SELECTED CARCINOGENIC ORGANIC MICROCONTAMINANTS AND HEAVY METALS IN THE VENICE LAGOON. II. CONTAMINATION LEVELS OF BIOTA SAMPLES

Alessandro di Domenico,[a] Luigi Turrio Baldassarri,[a] Giovanni Ziemacki,[b] Elena De Felip,[a] Fabiola Ferri,[a] Nicola Iacovella,[a] Cinzia La Rocca,[a] Fabrizio Rodriguez,[a] Fabrizio Volpi,[a] Ottavio D'Agostino,[a] Raffaela Sansoni[a]

(a) Laboratory of Comparative Toxicology and Ecotoxicology, and *(b)* Laboratory of Environmental Hygiene — Istituto Superiore di Sanità, 00161 Rome, Italy

ABSTRACT

In the fall of 1995, the Italian national health authority set out an investigation to obtain data for a health risk assessment concerning the presence of toxic microcontaminants in foodstuffs from the Venice lagoon. Based on the analytical findings, two major provisional conclusions were later reached: *(a)* biota seemed to exhibit increasing contamination contents with increasing anthropic impact, and *(b)* no human health risks of particular concern could be associated with consumption of lagoon foodstuffs.

INTRODUCTION

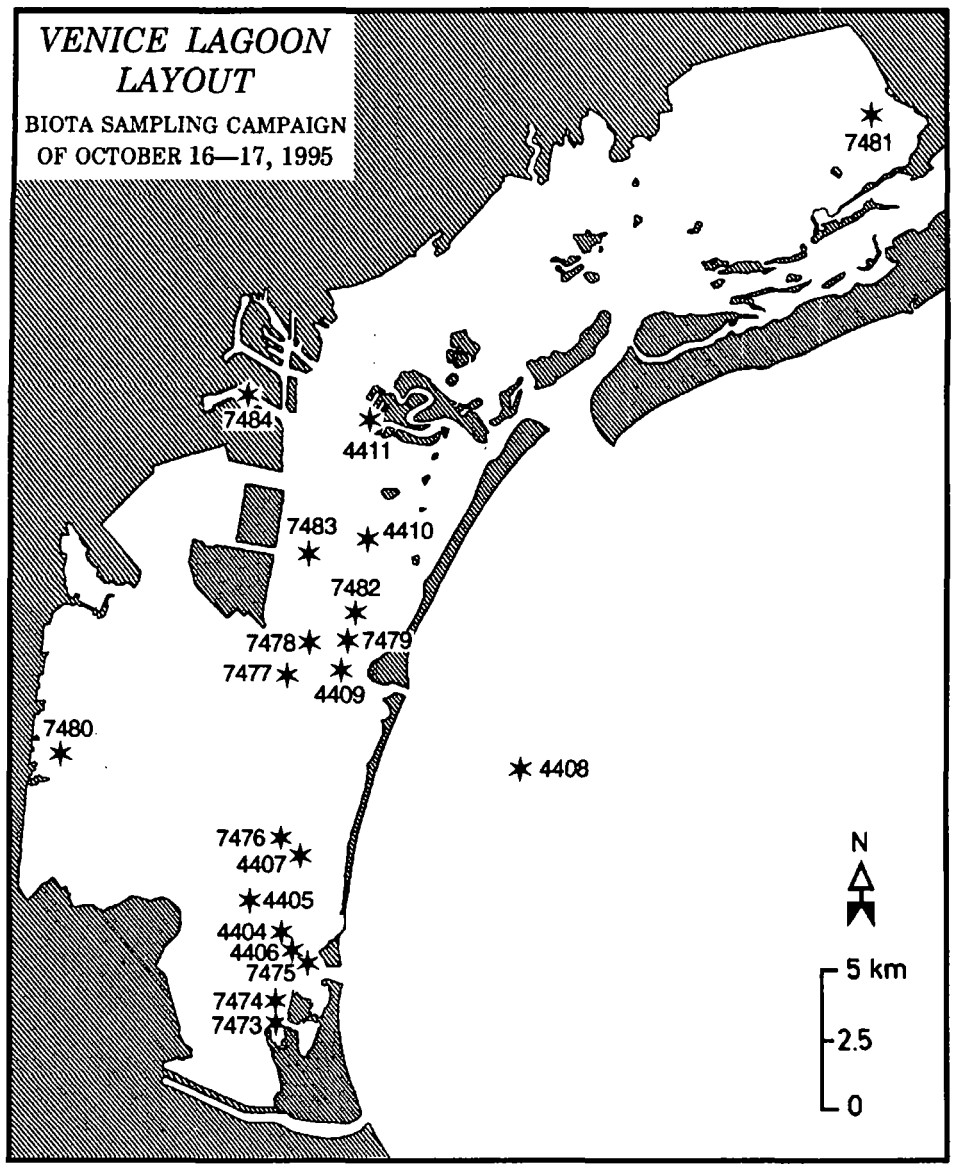
The contents of several toxicologically important microcontaminants in the bottom marine sediments from the Venice lagoon were extensively examined by the authors between 1992 and 1995.[1-5] The microcontaminant group comprised: selected compounds of the families of PAHs (polycyclic aromatic hydrocarbons), PCBs, PCDDs, and PCDFs; chlorinated pesticides, such as DDE, DDT, and HCB; the five heavy metals Cd, Cu, Hg, Pb, and Zn. Investigations confirmed that contamination was quite unevenly distributed, in general its quality and quantity strongly reflecting the type and magnitude of the anthropic impacts prevailing locally.[1-5] On the whole, a remarkable increase of contamination levels was observed in the industrial zone of Porto Marghera and, second next, in the urban environment of Venice itself; on the contrary, authorized fishing areas exhibited mean values comparable to open sea background. Oddly enough, some sediments from the nearby open sea, facing the lagoon, presented unexpected high levels of contamination.

