# Determination of Chlordane in Human Adipose Tissue by SFE-LC off-line MS

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## 1. Introduction

Chlordane has been used as an insecticide and termiticide since the 1950's. Its use was banned in Sweden, the United States and Japan in the 1980's. Because chlordane's half-life is at least 5, possibly 15, years and its metabolites as well have long half-lives people will be exposed to this group of toxic xenobiotics for another generation. There is inadequate evidence in humans for the carcinogenicity of chlordane and limited evidence in animals. The International Agency for Research on Cancer (IARC) has evaluated chlordane as a possible human carcinogen (Group 2B)[1].

The chlordanes are non-aromatic, chlorinated (7-9 chlorines) and have a tricyclic configuration. The major components of technical chlordane are *cis*- and *trans*-chlordane, *cis*- and *trans*-nonachlor and heptachlor. They are, in mammals, mainly metabolised to two persistent epoxides, e.g. heptachlor epoxide and oxychlordane[2]. They have been found as enantiomeric pairs in environmental samples[3].

The main components of technical-grade chlordane have all been identified in human tissue indicating that all are absorbed following exposure. Chlordane has a global distribution with relatively high concentrations in the Arctic food-chain[4].

An analytical technique, based on supercritical fluid extraction (SFE) using $CO_2$, for determination of chlordane was successfully used in the study of eight chlordane compounds in adipose tissue. All samples were taken in 1994/95 from a Swedish population group, an integral part of an case-control study on non-Hodgkin lymphoma (NHL) associated with exposure to organohalogen compounds[5]. The supercritical fluid extraction was carried out on an instrument equipped with a carbon trap liquid chromatography (LC) system[6]. Eight chlordane components; *trans*-nonachlor, *cis*-nonachlor, MC 6, *trans*-chlordane, oxychlordane, *trans*-heptachlor epoxid and *cis*-heplachlor epoxid were determined by this technique. The MDL was 0.1 ppb (ng/g lipid). The arithmetic mean value (n= 49) of total chlordane (sum of 8 components) was 134 ppb with a minimum of 31 ppb and a maximum of 678 ppb. Further, the correlations of levels with respect to *trans*-nonachlor (as a marker for total chlordane), age and sex were studied.

The carbon trap LC made it possible to simultaneously separate non-planar (pesticides) and planar organochlorine compounds (dioxins and PCBs) in the same extracts. This technique was reported on at *Dioxin 95'*[7].

## 2. Experimental

*The SFE Extraction*

Human adipose tissue, approximately 2 grams, was homogenised and mixed with activated $Na_2SO_4$. An internal standard (IS) consisting of 21 $^{13}C$ labelled planar and non-planar organochlorines (PCDDs, PCDFs and PCBs) was added to the homogenate which was then placed in an SFE extraction thimble. The extraction was carried out with a Hewlett-Packard 7689T SFE extractor. The SFE-LC/ MS parameters used for the chlordane determination are listed in Table 1.

Table 1: Parameters used for determination of chlordane by SFE-LC/ MS

| SFE | LC | HRGC-MS |
|---|---|---|
| $CO_2$ (N48 grade) | Carbon trap (PX-21) | SIR-LRMS |
| 2 ml/min ($CO_2$ flow) | Hexane/MeCl | DB-5 |
| 40°C (chamber temp) | 40°C (trap temperature) | 60 min/run |
| 0.9 g/ml ($CO_2$ density) | Reconditioning (35 min) | |
| Dynamic mode (34 min) | | |

The 7 ml-volume extraction chamber was filled with aluminium oxide as a fat retainer. The extraction of the chlordanes (fraction 1) required 68 ml $CO_2$ and 6 ml hexane/MeCl (50:50). A planar fraction (fraction 2; dioxins, non-o-PCBs and naphtatenes) was eluted from the carbon column with xylene before reconditioning of the carbon trap with 10 ml xylene and 10 ml hexane /methylene chloride. Fraction 2 was further analysed by HRGC-HRMS, and the results will be reported separately[8].

Some of the chlordane extracts were further cleaned-up by the use of a small acidic silica column before HRGC-MS analyses. This was required only if the sample had a high fat content. A recovery standard (RS) was added to the extracts and they were concentrated to 30 µL in tetradecane before analysed by Selected Ion Monitoring (SIM).

