion by-products polychlorinated dibenzofurans (PCDFs) and dibenzo-*p*-dioxins (PCDDs) are also important members of this class of chemical pollutants.[5,6]

In the course of biological transformation and environmental degradation, drastic changes in congener and isomer composition of various POPs have been observed; while some compounds are rapidly degraded and not detectable in biological or environmental samples, others are highly persistent.[7] This implies that recalcitrance of the individual compound against biotic or abiotic processes is related to the number and, especially, position of the halogen substituents.[8,9] Beside isomeric differences, enantioselectivity and chiral discrimination of optically active chemicals may influence

the degree of accumulation.[9-14] In addition, isomers, diastereomers and even enantiomers of the various POPs may exhibit different toxicity.[15-19] From the above it is clear that chemical structures cause the molecular interactions that govern the various biotic and abiotic processes which POPs undergo in the environment. Therefore, a detailed characterization and structure elucidation of these compounds is necessary for their toxicological studies and for a correct assessment or prediction of their degradation or accumulation in the environment.

The identification of POPs and their metabolites and/or environmental degradation products is most often based on gas chromatographic/mass spectrometric (GC/MS) techniques.[20] In addition, infrared (IR) spectral data can be used to complement mass spectral ones,[20] but these methods alone do not offer a reliable way for isomer-specific (including configurational isomers) structure elucidation of POPs.

However, modern nuclear magnetic resonance (NMR) spectroscopy is capable of providing unique insights into structural, stereochemical, and conformational details of this class of compounds. Furthermore, NMR spectroscopy offers an experimental versatility virtually unsurpassed among non-destructive analytical methods for identifying the structure of both pure compounds and mixtures. While simple one-dimensional NMR spectra can provide sufficient information for the structure elucidation, modern two-dimensional NMR experiments are often needed for the assignment of chemical shifts and couplings of relatively complicated spin systems.

An extraction of NMR parameters from the measured spectra is not always straightforward. Therefore, a variety of computer software for spectral analysis has been developed.[21] Unfortunately, NMR significantly trails MS and IR techniques in terms of minimum sample amount for an analysis. This drawback can be partly compensated, for example, by using smaller sample tubes or tube inserts to decrease the volume required for an experiment.[22]

In addition to structure determination by NMR, quantum chemical studies can be used in describing the molecular structures, energies, and spectroscopic properties, such as NMR shieldings. It has been suggested that the combination of experimental NMR data, high level *ab initio* optimized geometries, and theoretically computed NMR chemical shifts provides a tool that can be routinely applied for structural elucidation and characterization of new compounds.[23] Finally, X-ray crystallographic investigations can provide the complete molecular structure of pure, crystalline materials.

2 REVIEW OF THE LITERATURE

2.1 COMPONENTS OF TECHNICAL CHLORDANE

2.1.1 General remarks

Technical chlordane, a mixture of at least 147 compounds, is a pesticide that has had both residential and agricultural uses.[24] It has been estimated that more than 70 000 tons of technical chlordane have been produced and used since 1946.[24] Technical chlordane is persistent in the environment, with a half-life of 10-20 years.[25] Since mid 1970s, the use of technical chlordane has been increasingly restricted in many countries.[26] Based on the assumption that its production peaked in the 1970s, it has been estimated that 25-50% of all the technical chlordane ever produced still exist unaltered in the environment.[24]

Technical chlordane is synthesized by a Diels-Alder condensation of cyclopentadiene and hexachlorocyclopentadiene, followed by chlorine addition.[27] The most abundant components of the mixture are tricyclic polychlorinated C_{10}-based compounds with the cyclodiene-type (in this thesis, the term "cyclodiene-type" refers to the Diels-Alder condensation products of cyclopentadiene with polychlorocyclopentadiene) tetrahydro-4,7-methano-1*H*-indene or tetrahydro-4,7-methanoindane skeleton.[24,28-31] Another group of C_{10}-based products, which results from Wagner-Meerwein rearrangements of chlordene, possesses a so-called "caged" structure.[24,28-32] Among these are compounds with the tetrahydro-1,6-methano-1*H*-indene, hexahydro-1,6-methano-1*H*-indene,

hexahydro-1,4-ethenopentalene and hexahydro-1,3-methano-1*H*-cyclobuta[*cd*]pentalene skeletons. In addition, some C_5-, C_9-, C_{11}-, and C_{15}-based compounds have been observed in technical mixtures.[24,33] Most chlordane components are chiral and thus exist as two enantiomers (optical isomers).[34-37]

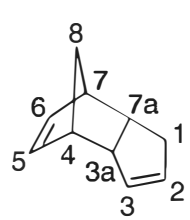
2.1.2 Nomenclature

The term chlordane commonly refers to a technical mixture of chlordane isomers, other chlorinated hydrocarbons and by-products. In this work, single components of technical chlordane are named according to the IUPAC semisystematic nomenclature[38] for fused polycyclic hydrocarbons. Furthermore, trivial names are used as far as such names exist. The IUPAC numbering for the tetrahydro-4,7-methano-1*H*-indene, tetrahydro-4,7-methanoindane, tetrahydro-1,6-methano-1*H*-indene, hexahydro-1,6-methano-1*H*-indene, hexahydro-1,4-ethenopentalene, and hexahydro-1,3-methano-1*H*-cyclobuta[*cd*]pentalene skeletons is given in Scheme 1.

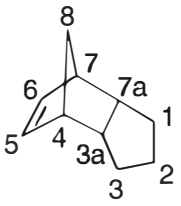
2.1.3 Cyclodiene-type compounds

In 1977, Wilson and Sovocool[39] examined the NMR parameters of a series of chlorinated cyclodiene-type compounds whose chemical structures were well characterized[27,30,40]. These were chlordene (**1**) (4,5,6,7,8,8-hexachloro-3a,4,7,7a-tetrahydro-4,7-methano-1*H*-indene), *cis*-chlordane (**2**) (1-*exo*,2-*exo*,4,5,6,7,8,8-octachloro-3a,4,7,7a-tetrahydro-4,7-methanoindane), *trans*-chlordane (**3**) (1-*exo*,2-*endo*,4,5,6,7,8,8-octachloro-3a,4,7,7a-tetrahydro-4,7-methanoindane), *cis*-nonachlor (**4**)

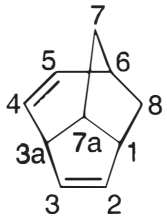
(1-*exo*,2-*exo*,3-*exo*,4,5,6,7,8,8-nonachloro-3a,4,7,7a-tetrahydro-4,7-methanoindane), *trans*-nonachlor (**5**) (1-*exo*,2-*endo*,3-*exo*,4,5,6,7,8,8-nonachloro-3a,4,7,7a-tetrahydro-4,7-methanoindane), and the chlordane metabolite, 1,2-dichlorochlordene (**6**) (1-*exo*,2,4,5,6,7,8,8-octachloro-3a,4,7,7a-tetrahydro-4,7-methano-1*H*-indene) (Scheme 2).



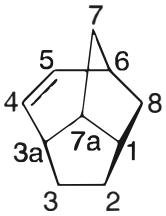
Tetrahydro-4,7-methano-1*H*-indene



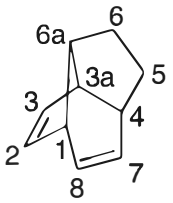
Tetrahydro-4,7-methanoindane



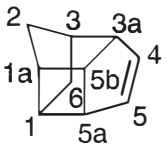
Tetrahydro-1,6-methano-1*H*-indene



Hexahydro-1,6-methano-1*H*-indene



Hexahydro-1,4-ethenopentalene

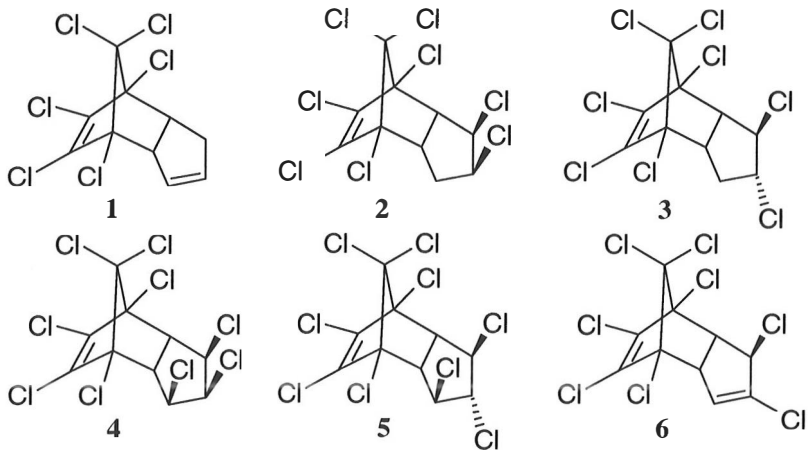


Hexahydro-1,3-methano-1*H*-cyclobuta[*cd*]pentalene

Scheme 1

For compounds **1**, **2**, **4** and **6**, the ^{1}H NMR parameters were extracted from the experimental spectra using the iterative spectral fitting program LAOCOON3[41,39] For **3** and **5**, the spectral resolution did not permit a complete analysis, although the spectrometer operated at 270 MHz. Therefore, only the approximate chemical shifts were reported for these compounds. The olefinic protons, H2 and H3 in **1** and H3 in **6**, resonated in the range of 5.5-6.0 ppm. The signals of the geminal methylene protons on C1 in **1** and on C3 in **2** and **3** were in the range of 1.5-2.5 ppm. In each case, the *endo* methylene protons resonated at a higher field than the *exo* protons. A chloromethylene

proton in the 2-*endo* position in compounds **2** and **4** was substantially deshielded relative to those in the 1- or 3- positions. This was attributed to proximity of the 2-*endo* proton to the chlorinated double bond on C5-C6. The bridgehead protons, H3a and H7a, had chemical shifts that ranged from 3.3 to 4.0 ppm.



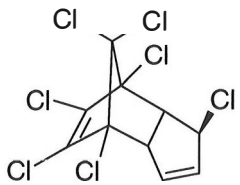
Scheme 2

$^{1}H,^{1}H$ coupling constants in **1-6** were within the usual ranges[42] for this type of compounds and the vicinal coupling constants showed the expected Karplus-type[43,44] dihedral angle dependence.[39] Several significant long-range couplings were observed in **1** and **6**: *J*(1-*endo*,3), *J*(1-*exo*,3), *J*(2,3a), *J*(1-*endo*,3a) and *J*(1-*exo*,3a) in **1**, and *J*(1,3), *J*(1,3a) and *J*(3,7a) in **6**.

In the ^{13}C NMR spectra of **1-6**, the sp^2 carbons had chemical shifts in the range of 125-139 ppm.[39] Predictably, those carbons with chlorine substituents resonated in a lower field than similar carbons with hydrogen substituents. Of the sp^3 carbons, the dichloromethylene carbon C8 had the most distinctive resonance range, 102-105 ppm.

Chemical shifts for the bridgehead chloromethine carbons C4 and C7 were in the range of 79-83 ppm, while chemical shifts for the bridgehead methine carbons C3a and C7a were in the range of 49-63 ppm. Bridgehead methine carbons C3a and C7a with α CHCl groups were deshielded in comparison to those carbons without α CHCl groups. The CHCl groups themselves had resonances that ranged from 58 to 71 ppm.

In 1978, Cox and McKinney[45] published the ^{13}C NMR chemical shifts for heptachlor (**7**) (1-*exo*,4,5,6,7,8,8-heptachloro-3a,4,7,7a-tetrahydro-4,7-methano-1*H*-indene), (Scheme 3). The spectrum of this well-established compound (X-ray structure published in 1973[46]) showed the same characteristics as the carbon spectra of **1**-**6**.



7

Scheme 3

Four years later, ApSimon *et al.*[47] reported the ^{1}H and ^{13}C NMR parameters for chlordene (**1**) and heptachlor (**7**). The ^{1}H NMR parameters were extracted from experimental 250 MHz spectra using the spectral fitting program LAOCOON3[41]. The ^{1}H and ^{13}C chemical shift assignments for **1** differed from those of Wilson and Sovocool[39] in the case of protons H2 and H3 and carbons C3a, C7a, C4 and C7. For compound **7**, the shift order of carbons C3a and C7a was reversed in comparison to that reported by Cox and McKinney[45].

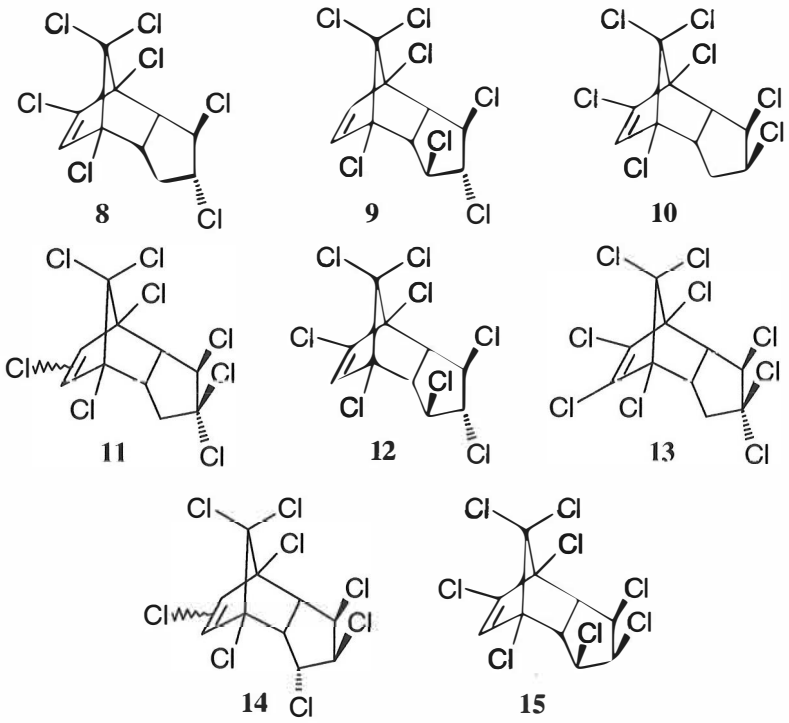
In 1985, Miyazaki *et al.*[48] published the ^{1}H and ^{13}C NMR parameters for *cis*-chlordane (**2**), *trans*-chlordane (**3**), *cis*-nonachlor (**4**) and *trans*-nonachlor (**5**). The ^{1}H NMR chemical shift assignments for **2-5** were in agreement with those given by Wilson and Sovocool[39]. On the contrary, the ^{13}C NMR results differed from those of Wilson and Sovocool[39] in the assignments of C1 and C7a in **2** and **3**, and C3a/C7a and C1/C3 in **5**.

Miyazaki *et al.*[48] also isolated seven unknown components from technical chlordane by liquid chromatography. In addition, a photodechlorination product of *cis*-nonachlor was found in the chlordane mixture by GC/MS. The structures of these compounds were examined by means of ^{1}H and ^{13}C NMR spectroscopy combined with MS experiments.

The data from ^{13}C NMR proved the presence of only one double bond in all the unknown compounds.[48] Furthermore, the spectroscopic data indicated the characteristics of a tetrahydro-4,7-methanoindane structure. Based on the NMR and MS experiments the following structures were proposed: 1-*exo*,2-*endo*,4,6,7,8,8-heptachloro- (**8**), 1-*exo*,2-*endo*,3-*exo*,4,7,8,8-heptachloro- (**9**), 1-*exo*,2-*exo*,4,6,7,8,8-heptachloro- (**10**), 1-*exo*,2,2,4,5(or 6),7,8,8-octachloro- (**11**), 1-*exo*,2-*endo*,3-*exo*,4,6,7,8,8-octachloro- (**12**), 1-*exo*,2,2,4,5,6,7,8,8-nonachloro- (**13**), 1-*exo*,2-*exo*,3-*endo*,4,5(or 6),7,8,8-octachloro- (**14**), and 1-*exo*,2-*exo*,3-*exo*,4,6,7,8,8-octachloro-3a,4,7,7a-tetrahydro-4,7-methanoindane (**15**) (Scheme 4).

