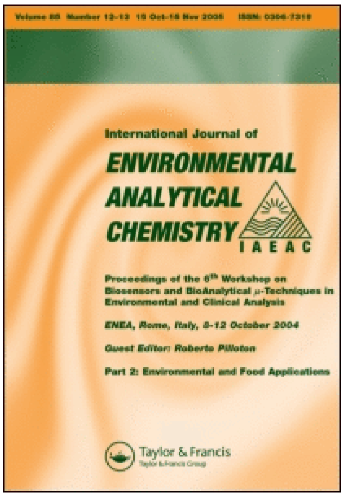

International Journal of Environmental Analytical Chemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713640455

GC Analysis of Some Organochlorine Pesticides using a Brominated Internal Standard

M. Natangelo[a]; S. Romagnano[a]; L. Guzzella[b]; E. Benfenati[a]
[a] Laboratory of Environmental Pharmacology and Toxicology, Istituto di Ricerche Farmacologiche "Mario Negri", Milano, Italy [b] Istituto di Ricerca sulle Acque, Consiglio Nazionale delle Ricerche, Brugherio, Milano, Italy

To cite this Article Natangelo, M. , Romagnano, S. , Guzzella, L. and Benfenati, E.(1995) 'GC Analysis of Some Organochlorine Pesticides using a Brominated Internal Standard', International Journal of Environmental Analytical Chemistry, 58: 1, 55 — 66

To link to this Article: DOI: 10.1080/03067319508033112

URL: http://dx.doi.org/10.1080/03067319508033112

Intern. J. Environ. Anal. Chem., Vol. 58, pp. 55-66
Reprints available directly from the publisher
Photocopying permitted by license only

GC ANALYSIS OF SOME ORGANOCHLORINE PESTICIDES USING A BROMINATED INTERNAL STANDARD

M. NATANGELO[1], S. ROMAGNANO[1], L. GUZZELLA[2]
and E. BENFENATI[1]*

[1]*Laboratory of Environmental Pharmacology and Toxicology, Istituto di Ricerche Farmacologiche "Mario Negri", Via Eritrea, 62-20157 Milano, Italy;* [2]*Istituto di Ricerca sulle Acque, Consiglio Nazionale delle Ricerche, 20047 Brugherio (Milano), Italy*

(Received, 10 September 1993; in final form, 17 March 1994)

The brominated compound 1,1-bis(4-chlorophenyl)2-bromoethane was specifically synthesized for use as internal standard (IS) in the GC analysis of some organochlorine pesticides. The IS was prepared by reacting commercially available 2,2-bis(4-chlorophenyl)ethanol with triphenylphosphine and tetrabromomethane.

Organochlorine pesticides were tested together with this IS for the linearity of the analytical method in the pg range of injected pesticides (HCB, α- and γ-HCH, heptachlor, aldrin, op′-and pp′-DDT and its metabolites: op′-and pp′-DDE, op′- and pp′-DDD), using MS detection in the negative ion chemical ionization mode (NICI). GC-ECD was also used to test the IS response and linearity of the method for some of the above pesticides in the pg range of injected analytes.

The synthesized IS was added and analyzed in vegetable samples (spruce needle and branch) to prove the applicability of this compound in a GC-NICI-MS analytical method for organochlorine pesticides in such samples.

KEY WORDS: Organochlorine pesticides, gas chromatography-mass spectrometry, spruce needles, internal standard, gas chromatography-electron capture detection

INTRODUCTION

Organochlorine pesticides are among the neutral organic chemicals most commonly of concern as persistent and widespread environmental contaminants on account of their stability and toxicity. It is generally best to use an internal standard (IS) for the standardization of the extraction and GC analysis of these compounds. An IS in GC quantitative analysis presents advantages in accuracy over direct quantification methods, but problems arise in the choice of an appropriate chemical with structural similarities to the analytes of interest.

---

*To whom correspondence should be addressed.

Some authors[1,2] based their recovery experiments on endrin or op′-DDE quantification. These organochlorines are often but not always absent in environmental experiments. Other pesticides like aldrin and heptachlor were used as IS[3,4]. Other authors[5] used polychlorinated biphenyl (PCB) congener no. 30, whose retention time is closer to the most common organochlorine pesticides. Jensen *et al.*[6] used congener no. 189, whose retention time is longer than the analytes considered. Driscoll *et al.*[7] added to the extracts an octachlorobiphenyl, but problems arised from the presence of some interferences. Larsen and Riego[8] preferred 2,4-dichlorobenzyl heptyl ether and 2,4-dichlorobenzyl tetradecanyl ether. In all these studies, it was clearly difficult to find an IS for the determination of organochlorine pesticides.

Lopez-Avila *et al.*[9] selected various compounds suitable for the use as IS in the analysis of 45 organochlorines with a dual column GC system; other authors[10] used 2,2′,5-tribromobiphenyl; all these IS were added to the extracts just prior the instrumental analysis. Knickmeyer *et al.*[11] used the ε-HCH, a reliable IS for the analysis of compounds that exhibit low retention times.

