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Gas Chromatographic Determination of Organochlorine Pesticides; Contamination of Dicofol, Fenson, and Tetradifon in Fish and Natural Waters of a Wet Area Beside the Mediterranean Sea

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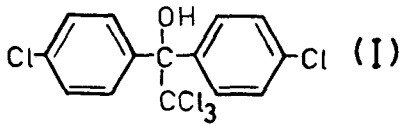
Large-scale application of pesticides to agricultural and forest areas may contribute to the presence of these toxic substances in the environment. Among their different kinds, that of organochlorines requires larger attention because of the high stability and toxicity it displays as regards aquatic fauna. Its toxicity is a hundred times greater than that organophosphorus pesticides (Rodier 1978).

In November 1984 a large number of dead Mugil spp. appeared in a wet area on the Mediterranean shore (Province of Castellon, Spain), known as "Clot de la Mare de Deu" (Map in Figure 1). Both the mainly agricultural character of the province and a recent red-mite pest directed the present work towards the study of organochlorine acaricides in water as well as organisms from the affected zone. Dicofol, Fenson and Tetradifon were identified as main components.

Dicofol is 1,1-bis(p-chlorophenyl)-2,2,2-trichloroethanol (I) and also known as DMTC, Kelthane, Mitigan...(Merck Index 1983). It is a compound of a very similar structure to that p,p'DDT. It has been shown that Dicofol is a metabolic product of DDT (Fishbein 1974). On the other hand, it has been suggested that 4,4'-dichloro benzophenone, 4,4'-dichlorobenzhydrol (Fishbein 1974) and DDE (Fishbein 1974; McKinley et al. 1960) were metabolites of Dicofol. The GLC determination of p,p'kelthane has revealed the presence of seven peaks (Burke et al. 1965) in the techical grade material and only one (Gudzinowicz 1965) or two peaks in the purified samples. Some of the impurities identified in a typical chromatogram of technical Kelthane are pp'DDT, op'DDT, pp'DDE, op'DDE, op' and pp'-dichlorobenzophenone (Black et al. 1971). The latter compound has been shown as alkaline degradation product of Kelthane (Krause 1972). Several authors have previosly studied its toxicology and metabolism (Smith et al. 1959; Brown et al. 1969; Tabata et al. 1979). This acaricide, together with Tetradifon is widely used on citrus within the studied area.



(I)

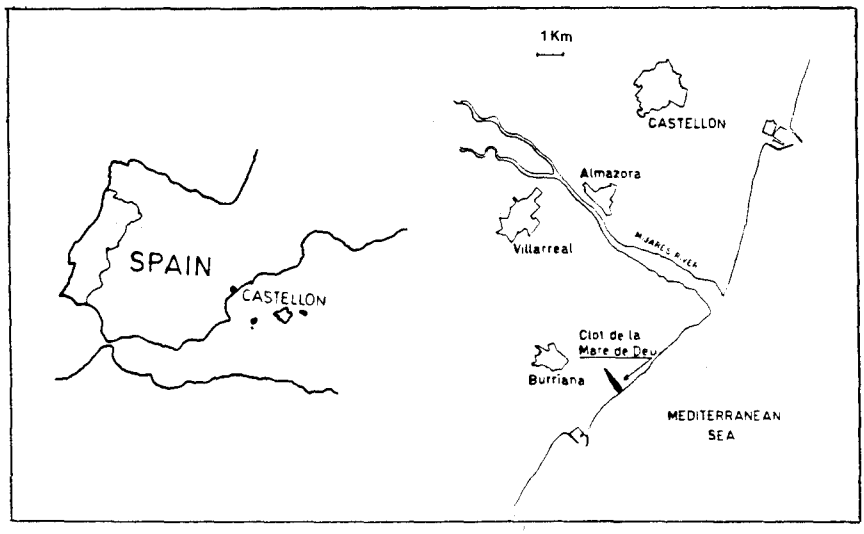
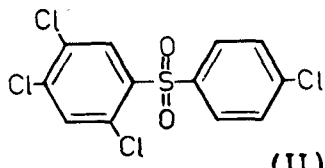


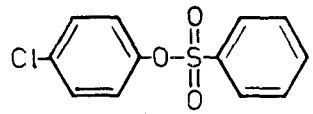
Figure 1. Map of the studied area

Tetradifon (1,2,4-trichloro-5-((4-chlorophenyl)sulfonyl)benzene) (II) is known also as Duphas and Tedion (Merck Index 1983), its toxicology having been studied by Ben-Dyke et al. (1970).

Finally, Fenson (p-chlorophenyl benzene sulfonate) (III) is an acaricide as well, though lees frequently used. Commercial products such as Sigmaton (Celamerck) contain these pesticides and are commonly employed to combat mite pests.