Based on the mass spectra, compounds **8** and **10** were the isomers of dihydroheptachlor ($C_{10}H_7Cl_7$) derived from pentachlorocyclopentadiene.[48] In addition, compound **9** was

suspected to be an isomer of dihydroheptachlor derived from tetrachlorocyclopentadiene.



Scheme 4

The ^{1}H NMR spectrum of **8** resembled that of *trans*-chlordane (**3**) except the singlet signal due to the olefin proton.[48] The ^{13}C NMR spectrum confirmed the presence of an olefin structure. Consequently, compound **8** was assigned the structure of *trans*-chlordane monodechlorinated at the double bond (C5=C6). The spectroscopic data of compound **10** resembled those of *cis*-chlordane (**2**) except for the presence of an olefin proton. Similarly, the structure of **10** was possibly due to a monodechlorinated

derivative of *cis*-chlordane. The relative configuration at the C5 and C6 positions of **8** and **10** was elucidated by NOE experiments.

Based on the ^{1}H and ^{13}C NMR data it was postulated that compound **9** had a symmetric structure.[48] The singlet peak (2H) in the olefinic region in ^{1}H NMR and the signal in the sp^2 range in ^{13}C NMR spectrum indicated the partial structure of –CH=CH– at the C5 and C6 positions. The chemical shifts and coupling constants between C1/C3 and C2 were similar to those of *trans*-nonachlor (**5**) and compound **12**. From the data, compound **9** was assigned a doubly dechlorinated structure at the olefin position of *trans*-nonachlor.

The MS data showed that compounds **12**, **14**, and **15** were isomers of chlordane derived from pentachlorocyclopentadiene.[48] For compound **12**, the pattern of ^{1}H NMR signals, except that of the olefin proton, was similar to that of *trans*-nonachlor (**5**). These data combined with data of the ^{13}C NMR spectrum suggested that **12** was the monodechlorinated (at the olefin position C5=C6) derivative of *trans*-nonachlor. Similarly, the spectroscopic data allowed the authors to define the structure of compound **15** as the monodechlorinated derivative of *cis*-nonachlor (**4**).

For compound **14**, the ^{1}H NMR and ^{13}C NMR spectra confirmed the presence of an olefinic proton.[48] The proton chemical shifts and coupling constants due to the cyclopentane ring (C1-C2-C3-C3a-C7a) were somewhat different from those of *cis*- and *trans*-nonachlor. However, the spin decoupling experiments showed that there was one proton on each carbon of the cyclopentane ring. Based on the relationship between the

vicinal coupling constant and the dihedral angle (Karplus equation[43,44]) the proton configuration of 1-*endo*,2-*endo*,3-*exo* on the cyclopentane ring was proposed.

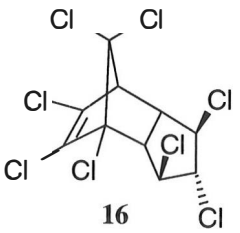
The mass spectra indicated that compound **13** was an isomer of nonachlor similar to the structures of *cis*- and *trans*-nonachlor.[48] Based on these observations combined with the ^{1}H and ^{13}C NMR data it was postulated that compound **13** had two chlorine substituents on C2 and two protons on C3.

From the mass spectra, it was concluded that compound **11** was an isomer of chlordane derived from pentachlorocyclopentadiene.[48] The chemical shifts and coupling constants in the ^{1}H NMR spectrum of compound **11** resembled those of compound **13** except a singlet signal due to the olefin proton. The ^{13}C NMR spectrum confirmed the presence of the olefin proton. Consequently, compound **11** was assigned the structure of compound **13** monodechlorinated at the double bond (C5=C6).

Recently, Karlsson *et al.*[49] isolated an unknown octachloro isomer designated U82 from technical chlordane. The structure of U82 was elucidated by ^{1}H NMR and mass spectrometry. The ^{1}H NMR parameters were extracted from experimental 500 MHz spectra using the PERCH program package[50,51].

Based on the mass spectra, U82 had a tetrahydro-4,7-methanoindane skeleton with five chlorine atoms at the 6-membered ring and three at the 5-membered ring.[49] The ^{1}H NMR spectrum indicated that there were no olefinic or geminal protons since the chemical shifts for all six protons were in the range of 3.4-4.2 ppm and all coupling

constants were below 11 Hz. In chlordane structures olefinic protons have shifts around 6 ppm.[39,48] Furthermore, in these molecules geminal protons have resonances in the range of 1.5-2.6 ppm with coupling constants of *ca.* -14 Hz. Homonuclear decoupling experiments were performed to assign the coupling partners in the spin system.[49] In addition, neighbourhood correlations were partly established by two-dimensional NOESY experiments. The overall NMR information allowed the authors to define the structure of U82 as 1-*exo*,2-*endo*,3-*exo*,4,5,6,8,8-octachloro-3a,4,7,7a-tetrahydro-4,7-methanoindane (**16**, Scheme 5). So far, U82 is the only identified cyclodiene-type chlordane component having a hydrogen atom instead of a chlorine at the bridge head atom C7.



16

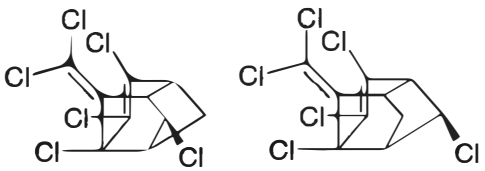
Scheme 5

2.1.4 "Caged" compounds

2.1.4.1 α-, β- and γ-chlordene

In 1972, Parlar and Korte[52] proposed structures for the two UV irradiation products of chlordene (**1**) arising from specific ring-opening reactions. Based on the ^{1}H NMR

parameters combined with the infrared (IR) and mass spectral (MS) data it was postulated that the compounds had structures **17a** and **18a** (Scheme 6).



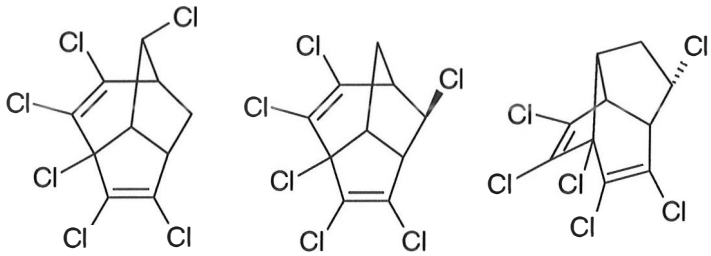
17a **18a**

Scheme 6

Three years later, Cochrane *et al.*[32] reported on the structural analysis of the technical chlordane components designated β- (**17**), γ- (**18**), and α-chlordene (**19**). These compounds are isomers of chlordene (**1**) but they do not possess the cyclodiene-type structure. The authors established by melting point studies and comparison of ^{1}H NMR and IR spectra that the two photoproducts, **17a** and **18a**, reported by Parlar and Korte[52] were identical with β- (**17**) and γ-chlordene (**18**).

Based on the chemical reactions and ^{1}H NMR and MS data, Cochrane *et al.*[32] concluded that the carbon skeleton of chlordenes **17**, **18** and **19** did not contain an exocyclic 1,1-dichloro double bond, as originally proposed by Parlar and Korte[52]. Additional evidence against the exocyclic double bond was obtained from ^{13}C NMR studies on UV irradiation products of **18** and **19**. In these spectra, no signals typical for a dichloromethane carbon occurred in the range of 96.8 to 107 ppm.

Cochrane *et al.*[32] postulated that both β-chlordene (**17**) and γ-chlordene (**18**) possess a tetrahydro-1,6-methano-1*H*-indene skeleton. In addition, it was found that **17** and **18** were stereoisomers differing only in the positioning of one chlorine atom. Consequently, the structure of **17** was defined as 2,3,3a,4,5,7-hexachloro-3a,6,7,7a-tetrahydro-1,6-methano-1*H*-indene and that of **18** as 2,3,3a,4,5,8-hexachloro-3a,6,7,7a-tetrahydro-1,6-methano-1*H*-indene (Scheme 7). Further, based on the above-mentioned studies it was postulated that α-chlordene (**19**) was 1,2,3,6,7,8-hexachloro-1,3a,4,5,6,6a-hexahydro-1,4-ethenopentalene (Scheme 7).



17 **18** **19**

Scheme 7

In 1976, as a continuation of their previous work[32], Gäb *et al.*[53] published ^{1}H and ^{13}C NMR spectral analyses of chlordenes **17**, **18** and **19** and their derivatives. The authors also employed additional spectroscopic methods such as MS, IR and ^{35}Cl nuclear quadrupole resonance (NQR). In this study, several of the ^{13}C NMR resonances for **17**, **18** and **19** were not observed or were not assigned. In addition, as in their earlier paper[32], the $^{1}H,^{1}H$ coupling constants below $J \leq 1.6$ Hz were not considered.

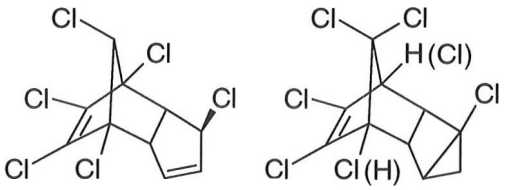
The following year Wilson and Sovocool[39] examined the ^{1}H and ^{13}C NMR spectra of chlordenes **17**, **18** and **19**. These results confirmed the structures originally proposed by Cochrane *et al.*[32] The ^{1}H NMR parameters presented by Wilson and Sovocool[39] differed from those of Gäb *et al.*[53] in the assignments of the signals of the protons H3a and H6a in **19**. In addition, Wilson and Sovocool[39] observed a long-range coupling between H6 and H7a in **17** (^{4}J = 2.1 Hz) but this coupling was not apparent between analogous protons in **18**. On the contrary, Gäb *et al.*[53] observed this coupling both in **17** (^{4}J = 2.3 Hz) and in **18** ($^{4}J \approx 2$ Hz).

Furthermore, the ^{13}C NMR chemical shift assignments for **17**, **18** and **19** had several deviations in comparison with results presented by Gäb *et al.*[53] These differences were in the assignments of the protonated carbons C3a, C4 and C6a in **19**, and C1, C6 and C7a in **17** and **18**. Since their carbon assignments were based on selective proton decoupling after complete analyses of the proton spectra, Wilson and Sovocool[39] claimed that their assignments were unequivocal. Later, the structures originally proposed by Cochrane *et al.*[32] for β- (**17**), and γ- (**18**), and α-chlordene (**19**) were confirmed by means of X-ray crystallography.[24,54,55]

2.1.4.2 Compound C, compound '2' and compound K

Originally, it was postulated that the constituent of technical chlordane designated compound C was a monochlorinated adduct of pentachlorocyclopentadiene and cyclopentadiene possessing the structure depicted by **20a** (Scheme 8).[56] In 1976, Cochrane and Greenhalgh[31] proposed the structure **20b** instead of **20a** based on a

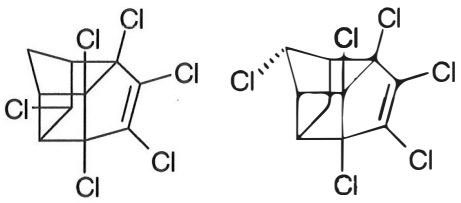
preliminary ^{1}H NMR experiment combined with IR, MS and synthetic studies (Scheme 8).



20a **20b**

Scheme 8

The following year, Gäb *et al.*[57] studied the structure of compound C by means of ^{1}H and ^{13}C NMR spectroscopy combined with IR and MS experiments. Up to that time retro Diels-Alder (RDA) fragments at *m/z* 236 and *m/z* 100 in the mass spectrum of compound C were considered as sufficient evidence for the presence of a cyclodiene-type structure.[31,56] However, Gäb *et al.*[57] concluded from the spectroscopic data that compound C was not a chlorinated 4,7-methano-1*H*-indene derivative, as was previously assumed[31,56] (structures **20a** and **20b** in Scheme 8). The data from ^{13}C NMR and IR showed that there was only one double bond in compound C, in contrast to two double bonds in **20a**.[57] In addition, the lack of olefinic resonances in the ^{1}H NMR spectrum indicated a chlorinated double bond. Based on these observations, Gäb *et al.*[57] proposed that compound C had a structure consistent with 3a,4,5,5a,5b,6-*exo*-hexachloro-1a,2,3,3a,5a,5b-hexahydro-1,3-methano-1*H*-cyclobuta[*cd*]pentalene (**20**, Scheme 9). It was noted that the ^{1}H NMR data did not distinguish between the structures **20** and **20c** (Scheme 9). However, the structure **20** was considered to be reaction mechanistically more probable.



20 **20c**

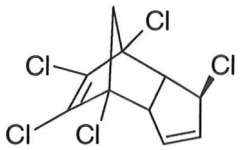
Scheme 9

Later, Smith *et al.*[58] and Dearth and Hites[24] confirmed the correctness of the structure **20** by X-ray crystallographic analysis. In addition, Smith *et al.*[58] applied two-dimensional ^{1}H,^{1}H and ^{13}C,^{1}H correlation techniques to ascertain the ^{1}H and ^{13}C NMR chemical shift assignments of compound C proposed by Gäb *et al.*[57] The ^{1}H NMR shift assignments were in agreement with those given by Gäb *et al.*[57] whereas the ^{13}C NMR chemical shift assignments had several deviations. However, both Gäb *et al.*[57] and Smith *et al.*[58] noted that their carbon assignments were not unequivocal.

In 1977, Sovocool *et al.*[30] proposed that a pentachlorinated substance known as compound '2' was a tetrahydro-4,7-methano-1*H*-indene derivative depicted by **21a** (Scheme 10). The intense RDA fragments at *m/z* 100 and *m/z* 202 in the mass spectrum were considered as sufficient evidence for the presence of a tricyclic cyclodiene-type structure.

In the early 1990s, Smith *et al.*[58] studied the structure of compound '2' by using ^{1}H and ^{13}C NMR techniques, including two-dimensional ^{1}H,^{1}H and ^{13}C,^{1}H, correlations. It was found that the ^{13}C NMR chemical shifts established the presence of a single double

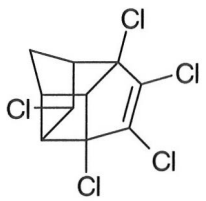
bond, while the $C_{10}H_7Cl_5$ formulation required a total of five rings or double bonds. Therefore, it was concluded that compound '2' was tetracyclic, rather than tricyclic.



21a

Scheme 10

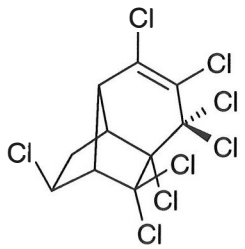
Smith *et al.*[58] noticed that the two-dimensional $^1H,^1H$ COSY spectrum of compound '2' included an array of cross peaks analogous to those observed for compound C (**20**, Scheme 9), together with cross peaks which indicated that H1 and H1a were coupled with the proton on C5b. Based on the above-mentioned couplings and the ^{13}C NMR data the authors proposed that the structure of compound '2' should be revised from **21a** (Scheme 10) to that of 3a,4,5,5a,6-*exo*-pentachloro-1a,2,3,3a,5a,5b-hexahydro-1,3-methano-1*H*-cyclobuta[*cd*]pentalene (**21**, Scheme 11).