The Venice lagoon is an important source of edible marine fish and shellfish: they not only are consumed locally but also find their way into a much larger distribution network. Following a release of preliminary results — later confirmed — by the fall of 1995, the Italian national health authority grew concerned that the lagoon biota might contain microcontaminant levels so high as to render lagoon foodstuffs unsuitable for human consumption; thereby, an investigation was set out to obtain data for a pertinent risk assessment. This report is a brief account of the major investigation findings, focused primarily on risk analysis and management.

EXPERIMENTAL METHODS

Samples of molluscs and fish were collected on October 16—17, 1995, at 19 sites within the lagoon and one in the nearby open sea (see map).

Once delivered to the analytical laboratory, samples were immediately subjected to pretreatment. Specimen exteriors were individually rinsed with distilled water; shellfish individuals, once opened, were also rinsed thoroughly inside. Subsequently, the edible parts of the specimens of a same sample were carefully excised and pooled together (pool amounts, >200 g of fresh tissue): for molluscs, a pooled matrix contained a multitude of individuals, whereas fish matrices were



Venice lagoon layout. Once collected, the 20 live mollusc and fish samples were quickly refrigerated at ≈0 °C, and kept stored on ice until they were delivered to the analytical laboratory in Rome (October 18). Here, within the next two days from delivery, analytical pretreatment (see text) was promptly carried out. It may be recalled that, in previous investigations,[3,5] the lagoon environment was broadly subdivided into six exposure areas (*AREAS 1 — 6*). The subdivision was intended to provide a pragmatic grouping criterion to facilitate interpretation of sediment data: areas were chracterized on the basis of prevailing anthropic impacts they were presumably subjected to. As a detailed map of contamination distribution was not available, areas were left topographically unidentified and referred to as "virtual". However, from the application of the grouping criterion — first to sediment[3,5] and later to biota (see tables) data — the locations of virtual areas turn out to be coarsely indicated by the sites (and zones) assayed.

made of 12 to 20 fillets taken from three to five individual specimens. Pools were gently drained off to remove loose water; matrices were then stored in plastic bags for food use at –20 °C.

