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Clean-up and confirmatory procedures for gas chromatographic analysis of pesticide residues. Part II

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Abstract

The behaviour of standard solutions of fourteen simple organohalogenated pesticides, nine individual polychlorinated biphenyls (PCBs) and Aroclor 1242, 1248, 1254 and 1260 on treatment with sulphuric acid, potassium hydroxide and chromium(IV) oxide was studied by capillary gas–liquid chromatography (cGC) using electron-capture detection. These methods were applied to confirm the presence of organochlorine residues in river water and human milk. Positive confirmation with the three treatments were in agreement with capillary GC(cGC)–MS determinations carried out in the electron impact and selected-ion monitoring mode. After cGC analyses, the extracts containing possible pesticides or Aroclors were treated with the three chemicals and re-analysed under the same cGC conditions. The new chromatographic profiles showed many missing artifact peaks, and some pesticides or PCBs were also destroyed. The presence or disappearance of the peaks after chemical attack makes it possible to identify the specific pesticides and PCBs analysed. PCBs resist both acid and alkali attacks, but some low-chloride PCBs are totally or partially destroyed by oxidative treatment. The methods studied are useful for intralaboratory purification and confirmation of residues of pesticides and PCBs, although they can be insufficient for identifying organochlorine pesticide residues from some very polluted samples.

1. Introduction

Capillary gas chromatography (cGC) with electron-capture detection (ECD) for the determination of organochlorine compound residues is a sensitive and selective method that is used in most research laboratories. However, extracts of plant, animal or environmental origin can contain electron-capturing materials other than pesticides or polychlorinated biphenyls (PCBs), and this can lead to incorrect identifications even if two different polarity capillary columns are employed.

To eliminate interferences normally occurring in halogenated residue analyses, several methods have been proposed. Most of them include adsorption column chromatography to clean up the extracts before cGC determination. This additional step is a major factor affecting the reproducibility of the overall analytical procedure and it is time, solvent and adsorbent consuming.

Adsorbents for column chromatography have also been mixed or impregnated with other compounds, such as acids, alkalis or oxidizing

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reagents, to help in the clean-up process. For example, Extrelut-1 was impregnated with sulphuric acid [1], Celite with sulphuric acid or magnesium oxide [2], alumina with potassium hydroxide or *tert.*-butoxide [3] and Florisil with silver nitrate [4].

Alternative methods for purifying extracts containing organochlorine residues or confirming some of the possible identified residues include chemical treatments. These treatments can be carried out on-line in a gas chromatographic system, with a liner filled with the chemical reagent, generally sodium or potassium hydroxide [5–7], magnesium oxide [8] or reducing, oxidizing, Lewis acid or weak alkali agents [9]. Most chemical treatments, however, are carried out off-line by mixing the extracts with acid, alkali, oxidizing or reducing reagents. These procedures do not require any modification of the chromatographic system, are inexpensive and are applicable in most research laboratories. However, they are not fully utilized by residue laboratories in routine confirmatory analyses, and no studies on the behaviour toward potassium hydroxide, sulphuric acid and chromium(VI) oxide treatments of some interesting pesticide metabolites such as endrin aldehyde and endrin ketone and individual PCBs were found in the literature.

Chemical treatments were originally applied to confirm organochlorine pesticide peaks in residue analyses when they were determined on packed columns [10–13]. Sulphuric acid dissolves many organic compounds other than saturated or chlorinated hydrocarbons. For this reason, it is used to purify food extracts in organochlorine pesticide and PCB analyses [14–20]. Alkali metal hydroxides in ethanolic solution dehydrochlorinate pesticides from the bis (phenyl) chloroethane group [21,22]. This effect has been employed to distinguish DDT and its metabolites from PCB residues [1,23,24]. Chromium(VI) oxide in acetic acid solution makes it possible to determine Aroclors in the presence of DDT and its analogues [25,26], and to determine total DDT metabolites as dichlorobenzophenones present in the interfering Aroclors [27]. The reactions between cyclodiene pesticides and different acidic, basic and derivatization agents have investigated to identify the mechanisms of the reactions [28–30].