21

Scheme 11

In 1977, Wilson and Sovocool[39] reported on the structure of a chlordane component referred to as compound K. Based on the ^{1}H NMR, ^{13}C NMR, and MS data combined with dechlorination experiments, the structure **22a** (Scheme 12) was suggested.



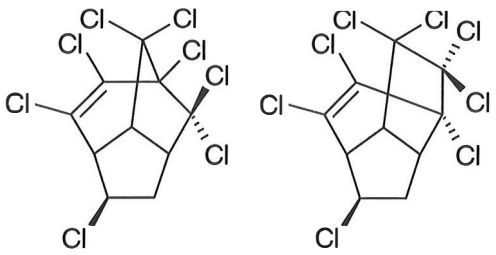
22a

Scheme 12

Later that year, Gäb *et al.*[59] examined the structure of compound K and its photodechlorination products by means of spectroscopic analysis (MS, ^{1}H NMR, and ^{13}C NMR) as well as by X-ray diffraction. Comparing the ^{1}H NMR data with those of α-chlordene (**19**), a precursor of compound K, only slight differences in the respective chemical shifts and coupling constants were observed. From the ^{13}C NMR data it was concluded that compound K had two chlorine substituted olefinic carbons in addition to two dichloromethylene, one chloromethylene and five protonated carbons.

The authors noted that the structural elements proved by the NMR data of compound K and its photodechlorination derivatives can be combined in a chemically reasonable way in many different structures.[59] However, the structures **22** and **22b** (Scheme 13) were considered most consistent with the spectroscopic data. The structure of compound K

was confirmed to be that of 3,4,5,6,7,7,8,8-octachloro-2,3,3a,6,7,7a-hexahydro-1,6-methano-1*H*-indene (**22**, Scheme 13) by X-ray crystallographic analysis.[59]



22 **22b**

Scheme 13

2.2 POLYCHLORINATED DIBENZOTHIOPHENES

2.2.1 General remarks

Polychlorinated dibenzothiophenes (PCDTs) are sulfur analogues of polychlorinated dibenzofurans (PCDFs).[60-62] PCDTs are environmentally and toxicologically interesting due to their structural resemblance to polychlorinated dibenzo-*p*-dioxins (PCDDs) and PCDFs, which are well-known artefacts ubiquitous in the present environment.[5]

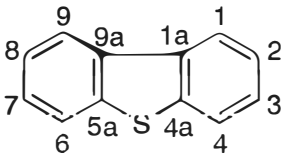
PCDTs can be formed in chemical processes similar to those that result in the formation of PCDFs.[60-62] Condensation of chlorothiophenols instead of chlorophenols could lead to the formation of PCDTs. Other possibilities are reactions of elemental sulfur or some reactive sulfur compounds with polychlorinated biphenyls (PCBs). The total number of

possible PCDT congeners is 135, from mono- to the octachlorinated compounds, including 38 TeCDTs and 28 PeCDTs.

The major known sources of PCDTs in the environment are combustion and metallurgy.[60-62] PCDTs are known to be formed in waste incineration,[63,64] thermal reaction of PCBs,[63] and in metal reclamation processes[65]. In addition, PCDTs are found, though in very low concentrations, in pulp mill effluents.[66,67] Among the other potential sources of PCDTs are automobile exhaust, wood combustion, oil/gas heating, chemical production of PCBs and trichlorobenzene sulfonates, and sewage sludge.[68]

2.2.2 Nomenclature

The IUPAC numbering[38] of the dibenzothiophene skeleton is given in Scheme 14.



Scheme 14

2.2.3 NMR spectroscopy

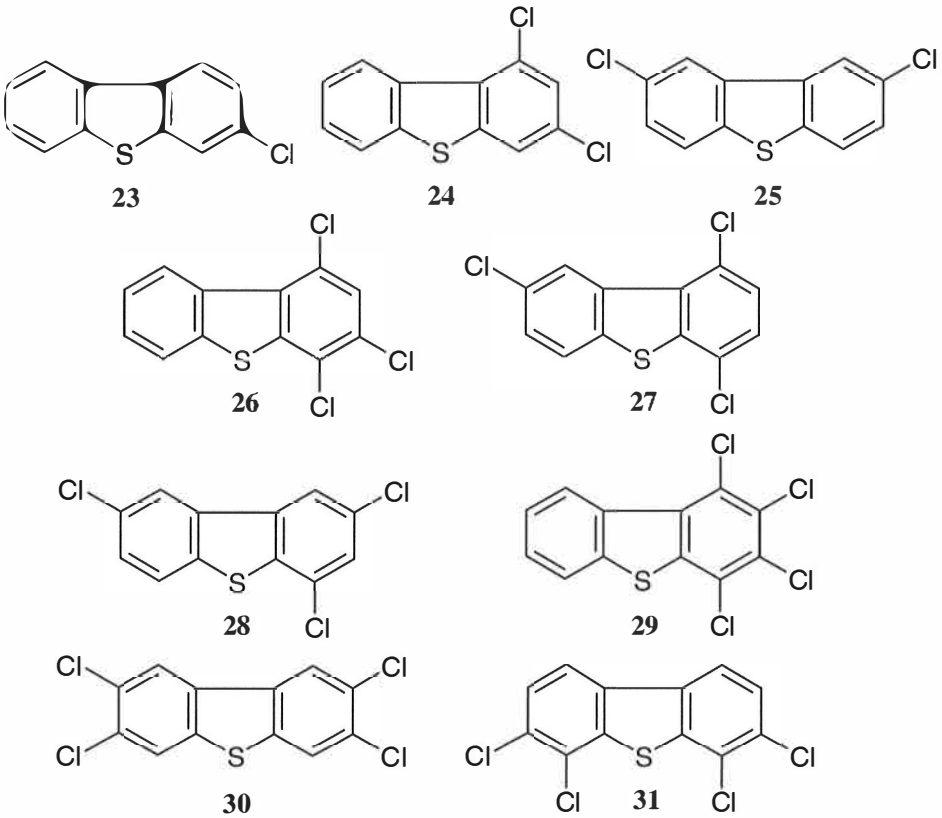
NMR spectroscopy offers a good method for differentiating polychlorinated DBTs.[60] The greatest drawback of ^{1}H and even more of ^{13}C NMR spectroscopy is their insensitivity in comparison with GC/MS. This insensitivity can be problematic, because

the synthetic work of these potentially harmful compounds is usually performed at milligram levels for safety reasons. Furthermore, interferences from solvent and/or impurity signals may strongly disturb the reliable detection and analysis of the NMR spectra. Therefore, special attention should be paid to the selection of a proper NMR solvent and optimization of other measuring conditions.

The NMR studies of PCDTs have been quite scarce. According to a literature search, only Sinkkonen *et al.*[67,69-71] have extensively used ^{1}H NMR spectroscopy in the identification of different PCDT congeners. The authors employed a method[72] originally developed for terpenoid type off-flavour compounds. By this method, a ^{1}H NMR spectrum was measured from *ca.* 5 µl of monoterpenoid compound collected directly in the NMR solvent by preparative GC. A thick-wall sample tube instead of a standard NMR tube was selected for the measurements in order to reduce the solvent volume from 700 µl to 100 µl. CD_2Cl_2 was found to be a suitable medium instead of $CDCl_3$, because its residual ^{1}H NMR signal is located at 5.30 ppm from TMS. Consequently, it did not overlap the ^{1}H NMR lines of aromatic protons, which resonated characteristically at 6.9-8.9 ppm. The ^{1}H NMR spectral assignment was mainly based on the synthetic procedure used, symmetry considerations of possible products, and characteristic intra-aromatic couplings. The ^{1}H NMR parameters were extracted from the experimental 270 MHz spectra using the spectral analysis program MAOCON[73].

By the above-mentioned method, the structures of nine chlorinated DBTs were determined.[67,69-71] These were 3-chlorodibenzothiophene (**23**), 1,3- (**24**) and 2,8-dichlorodibenzothiophene (**25**), 1,3,4- (**26**), 1,4,8- (**27**) and 2,3,8-

trichlorodibenzothiophene (**28**), and 1,2,3,4- (**29**), 2,3,7,8- (**30**) and 3,4,6,7-tetrachlorodibenzothiophene (**31**) (Scheme 15).



Scheme 15

Suárez *et al.*[74] have reported the ^{1}H and ^{13}C NMR parameters for 2,8-dichlorodibenzothiophene (**25**) as part of a study of solvent effects on the chemical shifts of halogenated sulphur containing aromatics. The ^{1}H NMR spectrum of **25** was recorded in DMSO-d_6 and $CDCl_3$ whereas the ^{13}C NMR experiment was carried out only in $CDCl_3$. The ^{1}H NMR spectrum was analyzed using the spectral fitting program LAOCOON3[41].

2.3 COMPONENTS OF TOXAPHENE MIXTURE

2.3.1 General remarks

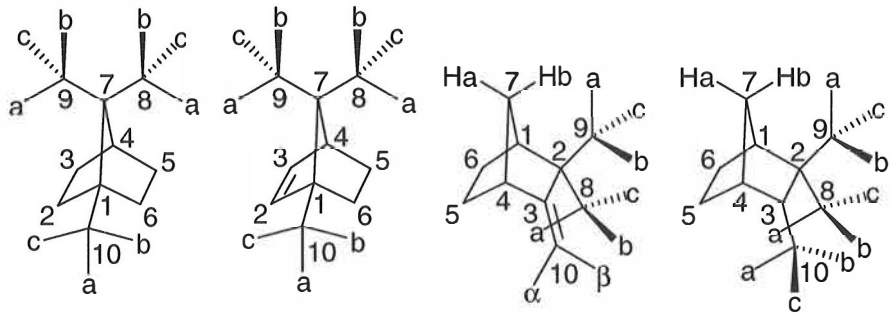
Toxaphene is a complex mixture of polychlorinated C_{10}-terpenes with a broad spectrum of pesticidal activity.[75-77] GC/ECD and GC/MS analyses revealed from at least 177[78] up to 670[79] compounds in the technical toxaphene mixture. These numbers are high but low in comparison to the theoretically possible number of congeners which exceeds 30 000.[80] Formerly, toxaphene has been one of the most widely used organochlorine insecticides in many parts of the world. During the years following its introduction in 1946, cumulative world use up to 1974 was 450 000 tons and cumulative use between 1950 and 1993 has been estimated at 1.33 million tons.[81] At present, use of toxaphene is restricted in many countries.[75-77] Despite its high use, toxaphene is one of the least chemically and toxicologically understood agrochemicals. Because of its persistence in the environment and its mobility, toxaphene nowadays can be considered as being dispersed throughout the world.[75]

Toxaphene is produced by the controlled chlorination of camphene.[75-77] The initial step is the ionic chlorination of the primary olefinic carbon of camphene, which leads via a Wagner-Meerwein pathway to a bornane skeleton.[82,83] The main intermediate products, 2-*exo*,10-dichlorobornane and 2-*exo*,10,10-trichlorobornane, are accompanied by other low chlorination products. Further radical chlorination of the mixture under UV light leads to highly chlorinated bornanes, the major part of toxaphene.[83] Polychlorinated camphenes and dihydrocamphenes are the most plausible side products. In addition,

some traces of polychlorinated bornenes have been observed in technical mixtures.[84] Finally, the presence of tricyclic components has also been discussed.[85] Most toxaphene congeners are chiral and exist in two enantiomeric forms.[77,86]

2.3.2 Nomenclature

Initially, toxaphene was the trademark of the product manufactured by Hercules Inc.[77] However, due to the non-restricted use of the trademark, toxaphene has become a general term for this pesticide. In this work, single components of toxaphene are named according to the IUPAC nomenclature[38,77] for terpene hydrocarbons. The orientations of the substituents on C8, C9, and C10 are distinguished with letters a, b, and c.[86] The IUPAC numberings of bornane, bornene, camphene and dihydrocamphene skeletons are given in Scheme 16.



Scheme 16

2.3.3 Polychlorinated bornanes and bornenes

2.3.3.1 Structural analysis

^{1}H NMR spectroscopy is the most commonly applied tool for the structure elucidation of toxaphene congeners.[77] Since the early 1970s, the structures of about forty polychlorinated bornanes have been elucidated by ^{1}H NMR.[87-104] In some publications, ^{13}C NMR data are also given.[88,90,91,99,102] Some of these structures have been confirmed by X-ray crystallographic investigations.[105,106]

Several suggestions concerning experimental conditions and rules concerning ^{1}H NMR chemical shifts and coupling constants of polychlorinated bornanes have been published.[75,77,87,88,93] This information is summarized in the following.

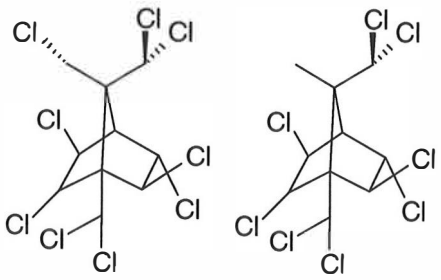
Because many resonances and couplings exist in the range of 2-7 ppm, the use of minimum 300 MHz (7.1 T) instruments and preferably 500 MHz (11.8 T) or higher field systems has been suggested to achieve an adequate signal separation.[77,93] Two coupling systems independent from each other are present in the bornane molecule, the six-membered ring (C1 to C6) and the carbons C8 and C9 at the bridge. Interactions between protons close in space from two coupling systems can be studied by NOE experiments. It has been reported that the solvent exerts a large influence on the chemical shifts of the protons in ^{1}H NMR spectra of polychlorinated bornanes.[75,77,93] $CDCl_3$ has been suggested since it usually results in a better dispersion of the signals at lower ppm values than C_6D_6.[75,77]

Typical ranges of the ^{1}H NMR resonances and coupling constants of polychlorinated bornanes are given in Table 1. Generally, protons of the $CHCl_2$ groups on bridge carbons C8 and C9 resonate at a lower field than $CHCl_2$ protons on the bridgehead carbon C10. For compounds containing one CH_2Cl and one $CHCl_2$ group on bridge carbons C8 and C9, it is possible to select the right position of the two groups using the following rule.[87] If there is an *exo*-proton on C2 or C6, "below" the CH_2Cl group, the doublet of doublets from the CH_2Cl group is located at a higher field than the doublet. If the doublet is located at a higher field, there is no *exo*-proton "below" the CH_2Cl group. In geminal CH_2 groups in the six-membered ring, *endo* protons usually resonate about 0.4 ppm downfield relative to *exo* protons. Adding one chlorine atom to the C8 and C9 leads to a downfield shift of the proton on the bridgehead carbon C4. For example, the (2 + 1) substitution at 2-*endo*,3-*exo*,5-*endo*,6-*exo*,8,8,9,10,10-nonachlorobornane (TOX9) (**32**, Scheme 17) leads to a downfield shift of *ca.* 0.6 ppm compared with 2-*endo*,3-*exo*,5-*endo*,6-*exo*,8,8,10,10-octachlorobornane (TOX8) (**33**, Scheme 17) with a (2 + 0) substitution.[93]

The long-range coupling between H8a and H9a on non-rotating chloro- and dichloromethyl groups varies from 1.9 to 3.1 Hz (Table 1). On the contrary, the compounds with a rotating methyl group only exhibit a small average coupling, typically less than 1.0 Hz. Bulky chlorine atoms require more space than hydrogen, and upon addition of chlorine, the bornane backbone may be deformed. Therefore, for instance a vicinal coupling between H3-*endo* and H4 is evident in some cases in spite of the unfavourable dihedral angle. In addition, a ^4J coupling between H4 and H6-*exo* is evident only in some polychlorinated bornanes.

