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Assessment of Exposure to Environmental Microcontaminants and Pesticide Residues in *Scapharca inaequivalvis*

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Polychlorinated biphenyls (PCBs), dibenzodioxins (PCDDs), and dibenzofurans (PCDFs), as well as a number of chlorinated pesticides and other related compounds are characterized by a wide distribution in the environment. This is due to the combination of their large scale commercial production or widespread formation with their generally long environmental persistence. Some of the above compounds are also highly toxic and have carcinogenic and teratogenic activity in animal species even at trace level exposure (US EPA 1985). The above compounds exhibit a high affinity for lipophilic matrices and may concentrate in the biota reaching levels remarkably higher than those in the surrounding media (Ellgehausen et al. 1980; Hawker and Connell 1986; Miyata et al. 1987). Due to bioconcentration, compounds which are not harmful per se at environmental dilution may become a major source of toxicologic risk.

Scapharca inaequivalvis (Ghisotti and Rinaldi 1976) is not a component of Italians' regular diet. However, in recent years the mollusc has become a thriving part of Italian coastal fauna and, because of that, an intriguing scientific subject. Little doubt exists that it was accidentally introduced from its native Japanese waters into the Mediterranean and, specifically, Adriatic seas where it quickly adapted and started to proliferate. Indeed, Scapharca inaequivalvis seems to survive in highly eutrophic media - such as some zones of the Adriatic sea (Justic 1987; Sfriso et al. 1987) - better than most indigenous molluscs: the higher adaptation capability may be provided by its hemoglobin-based blood different from that of common molluscs. In fact, hemoglobin could provide a better survival potential in particular when oxygen concentration in the medium is low (Carpené et al. 1985; San George and Nagel 1985; Chiancone et al. 1986).

For its respiratory charactistics and thriving power even in adverse conditions, Scapharca inaequivalvis has been considered an interesting subject for this investigation also in order to estimate its use as a biological indicator and/or accumulator of environmental contamination. The results presented are the prelimi-

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nary outcome of a study in progress aimed at the definition of criteria and methodologies to: (a) detect microcontaminants (including polynuclear aromatics) and pesticide residues in marine ecosystems, and (b) assess their impact on the ecosystems involved. A more exhaustive investigation to estimate man's exposure to said compounds through the food chain is expected to be developed.

MATERIALS AND METHODS

Acetone and hexane were high purity solvents provided by Rudi Pont Eurobase (Milan, Italy). iso-Octane, diethyl ether, and anhydrous ethyl alcohol were furnished by Fluka (Buchs, Switzerland) or BDH Italia (Milan, Italy). Except for hexane which was distilled in a glass apparatus prior to use, all other solvents were used as supplied. Orthophosphoric acid, concentrated sulfuric acid, and anhydrous sodium sulfate were purchased from Carlo Erba (Milan, Italy) or BDH Italia (Milan, Italy). Sodium chloride and sodium carbonate were furnished by E. Merck (Darmstadt, FRG).

Fenclors (PCBs) came from Caffaro (Genoa, Italy). Individual 2,4,4'-tri-, 2,2',5,5'-tetra-, 2,3',4,4',5-penta-, 2,2',3,4,4',5-hexa-, and 2,2',3,4,4',5,5'-heptachloro PCB isomers were provided by CIL (Woburn, Massachusetts). 2,3,7,8-Tetra-, 1,2,3,6,7,8-hexa-, and octachloro dibenzodioxins and dibenzofurans were also provided by CIL. Hexachlorobenzene (HCB) was purchased from Alltech (Deerfield, Illinois); lindane, DDD, and DDT were provided from Supelco (Bellefonte, Pennsylvania). Standard substances were GC analytical grade for environmental assessment (Figure 1).

For liquid-liquid extraction, funnels, 250-mL separatory funnels, 100- and 250-mL beakers, glass-stoppered 100- and 300-mL Erlenmeyer flasks were used. For treatment and storage, glass-stoppered 10-mL centrifuge tubes and 8-mL conical-bottom vials sealed with Teflon-lined screwcaps were employed. Borosilicate glassware was cleansed very carefully, and kept at 250 °C overnight prior to use.

