I - Executive Summary

1. Introduction

Changing land use patterns, global climate change, and expanding human populations along coast lines, including those of the Mediterranean and Black Seas, are putting increased stress on coastal regions and may have serious impacts on public health. Sewage, both treated and raw, which enters into receiving waters may carry diverse inorganic and organic contaminants as well as pathogenic microorganisms, all of which may affect both marine populations as well as their human consumers. While a number of such contaminants (*e.g., chlorinated hydrocarbons, methylmercury, polycyclic aromatic hydrocarbons*) are relatively well documented in their effects on marine and human populations, other more novel contaminants are starting to be recognized as having worrisome effects. These may include a new generation of insecticides, herbicides (*e.g. carbamate and organophosphate pesticides)*, and other novel organic compounds used in industry *(e.g. food additives)*, pharmaceutically active compounds which may influence hormonal function in marine animals and in human consumers, antibiotics which may enter coastal communities through sewage and mariculture applications, long-lived radionuclides associated with nuclear power installations, diverse metals and metalloids which may be associated with new emerging industries, and emerging pathogens that may arise from changing land use patterns and new forms of sewage treatment and disposal. The impacts of these new contaminants on coastal ecosystems and on human consumers are largely unstudied. Indeed, even the links between the best studied contaminants entering marine ecosystems and human health are only now starting to be drawn, and clearly there is much new research that is needed even for these contaminants.

The Mediterranean and Black Sea are ringed by densely populated coastal communities that are often in a state of flux in sheer numbers, in socio-economic development and in agricultural and industrial activity. Moreover, river diversion in some areas, for transportation, hydroelectric power, and irrigation purposes can greatly influence the entry of contaminants and pathogens into estuarine waters. Increasingly we are recognizing that industrial shipping and pleasure craft can also transport toxic microorganisms from one region to another. Different geographic regions are often facing similar types of environmental and public health problems, and yet there are relatively few coordinated efforts to address these problems or even summaries of the current state of knowledge in these areas that managers or scientists can be guided by.

To assess the scientific dimension of such challenges, CIESM organized a Workshop in May 2004 in Neuchatel. In welcoming the participants Frédéric Briand expressed his gratitude to Nicholas Fisher and Gerhard Herndl, Presidents respectively of the Committees on Marine Biogeochemistry and Marine Microbiology, who had suggested the central theme and agreed to co-chair this event, and to François Nyffeler, Representative of Switzerland on the Commission, for his efficient assistance on the logistic side. Thirteen scientists from ten countries attended the brainstorming sessions at the invitation of CIESM. This summary - a synthesis of the discussions and of follow-up consultations within the group – must be considered essentially as a team effort.

2. NOVEL CHEMICAL CONTAMINANTS

2.1 Contaminants of concern

The history of investigations and discussions on chemical pollutants now covers more than 40 years. Major political and scientific milestones were Rachel Carson's book "Silent Spring" in 1962, James Lovelock's Gaia Hypothesis published in 1989, the book entitled "Our Stolen Future" by Theo Colburn, and the Stockholm Persistent Organic Compounds (POP) Convention adopted on May 22, 2001. The latter encompasses 12 polychlorinated chemicals (hydrocarbons, biphenyls, benzodioxins and benzofurans), the so-called dirty dozen and have become legally binding on May 17, 2004 following the ratification by more than 55 countries.

In the last two decades environmental scientists provided evidence for the spread throughout the global environment of certain persistent organic compounds (such as many chlorinated hydrocarbon compounds) and quantitative understanding of the mechanisms of the distribution of these and other compounds in environmental reservoirs, including living organisms and human populations. The scientific community also has developed an expanded data base on the biochemical reactivity and potential for biological effects (e.g. immunotoxicity, endocrine disruption and carcinogenicity) of various organic pollutants in animals - including humans. Recently, for instance, the endocrine disrupting effects of some organic contaminants have been recognized as having great potential for detrimentally affecting wildlife.

The "conventional" contaminants of the 1960s to 80's have been eclipsed by a long list of "potential bad actors." Compounds like PCBs, PAHs, and organochlorine pesticides are well-investigated, while environmental data for emerging contaminants such as pharmaceuticals (e.g., analgesics, blood-lipid regulators, beta-blockers, anti-epileptics, X-ray contrast media), antibiotics (e.g. fluoroquinolones, macrolides, sulfonamides, tetracyclines), steroidal hormones, flame retardants (e.g., polybrominated diphenyl ethers, hexabromocylododecane, tetrabromobisphenol A, organophosphorous flame retardants), polyfluorinated surfactants, and constituents of personal care products are rather rare. The paradigm of cancer causing compounds, such as PAHs and PCBs, has been supplemented to contaminants that cause endocrine disruption, act as "gender benders" or trigger microbial resistance. Thus, "New Environmental Quality" standards are evolving that are linked to the analytical determination of many of these new polar contaminants. A classification of classic vs. novel contaminants in relation to their environmentally relevant physicochemical properties is attempted in Figure 1. Table 1 presents some information on classification and environmental behavior of novel chemical contaminants that enter coastal waters.

Table 1. Incomplete overview on currently emerging contaminants.

Types	**Chemical compound classes**	**Current environmental knowledge**	**Chapters of this report* and literature**
Pharmaceuticals and metabolites	Broad range of chemical composition	Wide input via municipal wastewaters and hospital effluents As above, plus aquaculture	... Kolpin *et al.,* 2002 Heberer *et al.,* 2002 Sacher *et al.,* 2001 Snyder *et al.*, 2003
Antibiotics	fluoroquinolones macrolides sulfonamides		Giger *et al.** Giger *et al.*, 2003 Golet *et al.*, 2003
Endocrine disruptors	17β-estradiol, ethinylestradiol, bisphenol A, nonylphenol	Wide input via municipal wastewaters	......
Flame retardants	Polybrominated diphenylethers (PBDEs) Hexabromocyclocdodecane (HBCD) Tetrabromobisphenol A (TBBPA) organophosphates	Ubiquitous POPs atmospheric transport	Hites, 2004
Polyfluorinated surfactants	perfluorooctane sulfonate (PFOS) perfluorooctanoic acid (PFOA)	New POPs, unclear input	Schultz *et al.*, 2003
Polar pesticides and metabolites			
Nonagricultural biocides	organotins, irgarol, dichlorooctyl-isothiazolinone triclosan	Antifungal chemicals for boats. Biocidal products	
Additives			
Plasticizers	Phthalates, organophosphates		
anticorrosives	benzotriazoles		
preservatives	isothiazolinones		
	polychlorinated paraffins		Marvin *et al.*, 2003
Surfactants and metabolites	nonlyphenolpolyethoxylates, nonylphenol	EU risk assessment report for NP	Ahel and Terzic* Stephanou* Montgomery and Reinhard, 2003
Gasoline additives, oxygenates	MTBE		Deeb *et al.*, 2003

The identification and determination of concentrations of many previously undetected organic anthropogenic compounds in the environment has progressed rapidly in recent years with the development of new and improved analytical techniques. Furthermore, it has been found, that the detection of certain of these "novel" contaminants such as polybrominated diphenyl ethers (PBDEs); perfluorochemicals: perflurooctane sulfonates (PFOS) and perfluorooctanoic acid (PFOA); alkylphenolic compounds – nonyl- and octylphenol; many pesticides (e.g., triazine and phenylurea herbicides); veterinary and human pharmaceuticals; biocides and bactericides (e.g., irgarol and triclosane); and phthalate esters, among others might be of environmental concern, because they have been shown to be mobile, persistent and toxic and some are bioaccumulative. Studies have also shown that the levels of at least some of these chemicals have increased in recent decades and that their presence in the environment is widespread (e.g., PBDEs).