Table 1 Typical ^{1}H NMR chemical shifts and coupling constants of polychlorinated bornanes.[75,77,88,93]

Proton position	Range of the chemical shift [ppm]	$^{1}H,^{1}H$ coupling	Range of the coupling constant [Hz]
$-CHCl_2$	6.0 – 7.2	Geminal on 2° carbons	>-15.0
$-CH_2Cl$	3.7 – 5.1	Geminal on 1° carbons	-11.0 – -14.5
$-CH_3$	1.8 – 2.0	Vicinal *syn* (H2-H3)	8.5 – 9.5
$-CH_2-$	2.7 – 3.8	Vicinal *anti* (H2-H3)	4.0 – 6.5
-C(H-*endo*)Cl-	4.1 – 6.0	Vicinal H4-H3-*exo*	2.5 – 5.0
-C(H-*exo*)Cl-	4.6 – 5.2	Vicinal H4-H3-*endo*	0.3 – 2.1
H4	2.6 – 3.7	Long-range W H8-H9	0.5 – 3.1
		Long-range H4-H6-*exo*	0.3 – 0.9
		Long-range H3-*exo*-H5-*exo*	1.8 – 2.2



32 **33**

Scheme 17

^{13}C NMR data of polychlorinated bornanes are quite scarce. In 1995, Hainzl *et al.*[88] reported that it was difficult to distinguish between the signals of the two quaternary carbons in position 1 and 7 even with DEPT experiments. It was observed that the chemical shift of C7 was generally in the range of 60-64 ppm in the case of compounds

with one Cl on C8 and one Cl on C9 or two Cl on C9, while that of compounds with one Cl on C8 and two Cl on C9 was in the range of 64-68 ppm. The chemical shift of a CCl_2 group in position 2 (90-98 ppm) was always shifted to higher ppm values than that of a group in position 5 (84-90 ppm). This was attributed to the direct neighbourhood of a quaternary carbon atom in the case of C2. Compounds with a CH_3 in position 8 or 9 were recognized by a signal at 10-20 ppm.

Polychlorinated bornenes have been detected by GC/MS[78,107,108] and by LC/FTIR[84], although no single polychlorobornene has been isolated directly from the technical mixture. According to a literature search, Turner *et al.*[103] and Parlar[109] have reported some preliminary ^1H NMR spectral analyses for polychlorinated bornenes produced by dehydrochlorination of individual polychlorinated bornanes.

2.3.3.2 Structural constraints and conformation on C8, C9, and C10

In 1993, Vetter[80] calculated the number of theoretically possible polychlorinated bornanes, including bornane itself, as 32768 (16128 pairs of enantiomers and 512 achiral structures). This number was obtained as the product of the possibility to chlorinate nine positions in the six-membered ring with a variety of 2 and three positions of the primary carbons C8, C9, and C10 with a variety of 4 giving $2^9 \times 4^3 = 32768$ possible compounds. However, the real number in technical toxaphene is smaller due to the synthetic mechanisms and steric hindrance as discussed by Hainzl *et al.*[110] and Vetter and Scherer[86].

For example, three chlorine atoms on primary carbons C8, C9, and C10 are not likely and neither are two chlorine atoms on both C8 and C9.[86,110] A chlorine atom on bridgehead carbon C4 has not been observed and four chlorine substituents on C2/C3 as well as C5/C6 have not been found either. The synthesis pathway via 2-*exo*,10-dichlorobornane means that it is only possible to form bornanes possessing one chlorine atom in the 2-*exo* position and at least one chlorine atom in position 10. The so-called "bridge and *exo*" rule states that $CHCl_2$ groups on C8 require an *exo*-chloro on C6, and $CHCl_2$ groups on C9 an *exo*-chloro on C2.[86] Furthermore, two *endo*-chlorine atoms next to each other or localized directly opposite each other in the six-membered ring are not likely.[110] These restrictions permit a maximum degree of chlorination of only eleven in a bornane molecule. However, Saleh[75] has reported the presence of dodecachlorobornanes in a technical toxaphene mixture.

In 1994, Frenzen *et al.*[105] published an X-ray structure analysis of three polychlorinated bornanes. The authors suggested that an assignment of the orientation of the chlorine substituents on the primary carbons C8, C9, and C10 is necessary for an exact description of the structure. These orientations can be distinguished with letters a, b, and c (see Scheme 16, p. 34). However, other studies claim that this is not necessary because rotations about the C7-C8, C7-C9, and C1-C10 bonds are not restricted at physiological temperatures (no stable atropisomers exist).[86,111] Nevertheless, these conformers on the primary carbons seem to be the key to the understanding of the persistence of polychlorinated bornanes, as discussed by Vetter and Scherer[86].

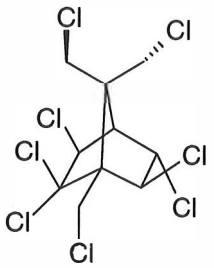
Hainzl *et al.*[110] and Vetter and Scherer[86] have formulated restrictions on the variety of conformations on the primary carbons C8, C9, and C10. For example, the positions towards the six-membered ring, *i.e.* 8a and 9a, are always substituted with a proton. Consequently, chlorine atoms are only possible at 8b, 8c, 9b, and 9c. The "a"-protons and *exo*-protons in the six-membered ring are close in space. Therefore, their interactions can be studied by NOE experiments. Furthermore, if there are two chlorine atoms on a bridge carbon (C8 or C9) and two on bridgehead C10, the following influence on the orientation is seen: (i) two chlorine atoms on C8 result in 8b,8c orientation together with 10a,10c and (ii) two chlorine atoms on C9 result in 9b,9c orientation together with 10a,10b. If a third chlorine atom is added on the second carbon of the bridge, it is always positioned behind the C8-C7-C9 plane, *i.e.* it has a "c"-orientation. According to Vetter and Scherer[86], this is a consequence of the "bridge and *exo*" rule (see above). The additional chlorine atom is preferably as far as possible away from the *exo*-chlorine atom vicinal to C1.

Based on the above-mentioned observations, Vetter and Scherer[86] assigned the optimal conformers for polychlorinated bornanes with five chlorine substituents on primary carbons as follows: (i) if there is an 8,8,9,10,10 substitution, the optimal conformation is 8b,8c,9c,10a,10c and (ii) in the case of an 8,9,9,10,10 substitution, the optimal conformation is 8c,9b,9c,10a,10b. Furthermore, polychlorinated bornanes with one chlorine atom on both C8 and C9 (8,9,10 or 8,9,10,10 substitution) have one in front of the C8-C7-C9 plane ("b"-orientation) and the other behind it ("c"-orientation). The chlorine substituent on a bridge carbon above 2-*exo* or 6-*exo* chlorine prefers "b"-orientation, while the other chlorine is in "c"-orientation.

2.3.3.3 Semiempirical calculations

In addition to NOE experiments and X-ray crystallographic investigations, information about the orientation of the chlorine substituents on C8, C9, and C10 can be obtained by molecular modelling. In 1994, Vetter *et al.*[93] performed semiempirical calculations for 2,2,5-*endo*,6-*exo*,8,9,10-heptachlorobornane (Toxicant B) (**34**, Scheme 18), 2-*endo*,3-*exo*,5-*endo*,6-*exo*,8,8,10,10-octachlorobornane (TOX8, **33**) and 2-*endo*,3-*exo*,5-*endo*,6-*exo*,8,8,9,10,10-nonachlorobornane (TOX9, **32**) using the AM1 method[112]. In order to obtain structures with minimum heat of formation (ΔH_f) values, energy profiles were recorded by rotation of the methyl groups in steps of 120° about the C7-C8, C7-C9, and C1-C10 bonds, respectively. A total of $3^3 = 27$ possible conformers were modelled. The geometries of the resulting starting structures were freely optimized by minimizing the heat of formation with the AM1 method.

The optimal conformer of Toxicant B (**34**) had an 8c, 9b, and 10a orientation of the chlorine atoms on the primary carbons.[93] This orientation agreed with the published X-ray data of **34**.[106] A second energy minimum had an 8b, 9c, and 10a orientation.[93] This conformer was only 2.3 kJ mol^{-1} higher in energy compared with the optimal conformer of **34**.



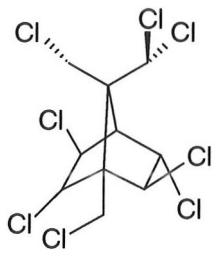
34

Scheme 18

An activation barrier of *ca.* 40 kJ mol^{-1} separated the most stable conformation of TOX9 (**32**) and TOX8 (**33**) from the next deepest local minimum.[93] This second minimum was 17 kJ mol^{-1} higher in energy than the structure of minimal energy. In both cases, the rotation about C1-C10 resulted in an energy minimum with hydrogens in position 10b and the chlorine atoms in positions 10a and 10c. The distance information extracted from the NOE experiments was in agreement with the energy minimized AM1 structures, *i.e.* NOE effects were found for all calculated H-H distances that were shorter than 3 Å. In addition, all $^{1}H,^{1}H$ coupling pathways correlated with the calculated structures. Later, Frenzen *et al.*[105] confirmed the predicted conformations for **32** and **33** by X-ray analysis.

In 1997, Vetter *et al.*[92] determined the optimal conformations for 2-*endo*,3-*exo*,5-*endo*,6-*exo*8,8,9,10-octachlorobornane (**35**, Scheme 19) using the semiempirical AM1 method. The conformer with an 8b,8c,9c,10c orientation of the chlorine atoms represented the structure with the lowest heat of formation. Two further conformers with 8b,8c,9c,10a and 8b,8c,9b,10a orientation were in energy only 2.76 and 5.23 kJ mol^{-1}, respectively, higher than the optimal conformer. The remaining 24 conformers

were not considered relevant as they had ΔH_f values more than 60 kJ mol^{-1} higher. The Boltzmann distribution of the three conformers mentioned above was predicted to be 69% (8b,8c,9c,10c), 23% (8b,8c,9c,10a), and 8% (8b,8c,9b,10a) at 295 K (22 °C). However, due to a lack of stable atropisomers, only one set of signals was found in the ^{1}H NMR spectrum of **35** at 21 °C. Furthermore, this interconversion was proposed as an explanation for the absence of NOE from H10b to H8a although the distance through space was only 2.7 Å, like in the case of TOX9 (**32**).

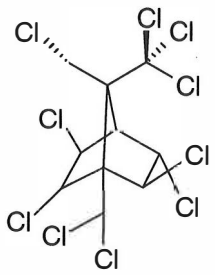


35

Scheme 19

Because of steric hindrance, three chlorine atoms on C8, C9, and C10 are not likely to exist on polychlorinated bornanes as discussed by Hainzl *et al.*[110] and Vetter and Scherer[86] (see above). To prove this assumption with the AM1 method, Vetter and Scherer[86] started from TOX9 (**32**) and added one more chlorine atom to C8 resulting in the hypothetical 2-*endo*,3-*exo*,5-*endo*,6-*exo*,8,8,8,9,10,10-decachlorobornane (**36**, Scheme 20). The favourable conformation of **36** had a heat of formation of –173.4 kJ mol^{-1}. This value was much higher than that of **32** (-245.7 kJ mol^{-1}). The authors found significant deformation of the bornane skeleton for the hypothetical decachlorobornane (**36**). The deformation in **36** resulted in additional great overlaps of the van der Waals

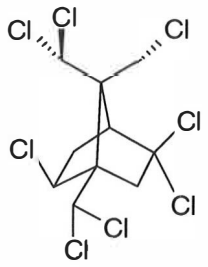
radii of neighbouring chlorine atoms over the whole molecule. It was speculated that low-chlorinated bornanes might be able to compensate for additional chlorine atoms by minor deformations or making space by going to another local minimum. On the contrary, high-chlorinated bornanes are greatly affected by minor changes and one additional chlorine atom may cause great strains in the molecule and a significant increase in energy.



36

Scheme 20

In 1999, Vetter and Scherer[113] used ΔH_f values to explain the persistence and lability of compounds of technical toxaphene. After determination of the energetically favoured conformations, ΔH_f values were obtained for 14 polychlorinated bornanes using the AM1 method. Low heats of formation were found for molecules with exclusive *endo-exo-endo-exo* substitution on the six-membered ring. All polychlorinated bornanes identified as persistent congeners had low ΔH_f values, except 2-*exo*,5,5,8,9,9,10,10-octachlorobornane (**37**, Scheme 21). Furthermore, it was found that geminal chlorine atoms significantly reduce the heat of formation.



37

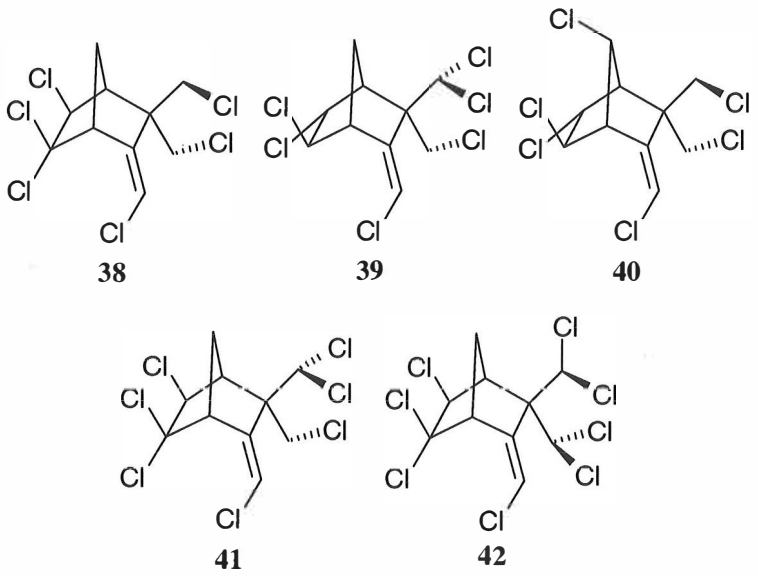
Scheme 21

2.3.4 Polychlorinated camphenes and dihydrocamphenes

Polychlorinated camphenes are the most important unsaturated C_{10}-components in technical toxaphene.[84] In 1995, Hainzl *et al.*[88] reported the isolation and identification of five polychlorinated camphenes by spectroscopic methods (^{1}H and ^{13}C NMR, IR, MS) from photochemically modified technical toxaphene. These were 5,5,6-*exo*,8,9,10α- (**38**), 5-*exo*,6-*endo*,8,9,9,10α- (**39**) and 5-*exo*,6-*endo*,7a,8,9,10α-hexachlorocamphene (**40**), 5,5,6-*exo*,8,9,9,10α-heptachlorocamphene (**41**), and 5,5,6-*exo*,8,8,9,9,10α-octachlorocamphene (**42**) (Scheme 22). Later, the structure of **41** was confirmed by means of X-ray analysis.[114]

The ^{1}H and ^{13}C NMR spectra of **38-42** revealed the presence of olefin structure.[88] The chemical shift of the olefinic proton H10β was in the range of 6.5-6.7 ppm. The quaternary sp^2 carbon C3 had resonances that ranged from 135 to 140 ppm, while the tertiary sp^2 carbon C10 had resonances that ranged from 119 to 126 ppm. Of the sp^3 protons, the geminal protons in position 7 resonated in the range of 1.9-2.5 ppm.

Otherwise, the ^{1}H and ^{13}C chemical shift ranges were very similar to those of polychlorinated bornanes.