Later, matrices were allowed to thaw out in the laboratory and combined with fully C^{13}- and H^2-labelled standards to quantitate chlorinated and PAH analytes, respectively.[6,7] Spiked matrices were homogenized and lyophilized. A portion (<1 g) of the freeze-dried material of each matrix was subjected to CO_2 supercritical fluid extraction (SFE) for the assessment of PAHs, PCBs, and chlorinated pesticides; cleanup entailed a chromatographic filtration on silica gel. A larger portion (<15 g) of freeze-dried material was used for PCDD and PCDF determination by accelerated solvent extraction (ASE), for which a 1:1 (v/v) mixture of *n*-hexane-acetone was utilized; cleanup required a sequence of canonical steps, ending with chromatographic filtration on activated alumina.[6-9] An account of the analytical procedures adopted is in preparation.

Quantitative determination of organic analytes was carried out by HRGC-LRMS(SIM) or -HRMS(SIM),[6,7] the latter being used to measure PCDDs and PCDFs or, else, as a confirmatory technique. GLP and QA/QC protocols were applied throughout; procedural blanks were run at the beginning, during, and at the end of a daily assessment sequence.

Heavy metals were measured by a canonical AAS technique,[10] after appropriate acid digestion of fresh tissue portions (≤5 g) taken from the (untreated) pooled matrices. Measurements were replicated at least thrice.

RESULTS AND DISCUSSION

The data obtained are summarized in Tables 1 and 2; for PAHs, PCBs, and PCDDs and PCDFs, they are expressed as cumulative values. PCDD and PCDF results are also presented after conversion to 2,3,7,8-T_4CDD toxicity equivalents; B[a]P data are singled out due to the toxicologic importance of the compound (in this work, B[a]P is also used as a PAH indicator). As tables are largely self-explanatory, only a few interpretative hints are provided below. It may be noticed that at the end of the study, while recognizing its limits, two major provisional conclusions were reached: *(a)* biota did appear to show, as expected, a trend towards higher contamination levels with increasing anthropic impact, and *(b)* based on the analytical data, risk analysis did not provide indications of particular health risks associated with consumption of lagoon foodstuffs.

As a substantially low space frequency and a lack of randomness characterize the samplings of lagoon sediment studies,[1-5] the contamination distribution map obtained — lacking detail — can only be taken as a general reference; moreover, because of the large lagoon areas left unassessed, limited deposits of chemical contaminants might have gone undetected even if highly contaminated ("local contamination peaks"). These observations also apply to the biota investigated in this work, primarily collected in authorized fishing areas and therefore expected to be biased towards low contamination contents.

Indeed, the levels of organic microcontaminants in molluscs from *AREA 4* appear to be relatively low: they are comparable with those measured in the open sea Sample 4408 (*AREA 6*) and, more generally, in specimens from marine zones under modest-to-negligible anthropic impact.[12-22] Accordingly, fish Samples 7480 and 7481 (*AREA 5*) also exhibit modest-to-low organic microcontaminant levels;[12,17-19] however, PAHs, PCBs, PCDDs and PCDFs, and DDE are approximately three to nine times lower in the omnivorous grey mullet than in the sea bream, a fact that may provisionally be accounted for by the carnivorous habits of the latter. Lastly, the higher contents of some microcontaminants in the clams from *AREAS 1*, *2*, and *3* (Sample 7483) should reflect the higher exposure levels one would possibly expect there.[23-25]

As to the heavy metals, the magnitude of Cu and Zn presence in the samples assayed is of little relevance for toxicological consideration here; on the other hand, the Cd, Hg, and Pb concentration levels found in the lagoon biota are in general agreement with the pertinent data reported by an extensive investigation of the Italian coastal waters.[10] These levels are also well within the existing regulatory limits; however, the Hg content in fish Sample 7481 stands out as an exception in that it comes quite near its own limit of 0.5 μg/g. It may be pointed out that Hg levels in lagoon mussels from *AREAS 4* and *6* appear to be consistently slightly higher (0.029—0.098 μg/g) than those reported in the aforecited reference (0.010—0.069 μg/g).

Table 1. Concentration levels of selected organic microcontaminants in samples of biota from the Venice lagoon collected on October 16—17, 1995. Analyte values are grouped by virtual risk areas (*AREAS 1—6*)[3,5] and expressed per unit fresh tissue weight.

