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ASSESSMENT OF THE STATE OF POLLUTION OF THE MEDITERRANEAN SEA BY CARCINOGENIC, TERATOGENIC AND MUTAGENIC SUBSTANCES

In cooperation with:

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1. BACKGROUND

Article 8 of the Convention for the Protection of the Mediterranean Sea against Pollution, adopted by the coastal states of the region in Barcelona on 16 February 1976, and in force since 12 February 1978, stipulates that Contracting Parties shall take all appropriate measures to prevent, abate and combat pollution of the Mediterranean Sea area caused by discharges from rivers, coastal establishments or outfalls, or emanating from any other land-based sources within their territories (UNEP, 1982).

In conformity with the provisions of this article and others of a more general nature contained in the Convention, Mediterranean coastal states adopted the Protocol for the Protection of the Mediterranean Sea against Pollution from Land-based Sources in Athens on 17 May 1980. The Protocol entered into force on 17 June 1983.

Article 5 of the Protocol stipulates that Contracting Parties shall undertake to eliminate pollution of the Protocol Area from land-based sources by substances listed in Annex I to the Protocol and, to this end, shall elaborate and implement, jointly or individually as appropriate, the necessary programmes and measures. The same article also stipulates that these programmes and measures shall include, in particular, common emission standards and standards for use, and that the standards and time-tables for the implementation of the programmes and measures aimed at eliminating pollution from land-based sources shall be fixed by the Parties and periodically, if necessary, every two years, for each of the substances listed in Annex I, in accordance with the provisions of Article 15 of the Protocol.

Annex I to the Protocol includes, as one of its items, substances having proven carcinogenic, mutagenic or teratogenic properties in or through the marine environment.

Article 7 of the Protocol stipulates that Contracting Parties shall progressively formulate and adopt, in cooperation with the competent international organizations, common guidelines and, as appropriate, standards or criteria dealing in particular with, *inter alia*, the quality of seawater used for specific purposes that is necessary for the protection of human health, living resources and ecosystems.

As of 31 March 1991, the 1976 Barcelona Convention has been ratified, acceded to, or approved by all eighteen Mediterranean states and by the European Community, and the 1980 Athens Protocol by sixteen Mediterranean coastal states and by the European Economic Community.

At their Fourth Ordinary Meeting held in Genoa from 9 to 13 September 1985, Contracting Parties to the Convention and its related Protocols agreed that, with regard to the technical implementation of the Protocol for the Protection of the Mediterranean Sea against Pollution from Land-based Sources, the Secretariat would propose an order of priority and a realistic time-table for the development of programmes and measures for at least two substances (or groups of substances) annually, including proposed common emission standards and standards for use, required for the implementation of the Protocol and that, in preparing such a proposal, substances listed in Annex I to the Protocol should be accorded priority (UNEP, 1985a). In terms of this decision, a meeting of technical experts on the technical implementation of the Protocol was convened by UNEP in Athens from 9 to 13 December 1985. The meeting agreed on a workplan and time-table which included the phased preparation of assessments of the state of pollution of the Mediterranean Sea by substances listed in Annex I and II to the Protocol, together with proposed control measures

on the basis of such assessments (UNEP, 1985b). It was agreed that such assessment documents should include, *inter alia*, chapters on:

- sources, points of entry and amounts from industrial, municipal and other discharges into the Mediterranean Sea;
- levels of pollution;
- effects of pollution;
- current legal, administrative and technical measures at national and international level.

The workplan and time-table for implementation of the Protocol was approved by Contracting Parties at their Fifth Ordinary Meeting in Athens from 7 to 11 September 1987 (UNEP, 1987).

As part of the preparations for assessment of the state of pollution of the Mediterranean Sea by carcinogenic, mutagenic and teratogenic substances, a Consultation on carcinogenic and mutagenic marine pollutants in the Mediterranean was jointly convened by WHO and UNEP in Athens from 23 to 25 June 1988 (WHO/UNEP, 1988). The meeting agreed on the outline content of the document, and also consolidated preparations for a pilot monitoring study on priority substances. This study was carried out between 1989 and 1991 in selected coastal areas of Italy, Spain and Yugoslavia, by the Institute of Hygiene and Preventive Medicine of the University of Genoa, the Environmental Chemistry Laboratory of the National Institute of Cancer Research, Genoa, the Department of Environmental Biology of the University of Siena, the Department of Environmental Chemistry, Centre for Research and Development, CSIC, Barcelona, and the Department of Nuclear Chemistry, Josef Stefan Institute, Ljubljana.

The present document, overall technical responsibility for which was entrusted to the World Health Organization, has been mainly prepared by Professor S. De Flora, Institute of Hygiene and Preventive Medicine, University of Genoa, Italy, and Professor P. Grasso, Robens Institute, University of Surrey, United Kingdom. It attempts to provide an assessment of the state of pollution of the Mediterranean Sea by selected carcinogenic, mutagenic and teratogenic substances, together with the effects of Mediterranean environmental factors on the fate of such substances, on the basis of information available to date, and to outline the main risks to marine organisms and to man. Some of the substances have already been the subject of previous assessments on overall toxicological grounds. In this document, their treatment has been limited to actual or potential carcinogenic, mutagenic and/or teratogenic effects.

The document also proposes action which could be taken by Mediterranean States within the framework of the Protocol for the Protection of the Mediterranean Sea against Pollution from Land-based Sources, to alleviate the situation.

2. INTRODUCTION

Assessing the state of pollution of the Mediterranean Sea by carcinogenic, mutagenic, and teratogenic agents represents an exceedingly complex exercise. Even more

difficult is the task of delineating the possible adverse consequences of such pollution in exposed marine biota and, indirectly, in the human organism.

The first scientific problem is to categorize marine pollutants as carcinogens, mutagens, and/or teratogens. Provided this first step may be overcome, identification by chemical analysis of a compound allocated into one or more of these categories will not be sufficient to predict the toxicological hazards of seawater, sediments, or biota. In fact, pollutants of marine ecosystems are just components of complex mixtures, and are known to undergo a variety of interactions and biotransformations both in the marine environment and in host organisms. These mechanisms are expected to affect their toxicological properties, either in the sense of activation or, more often, of detoxification.

In addition to these and other problems, which will be discussed in the next sections of this document, an objective approach to the issue of the marine environment as a possible source of carcinogenic, mutagenic, and teratogenic hazards to marine organisms and to humans should take into account the existence of counterbalancing agents in the same *milieu*. This is particularly important in the marine environment, because of the very low concentrations of these types of pollutants. In general, all arguments which are usually forwarded in order to justify the importance of risk factors of environmental source in the pathogenesis of certain diseases, such as cancer, may hold equally well in support of the role of protective factors in the environment. Indeed, the occurrence of chronic-degenerative diseases is the result of the interplay between risk factors and anti-risk factors, and of the impact of these antithetic forces on the homeostasis of the host organisms. For instance, skeletal anomalies in fish can occur not only following exposure to toxic chemicals but also as a deficiency of protective agents, e.g. ascorbic acid, during spinal development (Hodson, 1987).

Organisms possess a formidable defensive machinery against toxic agents, including mutagens, carcinogens and teratogens and interestingly, most, if not all, defense physiological processes can be modulated exogenously. Tens of different mechanisms have been discovered by which antimutagens and anticarcinogens can inhibit the development of these multifactorial and multistep pathological conditions (De Flora and Ramel, 1988). In humans, these mechanisms can be exploited for chemo-preventive measures, providing an additional strategy of primary prevention, which complements the goal of reducing exposure to risk factors.

Under natural conditions, xenobiotics can modulate the host response to aggression by toxic agents. This is also true for organisms living in the marine environment, which have been even proposed as models for assessing the efficacy of antimutagens and anticarcinogens. For instance, fish have been rather extensively used not only in experimental carcinogenicity studies (see section 4.1.2.1) but even in large-scale anticarcinogenicity assays, e.g., in *Salmo gairdneri*, where dietary indole-3-carbinol, butylated hydroxyanisole and b-naphthoflavone were found to affect metabolism, DNA adduct formation and hepatocarcinogenicity by aflatoxin B1 (Nixon *et al.*, 1984; Goeger *et al.*, 1986 and 1988; Dashwood *et al.*, 1988).

A variety of potential inhibitors of mutagenesis and carcinogenesis have been isolated from the marine environment. For instance, more than 20 thiols were found to occur in sediment pore-water samples from Biscaney Bay (Florida) as the result of geochemical formation due to reaction between hydrogen sulphide and sedimentary organic matter. 3-Mercaptopropionic acid was detected as a major thiol, but methane thiol and glutathione were also present in significant concentrations (Vairavamurthy and Mopper, 1987). These

organosulphur compounds are among the most promising protective agents, displaying a large variety of antimutagenic and anticarcinogenic mechanisms (De Flora *et al.*, 1991a). Prenylated hydroquinone derivatives isolated from the marine urochordate *Aplidium californicum* showed antioxidant properties, and protected against cancer and mutation in experimental systems (Howard *et al.*, 1979). Bryostatin 1, a macrocyclic lactone isolated from the marine bryozoan *Bugula neritina*, activated protein kinase C, the major receptor for tumour promoting phorbol esters, and inhibited tumour promotion by these agents in mouse skin (Hennings *et al.*, 1987). An antitumour glycoprotein, aplysianin E, inducing tumour lysis, was purified from the sea hare (*Aplysia kurodai*) eggs (Kisugi *et al.*, 1987). Eggs of marine invertebrates, such as sea urchins, contain about 2 mM glutathione and 5 mM 4-mercaptohistidine, along with its amino- and N1 ring nitrogen-methylated derivatives, referred to as ovothiols (Hartman and Shankel, 1990). These compounds act as non-enzymatic glutathione peroxidases, serving as antioxidants (Shapiro and Turner, 1988). Of particular interest is the finding that certain aquatic invertebrates appear to be simultaneously resistant to multiple toxic xenobiotics, and are capable of surviving and reproducing in heavily polluted waters. Such a phenomenon has been linked to the production of the membrane glycoprotein P-170, coded by gene mdr-1. As also shown in the freshwater mussel *Anodonta cygnea* and in the marine invertebrate *Phylla*, multi-drug resistance is associated with a decreased expression of Phase I metabolizing enzymes (cytochrome P450-dependent monooxygenases) and increased expression of Phase II conjugating enzymes, such as glutathione S-transferase and glutathione peroxidase (Kurelec and Pivcevic, 1989). It is also well known that fish contains large amounts of protective vitamins and long-chain n-3 polyunsaturated fatty acids, such as eicosapentenoic, docosapentenoic, and docosahexaenoic acids. In rodent studies, feeding fish oil resulted in inhibition of both transplanted and carcinogen-induced mammary tumours, which can be possibly ascribed to displacement of linoleic acid and arachidonic acid in membrane lipids and to inhibition of arachidonic acid metabolism (Karmali, 1989).

Unfortunately, protective factors can quite often behave like double-edge swords and, depending on peculiar conditions, such as route of administration, doses, time and sequence of intake, etc., they can trigger adverse effects. This depends on the multiple properties of several potential inhibitors and on the extreme complexity of the mechanisms regulating the host response to xenobiotics. Thus, essential nutrients having a well known protective role can become a cause of disease and, vice versa, typical pollutants can act as inhibitors of disease development. An example of the former situation is provided by selenium which, mainly as a constituent of the enzyme glutathione peroxidase, is a known antioxidant and anticarcinogen. In fact, a low selenium intake is associated with an increased incidence of certain forms of cancer (Diplock, 1984). On the other hand, an excess of this nutrient is toxic to most organisms, including marine biota (GESAMP 28, 1986). An example of typically hazardous marine pollutants which may protect from cancer is provided by polychlorinated biphenyls (PCBs), which are potent inducers of mixed function oxidases not only in mammals but also in fish. Since this enzyme system shares activation and detoxification properties, in some cases its stimulation can inhibit mutagenesis and carcinogenesis. For instance, the mutagenicity of aflatoxin B1 in the Ames reversion test, in the presence of liver post-mitochondrial fractions from *Salmo gairdneri*, was significantly decreased when fish were pretreated with the PCBs Aroclor 1242, Aroclor 1254, or Aroclor 1260 (Stott and Sinnhuber, 1978). These results are in agreement with several anticarcinogenicity studies in the same fish species, showing the ability of Aroclor 1254 to inhibit liver carcinogenesis and formation of DNA adducts by aflatoxin B1 (Shelton *et al.*, 1986). Another example is arsenic, for which seafood represents the dominant source of intake in humans. Arsenic may potentially cause adverse effects in humans following long-term exposure (GESAMP 28, 1988), but experimental studies in mice have also pointed

out its ability to significantly suppress spontaneous lung tumours (Kanisawa and Schroeder, 1967).

All these considerations are important in estimating the risk from carcinogenic, mutagenic and teratogenic substances in the marine environment.

3. ASSESSMENT OF POLLUTION

3.1 Carcinogenic, mutagenic and teratogenic substances relevant to marine pollution

3.1.1 Naturally occurring substances

Although it is apparent that man-made chemicals are those causing major concern in environmental (including marine) pollution, it should be preliminarily noted that natural agents are not exempt from carcinogenic, mutagenic and teratogenic hazards. At least according to some views, "natural pesticides" are much more important than "man-made pesticides" as carcinogenic risk factors (Ames *et al.*, 1987). Similar considerations have been specifically addressed to the marine environment (Payne and Rahimtula, 1989). For instance, marine plants produce mutagenic haloforms such as bromoform and dibromochloromethane, as well as the carcinogen chloroform. Polyhalogenated compounds isolated from a marine alga were found to be mutagenic, one of them being 200 times more potent than a typical synthetic mutagen and carcinogen, i.e. EMS (ethyl methanesulfonate), (Leary *et al.*, 1979). It has be considered that the global production of the mutagen and carcinogen methyl iodide may exceed the amount resulting from industrial pollution by a factor of 80 (Payne and Rahimtula, 1989). It has been also suggested that marine algae may be a significant source of methyl chloride, which is the principal halocarbon in the atmosphere (Zafiriou, 1975). A variety of possibly hazardous substances have been detected in species of the seaweed *Asparagopsis* collected from the Gulf of California, from the Caribbean, from Hawaii, and *A. armata* from the Spanish Mediterranean coast (Mower, 1983).

Of great scientific interest is the recent finding that, using the 32_P postlabeling technique (see section 4.1.3.2), natural populations of the freshwater fish species *Leuciscus cephalus*, *Barbus barbus*, *Abramis brama*, *Vimba vimba carinata* and *Cyprinus carpio*, and of the marine fish *Mugil auratus*, caught from the Adriatic Sea, revealed the presence of 4 to 9 qualitatively similar adducts to liver DNA. The presence of these carcinogen-DNA adducts was detected irrespective of whether fish was caught from unpolluted or polluted waters, which suggests that a vast majority of DNA modification in fish are caused by natural factors rather than man-made chemicals (Kurelec *et al.*, 1989). Another study, however, reported that the liver DNA of brown bullheads caught from polluted rivers contained several DNA adducts which were undetectable in the liver DNA of aquarium-raised fish (Dunn *et al.*, 1987).

3.1.2 Substances of anthropogenic origin

It is virtually impossible to compile an exhaustive list of carcinogens, mutagens and teratogens relevant to marine pollution. In general, the assessment of these properties is extremely difficult and often controversial from a scientific point of view (see section 4). Chemical families generally include a broad variety of structurally related compounds, whose differential toxicological impact is hardly predictable. To give an example, GESAMP identified as many as 800 chlorinated hydrocarbons having relevance to the marine environment, of

which 58 allocated in the group of low molecular weight (C1 to C3), 249 in the medium molecular weight group (C4 to C6), and 413 in the high molecular weight group (greater than C6). Even disregarding carcinogenicity, mutagenicity, and teratogenicity, there is a gradient of harmful properties of these compounds, without any clear interface or separation between the more harmful and the less harmful (GESAMP, 1990). The analytical identification of potentially hazardous substances in the marine environment is not sufficient to claim that real hazards may result from the same environment. In addition, some of the suspected pollutants, such as metals, are also essential nutrients. Speciation of chemicals, e.g., valence of metals or complexation with organic ligands, should be also taken into account. Finally, it should be also stressed that several recognized carcinogens are hazardous only when inhaled and not following ingestion.