Our interest centres on the ability of the most widely accepted chemical treatments, such as with concentrated sulphuric acid, ethanolic potassium hydroxide and chromium(VI) oxide in acetic acid solution, to purify environmental extracts and identify pesticide residues. A preliminary report gave the results for the three cited treatments when they were applied to eleven organochlorine and ten organophosphorus pesticides [31].

2. Experimental

2.1. Reference materials

Aldrin (purity 98%), endrin (95%), endrin ketone (98%), heptachlor (99%), heptachlor epoxide (99%), hexachlorobenzene (HCB) (99%), lindane (99%) and methoxychlor (99%) were purchased from Promochem (Wesel, Germany), *o,p'*-DDD (99%), *p,p'*-DDD (99%) from Aldrich, (Alcobendas, Spain) and *P,p'*-DDE (99%), *p,p'*-DDT (99%), dicofol (99%), endrin aldehyde (98%) and individual PCBs from Riedel-de Haën (Seelze, Germany). Commercially available PCB mixtures, Aroclor 1242, 1248, 1254 and 1260, were purchased from Supelco (Bellefonte, PA, USA).

2.2. Solvents

Ethyl acetate, *n*-hexane, ethanol and methanol (Nanograde quality) were purchased from Promochem.

2.3. Reagents

Sulphuric acid (sp. gr. 1.84), glacial acetic acid, potassium hydroxide and chromium(VI) oxide were of analytical-reagent grade from Merck (Darmstadt, Germany). Reagent solutions were as follows: acidic solution, 90% sulphuric acid; alkaline solution, 2 *M* potassium hydroxide in ethanol; and oxidative solution, 5 g

of chromium(VI) oxide dissolved in 3 ml of distilled water with addition of 60 ml of glacial acetic acid.

2.4. Apparatus

A Konik KNK 2000C gas chromatograph (Sant Cugat del Vallés, Barcelona, Spain) equipped with a Ni^{63} electron-capture detector and a Spectra-Physics SP 4290 integrator were used. A Hewlett-Packard HP 5890 gas chromatograph equipped with an HP 5970 mass-selective ion detector (quadrupole), HP 59970 MS-CHEM station and HP 59973 NBS mass spectral library was also used.

The working fused-silica capillary column for both gas chromatographs was 0.25 μm bonded-phase BP-5 (5% phenyl–methylsiloxane) (25 m × 0.22 mm I.D.) provided by Scientific Glass Engineering (Ringwood, Victoria, Australia). For confirmatory purposes a 0.25-μm bonded-phase DB-17, (50% phenyl–methylsiloxane) column (30 m × 0.24 mm I.D.) provided by J & W Scientific (Folsom, CA, USA) was employed.

2.5. Gas chromatographic conditions

With the KNK 2000C system, the injector temperature, operating in splitless mode (0.7 min), was set at 285°C, the detector temperature was set at 300°C and the oven temperature was programmed as follows: initial temperature 50°C (0.8 min), increased at 30°C min^{-1} to 140°C, held for 2 min, then increased at 5°C min^{-1} to 280°C, the final temperature being held for 12 min.

With the HP 5890 system, the injector and oven temperatures were the same as for the KNK 2000C system, the transfer line was set at 300°C, the mass spectrometric source was set at 200°C, the electron impact (EI) energy was set at 70 eV and selected-ion monitoring (SIM) was performed according to characteristic ions of the pesticides and PCBs to be analysed.

2.6. Extraction procedures

Water analysis was based on solid-phase extraction (SPE) with preparative octadecylsilica placed in a glass minicolumn, as in previous work [32–34].

Human milk was analysed as described by Mañes and co-workers [35,36]. The samples were treated with methanol and distilled water to destroy the fat globules and then extracted with a glass minicolumn of octadecylsilica.