SAMPLING SITE AND TYPE[a]		ANALYTE LEVELS[b] (ng/g, except where noticed)							
		PAHS[c]	B[a]P	PCBs[d]	PCDDs+PCDFs[e] *pg/g*	PCDDs+PCDFs[e] *pgTE/g*	DDE	DDT	HCB
AREA 1 - INDUSTRIAL OR PREVAILING INDUSTRIAL EXPOSURE									
7484	clam[f]	62	5.5	23	42	1.3	2.1	<1[g]	≈4
AREA 2 - URBAN OR PREVAILING URBAN EXPOSURE									
4411	clam[f]	26	2.4	29	13	0.87	1.2	<0.5	0.73
AREA 3 - MIXED EXPOSURE									
4410	clam	14	1.1	19	7.8	0.54	0.73	<0.3	0.59
7473	clam	4.7	0.65	4.7	3.2	0.27	0.29	<0.3	0.17
7474	clam	5.4	0.55	8.0	4.5	0.40	0.40	<0.3	0.44
7483	clam	28	3.2	29	26	1.4	1.2	<1	2.5
AREA 4 - LOW EXPOSURE, OPEN FISHING AREAS									
7475	clam	2.8	<0.2	1.9	1.4	0.079	0.18	<0.3	0.29
4404	mussel[h]	11	0.77	16	3.6	0.23	1.8	<0.3	<0.08
4405	mussel	17	1.8	23	4.3	0.32	2.0	<0.3	0.17
4406	mussel	10	<0.2	32	4.3	0.46	3.8	0.72	0.12
4407	mussel	14	≈0.3	46	4.7	0.52	3.8	0.62	0.12
4409	mussel	8.7	0.50	34	4.3	0.47	3.1	0.51	0.23
7476	mussel	13	<1	37	5.8	0.54	3.4	0.76	0.28
7477	mussel	5.2	≈0.2	14	3.1	0.24	1.5	0.37	0.11
7478	mussel	6.7	0.45	16	4.1	0.31	1.5	0.42	0.14
7479	mussel	8.8	<0.2	35	6.2	0.57	3.3	0.59	0.55
7482	mussel	9.3	<1	36	6.3	0.63	3.1	0.76	0.50
AREA 5 - LOW EXPOSURE, PRIVATE FISHING AREAS									
7480	grey mullet	<2	<0.2	7.3	0.78	0.15	2.1	1.5	0.60
7481	sea bream	4.6	<0.2	26	6.5	1.3	6.4	0.92	0.83
AREA 6 - GENERAL ENVIRONMENT, OPEN SEA									
4408	mussel	3.2	<0.5	15	3.4	0.25	1.6	0.56	0.88

(a) To locate sampling sites, see map. Biota species: clam, *Tapes philippinarum* or *semidecussatus*; mussel, *Mytilus galloprovincialis*; grey mullet, *Liza ramada*; sea bream, *Sparus aurata*. Except when noticed, biota was collected from breeding areas. *(b)* All values corrected for analytical recovery and rounded off to two figures. Estimated analytical uncertainty, CV < |±30| %. *(c)* B[a]A, B[b+j+k]Fl, B[a]P, B[ghi]Pe, Chr, DB[ah]A, and IP. *(d)* Approximately 50 analytically relevant congeners of the tri- to octaclorosubstituted homologous groups. *(e)* All 17 2,3,7,8-chlorosubstituted congeners. Cumulative results are expressed in analytical units and as 2,3,7,8-T_4CDD toxicity equivalents (I-TEF system).[11] *(f)* Wild specimens. *(g)* The sign < indicates below limit of quantification (S/N ≈ 3; N ≈ 4 σ_N); figures preceded by this sign were entered as half their nominal value in calculations. *(h)* Specimens grown uncontrolled on breeding area poles.

Lastly, it might be observed that, given a certain analyte, contamination levels in biota (molluscs) do not generally appear to follow closely the variations of concentration values detected in sediments.[1-5] For instance, the analytes covering the largest concentration ranges in sediments — namely, the organic compounds PAHs, PCDDs and PCDFs, and HCB ($\Delta \approx 10^3 - 10^4$, where $\Delta = [C_{MAX}] \cdot [C_{MIN}]^{-1}$), and the metals Cd, Hg, and Zn ($\Delta \approx 10^2 - 10^3$) — exhibit variations in molluscs that are visibly not as extended ($\Delta < 100$ and $\Delta < 10$, respectively).