Exposure assessment in Europe is hampered by the lack of monitoring data for the above-mentioned compounds. In addition, wastewater is targeted in some Mediterranean regions with an important lack of water resources, a susceptible aquatic environment and the important need of water re-use. The presence and the behavior of these compounds in sewage treatment plants will determine their occurrence not only in Mediterranean fresh waters (rivers, groundwater, etc.) but also in the marine environment, and possibly in the coastal atmosphere.

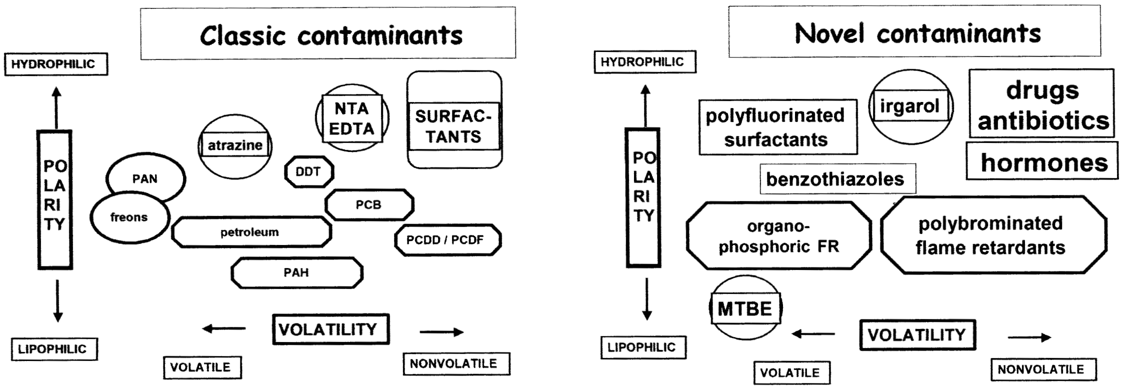

Fig. 1. Comparison of "classic" and "novel" contaminants with respect to polarity and volatility.

2.2 Analytical methods for measuring novel contaminants

A sophisticated operation of instruments and data evaluation is a prerequisite for successful analysis of emerging contaminants. Solid-phase extraction in combination with liquid chromatography (LC) on reversed phase columns have been mostly used for sample pre-concentration and for compound classes like pharmaceuticals or estrogens. The introduction of liquid chromatography directly coupled to mass spectrometry (LC/MS) has enabled the detection and quantitative determination of trace amounts of more polar organic contaminants. Electrospray ionization (ESI) and atmospheric pressure ionization (APCI) are the most frequently used ionization techniques for polar and ionic compounds, as well as for less polar nonionic ones. Quantitative assessment of the environmental occurrence and behavior of these emerging contaminants will require further development of sensitive analytical methods, based on the above mentioned techniques. Thus, the forefront analytical technologies that are suited for these mostly polar compounds are LC directly coupled to multistage mass spectrometry (MS^n), time-of-flight mass spectrometry (TOF-MS) and inductively coupled plasma mass spectrometry (ICP-MS).

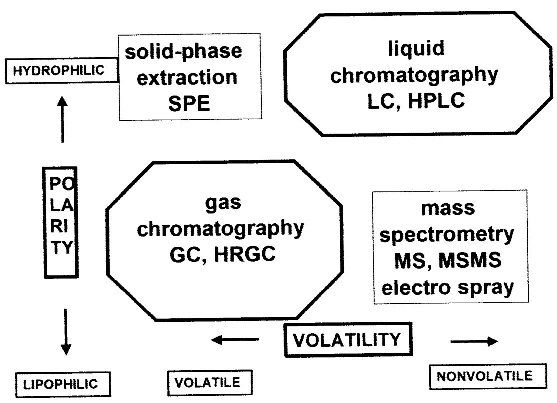

Fig. 2. Analytical approaches appropriate for analysis of novel chemical contaminants.

Multistage mass spectrometric techniques and the use of diagnostic ions reveal their usefulness for compound-class specific screening. In addition, several emerging contaminant classes can also be investigated directly by GC/MS without or after derivatization. An overview of the analytical techniques to be used for the environmental analysis of novel contaminants, in relation to the environmentally relevant physicochemical properties, is presented in Figure 2.

2.3 Sources of novel contaminants in coastal waters

As noted above, an increasing diversity of chemicals is entering the environment, including coastal waters. Sources include urban, industrial and agricultural areas or activities, and include direct discharges at point sources (such as water treatment plants and industrial waste waters) and more diffuse sources like runoff of drainage from agricultural areas. Discharges from some point sources, such as hospital waste waters entering urban sewage systems (Kimmerer, 2001), can present a unique suite of unusual contaminants. In addition, to land-based sources, the direct inputs of chemicals used for example in aquaculture should also be taken into account, most particularly certain types of pesticides (pyrethroids, for example) and antibiotics (see below).

The time course and frequency of inputs of these sources can vary considerably ranging from continuous emissions, seasonal uses and discharges, peak discharges and accidental events. Clearly, studies should take into account the spatial and temporal distribution of sources to allow better prediction and modeling of the distribution of these emerging chemicals in the environment.

Some specific examples of emerging contaminants in coastal waters can be given. Natural and synthetic steroid estrogen (e.g., 17a-ethinylestradiol) is present in sewage effluent and in receiving waters at levels that elicit biological effects such as endocrine disruption (Garcia-Reyero *et al*., 2001). Similarly Bisphenol A has been detected in wastewater effluents and sewage sludge (Furhacker *et al*., 2000). Phathalates have been used for more than 40 years in very large amounts for the manufacturing of different kind of plastics (e.g., PVC) and other resins (Fromme *et al*., 2002). DEHP has been detected in surface waters, sewage effluents and sediments (Watanabe *et al*., 1987). Antifouling agents such as TBT are used in marine paints on boats and vessels; marinas and shipyards are point sources for these contaminants in the marine environment. Surfactants are mainly used in detergents. The surfactants of most concern are the alkylphenols (AP) and their ethoxylates (APEO), linear alkylbenzene sulfonates (LAS), perfluorochemicals: perflurooctane sulfonates (PFOS) and perfluorooctanoic acid (PFOA), flame retardants and especially brominated compounds that have been used in plastics, textiles, electronic circuitry, and as chemical additives. This group includes polybrominated biphenyls (PBB), polybrominated diphenyl ethers (PBDEs), hexabromocyclododecan (HBCD) and tetrabromobisphenol A (TBBPA). Many diverse pesticides are used in agriculture as well as in urban and domestic settings. These include insecticides, herbicides, and fungicides. Pharmaceuticals, including human and veterinary drugs are highly variable and are used in large amounts. Prescription amounts for some high volume pharmaceuticals in Europe, e.g. anti-inflammatory agents or some antiepileptics, reach several hundred tons per year.