Homogenates were prepared with an Omni-Mixer model 17106 apparatus made by OCI Instruments (Waterbury, Connecticut) and equipped with 50-mL stainless steel blending containers.

Gas chromatographic analyses were performed on a Hewlett-Packard model HP 5840 unit equipped with a Ni-63 electron-capture detector. An HP-5 30-m-long 0.53-mm-i.d. macrobore fused-silica column and an Ar-10% methane carrier (2 mL/min) were used for this work. Operating temperatures were: (a) injector, 250 °C; (b) oven, program from 170 to 270 °C at a rate of 1.8 °C/min; (c) final isotherm, 40 min; (d) detector, 300 °C.

Gas chromatographic-mass spectrometric analyses were performed with a Finnigan model 5100 unit equipped with a split-splitless injector and an HP Ultra-2 50-m-long 0.32-mm-i.d. fused-silica capillary column entering directly the ion source. Helium was used as a carrier (1 mL/min). Operating conditions were: (a) injector temperature, 290 °C (splitless mode); (b) oven, initial temperature at 60 °C followed by program from 160 to 290 °C; (c) temperature rates,

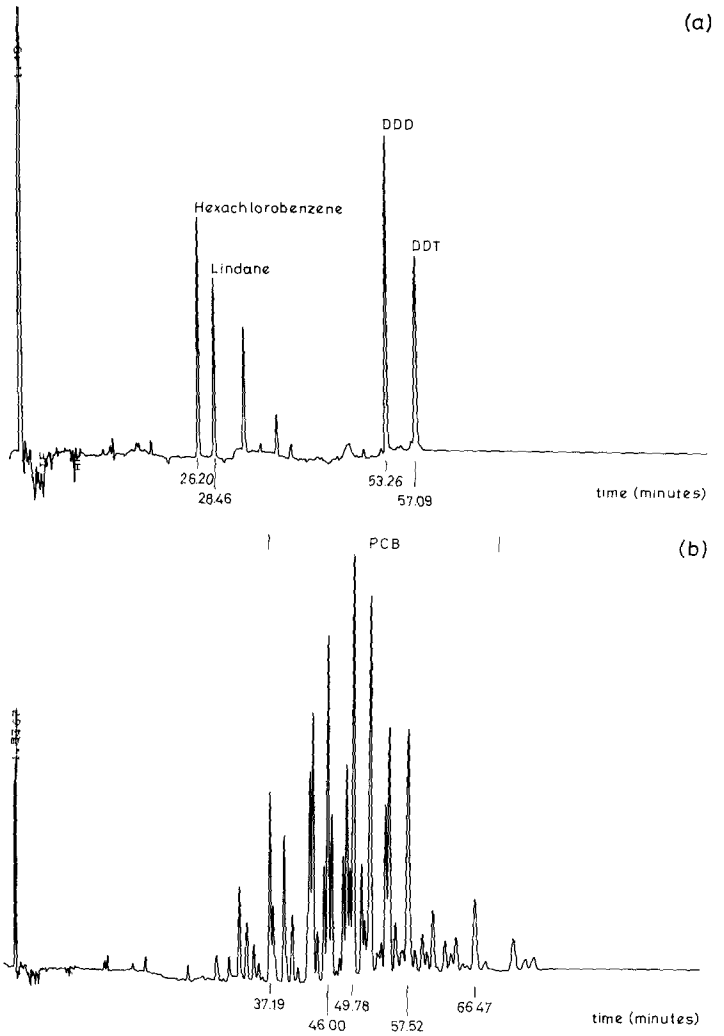

Figure 1. (a) Pesticide standard mixture. Gas chromatogram obtained by injecting 32-pg HCB, 48-pg lindane, 160-pg DDD, and 96-pg DDT; attenuation 6. (b) Reference mixture for PCB assay. ECD gas chromatogram of 4.6-ng Fenclor 54; attenuation 7.