Table 2. Concentration levels of selected heavy metals in samples of biota from the Venice lagoon collected on October 16—17, 1995. Analyte values are grouped by virtual risk areas (*AREAS 1—6*)[3,5] and expressed per unit fresh tissue weight.

SAMPLING SITE AND TYPE[a]		ANALYTE LEVELS[b] (μg/g)				
		Cd	Cu	Hg	Pb	Zn
AREA 1 - INDUSTRIAL OR PREVAILING INDUSTRIAL EXPOSURE						
7484	clam[c]	0.220	1.96	0.145	0.44	27.2
AREA 2 - URBAN OR PREVAILING URBAN EXPOSURE						
4411	clam[c]	0.179	2.17	0.076	0.24	29.9
AREA 3 - MIXED EXPOSURE						
4410	clam	0.257	2.04	0.055	0.46	40.5
7473	clam	0.165	1.36	0.040	0.16	25.4
7474	clam	0.425	1.78	0.027	0.42	31.2
7483	clam	0.375	1.81	0.118	0.78	56.3
AREA 4 - LOW EXPOSURE, OPEN FISHING AREAS						
7475	clam	0.128	1.68	0.056	0.11	16.0
4404	mussel[d]	0.157	1.21	0.059	0.20	22.3
4405	mussel	0.116	1.19	0.061	0.16	16.4
4406	mussel	0.133	1.16	0.045	0.13	18.9
4407	mussel	0.174	0.94	0.058	0.24	22.2
4409	mussel	0.178	0.79	0.029	0.19	19.8
7476	mussel	0.214	2.10	0.098	0.20	24.7
7477	mussel	0.191	1.22	0.034	0.13	23.6
7478	mussel	0.173	1.41	0.034	0.16	24.4
7479	mussel	0.339	1.36	0.039	0.17	30.0
7482	mussel	0.310	1.59	0.081	0.24	30.0
AREA 5 - LOW EXPOSURE, PRIVATE FISHING AREAS						
7480	grey mullet	≈0.007	0.43	0.355	≈0.03	25.3
7481	sea bream	≈0.002	0.50	0.475	≈0.01	13.3
AREA 6 - GENERAL ENVIRONMENT, OPEN SEA						
4408	mussel	0.193	1.43	0.052	0.14	22.8

(a) To locate sampling sites, see map. Biota species: clam, *Tapes philippinarum* or *semidecussatus*; mussel, *Mytilus galloprovincialis*; grey mullet, *Liza ramada*; sea bream, *Sparus aurata*. Except when noticed, biota was collected from breeding areas. *(b)* Values rounded off to two or three figures. Estimated analytical uncertainty, CV < |±10| % (CV < |±30| % where marked ≈). *(c)* Wild specimens. *(d)* Specimens grown uncontrolled on breeding area poles.