Tentative lists of carcinogens in the marine environment have been prepared by using the data-base available in the IARC Monographs on the Evaluation of the Carcinogenic Risks to Humans. Depending on the degree of evidence resulting from epidemiologic surveys in humans and from carcinogenicity assays in animals, and with the support of the indications of short-term tests, suspected agents are categorized by IARC as carcinogenic (Group 1), probably carcinogenic (Group 2A), or possibly carcinogenic (Group 2B) to humans. Compounds for which there is inadequate evidence are classified in Group 3. It should be stressed that such categorization only reflects the strength of evidence of carcinogenicity to humans, and should not be taken as an indication of carcinogenic potency. For instance, it is meaningful that none of the polycyclic aromatic hydrocarbons, which are often potent mutagens *in vitro* and potent carcinogens in animals, can be allocated to Group 1 due to the lack of epidemiologic data for individual compounds.

Based on Supplement 7 of Volumes 1 to 42 of the IARC Monographs (1987), L & IS (1988) reported the following list of 29 suspected carcinogens present in the marine environment:

1. **Metals:** arsenic and nickel (Group 1); cadmium (Group 2A); lead (Group 2B).

2. **Polycyclic aromatic hydrocarbons:** benz(*a*)anthracene and benzo(*a*) pyrene (Group 2A); benzo(*b*)fluorantrene, benzo(*k*)fluorantrene and indeno(1,2,3-*cd*) pyrene (Group 2B); anthracene, benzo(*ghi*)perylene, benzo(*e*)pyrene, chrisene, phenanthrene, pyrene and triphenylene (Group 3).

3. **Chlorinated organics:** polychlorinated biphenyls (Group 2A); DDT, 1,2-dichloroethane, lindane; tetrachloromethane, trichloromethane, toxaphene (Group 2B); aldrin, chlordane, dieldrin, heptachlor, trichloroethylene and vinylidene chloride (Group 3).

Using the same IARC data, Wilbourn and Kauppinen (1989) evaluated that the following Group 1 chemicals or complex mixtures could potentially occur as pollutants in seawater or in marine organisms: coal-tar pitches, coal tar, and mineral oils (local pollution), and soots. For asbestos, benzidine, hexavalent chromium compounds, and nickel compounds, the situation was considered to be uncertain. The most important potential marine pollutants belonging to Group 2A were reported to be polychlorinated biphenyls (PCB), cadmium and cadmium compounds, the polycyclic aromatic hydro-carbons benz(*a*)anthracene, benzo(*a*)pyrene and dibenz(*a,h*)anthracene, and the chreosotes. The group 2A nitrosamines *N*-nitrosodimethylamine and *N*-nitrosodiethylamine have been also found in some fish samples. Among Group 2B compounds, the chlorinated hydrocarbons chloroform, carbon tetrachloride, 1,2-dichloroethane, dichloromethane, and tetrachloro-

ethylene have been detected in various water samples, but only chloroform and tetrachloroethylene have been found in marine organisms. Acrylamide could occur in water when polyacrylamides are used in drilling operations. Pesticides such as lindane, DDT, mirex, and toxaphene can accumulate in fish. The chlorophenols, particularly pentachlorophenol, can be found in biota and could occur as marine pollutants. 2,3,7,8-Tetrachlorodibenzo-para-dioxin (TCDD) could occur in organisms in areas of local pollution. Many polycyclic aromatic hydrocarbons, several of which have been detected in polluted waters, are classified in Group 2B, which also includes complex mixtures such as diesel fuel (marine), fuel oil (residual), and gasoline (Wilbourn and Kauppinen, 1989).

A primary list of chemicals, groups of chemicals, and industrial process products which are considered to be carcinogenic for humans has been prepared by GESAMP Working Group 13. This list has been expanded by also including animal carcinogens and possible or suspected human carcinogens. Many of these agents, such as arsenic, chlordane, creosote, DDT, dieldrin, hepatochlor, lindane and polychlorinated biphenyls, have been found in marine sediments, waters, and biota (Malins and Jensen, 1988), although this does not automatically imply that they also pose a carcinogenic risk to marine organisms, or to man through the marine route.

Paradoxically, even substances used to abate marine pollution may possess genotoxic properties. This may be the case for oil dispersants, consisting of various mixtures of surfactants, hydrocarbon solvents, and stabilizing agents, which are used as antidotes in case of oil spills. Moreover, although new generation dispersants have a lower toxicity, these products can exert toxic effects to marine biota. For instance, an oil dispersant caused spinal deformities in hatched larvae of sea bass (*Dicentarchus labrax*) (Tudor and Katavic, 1987). Several samples of oil dispersants, either *per se* or mixed with crude oil, were non-mutagenic in the Ames reversion test (Petrilli *et al.*, 1980). Further studies confirmed the inactivity of oil dispersants in the same test as well as in the SOS chromotest in *E. coli*, and in a genotoxicity test using the yeast *Saccharomyces cerevisiae* (strain D5), evaluating mitotic crossing-over and other genetic effects. However, they produced DNA damage in *E. coli*, as evaluated by assessing the differential toxicity in repair-proficient and -deficient strains (De Flora *et al.*, 1985).

Another important example is provided by nitrilotriacetic acid (NTA), which has been proposed and used, often in controlled amounts, as a substitute for polyphosphates in household laundry detergents. As such, it is a valuable tool for preventing seawater eutrophication, but is also a massive contaminant of marine bodies receiving domestic sewage effluents. NTA is an established animal carcinogen (IARC, Vol. 48, 1990). Although NTA is generally considered to be a non-genotoxic carcinogen, it yielded positive responses in some short-term tests, such as induction of micronuclei in *Vicia faba* and *Allium cepa* roots (De Marco *et al.*, 1986), a DNA-repair test in *E. coli* (Venier *et al.*, 1987), and induction of aneuploidy in Drosophila (Costa *et al.*, 1988). With this and other important exceptions, such as halogenated hydrocarbons and certain metals, most carcinogenic pollutants are also genotoxic. On the other hand, there are also several examples of mutagenic substances which have been negative or equivocal in animal carcinogenicity assays, often because they tend to be detoxified in the host organism (De Flora *et al.*, 1989b). The issue of detection of mutagens in seawater, sediments, and marine biota will be discussed in section 4.1.3.1.

According to some views, the processes of mutagenesis, carcinogenesis and teratogenesis may have some common denominators. However, the contribution of genetic damage to teratogenesis is also still under debate within some series of structurally-related pollutants, such as organochlorine and organophosphorus pesticides (Lansdown, 1990). By

late 1985, the Registry of Toxic Effects of Chemical Substances (RTECS) included as many as 4,508 names of chemicals associated with reproductive effects. In early 1987, this list had grown to 6,917 entries (Kolb Meyers, 1988). Although the registry also includes typical marine pollutants, a specific list of teratogens in the marine environment is not available. In a survey of the literature on the possible association between malformations and 23 exposures, PCBs and methylmercury were two of the 3 chemicals exhibiting clear evidence for teratogenicity in experimental animals, as well as for a high risk in humans, as inferred from epidemiologic studies (Hemminki and Vineis, 1985). Organophosphorus pesticides were found to possess teratogenic properties, also in aquatic organisms (see section 4.1.4). Several organochlorine pesticides are known to be embryotoxic and carcinogenic in rodents, but it is noteworthy that DDT had opposite effects, by prolonging reproductive life and protecting against the teratogenic effects of other chemicals (Lansdown, 1990). Again, as in the case of carcinogens and mutagens, it should be taken into account that the levels of teratogenic substances in the marine environment would be expected to be very low.

3.2 Effects of environmental factors on transformation and degradation processes

3.2.1 Fate of carcinogens, mutagens and teratogens in the marine environment

Once introduced into the marine environment, the fate of carcinogens, mutagens and teratogens depends on a variety of factors, which may lead to their activation or, more frequently, to detoxification. First of all, stability depends on the chemical nature of the molecules of pollutants. In general, direct-acting genotoxins are reactive molecules and hence tend to degrade readily via chemical, biochemical or photochemical decomposition. Much more stable are procarcinogens, promutagens, and proteratogens, which are *per se* inert molecules, requiring conversion into proximal and ultimate metabolites in order to acquire electrophilic properties. Such a conversion usually occurs intracellularly in host organisms, including marine organisms, which possess the inducible metabolic machinery needed for the biotransformation of xenobiotics (see section 4.1.1). However, activation to electrophilic derivatives can also be photodynamically achieved (see section 3.2.4). Non-genotoxic carcinogens, such as the organochlorine pesticides, biodegrade with great difficulty and persist for a long time (up to 15 years) in the environment (Grasso, 1989). Usually, organic and inorganic compounds of metals are also stable. However, their stable forms are often non-toxic, as it is the case with chromium, which is widespread in the environment in its non-toxic, trivalent form.

Hazardous xenobiotics can interact in the marine environment with microorganisms (section 3.2.2), other chemicals (3.2.3), or solar irradiation (3.2.4), to produce either detoxified or activated derivatives, often characterized by an enhanced bioavailability compared to the parent compounds. These processes can be also associated. For instance, it has been suggested that photooxidation of PAHs may enhance their susceptibility to microbial mineralization (McElroy *et al.*, 1989). Another important mechanism is represented by absorption/desorption phenomena between xenobiotics and sediments or suspended particulate matter having a central role as a transport vehicle for toxic pollutants (Landner, 1976). In particular, various mechanisms can account for the association of pollutants with particles in seawater, i. e.:

(i) precipitation or hydrophobic interactions with the particle surface;

(ii) co-precipitation with hydrous oxides of iron and manganese either as coatings, or as flocs of the precipitate;

(iii) incorporation into mineral lattices, organisms or faecal material; or

(iv) flocculation of colloidal organic and inorganic matter during river and sewage mixing (Olsen *et al.*, 1982).

3.2.2 Microbiological transformations

Several pollutants are accumulated by the bacterial flora populating seawater and marine sediments, and they tend to be transformed intracellularly by various metabolic pathways (Cerniglia and Heitkamp, 1989). Usually, such a transformation leads to detoxified products. Biodegradation is so efficient that bacteria are often exploited in the treatment of both domestic and industrial effluents, as well as in the abatement of specifically metabolized pollutants.

However, in some cases, the products released into the aquatic environment following lysis of bacterial cells may be toxic to higher organisms. The most typical and extensively investigated situation of this type is that of inorganic mercury, which is methylated by a variety of marine bacterial and fungal species to yield methyl mercury. As well known, bioaccumulated organic mercury is more toxic than inorganic mercury to higher organisms, including humans, mainly by affecting their central nervous system, following exposure after birth, especially in early childhood, or via the placenta (GESAMP 28, 1986).

3.2.3 Chemical interactions

Pollutants of the marine environment undergo a large variety of interactions with other pollutants as well as with normal chemical components of the contaminated ecosystem. Combinations of different substances may result either in the sum of their toxic properties (additive effect), or in an enhanced toxicity (synergism), in some instances with multiplicative effects, or in a decreased toxicity (antagonism). The outcome of chemical interactions, often with an extremely high number of combination key is, in the majority of cases, hardly predictable.

Several interactions occurring in the seawater and marine biota bear relevance as far as carcinogenic, mutagenic and teratogenic problems are concerned. For instance, interaction between normal seawater constituents and the chlorine discharged by the cooling system of power plants leads to the formation of a variety of toxic products. The total demand of the Mediterranean Seawater for chlorine is low, in the range of mg/l (Rav-Acha *et al.*, 1989). The chemistry of seawater chlorination is even more intricate than the chemistry of freshwater or effluent chlorination, mainly due to the high bromide concentration in seawater. The reaction of chlorine with bromine produces various brominated species, such as Br_2, HBrO, BrO- and BrO_3, and interhalogenated compounds are also produced. Instead of chloroamines, bromoamines are formed, with the predominance of dibromoamine. The derivatives resulting from seawater chlorination have various toxic properties, and are known or suspected to behave as mutagens and/or carcinogens (Davies and Middaugh, 1978; Rav-Acha *et al.*, 1989).

The simultaneous occurrence of various halogenated compounds in seawater, such as planar polychlorinated biphenyls, brominated/chlorinated dioxins, dibenzofurans, polychlorodibenzodioxins and polychlorodibenzofurans, may be expected to result in additive

effects, but the problem warrants more exhaustive studies, also assessing the possibility of interactions with other contaminants (Nordisk Expertgrupp, 1988). Strong synthetic chelators such as EDTA and DTPA appear to decrease the toxicity of heavy metals to fish. This effect has been ascribed to the translocation of metals from gills to other parts of the body where they cause less damage, as well to an increased excretion of metals (Landner, 1976). The interaction of oil dispersants with oil may enhance the toxic effect of the oil by releasing its degradation products (Marine Biological Association of the United Kingdom, 1970) which, on the other hand, are more readily diluted in seawater. High doses of NTA can release toxicologically active metal ions from insoluble compounds (e.g., lead chromate), thereby causing a variety of genetic effects, including induction of micronuclei in gill cells of *Mytilus galloprovincialis* (Gola *et al.*, 1986).

In contrast with the experimental studies carried out with individual polycyclic aromatic hydrocarbons (PAHs), for which an enormous literature is available, these compounds always occur as complex mixtures in polluted environments. As many as 500 distinct PAHs have been identified at a single site, together with many other organic and inorganic compounds, in sediments near urban areas (Malins *et al.*, 1984). The carcinogenicity of individual PAHs and of complex mixtures containing PAHs has been evaluated in a series of IARC Monographs (Volumes 32-35, 1983-1985), together with indications on their toxic, mutagenic and teratogenic properties. It is a matter for debate whether combinations of different PAHs may lead to additive, synergistic, or antagonistic effects, depending on a variety of factors, such as amount and chemical features of compounds, and availability of metabolizing enzymes and/or of substrates in the host organism. It is noteworthy that the mutagenicity of benzo(*a*)pyrene in the *Salmonella* reversion test was not affected by seawater nor by oil dispersants, whereas it was inhibited by adding crude oil and its extracts, irrespective of the presence of oil dispersants (Petrilli *et al.*, 1980). Suppression of benzo(*a*)pyrene mutagenicity was also observed in the presence of other complex mixtures, such as tar sand fractions (Shahin and Fournier, 1978), shale oil fractions (Pelroy and Petersen, 1979), and mineral oils (Hermann *et al.*, 1980), and ascribed to inhibition of the metabolic activation of this promutagen (Haugen and Peak, 1983). The same mechanism is likely to explain inhibition of benzo(*a*)pyrene mutagenicity by a metal compound, i.e., the hexavalent chromium salt sodium dichromate (Petrilli and De Flora, 1982).

An issue of particular interest is the possible reaction between nitrite and aminocompounds to form *N*-nitrosoderivatives. Out of more than 300 components of this chemical family tested for carcinogenicity, as many as 90% produced tumours in 40 animal species (Bartsch *et al.*, 1985). Formation of *N*-nitrosocompounds typically occurs in the acidic environment of the stomach, and can be prevented in the presence of ascorbic acid or various other inhibitors (Bartsch *et al.*, 1988). Fish represent one of the most typical sources of nitrosatable precursors, especially due to the presence of significant amounts of dimethylamine and of trimethylamine. The latter compound results from the bacterial metabolization of trimethylamine oxide, an end-product of nitrogen metabolism in fish, after death (Jebsen and Riaz, 1977). The human consumption of fish was shown to produce a significant increase in the urinary excretion of methylamines (Zeisel and DaCosta, 1986), and mixture of nitrite with fish homogenates in simulated acidic environment resulted in the ascorbate-inhibitable formation of mutagenic and carcinogenic derivatives (Marquardt *et al.*, 1977; Weisburger *et al.*, 1980; Stich *et al.*, 1982). It may be argued whether a significant formation of N-nitrosocompounds may also occur in seawater and marine organisms, taking into account that both nitrite and secondary amines occur in natural waters in very low concentrations. Formation of dimethylnitrosamines was demonstrated in sewage or lake water simultaneously receiving high concentrations of nitrite and dimethylamine or

trimethylamine (Ayanaba and Alexander, 1974). Moreover, it was shown that the *in vivo* exposure of *Salmo gairdneri* to lake water enriched with sublethal amounts of sodium nitrite resulted in the formation of mutagenic and DNA-damaging derivatives in fish muscle (De Flora and Arillo, 1983). Whether these findings may also apply and bear relevance to the marine environment under realistic conditions remains to be established.

3.2.4 Light-mediated transformations

The UV component of solar irradiation is likely to represent the most widespread mutagen existing in nature. In fact, its impact on human cancer is well established, although rather undefined in some qualitative and quantitative aspects. Due to the low penetration capacity of DNA-damaging wavelengths in an aqueous medium, it is unlikely that sunlight may have important direct consequences on marine biota, although potential effects cannot be ruled out.