When negative ion chemical ionization mass spectrometry (NICI-MS) detection was used, ^{13}C labelled HCB was employed as IS in the quantification of the unlabelled pesticide in vegetable samples[12].

In this work we present the chemical synthesis of 1,1-bis(4-chlorophenyl)2-bromoethane (Figure 1), together with its application as IS in the GC analysis of some organochlorine pesticides. Two different detection systems are used: NICI-MS and electron capture detection (ECD). The method is applied for the analysis of vegetable samples.

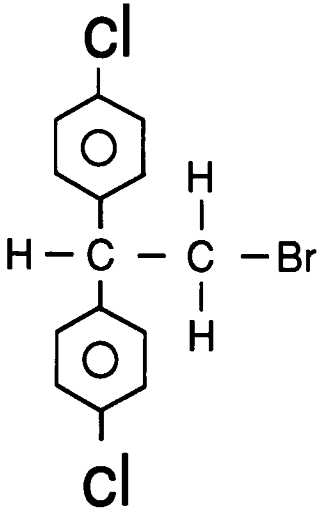

Figure 1 Chemical structure of 1,1-bis(4-chlorophenyl)2-bromoethane

EXPERIMENTAL

Reagents

2,2-bis(4-Chlorophenyl)ethanol, carbon tetrabromide (CBr_4) and triphenyl phosphine (PPh_3) were from Aldrich (Milwaukee, WI, USA). Standard solutions of 11 organochlorine pesticides (α-HCH, γ-HCH, HCB, heptachlor, aldrin, op′- and pp′-DDE, op′- and pp′-DDD, op′- and pp′-DDT) in *n*-hexane were from Supelco Inc. (Bellefonte, PA, USA). All solvents were for pesticide analysis from Carlo Erba (Milano, Italy) and Merck (Darmstadt, Germany).

Synthesis of 1,1-bis(4-chlorophenyl)2-bromoethane

One hundred mg of 2,2-bis(4-chlorophenyl)ethanol were dissolved in 4 ml of methylene chloride, and 156 mg of CBr_4 were added; the mixture was subsequently held at 0°C, adding 123 mg of PPh_3 in small portions, then the temperature was raised to the ambient. After 2 hours, the bromination mixture (100 mg of CBr_4 plus 100 mg of PPh_3) was added in the reaction flask and 12 hours later, the reaction solvent was evaporated using a Rotavapor. The product was purified with silica gel column chromatography, using n-hexane as elution solvent.

The purity of the compound was assessed using electon ionization mass spectrometry (EI-MS), introducing the sample directly into the ion source (direct inlet system, DIS), or by means of the gas chromatographic column.

Calibration curves

Calibration curves were obtained using standard solutions prepared in *n*-hexane at increasing concentrations for the analytes plus a constant level of the IS. For GC-NICI-MS analysis, standard mixtures were at concentrations of 0, 50, 100, 200 pg/μl for the analytes, and IS was 200 pg/μl.

For GC-ECD analysis, standard mixtures of α-HCH, γ-HCH, HCB, pp′-DDE, pp′-DDD and pp′-DDT plus the IS were made at concentrations of 0, 12.5 and 25 pg/μl (α-HCH, pp′-DDE, pp′-DDD), 0, 2.5 and 5 pg/μl (HCB), 0, 6.75 and 12.5 pg/μl (γ-HCH), 0, 25 and 50 pg/μl (pp′-DDT), the concentration of IS was always 50 pg/μl.

Standard solutions were stored at –4°C in the dark.

Spruce needle and branch analysis

About 40 g of spruce needles or branches were fortified with 200 ng of the IS. After an equilibration period of 16 hours at room temperature, samples were partially dried at 35°C for 16 hours, then homogenized with an Omni-mixer, and the sample was extracted with *n*-hexane in a Soxhlet apparatus for 16 hours. The extract was purified by adding concentrated sulfuric acid; after this, the organic layer was separated and passed through a column of anhydrous sodium sulfate. After final concentration to a small volume (typically 0.3–0.5 ml), 2 µl portions of the extracts were injected into the GC-NICI-MS system for analysis.

For the GC-ECD analysis, further purification of the sample involved Florisil column chromatography; the final sample volume was 3 ml.

Instrumental

EI-MS A VG TS-250 mass spectrometer interfaced with an HP-5890 gas chromatograph was used in the following conditions: GC column: Cp-Sil 8 CB (Chrompack, Middelburg, The Netherlands), 25 m length, 0.25 mm internal diameter, 0.25 µm film thickness; GC column head pressure 80 kPa (helium carrier gas); chromatographic program 85°C initial for 1 min; then from 85 to 230°C, first programmed temperature at 15°C/min; from 230 to 270°C, second programmed temperature at 7°C/min; from 270 to 300°C, third programmed temperature at 30°C/min; injector: split, split flow of 20 ml/min, temperature 240°C; GC-MS transfer line temperature 280°C; MS acquisition: full scan from m/z 45 to m/z 700, scan speed, 1 decade/sec.; EI source: electron energy, 36 eV, temperature 180°C.