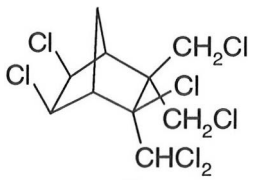


Scheme 22

The geminal and vicinal $^{1}H,^{1}H$ couplings were within the typical ranges[42] for this type of bicyclic compounds.[88] Due to several planar W arrangements, many significant long-range (^{4}J) couplings were observed in **38-42**: J(1,4), J(5-*endo*,7b), J(6-*endo*,7b), and J(8a,9a). In addition, the four bond allylic coupling between H4 and H10β with the range of 0.5 to 0.8 Hz was apparent in all camphenes examined.

Polychlorinated dihydrocamphenes are a further class of compounds detected in toxaphene.[115] In the 1970s, Landrum *et al.*[85,116] isolated the first isomer from technical toxaphene. Its structure was identified as 3-*exo*,5-*exo*,6-*exo*,8,9,10,10-

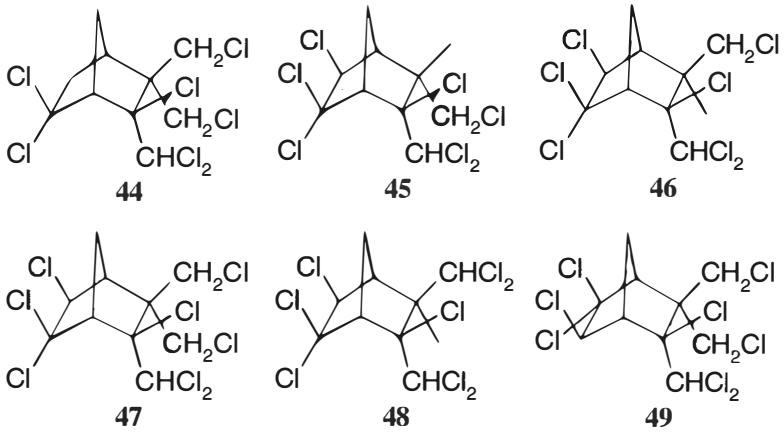
heptachlorodihydrocamphene (**43**, Scheme 23) by X-ray crystallography.[85] In addition, the ^{1}H NMR parameters with tentative chemical shift assignments were presented.



43

Scheme 23

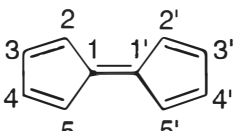
In 1996, Tribulovich *et al.*[115] examined the NMR spectra of six polychlorinated dihydrocamphenes (**44-49**, Scheme 24). The structure of compound **44** was unambiguously determined as 3-*exo*,5,5,8,9,10,10-heptachlorodihydrocamphene. For compounds **45-49** the positions of the CH_3, CH_2Cl, and $CHCl_2$ groups, as well as that of a remaining proton at 5-*endo* or 6-*endo* position, were not finally determined. ^{1}H NMR spectra of **44-49** differed from those of polychlorinated bornanes. A characteristic pattern was observed for all compounds: (i) singlet of H10 at 6.77-6.89 ppm, (ii) two broad singlet signals of H1 and H4 at 3.49-3.73 ppm and at 2.43-2.97 ppm, respectively, and (iii) two broad doublets of H7a and H7b at 2.28-2.49 ppm and at 2.67-2.70 ppm, respectively. Such behaviour was reported to be in accordance with the NMR spectra of polychlorinated camphenes. However, the ^{13}C NMR spectra of compounds **46-49** showed an absence of sp^2 carbons. Furthermore, DEPT and ^{1}H,^{1}H decoupling experiments for compounds **46** and **48** also were in accordance with the given dihydrocamphene structures.



Scheme 24

2.4 PENTAFULVALENE AND ITS DERIVATIVES

Pentafulvalene (1,1'-bicyclopentadienylidene) (**50**, Scheme 25) is a challenging compound especially with respect to aromaticity, π-electron delocalization and charge transfer distribution in cross-conjugated molecules.[117] From a theoretical point of view, fulvalene systems show interesting electronic excitation properties.[118] Furthermore, because electronic excitation energy is also included in the paramagnetic term of the NMR shielding tensor, the ^{13}C NMR chemical shifts of pentafulvalene (**50**) are particularly interesting.[119]



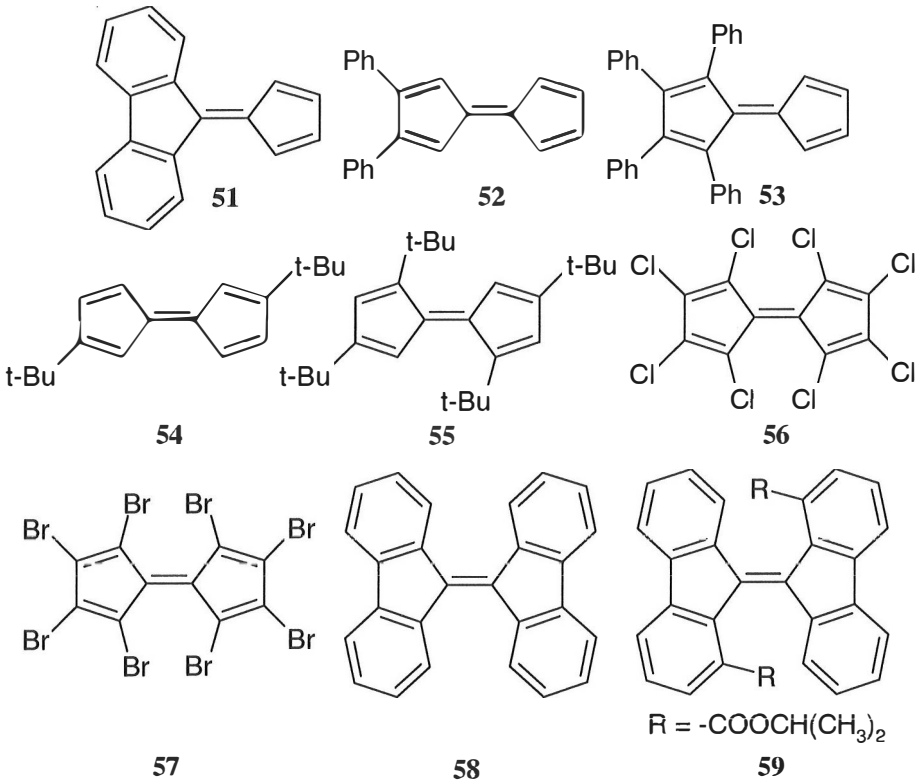
50

Scheme 25

Synthetic routes leading to pentafulvalene (**50**) are (i) dimerization of cyclopentadienylidene (carbene) generated by UV-photolysis from 1-diazocyclopenta-2,4-diene in various matrices at low temperatures,[120-122] (ii) oxidative coupling of cyclopentadienide with copper(II) chloride,[123,124] (iii) reaction of cyclopentadienide with iodine, converting the resulting unstable dihydrofulvalene to the dilithium salt with butyllithium, and finally treating the salt with oxygen[125] and (iv) oxidation of dicyclopentadiene in alkaline methanol by exposure to air[126]. Pentafulvalene (**50**) is an extremely unstable compound that can only be handled in very dilute solutions under an inert atmosphere at very low temperatures.[122] However, substituted pentafulvalenes are known to be stable at room temperature. The structures of some of these compounds have been determined by means of NMR spectroscopy[127-129] and/or X-ray crystallography[129-134] (**51**-**59**, Scheme 26).

NMR studies of pentafulvalene (**50**) were practically impossible until 1970 due to its instability and the low sensitivity of early continuous wave (CW) NMR spectrometers.[117] In 1982, Brand *et al.*[129] reported a "^{1}H NMR signal" at 6.47 ppm (in THF-d_8 at –60 °C) without analysis, whereas ^{13}C NMR data were missing. In the early 1980s, a new synthetic method made available solutions of pure **50** more concentrated than 0.3 M and allowed an investigation of its NMR spectra in more detail.[123]

In 1986, Escher *et al.*[117] published the complete set of ^{1}H and ^{13}C NMR parameters for **50**. In the 300 MHz ^{1}H NMR spectrum in CD_2Cl_2, complex multiplets were centered at 6.69 and 6.59 ppm. In the ^{13}C NMR spectrum in $CDCl_3$, three signals at 122.0 (C2, C5), 136.0 (C3, C4) and 147.9 ppm (C1) were observed.



51 **52** **53**

54 **55** **56**

57 **58** **59**

Scheme 26

Bond length alternation is a good qualitative criterion for deciding whether a cyclic conjugated molecule is olefinic or aromatic.[135] Information about bond length alternation may be provided by NMR spectroscopy since the size of the vicinal coupling constant, $^{3}J(^{1}H,^{1}H)$, is strongly dependent on bond lengths.[42] In the case of **50**, the difference of $^{3}J(^{1}H,^{1}H)$ values (J(H1,H2) = 5.41 Hz and J(H2,H3) = 1.99 Hz) was very pronounced.[117] Therefore, pentafulvalene (**50**) can be classified as a non-aromatic compound with strongly alternating bond lengths and a highly localized π-system.[117,124,135,136]

In the early 1990s, Neuenschwander and Bönzli[135] studied the NMR spectra of a series of pentafulvenes and pentafulvalenes. The authors proposed a simple criterion for estimating the extent of π-bond delocalization (or aromaticity) in this type of molecules. The proposition was based on the fact that changes of bond lengths (induced by exocyclic substituents) were reflected by systematic changes of $^{3}J(^{1}H,^{1}H)$ values, so that linear correlations of Hammett substituent constants (σ_p^+) *vs.* $^{3}J(^{1}H,^{1}H)$ were obtained. Plots of that type were found very useful for determining the extent of π-delocalization of various pentafulvenes and pentafulvalenes.

3 EXPERIMENTAL

3.1 AIM OF THE PRESENT STUDY

The aim of the present study was to investigate the structural properties of selected POPs and related compounds using NMR spectroscopy. For compounds whose chemical structures are well characterized, the intent was to confirm the previously reported ^{1}H and ^{13}C NMR chemical shift assignments. The basic approach in the structural analysis involved utilization of modern one- and two-dimensional NMR techniques to obtain experimental data. For the precise ^{1}H NMR parameters, computer aided spectral analysis was employed. The NMR data, comprising the measured chemical shifts, the spin-spin coupling constants and the NOE/ROE enhancement correlations, formed the basis for the structural analysis. In some cases, quantum chemical methods were used to support the structural elucidation. Whenever good quality crystals were available, the structures were determined by means of single crystal X-ray crystallography.

3.2 METHODS

3.2.1 NMR spectroscopy

All NMR experiments (DQF $^{1}H,^{1}H$ COSY,[137] PFG[138] $^{1}H,^{13}C$ HMQC,[139,140] PFG $^{1}H,^{13}C$ HMBC,[141] 2D INADEQUATE,[142,143] $^{1}H,^{1}H$ NOESY,[144,145] and $^{1}H,^{1}H$ ROESY[146]) were performed on $CDCl_3$ solutions with a Bruker Avance DRX 500 NMR spectrometer

equipped with a z-gradient accessory and an inverse (or a direct) detection 5 mm diameter probehead working at 500.13 MHz for ^{1}H and 125.77 MHz for ^{13}C, respectively. The detailed experimental conditions for the NMR measurements are described in publications I-V. The ^{1}H NMR spectral analyses were performed with WIN-NMR[147] and WIN-DAISY[148] software (publications I and III) or with the PERCHit iterator under PERCH software[50,51,149] (publications IV and V) using a Pentium 233 MHz personal computer. The sensitivity tests described in publication I were carried out using 5 mm diameter and 15 mm bottom length symmetrical NMR microtubes (Shigemi) which are magnetic susceptibility matched for $CDCl_3$.

3.2.2 Single crystal X-ray crystallography

The crystal structure data were recorded with a Nonius KappaCCD diffractometer. The structures were solved by direct methods (SHELXS-97)[150] and refined on F^2 by full-matrix least-squares techniques (SHELXL-97)[151]. The detailed X-ray crystallographic data are given in publications II, IV and V.

3.2.3 Quantum chemical methods

All full geometry optimizations were done at the *ab initio* HF/6-31G* level.[III,IV,V] Rotation barriers were characterized at the HF/6-31G* level (Publication IV) or with the semiempirical AM1 method[112] (Publication V). In publication III, the gauge-including atomic orbital (GIAO)[152] method was employed at the DFT BPW91/6-311G* and *ab initio* HF/6-311G* levels to calculate ^{13}C isotropic shielding constants (σ) for TMS and

for three PCDTs. In publication V, the GIAO method was used at the DFT B3LYP/6-311G* level to calculate $\sigma(^{13}C)$ for TMS and for nine polychlorinated dihydrocamphenes. All calculations were performed using the Gaussian 98 software[153] on Silicon Graphics Origin2000 or IBM RISC/6000 320 workstations. The detailed procedures are given in the above-mentioned publications.

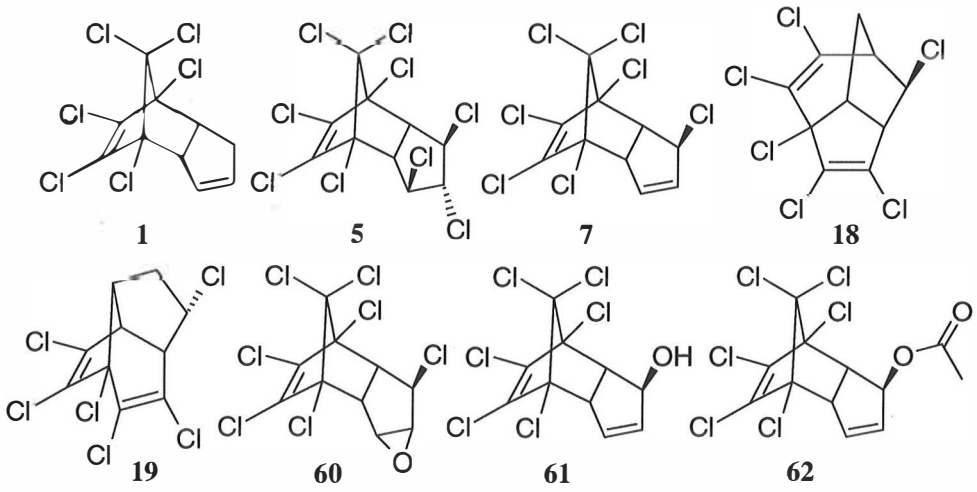
3.3 COMPONENTS OF TECHNICAL CHLORDANE

3.3.1 NMR spectroscopy

Two-dimensional homonuclear NMR techniques, DQF COSY, NOESY and INADEQUATE, and proton detected, two-dimensional heteronuclear chemical shift correlation techniques, HMQC and HMBC, were applied in the characterization of five C_{10}-based chlordane compounds, chlordene (**1**), *trans*-nonachlor (**5**), heptachlor (**7**), γ-chlordene (**18**) and α-chlordene (**19**).[I] In addition, complete ^{1}H and ^{13}C NMR parameter sets of three oxy derivatives, heptachlor *exo*-epoxide (**60**), 1-*exo*-hydroxychlordene (**61**) and its acetate (**62**), were determined. For compounds **61** and **62**, the NMR parameters were reported for the first time. Further, the $^{n}J(^{1}H,^{1}H)$ data for **5** have not been previously published. The structures of the above-mentioned compounds are given in Scheme 27.