(II)



(III)

Determination of organochlorine pesticides is usually carried out by gas chromatography with an electron-capture detector. FAO recommendations, document No. 158 (Bernhard 1976), have been followed to develop this work. Pesticides extraction is carried out in soxhlet using hexane as solvent, already employed by other authors (Addison et al. 1972; Murphy 1972; Harvey et al. 1974). An easy treatment with sulfuric acid after the extraction (Murphy 1972) removes the biological substances interfering with pesticide residue analysis.

MATERIALS AND METHODS

Water samples were collected in glass bottles, previously cleaned and rinsed out several times with the sample likely to be analysed and stored at 4ºC until they were analysed (American Public Health Association 1981).

Organism samples were collected in the area concerned and stored in a frrezer at -18ºC until preparations and analyses. They were weighed and measured; viscera and muscular parts to be used in the analyses were separated, lyophilised and homogenised.

The reagents were of a high purity, appropriate for organic residue analysis.

The commercial product "Sigmaton" (Celamerck) was used as a standard which is a mixture of Dicofol (223 g/l), Fenson (223 g/l) and Tetradifon (56 g/l) pesticides.

Extraction of organochlorine pesticides contained in water was performed making use of 2-liter separatory funnels, with a sample volume of 1500 ml and 60 ml of 15% diethyl ether in hexane. It was repeated a second and a third extraction with 60 ml of mixed solvent, the organic phase being carefully poured through a 2-cm diameter column containing 8 to 10 cm of anhydrous Na_2SO_4 (American Public Health Association 1981). Organic extract volume was reduced to about 10 ml in an evaporative concentrator and then cleaned up with 1 ml conc H_2SO_4, shaking it for 2 min. It was centrifuged for 5 min and finally the hexane layer was removed and dried out in the evaporative concentrator. The residue was dissolved in 1 ml hexane and analysed by G.C. with E.C.D.

Extraction of organochlorine pesticides in Mugil spp. was carried out in soxhlet for 5 hours, using hexane as solvent, from 2-3 g mixed-lyophylised organism and equal quantity of anhydrous Na_2SO_4. The hexane extract was transferred to a 50 ml centrifuge tube and its volume reduced down to 5 ml in an evaporative concentrator. Then it was cleaned up with H_2SO_4, according to the above-mentioned procedure on water. The hexane layer was removed and dried out in an evaporative concentrator. The residue was dissolved in 10 ml hexane and analysed by G.C. with E.C.D.

Chromatograms obtained for samples were compared to those of standards previously treated with H_2SO_4; retention times (relative to aldrin and methoxychlor) were used for the identification of peaks.

Quantification was performed by integration of areas, using the external standard method and taking into account the addition of all peak areas corresponding to the same pesticide.

For identification of peaks, alkaline hydrolisis was carried out with KOH-ethanol: a KOH pellet dissolved in 1 ml ethanol was added to 2 ml hexane extract, shaking for 5 min and letting it settle for 30 min.

Analyses were performed on a Perkin-Elmer Model Sigma 3B ECD Chromatograph equipped with a ^{63}Ni detector and a recorder Shimadzu Model C-R3A Chromatopac. Instrument parameters and operating conditions follows:

Temp. of column 210ºC; Temp. of injector 250ºC; Temp. of detector 300ºC.

Carrier gas N_2 at 25 ml/min; make up carrier gas N_2 at 35 ml/min. Glass column packing: 1/4", 2m, OV-17 1.5% and QF-1 1.95% on Chromosorb W-HP 80/100 mesh.

RESULTS AND DISCUSSION

Chromatograms obtained for live (2A) and dead (2B) Mugil spp. viscera are shown in Fig 2, as well as those of muscular parts (2C in live and 2D in dead). The most significant peaks correspond to Dicofol (D), Fenson (F) and Tetradifon (T).