DIS-EI mass spectra of the IS were recorded with the ion source held at 180°C.

GC-NICI-MS GC-NICI-MS determinations were made with the same instrument, GC column and chromatographic conditions as for GC-EI-MS analyses. MS operated in the selected ion recording (SIR) mode monitoring the ions at m/z 35 and 37 (corresponding to the chlorine isotopes) for all the analytes, the ion at m/z 71 (ion $H^{35}Cl_2$) specifically for α-HCH and γ-HCH, the ions at m/z 284 and 286 specifically for HCB (molecolar ion cluster), the ions at m/z 79 and 81 (bromine anion isotopes) for the IS. Positive identification of the analytes in the samples was based on retention times and correct ratios between peak areas in the different traces used to monitor the compounds, compared to the values found in the standards.

GC-ECD A Carlo Erba GC 8000 gas chromatograph equipped with a ^{63}Ni ECD detector was used in the following conditions: GC column: Cp-Sil 8 CB, 50 m length, 0.25 mm internal diameter, 0.25 µm film thickness; helium carrier gas at 1 ml/min; nitrogen auxiliary gas at 60 ml/min for the detector; chromatographic program 100°C initial; from 100 to 180°C, first programmed temperature at 20°C/min; from 180 to 250°C, second programmed temperature at 2°C/min; final temperature held for 30 min; injector: on-column; ECD detector temperature 270°C.

RESULTS

The total ion current profile relative to the GC-EI-MS analysis of the synthetized product is shown in Figure 2. The EI mass spectrum of the IS is shown in Figure 3: the molecular ion is present (peak at m/z 330), the base peak at m/z 235 corresponds to the $(M\text{-}CH_2Br)^+$ ion.

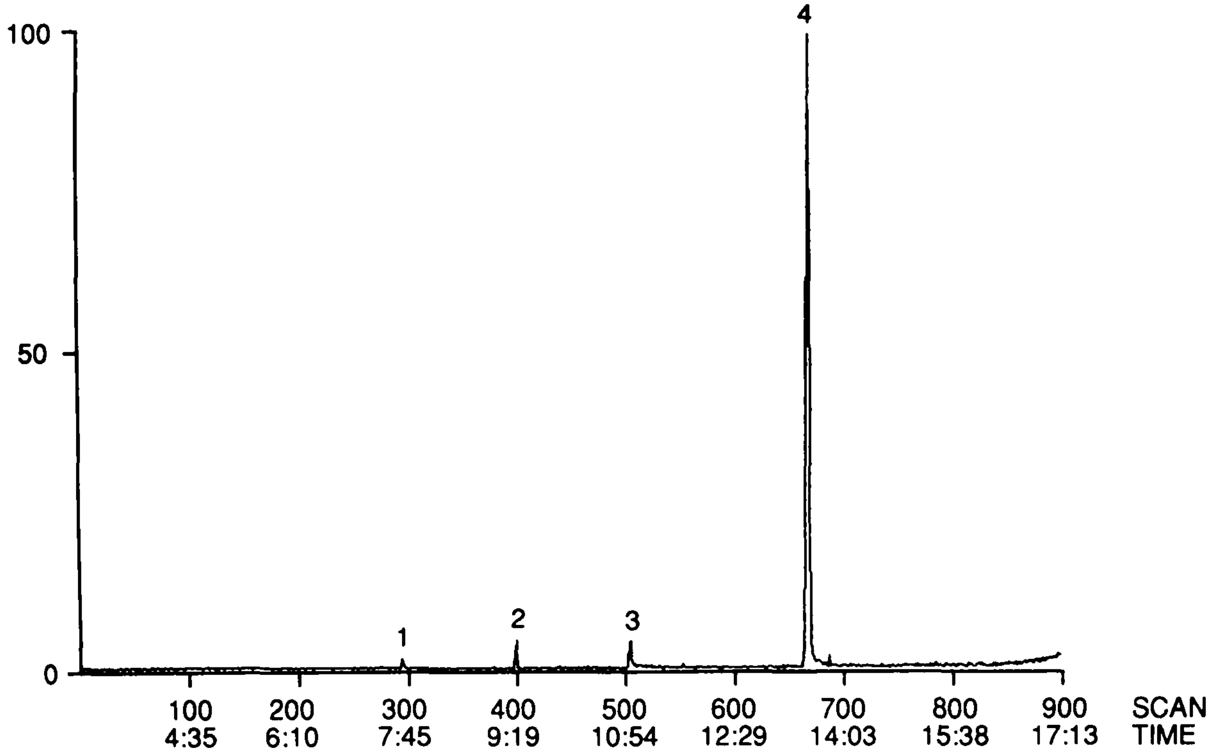

Figure 2 GC-EIMS total ion current profile relative to 1 μg injection of the synthesized brominated internal standard. 1 = butylated hydroxy toluene (BHA, probably a contaminant from the plastics), 2 = unknown, 3 = 1,1-bis(4-chlorophenyl)ethene (elimination by-product), 4 = 1,1-bis(4-chlorophenyl)2-bromoethane