As discussed in Chapter 2.1, a variety of NMR chemical shift assignments have been published for compounds **1**, **5**, **7**, **18** and **19**.[32,39,45,47,48,53] These results partly contradict those obtained in this work.[I] In order to obtain a reliable basis for our spectral

assignments, a COSY-like[142] 2D INADEQUATE spectrum was recorded for heptachlor (**7**) (Publication I, Figure 2). In this experiment, almost the entire carbon skeleton was mapped. However, due to the extremely low sensitivity of 2D INADEQUATE, no cross peaks indicating carbon-carbon connectivity were observed for C5, C6 and C8. An HMBC correlation map revealed connectivity between H3a and C5 and verified the correct assignment for C5 and C6 of **7**. This and the ^{1}H NMR chemical shift assignment for **7** are in agreement with those given by ApSimon *et al.*[47] Furthermore, the ^{1}H and ^{13}C NMR parameters for chlordene (**1**) presented by ApSimon *et al.*[47] and those for heptachlor epoxide (**60**) reported by Donnelly *et al.*[154] are consistent with our results.



Scheme 27

On the contrary, the ^{1}H and ^{13}C NMR chemical shift assignments for *trans*-nonachlor (**5**), γ-chlordene (**18**) and α-chlordene (**19**) reported by Wilson and Sovocool[39] differ from our results in several points.[I] These differences are in the assignments of carbons

C3a/C7a and C1/C3 in **5**, carbons C1 and C8 in **18**, and protons H6a and H3a in **19**. Furthermore, the ^{13}C NMR data for **18** and **19** given by Gäb *et al.*[53] differ from our observations in the assignments of carbons C7a, C6, C1 and C8 in **18**, and C6a, C3a and C4 in **19**.

Among the compounds studied, **1**, **7**, **61** and **62** have the same carbon skeleton, thus forming a basis for the comparison of the substituent effects.[I] Introduction of an electronegative substituent at position 1' in **1** causes a clear downfield shift or α-effect on the proton H1, being 2.46 ppm in **7** (-Cl), 2.39 ppm in **61** (-OH) and 3.20 ppm in **62** (-$OCOCH_3$) (Publication I, Table 1). The corresponding β-effects induced at protons H2 and H7a in compounds **1**, **61** and **62** are less than 0.22 ppm. The same trends in the substituent effects are also apparent in the ^{13}C NMR data (Publication I, Table 2).

Since they form a proper subgroup, compounds **1**, **7**, **61** and **62** may be used to estimate the substituent effects on the spin-spin coupling constants (Publication I, Table 3).[I] Typically, introducing an electronegative substituent, chlorine or hydroxy, into position 1' in chlordene (**1**) decreases the absolute values of all spin-spin coupling constants.[44] The most significant change was observed in the allylic coupling between H1 and H3.[I] The vicinal coupling between H1 and H7a also exhibited large changes upon substitution at C1.

3.3.2 Sensitivity tests

Sensitivity tests (^{1}H,^{13}C, HMQC and HMBC) were performed for heptachlor (**7**), γ-chlordene (**18**) and α-chlordene (**19**) using symmetrical $CDCl_3$ matched Shigemi micro-NMR tubes.[I] A 500 MHz (^{1}H) spectrometer was equipped with a direct 5 mm diameter multinuclear probehead. The concentrations of the samples were 4.39 mM for **7**, 3.07 mM for **18** and 3.10 mM for **19**. This means that the amount of each sample was less than 0.5 mg. The ^{1}H NMR experiments gave good quality spectra in all cases. In ^{13}C NMR experiments run overnight, all carbons of **18** and **19** were clearly detected whereas in **7** carbons C4, C6, C7 and C8 did not gave reliable resonance lines and C5 was only poorly visible. In the case of compound **7**, HMBC experiment revealed the signals of carbons C6 and C7. In the case of **18** and **19**, HMQC and HMBC gave complete shift correlation maps. By using an inverse 5 mm broad band probehead, reliable HMQC (experiment time 3 h) and HMBC (experiment time 12 h) shift correlation data for **7** were obtained.

The ^{1}H NMR spectral lineshape measured in micro-NMR tubes differs from a pure Lorenzian line.[I] This causes an increase in the percentage difference (*R*-factor) between the experimental and calculated spectrum of a computer based ^{1}H NMR spectral analysis performed by WIN-DAISY[148] program. The main reason for increased *R*-values is broad humps in spectral lines that, in spite of prolonged shimming, cannot be removed totally (Publication I, Figure 3). In the case of micro-NMR tubes, the *R*-values were below 3%, which according to WIN-DAISY manual are acceptable.

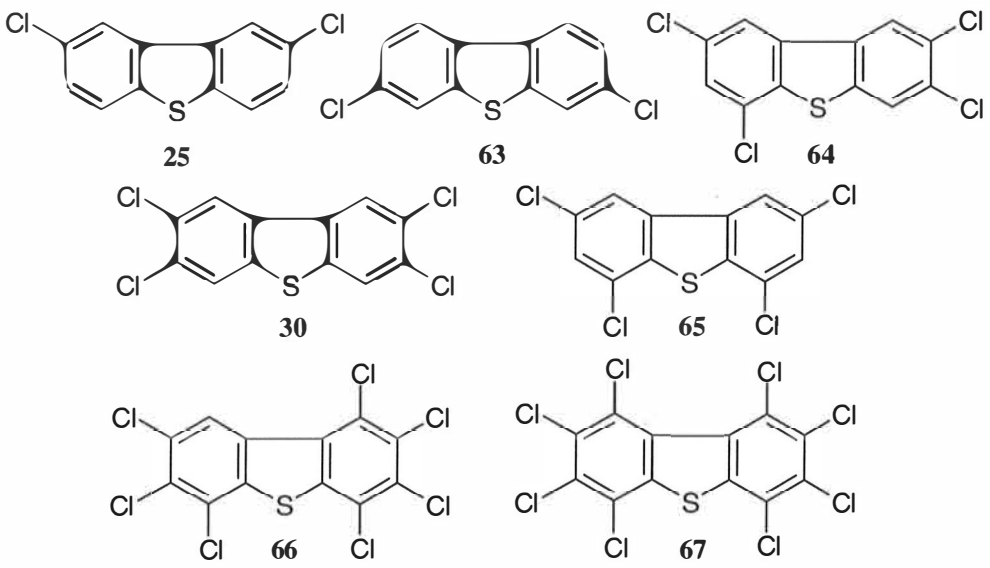
3.4 POLYCHLORINATED DIBENZOTHIOPHENES

3.4.1 NMR spectroscopy

^{1}H and ^{13}C NMR spectra for seven PCDTs, 2,8- (**25**) and 3,7-dichlorodibenzothiophene (**63**), 2,3,6,8- (**64**), 2,3,7,8- (**30**) and 2,4,6,8-tetrachlorodibenzothiophene (**65**), 1,2,3,4,6,7,8-heptachlorodibenzothiophene (**66**), and 1,2,3,4,6,7,8,9-octachlorodibenzothiophene (**67**), were measured.[III] The structures of these PCDTs are pictured in Scheme 28. For compounds **25**, **30** and **63**-**65**, complete ^{1}H and ^{13}C NMR chemical shift assignments were based on z-gradient selected inverse (proton detected) two-dimensional heteronuclear chemical shift experiments, HMQC and HMBC. For hepta- (**66**) and octachloro (**67**) congeners, the ^{13}C NMR chemical shift assignments were not possible solely on the basis of spectral data. The two main reasons for this were (i) low solubility and (ii) inefficiency of polarization transfer experiments. Therefore, in order to assign the ^{13}C NMR chemical shifts of **66** and **67**, quantum chemical methods were applied.

The ^{1}H NMR chemical shift assignment of symmetrically substituted 2,8- (**25**) and 3,7-dichloro-DBT (**63**) was straightforward via recognition of typical couplings between aromatic protons (Publication III, Table 1).[III] The $^{1}H,^{13}C$ HMQC correlation maps of **25** and **63** gave directly the assignment for protonated carbons (Publication III, Table 2). The assignment of *ipso*-carbons of **25** and **63** was based on $^{1}H,^{13}C$ HMBC experiments. It is known that in chlorobenzene all vicinal $^{1}H,^{13}C$ couplings are substantially larger than geminal $^{1}H,^{13}C$ couplings.[155] Therefore, it was possible to distinguish the

correlations transmitted by the vicinal and geminal $^{1}H,^{13}C$ couplings by selecting a 50 ms delay (corresponding to a 10 Hz coupling) for evolution of multiple bond coherences in **25** and **63**.



Scheme 28

In the unsymmetrically substituted 2,3,6,8-tetrachloro-DBT (**64**), the two coupling systems independent from each other were easily recognized by their different coupling constants (Publication III, Table 1).[III] A direct comparison of the chemical shifts of H1 and H4 of **64** with those of 2,3,7,8-tetrachloro-DBT (**30**) and H7 and H9 of **64** with those of 2,4,6,8-tetrachloro-DBT (**65**) revealed that substitution in one ring does not significantly influence the shifts in the other ring. This is also the case for the ^{13}C NMR chemical shift differences of these compounds. Jayalakshmi *et al.*[156] have reported similar findings for series of 2-substituted DBTs. By mutual comparison within the

subgroup of these tetrachlorinated DBTs, their chemical shifts were unambiguously assigned.

3.4.2 Quantum chemical methods

The ^{13}C NMR chemical shifts of **66** and **67** were calculated by the GIAO[152] method at the *ab initio* HF/6-311G* and DFT BPW91/6-311G* levels (Publication III, Table 3).[III] The mean deviation between the observed and predicted values was much less when calculated by DFT than by the *ab initio* method. However, both calculations predicted identical shift orders with the exception of C8 in **66** and C2 in **67**. For the sake of comparison, the shift order for 1,2,3,4,7,8,9-heptachloro-DBT was also calculated. It was found that 1,2,3,4,6,7,8-heptachloro-DBT (**66**) agreed better with the experimental NMR spectra than the 1,2,3,4,7,8,9-heptachloro isomer. In **67**, the most deshielded value predicted by both methods for C4a/C5a was in a good agreement with the experimental value. Unfortunately, we were not able to estimate the reliability of the assignment for the other five signals. The geometry optimization performed at the HF/6-31G* level predicted that the molecular skeleton of **67** is strongly twisted with a 29° C1-C1a-C9a-C9 dihedral angle. Therefore, the ^{13}C NMR chemical shifts of fully chlorinated rings in **66** and **67** are not directly comparable. However, the GIAO method even at the HF/6-311G* level correctly predicted the ^{13}C NMR chemical shift order of dibenzothiophene itself thus giving evidence for its reliability.

3.5 COMPONENTS OF TOXAPHENE MIXTURE

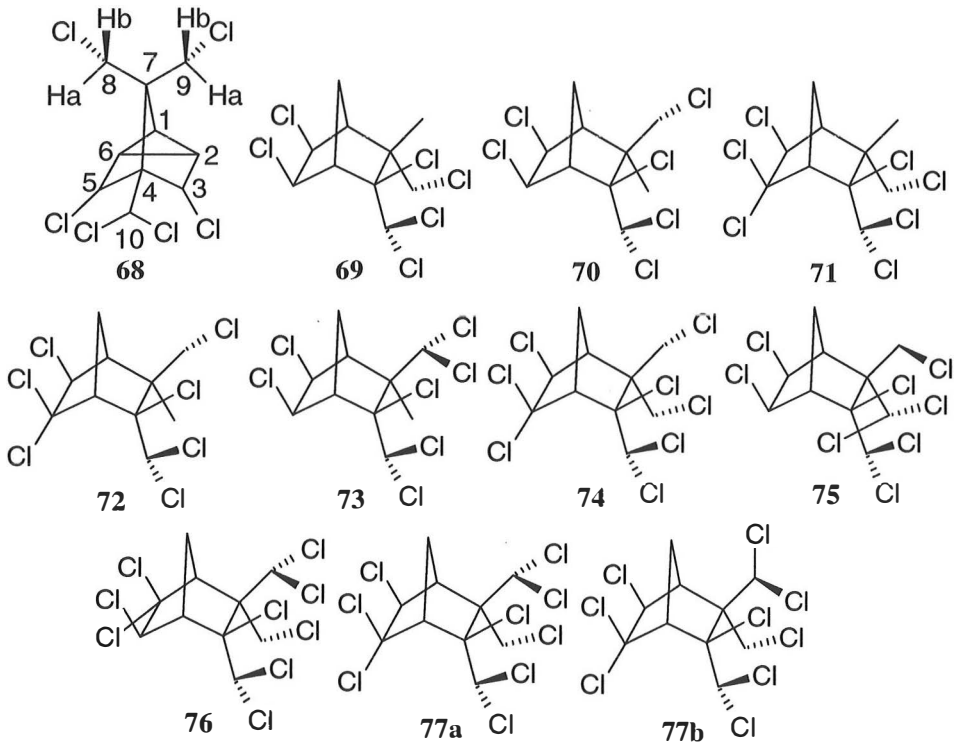
The structures of ten potential toxaphene congeners were studied. These were 3-*endo*,5-*endo*-dichloro-7,7-bis-chloromethyl-4-dichloromethyl-tricyclo[2.2.1.$0^{2,6}$]heptane (**68**)[IV], 3-*exo*,5-*exo*,6-*exo*,8,10,10- (**69**) and 3-*exo*,5-*exo*,6-*exo*,9,10,10-hexachlorodihydrocamphene (**70**), 3-*exo*,5,5,6-*exo*,8,10,10- (**71**), 3-*exo*,5,5,6-*exo*,9,10,10- (**72**) and 3-*exo*,5-*exo*,6-*exo*,9,9,10,10-heptachlorodihydrocamphene (**73**), 3-*exo*,5,5,6-*exo*,8,9,10,10- (**74**) and 3-*exo*,5-*exo*,6-*exo*,8,8,9,10,10-octachlorodihydrocamphene (**75**), and 3-*exo*,5-*exo*,6,6,8,9,9,10,10- (**76**) and 3-*exo*,5,5,6-*exo*,8,9,9,10,10-nonachlorodihydrocamphene (**77a**, major conformation; **77b**, minor conformation)[V]. The structures of these compounds are shown in Scheme 29.

3.5.1 Crystal structure

The crystal structures of compounds **68**[IV] and **70**[V] were determined by single crystal X-ray analysis (Figure 1). Suitable crystals were obtained by slow evaporation of a $CDCl_3$ solution.[IV,V]

The X-ray analysis of compound **68** revealed an unusual tricyclic structure where the two *endo*-position chlorine atoms, Cl3 and Cl5, are in close spatial proximity with each other (3.1951(7) Å) and near the neighbouring $CHCl_2$ group (Figure 1).[IV] In addition, both Cl8 and Cl9 are positioned behind the C8-C7-C9 plane. These findings are contradictory to those previously reported on polychlorinated bornanes (see section 2.3.3.2, p. 38).[86,110]

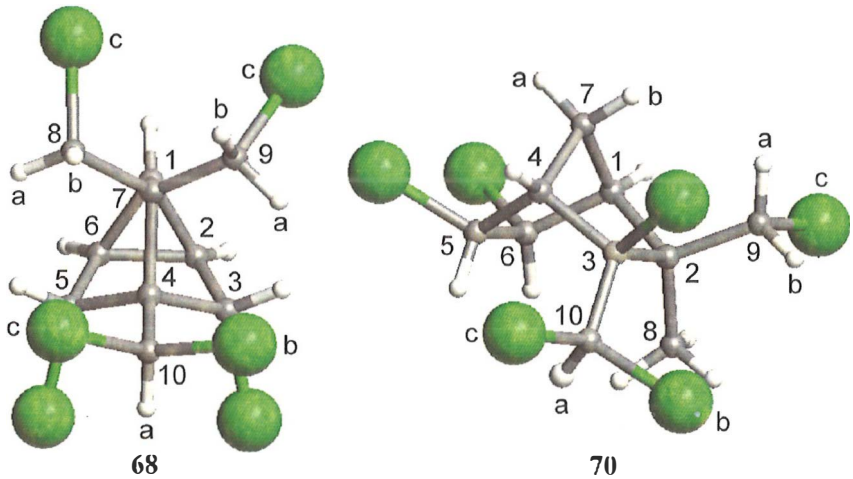
The X-ray analysis also revealed that the symmetry of **68** is distorted.[IV] A comparison of the dihedral angles C8-C7-C9-Cl9 (-102.53(16)°) and Cl8-C8-C7-C9 (-65.47(18)°) showed that Cl8 and Cl9 are twisted by different amounts from the C8-C7-C9 plane (Publication IV, Table 5). Furthermore, the angle C7-C9-Cl9 (114.28(14)°) is slightly larger than the angle C7-C8-Cl8 (110.47(13)°). A distortion of symmetry is also discernible on ^{1}H NMR parameters (liquid phase) and on the calculated structure (gas phase). Due to this asymmetry, compound **68** is chiral.