2.7. Acid, alkali and oxidative treatment procedures

These procedures were fully described in a previous paper [31], and can be summarized as follows: extract-containing pesticides or PCBs were mixed with 90% sulphuric acid, chromic (VI) oxide in glacial acetic acid at 75–80°C or 2 *M* ethanolic potassium hydroxide, shaken for a few minutes, washed to eliminate the excess of the reagents and then the organic layers were recovered and re-analysed by cGC.

3. Results and discussion

Tables 1 and 2 give the recoveries of the studied organochlorine pesticides and PCBs, respectively, after the chemical treatments at two concentration levels. The results show the applicability of the three chemical treatments at trace levels of the studied compounds.

Of the three treatments, the sulphuric acid treatment gives the least degradation. It is usually applied to purify extracts containing lipids in organochlorine pesticide analyses [4] and in PCB analyses [19].

Our results agree with other reports of the use of sulphuric acid in all instances except for heptachlor epoxide, which some workers [12,17,37] state is not degraded by sulphuric acid attack. Another report [18] describes a lower recovery for heptachlor epoxide than for other pesticides that do not contain oxygen. In some studies heptachlor epoxide was destroyed by a mixture of acetic anhydride in hydrobromic acid [10], hydrochloric acid [29] and trifluoroacetic acid [38]. Under our experimental conditions the degradation occurs at both levels of concentra-

Table 1
Recoveries (% not destroyed by acid, alkali and oxidant treatments) of standard organochlorine pesticide solutions at two concentration levels

Pesticide	Working solution (μg/ml)		Recovery after treatment (%)					
			Acid		Alkali		Oxidant	
	Level 1	Level 2	Level 1	Level 2	Level 1	Level 2	Level 1	Level 2
Aldrin	0.10	1.00	57 ± 12	60 ± 5	84 ± 15	87 ± 3	0	0
o,p'-DDD	0.35	2.55	92 ± 10	95 ± 2	75 ± 13	79 ± 6	78 ± 11	81 ± 4
p,p'-DDD	0.60	5.50	92 ± 10	94 ± 4	0	0	0	0
p,p'-DDE	0.15	1.50	85 ± 14	88 ± 2	85 ± 12	90 ± 5	0	0
p,p'-DDT	0.25	2.00	80 ± 14	88 ± 5	0	0	49 ± 17	50 ± 9
Dicofol	0.75	6.00	65 ± 15	79 ± 8	0	0	0	0
Endrin	0.25	2.00	0	0	85 ± 12	89 ± 6	0	0
Endrin aldehyde	0.30	2.50	87 ± 10	94 ± 3	69 ± 15	72 ± 5	0	0
Endrin ketone	0.30	2.50	94 ± 11	96 ± 6	0	0	94 ± 10	98 ± 6
HCB	0.10	0.50	72 ± 12	75 ± 5	70 ± 14	78 ± 4	73 ± 14	77 ± 4
Heptachlor	0.10	1.00	79 ± 12	89 ± 8	85 ± 16	94 ± 7	0	0
Heptachlor epoxide	0.10	1.00	0	0	71 ± 13	76 ± 8	85 ± 12	91 ± 8
Lindane	0.10	1.00	90 ± 13	92 ± 8	0	0	50 ± 14	50 ± 7
Methoxychlor	0.35	2.95	50 ± 15	53 ± 9	80 ± 11	83 ± 7	0	0

See Experimental for details of the treatments. Results are means ± relative standard deviations for quintuplicate analyses.

Table 2
Recoveries (% not destroyed by acid, alkali and oxidant treatments) of standard PCBs and Aroclors at two concentration levels