A more realistic possibility is that components of sunlight may interact with pollutants spread on the seawater surface or in the upper part of the water column, thereby producing changes in their molecular structure and biological activity (Payne and Phillips, 1985). In some cases, photodegradation of certain compounds may occur, as has been shown, for instance, with polycyclic aromatic hydrocarbons (Fox and Olive, 1979; Valerio and Lazzarotto, 1985; Holloway *et al.*, 1987). Another example is provided by oil dispersants, whose ability to induce a non-reparable DNA damage in *E. coli* was decreased following exposure to sunlight (De Flora *et al.*, 1985), which correlates with the finding that the toxicity of oil dispersants to *Artemia* was reduced in the presence of sunlight (Moraitou-Apostolopoulou and Verriopoulos, 1987).

Even more interesting is the possibility of conversion of inactive molecules into genotoxic derivatives due to photodynamic effects. Far-UV radiation converted dieldrin and p,p'-DDE, but not p,p'-DDT, which are generally classified as non-genotoxic carcinogens, into weak direct-acting mutagens in the *Salmonella* reversion test (De Flora *et al.*, 1989a). This kind of radiation does not normally reach the earth's surface, but is sometimes used in small water-treatment devices. Solar irradiation is capable of activating typical promutagens/ procarcinogens into direct-acting derivatives, as has been shown with several polycyclic aromatic hydrocarbons, aromatic amines and aflatoxins (reviewed by De Flora *et al.*, 1989a). Complex mixtures, such as coal- and shale-derived synthetic fuels, were also activated by light (Selby *et al.*, 1987). Photoactivation, which could be mainly ascribed to near-UV wavelengths, did not occur in nitrogen atmosphere, and was magnified in pure oxygen atmosphere. The interaction between radiation and oxygen is known to lead to the formation of singlet oxygen, which may oxidize promutagens to reactive intermediates. Photoactivation of mutagens is related to the length and intensity of sunlight exposure, in that the phenomenon is followed by degradation of direct-acting mutagens after a prolonged exposure to light. Nevertheless, once formed and transferred into the dark, photoactivated mutagens are extremely stable, as shown for instance with 2-amino-3,4-dimethylimidazo [4,5-*f*]quinoline (MeIQ), whose photoderivative maintained the direct mutagenicity unchanged even after 2 years and 3 months of storage at room temperature (De Flora *et al.*, 1989a).

Photoactivation is related to structural features of irradiated compounds. For instance, the analysis of a series of structurally related aromatic amines, i.e., 2-aminofluorene, 2-acetylaminofluorene, 4-acetylaminofluorene, 1-aminoanthracene, 2-aminoanthracene, 1-naphtylamine and 2-naphtylamine, showed that activation by sunlight requires the amino group in position 2 of the fluorene molecule. Likewise, the analysis of two pairs of heterocyclic arylamines, i.e., 3-amino-1,4-dimethyl-5H-pyrido[4,3-*b*]indole (Trp-P-1), 3-amino-

1-methyl-5H-pyrido[4,3-*b*]indole (Trp-P-2), 2-amino-3-methylimidazo[4,5-*f*]quinoline (IQ), and MeIQ, showed that Trp-P-1 and Trp-P-2 are not photoactivated, whereas IQ and MeIQ are extraordinarily sensitive to sunlight (De Flora *et al.*, 1989a). Both in the case of aromatic amines (Strniste *et al.*, 1986) and heterocyclic amines (Hirose *et al.*, 1990), acquisition of direct mutagenicity depends on conversion into the corresponding nitroderivatives.

The phenomenon of photoactivation is likely to have obvious consequences on the environmental spread of mutagens and carcinogens that, at variance with their unirradiated precursors, are expected to have a more direct impact on exposed tissues, without any need for further metabolic activation in the host organism. However, applicability of the reported laboratory findings to field conditions and in particular to the marine environment warrants further studies. It is noteworthy that binding of benzo(*a*)pyrene to DNA and other macromolecules in the sponge *Tethya lyncurium*, collected from the Northern Adriatic Sea, and from Californian coastal waters in the Pacific Ocean, only occurred in the presence of light (Zahn *et al.*, 1981, 1982 and 1983). Since sponges have no detectable mixed-function oxygenase activity, the hypothesis has been raised that stable benzo(*a*)pyrene photoderivatives may be transported to bottom layers and react with sponge macromolecules in the dark (Zahn *et al.*, 1982).

3.2.5 Bioaccumulation and biomagnification processes

Trace chemicals, including hazardous substances, can be present in higher aquatic organisms as a result of food chain biomagnification. Methylmercury is the prototype compound for this kind of bioaccumulation process, which is also typical for long-living radionuclides. Specific information on carcinogenic, mutagenic and teratogenic substances is relatively scanty, and a variety of complex phenomena renders the understanding of the underlying mechanisms rather uncertain. Since migratory fish represent one of the top predators in the aquatic environment, occurrence of bioconcentration processes may contribute to the spread of hazardous substances in marine organisms even at a distance from polluted environments (Kurelec *et al.*, 1989). Concentration of pollutants in marine biota not only depends on the trophic level of the organisms concerned in the food web but also on their longevity, growth rate, and body weight, as well as on features of the pollutants themselves, such solubility and water/lipid partition coefficients in the host tissues, persistence, metabolic rate, excretion, etc. Moreover, it should be taken into account that for certain pollutants the intake through ingestion is less important than intake from the water passing over the gills. This is the case for petroleum hydrocarbons, for which the direct uptake from seawater or sediments appears to be more important than accumulation through the food chain (Landner, 1976). In contrast, organochlorine pesticides are poorly available from seawater, due to their very low solubility, and are preferably accumulated through the food web (Kerr and Vass, 1973). However, in the light of more recent studies, the situation is unclear even for chlorinated hydrocarbon pollutants (UNEP/FAO/WHO/IAEA, 1990). As an example applying to hazardous metals, arsenic does not appear to be biomagnified in marine foodchains, although it is bioaccumulated by several species. For instance, marine algae contain arsenic at concentrations 2,000 to 5,000 times greater than those in seawater (GESAMP No. 28, 1986).

Another well-known mechanism of bioconcentration of pollutants from seawater is provided by bivalves such as mussels, which can act as non-selective filter feeders filtering as much as 1.5 litres of seawater per hour, thereby accumulating in significant amounts those microorganisms and noxious substances which are present in trace amounts in the surrounding environment (Mix, 1986). For this reason, as reported in section 4.1.3.4., mussels can be used not only as indicators of chemical, radioactive, or microbiological

pollution, but also as targets for seawater genotoxins. Similar considerations may apply to sponges, which are filter feeders living in the benthic regions of the continental shelf, and filter every hour 1 litre water per 10 grams of these biota (Vogel, 1977).

3.3 Sources and input

Natural processes, general urban sewage, and specific industrial or agricultural effluents can account for seawater pollution by carcinogenic, mutagenic and teratogenic substances. The relative importance of natural and anthropogenic sources varies depending on the type of pollutant, often with mixed contribution. However, the source of pollution by synthetic organic chemicals is always anthropogenic (Magos, 1989). The annual pollution load of the Mediterranean from land-based sources, either originating in the coastal zone from domestic, industrial, or agricultural sources, or carried by rivers, has been tentatively estimated for a number of pollutants. The estimated parameters included the total discharge volume, organic matter, nutrients (phosphorus and nitrogen), some specific organics (detergents, phenols, mineral oils), some metals (mercury, lead, chromium, zinc), suspended matter, organochlorine pesticides, and radionuclides. It was concluded that 60 to 65% of the total load comes from coastal sources, half of which from industry and about a quarter each from domestic sewage and agriculture (Helmer, 1977; UNEP/ECE/UNIDO/FAO/UNESCO/WHO/IAEA, 1984). It is evident that, for a variety of reasons, these estimates are uncertain, and may be considered accurate within an error range of about one order of magnitude (Helmer, 1977). It is also evident that it is extremely difficult to quantify the specific input of carcinogenic, mutagenic, and teratogenic substances, also due to the uncertainties in the classification of these hazardous substances and to the lack of systematic surveys.

Atmospheric input of pollutants is an additional source of seawater contamination. According to GESAMP (1980), exchange of matter across the air/sea interface can occur as follows:

(a) Downward transport

Gaseous

(i) Wet - incorporation in precipitation
(ii) Dry - direct transfer across air/sea interface

Particulate

(iii) Rainout
(iv) Washout

Dry

(v) Gravitational/Brownian deposition
(vi) Trapping by whitecap bubbles

(b) Upward transport

Gaseous

(vii) Molecular evaporation from surface
(viii) Purging by bubbles

Particulate

(ix) Bursting bubbles and spray

The following are the major sources of oil pollution in the Mediterranean, according to UNEP/IMO/IOC (1987):

(1) natural seeps and erosion of sedimentary rocks

(2) spills and operational (produced water) discharges from offshore petroleum production facilities

(3) refinery and oil storage wastes

(4) marine transportation, including:

(a) operational discharges from tankers (ballast, slop tanks and tank washing water)

(b) terminal and bunkering operations (e.g. spillages, pipeline or storage tank ruptures)

(c) dry-docking

(d) bilges and fuel oil from ships (machine space bilges, fuel oil sludges, oily ballast from fuel tanks)

(e) accidental spills from tankers and other ships

(5) pleasure watercraft

(6) ocean dumping

(7) precipitation from the atmosphere

(8) municipal waste waters

(9) industrial waters (non-refinery)

(10) urban runoff

(11) river-borne pollution.

It has been estimated that the total input of petroleum hydrocarbons in the Mediterranean is 635,000 tonnes/year. More than half of such input (330,000 tonnes) is

accounted for by spilled oil from tankers, ballasting and loading operations, bilge and tank wastings. Run-off from municipal and industrial discharges are responsible for 160,000 and 110,000 tonnes, respectively, whereas a less important yet appreciable contribution (35,000 tonnes) is ascribed to atmospheric deposition (UNEP/IOC, 1988).

Halogenated hydrocarbons can contaminate the marine environment through agricultural run-off, rivers and discharge of industrial and municipal wastes. According to the conclusions of project Med X of MED POL-PHASE I, the total load of organochlorine pesticides carried into the 10 regional Mediterranean sea areas by surface run-off, either directly or through rivers, was 90 tonnes/year (range 50-200) (UNEP/ECE/UNIDO/FAO/UNESCO/WHO/IAEA, 1984). In addition, organochlorine pesticides and polychlorinated biphenyls can occur in seawater due to atmospheric deposition. Air/sea exchanges, according to the aforementioned mechanisms, can be responsible for seawater contamination by halogenated hydrocarbons even at a distance from pollution sources (UNEP/FAO/WHO/IAEA, 1990).

Metals can be released into the marine environment from both natural and anthropogenic sources. For instance, cadmium, like other trace metals, reaches seawater through rivers and surface runoff, as a consequence of geologic weathering and erosion of the earth's crust. Deep sea volcanic activity and the atmosphere can also contribute to its natural spread. The main anthropogenic sources are metallurgical industries, ore mines, and sewage sludges, but domestic and mixed sewage in which cadmium occurs in high proportions relative to other trace metals, gives a contribution as well. The range of cadmium concentration in the sewage of some Mediterranean towns was 0.1-24 µg ℓ^{-1} (UNEP/FAO/WHO, 1989). Arsenic is released into the environment as a component of pesticides, as the result of smelting or roasting sulphide minerals, combustion of fossil fuels, leaching of exposed wastes from mining activity, and accelerated erosion of land. River drainage of areas with substantial arseniferous ore deposits are significant sources of arsenic (GESAMP 28, 1986). Although the presence of arsenic in the marine environment can also result from volcanoes, burning of vegetation and continental weathering, its release from anthropogenic sources seems to exceed those from natural processes (MacKenzie *et al.*, 1979).

3.4 Recorded levels in the Mediterranean

The Mediterranean has a volume of 3.7 million Km^3, with a narrow communication (15 km width and 365 m depth) with the Atlantic Ocean through the Strait of Gibraltar. Hence, the Mediterranean is almost an enclosed sea, with a renewal period of 80 years for its waters, which hampers dilution into oceanic waters and favours accumulation of long-living hazardous pollutants. For instance, in spite of the fact that the Mediterranean has so far been spared by major oil spills, it is considered to be relatively more polluted by oil than any other sea for which data are available (UNEP, 1980). However, as will be seen later in this document, such an assumption is not supported by quantitative data on petroleum hydrocarbons in sediments.

Several studies, many of which were carried out within the framework of the MED POL programme, have investigated the concentration of compounds quoted in the list of carcinogenic, mutagenic, and teratogenic substances in Mediterranean seawater, sediments and biota. Data concerning some hazardous pollutants have been recently reviewed in detail in specific issues of the MAP Technical Reports Series, e.g., petroleum hydrocarbons (UNEP/FAO/WHO, 1989), and organohalogen compounds (UNEP/FAO/WHO/IAEA, 1990), as well as in a recent Report on the State of the Mediterranean Marine Environment (UNEP, 1989).

In synthesis, a large number of potentially harmful trace elements have been studied in the Mediterranean. In MED POL studies, priority was given to mercury and cadmium, since preliminary surveys had shown that both elements occur in marine biota in high concentrations. Older analytical data for trace elements, indicating in the case of cadmium, a range from less than 0.05 up to 0.60 µg ℓ^{-1} in open Mediterranean waters (UNEP, 1978), must be considered with some reserve, due to several technical drawbacks. More recent analyses report ranges from 0.004 to 0.017 µg ℓ^{-1} for open waters. However, certain coastal areas of Spain and Italy have cadmium concentration higher than those from the open sea ((UNEP/FAO/WHO, 1989). Cadmium concentrations in coastal sediments from areas receiving industrial effluents, solid wastes and domestic sewage, as well as in river deltas and estuaries, are considerably higher than Mediterranean background values. Concentrations in marine biota were found to vary broadly depending on the food-chain position of the organism, on the tissues examined, and on concentration and chemical species (e.g., ionic species) of cadmium in seawater (UNEP/FAO/WHO, 1989). Similar problems of speciation and chemical analysis also occur with other metals which may become harmful under certain conditions, e.g., arsenic and chromium.

An extensive literature is available on the presence of petroleum hydrocarbons in marine ecosystems, and the number of reported data has also been steadily increasing for the Mediterranean area during the last 10 years, mainly as a result of the activities generated by MED POL projects. In general, dissolved/dispersed petroleum hydrocarbons (DDPH) in off-shore Mediterranean waters have concentrations below 10 µg ℓ^{-1}, the aliphatic fraction of petrogenic hydrocarbons being more abundant than the aromatic one. DDPH concentrations are much higher, i.e., above 10 µg ℓ^{-1} near the shore, particularly near industrialized areas or river mouths (UNEP, 1989). Comparing DDPH data reported for other regions, the distribution of results in Mediterranean regions suggested the present of two different groups, with concentrations lower and higher than 0.4 µg ℓ^{-1}, respectively (IOC, 1981). Available data on pelagic tar show than, between 1969 and 1983, mean concentrations in the Mediterranean ranged from 0.5 to 130 mg m^{-2}, the Ionian Sea being the most polluted area. Normal values for off-shore areas appear to be up to 5 mg m^{-2}, while in nearshore waters concentrations are in the 10-100 mg m^{-2} range (UNEP/IOC, 1988). The mean amounts of tar on Mediterranean beaches ranged between 0.2 and 4388 g m^{-2} (Golik, 1986). Similar to floating tars, the tar on beaches tended to decrease drastically during the last years, as a consequence of prohibition since 1978 of oily water deballasting and release of oily compounds into the sea (UNEP/IOC, 1988). Too few data are available to obtain a distribution pattern of petroleum hydrocarbons in the Mediterranean sediments. In general, the analytical results so far reported suggest a moderate contamination of sediments, compared with other regions (UNEP/IOC, 1988). Very few studies are available for petroleum hydrocarbons in Mediterranean marine organisms, most of them sampled from the Spanish coast. Mussels contained much higher concentration than fish collected from the same area. The levels found in mussels (*Mytilus galloprovincialis*) from the Ebro delta were in the order of 100-300 µg g^{-1} (Risebrough *et al.*, 1983), which are equivalent to those recorded in the most polluted harbours and bays of California analyzed by the same technique.