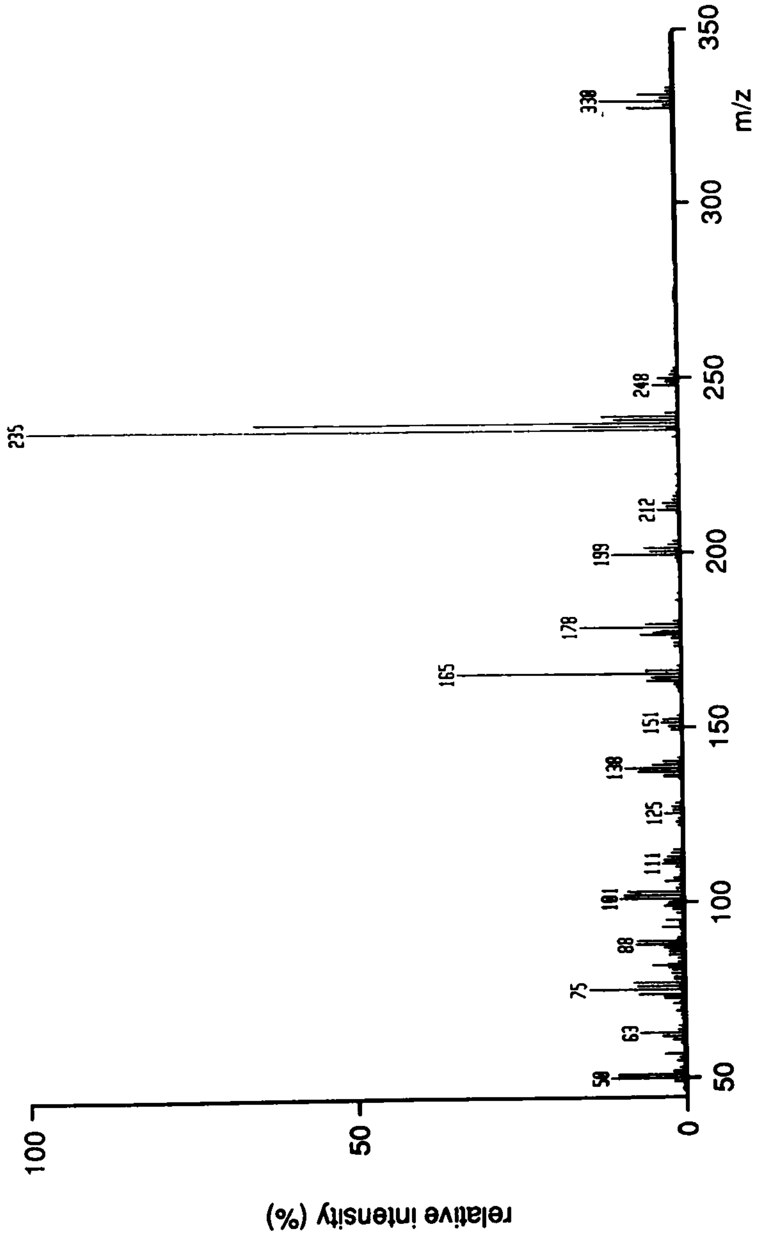

Figure 3 Electron ionization mass spectrum of 1,1-bis(4-chlorophenyl)2-bromoethane

The chromatogram presented a peak corresponding to the elimination by-product of bromination, 1,1-bis(4-chlorophenyl)ethene, identified by its mass spectrum; based on the peak area, this product accounts for 3.5% of the brominated compound.