*The HRGC-MS Analyses*

The extracts were analysed on a Fisons MD 800 mass spectrometer coupled to a Fisons GC 8000 gas chromatography. The sample, 2 µL, was introduced by splitless injection at 250°C. The HRGC-MS parameters used for the determination of the eight chlordane components are listed in Table 2.

In the SIR mode two of the most abundant ions in the chlorine cluster of the native components were monitored and one ion for each of the $^{13}C$-labelled internal and recovery standards. Quantification was performed against an external chlordane $^{12}C$ standard consisting of the eight chlordane components determined in the samples. In Figure 1, a SIM mass chromatogram of one of the adipose sample shows the signals of the six detected chlordanes.

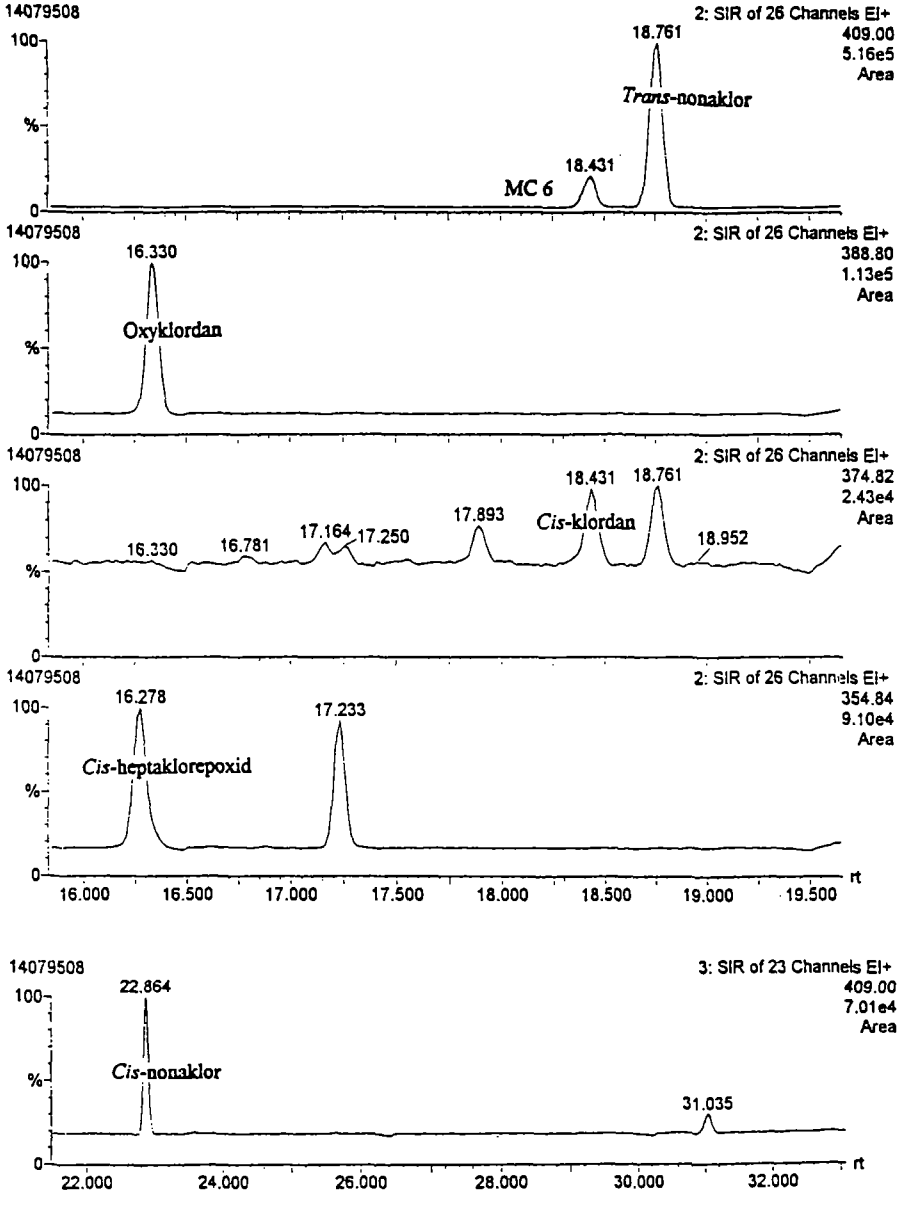


Figure 1. SIM Chromatograms of six chlordanes, MC 6, *trans*-nonachlor, oxychlordane, *cis*-chlordane, *cis*-heptachlor epoxide and *cis*-nonachlor in human adipose tissue.