A number of data are available on contamination of the Mediterranean by halogenated hydrocarbons. Again, analytical problems complicate the comparison of data generated by different laboratories. Moreover, concentrations in seawater of several components of this chemical family are below detection limits or too low for a quantitative determination. PCBs concentrations in seawater samples ranged between 0.2 and 38 ng ℓ^{-1}. In the Northern Adriatic coastal waters most samples were below the detection limit for PCBs (0.1 ng ℓ^{-1}) and p,p'-DDT (0.05 ng ℓ^{-1}). Lindane levels off-shore in the Eastern basin ranged from 0.06 to 0.12 ng ℓ^{-1} , with higher concentration in particulate matter than in the seawater

dissolved phase (UNEP, 1989; UNEP/FAO/WHO/IAEA, 1990). Although PCBs remain an important class of halogenated pollutants in the Mediterranean, comparable data showed that a decrease of their concentration in seawater has occurred with years, presumably as a consequence of restrictions of industrial discharges in many countries. In the meantime, however, other chlorinated hydrocarbons such as lindane and hexachlorobenzene are acquiring a growing importance in the Mediterranean area (Burns *et al.*, 1985). Concentration of PCBs in open sea sediments in the Mediterranean were in the 0.8-9.0 µg Kg^{-1} range, whereas those in coastal sediments were affected by "hot spots", such as sewage outfalls, accounting for concentrations in the order even of few mg Kg^{-1}. Mean concentrations in coastal sediments from the Central Mediterranean were in the 4.5-390 µg Kg^{-1} range for p,p'-DDT and in the 0.1-2.5 µg Kg^{-1} range for hexachlorocyclohexane (HCH). Again, the recorded levels were affected by occurrence of "hot spots" in the analyzed areas (UNEP, 1989; UNEP/FAO/WHO/IAEA, 1990). The same is true for data concerning Mediterranean biota, with PCBs means ranging from 1.5 to 815 µg Kg^{-1}, as mainly assessed in the mussel and the red mullet. The highest levels of organochlorine pesticides were observed in tuna (*Thunnus thynnus*). The observed ranges were 0.1-343 µg Kg^{-1} for p,p'-DDT, 0.4-325 µg Kg^{-1} for p,p'-DDD, 1.5-600 µg Kg^{-1} for p,p'-DDE, 0.4-6.2 µg Kg^{-1} for dieldrin, 0.2-2 µg Kg^{-1} for aldrin, 0.7-20 µg Kg^{-1} for hexachlorocyclohexane, and 0.4-19 µg Kg^{-1} for lindane (UNEP, 1989).

The consultation meeting held in Athens in June 1988 agreed on a list of priority pollutants for the pilot monitoring study which was carried out in selected areas in the northern Mediterranean coast of Spain, the Ligurian Sea, the Ebro delta and the eastern Adriatic coast between 1989 and 1990. This list is reproduced in Table 1 (WHO, 1988).

Concentrations of total Arsenic in mussels ranged from 11.00 to 18.6 µg g^{-1} in the Ebro delta, from 11.2 to 23.2 µg g^{-1} in the Ligurian Sea and from 0.91 to 49.1 µg g^{-1} in the Adriatic. A considerable variation was also observed in fish. In *Mullus barbatus*, concentrations ranged from 34.6 to 74.2 µg g^{-1} in the Ligurian Sea and from 21.9 to 160.0 µg g^{-1} in the Adriatic. Ranges recorded for other species were 7.54 to 61.7 µg g^{-1} (*Merluccius merluccius*), 15.3 to 71.2 µg g^{-1} (*Pagellus erythrinus*), 18.1 to 55.3 µg g^{-1} (*Solea vulgaris*) and 2.04 to 15.2 µg g^{-1} (*Diplodus annularis*), all in the Adriatic. Concentrations recorded in sediments ranged from 14.2 to 30.5 µg g^{-1} in the Ebro delta and from 1.70 to 44.2 µg g^{-1} in the Adriatic. Results are reproduced in Table 2 (Stegnar, 1991).

The concentrations of various halogenated hydrocarbons, i.e., PCBs, PCCs (Toxaphene), Mirex, DDT isomers, hexachlorobenze (HCB), and hexachloro-cyclohexane (HCH) isomers, were analysed by Kanitz *et al.*, (1990) in *Mullus barbatus*, *Xiphias gladius* and *Mytilus galloprovincialis* in various areas of the Ligurian Sea. With the exception of delta-HCH and Mirex, all the pollutants were consistently found in the samples analysed, with remarkable quantitative variations depending on the organisms, the sampling locality and the season. Results are given in Table 3.

Concentrations of a number of Polycyclic aromatic hydrocarbons (PAHs) in mussels were analysed from 13 sites along the Ligurian coast between 1989 and 1991 (Piccardo and Valerio, 1991). PAHs analysed were Anthracene, Pyrene, Flouranthene, Benzo(*a*)anthracene, Benzo(*b*)fluoranthene and Dibenzo(*a,h*) anthracene. Concentration of each PAH showed wide variations between the different sites. Results are given in Table 4.

Table 1

Selected substances/groups of substances of relative importance as carcinogenic marine pollutants

AGENT	COMMENTS ON ANALYSIS	SOURCE	MONITORING
1. As III + As V	Tot. As. speciation	Pesticides Chemical wastes	Sediments Benthic biota
2. Cd + compounds			NO
3. Cr (VI) compounds			NO
4. Ni + compounds			NO
5. Be + compounds	Literature search	Incineration	Sediments, biota
6. Pb + compounds (inorganic)			NO
7. PAHs	4-7 ring fraction compounds	Used oils, coal tars, street runoff	sediments, benthic biota
8. Low mol. hal. HCs	**	Solvents	?
9. PCBs	Individual congeners *	Industrial and urban effluents	Biota *
10. PBBs	*		*
11. PCCs	Toxaphene *		*
12. Mirex	*		*
13. DDT	Incl. isomers and derivatives *	Agriculture	*
14. HCB	All isomers *		*
15. HCH	All isomers *		*
16. PCDDs + PCDFs	Individual congeners	Incineration	Biota
17. Chlorophenols	**		?
18. Benzene			NO
19. 1,4 Dioxane			NO
20. Amitrole			NO
21. Aromatic amines		Dyestuffs	Sediments
22. NTA ***		Households	Seawater

* Can be determined by a single analytical procedure (group analysis)
** Broad-spectrum analysis to define individual compounds
*** Not included in IARC list, but identified as non-carcinogenic mutagen
? Undecided

Table 2

Concentrations of total Arsenic in mussels, fish and sediments from selected Mediterranean areas, 1989-1990
Results (ranges and means) are expressed in μg g^{-1} dry weight (Stegnar, 1991)

	EBRO DELTA	LIGURIAN SEA			ADRIATIC SEA		
		West	Central	East	North	Central	South
Mytilus galloprovincialis	11.0-12.0 (11.2) 15.2-18.6 (17.2) 10.2-12.5 (11.2) 14.6-16.3 (15.5)	21.7-23.2 (22.6)	13.3-15.2 (14.9)	11.2-12.2 (12.0)		0.91-20.5 (10.6) 11.7-49.1 (25.9)	
Mullus barbatus		66.4-74.2 (68.8)	45.6-53.9 (51.2)	34.6-39.2 (36.6)	52.6-71.7 (61.1)	21.9-60.4 (40.1)	125.0-160.0 (145.0)
Merluccius merluccius					48.4-61.7 (56.2)	7.54-36.6 (20.92)	18.0-25.1 (21.75
Pagellus erythrinus					55.9-71.2 (64.9)	15.3-42.2 (28.0)	
Solea vulgaris						18.1-23.2 (21.0)	43.4-55.3 (50.4)
Diplodus annularis						2.04-15.2 (16.16)	
Sediments, 150 μm	14.2-19.9 (17.3)				1.70-7.20 (4.18)	6.6-27.6 (18.6)	2.9-5.4 (4.1)
Sediments, 80 μm	24.4-30.5 (27.9)					20.3-44.2 (30.37)	

Table 3

Levels of chlorinated hydrocarbons in fish and mussels from three areas in the Ligurian Sea, 1989. Data expressed as ng g^{-1} dry weight
M = May, N = November, nd = below detectable limits
(Kanitz *et al.*, 1990)

		Mullus barbatus			*Xiphias gladius*	*Mytilus galloprovincialis*		
		East	Central	West	West	East	Central	West
Toxaphene	M	30.10	18.78	34.75	26.92	168.29	89.97	109.31
	N	10.33	54.76	29.58	-	155.61	87.74	173.55
Aroclor 1260	M	153.85	705.27	576.28	619.85	221.83	117.39	52.57
	N	162.77	1,273.15	2,519.03	-	85.19	70.49	53.82
Aroclor 1254	M	244.34	698.62	261.93	983.70	341.06	313.05	203.36
	N	110.51	718.22	2,915.61	-	1,060.52	677.06	399.18
Total PCB	M	398.18	1,403.89	848.77	1,603.55	562.89	430.44	255.94
	N	273.29	1,991.37	5,434.64	-	1,145.70	747.55	452.99
Alpha-HCH	M	1.14	0.86	0.08	0.22	2.45	2.22	2.55
	N	0.82	0.99	1.81	-	0.68	1.73	1.60
Beta-HCH	M	2.09	1.49	0.08	0.13	3.98	3.71	1.50
	N	1.47	1.78	3.72	-	1.62	4.14	2.79
Gamma-HCH	M	0.99	0.97	0.15	0.32	1.69	1.87	1.91
	N	1.47	1.65	3.58	-	0.49	2.30	1.81
Delta-HCH	M	0.49	0.22	nd	0.13	0.77	nd	nd
	N	nd	nd	nd	-	0.23	nd	nd
Total HCH	M	5.86	3.53	0.31	0.80	8.89	7.80	5.96
	N	3.75	4.43	9.11	-	3.02	8.17	6.20
HCB	M	0.08	12.13	0.27	1.08	0.77	0.90	0.71
	N	1.04	1.12	1.54	-	2.38	3.28	3.00
Mirex	M	nd	nd	102.52	nd	nd	nd	nd
	N	nd	nd	nd	-	54.18	nd	nd
p,p'-DDE	M	35.62	40.32	9.75	250.57	32.94	24.38	15.79
	N	58.35	54.29	172.52	-	67.37	23.86	29.83
o,p'-DDD	M	3.43	0.33	0.81	4.27	3.06	2.85	2.89
	N	0.52	0.53	0.34	-	4.72	4.60	4.24
o,p'-DDT	M	1.94	0.97	0.27	21.28	0.36	0.23	0.30
	N	0.23	0.59	0.84	-	0.27	0.46	0.47
p,p'-DDD	M	6.97	5.47	3.79	19.81	11.15	12.28	7.88
	N	2.97	13.23	4.19	-	14.81	8.63	2.27
p,p'-DDT	M	0.34	12.87	3.02	88.55	9.92	3.28	1.57
	N	5.67	13.23	9.14	-	1.62	2.07	0.77
Total DDT	M	48.31	59.97	17.65	384.49	57.37	43.02	28.42
	N	67.74	81.87	186.73	-	88.79	39.62	37.59

Table 4

Concentrations of PAHs (ng g^{-1}) in mussels collected along the Ligurian coast between 1989 and 1991. (Piccardo and Valerio, 1991)

SITE	An	Py	Flu	BaA	BbF	BaP	BkF	BP-DBA
1	3.33	48.95	80.24	39.26	28.86	8.27	9.95	6.20
2	1.72	15.07	26.69	10.24	13.84	6.90	4.27	3.67
2	0	18.65	32.01	26.22	14.90	4.88	0.51	3.76
3	0.88	8.66	8.48	4.60	7.50	3.95	4.73	1.16
4	1.93	13.37	11.10	6.02	7.80	3.47	2.77	1.48
5	0	2.73	1.80	1.39	1.68	0.57	0.16	1.1
6	0.95	3.07	2.31	1.46	3.07	1.47	0.66	0.72
6	0.58	4.29	6.29	0	0.25	0.17	0.28	1.08
7	0.87	2.72	0.80	0	0.65	0.31	0.08	0.29
8	0.55	3.39	0.62	0	2.12	0.62	0.02	0.89
9	0	10.27	3.20	6.19	6.50	2.85	4.45	4.29
10	0	1.23	0.32	0	0.49	0.13	0.34	1.39
11	0.75	3.21	5.24	0	1.27	0.21	0.55	1.76
12	0	2.94	4.96	0	0.75	0.20	0.59	0.97
13	0.70	2.46	2.02	0	1.57	0.63	0	0.44

Sites 2 and 6 sampled twice
Site 13 - "control" site

An = Anthracene
Py = Pyrene
Flu = Fluoranthene
BaA = Benzo(*a*)anthracene
BbF = Benzo(*b*)fluoranthene
BaP = Benzo(*a*)pyrene
BkF = Benzo(*k*)fluoranthene
BP-DBA = Benzo(*g,h,i*)fluoranthene, Dibenzo(*a,h*)anthracene

Within the framework of the same pilot project, as number of marine organisms, as well as sediments, from the Ebro delta and the coast of Barcelona were analysed for PCBs (individual congeners), organochlorine pesticides, and PAHs. A number of aromatic amines were also identified in sediments. Samples of fish and mussels collected from the Ligurian Sea (*vide* Table 3) were also cross-analysed (Albaiges and Bayona, 1991).

A total of 18 individual PCB congeners were determined in tissues of *Mytilus galloprovincialis* and *Mullus barbatus*. In general, the latter species exhibited a higher burden than the former, comparing species collected from the same area. The pattern of distribution of PCB congeners on these two species differed from that observed in eggs of the marine bird *Ardea purpurea*. Results are given in Table 5. The total PCB values found in *Mytilus* and *Mullus* species during the present study were lower than those quoted in reports published during the last decade and, assuming the inter-comparability of analytical procedures, this could afford an indication of reductions in the emission of these compounds into the coastal areas in question.

A total of 20 organochlorine pesticides were also systemically determined. Results are given in Table 6. In most cases, a generally cosmopolitan distribution was found, reflecting a widespread scale of pollution, though in the case of some DDT isomers, HCHs and HCB, concentrations were lower than those reported in earlier literature. In the case of PAHs, a variety of parent compounds ranging from 3 to 6 aromatic rings and their alkylated derivatives were identified in *Mytilus galloprovincialis* and in sediments. Environmental levels were generally comparable with others previously reported from the Mediterranean region. Results are given in Table 7, where the ratio between parent and monoalkalated phenanthrenes reflects the contribution of pyrolytic versus fossil sources of pollution. The latter were more predominant in samples from areas under river influence. Conversely a significantly high predominance of pyrolytic sources was evident in the rest of the sampling sites.

4. ASSESSMENT OF RISK TO MARINE ORGANISMS

4.1 Effects on marine organisms

In evaluating the possible harmful effects of pollutants on marine biota, the route of exposure should be taken into account along with toxicokinetic and metabolic features inherent to the pollutant itself and to the complexity of the host organism machinery. Exposure of fish may occur either by the respiratory route following absorption of waterborne chemicals through the gills or by the digestive route through ingestion of dietary chemicals. Accordingly, the different distribution of pollutants in the water and in the diet is a critical factor affecting toxicokinetics in fish and its susceptibility to harmful substances, which should be kept in mind in the interpretation of both laboratory and field studies. While there are some phylogenetic variations in metabolic pathways, the responses in fish actually mimic those in mammals very closely (Hodson, 1987). For this reason, as it will be discussed in next sections, long-term effects in fish are largely comparable with those occurring in mammals.