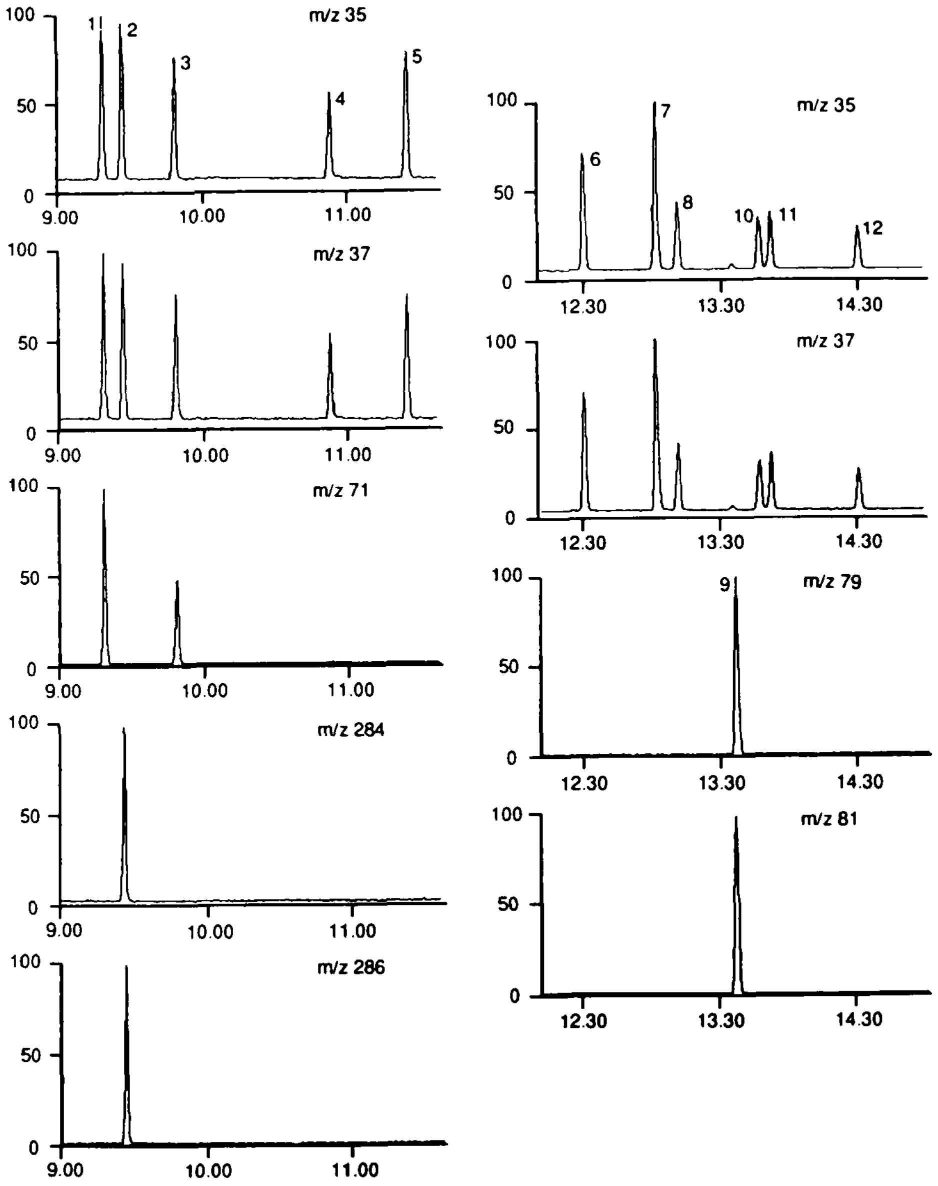

Figure 4 GC-NICI-MS selected ion recording traces relative to the injection of 50 pg for each organochlorine pesticide plus 200 pg of 1,1-bis(4-chlorophenyl)2-bromoethane. 1 = α-HCH, 2 = HCB, 3 = γ-HCH, 4 = Heptachlor, 5 = Aldrin, 6 = op′ DDE, 7 = pp′ DDE, 8 = op′ DDD, 9 = 1,1-bis(4-chlorophenyl)2-bromoethane, 10 = pp′ DDD, 11 = op′ DDT, 12 = pp′ DDT. On X-axis, time expressed in minutes

DIS analysis showed no impurities besides that identified in the above experiments.

GC-ECD analysis for the IS showed a limit of detection in the order of 5 pg injected, based on a signal-to-noise ratio of 3:1; the limit for the organochlorines ranged from 0.5 pg for α-HCH and γ-HCH to 1 or 2 pg for the other analytes.

The NICI-MS behaviour for IS was similar to that observed for most halogenated compounds[13]; major peaks of the spectrum are the isotopic signals due to the bromine anion produced. We also observed small signals corresponding to the chlorine anion and the ion-molecule association $(M+Br)^-$, these not being of analytical interest.

An example of SIR analysis after injection of 50 pg for each pesticide and 200 pg of IS is shown in Figure 4: chromatographic separation of the IS from the analytes was good in these conditions. The signal at m/z 71 used for monitoring the hexachlorocyclohexanes provided better specificity and a lower noise level than the m/z 35 and 37 fragments. In all the pesticides considered, only HCB presented a molecular ion signal in its spectrum, useful for SIR analysis.

Using this technique, limits of detection were of the order of 0.5 pg for α-HCH and γ-HCH, 2 pg for HCB, and 5 pg for the other analytes.

Table 1 summarizes the calibration curves using the two detection systems. Linearity was good in the concentration ranges considered.

Other halogenated compounds similar to some of the organochlorines analysed were tested as IS in GC-NICI-MS, but the results were disappointing: 2,2-bis (4-methyphenyl) hexafluoropropane presented poor stability at room temperature in the solvent solution, and chlorobis (4-fluorophenyl) methane gave insufficient one-day reproducibility because of its thermal instability in the GC split/splitless injector.