Scheme 29

The mean C-C bond length (1.514(3) Å) in the three-membered ring of **68** equals the mean bond length for cyclopropane (1.509(2) Å)[157].[IV] In the nortricyclene part of **68**,

bonds to C4 are longer and the bonds C2-C3 and C5-C6 are shorter than the single bond value of 1.541(3) Å[158] (Publication IV, Table 4). The most probable reason for this is the steric effect of the $CHCl_2$ group in position 10. The C7-C8 bond (1.534(3) Å) is considerably shorter than the C7-C9 bond (1.551(3) Å). Further, the C-Cl bonds (1.7754(19)-1.8030(18) Å) in **68** are appreciably longer than the single bond value of 1.767(2) Å[158]. The above-mentioned observations were confirmed by *ab initio* calculations.



68 **70**

Figure 1 Crystal structures of 3-*endo*,5-*endo*-dichloro-7,7-bis-chloromethyl-4-dichloromethyl-tricyclo[2.2.1.$0^{2,6}$]heptane (**68**)[IV] and 3-*exo*,**5**-*exo*,6-*exo*,9,10,10-hexachlorodihydrocamphene (**70**).[V]

The X-ray analysis of hexachlorodihydrocamphene **70** showed that there are three crystallographically independent molecules in an asymmetric unit.[V] Two of these molecules are identical and the third one is their enantiomer. Typically, the C-C and C-Cl bonds in **70** are longer than the single bond values (Publication V, Table 1). The

orientation of the chlorine atoms, 3-*exo*, 5-*exo*, 6-*exo*, 9c, 10b, and 10c (Figure 1), determined by X-ray analysis, is in agreement with that determined by NMR and quantum chemical methods.

3.5.2 NMR spectroscopy

The ^{1}H and ^{13}C NMR spectra of the tricyclic compound **68**[IV] and dihydrocamphenes **69-77**[V] were assigned by means of DQF $^{1}H,^{1}H$ COSY, PFG $^{1}H,^{13}C$ HMQC, $^{1}H,^{13}C$ HMBC and $^{1}H,^{1}H$ ROESY experiments. The ^{1}H NMR chemical shifts and coupling constants for these compounds were solved precisely with the PERCHit iterator under PERCH software[50,51,149].

3.5.2.1 ^{1}H NMR

A distortion of symmetry in compound **68** was discernible in the ^{1}H NMR parameters but not in the ^{13}C NMR spectra (Publication IV, Tables 1 and 2).[IV] In order to achieve the correct solution in the computer-based spectral analysis of the ^{1}H NMR parameters, it was necessary to use all ten spins in the iteration process without any symmetry descriptions. In the nortricyclene part of **68**, the chemical shifts of the protons across the symmetry plane C1-C7-C4 are practically identical. However, the difference between the shifts of the protons H8a and H9a are approximately 0.6 Hz, and that is true for the protons H8b and H9b as well. The best RMS value in the iteration process was achieved when the shift order was 8b>9b>8a>9a. Unfortunately, the lack of a planar W pathway between the protons in positions 8 and 9 prevented the assignments based on ^{4}J

coupling constants. Furthermore, attempts to distinguish the a- and b-protons by $^{1}H,^{1}H$ ROESY experiment were unsuccessful. Therefore, ambiguities in their assignments remain.

Due to the planar zigzag arrangement, the ^{5}J coupling between H1 and H10 in **68** is relatively large (0.92 Hz).[IV] On the contrary, the ^{3}J coupling between H2 and H3 (1.03 Hz) and that between H5 and H6 (1.09 Hz) is small. According to the Karplus-type[44] equation,[43] these small ^{3}J values result from the unfavourable dihedral angles H2-C2-C3-H3 (62.20(2.15)°) and H5-C5-C6-H6 (61.99(2.11)°). In addition, an electronegative chlorine atom in positions C3 and C5 can further decrease the values of these couplings.[44]

Typical ranges of the ^{1}H NMR chemical shifts and coupling constants of dihydrocamphenes **69-77** are given in Table 2. The chemical shift of H10a is in the range of 5.99-6.09 ppm in compounds with proton in the 5-*endo* position (**69**, **70**, **73** and **76**), while that of compounds with an electronegative chlorine substituent in the 5-*endo* position (**71**, **72**, **74** and **77**) is in the range of 6.79-6.90 ppm (Publication V, Table 2).[V] In the case of compound **75**, H10a (signal at 7.10 ppm) is deshielded by a chlorine atom in position 8a (Publication V, Figure 3). Typically, the b-protons of the CH_2Cl groups on C8 resonate at a lower field than the a-protons.

In general, the *endo* protons on C5 and C6 have resonances that range from 4.27 to 4.91 ppm.[V] However, in compound **75** the *endo* proton H6 signal is at 5.70 ppm. This can be attributed to spatial proximity of a chlorine substituent in position 8a. The chemical

shift order of H5 and H6 was based on dipolar interactions revealed by two-dimensional $^1H,^1H$ ROESY experiments.

Table 2 Typical ^1H NMR chemical shifts and coupling constants of polychlorinated dihydrocamphenes **69**-**77**.[V]

Proton position	Range of the chemical shift [ppm]	$^1H,^1H$ coupling	Range of the coupling constant [Hz]
$-CHCl_2$	6.0 – 7.1	Geminal on 2° carbons	-12.0 – -13.3
$-CH_2Cl$	3.5 – 4.9	Geminal on 1° carbons	-11.2 – -12.8
$-CH_3$	1.4 – 1.6	Vicinal H5-H6	6.6 – 6.9
H5/H6	4.3 – 5.7	Vicinal H1/H4-H7a/H7b	1.3 – 1.9
H1/H4	2.4 – 4.0	Long-range W H5/H6-H7b	2.1 – 3.6
H7a/H7b	2.2 – 3.4	Long-range W H1-H4	2.7 – 3.1
		Long-range W H8-H9	0.6 – 2.0
		Long-range near W H7a-H8b	0.5 – 1.1

In compounds **69**-**75** and **77**, the electronegative chlorine substituents on C3 and C5 result in a downfield shift for the bridgehead proton H4 with respect to H1 that has only one nearby chlorine atom.[V] In the case of compound **76** the shift order for these protons is reversed, probably because of the CCl_2 group in position 6.

At 30 °C the ^1H NMR spectrum of compounds **75** and **77** show significant broadening of the signals of several protons.[V] These poorly resolved spectra can be attributed to the existence of several conformers in equilibrium due to the rotation of the CH_2Cl and $CHCl_2$ groups. The ^1H NMR parameters of compound **75** were determined from the spectrum measured at –50 °C. The favourable conformation was based on the $^1H,^1H$

ROESY experiment at the same temperature. At low temperature no sign of another conformation appeared. Consequently, the most stable conformation is predominant (>90%) in the equilibration mixture. The preferred conformation, with the chlorine atoms in 8a, 8c, 9b, 10b and 10c positions, is identical to that obtained from theoretical calculations performed at the HF/6-31G* level (Publication V, Figure 3).

The ^{1}H NMR parameters and the conformational analysis of compound **77** were based on experiments performed at 0 °C.[V] The ^{1}H NMR spectra of **77**, recorded at 0 °C and 30 °C, are shown in Figure 2. At 0 °C, the fine structure of two sets of signals is clearly visible. At this temperature, compound **77** exists in two conformations that are contributing in the proportion of *ca.* 71 (**77a**) to 29 (**77b**). The only difference between the major conformation **77a** and the minor conformation **77b** is the orientation of the chlorine atoms on C9 (9b, 9c in **77a** and 9a, 9b in **77b**). These two conformations are completely consistent with those obtained from the geometry optimizations performed at the HF/6-31G* level (Figure 3).

In compounds **74**, **76** and **77** (major conformation **a**), the long-range coupling between H8a and H9a on nonrotating CH_2Cl and $CHCl_2$ groups range from 1.90 to 2.10 Hz (Table 2).[V] On the contrary, dihydrocamphenes with a rotating methyl group (compounds **69**, **70**, **71**, **72** and **73**) show somewhat smaller average coupling between 0.59 and 1.25 Hz. A vicinal coupling between H1 and H6-*endo* and that between H4 and H5-*endo* is missing in all dihydrocamphenes examined, mainly because of unfavourable dihedral angle(s) H1-C1-C6-H6 and/or H4-C4-C5-H5 (*ca.* 75-84°).

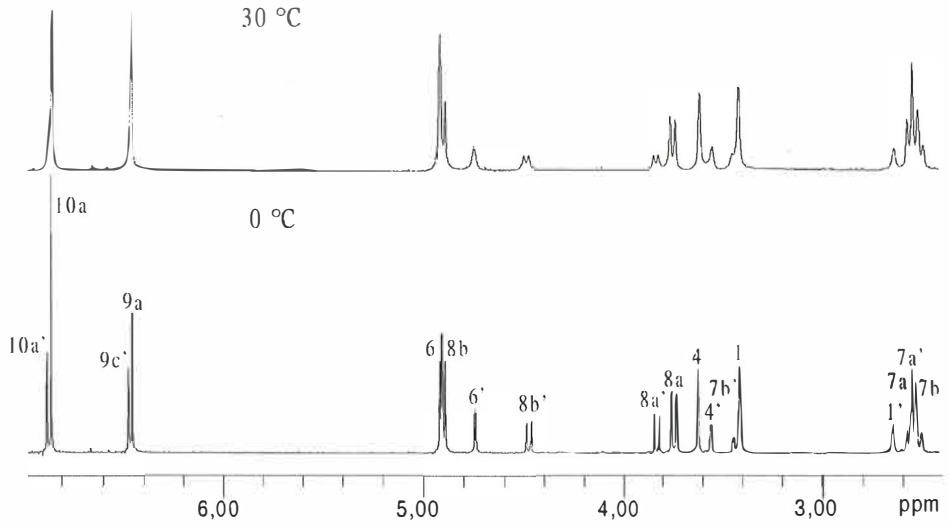
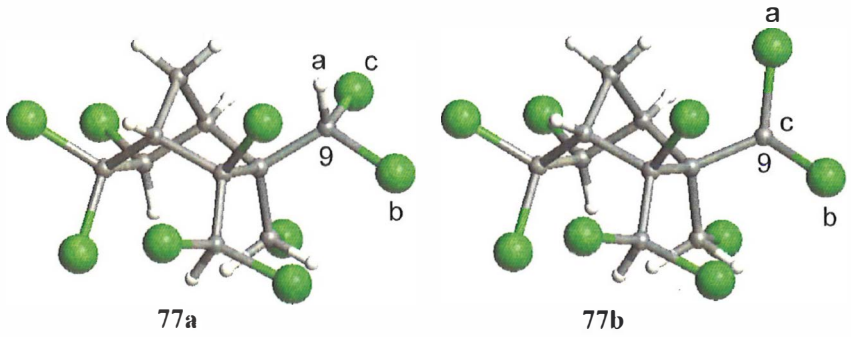


Figure 2 ^{1}H NMR spectra of **77** recorded at 0 °C (bottom) and 30 °C (top). The signals labelled with dotted numbers belong to the minor conformation **b**.[V]



77a **77b**

Figure 3 HF/6-31G* optimized rotamers (at 273 K), **a** (major) and **b** (minor), of compound **77**.[V]

3.5.2.2 ^{13}C NMR

The PFG ^{1}H,^{13}C HMQC correlation map of the tricyclic compound **68**[IV] and dihydrocamphenes **69-77**[V] provided an assignment of the ^{13}C NMR chemical shifts of

protonated carbons. The assignments of the quaternary carbons were based on PFG $^{1}H,^{13}C$ HMBC experiments.

At 30 °C, the ^{13}C NMR spectra for dihydrocamphenes **69-74** and **76** show ten sharp signals according to a single conformation. In the case of compound **75**, the spectrum shows sharp signals for nine of the carbons, while C9 presented a signal of low intensity and broadened linewidth. For compound **77**, two sets of signals with different intensities were observed, enabling assignment of the chemical shifts to conformers **77a** and **77b**.

Typical range of the ^{13}C NMR chemical shifts of dihydrocamphenes **69-77** are given in Table 3. Generally, the chemical shift of a $CHCl_2$, CH_2Cl or CH_3 group in position 9 (*exo*) is shifted to a higher ppm value than a group in position 8 (*endo*) (Publication **V**, Table 4).[V] In the case of bridgehead carbons, C1 (56.5-64.5 ppm) always resonates at a higher field than C4 (64.5-72.0 ppm).

Table 3 Typical ^{13}C NMR chemical shifts of dihydrocamphenes **69-77**.[V]

Carbon position	Range of the chemical shift [ppm]
-$CHCl_2$	73 – 79
-CH_2Cl	41 – 52
-CH_3	13 – 25
-CCl_2-	91 – 93
C7	30 – 36
C1/C4	57 – 72
C2	50 – 62
C3	84 – 89

3.5.3 Quantum chemical methods

Full geometry optimizations for tricyclic compound **68**[IV] and dihydrocamphenes **69**-**77**[V] were performed at the *ab initio* HF/6-31G* level for a comparison with the conformational and structural properties obtained from the NMR and X-ray experiments. In all cases, the optimized geometries were in excellent agreement with the experimental structures.

For compound **68**, the influence of the $CHCl_2$ group on *ab initio* optimized bond lengths was studied by replacing the bulky substituent either with a CH_3 group (3-*endo*,5-*endo*-dichloro-7,7-bis-chloromethyl-4-methyl-tricyclo[2.2.1.$0^{2,6}$]heptane) or with a hydrogen atom (3-*endo*,5-*endo*-dichloro-7,7-bis-chloromethyl-tricyclo[2.2.1.$0^{2,6}$]heptane).[IV] It was found that the bulky substituent in C4 deforms the nortricyclene part of **68** by stretching the bonds to C4 and shrinking the bonds C2-C3 and C5-C6 (Publication IV, Table 4).

HF/6-31G* optimized structures of symmetrically substituted compounds mentioned above and those of 7,7-bis-chloromethyl-tricyclo[2.2.1.$0^{2,6}$]heptane and 7,7-bis-chloromethyl-4-dichloromethyl-tricyclo[2.2.1.$0^{2,6}$]heptane show similar distortion of the symmetry as compound **68**.[IV] An explanation for this asymmetry is complicated. Preliminary calculations on the electrostatic potential energy surfaces of these compounds revealed that the three-membered ring causes a π character in the carbon skeleton. As a result, the CH_2Cl group in position 9 (or 8) is twisted and a weak hydrogen bond between Cl9c and H3 (or Cl8c and H5) is formed.[159] Only one CH_2Cl

group can be twisted at a time. Otherwise, the a-protons of these groups become too close to each other. Apparently, a second hydrogen bond is formed between Cl8c and H9b (or Cl9c and H8b).