2.4 Environmental pathways, behavior and fates of novel contaminants

Many of these emerging chemical contaminants enter the environment by various pathways. Perhaps the most important inputs into coastal waters of novel contaminants are through riverine fluxes, where large drainage basins (e.g., Rhone, Po) contribute to their transport into the Western Mediterranean. In addition, the direct inputs of big urban centers near coastlines through sewage treatment plants and other non-point sources can also contribute to chemical loading in the coastal Mediterranean. Estuaries constitute an interface between continental drainage basins of the surface waters and coastal waters. They integrate fluxes from riverine basins and constitute transitional waters where dissolved and particulate chemical constituents are transported from continental to marine environments. Because estuaries and other coastal regions are often areas of active biological recruitment or nurseries, elevated concentrations of contaminants in these regions pose potential dangers to diverse populations far more than in open ocean waters. Moreover, these are areas of active harvesting of seafood by human consumers, hence the potential for public health impacts are more pronounced by contamination of estuaries than more remote waters. Atmospheric fallout through dry and wet deposition could be important for certain compounds, but this route may be more likely to contribute contaminants to open waters.

Once in the marine environment many of these contaminants are mainly present in the water phase since they are medium to polar compounds. For a considerable number of novel contaminants, however, specific sorption mechanisms can lead to a significant mass transfer into sediments. Their solubility in water makes them mobile in the environment and enables their long transport via currents. They appear to be persistent in the environment, although detailed studies on their degradation rates in the environment at trace levels have not been undertaken.

Information about the occurrence and concentrations of novel contaminants in the coastal Mediterranean is still very limited. The occurrence of contemporary pesticides and antifouling agents has been reported in the coastal waters of the Mediterranean and other marine areas. The distribution and levels of selected compounds are summarized in this volume by Theobald, Zuccato, and Tronczynski. New data about the identification and concentrations of chemicals in river systems entering the Mediterranean provide evidence of the presence of diverse organic substances including pesticides, pharmaceuticals, surfactants and others that probably enter coastal regions of the Mediterranean. Further, more advanced data bases describing contamination of other marine environments (North Sea, Baltic Sea, coastal Atlantic Ocean) by previously undetected chemicals are now available. The concentrations of these emerging contaminants (e.g., pesticides, surfactants, antifouling agents) in the North and Baltic Seas indicate that large amounts of these chemicals are introduced into coastal waters, that the compounds are persistent, and their dissolved concentrations rise to levels that are higher than those of some "classic" contaminants (e.g., hexachlorocyclohexane). It is likely that processes leading to coastal contamination in other waters are also active in the Mediterranean. Indeed, human and veterinary pharmaceuticals have been detected at elevated levels (on the order of 10 to 100 ng L^{-1}), in Po river water, including one station at the mouth of the river (see Zuccato contribution, this volume), and levels and spatial and temporal distribution of antifouling agents and selected herbicides have also been reported for the coastal Mediterranean.

2.5 Bioavailability, bioaccumulation of novel contaminants

Clearly, concern about the introduction of contaminants of any type into marine waters stems from their possible danger to living organisms (including people). While we can measure the presence of contaminants in aquatic ecosystems, this does not indicate that these contaminants are influencing living organisms. Indeed, it is at present difficult to quantify the risks associated with many novel emerging contaminants, either to aquatic organisms or to human populations that consume seafood, because there is considerable uncertainty regarding the biological uptake and toxic effects of these contaminants in marine ecosystems.

As a first principle, it is well established that chemical contaminants can exert toxic effects on organisms only after they have been taken up; that is, contaminants that remain in water or bound to sediment, for example, and are not taken into living organisms can not be toxic (Newman, 1998). It is also known that contaminants may speciate chemically and physically in the environment in ways that affect the extent to which they are available for uptake by aquatic organisms (Campbell, 1995; organic reference). Therefore, the bioavailability of contaminants for aquatic organisms must be assessed in order to evaluate their bioaccumulation potential. Once a contaminant is associated with an organism it can exert toxicity (either lethal or sublethal) and/or it can be transferred to another trophic level where it may exert toxic effects. Such trophic transfer is well known for many inorganic (e.g., metals and metalloids) and organic (e.g., chlorinated hydrocarbons) contaminants (Fisher and Reinfelder, 1995). Thus, animals can obtain their contaminants both through dietary pathways as well as directly from the dissolved phase, whereas plants, which do not eat, only accumulate contaminants from the dissolved phase. The overall issue of metal and radionuclide bioaccumulation in marine organisms has been treated in a separate CIESM workshop (CIESM, 2002b), and many of the processes discussed in that document are relevant to other contaminants as well. Briefly, it can be stated here that kinetic models now exist to quantitatively distinguish the relative importance of solute vs. dietary sources of contaminants for aquatic animals (Landrum *et al*., 1992; Wang and Fisher, 1999a), and model predictions have been verified with independent field measurements for diverse contaminants in marine, estuarine, and freshwater invertebrates and vertebrates (Wang *et al.*, 1996; Fisher *et al.*, 2000; Roditi *et al.*, 2000; Baines *et al.*, 2002). These models rely on measuring the assimilation efficiency of ingested contaminants and their efflux rates from the animals, and experimental protocols for measuring these parameters have been well developed (Fisher *et al.*, 1996). These parameters have already been evaluated for diverse inorganic and organic contaminants in marine and freshwater organisms (Wang and Fisher, 1999b).

The extent to which contaminants get accumulated out of water into organisms can be quantitatively compared among contaminants and among organisms using bioconcentration factors. These values essentially reflect the extent to which contaminants are enriched in organisms relative to ambient water. Such bioconcentration factors are readily used in risk assessment models but complications arise with organic compounds, which can be metabolized by living organisms. Nevertheless, striking patterns have been demonstrated for measuring the bio-accumulation of many organic compounds across many types of organisms. Generally, it appears that the hydrophobicity of a compound, assessed as its octanol-water partition coefficient, can be related to the degree to which it is enriched in organisms (Schwarzenbach *et al.*, 2003). Thus, for a compound whose biological uptake is not studied, knowledge of a compound's octanol-water partition coefficient (k_{ow}) may help in estimating the extent to which it is likely to bio-concentrate in organisms. However, for some of the ionizable compounds such as many of the pharmaceuticals released into the environment, log D (log K_{ow} adjusted for speciation to correct for ionized species) may be more useful as an organizing principle for comparing chemicals.

As noted above, once associated with an organism, a contaminant could exert toxicity either to that organism or to another organism that eats it. It appears that most contaminants in natural water do not reach sufficiently high concentrations to elicit acutely lethal effects. However, diverse sublethal effects are possible and might include diminished growth rates, impaired reproductive capability, altered metabolic pathways, and altered behavioral patterns in affected organisms. Such reduced fitness could have pronounced effects on the viability of populations in natural waters, leading to changes in community structure. It is widely acknowledged that sub-lethal toxicity tests need to be conducted to greatly expand the data base for effects on marine organisms, especially for contaminants that have only recently been measured in natural waters. Moreover, there is a need to improve toxicological testing protocols for aquatic organisms. For example, often the toxicity of a contaminant to a test animal is assessed by exposing the animal to dissolved contaminant, but of course animals (including man) can also accumulate contaminants through their diet, and the resulting tissue distribution of contaminants following different uptake pathways can be sufficiently different to greatly affect the biological response (Fisher and Hook, 2002). Thus, contaminant toxicity to animals also needs to consider dietary exposure.