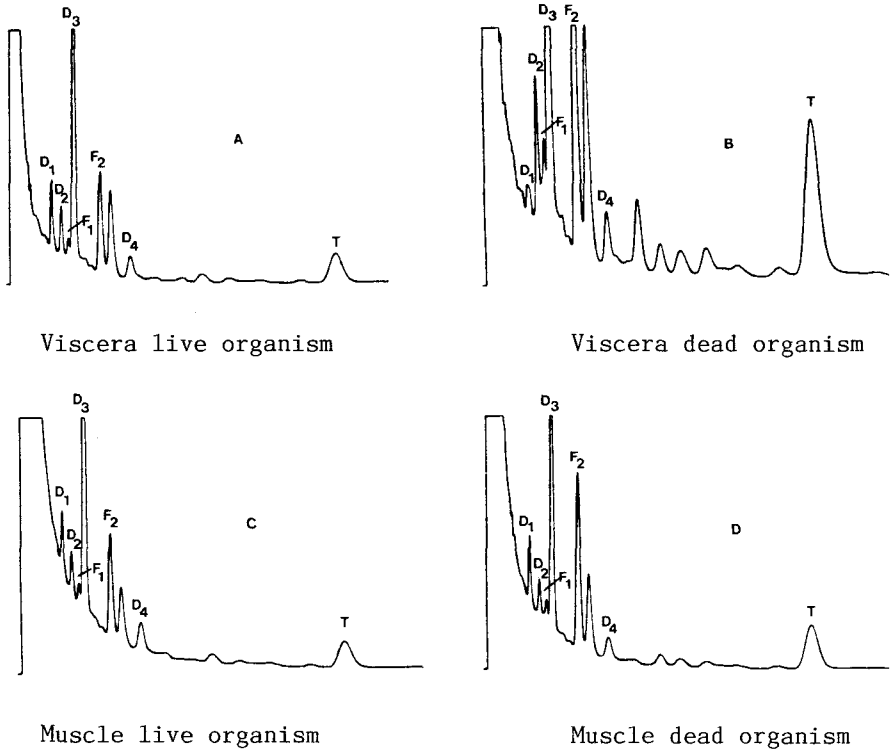


Figure 2. Gas chromatograms of Mugil spp. extracts treated with sulfuric acid.

A minor degree of contamination in viscera of alive organisms has been verified; and in muscular parts, both in alive and dead organisms, it has proved similar.

In Fig 3, Sigmaton standard chromatograms (Dicofol, Fenson and Tetradifon) are shown before (3a) and after (3b) having been subjected to a cleanup with sulfuric acid equal to the one applied to samples. Then retention times relative to aldrin and methoxychlor are similar for standards and for samples (Table 1). While performing H_2SO_4 cleanup, Fenson (F_1,F_2) and Tetradifon (T) peaks are observed to maintain their retention times. Average recoveries for each of the peaks were: 55% (F_1), 97.8% (F_2) and 46.2% (T). Dicofol remains with a recovery of 83.0% (D_1), although three new peaks

appear: D_2, D_3 and D_4. Relative retention times of peaks D_3 and D_4 are verified to coincide with those of pp'DDE and pp'DDT respectively.

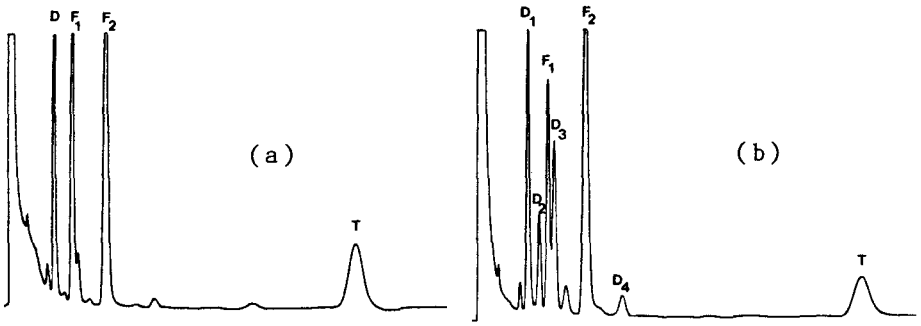


Figure 3. Gas chromatograms of Sigmaton standard before (a) and after (b) sulfuric acid cleanup

Table 1. Retention times relative to aldrin and methoxychlor of Sigmaton standard and Mugil spp. samples treated with sulfuric acid

	STANDARD (SIGMATON)			SAMPLES		
	peak	RRT (aldrin=1)	RRT (metoxychlor=1)	peak	RRT (aldrin=1)	RRT (metoxychlor=1)
DICOFOL	D_1	1.41	0.22	D_1	1.41	0.22
	D_2	1.70	0.27	D_2	1.70	0.27
	D_3	2.08	0.33	F_1	1.91	0.30
	D_4	3.80	0.60	D_3	2.08	0.33
FENSON	F_1	1.92	0.30	F_2	2.87	0.45
	F_2	2.87	0.45	D_4	3.76	0.59
TETRADIFON	T	9.83	1.55	T	9.83	1.55

Peak verification has been carried out by KOH-ethanol treatment, proving Tetradifon to be stable while performing KOH-ethanol hydrolysis. On the other hand peaks corresponding to Fenson practically disappear as a whole. In respect to Dicofol, stability of peaks D_1 & D_2 is observed, peak D_4 disappears and D_3 increases (Fig 4)

Table 2 shown the recovery of each peak after KOH-ethanol treatment. Peaks D_3 and D_4 may correspond with pp'DDE and pp'DDT; not only are their relative retention times the same, but the effect of alcoholic potassium hydroxide on them is similar. This has been verified by means of pp'DDT standard (Supelco), taking into account the relative response factors and molecular weights. In fact, the 158.8% recovery of D_3 is due to the disappearance of D_4.