Pesticide	Working solution (μg/ml)		Recovery after treatment (%)					
			Acid		Alkali		Oxidant	
	Level 1	Level 2	Level 1	Level 2	Level 1	Level 2	Level 1	Level 2
2'-PCB	5.00	35.00	97 ± 11	97 ± 4	96 ± 13	97 ± 4	64 ± 17	66 ± 6
2'2'-PCB	4.00	28.00	92 ± 10	95 ± 3	95 ± 14	97 ± 3	68 ± 15	68 ± 5
2,4'-PCB	0.60	3.60	97 ± 13	97 ± 4	98 ± 10	98 ± 3	0	0
4,4'-PCB	4.00	24.50	97 ± 12	98 ± 4	99 ± 14	99 ± 3	0	0
2,4,5'-PCB	0.40	2.40	98 ± 14	98 ± 6	97 ± 10	98 ± 5	60 ± 14	62 ± 7
3,3',4,4'-PCB	0.35	1.80	96 ± 10	97 ± 3	96 ± 12	97 ± 4	0	0
2,2',4,5,5'-PCB	0.25	1.25	94 ± 14	97 ± 5	96 ± 15	97 ± 6	95 ± 13	96 ± 5
2,2',4,4',5,5'-PCB	0.15	0.60	96 ± 12	98 ± 7	95 ± 11	97 ± 8	97 ± 10	98 ± 6
Decachlorobiphenyl	0.10	0.50	95 ± 10	99 ± 7	98 ± 14	99 ± 5	98 ± 11	99 ± 4
Aroclor 1242	0.35	2.55	97 ± 10	98 ± 5	96 ± 12	96 ± 10	87 ± 14	88 ± 8
Aroclor 1248	0.20	2.00	97 ± 12	99 ± 5	97 ± 14	96 ± 9	91 ± 14	95 ± 5
Aroclor 1254	0.20	2.00	96 ± 11	97 ± 7	95 ± 14	96 ± 6	95 ± 10	95 ± 6
Aroclor 1260	0.10	1.50	97 ± 11	97 ± 8	96 ± 12	97 ± 8	98 ± 11	97 ± 8
Aroclor 1262	0.10	1.60	97 ± 10	98 ± 7	97 ± 13	95 ± 4	97 ± 12	97 ± 4

See Experimental for details of the treatments. Results are means ± relative standard deviations for quintuplicate analyses.

tion studied. The 20-μg concentration level was also studied by cGC–MS in the scanning mode, and the profile clearly showed the disappearance of the heptachlor epoxide peak. The discrepancy can be attributed to the presence of an artifact peak when packed columns are used with ECD detection in place of capillary columns with a highly stable bonded phase in an MS detector, or more probably to a longer reaction time with sulphuric acid under our analytical conditions. The behaviour of endrin aldehyde and endrin ketone towards sulphuric acid treatment has not been reported in the literature so no comparison can be made.

The results of potassium hydroxide treatment are substantially different from those reported in the literature because all the reference studies were carried out at 100°C whereas our treatment was done at room temperature; at 100°C *o,p'*-DDD [12,21–22], heptachlor [29], dicofol [11] and methoxychlor [11,21,23] were destroyed. This destruction does not occur at room temperature (see Table 1) even if 5 *M* KOH is employed instead of 2 *M* KOH. These results suggest that KOH treatment is highly temperature dependent and this parameter must be controlled carefully. For example, carrying out the reaction in steam of water destroys only 30% of the heptachlor [21].

