

Dioxin '97, Indianapolis, Indiana, USA

DEVELOPMENT OF AN ISOTOPE DILUTION GC/NCI-MS METHOD FOR THE ANALYSIS OF ORGANOCHLORINE PESTICIDES IN HUMAN BREAST ADIPOSE TISSUE

L. She, J. Winkler, M. McKinney, P. Visita, M. Petreas

Hazardous Materials Laboratory, California Department of Toxic Substances Control, Cal/EPA, Berkeley, CA 94704, USA

Abstract

An isotope dilution GC/NCI-MS method for the determination of organochlorine pesticides in human breast adipose tissue was developed. ^{13}C labelled β -HCH, HCB, p,p'-DDT, p,p'-DDE, Dieldrin and Mirex were used as internal standards, and ^{13}C -labelled α -HCH, PCB-128 and PCB 178 as recovery standards. The MS was operated in the electron capture, negative ionization, multiple ion monitoring mode. Standard Reference Material (SRM-1945) was analyzed with this method to evaluate performance. Ten human breast tissue samples were also analysed. In decreasing concentration, p,p'-DDE, trans-nonachlor, oxychlorane, HCB, dieldrin, β -HCH and sometimes p,p'-DDT were found in the tissue samples.

Introduction

Certain organochlorine pesticides (OCPs) cause cancer in laboratory animals, but evidence for human carcinogenicity is uncertain. It has been reported that some of these compounds possess estrogenic activity, suppress immune function, and induce hepatic microsomal enzymes¹. To study the relationship between human body burden and breast cancer, a reliable analytical method is needed. Traditionally, OCPs at residue level have been identified by GC/ECD. This method requires a verification analysis by a second column, and confirmation by GC/MS. The advantages of NCI MS over EI and positive CI include selective ionization in the presence of complex matrices, a higher proportion of molecular ions, and greater sensitivity. Use of the ^{13}C -labelled compounds as internal standards compensates for the loss of analytes during cleanup and variation inside the ion source.

Experimental methods

Sample clean-up. Breast adipose samples (1-3g) were homogenized and extracted into 1:1 hexane:methylene chloride, shaken and centrifuged to separate phases. Nine tenths of the extract were analyzed for polychlorinated dibenzodioxins and furans, and one tenth of the extract was cleaned up for pesticide analysis. The extract was spiked with 10ng each of $^{13}\text{C}_6$ β -HCH, $^{13}\text{C}_4$ dieldrin and $^{13}\text{C}_8$ mirex and 20 ng each of $^{13}\text{C}_6$ HCB, $^{13}\text{C}_{12}$ p,p'-DDT and $^{13}\text{C}_{12}$ p,p'-DDE. The fat in the sample was removed by gel permeation and Florisil chromatography in a single automated system (FMS). The analytes were concentrated in the presence of 4 ng of $^{13}\text{C}_{12}$ PCB 128 and 178 and $^{13}\text{C}_6$ α -HCH (recovery standards) to 10 μL for GC/ECNI analysis.

CHIRAL COMPOUNDS

Sample analysis. The analysis was carried out using a Finnigan-4510 GC/MS system. Samples were introduced through a splitless injector connected to a 60m x 0.25 mm, 0.25 μm film thickness, DB-5ms column with helium as the carrier gas. Research grade methane was used as the reagent gas. The MS was operated in the electron capture, negative ionization, multiple ion monitoring mode. The ion source pressure was held at 0.6 Torr and ion source temperature was 100°C. The electron energy was typically 70eV and the electron emission current was kept at 0.3 mA. The quantitative mixture of ^{13}C - and ^{12}C -organochlorine compounds at concentrations of 125 to 400 pg / μL was injected every day to get the response factors and evaluate instrument performance prior to sample analysis.

Results and discussion

Ions monitored. Table 1 lists the ions monitored for the determination of OCPs in the SRM-1945 and human breast tissue samples. The ions were both representative of the structure and intense peaks from the full scan mass spectra.