The vegetable extracts analysed with GC-NICI-MS present the SIR profiles shown in Figures 5 and 6, for spruce needle and spruce branch from an urban area (Milan). Most of the interfering peaks in the chromatograms are due to chlorinated compounds, responding to the chlorine ion traces with the characteristic isotope ratio; some of these might be PCBs.

Table 1 regression analysis relative to the calibration curve experiments; linear model: $Y = a + kX$. Y = peak area analyte/peak area IS. X = analyte concentration/IS concentration. Analysis of variance parameters: r^2 = coefficient of determination, SQ(e) = residual, P = probability level; n (number of replicates per concentration point) = 2

	ECD determinations					*NICIMS determinations*				
ANALYTE	*k*	*a*	r^2	*SQ(e)* $(\times 10^{-3})$	*P* $(\times 10^{-4})$	*k*	*a*	r^2	*SQ(e)*	*P* $(\times 10^{-4})$
α-HCH	3.912	–0.032	99.58%	11	1.2	4.291	–0.14	86.49%	2.6	25
HCB	3.799	0.018	98.62%	1.4	6.9	2.297	–0.11	82.81%	0.97	46
γ-HCH	2.441	0.017	99.57%	1.1	1.2	2.089	–0.08	88.36%	0.53	18
Heptachlor						5.717	–0.12	94.09%	1.6	2
Aldrin						9.223	0.029	94.11%	4.2	2
op′ DDE						9.649	0.053	98.01%	1.3	1
pp′ DDE	2.584	0.012	99.46%	6.3	1.7	15.12	0.17	98.05%	3.7	1
op′ DDD						6.3	0.15	96.04%	1.7	2
pp′ DDD	1.987	0.021	99.26%	5.1	2.7	4.746	0.091	94.10%	1.3	3
op′ DDT						5.011	–0.16	98.02%	0.47	1
pp′ DDT	1.555	–0.056	99.31%	12	2.4	3.42	–0.13	90.25%	1.8	12