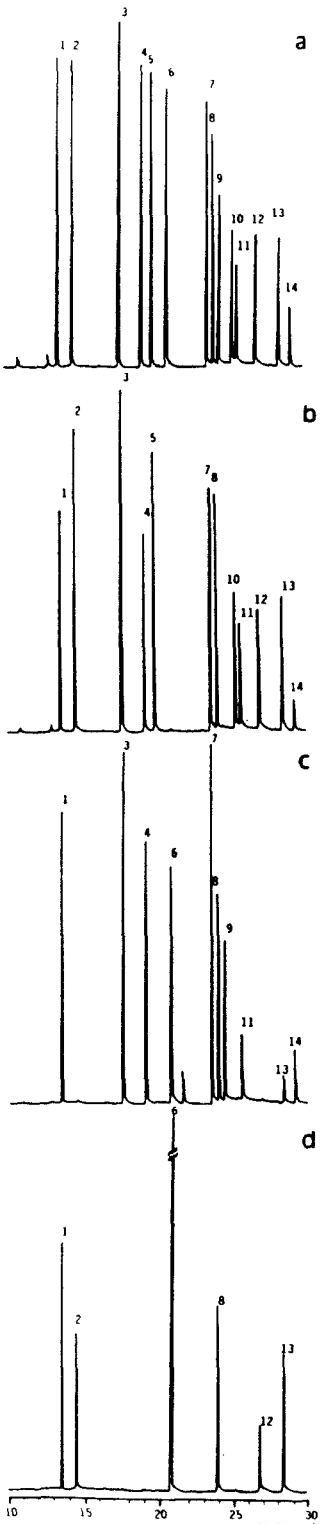
When the alkaline reaction is carried out at 100°C, it is more destructive than the same reaction at room temperature. At room temperature *o,p'*-DDD, *o,p'*-DDT [31], β-HCH [31], heptachlor, dicofol and methoxychlor were not destroyed and could be determined, but the purification power was also diminished. No references to reaction products of alkaline attack on endrin aldehyde and endrin ketone were found. The stability of the cyclodienes aldrin, endrin, dieldrin [31] and isodrin [31] when subjected to alkaline attack at room temperature is remarkable (Table 1). Aldrin [3,21], dieldrin [3], endrin [3] and isodrin [3,21] remain unaltered under alkaline attack at 70–100°C. Endrin aldehyde is slightly affected at room temperature and endrin ketone is virtually destroyed (Table 1).

Fig. 1a shows the chromatographic profiles of a standard mixture of organochlorine pesticides. Peaks remaining after the acidic, alkaline and oxidative treatments are shown in Fig. 1b, c and d, respectively. Chromium(VI) oxide is the most destructive of the three treatments.

Chromium(VI) oxide treatment yields the cleanest chromatographic profiles (see Fig. 2), although a large part of the pesticides studied were partially or totally destroyed. For this reason chromium(VI) oxide is frequently applied to determine residues of PCBs from Aroclors in environmental samples [24–27], but it degrades some PCBs that have low or medium chlorine contents (see Table 2). This means that the determination of Aroclors is carried out with losses, which are higher for low chlorine-content Aroclors. This effect has been reported by most researchers [24,26,27] but not reproduced by some [25]. It can be seen from Table 2 that Aroclors with a low chlorine content are degraded more than those with a high chlorine content. In addition, individual PCBs were studied, but the effects of the position of chlorine and the number of chlorine atoms on the rings and the degradation relationship were not evident. Of the individual PCBs studied, only those containing less than five chlorine atoms were destroyed. A lower chlorine presence and degradation were not directly correlated. For example, 2-PCB containing only one chlorine was not destroyed. More chlorine substitution on the same ring does not always protect against degradation (e.g., 2,4,5-PCB was not degraded whereas 2,4-PCB was virtually destroyed).

Some compounds from the degradation of the studied compounds after such treatments are well known. Sulphuric acid degrades endrin to its metabolites endrin aldehyde and endrin ketone [1,18,30]. This conversion was not quantitative, and the metabolites only appeared on the chromatogram if a sufficient amount of endrin was present in the extract. The ability of strong acids to destroy endrin has been well established [30,37]. Trifluoroacetic acid also destroys endrin and partially destroys endrin aldehyde [38].

Alkaline treatment degrades *p,p'*-DDD to *p,p'*-DDMU, dicofol to dichlorobenzophenone, *p,p'*-DDT to *p,p'*-DDE at room temperature and *o,p'*-DDD to *o,p'*-DDMU [12,21], *o,p'*-



DDT to o,p'-DDE [12] and methoxychlor to its corresponding olefin [11,23] at 100°C.

Chromium(VI) oxide reacts with p,p'-DDE and dicofol to form dichlorobenzophenones [25] and with heptachlor to from heptachlor epoxide [11]. Trichlorobenzoic acids were found via NBS mass spectral standards to be possible degradation products of some individual PCBs, and endrin ketone was partially formed from endrin on chromium(VI) oxide attack.