Table 2: HRGC-MS parameters used for determination of chlordanes in SFE-LC/MS.

| HRGC | MS |
|---|---|
| Splitless | LR |
| DB-5 (J&W) | SIR |
| 60 m | 407.0 $(M+2-Cl)^-$ nonachlor |
| 0.32 mm i.d. | 409.0 $(M+4-Cl)^+$ |
| 0.25 µm film | 372.8 $(M+2-Cl)^-$ octachlor |
| 180-300°C | 374.8 $(M+4-Cl)^+$ |
| 60 min | 386.8 $(M+2-Cl)^-$ oxychlordane |
| IS PCB #153 | 388.8 $(M+4-Cl)^-$ |
| RS PCB #80, #128 | 352.8 $(M+2-Cl)^+$ heptachlor epoxide |
| | 354.8 $(M+4-Cl)^-$ |

### 3. Results

The arithmetic averages, range and percentage distribution of the eight chlordane components in adipose tissue of 49 Swedish individuals, with an average of 62 years, are listed in Table 3.

Table 3. Average level in ppb (ng/g lipid), range and % distribution of chlordane components in a Swedish population group (n=49) in 1994/95.

| Component | Average | Range | Distribution |
|---|---|---|---|
| *Trans*-nonachlor | 70.8 | 13.0-388.6 | 51.5% |
| *Cis*-nonachlor | 11.7 | 1.7- 68.3 | 8.0% |
| MC 6 | 13.1 | 3.5- 67.6 | 10.0% |
| *Trans*-chlordane | ND | - | - |
| *Cis*-chlordane | 0.8 | - | - |
| Oxychlordane | 32.8 | 8.5-143.9 | 25.7% |
| *Trans*-heptachlor epoxide | ND | - | - |
| *Cis*-heptachlor epoxid | 4.6 | 0.2- 14.1 | 4.8% |
| Sum of chlordanes (ppb) | 133.8 | 30.8-678.1 | 100.0% |
| Age (years) | 62 | 31-90 | |

The correlation between *trans*-nonachlor and the sum of chlordanes (total chlordane) (See Figure 2) was found to be strong (r=0.99). The ratio of the total chlordane and *trans*-nonachlor is 1.95 (SD=0.16). As with all persistent organohalogens also the levels for chlordane were well correlated l with age (r=0.78) for the control group (n=17) and less so (r=0.39) for the cases (n=32). The age correlation for the first group (healthy controls) is plotted in Figure 3.