20 °C/min between 60 and 160 °C, 3 °C/min between 160 and 290 °C; (d) final isotherm, 5 min; (e) electron energy, 50–70 eV; (f) electron multiplier, 2300 V. Scan range for total ion acquisition: 90–450 amu at a 360 amu/s rate. Isotopic clusters for multiple ion detection or MID (underlined masses used for quantitation):

- PCBs (degree of chlorosubstitution, m/z): tetra-, 290 292 294; penta-, 324 326 328; hexa-, 358 360 362; hepta-, 392 394 396; octa-, 428 430 432;
- PCDDs (degree of chlorosubstitution, m/z): tetra-, 320 322 324; penta-, 354 356 358; hexa-, 388 390 392; hepta-, 422 424 426; octa-, 458 460 462;
- PCDFs (degree of chlorosubstitution, m/z): tetra-, 304 306

308; penta-, 338 340 342; hexa-, 372 374 376; hepta-, 406 408 410; octa-, 442 444 446;
pesticides (name, m/z): hexachlorobenzene, 282 284 286; DDD, 165 235 237; DDT, 165 235 237; aldrin, 261 263; heptachlor, 100 272 274; dieldrin, 261 263; endrin, 261 263; chlordane, 373 375 377.

The analytical procedure was adapted from the pertinent Directive of the European Communities (EEC 1987, and references therein) for accumulation tests in fish.

Fresh molluscs were kept on ice until used (up to 24 h). In one case, shells were opened and, if the inside looked clean, animals were thoroughly removed avoiding bleeding until a 65–70-g weight (8–10 specimens) was reached. Alternatively, shells were opened, and animal bodies pierced and let bleed exhaustively. Bloodless bodies were cleansed with distilled water, dried with laboratory paper, and weighed to obtain a 65–70-g sample (16–18 specimens). If necessary, samples were stored at -20 °C; when frozen, samples were allowed to thaw for some time at room temperature before use.

For each sample, bodies were cut into small pieces. One third of the sample was then transferred to a blending container, added with 10-mL 5:2 (v/v) acetone-hexane mixture, and blended for 5 min at a rotating speed increasing from 4000 to 7000 rpm while cooling at 0 °C. Mixing was stopped and the blended mixture poured on a quantitative paper filter-lined funnel held above a separatory funnel. The latter contained 50-mL 0.9% sodium chloride 0.1-M phosphoric acid aqueous solution. Blending was repeated two more times: each time the blended mixture was filtered and the solvent phase pooled with the previous fraction(s).

The empty container was filled with 25 mL of the same solvent mixture and then subjected to mixing for 1 min at 3000-rpm on ice bath. Mixing was stopped and solvent slowly dispersed over the finely minced material on filter. Container blades were cleansed carefully with few small cotton swabs saturated with the acetone-hexane mixture, and then rinsed; swabs were combined with extracted matrix on filter. On the whole, 35-mL acetone-hexane mixture was used to cleanse the container; solvent used for rinsing was dispersed over the biological matrix as described. A final 50-mL 10:1 (v/v) hexane-ether mixture was allowed to percolate through the matrix. All organic fractions were pooled in the separatory funnel.

The separatory funnel was shaken vigorously for 2 min. The system was then allowed to rest and separate overnight. The organic phase was collected in a tared Erlenmeyer flask, whereas the aqueous phase was transferred back to the same separatory funnel.

The dry extracted biological material was recovered from filter and reextracted in the blending container with 20-mL acetone-hexane mixture for 2 min at 5000 rpm. The homogenate was poured on the paper filter and the liquid phase gathered on the aqueous layer. A new liquid-liquid extraction was performed after adding extra 30-mL 2:1 (v/v) ether-hexane mixture and 4-g sodium chloride directly to