Some degradation products must be mentioned because they are interesting organochlorine residues and present retention times similar to those of the other compounds studied. Other degradation products such as benzophenones or trichlorobenzoic acids show retention times shorter than that of lindane under our cGC conditions and they are poor indicators of the presence of their precursors because elution occurs in a peak-rich zone of the chromatogram when real samples are processed. The presence of benzophenones is therefore poorly selective because they are known degradation compounds of many diphenyl-substituted compounds such as drugs [39,40].

In Table 1 it can be observed that the behaviour of pesticides (cyclodienes, diphenylethane derivatives, HCH isomers) on chemical treatments of the same chemical kind are dissimilar. With the same chemical treatment, some of the pesticides in a family are degraded whereas others persist. It should be pointed out that all of the diphenylethane derivatives are acid resistant and only o,p'-substituted diphenylethane derivatives are more chemically resistant than their p,p'-analogues (Table 1) [31].

This chemical resistance could be a partial reason for the remaining of o,p'-DDT metabolites in environmental samples even when gener-

Fig. 1. Chromatogram of working organochlorine pesticide solution obtained (a) without any treatment, (b) after acid treatment, (c) after alkali treatment and (d) after oxidant treatment. Peaks: 1 = HCB; 2 = lindane; 3 = heptachlor; 4 = aldrin; 5 = dicofol; 6 = heptachlor epoxide; 7 = p,p'-DDE; 8 = o,p'-DDE; 9 = o,p'-DDD; 10 = endrin; 11 = p,p'-DDD; 12 = endrin aldehyde; 13 = endrin ketone; 14 = methoxychlor.

Fig. 2. (a) Chromatograms for a human milk sample, obtained by GC–ECD, (a) without any chemical treatment and after (b) acid, (c) alkali and (d) oxidant treatment. ★ = Peak at the same retention time as HCB; ■ = peak at the same retention time as p,p'-DDE; ● = peak at the same retention time as methoxychlor. See text for operating conditions.

ally high-purity p,p'-DDT was utilized as pesticide.

Minor changes in the methods can change the results. In the sulphuric acid treatment, a small variation in the acid concentration strongly affects the recovery of methoxychlor [1]. This explains the irregular recoveries and poor R.S.D.s with the sulphuric acid treatment (see Table 1).

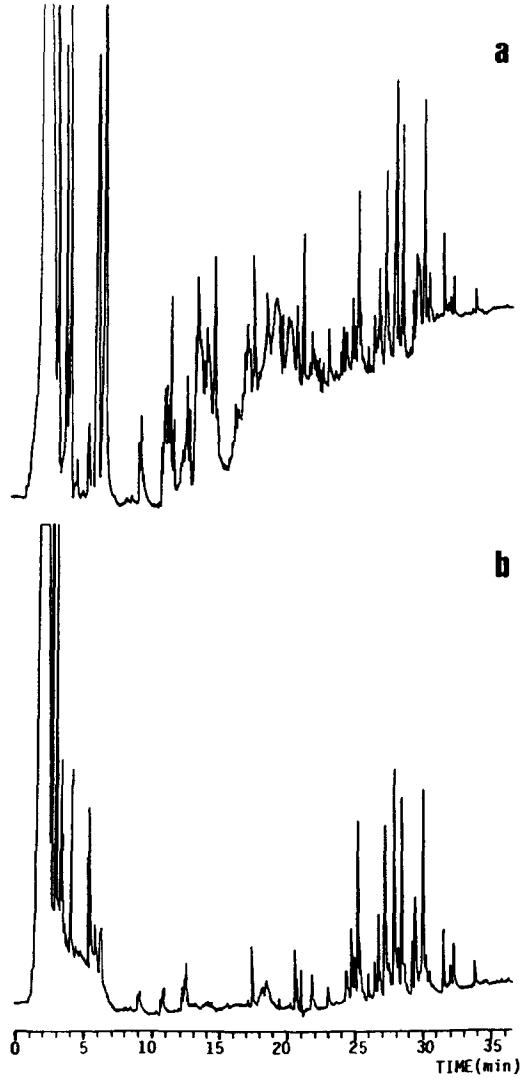


Fig. 3. Chromatograms obtained from a water sample containing Aroclor 1254, (a) before treatment and (b) after treatment with chromium(VI) acid.