LITERATURE CITED

(1) Turrio Baldassarri, L.; di Domenico, A.; Iacovella, N.; La Rocca, C.; Rodriguez, F. *Organohalogen Compounds*, Vol. 20, pp. 183-186, **1994**. Kyoto University (Kyoto).
(2) di Domenico, A.; La Rocca, C.; Rodriguez, F.; Conti, L.; Crebelli, R.; Crochi, B.; Ferri, F.; Iacovella, N.; Turrio Baldassarri, L.; Ziemacki, G. *Ecotossicologia ed Effetti Biologici di Inquinanti Inorganici ed Organici nel Sistema Lagunare Veneziano. Caratterizzazione dei Microinquinanti Chimici a Maggiore Potenziale Mutageno nei Mitili e nel Loro Habitat.* ISTISAN 95/3, **1995**. Istituto Superiore di Sanità (Rome).
(3) di Domenico, A.; Turrio Baldassarri, L.; Ziemacki, G. *Relazione di Perizia Tecnica sulla Qualità e la Quantità dell'Impatto Antropico nella Laguna di Venezia*, Vol. 1, **1996**. Case No. 7105/95/N RGNR, Procura della Repubblica, C/O Pretura Circondariale (Venice).
(4) La Rocca, C.; Conti, L.; Crebelli, R.; Crochi, B.; Iacovella, N.; Rodriguez, F.; Turrio Baldassarri, L.; di Domenico, A. *Ecotoxicol. Environ. Saf.* **1996**, 33, 236-245.
(5) di Domenico, A.; Turrio Baldassarri, L.; Ziemacki, G.; De Felip, E.; Ferri, F.; Iacovella, N.; La Rocca, C.; Rodriguez, F.; Volpi, F.; Ferrari, G.; Sansoni, R.; Settimo, G. *This Symposium.*
(6) di Domenico, A.; De Felip, E.; Ferri, F.; Iacovella, N.; Miniero, R.; Scotto di Tella, E.; Tafani, P.; Turrio Baldassarri, L. *Microchem. J.* **1992**, 46, 48-81.
(7) Turrio Baldassarri, L.; di Domenico, A.; Fulgenzi, A.; Iacovella, N.; Bocca, A.; Larsen, B.R. *Fresenius Environ. Bull.* **1993**, 2, 370-374.
(8) De Felip, E.; di Domenico, A.; Grande, M.; Pazzaglia, R.; Falleni, M. *Intern. J. Environ. Anal. Chem.* **1990**, 38, 607-616.
(9) De Felip, E.; di Domenico, A.; Turrio, L.; Volpi, F.; Merli, F. *Toxicol. Environ. Chem.* **1990**, 29, 37-46.
(10) Costantini, S.; Giordano, R.; Ciaralli, L.; Vernillo, I.; Rubbiani, M.; Rinaldi, S.; Beccaloni, E.; Musmeci, L.; Chirico, M.; Piccioni, A. *Valutazione Sperimentale dei Livelli di Mercurio, Cadmio, e Piombo in Alcuni Componenti dell'Ecosistema Marino Italiano.* ISTISAN 92/20, **1992**. Istituto Superiore di Sanità (Rome).
(11) US EPA *Interim Procedures for Estimating Risks Associated with Exposures to Mixtures of Polychlorinated Dibenzo-p-dioxins and Dibenzofurans (CDDs and CDFs) and 1989 Update.* EPA/625/3-89/016, **1989**. Risk Assessment Forum, US Environmental Protection Agency (Washington).
(12) Bocca, A.; Fabietti, F. *Idrocarburi Policiclici Aromatici: Basi Scientifiche per la Proposta di Linee-Guida.* ISTISAN 91/27, pp. 57-68, **1991**. Istituto Superiore di Sanità (Rome).
(13) Maher, W.A.; Aislabie, J. *Sci. Total Environ.* **1992**, 112, 143-164.
(14) Shchekaturina, T.L.; Khesina, A.L.; Mironov, O.G.; Krivosheeva, L.G. *Mar. Poll. Bull.* **1995**, 30, 38-40.
(15) WHO *Polychlorinated Biphenyls and Terphenyls (Second Edition).* EHC 140, **1993**. IPCS, World Health Organization (Geneva).
(16) Picer, M.; Picer, N. *Chemosphere* **1995**, 30, 31-38.
(17) Giouranovits-Psyllidou, R.; Georgakopoulos-Gregoriades, E.; Vassilopoulou, V. *Mar. Poll. Bull.* **1994**, 28, 121-123.
(18) Fürst, P.; Fürst, C.; Groebel, W. *Chemosphere* **1990**, 20, 787-792.
(19) Haynes, D.; Toohey, D. *Mar. Poll. Bull.* **1995**, 30, 885-891.
(20) Cowan, A.A. *Environ. Poll. (Series B)* **1981**, 2, 129-141.
(21) Krämer, W.; Buchert, H.; Reuter, U.; Biscoito, M.; Maul, D.G.; Le Grand, G.; Ballschmiter, K. *Chemosphere* **1984**, 13, 1255-1267.
(22) Granby, K.; Spliid, N.H. *Mar. Poll. Bull.* **1995**, 30, 74-82.
(23) WHO *Polychlorinated Dibenzo-p-dioxins and Dibenzofurans.* EHC 88, **1989**. IPCS, World Health Organization (Geneva).
(24) Knutzen, J.; Oehme, M. *Chemosphere* **1989**, 19, 1897-1909.
(25) Rappe, C.; Bergqvist, P.-A.; Kjeller, L.-O. *Chemosphere* **1989**, 18, 651-658.