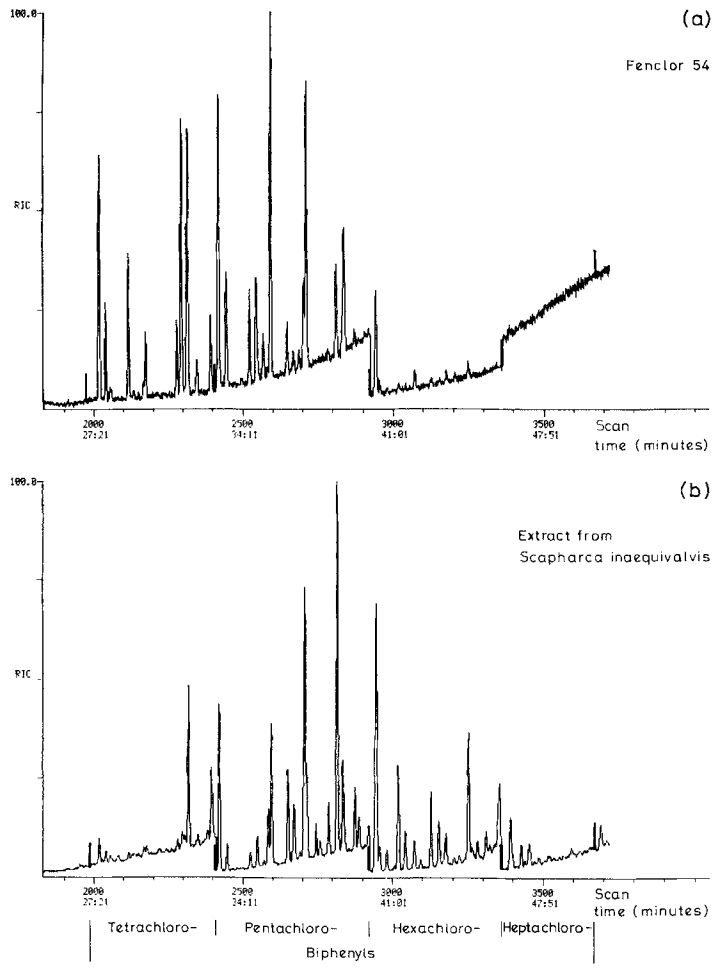

Figure 2. Reconstructed mass chromatograms of (a) injected 9.3-ng Fenclor 54 (PCB standard), and (b) whole-body Scapharca inaequivalvis extracts. Individual MID scan times of tetra- to heptachlorinated isomer groups are visible at the bottom.

the separatory funnel contents. The separatory funnel was shaken as described and allowed to rest until separation occurred (<1 h). Again, the two phases were separated: the organic one was combined with the organic phase from previous extraction; the aqueous phase was retransferred to the separatory funnel. Extraction was performed three more times. Extraction phases were pooled in the same tared Erlenmeyer flask; the pool was combined with 10-mL anhydrous ethanol and evaporated under nitrogen flow until dry.

Dry residues were kept in a desiccator until constant weight (<72 h). Mean fat contents were found to be 0.43% and 0.14% (n = 3) for whole-body and bloodless matrices, respectively.

The dry fat residue was dissolved with <4-mL hexane and transferred to a calibrated test tube. Additional 2-mL solvent portions were used to achieve a quantitative transfer. In the test tube the over-