Many contaminants associate with sediments, which have often been thought to be the final repository for particle-reactive metals and organic compounds. While estuarine sediments are often greatly enriched (relative to the water) in many contaminants, they can also be thought of as a source, not just sink, of contaminants for marine systems. There is now unequivocal evidence of biological uptake of diverse organic and inorganic contaminants bound to sediments by a wide variety of benthic invertebrates (Mayer *et al.*, 1996; Griscom *et al.*, 2000). These studies suggest that sediment-dwelling animals (both infaunal and epifaunal) can assimilate contaminants from ingested sediment as well as absorb contaminants from pore water and overlying water, and these animals may transfer contaminants to predators who consume them (Luoma and Fisher, 1997). The extent to which many of the emerging organic compounds get assimilated out of sediment into benthic animals and get subsequently transferred up the food chain has largely gone unstudied.

2.6 Biological effects of novel contaminants

Many of the novel contaminants studied in this report have endocrine disrupting properties, and are known to have carcinogenic, reprotoxic or neurotoxic effects on living organisms, often at extremely low concentrations. For example, among natural and synthetic steroid estrogens, ethinylestradiol (EE2) has been shown to induce vitellogin production in male rainbow trout at 0.1 ng l^{-1} levels (Purdom *et al.*, 1994) and the growth and development of testes in maturing male trout has been shown to be retarded by 50% due to a single dose of EE2 at 2 ng l^{-1} (Jobling *et* *al*., 1996) resulting in sex reversal in fish (Martin-Robichaud *et al*., 1994). Similarly, reproductive effects of Bisphenol A (BPA) have been observed on fathead minnow (*Pimephales promelas*), including inhibition of spermatogenesis (Sohoni *et al*., 2001). Still, BPA levels in surface water are typically one to several orders of magnitude lower than those which cause chronic effects in test organisms (Staples *et al*., 1998), although bioaccumulation in biota may occur), and the risk associated with contaminated sediment is likely to be much greater. Generally, the toxicity of phthalates to aquatic organisms increases with increasing alkyl chain length due to a corresponding increase in log Kow and decreasing aqueous solubility (Call *et al*., 2001); calculated predicted no effect concentrations (PNECs) for PAEs of varying alkyl chain length suggest that there is some concern for aquatic organisms and benthic species (Staples *et al*., 2000).

Tributyltin (TBT) residues provide one of the best examples of population level effects on wildlife (Van der Kraak, 1998). Both lethal and sub-lethal effects have been observed in biota (Voulvoulis, 1999), including severely retarded larval development, masculinization of females (imposex), behavioral changes and shell deformation in oysters. In some areas TBT exposure has resulted in sterility leading to species extinction (Minchin *et al*., 1996). Imposex has been determined at levels of 3 ng l^{-1} TBT, and a no effect concentration (NOEC) has yet to be identified (Burton and Scott, 1992). Generally, among surfactants, toxicity is greater to aquatic organisms than mammals, with toxicity for alkylphenolethoxylater (APEO) increasing with decreasing number of ethoxylate units and increasing hydrophobic chain length (Fent, 1995). A recent survey of wild roach in U.K. rivers has found a high percentage of males with eggs in their testes and female egg yolk protein in their blood attributed to exposure to such surfactants (Jobling *et al*., 1998). PAHs and their metabolites that are associated with sediment are bioavailable to both benthic and demersal aquatic organisms (Landrum *et al*., 1991), with the lifestyle of the organism affecting its exposure to sediment-associated PAH and the direct intake of particulates contributing a significant proportion (Woodhead *et al*., 1999).

Flame retardants and especially brominated compounds (PBDEs) are also of concern since they are persistent, lipophilic, have been shown to bioaccumulate (de Wit, 2002). Pesticides and other biocides are known to exhibit endocrine activity and have the potential to accumulate in the aquatic food chain due to their persistence and lipophilicity (Bulger *et al*., 1978; Ireland *et al*., 1980; Bedding *et al*., 1982; Bulger and Kupfer, 1983). There is much evidence linking organochlorine insecticide exposure to endocrine disrupting effects on the wildlife. Exposure results in both physiological abnormalities such as thinning of eggshells and damage to the male reproductive system, and to behavioral changes which are also potentially dangerous to survival (Colburn, 1995; LeBlanc, 1995). In addition, as a result of restrictions in the use of organotins in antifouling applications, alternatives to TBT paint such as copper based coatings containing organic booster biocides are of particular concern, because of limited data and information available on their occurrence, fate, toxicity, and persistence in the marine environment (Voulvoulis, 1999).

As a rule, there is currently little evidence to suggest that acute effects on aquatic systems are occurring. Exceptions can occur locally when untreated production or municipal waste is released directly into surface waters or estuaries. However, emerging contaminants such as pharmaceuticals may very well not be the most deleterious agents in these situations, where ammonia, toxic metals, and other anthropogenic, industrial or agricultural toxicants may control ecosystem health ("dead fish do not care for endocrine disruption"). There is, however, considerable scientific uncertainty regarding the environmental impact of pharmaceuticals due to their specific modes of action, the chronic exposure situation of aquatic life and the complex mixture situation (Seiler, 2002). Ecological effects data, taking into account chronic exposure and mixture effects, are being increasingly recognized (Cleuvers, 2004; Ferrari *et al*., 2003; Huggett, 2002; Ferrari, 2003; Laenge *et al*., 2001; Jones *et al*. in press).

An acute to chronic ratio (ACR) of 10-100 has been established for a large number of industrial chemicals. Some researchers claim now that for specific pharmaceutical classes, ACRs of 10,000 or higher might be more appropriate. For specifically acting substances (like many pesticides or pharmaceuticals) a recent review showed a median ACR of 10.6, with a minimum-maximum range of 1.33 – 34800 and a 95th percentile ACR of 191 (ECETOC, 2003). For comparison, a few available examples have been compiled below and the respective ACRs have been calculated.

Table 2. Acute to chronic ratios (ACRs) in vertebrates for a selection of human pharmaceuticals.

Substance	**Acute data**		**Chronic data**		**ACR**	**Ref. (chronic data)**
	Type	Value	Type	Value		
Ethinylestradiol	LC_{50}	1.5 mg L^{-1}	Chronic NOEC	1 ng L^{-1}	1.5 x 10^6	Laenge *et al.*, 2001
Estradiol	LC_{50}	6.1 mg L^{-1}	Chronic EC_{50}	0.12 µg L^{-1}	50,833	Kramer *et al.*, 1998
Propranolol	LC_{50}	24.3 mg L^{-1}	Chronic LOEC	0.5 µg L^{-1}	48,600	Huggett *et al.*, 2002
Fadrozol	LC_{50}	49 mg L^{-1}	Chronic LOEC	2 µg L^{-1}	18,500	Ankley *et al.*, 2002
Carbamazepine	LC_{50}	43 mg L^{-1}	Chronic NOEC	25 µg L^{-1}	1,720	Ferrari *et al.*, 2003
Diclofenac	LC_{50}	50 mg L^{-1}	Chronic NOEC	1 mg L^{-1}	50	Ferrari *et al.*, 2003
Thiabendazole	LC_{50}	0.56 mg L^{-1}	Chronic NOEC	0.012 mg L^{-1}	46	EPA online data [a]

[a] http://www.epa.gov/pesticides/reregistration/thiabendazole/

Contaminants do not reach the environment as individual chemicals but are present in complex and constantly changing mixtures. Current RA procedures do leave this aspect largely out of consideration. In the aquatic environment most organisms are continually exposed to numerous potentially toxic substances simultaneously with possibly only slight temporal and spatial variations in concentration levels (Schowanek, 1998). Recent work is beginning to demonstrate the significance of exposures to mixtures of chemical (and non chemical) stressors at low concentrations and this raises the concern whether additive effects might occur or whether synergy could magnify the effects of certain pharmaceuticals (Fox, 2001; Renner, 2002). Most available experimental evidence suggests concentration additivity as opposed to synergistic or antagonistic effects, but there is still a lot of work to do in this area.