Table 5

Distribution of individual PCB congeners in biota (ng g^{-1}, dry wt.)
from 3 Mediterranean areas, 1989-1990
(Albaiges and Bayona, 1991)

	COASTAL BARCELONA						EBRO DELTA					LIGURIAN SEA					
	Mytilus sp.			*Mullus sp.*			*Mytilus sp.*		*Ardea sp.* eggs			*Mytilus sp.*			*Mullus sp.*		
IUPAC No.	Max	Min	Ave*	Max	Min	Ave*	Max	Min	Max	Min	Ave*	West	Central	East	West	Central	East
28+31	3.5	0.08	3.0	8.7	0.1	3.0	1.6	0.6	120	0.4	80.7	n.d.	n.d.	0.6	n.d.	n.d.	n.d.
52	3.0	0.04	4.4	17.3	0.2	6.1	0.8	n.d.	41.5	0.3	18.7	n.d.	2.9	9.0	5.7	13.5	n.d.
44	4.5	n.d.	-	n.q.			1.6	n.d.	21.3	n.d.	-	-	-	1.3	5.7	1.8	1.4
101	11.5	0.4	8.5	37.6	0.5	13.1	1.4	0.3	46	n.d.	-	n.d.	5.3	4.9	0.6	8.2	4.1
118	4.5	0.98	8.6	60.6	2.3	22.7	n.d.		174	n.d.	-	n.d.	4.7	n.d.	1.6	17.5	n.d.
153	16.9	2.4	9.1	96.6	1.7	35.4	8.7	4.5	11.1	7.1	8.5	n.d.	21.6	10.1	7.4	17.3	33.8
138+163	21.7	3.1	14.7	145.	5.0	56.8	6.7	2.2	14.3	n.d.	-	24	17.8	10.7	6.5	21.9	n.d.
187	14.6	6.1	7.8	9			7.2	n.d.	6.4	n.d.	-	18	7.3	14.7	4.9	n.d.	51.8
128	7.3	0.7	3.4	n.q.			1.1	0.4	60	n.d.	-	10	n.d.	5.5	1.2	8.2	7.7
156	n.d.		-	n.q.		n.d.	n.q.		-		0.8	n.d.	n.d.	0.6	4.2	n.d.	
180	0.4	0.8	3.4	n q.	0.9	2.5	1.2	n.d.	0.3	n.d.	-	4.8	1.2	2.3	4.1	6.5	31.5
170	n.d		-	44.3		n.q.	n.q.		-		2.5	5	0.9	2.5	5	16.8	
				n.d.													
ΣPCB_{cong}	80	15	62.9		11	140	30	8	495	7.8	-	60.1	66	33	41	110	147
ΣPCB_{1254}			201	411		445	74	27	739	26		-	171	120	83	270	221

* Mean of three samples
n.d. Not detected
n.q. Not quantified

Table 6

Distribution of organochlorinated pesticides in biota (ng g^{-1}, dry wt.) from 3 Mediterranean areas, 1989-1990 (Albaiges and Bayona, 1991)

	COASTAL BARCELONA			EBRO DELTA				LIGURIAN SEA					
	Mytilus sp.			*Mytilus sp.*		*Ardea sp.* eggs		*Mytilus sp.*			*Mullus sp.*		
	Max	Min	Ave*	Max	Min	Max	Min	West	Central	East	West	Central	East
Methoxychlor	63.9	n.d.	-	n.d.		n.d.		8.3	n.d.	n.d.	n.d.	n.d.	n.d.
Dieldrin	n.d.			n.d.		181	7.9	2.3	0.5	n.d.	n.d.	0.9	n.d.
Heptachlor	20	n.d.		2.8	2.1	n.d.			n.d.	1.0	n.d.	n.d.	n.d.
Endosulfan I+II	23.9	5.3	15.5	6.1	n.d.	n.d.		n.d.	n.d.	10.2	n.d.	n.d.	n.d.
HCB	1.3	0.5	0.8	1.6	0.8	15.1	10.4	n.d.	n.d.	n.d.	0.2	0.4	n.d.
α-HCH	2.9	0.2	1.2	1.0	0.3	11	n.d.	n.d.	n.d.	n.d.	0.9	0.1	n.d.
γ-HCH	2.7	1.6	2.3	1.5	n.d.	11.3		n.d.	n.d.	n.d.	2.5	0.4	n.d.
o,p'-DDT	29.3	n.d.	-	10.7	n.d.	115	8.9	14.1	n.d.	5.7	3.1	n.d.	n.d.
p,p'-DDT	27.1	3.4	11.8	5.4	n.d.	84	n.d.	9.0	n.d.	4.1	1.7	n.d.	12.7
p,p'-DDD	11.5	n.d.	-	12.1	3.2	64	6.1	4.0	5.4	0.8	0.1	n.d.	n.d.
o,p'-DDE	17.1	n.d.	-	3.9	2.5	0.14	n.d.	8.3	n.d.	n.d.	n.d.	n.d.	3.9
p,p'-DDE	151	51	96	42	31	152	4.2	20.5	33.9	36	36	34	43

* Mean of three samples
n.d. Not detected
n.q. Not quantified

Table 7

Distribution of PAHs in sediments (ng µg g^{-1}, dry wt.) and biota (ng g^{-1}, dry wt.) from 3 Mediterranean areas, 1989-1990 (Albaiges and Bayona, 1991)

	COASTAL BARCELONA				EBRO DELTA			LIGURIAN SEA
	Sediments (depth, m)				Sediments (depth, m)			
	A	B	C		D	E		
COMPOUND (No.)	10	40	43	*Mytilus sp.*	30	520	*Mytilus sp.*	*Mytilus sp.*
Phenanthrene (1)	30	405	57	15	9.2	16	32	38
Anthracene	10	150	16	n.d.	0.6	1.3	n.d.	14
C_1-Phenanthrenes	107	271	71	113	14.1	17.1	195	89
Fluoranthene (2)	97	757	373	19	17.8	30.7	8	322
Pyrene (3)	42	715	314	34	12.6	25.4	7	571
Benz[*a*]anthracene (4)	68	274	270	7	6.1	15.2	3	372
Chrysene+Triphenylene (5)	45	451	338	54	13.9	36.5	8	448
Benzo[*b*+*j*+*k*]fluoranthenes (6)	98	749	797	31	2.6	69	n.d.	310
Benzo[*a*]fluoranthene (7)	n.d.	129	58	n.d.	0.5	24	n.d.	51
Benzo[*e*]pyrene (8)	29	364	369	20	3	30	n.d.	158
Benzo[*a*]pyrene (9)	33	525	452	6	0.2	15.2	n.d.	18
Perylene (10)	9	166	155	46	40	3	n.q.	n.q.
Indeno[1,2,3-cd]pyrene (11)	31	240	361	n.q.	8.7	40	n.q.	n.q.
Benzo[*ghi*]perylene (12)	82	749	474	n.q.	9.4	28	n.d.	n.q.
ΣPAHs	681	5945	4105	346	139	351	253	2391
Ph/ΣC_1Ph	0.28	1.49	0.8	0.13	0.66	0.93	0.16	0.42
Fl/Py	2.3	1.06	1.19	0.55	1.41	1.21	1.18	0.56

4.1.1 Metabolic effects

The earliest warning signal of exposure of marine organisms to potentially harmful pollutants is the induction of those metabolic pathways which are responsible for their biotransformation in host cells. It is well known that most xenobiotics undergo various pharmacokinetic and metabolic processes in the organism, which in principle tend to transform non-polar (lipid-soluble) compounds into more polar (water-soluble) derivatives, thereby favouring their excretion from the organism. However, the same mechanisms often lead to activation of inert precursors (procarcinogens/ promutagens/proteratogens) into intermediate (proximate) and ultimate metabolites, which in virtue of their electrophilicity can bind covalently nucleophilic sites of DNA and other cell macromolecules (e.g., RNA or proteins), thereby forming carcinogen-DNA or carcinogen-protein adducts. The balance between activating and deactivating mechanisms is extremely delicate and is governed by intricate biochemical reactions, often interconnected or in cascade-like sequence, which mainly occur in the endoplasmic reticulum (microsomal fractions), but also in the soluble fraction of the cytoplasm (cytosolic fractions), or in other cell structures, e.g., in mitochondria or in nuclei themselves. Without going into detail, two broad groups of reactions are involved, also in aquatic organisms, i.e., phase I reactions, such as oxidation, reduction, and hydrolysis, leading to creation of new functional groups (Buhler and Williams, 1989), and phase II reactions, involving conjugation of phase I products with endogenous polar or ionic moieties, consisting of large chemical groups or entire compounds, such as sugars or amino acids (Foureman, 1989). A central role in biotransformation is played by mixed-function oxygenases (MFO), having cytochromes P450, a family of iron-containing hemeproteins, as the terminal oxidase. The levels of cytochrome P450 and of benzo(*a*)pyrene hydroxylase activity in liver microsomes of various marine species are reported in Table 8.

Two main features of MFO and of other xenobiotic-metabolizing activities are worthy of attention in marine biota, with reference to the problem of marine pollution. The first one is that the "constitutive" levels of these enzymes vary not only according to the animal species and strain, but also at the individual level (Lech and Vodicnik, 1984). Typical promutagens and procarcinogens, such as aflatoxin B1 (Loveland *et al.*, 1987) and benzo(*a*)pyrene (Varanasi *et al.*, 1986) are known to be bioactivated by fish liver. In any case, a broad variability of the responsible enzyme activities occurs among different marine vertebrate species (Bend and James, 1979; Funari *et al.*, 1987), also as a function of sex and of the year period (Buhler and Williams, 1989; Lafaurie *et al.*, 1989). Fish is also able to repair DNA damage, as shown for instance by the presence of O^6-methylguanine DNA methyltransferase at levels comparable to those of rodents (Nakatsuru *et al.*, 1987). This enzyme plays an important role in repairing the lesions produced by alkylating chemicals, such as *N*-nitroso compounds, which are carcinogenic also in fish (see section 4.1.2).

In general, many invertebrates have a very low inherent capability for xenobiotic transformation (James, 1989). However, the MFO system has been reported in 18 species of marine invertebrates belonging to 4 phyla (Annelida, Arthropoda, Echinodermata and Mollusca). This multi-component system is located in the endoplasmic reticulum of various tissues, such as the stomach, hepatopancreas and green gland of crustaceans, and the intestine of polychaetes (Lee, 1981). Although molluscs, such as mussels, clams, and oysters, can attain very high body burdens of contaminants in polluted environments, the results of metabolic studies have been somewhat conflicting (James, 1989). In some studies typical spectra of cytochromes P450 were detected in digestive gland and gills of bivalve species, such as *Mytilus edulis*, *Macrocallista maculata* and *Arca zebra*, with highest levels of P450 and rate of benzo(*a*)pyrene metabolism in the digestive gland (Stegeman, 1985). However, cytochrome P450-dependent benzo(*a*)pyrene hydroxylase activity could not be

detected in these marine bivalves, as confirmed in several studies (Britvic and Kurelec, 1986). On the other hand, the digestive gland of *Mytilus galloprovincialis* was found to possess a FAD-containing MFO capable of activating aromatic amines, but not benzo(*a*)pyrene, as well as detoxifying enzymes, such as UDP-glucuronyl transferase and b-glucuronidase (Kurelec *et al.*, 1986). Some *in vivo* studies have shown that PHA metabolites are poorly excreted by certain marine invertebrates, which could therefore be dietary sources of potentially bioactive metabolites, even in the absence of parent compounds (James, 1989).

The second outstanding feature of carcinogen-metabolizing enzymes is that their activities can be modulated by exogenous factors, including dietary and environmental factors. This phenomenon is well known to occur in mammals and is also well established in marine organisms. In particular, induction by xenobiotics of MFO activities in fish liver, but also in other tissues or marine biota, has been investigated in a large number of laboratory or field studies carried out worldwide, including the Mediterranean area. Typical harmful pollutants, such as polycyclic aromatic hydrocarbons (PAH), polychlorinated biphenyls (PCB), polybrominated biphenyls (PBB), and petroleum hydrocarbons are quite effective in inducing MFO and other enzyme activities. MFO stimulation is generally accompanied by a less pronounced increase in total cytochromes P450. Monitoring of these biochemical parameters has been proposed as a tool for discriminating the quality of the aquatic environment and as an early warning system for assessing the impact of harmful pollutants. Such a "fast" adaptive response can be followed, after a prolonged exposure, by a "slow" adaptive response leading to fish liver hyperplasia (see Payne, 1984, and Payne *et al.*, 1987, for reviews).

Examples of induction of enzyme activities in fish caught from polluted Mediterranean seawaters or river waters include: stimulation of arylhydrocarbon hydroxylase (AHH) activity in blenny (*Blennius pava*) exposed to an oil spill (Kurelec *et al.*, 1977) or to the effluent of a petroleum industry (Rijavec *et al.*, 1981) in the Adriatic Sea, or in chub (*Leuciscus cephalus*), barbel (*Barbus barbus*), and nase (*Chondrostoma nasus*) living in the polluted river Sava in Yugoslavia (Kezic *et al.*, 1983); enhancement of AHH, glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase, shift on the left of cytochromes P450, and decrease of glutathione peroxidase and glutathione S-transferase in annular seabream (*Diplodus annularis*) living in a polluted portual environment in the Ligurian Sea (Bagnasco *et al.*, 1991). In the same study, seawater pollution markedly enhanced the metabolic activation by liver preparations of benzo(*a*)pyrene-trans-7,8-diol and of the arylamine 3-amino-1-methyl-5H-pyrido(4,3) indole (Trp-P-2), and at the same time decreased their ability to detoxify the direct-acting mutagen 2-methoxy-6-chloro-9-[3-(2-chloroethyl) aminopropylamino]acridine (ICR 191). A considerable liver hyperplasia was also observed. Of particular interest was the follow-up of benzo(*a*)pyrene monooxygenase (BPMO) recorded in blenniideae at a hospital site near Rovinj, in the Northern Adriatic, before and after the New Year 1977 oil spill accident (Kurelec *et al.*, 1977). An interlaboratory group, referred to as GICBEM has been created in France and Italy, with the objective of monitoring activities of bioprotection systems of marine organisms representative of coastal ecosystems in the Mediterranean Sea. Preliminary data concerning a MFO activity, the ethoxyresorufin-O-deethylase (EROD), and the detoxifying enzymes epoxide hydrolase and glutathione S-transferase in the red mullet (*Mullus barbatus*) and in Serranidae (*S. scriba* and *S. cabrilla*) caught from the French Mediterranean coastal area and Corsica, appear to indicate a correlation between EROD activity and the supposed levels of local pollution (Lafaurie *et al.*, 1989).

Table 8

Hepatic microsomal cytochrome P450 and benzo(*a*)pyrene (BaP)-hydroxylase activities of marine species. Reproduced from Buhler and Williams (1989).

SPECIES	Cytochrome P-450 (nmol/mg protein)	BaP-hydroxylase	
		(nmol/min/mg protein)	FU* /min/mg protein)
TELEOSTS			
Scup	0.62	0.69	-
	0.61	1.23	-
	0.27	3.0-3.8	3.1
Sheepshead	-	0.28	-
Coho salmon	-	0.13	-
	-	0.12	-
	-	0.027	-
Mullet	0.047	-	2.9
	-	0.053	-
Starry flounder	-	0.040	-
Mangrove snapper	0.025	-	6.3
Pigfish	-	-	5.1
Mummichog	-	-	4.1
Sea bass	-	-	3.6
Winter flounder	0.17	-	2.54
	0.12-0.60	-	0.7-6.4
Sculpin	-	0.90	-
Black drum	0.15	-	0.53
Codfish	-	-	0.51
Southern flounder	0.11	-	0.25
Eel	-	0.21	-
Mackerel	-	-	< 0.07
King of Norway	-	-	0.004
ELASMOBRANCHS			
Nurse shark	0.47	-	1.4
Atlantic stingray	0.50	-	0.77
Large skate	0.29-0.36	-	0.30
Little skate	0.32	-	0.17
Bluntnose ray	0.30	-	0.15
Thorny skate	-	-	0.12
Dogfish shark	0.23-0.29	-	0.07
Southern stingray	0.31	-	ND[b]
CRUSTACEA[c]			
Crabs:			
Uca pugnax	0.14-0.23	-	0.133-0.517
Callinectes sapidus	0.04-0.19	-	0.018-0.127
	0.18	-	0.008
	-	0.057 (F)[d]	-
	-	0.00075 (M)[d]	-
Menippe mercenaria	0.20-1.00	-	0.008
Libinia sp.	0.36-0.56	-	0.002-0.011
Sesarma cinerum	0.31-0.51	-	ND-0.003

SPECIES	Cytochrome P-450 (nmol/mg protein)	BaP-hydroxylase	
		(nmol/min/mg protein)	FU*/min/mg protein)
Crabs: (Continued...)			
Una minax	0.06-0.14	-	ND-0.001
U. pugilator	0.09-0.16	-	ND
Lobsters:			
Homarus americanus	-	-	0.025-0.065
	-	-	n.d.-0.02
	-	-	< 0.01
Spiny Lobster:			
Panulirus argus	0.91	-	0.03
MOLLUSCA[e]			
Barnacle:			
Balanus eburneus	0.11	0.043	-
Mussel:			
Mytilus galloprovincialis	0.047	0.024	-
M. edulis	0.047	0.054	-
	0.134	0.019-0.031	-
Periwinkle:			
Littorina littorea	-	0.046	-
Snail:			
Tegula funebralis	-	0.073	-
Thais haemastoma	-	0.001-0.013	-
Softshell clam:			
Mya arenaria	-	-	ND
European oyster:			
Ostrea edulis	-	-	ND
OTHER PHYLA			
Starfish:[e]			
Asterias sp.	-	-	0.08
Sea urchin:			
Strongylocentrotus sp.[e]	-	-	0.08
S. purpuratus[f]	-	-	0.040
Lugworm:[f]			
Arenicola sp.	-	-	ND

[a] Activity expressed in fluorenscence units (FU) defined somewhat by different authors; for example, 1 FU is the fluorescent intensity of hydroxylated BaP metabolites at excitation wavelength 400 nm and emission wavelength 525 nm that is equal in fluorescent intensity to a solution of 3 µg of guinine sulfate per millilitre in 0.1 N H_2SO_4.
[b] ND, not detected
[c] Hepatopancreas
[d] Male (M) and female (F)
[e] Digestive gland
[f] Larvae

Fish also possess detoxification mechanisms against inorganic pollutants, such as metals. One of these is due to the presence of metallothioneins, i.e., proteins binding certain heavy metals, whose presence in fish liver has been shown to be enhanced as an adaptive response to metal pollution (Roch *et al.*, 1982). Hexavalent chromium is reduced to the non-toxic trivalent form by fish skin and gill mucus, depending on protein-bound sulfhydryl groups (Arillo and Melodia, 1990), and the same process is accomplished in fish liver, as shown both in *Salmo gairdneri* (De Flora *et al.*, 1982) and *Diplodus annularis* (Bagnasco *et al.*, 1991), via both non-enzymatic (e.g., reduced glutathione) and inducible enzyme-catalyzed mechanisms.