The rotation energy profile for **68** was calculated at the HF/6-31G* level.[IV] The optimal conformer is equivalent with that obtained from the X-ray analysis. It was found that rotation of the $CHCl_2$ group and that of the CH_2Cl groups is synchronized. The *ab initio* calculated rotational barrier is 52.4 kJ mol^{-1} for the $CHCl_2$ group and 49.8 kJ mol^{-1} for the CH_2Cl groups. Therefore, rotation of the substituent about the C7/C8, C7/C9 and C4/C10 bonds is highly unlikely at room temperature.

For dihydrocamphene **75**, HF/6-31G* calculations indicate that only a single conformer is contributing significantly.[V] In the case of compound **77**, HF/6-31G* predicts that the two rotamers, **77a** and **77b** (see Figure 3, p. 68), are contributing in the proportion of 59.3 (**77a**) to 40.7 (**77b**) at 273 K. These observations are in agreement with the NMR experiments.

Rotation barriers of **75** and **77** were characterized with the semiempirical AM1 method[112] (Publication V, Figure 4).[V] For compound **77**, conformation **77a** (chlorine atoms in 8c, 9b, 9c, 10b and 10c positions; $\Delta H_f = -188.78$ kJ mol^{-1}) is predicted to be slightly more stable than conformation **77b** (8c, 9a, 9b, 10b and 10c; $\Delta H_f = -183.89$ kJ mol^{-1}). The barrier to rotation from the **a** conformation to **b** conformation is *ca.* 72 kJ mol^{-1} and the Boltzmann distribution 89.6% (**a**) and 10.4% (**b**). These findings are qualitatively consistent with the NMR experiments.

In the case of **75**, two stable conformations were again found (Publication V, Figure 4).[V] The second conformer (8a, 8b, 9c, 10b and 10c; ΔH_f = –183.89 kJ mol^{-1}) is only 6.52 kJ mol^{-1} higher in energy compared with the optimal conformer of **75** (8a, 8c, 9b, 10b and 10c; ΔH_f = -190.41 kJ mol^{-1}). An activation barrier of *ca.* 50 kJ mol^{-1} separates these two conformers. However, no signs of a second stable conformation were found in the *ab initio* and NMR data. After reoptimization at the HF/6-31G* level the energy difference is appreciably higher, 14.7 kJ mol^{-1}. Thus there is no need to consider the second conformation.

The ^{13}C NMR chemical shifts of dihydrocamphenes **69-77** were calculated using the GIAO[152] method at the DFT B3LYP/6-311G* level of theory and HF/6-31G* optimized structures as geometry input.[V] In general, B3LYP predicts shifts which are too deshielded. However, the overall correlation between calculated and experimental values is good (Publication V, Figure 5). The largest deviations are in the chemical shift range of 60-80 ppm, although the largest absolute errors occur with highly chlorinated carbons. From the data, the following linear correlation between experimental (δ_{obs}) and theoretical (δ_{calc}) shifts was derived:

$$\delta_{obs} = 0.791\delta_{calc} + 6.817 \qquad (1)$$

with a standard error of 2.314 ppm and a squared correlation coefficient r^2 = 0.985. The predictive ability of the model was improved by adding an indicator variable N_{Cl} (the number of chlorine atoms attached to each carbon) to the regression equation. The following adjusted correlation between δ_{obs} and δ_{calc} was obtained:

$$\delta_{obs} = 0.862\delta_{calc} - 3.170N_{Cl} + 4.316 \qquad (2)$$

The standard error decreased to 1.435 ppm, the squared correlation coefficient improved to 0.994 and the largest absolute error was only –4.0 ppm (Publication V, Table 4).

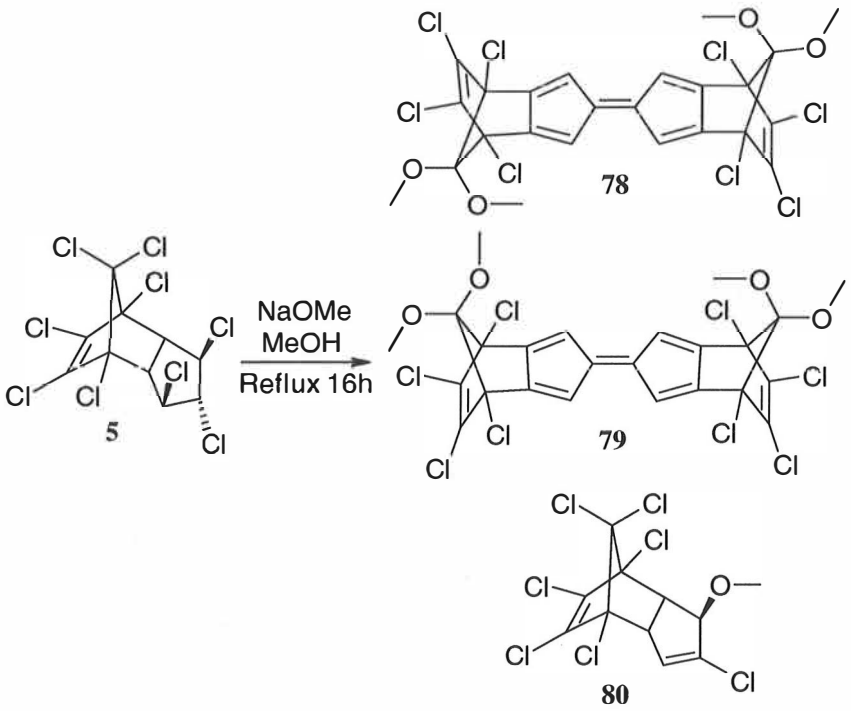
3.6 POP RELATED PENTAFULVALENE DERIVATIVES

Together with the synthesis of chlordane metabolite 1-*exo*,2-dichlorochlordene[40] we were able to prepare and purify novel POP related pentafulvalene derivatives, *trans*- and *cis*-isomers of bis-2,2'-(4,5,6,7-tetrachloro-8,8-dimethoxy-3a,4,7,7a-tetrahydro-4,7-methanoindan-1,3a-dienylidene) **78** and **79**.[II] The structures of these compounds are described in Scheme 30. The numbering of the methanoindano moieties in **78** and **79** is the same as in *trans*-nonachlor (**5**), the halves of the dimer being denoted by suffixes A and B. Also a monomeric compound was found in the reaction mixture. Its structure was identified as 2,4,5,6,7,8,8-heptachloro-1-*exo*-methoxy-3a,4,7,7a-tetrahydro-4,7-methano-1*H*-indene (**80**, Scheme 30) by NMR spectroscopy and X-ray crystallography.[160]

3.6.1 Synthesis

Pentafulvalene derivatives **78** and **79** were derived from *trans*-nonachlor (**5**) by treatment with sodium methoxide (Scheme 30).[V] The formation of **78** and **79** comprises three steps, (i) dehydrohalogenation of the partially chlorinated five membered ring C1-C2-C3-C3a-C7a with carbene formation,[161] (ii) dimerization of the formed carbene[162] and (iii) an anchimeric assistance to nucleophilic displacement at position 8 by methoxide ion resulting in the ketal[163]. The formation of the ketal could be rationalized

as follows. It is known that a simple S_N1 or S_N2 reaction of the bridge CCl_2 group in polychlorinated norbornenes is unlikely.[164-166] Therefore, in the case of **78** and **79** a neighbouring group effect of the 10π pentafulvalene moiety causes a displacement (as in the corresponding benzene derivative[163]) of a chlorine at C8 (*anti* to pentafulvalene).[II] Upon this the chloroether undergoes a normal S_N1 reaction with stabilization of the cationic centre at C8 by the OCH_3 substituent and the proximate π-system.



Scheme 30

3.6.2 Crystal structure

Suitable crystals for X-ray diffraction analysis of pentafulvalene derivatives **78** and **79** were obtained by slow concentration of $CHCl_3$ and $CHCl_3$/light petroleum solutions, respectively.[II] The X-ray structures of **78** and **79** revealed that pentafulvalene moieties are twisted in the crystalline state (Figure 4). The least square planes defined as C1A, C2A, C3A, C3aA, C4A, C7A, C7aA and as C1B, C2B, C3B, C3aB, C4B, C7B, C7aB have an angle of 4.04° in **78** and 14.3° in **79**. In addition, the torsion angles C1A-C2A-C2B-C1B show distortion from the planarity of 3.5° in **78** and 7.3° in **79**. The bond distances and angles in these compounds show no exceptional values.

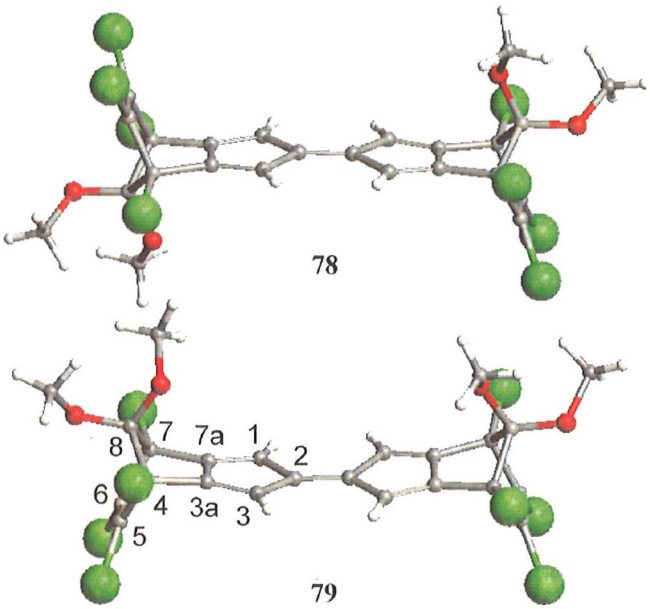


Figure 4 Crystal structures of pentafulvalene derivatives **78** and **79**.[II]

3.6.3 NMR spectroscopy

The ^{1}H and ^{13}C NMR chemical shift assignments for compounds **78** and **79** were based on two-dimensional z-gradient selected $^{1}H,^{13}C$ HMQC and $^{1}H,^{13}C$ HMBC experiments (Publication II, Table 1).[II] The methoxy group *anti* to the pentafulvalene moiety is situated above the C5=C6 double bond. Consequently, its resonance frequency is shifted towards higher field strength than that of the *syn* methoxy group. Distortion of symmetry found in X-ray analysis (solid state) is not discernible in the NMR parameters (liquid state).

4 SUMMARY AND CONCLUSIONS

The present investigation reports the characterization of 25 POPs by NMR spectroscopy. Additionally, the methods of quantum chemistry and single crystal X-ray crystallography were applied. For precise ^{1}H NMR parameters, computer aided spectral analysis was used. Furthermore, two POP related pentafulvalene derivatives were synthesized and their structures were studied by NMR and X-ray crystallography.

Approximated and partly contradictory ^{1}H and ^{13}C NMR chemical shifts previously reported for C_{10}-based chlordane compounds **1**, **5**, **7**, **18** and **19** were corrected in this work. Further, complete ^{1}H and ^{13}C NMR parameter sets of three oxy derivatives, **60**, **61** and **62**, were determined. For **61** and **62**, the NMR parameters were reported for the first time. Compounds **7**, **18** and **19** were reliably characterized at submilligram levels by their ^{1}H and ^{13}C NMR parameters with a 500 MHz (11.8 T) spectrometer and a 5 mm diameter standard probehead by using symmetrical $CDCl_3$ matched micro-NMR tubes and HMQC and HMBC experiments.

^{1}H and ^{13}C NMR spectra for seven PCDTs, **25**, **30** and **63**-**67**, were measured. Complete ^{1}H and ^{13}C NMR chemical shift assignments for **25**, **30** and **63**-**65** were based on proton detected PFG HMQC and HMBC experiments. For hepta- and octachloro congeners, **66** and **67**, where polarization transfer from proton to (all) carbons is not possible, the ^{13}C NMR chemical shift assignments were based on theoretical calculations using the GIAO method at the *ab initio* HF/6-311G* and DFT BPW91/6-311G* levels. The mean

deviation between the experimental and theoretical values was much less when calculated by DFT than by *ab initio* method.

The structural properties of ten potential toxaphene congeners, **68**-**77**, were studied. The X-ray analysis of **68** revealed an unusual tricyclic structure, where the two chlorine atoms occupying *endo*-positions are in close spatial proximity with each other and near to the neighbouring $CHCl_2$ group. Further, it revealed that the symmetry of **68** is distorted. This asymmetry was also discernible in ^1H NMR parameters (liquid phase) and in the HF/6-31G* optimized structure (gas phase). According to the rotation energy profile calculated at the HF/6-31G* level, rotation of the CH_2Cl and $CHCl_2$ groups is highly unlikely at room temperature. Further, it was found that rotation of these groups is synchronized. In addition, *ab initio* calculations showed that a bulky substituent in C4 deforms the nortricyclene part of **68** by stretching the bonds to C4 and shrinking the bonds C2-C3 and C5-C6. In this work, it was demonstrated for the first time that an enantiomer pair of a stable structure of **68** could be formed during synthesis of toxaphene.

For polychlorinated dihydrocamphenes **69**-**77**, an exact description of the structure was achieved by extensive one- and two-dimensional ^1H and ^{13}C NMR spectroscopy. In the case of **70**, the structure was determined by single crystal X-ray analysis. The experimental results were in excellent agreement with conformational and structural properties obtained from *ab initio* optimizations performed at the HF/6-31G* level. Rotation barriers of compounds **75** and **77** were calculated with the semiempirical AM1 method. These results were qualitatively consistent with the NMR experiments. The ^{13}C

NMR chemical shifts for dihydrocamphenes **69-77** were obtained using the GIAO method at the DFT B3LYP/6-311G* level of theory and HF/6-31G* optimized geometries. A comparison of the calculated and experimental data yielded a regression equation which, with an added indicator variable (the number of chlorine atoms attached to each carbon), is capable of accurate prediction of carbon chemical shifts for these compounds.

Synthesis, single crystal structures, and ^{1}H and ^{13}C NMR chemical shift assignments based on proton detected PFG HMQC and HMBC experiments were presented for novel POP related pentafulvalene derivatives, **78** and **79**, derived from *trans*-nonachlor (**5**) by treatment with sodium methoxide. The route for the formation of **78** and **79** was also discussed.

In conclusion, modern NMR spectroscopy provides an effective tool for isomer specific structure elucidation for POPs and related compounds. The structural information hidden in complex ^{1}H NMR spectra becomes easily accessible by using computer aided spectral analysis. Detection limits of the NMR experiment could be pushed down to submilligram levels by using solvent susceptibility matched micro-NMR tubes to decrease the volume required for an experiment. However, from an environmental analytical point of view, further increases in NMR sensitivity are often needed. The development of small-volume RF probes and their coupling to microseparation strategies, such as GC and HPLC, is likely to lead to further advances in the field of NMR analysis in the future.[22]

Additionally, quantum chemical studies can be used to support structural analysis. These methods enable the calculation of molecular properties such as geometries, energies and NMR shieldings. Finally, single crystal X-ray analysis offers a way to determine the three-dimensional arrangement of the atoms in a molecule in the crystalline state.

The structural data obtained in this investigation, using the above-mentioned methods, are offered as a contribution towards improving the understanding of the behaviour of POPs and their physiological and environmental effects.

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Errata

PAPER IV

Page 676, second column, second paragraph, line 7
"1.530(15)" should be "1.541(3)"

PAPER I

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PAPER II

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