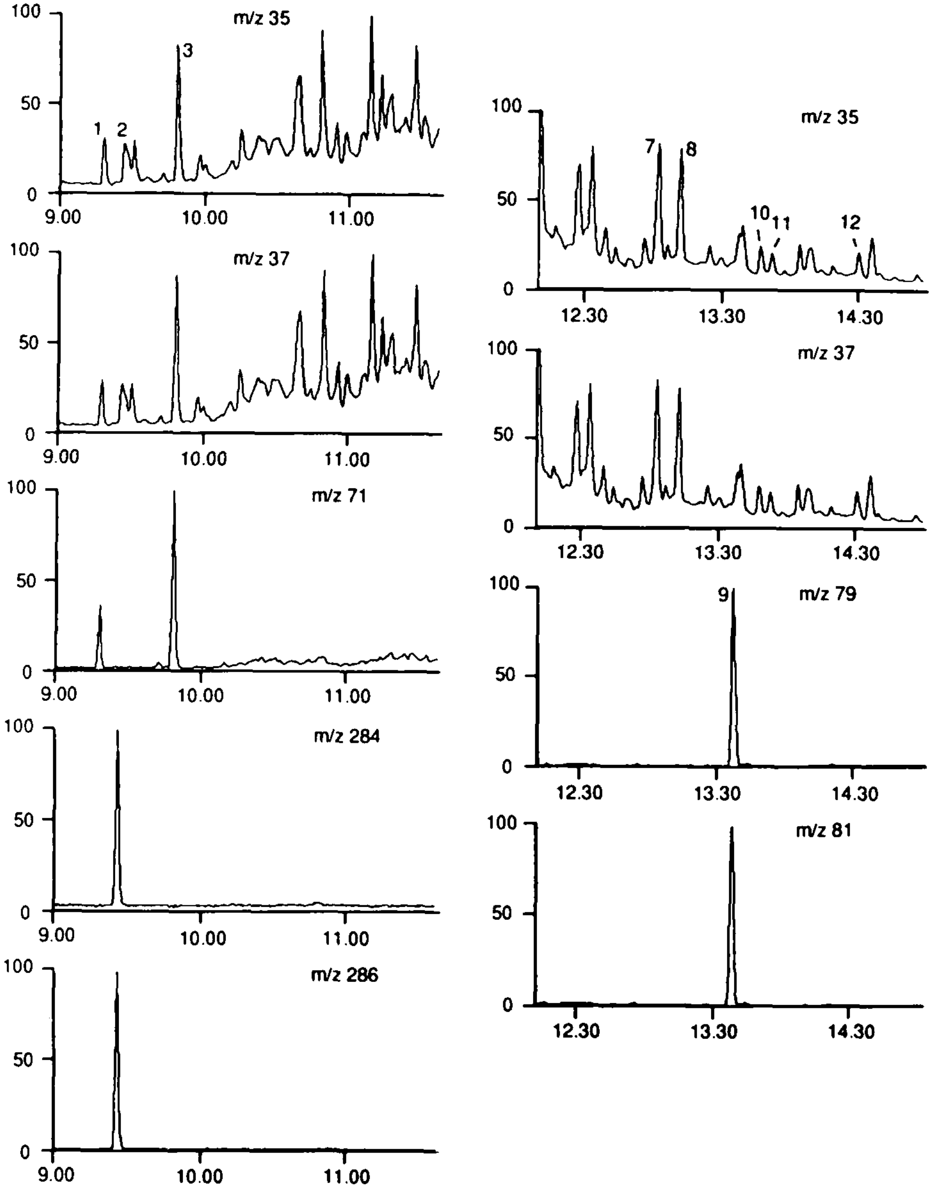

Figure 5 GC-NICI-MS selected ion recording traces of a spruce needle extract, sample from an urban area (Milan); see figure 4 for peak identification. On X-axis, time expressed in minutes

PCB have been detected in the needles of coniferous trees[14]. No significant interferences were observed in the m/z 79 and 81 SIR traces.

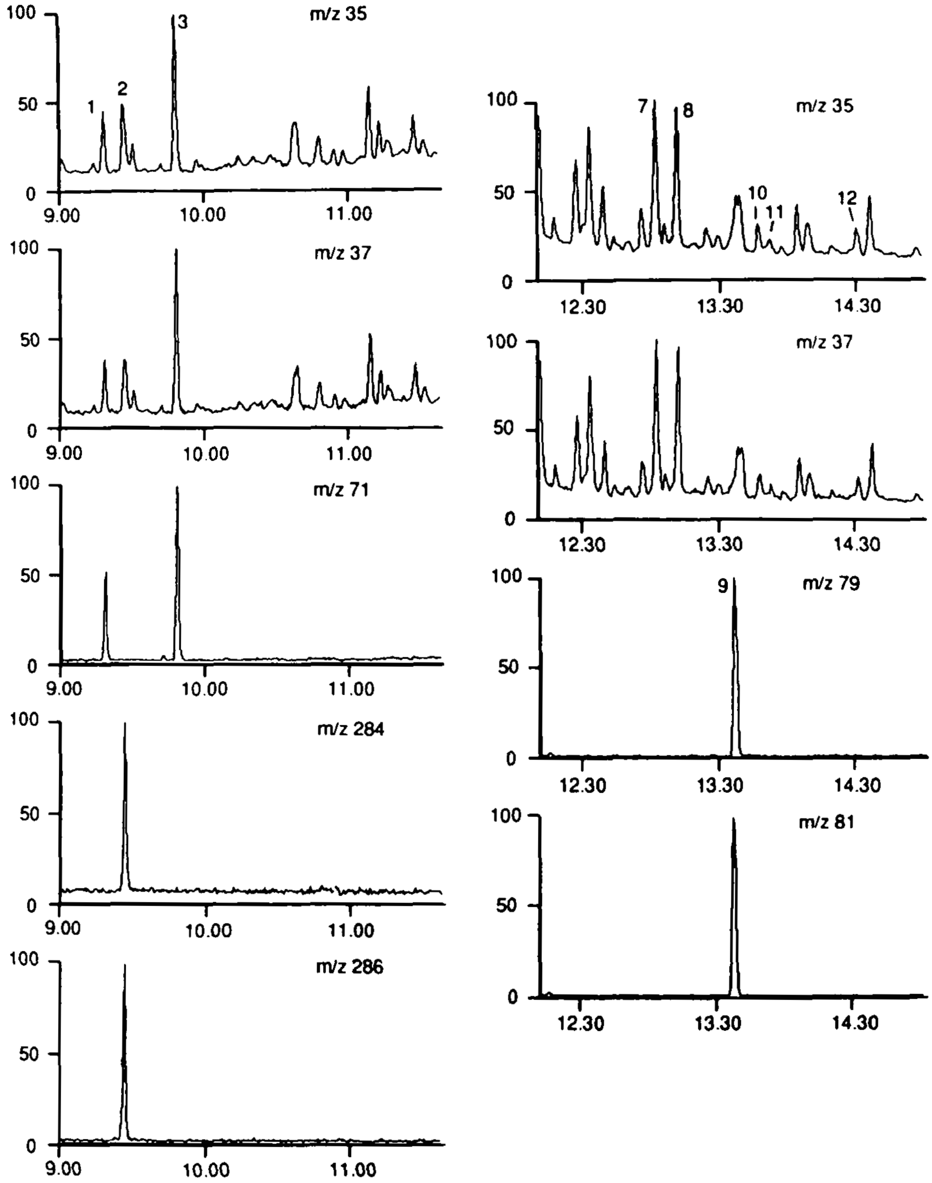

Figure 6 GC-NICI-MS selected ion recording traces of a spruce branch extract, sample from an urban area (Milan); see figure 4 for peak identification. On X-axis, time expressed in minutes

The GC-ECD chromatogram relative to an injection of the Florisil purified extract is show in Figure 7: our IS is well separated respect to other compounds eluted and observed with this detector.