Table 1: Ions monitored for the determination of OCPs in biological samples

Compounds	Ions	Fragment	Compounds	Ions	Fragment
aldrin	235, 237	C_5Cl_5^-	endosulfan I	404, 406	M^-
dieldrin	344, 346	$(\text{M}-\text{Cl}+\text{H})^-$	endosulfan II	404, 406	M^-
endrin	344, 346	$(\text{M}-\text{Cl}+\text{H})^-$	end. sulfate	384, 386	$(\text{M}-\text{HCl})^-$
endrin aldehyde	378, 380	M^-	mirex	402, 404	$(\text{M}-4\text{Cl})^-$
heptachlor	298, 300	$(\text{M}-72)^-$	α -HCH	253, 255	$(\text{M}-\text{Cl})^-$
heptachlor epo.	316, 318	$(\text{M}-2\text{Cl})^-$	β -HCH	253, 255	$(\text{M}-\text{Cl})^-$
α -chlordane	408, 410	$(\text{M}+2)^-$	γ -HCH	253, 255	$(\text{M}-\text{Cl})^-$
γ -chlordane	408, 410	$(\text{M}+2)^-$	δ -HCH	253, 255	$(\text{M}-\text{Cl})^-$
oxychlordane	350, 352	$(\text{M}-70)^-$	4,4'-DDT	281, 283	$(\text{M}-\text{HCl}_2)^-$
trans-nonachlor	442, 444	$(\text{M}+2)^-$	4,4'-DDE	316, 318	M^-
HCB	282, 284	M^-			

Dynamic linear range. Calibration standards of different concentrations (from 4 pg to 4000pg) were made and injected. Figures 1, 2 and 3 show the calibration curves for different groups of OCPs. It can be seen from Figure 2 that the response factors increased in the following sequence: β -HCH < δ -HCH < α -HCH < γ -HCH. The reason for this sequence may be the conformation of different HCH isomers. There are 3 chlorine atoms in the equatorial position for γ -HCH, 2 for α -HCH, 1 for δ -HCH and none for β -HCH. The more chlorine atoms in equatorial position, the more stable the corresponding isomer will be. The response factors reflect the base molecular stability. Comparing chlorinated diphenylethane derivatives (i.e. DDT and its analogs), it can be found (Figure 2) that DDE has the highest response, DDT the next highest, and methoxychlor the lowest (not shown here). Stemmler and Hites²⁾ suggested that conjugation between the aromatic rings in DDE gives intense molecular ions. DDT, due to its aliphatic character, shows weak molecular ions. Methoxychlor, with electron-donating substituents on the aromatic ring, gives the poorest response under the experimental condition and it was not included in this method. For the hexachlorocyclopentadiene derivatives, endosulfan I, II and endosulfan sulfate give the strongest response and mirex the weakest (Figures 1 and 3). At concentrations greater than 1 ng, the multiplier is saturated for most compounds.

Dioxin '97, Indianapolis, Indiana, USA

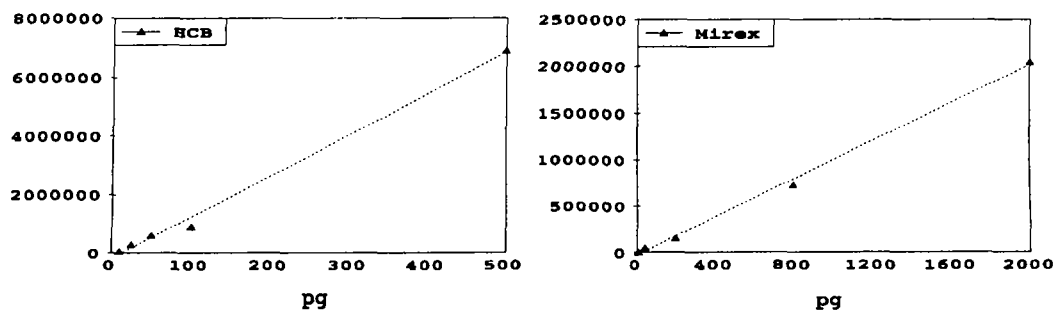


Figure 1: Calibration curves for HCB and mirex

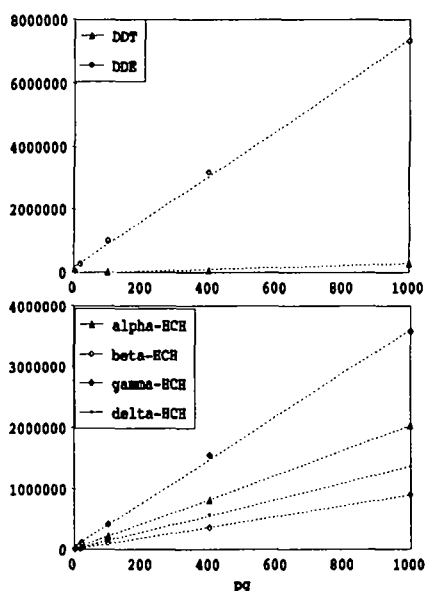


Figure 2: Calibration curves for HCH isomers, DDT and DDE.