2.7 Antibiotic resistance

Antibiotics are chemicals that have received considerable attention in recent years, in part because of potential public health concerns, and in part because of their known abundance in coastal waters from aquaculture operations as well as sewage inflow. Salmon farming uses large amounts of antibiotics to keep disease and parasite outbreak under control. Recently, antibiotic use has gone down considerably in some countries, while vaccinations have increased. Norway, for example - once considered a major user of antibiotics on fish farms - the consumption of antibiotics has been reduced by 96% in the course of the last ten years because the country vaccinates now every single farmed salmon. (Figure 3, source: www.fiskeoppdrett.no/akvakultur/html/Aquaculture.htm).

Some of the more common antibiotics that are used in aquaculture today include oxytetracycline and amoxycillin. The antibiotics are delivered to the target species in the form of medicated feed products. However, not all the feed is consumed, and hence some of the antibiotics are released into the environment, and often are transported into the sediments (Herwig *et al.*, 1997; Pouliquen and Le Bris, 1996; Munekage *et al.*, 2002). As a variety of antibiotics are used in both human medicine as well as aquaculture practices, their presence in the environment is of concern due to the possibility of increased bacterial drug resistance. The persistence of several antibiotics in marine sediments has been tested by Hektoen *et al.* (1995), and it was found that particularly oxytetracycline, as well as several quinolones (oxolinic acid and sarafloxacin), tend to be rather resistant (on the order of months) within sedi-

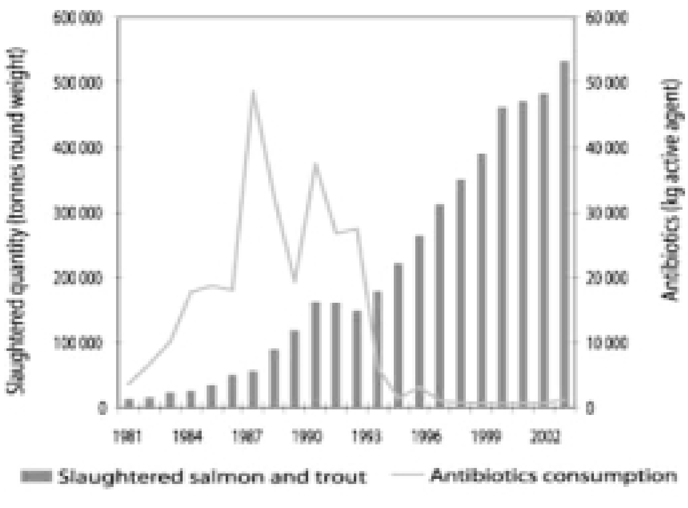

Fig. 3. Antibiotics use in relation to salmon and trout farms since 1981.

ments. Resistance of sediment bacterial populations to tetracycline has been investigated in a sturgeon farm by Forney *et al*. (1998). Their results indicated that antibiotic concentrations higher than the recommended therapeutic levels are necessary in order to produce a noticeable effect on the bacterial populations within the sediments. However, Herwig *et al*. (1997) found that antibiotic resistance in bacteria within the sediments increased in close proximity to salmon net cages in Puget Sound, WA, U.S.A. Furthermore, it is assumed that the extensive use of antibiotics within the shrimp industry in Ecuador may have resulted in the occurrence of the antibiotic-resistant *Vibrio cholerae* in humans (MacMillan, 2001). In addition, bacterial resistance to another type of antibiotic, flumequin, can be high so that only about 10% of the bacteria found in intensive aquaculture systems are unable to grow in its presence (Migliore *et al*., 2001). Another study by Schmidt *et al*. (2000) demonstrated the impact of trout farming on environmental bacteria as well as fish pathogens. Their findings show that antibiotic resistance levels were increased in those populations (especially those composed of aeromonads and Flavobacteria) that were exposed to the antibiotics.

Mediterranean concentrations of antibiotics, vaccines, and other chemotherapeutants have only been sporadically studied. In a study of coastal sediments in the Ligurian Sea (Western Mediterranean), Chelossi *et al*. (2003) found a threefold increase in bacterial density and biomass in sediments beneath the fish cages compared to control sediments. Antibiotic sensitivity tests showed a high percentage of resistant strains in both control and impacted sediments, but ampicillin resistance and multiple resistance patterns in bacterial isolates was greater in impacted sediments.

2.8 Risk assessment for novel chemicals

Environmental risk assessments for environmental contaminants are based on tiered decision trees with a constantly increasing level of detail and data. For pesticides and industrial chemicals, guidelines are quite detailed, however, for human pharmaceuticals, the proposed approach is quite simple (Straub, 2002). For human pharmaceuticals, as a prioritization tool, predicted exposure levels (PECs) are used prior to entering Tier 1. This is based on tonnage or use worst-case exposure estimation, refined by various depletion mechanisms such as metabolism or biodegradation. Effect assessment (PNECs, or predicted no-effects concentrations) is based on acute effects data in selected predefined species (e.g., algae, daphnia and fish). A next higher tier with a refined fate or effect assessment has to be entered if the risk ratio in an earlier tier indicates a concern (e.g., PEC/PNEC >1). While being an adequate screening tool, the approach has been criticized due to problems associated with the lack of data required for PEC and PNEC estimation, chronic toxicity data, and mixture effects.

In order to be able to properly assess the risks associated with the sources, use and disposal of these chemicals, several knowledge gaps need to be filled. These include data on:

- Sources
    - Tonnage (production)
    - Usage patterns
    - Disposal
- Behavior in relevant environmental compartments
    - Sewage Treatment Plants (STPs)
    - landfills
    - soils / runoff
- Exposure
    - Levels
    - Persistence
    - Bioaccumulation / Biomagnification
- Effects
    - Acute Toxicity
    - Chronic Toxicity

For conventional chemicals, where these data are often available, risks have been established and in a few cases, mitigation measures have even been introduced to reduce impacts (e.g., DDT, CFCs, lead in gasoline). For novel contaminants, despite considerable concern associated with some of them (e.g., estrogens), uncertainty is still too high because of the lack of data, to allow risk management strategies to be established. Moreover, for all classes of contaminants, sublethal toxicity testing with marine organisms needs to be improved, and must take into consideration the accumulation of contaminants in tissues (that is, effects should be expressed as a function of dose in the organism, not outside the organism) as well as toxic effects of contaminants accumulated through different uptake pathways.