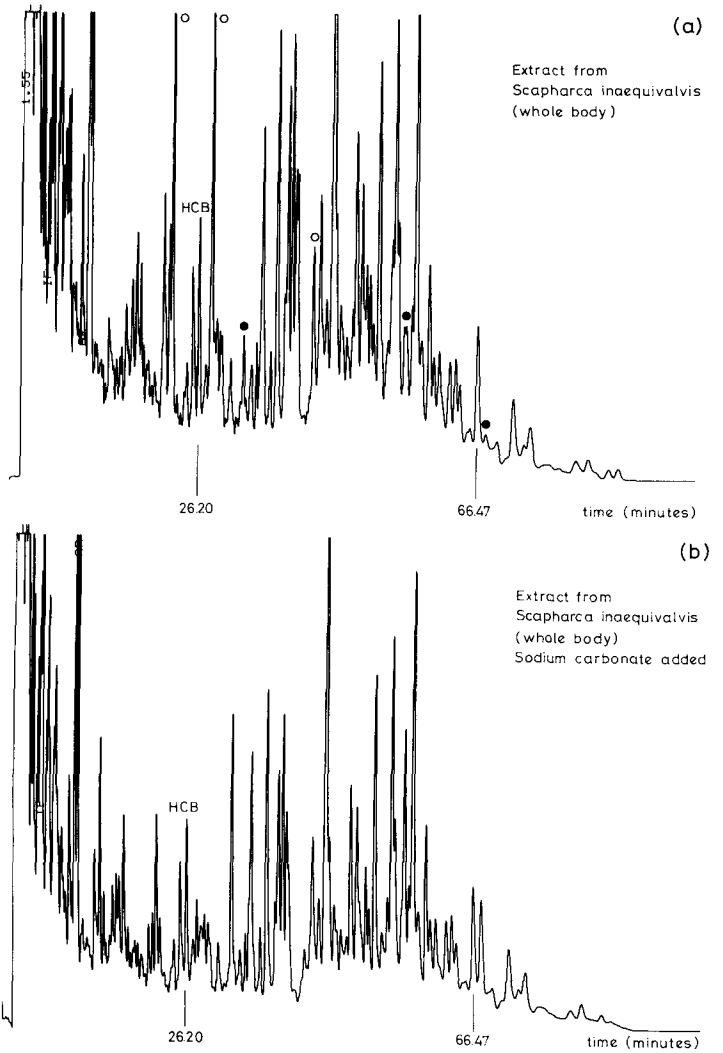

Figure 3. ECD gas chromatograms of Scapharca inaequivalvis extracts (whole-body samples): (a) without addition of sodium carbonate, (b) 3 days after sodium carbonate addition. Attenuation 7.

all volume during transfer was kept <5 mL by evaporation under nitrogen flow, and reduced to dryness in the end. The dry extract was taken up with 1-mL iso-octane and added with 4-mL concentrated sulfuric acid. The tube was sealed, inverted 20 times, and centrifuged to obtain separation of layers. The 1-mL organic supernatant was transferred to a conical-bottom vial and <0.2-g anhydrous sodium sulfate was added prior to instrumental analysis.

RESULTS AND DISCUSSION

Recovery yields were tested only for HCB, lindane, DDD, and DDT. They were added at two different levels (10 and 100 ppb) to freshwater test fish during homogenization. However, because of the

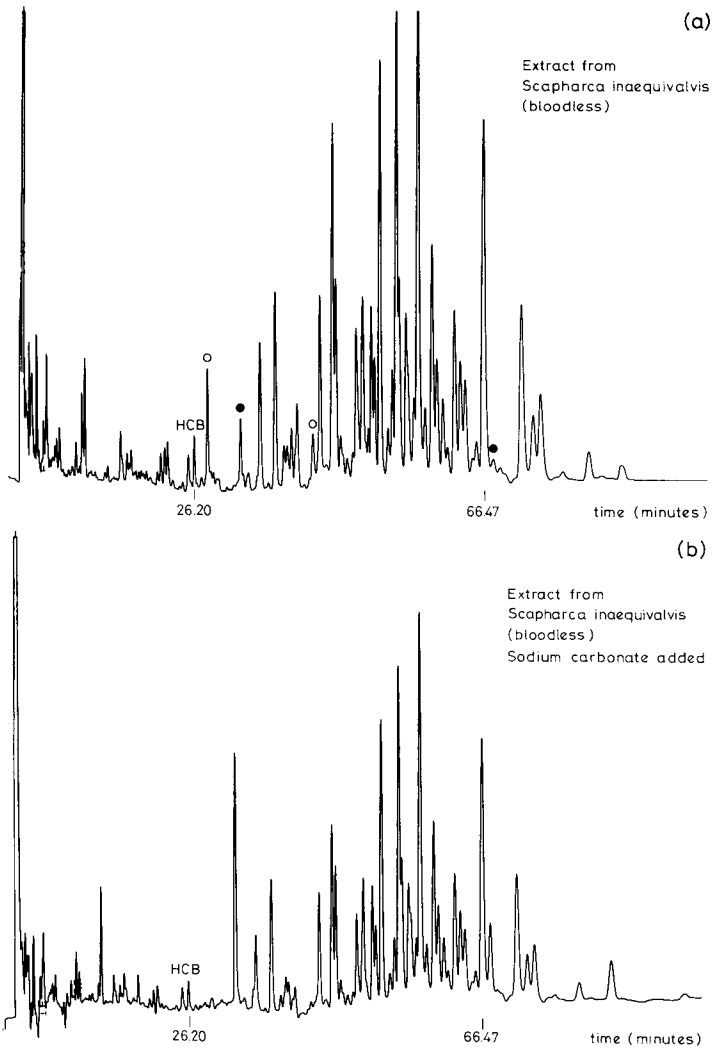

Figure 4. ECD gas chromatograms of Scapharca inaequivalvis extracts (bloodless samples): (a) without addition of sodium carbonate, (b) 3 days after sodium carbonate addition. Attenuation 7.