In marine invertebrates having low or undetectable enzyme activities, several studies were consistent with a poor inducibility by environmental factors, as assessed in mussels (*Mytilus edulis)*, clams (*Mya arenaria*), lobsters (*Homarus americanus*), sea urchins (*Strongylocentrosus droebachiensis*), snails (*Littorina littorea*), sandworms (*Nereis* spp.) and sea stars (*Asterias* spp.) (Payne, 1977; Payne *et al.*, 1983; Moore *et al.*, 1989), as well as in sponges (Zahn *et al.*, 1982). A slight induction was observed in oysters (*Crassostrea virginica*) exposed to PCBs (Anderson, 1977) and in mussels exposed to PCBs and PBBs (Payne *et al.*, 1983). An elevation of cytochromes P450 was detected in sandworms (*Nereis* spp.), fiddler crabs (*Uca pugilator* and *Uca rapax*) and blue crabs (*Callinectes sapidus*) exposed to oil spills (Lee *et al.*, 1981), and in mussels (*Mytilus galloprovincialis*) living in hydrocarbon polluted seawater (Gilewicz *et al.*, 1984). Moreover, an enhancement of NADP-neotetrazolium reductase activity occurred in the blood cells of mussels and littorines collected from an oil-polluted area (Moore, 1985), and an enhancement of cytochrome P450 and cytochrome b5 occurred in the digestive gland of mussels exposed to diesel oil (Livingstone, 1985). All the aforementioned studies have monitored biota living outside the Mediterranean area. Recently, Kurelec and Krca (1989) investigated the presence of glucuronides, the major end-products of the metabolism of most carcinogenic chemicals, in natural populations of mussels (*Mytilus galloprovincialis*) from polluted and unpolluted areas in the Northern Adriatic. However, they concluded that the mutagenicity testing of mussel glucuronides and of the corresponding aglucones does not seem to be useful as a biomonitor of aquatic carcinogens. Therefore, on the whole, in spite of their convenience as test organisms, invertebrates appear to be less sensitive than fish as metabolic indicators of exposure to harmful pollutants. Nevertheless, Rodriguez-Ariza *et al.* (1990) found significantly increased activities of some detoxifying enzymes (superoxide dismutase, catalase, glutathione transferase and glutathione peroxidase isozymes, and cytochrome P450) and ancillary enzymes (glutathione reductase and glucose 6-phosphate dehydro-genase) not only in fish (*Mugil* spp.) but also in mollusc species (*Chamaelea gallina*, *Ruditapes decussata*, and *Crassostroea gigas*) living in contaminated areas of Spanish (Andalusian) coastal waters.

The results reviewed indicate that induction of MFO or other xenobiotic-metabolizing enzyme activity is a sensitive index of pollution. These enzyme systems may either act as a protective mechanism or as an activation system for producing carcinogenic intermediate species. In addition, they may affect accumulation and bio-availability in edible marine organisms.

4.1.2 Carcinogenic effects

4.1.2.1 Experimental carcinogenicity studies

Animal carcinogenicity assays, which are usually performed in rodent species, provide an useful tool complementing or supporting the conclusions of epidemiologic studies in humans. Likewise, carcinogenicity assays in fish or other marine organisms may prove

useful either as an experimental model predictive of carcinogenicity in humans and/or as a target for assessing the harmful effects of marine pollutants in marine organisms themselves. The factors influencing experimental carcinogenesis in laboratory fish models have been reviewed (Bailey *et al.*, 1989). As already reported in section 2, fish has been also successfully used in anti-carcinogenicity studies, evaluating the cancer-protective properties of certain inhibitors.

Few carcinogenicity studies have been performed in aquatic invertebrates. Three types of neoplasms were induced in freshwater mussels treated with *N*-nitroso compounds (Khudoley and Sirenko, 1978). Suspicious lesions were observed in oysters exposed to both polycyclic aromatic hydrocarbons and diethylnitrosamine (Couch *et al.*, 1979; Winstead and Couch, 1988), and renal neoplasms developed in oysters experimentally exposed to heavily contaminated sediments (Gardner *et al.*, 1988). It is rather intriguing that in one case only (Khudoley and Sirenko, 1978) a blood-cell proliferative disorder was experimentally induced, such a disease being the most common neoplasm found in molluscs under natural conditions (see section 4.1.2.2).

A partial list of chemical compounds or complex mixtures that have been tested in marine or freshwater fish species under laboratory conditions is reported in Table 9 (Couch, 1989). This list also includes several compounds of anthropogenic source polluting seawater, sediments, and biota, which have been classified as potential carcinogens in section 3.1.2. It is evident that the results obtained in fish are similar to those obtained in mammalian species (Couch and Courtney, 1987; Hinton *et al.*, 1988; Prince Masahito *et al.*, 1988), which presumably reflects the analogies between fish and mammals in metabolizing carcinogens (see section 4.1.1).

Table 9

Spectrum of agents and agent types tested in fish carcinogen system.
Reproduced from Couch (1989)

COMPOUNDS	REPRESENTATIVE REFERENCES
Aromatic Amines	
Acetylaminofluorene (+)[1]	Pliss and Khudoley, 1975; Sato *et al.*, 1973
Azo Compounds	
0-aminoazotoluene (+) 4-dimethylaminoazobenzene (+) Aminotriazole	Halver, 1967; Hatanaka *et al.*, 1982; Pliss and Khudoley, 1975
Halogenated Organic Compounds[2]	Halver, 1967; Hawkins *et al.*, 1988: Walker *et al.*, 1985
Bis(2-chloroethyl)ether (S) Bromodichloromethane (m) Bromoform (m) Carbon tetrachloride (m,s) (+)) Chlorodibromomethane (m) Chloroform (m) Dichlorodiphenyltrichloroethane (DDT) (s) (+) Ethylene dichloride (s) Pentachlorophenol (s) Trichloroethylene (s)	

COMPOUNDS	REPRESENTATIVE REFERENCES
Mycotoxins	
Aflatoxin B-1 (+) Aflatoxin G.1 (+) Aflatoxin L/L-1 (+) Aflatoxin M-1 (+) Aflatoxib Q-1 (+) Sterigmatocystin (+) Versicolorin A (+) Ochratoxin A & B (-)	Doster *et al.*, 1972; Halver, 1967; Hatanaka *et al.*, 1982; Hendricks *et al.*, 1978, 1980a,b,c,d,e,f; Matsushima & Sugimura, 1976; Sato *et al.*, 1973; Schoenhard *et al.*, 1981; Sinnhuber *et al.*, 1974; Wales and Sinnhuber, 1972; Wolf & Jackson, 1967
***N*-Nitroso Compound**	
N-nitrosodiethylamine (+) N-nitrosododimethylamine (+) Nitrosomopholine (+) N-Methyl-N-nitrosourea (+) N-Ethyl-N-nitrosourea (-) Dibutylnitrosamine (-) N-Methyl-N-nitro-N-nitrosoguanidine (+)	Aydrin and Bulay, 1983; Couch & Courtney, 1987; Egami *et al.*, 1981; Halver, 1967; Hatanaka *et al.*, 1982; Hendricks *et al.*, 1980b, 1984; Ishikawa, *et al.*, 1975; Khudoley, 1984; Kimura *et al.*, 1981, 1982-83, 1984; Klaunig *et al.*, 1984; Koenig and Chasar, 1984; Kyono-Hamaguchi, 1984; Pliss and Khudoley, 1975; Sato *et al.*, 1973; Schwab *et al.*, 1978a,b; Schultz and Schultz, 1984; Simon and Lapis, 1984; Stanton, 1965
Plant Derivatives	
Braken (-) Cyclopropenoid fatty acids (+) Cycad nut meal (+) Cysasin (-) Gossypol (-) Methylmazoxymethanol acetate (+) Pyrrolizidine (*Senecio*) alkaloids (-)	Aoki and Matsudaira, 1977, 1984; Fournie *et al.*, 1987; Hawkins *et al.*, 1985a,b, 1986; Hendricks *et al.*, 1980c,d, 1981a, 1983, 1984; Herman, 1970; Lee *et al.*, 1968, 1971; Schoenhard *et al.*, 1981; Sinnhuber *et al.*, 1976; Stanton, 1965
Polynuclear Aromatic Hydrocarbons	
Benzo (*a*)pyrene (+) 7,12-Dimethylbenz(*a*)anthracene (+) 3-methylcholanthrene (+)	Ermer, 1970; Hendricks *et al.*, 1982, 1985; Kimura *et al.*, 1984; Pliss and Khudoley, 1975; Schultz and Schultz, 1984
Miscellaneous Compounds	
β-aminoproprinoitrile (-) Benzidine (?) Carbarzone (+) Diethylstilbestrol (+) Nifulpirinol (+) Nifurpirinol (+) Tannic acid (+) Thioacetamide (-) Thiourea (+) Trifluralin (-) Urethane (+)	Couch *et al.*, 1981; Halver, 1967; Hendricks *et al.*, 1980a, 1981b; Kimura *et al.*, 1984; Levy, 1962; Martin, 1982; Pliss and Khudoley, 1975

[1] **(+) - neoplasia experimentally induced on one or more fish species**
(-) - no neoplastic lesions experimentally induced
(?) - possible neoplastic, but results equivocable
[2] **Agents tested singly (2) or in mixtures (m)**

From a mechanistic point of view, it is of interest to note that studies using hybrids of freshwater fish suggested that a tumour gene, denominated Tu, which is present on distinct sites of specific chromosomes, may work as a suppressor gene in fish somatic cells. The molecular mechanism of carcinogenesis may involve an alteration of regulatory genes, leading to a depression of Tu, which may arise from translocations, deletions, and crossovers in key structural chromosomes and retroviral oncogene-related sequences (Anders *et al.*, 1984).

4.1.2.2 Field studies

Neoplastic diseases have been observed in a wide variety of aquatic animals, including bivalve molluscs, amphibians, bony fish and sharks (Payne and Rahimtula, 1989). The earliest observations were made in 1964 by Dawe *et al.*, who detected hepatic neoplasms in bottom-dwelling species of fish collected from Deep Creek Lake (MD, USA) and suspected an association with chemical pollution. Neoplasia in aquatic organisms has been even proposed as an indicator for carcinogenic hazards to man (Black, 1984). The biology and pathogenic significance of these tumours have recently been comprehensively discussed (GESAMP, 1992).

Among marine invertebrates, oysters, clams, and mussels have been reported to suffer from presumed epizootic neoplastic diseases in a number of studies (reviewed, e.g., by Lauckner, 1983; Sparks, 1985; Couch and Harshbarger, 1985; Mix, 1986; Bolognesi, 1989; Baumann, 1989; Couch, 1989). The reported tumours were of gonadal (ovarian and testicular) origin and, chiefly, of presumed blood cell origin. The latter tumours, which are comparable to leukemia forms in mammals, have variously been called sarcomas, hematopoietic neoplasms, or blood-cell proliferative disorders. A gill carcinoma has been also described in clams. Overall prevalences have ranged from 1 per 5,000 oysters examined to 8-12% of several hundred clams, oysters, or mussels (Couch, 1989). Suggested possible causes included genetic predisposition (Couch and Harshbarger, 1985), C-type retroviruses (Oprandy *et al.*, 1981), and carcinogenic chemicals (Khudoley and Syrenco, 1978). However, it is noteworthy that neoplasms have been found in bivalve molluscs collected from both contaminated and clean coastal waters (Lauckner, 1983; Couch and Harshbarger, 1985; Mix, 1986). Excepting the observation of blood tumours in European oyster specimens collected from Yugoslav coastal waters (Alderman *et al.*, 1977), all remaining reports refer to areas outside the Mediterranean, such as North Europe (England and Ireland), Japan, Australia, South America (Chile), Canada, and especially USA (Atlantic or Pacific coasts, and Gulf of Mexico) (Bolognesi, 1989; Couch, 1989).

Similarly, studies on the occurrence of neoplastic lesions in populations of marine bony fishes have been all carried out in areas other than the Mediterranean. Table 10 summarizes the findings obtained in North America and Japan (Couch, 1989). Further data are available, e.g., for Australia (Hard *et al.*, 1979), Ireland (Mulcahy, 1976), Sweden (Ljunberg, 1976; Falkmer *et al.*, 1976 and 1977), The Netherlands (Eggens and Vethaak, 1989). The prevalence of tumours in a variety of fish species ranged from 0 to 32% for hepatic tumours, from 0 to 58.6% for epidermal papillomas, from 0.01 to 2.8% for oral papillomas, from 0 to 47.3% for chromatophoromas, from 0.1 to 16% for lymphosarcomas, and from 0 to 11.4% for pseudobranchial tumours. More limited observations were made available in individual studies on epidermal carcinomas (0.03%), fibrosarcomas (0.7%), lipomas and osteomas (0.05%). It is clear that, in analogy to mammals, all these figures are affected by genetically determined interspecies, interstrain, and interindividual variations, as well as by environmental factors.

Table 10

Historic and significant occurrences of neoplastic lesions in marine bony fish from North America and the Pacific basin. Reproduced from Couch (1989)

HOST SPECIES	NEOPLASTIC LESIONS	GEOGRAPHICAL LOCATION	SOURCE
Genyonemus lineatus (white croaker)	Oral papillomas, epidermal papillomas, hepatic neoplasms	Southern California	Russel and Kotin, 1957; Young, 1964; Mearns and Sherwood, 1974, 1977; Malins *et al.*, 1988
Pseudopleuronectes americanus (Dover sole)	Epidermal papillomas	Southern California	Young, 1964; Mearns and Sherwood, 1977
Mugil cephalus (striped mullet)	Fibrosarcoma	Northern Gulf of Mexico	Edwards and Overstreet, 1976
Pleuronectids (flatfish)	Epidermal papillomas; lesions of internal organs, incl. hepatic neoplasms	Puget Sound	Stich *et al.*, 1976; McCain *et al.*, 1977, 1982, 1988; Pierce *et al.*, 1978; Malins *et al.*, 1980, 1982, 1984, 1988
Pleuronectids (flatfish)	Epidermal papillomas	Japan, Hokkaido Island	Oishi *et al.*, 1976; Stich *et al.*, 1977a, 1977b
Microgadus tomcod (Atlantic tomcod)	Hepatic neoplasms	Hudson River estuary	Smith *et al.*, 1979
Microgadus proximus (Pacific tomcod)	Hepatic neoplasms	Puget Sound	Malins *et al.*, 1980, 1982
Nibea mitsukurii (nibe croaker)	Chromatophoromas	Japan	Kimura *et al.*, 1984
Leptocottus aramatus (Pacific staghorn sculpin)	Hepatic neoplasms	Puget Sound	Malins *et al.*, 1984
Fundulus grandis (Gulf killifish)	Chromatophoroma	Northern Gulf of Mexico	Couch, 1985
Pseudopleuronectes americanus (winter flounder)	Hepatic neoplasms	Boston Harbour	Murchelano and Wolke, 1985

The tumours observed in fish have been variously ascribed to genetic factors, infective agents (viruses, parasites, or unspecified agents), specific chemical compounds, or pollution in general. Often, no association with suspected etiological agents could be pointed out. The suspected origin varied with the tumour type. Thus, viruses were suspected of being responsible for all lymphosarcomas, which is consistent with the notion that RNA viruses cause lymphomas and sarcomas in mammals. Infective agents were also suspected to play a role in almost half of the studies concerning epidermal papillomas. Liver tumours were conversely ascribed in almost all studies to pollution of seawater and/or sediments. Exposure of the liver to ingested substances has been associated, both in experimental and field studies, with various liver diseases, including hepatic neoplasms, such as hepatocellular carcinoma and cholangiolar carcinoma, and other hepatic lesions, such as cholangiofibrosis (adenofibrosis), spongiosis hepatitis, extreme fatty degeneration, and necrosis (Couch, 1989). The numerous studies carried out over the past 15 years in bottom-dwelling species having restricted territorial habits, such as Pleuronectids (flatfish), caught from the Puget Sound body water, are paradigmatic to this respect. It is demonstrative that none of the more than 300 specimens collected from unpolluted areas had liver tumours, whereas the 1.1-32.3 % of the specimens collected from Puget Sound areas receiving urban and industrial discharges was affected by the disease. A large variety of inorganic and organic chemicals was detected in the sediments of polluted areas, which, especially in the case of aromatic hydrocarbons, correlated with the prevalence of hepatomas. Moreover, the prevalence of hepatomas also correlated with the concentration of PAH metabolites in fish bile (Krahn *et al.*, 1986).