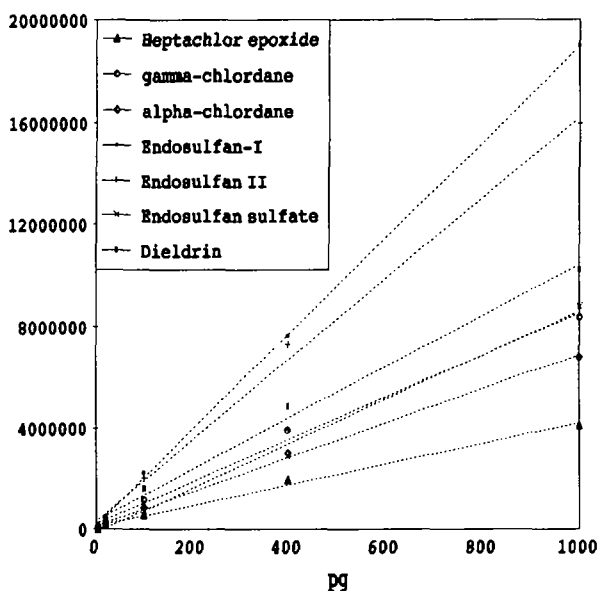


Figure 3: Calibration curves for some hexachlorocyclopentadiene derivatives

Results of SRM-1945. SRM 1945 was purchased from NIST and analyzed by this method to evaluate performance. SRM 1945^{3, 4)} is a frozen whale blubber homogenate intended for use in evaluating analytical methods for the determination of selected polychlorinated biphenyl (PCB) congeners and chlorinated pesticides. Briefly, about 1 gram SRM-1945 sample was homogenized and extracted in the same way the human breast tissue samples were. Four SRM-1945 samples in two batches (2 samples and 1 blank per batch) were analyzed. Table 2 lists the results obtained by this analysis and the comparison with NIST certified values.

Results of human breast adipose tissue. Ten human breast tissue samples were analyzed and the results are presented in Table 3. In decreasing concentration, p,p'-DDE, trans-nonachlor, oxychlordane, HCB, dieldrin, β -HCH and, sometimes, p,p'-DDT were found in the tissue samples. The other analytes were below the detection limit.

CHIRAL COMPOUNDS

Table 2: Concentrations of OCPs in four SRM-1945 whale blubber samples analyzed by isotope dilution GC/NCI MS (ng/g, wet)

OCPs	Our analysis						NIST values	RPD
	SRM-1	SRM-2	SRM-3	SRM-4	Mean	%CV		
HCB	39	44	ND	ND	42	9	32.9	23
α -HCH	12	ND	22	22	19	31	16.2	14
γ -HCH	ND	ND	3.2	4.7	4.0	27	3.3	18
Oxychlorane	ND	ND	22	30	26	22	19.8	27
t-Nonachlor	210	210	170	220	202	11	231	13
p,p'-DDT	270	260	280	310	280	8	245	13
p,p'-DDE	540	590	650	630	602	8	445	30
α -Chlordane	52	56	41	52	50	13	46.9	7
Mirex	34	40	24	34	33	20	28.9	13
Dieldrin	54	52	52	62	55	9	37.5	38

ND: Not determined; RPD: comparison of our mean and NIST value

Conclusions

1). The majority of OCPs show a linear response in the concentration range from a few pg to 1ng. The more stable the base molecule, the higher the response. Among those investigated, HCB shows the strongest response.

2). In most cases, negative ions were generated by undissociative resonance electron capture under experimental conditions (methane as the reagent gas). Ion-molecule reaction, forming $(M+Cl)^-$, can occur when the source is dirty, decreasing the sensitivity.

3). The performance of the method is satisfactory based on the analysis of the SRM-1945 whale blubber samples.

4). In human breast tissue, p,p'-DDE, trans-nonachlor, oxychlorane, HCB, dieldrin, β -HCH and, sometimes, p,p'-DDT were the most prevalent OCPs.

References

- 1). Mussalo-Rauhamaa H, Hasanen E, Pyysalo H, Kauppila AR, Pantzar P, Occurrence of beta-Hexachlorocyclohexane in breast cancer patients, *Cancer* 66, 2124-2128, 1990.
- 2). E.A.Stemmler and Ronald A. Hites, "Electron capture negative ion mass spectra of holo-genated diphenylethane derivatives", *Anal. Chem.*, 1988, 60, 787-792.
- 3). Standard Reference Materials Program, NIST, "Standard reference material 1945-organics in whale blubber", Technical Category 109.
- 4). Stephen A. Wise, Michele M. Schantz, et al, "Development of frozen whale blubber and liver reference materials for the measurement of organic and inorganic contaminants", *Fresenius J. Anal. Chem.*, 1993, 346, 766-778.

Table 3: Concentrations of the most prevalent OCPs measured in 10 human breast adipose tissue samples (ng/g, lipid basis)

OCPs	Min	Max	Mean	Median
p, p'-DDE	560	2200	1134	1080
trans-Nonachlor	20	155	81	74
Oxychlorane	26	116	73	76
HCB	23	120	63	52
Dieldrin	17	56	35	35
β -HCH	3	72	34	35