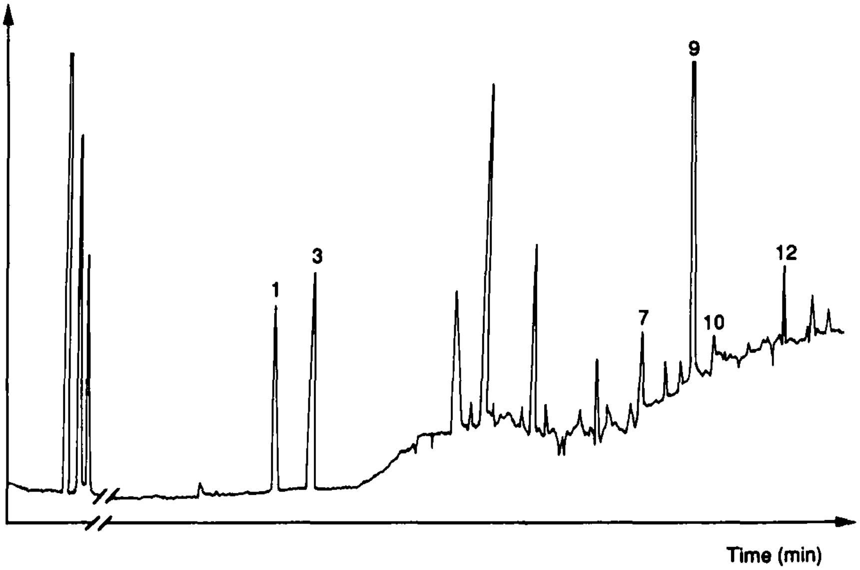

Figure 7 GC-ECD chromatogram relative to a Florisil purified conifer needle extract; the sample was spiked with the IS prior to its extraction. See figure 4 for peak identification.

The chemical stability of the IS to the sulfuric acid purification step was tested previously on a *n*-hexane solution of this compound enriched with the organochlorine pesticides, subsequently treated according to the purification step and analysed with GC-NICI-MS. No noteworthy differences were found from standard solutions at the same concentration ratio.

The stability of an IS solution in *n*-hexane was assessed after fourteen months of storage at –4°C in the dark; the GC-MS analysis did not reveal any new by-product respect to the fresh solution assay; the resulting purity of the material was not changed significatively over this storage time.

CONCLUSIONS

1,1-bis(4-chlorophenyl)2-bromoethane was successfully used as IS in the GC analysis of some organochlorine pesticides; linearity against the analytes was good using two different detection systems, NICI-MS and ECD. Application of this compound in analytical methods involving this latter detector is recommended, on the basis of the widespread use of ECD to assay organochlorine pesticides in most environmental samples. This brominated compound was easily synthetized using commercially available reagents, and the product had acceptable purity for our purposes.

Acknowledgements

This work was partly supported by the CNR-Comitato Ambiente, n° 91.02200.CT13.

References

1. D. J. Gregor and W. D. Gummer, *Environ. Sci. Technol.*, **23,** 561–565 (1989)
2. R. Guitart, J. L. Riu, A. Puigdemont and M. Arboix, *Bull. Environ. Contam. Toxicol.*, **44,** 555–560 (1990)
3. A. Ferrer, M. A. Bona, M. Castellano, J. To-Figueras and M. Brunet, *Bull. Environ. Contam. Toxicol.*, **48,** 561–566 (1992)
4. A. Borrell and A. Aguilar, *Bull. Environ. Contam. Toxicol.*, **45,** 46–53 (1990)
5. D. A. Verbrugge, R. A. Othoudt, K. R. Grzyb, R. A. Hoke, J. B. Drake, J. P. Giesy and D. Anderson, *Chemosphere*, **22,** 809–820 (1991)
6. S. Jensen, G. Eriksson, H. Kylin and W. M. J. Strachan, *Chemosphere*, **24,** 229–245 (1992)
7. M. S. Driscoll, J. P. Hassett, C. L. Fish and S. Litten, *Environ. Sci. Technol.*, **25,** 1432–1439 (1991)
8. B. Larsen and J. Riego, *Intern. J. Environ. Anal. Chem.*, **40,** 59–69 (1990)
9. V. Lopez-Avila, J. Benedicto, E. Baldin and W. F. Beckert, *J. High Resolut. Chromatogr.*, **15,** 319–328 (1992)
10. M. T. Galceran, F. J. Santos, J. Caixach and J. Rivera, *Chemosphere*, **27,** 1183–1200 (1993)
11. R. Knickmeyer and H. Steinhart, *Chemosphere*, **20,** 109–122 (1990)
12. K. Warman, *Chemosphere*, **20,** 1511–1516 (1990)
13. R. C. Dougherty, J. Dalton and F. J. Biros, *Org. Mass Spectrom.*, **6,** 1171–1181 (1972)
14. C. Gaggi, E. Bacci, D. Calamari and R. Fanelli, *Chemosphere*, **14,** 1673–1686 (1985)