2.9 Prioritizing, ranking of chemicals

Under Directive 2000/60/EC of the European Community, specific measures were decided at the Community level against pollution of water by individual pollutants or groups of pollutants presenting a significant risk to aquatic environment. Such measures are aimed at the progressive reduction, and for priority hazardous substances phasing out, of discharges, emissions and losses into environment of these substances. The ultimate aim for the marine environment is to achieve concentrations approaching background values for naturally occurring substances and close to zero for man-made substances. With a view to the adoption of these measures the European Commission has introduced a scientifically based methodology for selecting priority substances on the basis of their significant risk to or via the aquatic environment. This methodology includes the application of a simplified risk-based assessment procedure based on scientific principles taking into account:

(1) evidence regarding the intrinsic hazard of the substance concerned, and, in particular, its aquatic ecotoxicity and human toxicity via aquatic exposures routes;

(2) evidence from monitoring of widespread environmental contamination;

(3) other proven factors which may indicate the possibility of widespread environmental contaminantion, such as production, use volume and use pattern of the substance concerned.

The Commission has on this basis, developed a combined monitoring-based and modeling-based priority setting (COMMPS) scheme. The identification of priority hazardous substances should be made with regard to hazardous substances agreed for phase-out or for cessation of discharges, emissions and losses in international agreements such as the UN-ECE and OSPAR Convention, including hazardous substances identified by OSPAR DYNAMEC Selection (I and III) of substances that are persistent.

Estimate of pharmaceutical use provide some information as to the likeliest compounds that may reach coastal waters. The five leading therapeutic classes in 2000 according to IMS were antiulcerants, cholesterol lowering agents, antidepressants, blood pressure lowering agents and Ca^{++} antagonists. While detailed tonnage data are difficult to obtain, it

Table 3. Sale data of pharmaceuticals in 2001 (based on prescriptions in Italy). From Calamari *et al.* (2003).

Pharmaceuticals (Therapeutic categories)	**Sales in year 2001 (kg per million people)**
amoxycillin (antibacterial)	3680
ceftriaxone (antibacterial)	150
ciprofloxacin (antibacterial)	260
clarithromycin (antibacterial)	590
erythromycin (antibacterial)	72
spiramycin (antibacterial)	91
lincomycin (antibacterial)	130
atenolol (β - blocker)	390
bezafibrate (lipid regulating)	130
Enalapril (antihypertensive)	86
furosemide (diuretic)	18
hydrochlorothiazide (diuretic)	257
omeprazole (ulcer healing)	59
ranitidine (ulcer healing)	467

can be assumed that the tonnage range of high volume pharmaceuticals can reach several hundred tons annually worldwide. Another example of pharmaceutical use can be inferred from the sales figures for diverse compounds in Italy (Table 3).

3. Pathogens in coastal ecosystems

Global incidence of many water born infectious diseases is rising due to increased prevalence of existing pathogens and the emergence of novel pathogenic organisms. These emerging infectious diseases most likely result from a combination of environmental changes such as eutrophication, deforestation and climate change, as well as specific human behavior such as increased globalization and excessive use of antibiotics in agriculture and aquaculture (Rocourt *et al*., 2003; Theron and Cloete, 2002; Harvell *et al*., 2002; Ruiz *et al*., 2000).

In the Mediterranean Sea, global warming is expected to result in a spreading of subtropical species, including pathogens, into the Mediterranean climate zone. Desertification has also been identified as one of the key climatological problems that some Mediterranean countries will face in the coming decades, with accompanying changes in the terrestrial and freshwater biota. Pathogens originating from other seas may be introduced into the Mediterranean Sea with ballast water discharge by ships (Ruiz *et al*., 2000; CIESM, 2002c). Human demographics are changing as well. With rapidly increasing human populations along the Mediterranean coastline, many cities and towns still do not have sufficient sewage treatment. Along with increasing human populations, agricultural practices have been linked to increased incidences of outbreaks of classical waterborne disease as well as the development of new virulent strains of bacteria and viruses (Table 4). We are not aware of epidemiological data published from the Mediterranean region.

In response to these developments, there is a pressing need for 1) better risk assessment and improved monitoring programs for waterborne pathogens, 2) a better understanding of waterborne pathogens as components of aquatic ecosystems to help improve our understanding of their distribution and dynamics and 3) increased use of molecular technologies in monitoring and environmental research of waterborne pathogens.

Table 4. Incidence rate of notified diseases in developed countries (per 100,000 of human population).

Notified disease	Incidence rate /100 000 pop/year	Reference
Viral gastroenteritis	28 000	DeWit 2001
Gastroenteritis (norovirus)	14 000	Lopman 2002
Campylobacteriosis	100	"Foodborne" 1997
Salmonellosis	32	"Foodborne" 1997
Hepatitis A	12	"Foodborne" 1997
Shigellosis	5.6	"Foodborne" 1997
Vibriosis	2.5*	"Risk assessment" 2002
Yersiniosis	2.2	"Foodborne" 1997
Typhoid fever	0.5	"Foodborne" 1997
Listeriosis	0.4	Anon. 2002

* estimation from US data.

3.1 Better risk assessment

Monitoring programs currently practiced by Mediterranean countries test for *Escherichia coli* in seafood, drinking water, and waters used for recreational purposes (EU directive). Other bacterial pathogens such as *Vibrio cholerae, V. parahaemolyticus,* and *Shigella* are only searched for after outbreak incidents. In addition, sampling for viral pathogens is not yet routine (Butt *et al*., 2004; Wyn-Jones and Sellwood, 2001; Bosch, 1998).

The use of *E. coli*, as a reference indicator or culture techniques to detect pathogens, as presently done, fails to reduce risk. For example, shellfish responding to EU criteria (< 230 *E. coli*/100mg) were found to be responsible for significant outbreaks linked to shellfish consumption (Butt *et al*., 2004). Recommendations to improve monitoring efforts are:

- Include more major pathogens in routine sampling (Table 3).
- Couple risk assessment with the development and application of new molecular tools as proposed by Rose and Grimes (2001) that could provide a significant advance in protecting consumers.
- Reinforce the human population surveys for pathogens, especially in response to outbreaks in the populations close to beach or shellfish production areas. This will allow better prioritization of which pathogens to monitor depending on the epidemiological status of the coastal human population.
- Develop and support Mediterranean-wide data bases containing all the information on circulating strains, serotypes, emergence of mutants. Data base coordination and co-ordination of North and South shore countries is currently non-existent, but could be especially critical with emerging pathogens.
- Implement early warning systems to currently assess the water quality in bathing or seafood harvesting areas. This system would combine currently available data potentially relating to water quality degradation (i.e., rainfall events, real time monitoring of sewage treatment plants or sewage network failures, variations in river flow), with data from probes implemented in the environment (shellfish beds or buoys) able to provide real-time data on salinity, chlorophyll, pH. The data could be provided to central communication networks to model and predict the wastewater inflow and its impact on water quality. Combined with spatial information on pathogen outbreaks, this would allow predictions of pathogen concentrations in the water on a site-specific basis. Modeling the impact of possible events could also improve our understanding of the effects of sewage treatment plants and rivers in affecting water quality. This early warning system, if implemented, would be validated by sampling trials, for example to establish a link between decreased salinity and the presence of fecal pollution and pathogens.