absence of interfering GC signals, 0.5-ppb-level HCB was added to Scapharca inaequivalvis sample matrices during homogenization to provide an additional measure of recovery yield. For all pesticides, recovery yields were always >50% and in most cases between 70% and 100%.

GC-MS assessment of pesticides, PCBs, PCDDs, and PCDFs in whole-body and bloodless matrices provided similar findings. HCB, heptachlor, and lindane were absent at a detection threshold of <0.1 ppb; DDD and DDT exhibited levels (uncorrected) between 40 and 120 ppb. PCB amounts (uncorrected) varied according to the isomer group from 4 to 30 ppb, the more chlorinated terms exhibiting somewhat higher levels (Figure 2). No PCDDs and PCDFs were found at a detec-

tion threshold <0.2 ppb for the least sensitive terms (octachloro-derivatives). The presence of blood did not seem to change significantly the analytical patterns of the matrices analyzed (Figures 3 and 4). However, it may be preliminarily observed that addition of sodium carbonate to the iso-octane extract appeared to produce some changes in the GC patterns. In the (a)-sections of the figures, open (O) and solid (●) circles indicate disappearing/decreasing or appearing/increasing peaks, respectively; the retention times of added HCB and a PCB component have been marked for reference.

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REFERENCES

Carpené E, Zurburg W, Cortesi P, Isani G, Cattani O (1985) Biochemical effects of anaerobiosis in Venus gallina L. and Scapharca inaequivalvis (Bruguiere). Boll Soc It Biol Sper 61:707–720.

Chiancone E, Boffi A, Verzili D, Ascoli F (1986) Propietà delle emoglobine del mollusco Scapharca inaequivalvis in rapporto con le condizioni ecologiche del Mare Adriatico. In: Atti del 1° Simposio di Biochimica Marina, Cesenatico, September 19, 1985, published by Grasso (Bologna), pp 13–21.

EEC (1987) EEC Directive 79/831, Annex V, Part C: Methods for the determination of ecotoxicity - Accumulation - Flow-through test. Doc/87/258/XI.

Ellgehausen H, Guth JA, Esser HO (1980) Factors determining the bioaccumulation potential of pesticides in the individual compartments of aquatic food chains. Ecotoxicol Environ Safety 4: 134–137.

Ghisotti F, Rinaldi E (1976) Osservazioni sulla popolazione di Scapharca insediatasi in questi ultimi anni su un tratto del litorale romagnolo. Conchiglie 12:183–191.

Hawker DW, Connell DW (1986) Bioconcentration of lipophilic compounds by some aquatic organisms. Ecotoxicol Environ Safety 11: 184–197.

Justic D (1987) Long-term eutrophication of the northern Adriatic Sea. Mar Poll Bull 18:281–284.

Miyata H, Takayama K, Ogaki J, Kashimoto T, Fukushima S (1987) Monitoring of PCDDs in Osaka Bay using blue mussel. Chemosphere 16:1817–1822.

San George RC, Nagel RL (1985) Dimeric hemoglobins from the arcid blood clam, Noetia ponderosa. J Biol Chem 260:4331–4337.

Sfriso A, Marcomini A, Pavoni B (1987) Relationships between macroalgal biomass and nutrient concentrations in a hypertrophic area of the Venice lagoon. Mar Environ Res 22:297–312.

US EPA (1985) Health Assessment Document for Polychlorinated Dibenzo-p-dioxins. EPA/600/8-84/014F, US Environmental Protection Agency, Cincinnati (Ohio).

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