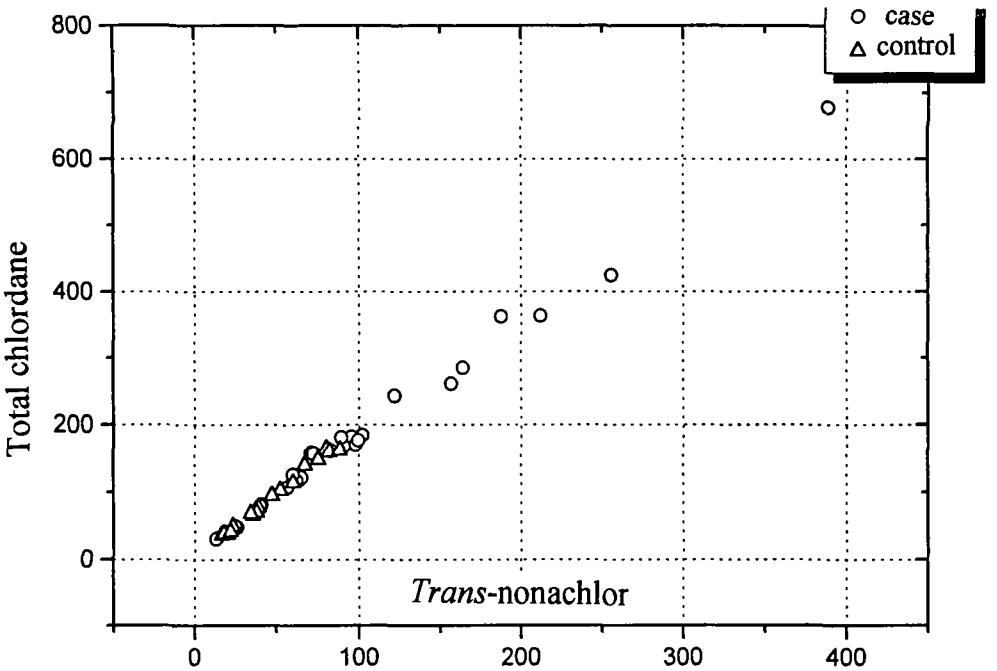


Figure 2. Correlation (r= 0.99) between *trans*-nonachlor and total chlordane in adipose tissue.

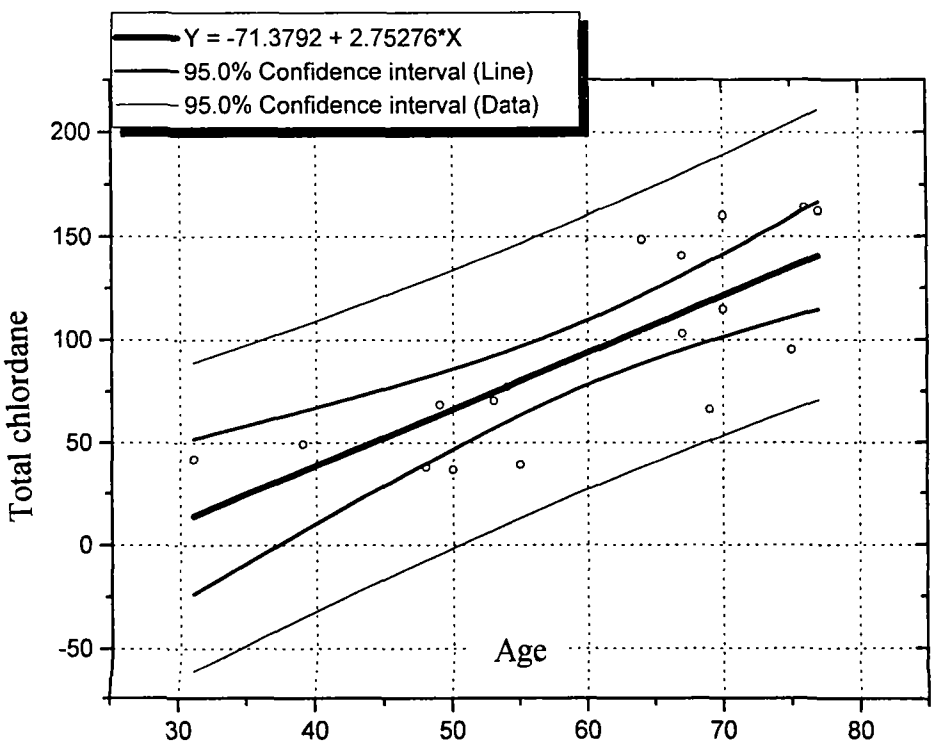


Figure 3. Age correlated (r=0.78) chlordane levels in 17 persons (control group).

The differences in levels of chlordane in women and men were also studied. The average level among men (n = 26, 19 cases and 7 controls) of the sum of chlordanes was 171.8 ng/g and among the women (n = 23, 16 cases and 7 controls) 117.6 ng/g.

## 4. Discussion

Determination of chlordane in human adipose tissue by SFE-LC off-line MS is a time and resource saving technique. The limited amount of solvents and other chemicals makes it a relatively 'green' technique in the environmemtal laboratory. And should as such be encouraged. It can further be carried out as integrated in the determination of other groups of chlorinated persistent compounds in biological tissue. And this adds to its use as a tool for the environmental scientist.

## Acknowledgement

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## 5. References

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