It is of interest that a new Mediterranean research project (CHOLCLIM) has been established for the quantification of emerging risks of cholera outbreaks in the Mediterranean basin in relation to climate change using spatial teledetection and epidemiological modeling. Headed by JF Gueguan, IRD, Centre de Montpellier, this project is based on observations of recent outbreaks on Mediterranean coasts in Italy, Greece, Turkey and Spain. The objectives are (1) to analyze the factors that contribute to outbreaks using information from satellites, modeling, and physical and biological oceanography, and (2) to propose different scenarios to assess and manage future outbreak risk. Scientific partners involved in the project are CNRS, IRD, Medias-France, IPLS, LMD, Univ. Princeton, Univ. Cambridge). For more information concerning the project and others on climate change, see htpp://medias.obs-mip.fr/gicc.

3.2 Considering pathogens in an ecological context

Waterborne bacterial and viral pathogens do not just interact with human hosts. They are also integral elements of the aquatic environment. Some human pathogens are naturally occurring organisms in aquatic ecosystems. For example, *Vibrio* spp., several of which are human pathogens, persist in aquatic ecosystems indefinitely as members of the bacterioplankton and are subject to various factors that impact the dynamics of plankton communities (e.g., Cottingham *et al.*, 2003). Other pathogens may exist outside the human host only in a transition stage, requiring input from infected humans to remain persistent in aquatic environments, but the spatial and temporal distribution of these elements will still be influenced by ecological parameters.

A better understanding of the ecology of human pathogens can lead to development of better risk assessment programs and may elucidate ecosystem management or human behavior options that reduce the risk of disease outbreaks. Environmental parameters such as temperature, salinity and nutrients, as well as interactions with other aquatic biota will affect the seasonal and spatial distribution and virulence of these pathogens. This will in turn affect when, where and how often humans will be at risk. For example, factors that affect filtering rates and lipid accumulation in shellfish will affect their uptake and retention of viral particles, which in turn can influence the risk of outbreaks from shellfish consumption (Rose and Sobsey, 1993; Lee and Morgan, 2003).

Of particular interest in the field of emerging and novel pathogens is a greater understanding of environmental conditions that may lead to the development of more virulent pathogen strains.

Understanding the links between aquatic reservoirs of pathogens (sewage treatment plants, lakes and rivers, estuaries, sediments, coastal ocean) is another crucial facet of disease ecology. Pathogens introduced into the Mediterranean originate largely from point sources such as rivers due to insufficient wastewater treatment. Thus, the distribution of pathogens is likely to be patchy and tightly linked to the overall hydrography of the coastal systems receiving the input.

Characteristic Mediterranean subsystems that are especially vulnerable to accumulation of pathogens are lagoonal systems. These typically have long water residence times accompanied by high receiving loads of wastewater and a generally shallow water column. High levels of biotic and abiotic particulates in these shallow systems might cause rapid settlement of pathogens via adsorption or active attachment and subsequent sedimentation. Coastal sediments might therefore act as temporary depositories of pathogens, becoming potentially mobilized again upon sediment resuspension.

3.3 Development and application of molecular technologies

Monitoring for many pathogens is still primarily done by culturing approaches (Moe, 2002; WHO, 2003). A well-documented drawback of this technique is that these pathogens are often not in a culturable state and will therefore escape detection (McDougald *et al*., 1998; Huq *et al*., 2000; Hot *et al*., 2003). Indicator pathogens such as *E. coli* are also often used to assess risk from other pathogens, but there is often poor correlation between indicator organisms and risk from other target pathogens (Toze *et al*., 1999; Hot *et al*., 2003; Butt *et al*., 2004).

Molecular techniques are increasingly applied to improve our ability to directly detect abundance of viral, bacterial and protozoan pathogens in water and wastewater. Often, these methods have the added advantage that they can potentially provide information about the viability, metabolic activity and virulence of pathogens *in situ*. These new methodologies are not yet widely used and most are still in developmental stages (Toze, 1999; Rose and Grimes, 2001; Theron and Cloete, 2002; Brinkman *et al*., 2003; Call *et al*., 2003; Straub and Chandler, 2003; Gruden *et al*., 2004). It is important for researchers and public health workers to be aware of these tools as they become available and to incorporate them into their monitoring and research practices whenever possible.

Fluorescent antibodies and molecular markers unique to a particular pathogen or even a particular strain of that pathogen can be used in conjunction with microscopy or, more efficiently, flow cytometry to directly assess pathogen abundance and viability in water samples (Huq *et al*., 1990, 2000; Theron and Cloete, 2002; Gruden *et al*., 2004). The transcription of specific genes may be indicative of the presence, metabolic activity, virulence or transmission risk of a pathogen. If these genes are unique to the pathogen in question, PCR (polymerase chain reaction), RT-PCR (reverse transcriptase-PCR) or QPCR (quantitative PCR; Brinkman *et al*., 2003) could be used to obtain qualitative or quantitative indications of pathogen abundance or activity from environmental and seafood samples. Microarray chips can simultaneously measure transcription of hundreds or thousands of genes to study whole genome response of a pathogen under environmental or experimental conditions. This approach may soon be adapted for pathogen monitoring by developing gene chips that can measure transcription products from multiple selected indicator genes in single pathogens or multiple pathogens (Rose and Grimes, 2001; Call *et al*., 2003).

Molecular approaches can also be applied to studying pathogen ecology. The genomes of many pathogens have been sequenced, and whole genome RNA transcription can be measured using microarrays. For many pathogens, the functions of myriad genes and genetic regulatory systems have been well studied in a medical context, but their activity and functions in aquatic environments are unknown. This makes microarrays a particularly powerful tool for understanding the detailed molecular response of pathogens to a wide range of environmental parameters and habitats. A more focused approach is also possible, using more specific microarrays or PCR techniques to follow expression of genes or protein products of particular interest. Finally, mutants can be constructed to examine the role of specific pathogen phenotypes in environmental growth and survival (such as attachment to plankton by *V. cholerae*; Chiavelli *et al*., 2001, this volume).

Major limitations to expanding monitoring programs and to implementing comprehensive research programs are the time and effort required to process samples, and the consistency and reliability of methodologies. A significant advance is the current development and testing of flow cytometry and multiplex-PCR methodologies capable of simultaneously and rapidly detecting multiple pathogens (Kong *et al*., 2002; Brinkman *et al*., 2003; Lee *et al*., 2003; Gruden *et al*., 2004). Ideally these methods could be developed into inexpensive, commercially available kits capable of high-throughput processing of either solid (e.g., sediments, shellfish) or liquid samples (Straub and Chandler, 2003).

3.4 Conceptual and practical links between novel contaminant and pathogen studies

Researchers and health workers involved in studying novel contaminants in Mediterranean systems would benefit from collaborating with those studying emerging pathogens and vice versa as there are a number of clear links between the fields. One of the most obvious link is sewage treatment plants, which can serve as the point sources for both chemical and pathogenic "contaminants" into aquatic ecosystems. Consequently, hydrological events could have similar effects on the distribution of both chemical contaminants and pathogens. Another particular concern with sewage treatment plants is that the co-occurrence in high concentrations of mutagenic and antibiotic contaminants and pathogens is very likely to be a potential contributor to emergence of new or resistant pathogen strains. Likewise, excessive use of antibiotics in aquaculture provides another potential direct link between novel contaminants and emerging pathogens and deserves further investigation.