4.1.3 Mutagenicity and other related effects

A large number of short-term tests, predictive of carcinogenicity and of genetic defects in the progeny, has been developed during the past two decades. These tests, evaluating various types of DNA damage or other end-points (e.g. cell transformation), have proven useful both as screening tools for hazardous substances and as experimental models for understanding their mechanisms. In addition, these and other laboratory methodologies have been exploited to provide biological exposure indices, i.e., for monitoring exposure of humans or other living organisms to genotoxic agents and, in some cases, for assessing mutagenic and carcinogenic hazards. It is likely that they have a similar connotation for marine organisms.

4.1.3.1 Detection of mutagens in seawater, sediments, and marine organisms

Various concentration methods, which have been more often used for freshwater, drinking water, or discharge effluents, have been also occasionally used for concentrating genotoxins from seawater, which thereafter have been assayed for mutagenicity in bacterial test systems. Limited surveys are also available for the Mediterranean. For instance, concentrated hexane extracts of seawater in the North Adriatic, 50m from the Yugoslav coast, induced a weak mutagenic response following metabolic activation with carp liver post-mitochondrial fractions, whereas a sample collected 500 m offshore was negative (Kurelec *et al.*, 1979). A weak direct mutagenicity was observed following concentration of chlorinated seawater collected from a power station at Tel Aviv by means of reverse phase silica C18 resins (Rav-Acha *et al.*, 1989). Estuarine waters and Tyrrhenian seawaters collected in the port of Leghorn and along the Tuscany coast, concentrated by means of Sep-pak C18 cartridges, displayed a direct mutagenic activity (Migliore *et al.*, 1989). Conversely, the blue cotton method (Hayatsu, 1990) was successful in concentrating aromatic amines from experimentally contaminated seawater, but failed to concentrate detectable mutagens both from an unpolluted area in the Ligurian Sea and from a polluted area in the port of Genoa (Bagnasco *et al.*, 1990).

Short-term test systems can be also used in order to detect the mutagenicity of polluted sediments. For instance, sediment extracts from coastal areas off Barcelona exhibited a positive response in the *Salmonella*/microsome test (Grifoll *et al.*, 1988). Extracts of heavily contaminated sediments from Black Rock Harbor (Connecticut, USA) were mutagenic in *S. typhimurium* strains and induced SOS repair in *E. coli* following metabolic activation, and eliminated metabolic cooperation in Chinese hamster V79 cells, which is an indicator of potential tumour-promoting activity (Jackim *et al.*, 1989).

Assessment of mutagenicity in marine organisms provides a reliable index of exposure to mutagenic and potentially carcinogenic substances. Bivalve molluscs seem to represent ideal indicators of in situ exposure, and have been proposed as biomonitors of the concentration of mutagens from polluted seawater, e.g., along Wales coastal waters (Parry *et al.*, 1976), US Atlantic estuarine waters (Sparks *et al.*, 1981), and the Adriatic Sea (Frezza *et al.*, 1982). Ethanolic extracts of 3 mollusc species living in Southern Spain (Andalusia) displayed a direct mutagenicity of oxidative type both in *his⁻ S. typhimurium* and in *E. coli* strains tested for the Ara forward mutation assay (Rodriguez-Ariza *et al.*, 1990). Monitoring of the mutagenicity of fish bile, which contains metabolites of pollutants, also correlating with the prevalence of liver tumours (see section 4.1.2.2), has been also proposed for assessing exposure to mutagens undergoing biotransformation in the liver (Van Kreijl *et al.*, 1982).

4.1.3.2 Detection of carcinogen-DNA adducts in marine organisms

As discussed in section 4.1.1, the covalent binding to DNA of an electrophilic chemical species to form addition products (adducts) represents the primary critical event in cancer initiation (Miller, 1978). Therefore, measurement of carcinogen-DNA adducts provides a direct indicator not only of exposure but also of the genetic damage produced by a given carcinogen, which does not have to be indirectly presumed from the local environmental pollution nor inferred from occurrence of tumours. Detection of carcinogen-DNA or carcinogen-protein adducts has opened new perspectives in fields such as molecular epidemiology and molecular dosimetry, reflecting the actual DNA damage irrespective of the interspecies, interstrain, interindividual, or even intraindividual variations of mechanisms (e.g., toxicokinetic, metabolism, DNA repair) involved in the initiation of cancer. In the framework of the large variety of techniques assessing biological exposure indices (De Flora, 1990), several methodologies have been set up in order to detect carcinogen-DNA adducts, and some applications to the study of marine organisms are already available.

The simplest method involves treatment of animals with radioactive carcinogens, whose ability to bind DNA is then determined. Such a technique has been used to detect binding of the major benzo(*a*)pyrene metabolite, i.e., 7,8-dihydroxy-7, 8-dihydrobenzo(*a*)pyrene, to the liver DNA of the benthic fish species *Parophrys vetulus* (English sole) and *Platichtys stellatus* (starry flounder) (Varanasi *et al.*, 1986), as well as binding of aflatoxin B1 to the liver DNA of *Salmo gairdneri* (rainbow trout) and *Oncorhynchus kisutch* (coho salmon), leading to formation of 8,9-dihydro-8-(N7-guanyl)-9-hydroxyaflatoxin B1 and other minor adducts. The higher ability to form specific adducts reflected the more efficient cytochrome P450 metabolism and correlated with the greater susceptibility to aflatoxin B1 carcinogenicity (Bailey *et al.*, 1988). Likewise, inhibition by Aroclor 1254 of the formation of the same aflatoxin B1 adducts in rainbow trout correlated with Aroclor anticarcinogenicity (Shelton *et al.*, 1986) (see section. 2). Radiolabelled benzo(*a*)pyrene was also used in order to investigate DNA binding of its photoderivatives in sponges (Zahn *et al.*, 1981) (see section 3.2.4).

Other more recent methodologies use immunoassays with specific antibodies to DNA adducts, or measure specific fluorescence spectra, e.g., in the case of the benzo(*a*)pyrene-DNA adduct. The most advanced technique available today is the ^{32}P postlabeling procedure (Gupta *et al.*, 1982), which has the advantages of being extremely sensitive, detecting 1 adduct per 10^9-10^{10} nucleotides, and of detecting also DNA adducts formed by unknown carcinogens, which in some cases can be later identified by means of analytical procedures. Such a technique has been used to detect nucleotide adducts formed by a variety of bulky hydrophobic aromatic environmental compounds with the liver DNA of *Ictalurus nebulosus* (brown bullheads) sampled from Great Lakes tributaries. This fish is exposed to high levels of sediment bound polycyclic aromatic hydrocarbons, and suffers from an elevated frequency of liver cancer (Dunn *et al.*, 1987). Diagonal radioactive zones and several distinct spots, indicative of exposure to genotoxic compounds, were also detected at autoradiography in liver DNA of English sole sampled from contaminated sites of Puget Sound, WA, and of winter flounder sampled from Boston Harbour, MA. These adducts were absent in the DNA of English sole sampled from a site of very low contamination in sediments (Varanasi *et al.*, 1989).

The experimental exposure of the mussel *Mytilus galloprovincialis* to 2-aminofluorene resulted in the formation of two adducts in the DNA of the digestive glands, with a frequency of 1 adduct per 1-4 x 10^9 nucleotides. In contrast, exposure to benzo(*a*)pyrene did not produce any adduct or a very weak adduct spot, which reflects the already discussed (section 4.1.1) differential ability of mussel tissues to metabolize these two carcinogens (Kurelec *et al.*, 1988). In the same study, it was noted that, contrary to the lack of adduct spots in brown bullheads raised in clean aquaria (Dunn *et al.*, 1987), samples of DNA from mussel, carp and bream collected from apparently clean waters of the Adriatic Sea had one to several weak adducts (Kurelec *et al.*, 1988). This aspect was further expanded by Kurelec *et al.* (1989), who found 4 to 9 adducts to liver DNA in several freshwater fish species and in *Mugil auratus* caught from two areas of the Northern Adriatic Sea. A dominant feature of the fish DNA adducts was a species specificity. Surprisingly, within each species, the specimens caught from the unpolluted area gave practically identical adduct profiles as those seen in specimens caught from the polluted area (see section 3.1.1). It is noteworthy in this respect that the analyzed fishes had restricted territorial patterns (Kurelec *et al.*, 1989). A further study with *Mytilus galloprovincialis* pointed out the presence of 6 to 10 adducts in the mussel digestive gland DNA, irrespective of pollution. However, pollution-related DNA adducts were found in juvenile mussels collected from an oil refinery site (Kurelec *et al.*, 1990).

It has been suggested that, in addition to analysis of hepatic DNA, the ^{32}P postlabeling technique should allow the detection of DNA adducts in extrahepatic tissues such as blood and gonads, which may be useful in studies of impaired reproductive processes in fish (Varanasi *et al.*, 1989).

4.1.3.3 DNA damage and repair in marine organisms

Various techniques can be used in order to evaluate alterations of DNA molecules produced by the *in vivo* exposure of organisms to genotoxins, and its subsequent repair. An example of this kind of evaluation is provided by studies in the sponge *Tethya lyncurium* collected from Northern Adriatic Sea, experimentally exposed to benzo(*a*)pyrene in the presence of light (see section 3.2.4 for a thorough discussion on the problem of photoactivation), showing that DNA damage and repair in these organisms seem to differ from that of most eukariotes (Zahn *et al.*, 1983). In particular, double-stranded (ds) DNA purified from the sponge after treatment with benzo(*a*)pyrene was comprised between single

stranded (ss) fragments. Treatment with nuclease S1, which attacks ss DNA, cut the intact strand opposite to the positions where the other strand had been nicked, and fragments of ds DNA were characterized by means of electronmicroscopy. Under conditions of possible repair, ss breaks completely disappeared from sponge DNA in the course of 3 weeks, during which a substantial DNA synthesis was observed (Zahn *et al.*, 1983).

In a recent study, benzo(*a*)pyrene was found to cause similar patterns of DNA damage, as evaluated by measuring single strand breaks with the alkaline elution technique, in the haemolymph of the crab *Maja crispata* and in the liver of the fish *Gambusia affinis*. This finding could be variously interpreted, as a function of the interplay between benzo(*a*)pyrene metabolism (activation/detoxification), interaction of active metabolites with DNA, and efficiency of repair of DNA damage (Bihari *et al.*, 1989).

4.1.3.4 Cytogenetic alterations in marine organisms

Cytogenetic analyses can detect gross genomic alterations, concerning either the number and/or the structure of chromosomes, which can be visualized at the optical microscope. Since many years these techniques have been used for evaluating exposure to clastogenic agents, both *in vitro* and *in vivo*, under laboratory or field conditions. In spite of some technical difficulties, the assessment of chromosomal aberrations (CA), sister chromatid exchanges (SCE) and micronuclei (MN) has been also applied to marine vertebrates and invertebrates (reviewed by De Flora *et al.*, 1991b). SCE arise as a consequence of a reciprocal exchange of DNA fragments between sister chromatids, which can be detected in cells undergoing two replicative cycles in the presence of bromodeoxyuridine. MN may derive either from DNA fragments resulting from chromosomal damage and/or segregation of genetic material during mitosis.

A number of laboratory studies aimed at developing and validating cytogenetic techniques in aquatic organisms experimentally exposed to known clastogens. Cytogenetic changes in polychaetes (*Neanthes arenaceodentata*) were proposed as a model for marine genetic toxicology (Pesch and Pesch, 1980). Several studies were carried out in bivalve mussels which, as already discussed for other end-points, seem to be ideal bioindicators of exposure due to their ability to concentrate local pollutants. The frequency of MN in the gill tissue of *Mytilus galloprovincialis* was persistently enhanced following exposure to known clastogens (Gola *et al.*, 1986; Majone *et al.*, 1987, 1990; Migliore *et al.*, 1989; Scarpato *et al.*, 1990). Methodological aspects, such as staining techniques, were also investigated (Majone *et al.*, 1988). SCE frequency was tested in larvae of *Neanthes arenaceodentata* (Pesch and Pesch, 1980), in larvae (Harrison and Jones, 1982; Jones and Harrison, 1987) and gill tissue (Dixon and Clarke, 1982) of *Mytilus edulis*, and in developing eggs (Brunetti *et al.*, 1986) and gill tissue (Brunetti *et al.*, 1989) of *Mytilus galloprovincialis*. Negative results have been also reported. For instance, the organotin antifouling compound bis(tributylin)oxide failed to induce chromosomal aberrations and SCE in larvae of *Mytilus edulis* (Dixon and Prosser, 1986). Other experimental studies have investigated cytogenetic alterations in various fish tissues. For instance, SCE induction was evaluated in *Umbra limi* (Kligerman, 1979) and in *Notobranchius rachowi* (van der Gaag and van de Kerkhoff, 1985); MN in the erythrocytes of *Heteropneustes fossilis* (Das and Nanda, 1986); CA in various tissues of *Boleophtalmus dussumieri* (Krishnaja and Rege, 1982); CA and SCE in *in vitro* cultured lymphocytes of *Anguilla rostrata* and of *Opsanus tau* (Ellingham *et al.*, 1986), and in the hematopoietic tissue of the latter species, exposed *in vivo* (Maddock *et al.*, 1986), as well as in cultured lymphocytes of *Leptococcus armatus* (Zahour *et al.*, 1984). On the whole, MN seem to be more suitable than SCE as a cytogenetic end-point in the gill tissue, because MN may be observed during interphase, whereas SCE can be only detected during

metaphases, which are infrequent in the gill tissue. In fact, only a low fraction of the gill cell population undergoes mitosis, as assessed in both mussels (Dixon and Clarke, 1982; Brunetti *et al.*, 1989) and fish species (Kligerman 1979; Alink *et al.*, 1980; van der Gaag and van der Kerkhoff, 1985). Mussel developing eggs are conversely a quite appropriate target for SCE induction, because they contain a population of actively proliferating cells with frequent mitoses (Brunetti *et al.*, 1986 and 1989). Use of antikinetochore antibody allowed the distinction between micronuclei resulting from acentric fragments or from lagging chromosomes in *Mytilus galloprovincialis* cells (De Flora *et al.*, 1991b).