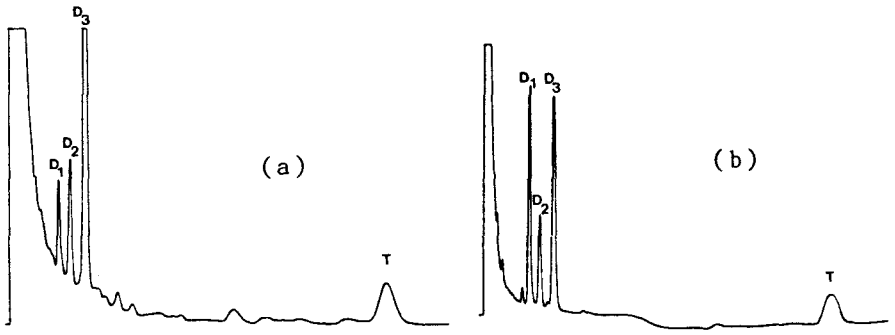


Figure 4. Gas chromatograms of (a) Sigmaton extract and (b) Mugil spp. extract treated with sulfuric acid and potassium hydroxide.

Table 2. Average recoveries of Dicofol, Fenson and Tetradifon after KOH-ethanol treatment.

SIGMATON	peak	recovery (%)
DICOFOL	D_1	88.8
	D_2	103.2
	D_3	158.8
	D_4	0
FENSON	F_1	2.2
	F_2	0.9
TETRADIFON	T	99.7

Water samples collected on two differents spots, have been analysed, verifying as well the presence of these pesticides (Fig 5). Their concentrations are 119 & 221 ng/l Dicofol, 203 & 72 ng/l Fenson and 81 & 113 ng/l Tetradifon.

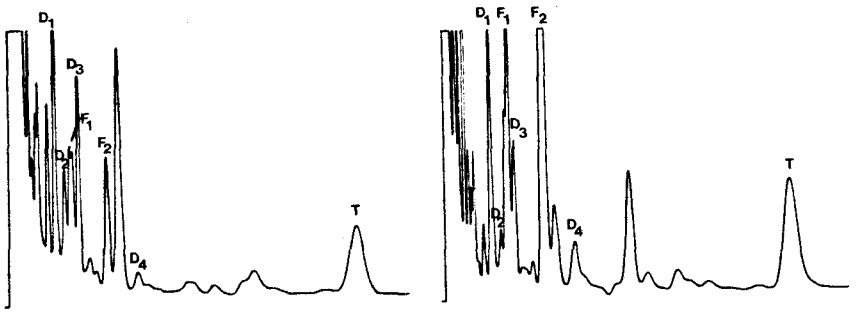


Figure 5. Gas chromatograms of two water extracts treated with sulfuric acid.

Results obtained from analyses on organisms are shown in Table 3, in which the number of individuals forming a sample are indicated, as well as the precision of analyses, as relative standard deviation for three replicates of one sample. Analyses outcomes are expressed as ng/g fresh organism.

Table 3. Concentration of organochlorine pesticides in Mugil spp. in ng/g fresh weight (mean value for three analyses). ( )* relative standard deviation for three analyses.

SAMPLE	INDIVIDUALS FOR SAMPLE	LENGTH OF ORGANISM (cm)	FRESH WEIGHT (g)	DICOFOL	FENSON	TETRADIFON
Viscera dead organism	27	13.1 - 17.9	41.3 - 105	3938 (3.9)	1057 (8.1)	1190 (2.8)
Muscle dead organism				869 (7.2)	383 (11)	467 (9.3)
Viscera live organism	18	12.0 - 15.6	36.9 - 96.8	1263 (6.5)	254 (13)	194 (9.2)
Muscle live organism				1201 (10)	570 (9.2)	456 (6.1)

Although no LD_{50} of these pesticides for Mugil spp. have been found in the bibliography, it is reasonable to suppose that the amounts found are large enough to provoke fish death, taking into consider ation the highly toxic nature of these organochlorine acaricides in relation to aquatic fauna in general. In "Handbook of Acute Toxicity of Chemicals to Fish and Aquatic Invertebrates" (Johnson et al. 1980) values of LD_{50} are indicated in other aquatic species for Dicofol, Fenson and Tetradifon. For one specie, the following toxicity relation is observed: Dicofol > Tetradifon > Fenson; in fact, the main component on analysed samples (Dicofol) is the most toxic of all three pesticides.

Significantly enough, the different concentrations found in dead and living organisms are a proof that the cause of death was a large temporary increase in the concentration of these pollutants in the environment. This was due to a heavy rainfall in a short period of time (26.5 quarts/yd^2 in approximately 5 hours, on 14th Nov 1984) (Instituto Nacional de Meteorologia 1984), which caused pesticides to be carried away towards the wet area. That aided an accumulation of pesticides because they could not reach the sea due to gravel and sandbanks.

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