When the alkaline treatment was carried out in the presence of water, DDT was not degraded to DDE [10]. On the other hand, important differences were found when the reactions were carried out at 100°C (results in the literature) instead of room temperature (this work). The reaction of *p,p'*-DDD with chromium(VI) oxide is reported to be temperature dependent and moisture sensitive [13]. To minimize these differences, treatments can be carried out in parallel with standards containing a pesticide or Aroclor at the suspected concentrations.

The purification power of the treatments studied may be insufficient for samples very highly contaminated with compounds other than pesticides or Aroclors. In such cases, chromium(VI) oxide is the best of the three treatments for Aroclor analyses and for the few organochlorine pesticides that resist oxidation, such as *o,p'*-DDD, *p,p'*-DDT, HCB, heptachlor epoxide, lindane and endrin ketone. *o,p'*-DDT, endosulfan sulphate, mirex and, to a certain extent, α-HCH, β-HCH and δ-HCH [31], can be also included in this group.

Another drawback with the proposed method is a poor limit of detection because three aliquots of sufficient concentration are required. This drawback can be minimized by working without fractionation of the extract and starting with the least destructive method, i.e., acid treatment, followed by alkali treatment and finally by the most destructive oxidative treatment.

To confirm the applicability of the treatments to real samples, the proposed method was used to confirm the presence of organochlorine compound residues in surface water and human milk extracts.

The water samples were extracted and analysed by cGC with ECD and the working BP-5 column. If peaks of possible pesticides or PCBs appear in the first chromatogram, a second analysis is carried out on the DB-17 column. If the retention times do not confirm the presence of the possible pesticides or PCBs, the result is negative. If the retention times coincide with those of possible identified compounds, aliquots of organic extracts are treated using chemical procedures. Only if the three results of the chemical treatments agree with the results in Table 1 are the analyses positive. Fig. 3 shows the chromatograms obtained from a water sample containing Aroclor 1254, (a) prior to any treatment and (b) after treatment with chromium(VI) oxide.

As can be in Table 3, the organochlorine pesticides aldrin and *o,p'*-DDD were confirmed after treatment in some water sample, whereas in two other samples, aldrin and heptachlor epoxide gave false-positive results.

The human milk analyses were performed in the same way as for water samples but samples containing possible residues were also analysed by EI-MS-SIM. In all instances, chemically positive identifications agreed with the EI-MS-SIM analyses.

In conclusion, chemical treatment offers a means of achieving residue analyses with significant savings of reagents, glassware and equip-

Table 3
Pesticides present in surface water samples from the Valencia area

Sample No.	Pesticide possible	Chemical treatment			Confirmation
		Acid	Alkali	oxidant	
1	Aldrin	+	–	–	Negative
2	Aldrin	+	+	+	Positive
	o,p'-DDD	+	+	+	Positive
3	Heptachlor epoxide	–	–	–	Negative
4	*o,p'*-DDD	+	+	+	Positive

+ = Unaltered; – = destroyed.

ment. If the analyses do not include chemical confirmation, it must be assumed that some of the positive analyses are false.

However, sulphuric acid treatment, only allows the determination of acid-stable compounds and column chromatographic clean-up has to be used if the determination of acid-labile compounds is required. For example, dieldrin, α-endosulfan, β-endosulfan, isodrin [31], endrin and heptachlor epoxide are degraded by sulphuric acid and cannot be determined by this technique.

Chromium(VI) oxide provides clean chromatographic profiles, but this technique is not recommended for the determination of low chlorine-containing Aroclors in environmental samples. Quantification errors can be diminished by carrying out parallel runs with similar concentrations of suspected Aroclors or choosing individual PCBs that are not degraded by this method.

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