There is a lack of comprehensive baseline monitoring programs and of research linking environmental parameters with abundance and distribution for both novel contaminants and pathogens in the Mediterranean. Given the expense of implementing effective monitoring and research programs, combined sampling efforts for waterborne contaminants and pathogens could be highly beneficial.

Finally, the molecular approaches available for studying pathogenic microorganisms can be applied to better understand sublethal biological impacts of contaminants in both laboratory and in situ studies (Purohit *et al*., 2003). Many aquatic pathogens have far more well-studied genomes than typical indicator organisms used in ecotoxicology. For example, genetic regulatory systems involved in such processes as cell division and growth, nutrient uptake, membrane function, antibiotic resistance, etc. are relatively well understood, making such organisms, which are also natural members of planktonic and sedimentary communities, potentially very useful in studying specific sublethal effects of novel contaminants. The same molecular techniques (PCR, microarrays, etc.) that can be used for specific environmental monitoring and assessing a pathogen's response to environmental parameters can be used to assess the response of these microorganisms to different types, concentrations, and combinations of contaminants.

Of course this approach is not limited to pathogenic microbes. The number of organisms with sequenced genomes is rapidly increasing. In addition to pathogens, genomes are now known or being obtained for a number of traditional ecotoxicology indicator organisms in other taxonomic groups such as the green alga *Chlamydomonas* and the microcrustacean *Daphnia*. Functional genetic information is typically less complete for these organisms than for pathogenic microorganisms, but this situation is changing rapidly.

4. Recommendations for Future Research

<u>Analysis of novel contaminants</u>

Analytical methods, including compound enrichment, separation and determination, should aim at limits of quantification at the level of ng L^{-1}. Enrichment techniques, such as solid phase extraction and semi-permeable membrane devices have to be improved with respect to their selectivity for polar compounds. TOF-MS for accurate identification, MS^n, and ICP-MS have to be optimized for polar analytes. Multistage mass spectrometric techniques and the use of diagnostic ions should be explored for compound-class specific screening to explore their usefulness for precise quantification of many novel contaminant compound classes, particularly in complex matrices such as marine sediments and sludge. Although LC directly coupled to the above mentioned mass spectrometric techniques is the best choice for polar contaminants, the use

GC/MS-MS and high-resolution mass spectrometry are useful techniques for some compounds classes such as brominated flame retardants. In addition, GC/MS-MS is still a powerful tool for the identification of unknown compounds. The spectra data bases for LC-MS/MS (APCI, ESI modes) should be further improved and made available for specialized laboratories. Due to the specific analytical techniques necessary and QA procedures, a joint cooperation of specialized laboratories is recommended.

The coupling of the quantifiable bioeffects of the species to be determined with their molecular structure will allow us to confront the challenging question of the relevance of the environmental analytical data obtained.

Environmental behavior of novel contaminants

Because of the considerable lack of field data in the Mediterranean Sea it is recommended that surveys must be conducted in selected coastal areas of the most important rivers entering the Mediterranean Sea to measure the concentrations of target compounds identified in this volume. Special attention should be paid to estuaries (especially those of the largest rivers), delta regions and lagoons, which are often close to areas of intensive human activity. The systematic monitoring of most relevant novel contaminants in the marine environment should be started as early as possible to establish appropriate baseline data and allow for estimates of temporal changes in coastal contamination. It is further recommended that joint sampling ventures and common analytical protocols be established between cooperating laboratories from different nations. CIESM may play an important federating role in this sector.

Biological Uptake and Effects of novel contaminants

(1) For those chemical contaminants identified as being present at above trace concentrations (ng/L) in water or sediment, biological uptake experiments should be conducted to measure their potential bioaccumulation. Experiments should focus on prominent taxonomic components of coastal waters, including phytoplankton, invertebrates, and possibly fish. Consideration should be given to using mussels as bioindicators of coastal contamination by emerging contaminants (much as they are used with conventional contaminants) to discern spatial and temporal patterns of bioavailable contaminant concentrations. CIESM Workshop Monograph No. 15 (2002a) describes a proposed network using mussels to assess spatial and temporal trends in coastal contamination by radionuclides in the Mediterranean, and inclusion of mussel samples for novel contaminant analyses could be coordinated with this ongoing effort. Biological uptake studies should be conducted to enable the application of kinetic models of contaminant uptake.

(2) Develop a classification /grouping approach for novel contaminants that exhibit the same mode of action and are structurally similar, e.g. pharmaceuticals, based on mechanisms of action.

(3) Given the unlikelihood of acute toxicity of the emerging chemical contaminants at environmentally realistic concentrations, toxicity tests should focus on sublethal or chronic effects. For many of the emerging contaminants, particular attention should be paid to consider endocrine disruption that may reduce the fitness of an individual organism and that could lead to a decline in a population. Toxicological assessments should primarily consider those contaminants which display bioaccumulation. Toxicity experiments involving animals should consider dietary as well as solute exposure. Since coastal waters are often contaminated with many co-occurring chemicals, some rational basis to evaluate the effects of mixtures of contaminants, in addition to the effects of individual compounds, should be attempted.

(4) For those radioisotopes that are released by hospitals that enter coastal waters via sewage treatment plants, emphasis should be placed on isotopes of elements that are known to accumulate in marine organisms. The radioactivity accumulated should be placed into the context of the natural radiation background experienced by those organisms (see Rose and Fisher, this volume).

Pathogens

(1) To improve risk assessments, looking for the pathogens potentially present in the area according to epidemiological information from the country;

(2) To develop and apply molecular techniques to directly detect pathogens;

(3) To implement an early warning system to mitigate against events (rainfall, sewage treatment plant failures, outbreaks, etc.) that may impact water quality and thus provide available tools for risk management.

5. Targeted recommendations

Analytical

- Limit of Quantitation below ng/L levels might be needed for marine systems.
- Improve method selectivity.
- Improve structure identification of environmental metabolites.
- Identify priority compounds for screening.
- Further develop methods for novel compounds and lower concentrations.
- Joint lab collaboration of sampling and method development and spectra databases.

Occurrence, Processes and Behavior

- Identify major sources of contaminants for coastal areas.
- Surveys in selected coastal areas (for target compounds).
- Special attention to estuaries, deltas, lagoons, and coastal outfalls.
- Start exploring if similarly acting compounds with similar structure can be grouped.

Biological Effects

- Assess sublethal, chronic effects.
- Assess biological uptake (invertebrates, phytoplankton, fish).
- Include dietary sources in addition to dissolved exposures.
- Evaluate effects of environmentally realistic concentrations (down to ng/L concentrations).
- Start to assess mixture toxicity.

Risk Assessment

- Establish databases with realistic data on: sources, behaviour, exposure and effects.
- Start exploring if similarly acting compounds with similar structure can be grouped.

Pathogens

- Improve risk assessment.
- Consider pathogenic microorganisms in an ecological context.
- Establish early warning systems.
- Improve data base on inputs, concentrations, effects in coastal Mediterranean.
- Combine sampling efforts for pathogens and pollutants.