Cytogenetic techniques have already been applied for monitoring fish or mussel exposure to pollutants under field conditions, or for assessing chromosomal alterations following exposure in the laboratory to polluted water. Induction of CA was observed in fish eggs and larvae collected from polluted areas of the Atlantic US coast (Longwell and Hughes, 1980). Increased SCE frequencies were observed in the worm *N. incisa* sampled from feral populations living on polluted sediments (Jackim *et al.*, 1989). The MN and SCE frequencies were monitored in the gill tissue of *Mytilus galloprovincialis* collected from coastal waters in Northern Italy, namely the Lagoon of Venice (Northern Adriatic) and the Gulf of La Spezia (Ligurian Sea) (Brunetti *et al.*, 1988). The frequency of MN and DNA single-strand breaks were significantly enhanced in the gills of *Mytilus galloprovincialis* collected from the Port of Genoa (Ligurian Sea), as compared with a reference area, which correlated with an enhanced concentration of PAH in the same tissue (Bolognesi *et al.*, 1990). Scarpato *et al.* (1990) collected adult mussels from a station in the Gulf of La Spezia, and transferred part of them into the port of Leghorn and the estuary of Fiume Morto (Dead River) in the Tyrrhenian Sea. MN in gill cells of mussel exposed to these polluted waters were significantly enhanced, compared to mussels kept in the original station, since the 2nd week of exposure and persisted for at least 16 additional weeks.

On the whole, cytogenetic analyses of natural fish or mussel populations provide a valuable biomonitoring end-point. However, several factors affect such evaluation, such as age, which often cannot be inferred, size of animals, which is also influenced by nutrients other than pollutants, and selection due to toxic pollutants leading to an apparent decrease of chromosomal damage in the surviving population (Brunetti *et al.*, 1989). Field monitoring may be also affected by the considerable interindividual variability, which has been pointed out, e.g., for SCE frequencies in unexposed larvae of *M. edulis* surveyed over a 2-year period (Jones and Harrison, 1987).

A target organism of special interest is the sea urchin. Cytogenetic alterations, which can be produced by genotoxicants in treated embryos or in embryos following adult or gamete exposure are one of the various sublethal end-points which can be monitored in these cosmopolitan metazoan systems. Mitotic aberrations investigated in the sea urchin include stray chromosomes, attached fragments, bridges, multipolar spindles, and acentric fragments (Pagano *et al.*, 1982a, 1982b, 1986; Hose and Puffer, 1983; Hose *et al.*, 1983; Hose, 1985; Dinnel *et al.*, 1988). Induction of MN has been also reported following exposure of the purple sea urchin to environmental levels of benzo(*a*)pyrene (Hose *et al.*, 1985).

4.1.4 Teratogenic effects

In its narrow sense, teratogenicity refers to the effects of any xenobiotic chemical or environmental condition on structural or functional development in the embryo/foetus (Lansdown, 1990). Some short-term tests for teratogens have been set up in aquatic organisms. In particular, fish embryos of various species, such as the Japanese medaka, zebra fish, rainbow trout, and fathead minnows, have been used in order to evaluate the

effects of chemicals on the development of eggs and embryos (Faustman, 1988; Anderson, 1990). The monitored end-points included lethality, specific malformations (mainly of the skeletal system), growth retardation, delayed hatching, and functional abnormalities, such as impaired swimming activity. A variety of compounds, including metals, pesticides, and complex mixtures, have been tested for developmental toxicity (reviewed by Faustman, 1988).

Among invertebrates, end-points relevant to teratogenesis have been investigated in brine shrimps, such as *Artemia salina* nauplii (Kerster and Schaffer, 1983; Sleet and Brendel, 1985), and especially in the sea urchin. Several species, including those living in the Mediterranean (e.g. *Paracentrotus lividus*, *Sphaerechinus granularis*, *Psammechinus microtuberculatus*), can be used for assessing teratogenic effects, in the framework of a large variety of parameters which can be monitored along the multiple life stages of these organisms. In particular, as detailed by Dinnel *et al.* (1988), sublethal exposures to toxicants can result in behavioural changes in sea urchin adults (food chemotaxis, righting response, predator avoidance), growth alterations (respiration, gonad maturation, spine regeneration, abnormal morphology), bioaccumulation (gonad concentrations, cytosolic concentrations, labeled protein incorporation into oocytes), and gamete effects (*in vivo* exposures during gametogenesis). Various developmental alterations can occur in embryos following exposure of adults, gametes, or embryos themselves, including retardation, abnormal morphology, reduced DNA synthesis and, as already reported in section 4.1.3.4, mitotic aberrations and micronucleus formation. *In vivo* exposure of adults or *in vitro* exposure of gametes can result in harmful effects on gametes, such as effects on development (sperm and/or egg exposure) and on fertilization (sperm motility, sperm morphology, oxygen consumption, chemotaxis, fertilization rate, membrane elevation) (Dinnel *et al.*, 1988).

The sea urchin bioassay was proposed for toxicity studies relevant to marine pollution by Kobayashi (1971) and Hangström and Lönning (1973), and has been later used for testing a number of physical and chemical agents (reviewed by Pagano *et al.*, 1986). One of the techniques suggested for assessing the toxicity of effluents discharged into marine and/or estuarine waters is the sea urchin sperm cell fertilization test (Dinnel *et al.*, 1987), which has been also recommended by the US Environmental Protection Agency in the form of a standardized protocol (Nacci *et al.*, 1987). The sea urchin embryo and sperm bioassay (*Sphaerechinus granularis*) has been applied, within a multidisciplinary toxicological approach, for assessing the effects of sewage pollution in French Mediterranean coastal waters (Bay of Toulon). The results obtained provided evidence that the frequency of larval malformations correlated with the levels of exposure to municipal sewage (Pagano *et al.*, 1989).

4.2 Estimated risks to marine organisms

The substantial achievements made in recent years in the fields of carcinogenesis, mutagenesis, and teratogenesis have resulted in a deeper understanding of the mechanisms involved and in a refinement of the available methodological tools for risk assessment. Nevertheless, several aspects of both conceptual and practical relevance need to be further explored and better elucidated.

From the available evidence, it is apparent that marine biota living in areas contaminated by harmful pollutants, at least those which are recognized to be carcinogenic, mutagenic, and/or teratogenic in the same organisms, are more likely to develop adverse effects. As in humans and other terrestrial species, such a kind of exposure in aquatic organisms does not automatically imply the occurrence of pathological effects. Due to interactions of pollutants with the host defense machinery and to possible simultaneous

exposures to protective agents (see section 2), the resulting health consequences to marine organisms should be viewed in terms of an increased risk in exposed, as compared to unexposed, individuals.

However, in the light of our present knowledge, any attempt to estimate and to predict specific risks for marine organisms living in the Mediterranean would be scientifically questionable for a variety of reasons, which have been discussed in previous sections of this document. They can be summarized as follows:

(a) The uncertainties and incompleteness of the present tentative identification and classification of carcinogens, mutagens, and teratogens in the marine environment as a whole (3.4).

(b) The lack of a systematic monitoring network of harmful pollutants in Mediterranean waters, sediments, and biota (4.1).

(c) The changes in biological properties of pollutants resulting from physical factors (3.2.1), microbiological transformations (3.2.2), chemical interactions (3.2.3), and light-mediated transformations (3.2.4).

(d) The possible impact of pollution at a distance from apparent sources, as a consequence of air/sea exchanges (3.3), and especially of bioaccumulation phenomena and food-chain biomagnification processes in migratory species (3.2.5).

(e) The considerable limitation of local surveys, and the paucity of scientific data available on adverse health effects in Mediterranean biota (4.1).

(f) The difficulty of assessing dose-effect relationships, and of extrapolating from high doses (experimental) to low doses normally encountered in the environment. This is a general drawback in toxicological studies and especially in the prediction of long-term effects.

(g) The marked variations in susceptibility to harmful chemicals, not only among different phyla, species, strains, and individuals, but even within the same individual, depending on the stage of life cycle, to circadian and seasonal cycles, to adaptive responses to pollutants, or to dietary or environmental factors other than pollutants (4.1).

(h) The still unidentified role of natural components of seawater as a confounding factor in the determination of certain adverse effects (3.1.1). For instance, in some studies, e.g., those concerning carcinogen-DNA adducts in both vertebrates and invertebrates (4.1.3.2) or neoplasms in molluscs (4.1.2.2), no differences were recorded between organisms sampled from apparently clean areas, and those sampled from polluted ones.

5. ASSESSMENT OF RISK TO MAN

5.1 General considerations

Toxicology is concerned with the recognition of the type of damage that may be produced by a particular chemical (hazard identification) and the likelihood that this damage may result from a particular type of exposure to the chemical (risk assessment).

In man and other mammalian species, environmental chemicals may enter the organixm by 3 major routes - ingestion (in water and food), inhalation or percutaneous absorption. On entry, the chemical is quickly distributed throughout the body, is metabolized and then excreted. Most chemicals are rapidly excreted, but some chemicals are disposed of with difficulty and are stored in the body, particularly in bone and body fat. Some storage in internal organs (e.g., liver and kidney) may take place.

Metabolism is an important step in rendering chemicals harmless but, in some instances, an intermediary toxic metabolite may be produced. Some chemicals do not need to be metabolized to exert a toxic effect.

Four major areas of hazard are recognized - systemic toxicity, mutagenicity, carcinogenicity and reproductive toxicity.

Systemic toxicity usually results from the accidental or intentional ingestion of a high dose of a particular chemical. In man such accidents occur from industrial exposure or from accidental contamination of food with large amounts of a toxic substance. Occasionally, large doses of chemicals may be ingested intentionally in an attempt to commit suicide. Episodes of this sort give some idea of the tolerance level in man for a particular substance.

Animal toxicity studies are conducted if human data on the systematic toxicity of a substance are inadequate or not available. There is a range of such studies from acute, single dose ones, such as LD_{50} to short-term (14-28 days) or longer-term studies lasting 90 days or up to one year. Studies of this sort are usually conducted in rodents but other species, particularly dogs, are used where appropriate.

Systematic toxicity data provide information on what organ or organs are affected by the chemical (target organ) and the smallest dose at which adverse effects are observed. From date of this sort, some indication of safe levels, e.g., ADI or PTWI may be inferred.

Mutagenicity is the ability of a chemical to induce changes in the genome (DNA) which are transmissible to the offspring. This property is assessed from *in vitro* tests on bacteria, fungi, yeasts, etc., and from *in vivo* tests in mice or rats. Test systems for the detection of mutagenicity in man are under development. Experience has shown that most animal and human carcinogenic chemicals are mutagenic in one or more of the tests mentioned. Such mutagenicity and related tests are employed as "short-term" tests to predict carcinogenic activity and other pathologic effects dependent on genomic changes either in somatic or germ cells.

Carcinogenicity is the term usually employed to indicate the property of a chemical to cause cancer in man or animals. Because cancer takes a long time to develop in man, it may not be possible to associate causally the development of a cancer with exposure to a specific chemical in any one individual. To overcome this difficulty, epidemiological studies on groups of people are conducted. These studies can often suggest some association

between an increased incidence of a particular type of cancer and exposure to some chemical or a mixture of chemicals.

Conclusive proof of a causal association between cancer and a suspected chemical can very rarely be provided by epidemiological studies, so that additional evidence is sought in the results of carcinogenicity studies in experimental animals, chiefly rats and mice. Animals, like man, are susceptible to cancer production, particularly as they approach old age. They also have been shown to be susceptible to cancer production by those chemicals which are known to be carcinogenic in man. Although interpretation of experimental carcinogenicity studies is often difficult, there is little doubt that useful information on carcinogenicity hazards is provided by investigations of this type. In general terms, the points looked for in carcinogenicity studies are:

1. dose-response relationship;

2. reduction of latency;

3. the biological nature of the tumour, whether benign and malignant, and

4. whether the carcinogen is genotoxic or not in short-term tests.

In carcinogenicity studies it is customary to administer the test chemical at very high doses to animals in order to ensure adequate exposure. By contrast, the concentrations of chemicals found in the environment to which man is exposed are very low. There is some discussion that, even at low levels, carcinogens may present a risk although, admittedly, a small one. This is particularly applicable to the so-called "genotoxic carcinogens" but is less so for the non-genotoxic carcinogens where calculation of a safe dose from a no-effect level is thought to be appropriate (Grasso *et al.*, 1991).

An estimate of the hazard presented by low doses of carcinogens is usually carried out by expert national and international committees on the basis of an acceptable risk usually cited to be one cancer in 10^8 of population. This approach is upheld by WHO (1989). An alternative approach, advocated by some environmental agencies, is to use mathematical formulae to estimate the incidence of tumours at low doses beyond the range of experimental observation from the tumour incidence obtained by the high doses employed in animal studies. Such mathematical estimates are known as "high dose-low dose extrapolation" and have been strongly criticised because they are based on unverifiable assumptions (e.g., "multihit" vs "one hit" hypothesis for cancer production; man is as sensitive as the rat to chemical carcinogens; shape of dose-response curve below the experimentally verifiable dose-levels). Because of these uncertainties, a very large number of mathematical formulae have been proposed. When a number of these formulae were applied to a particular set of date in order to estimate an acceptable dose, the figures obtained varied by several orders of magnitude (Butterworth, 1989). Experiences of this sort have discouraged the universal use of such formulae.

No attempt of mathematical extrapolation has been made in this document and the level of an acceptable dose is based on estimates made by various national and international committees.

Reproductive toxicology involves effects on fertility, foetotoxicity and teratology (foetal malformation). Such tests are usually conducted at high levels to maximise exposure, and

a safe exposure level is usually estimated from a no observable effect level (NOEL) in the experimental model.

Because of a lack of information of mutagenic and teratogenic effects in humans attributable to marine sources, the present section deals mainly with carcinogenic effects. Even in this area, information of risk to man is virtually non-existent and recourse to data from other sources had to be made for a tentative risk assessment.

5.2 Evaluation of priority pollutants

The following pollutants were accorded priority by the Consultation on carcinogenic and mutagenic marine pollutants in the Mediterranean, held in Athens in June 1989 to finalise arrangements for the pilot monitoring project referred to in previous sections, and to agree on the outline scope and content of the present document:

- Arsenic
- Polycyclic aromatic hydrocarbons (PAHs)
- Polychlorinated biphenyls (PCBs)
- Polybrominated biphenyls (PBBs)
- Toxaphene
- Mirex
- Dichlorodiphenyltrichloroethane (DDT)
- Hexachlorobenzene (HCB)
- Hexachlorocyclohexane (HCH)
- Nitrilotriacetic acid (NTA) and its salts
- Low molecular weight halogenated hydrocarbons
- Polychlorinated dibenzodioxin (PCDD) and polychlorinated Furans

In each case, the assessment of carcinogenicity to man is based on the results of short-term tests, animal carcinogenicity assays and epidemiological studies. Routes of exposure, and exposure levels, other than those directly related to the marine environment are also considered, to provide an indication of (a) the total exposure burden, and (b) the possible contribution to such from marine sources. Limits in the form of acceptable daily intakes recommended by various bodies are given in Table 11. It should be noted that such intakes take into account the sum total of health effects, not carcinogenicity risks alone.

5.2.1 Arsenic

Arsenic is widely distributed in the world's crust mainly as arsenides of copper, nickel and iron. The concentration of Arsenic is particularly low in seawater where arsenate prevails over arsenite, and tends to be metabolised by bacteria and fungi to form methylated derivatives (De Renzi *et al.*, 1989). Despite the low levels of Arsenic compounds found in seawater (0.001-0.008 mg/ℓ) (Penrose *et al.*, 1977) fish, and especially crustacea, contain appreciable amounts of this element in their tissues (0.2-200 mg/kg). In marine organisms, Arsenic is converted to organic forms so that inorganic Arsenic rarely exceeds 1 mg/kg and constitutes only 2-10% of the total Arsenic content in seafood (De Renzi *et al.*, 1989).

Despite its toxicological importance, no *in vitro* mutagenicity results were available to an IARC working group (IARC, 1980). A carefully conducted dominant lethal test in mice was negative (IARC, 1980). However, Arsenic compounds were found to induce an increased lethality in repair-deficient bacteria and chromosomal anomalies in *Drosophila* and in mammalian cells (De Renzi *et al.*, 1989).

Table 11

Acceptable daily intakes for selected chemicals

CHEMICAL	LIMITS OF TOLERABLE OR ACCEPTABLE ORAL INTAKE	AUTHORITY
Arsenic	0.015 mg/kg bw/week or 0.105 mg/person/week	JECFA 1989
PAHs	10-30 µg/person/day*	WHO 1989
PCBs	0.1-40 µg/person/day	Various countries in WHO 1985
PBB	20 µg/person/day	NTP 1982
Toxaphene	Calculated to be about 100 pg/person/day*	From EPA 1976, 1987
Mirex	Calculated to be about 7 µg/person/day*	From EPA 1976
DDT	0.02 mg/kg bw/day or 1.4 mg/person/day	FAO/WHO 1985
HCB	0.6 µg/kg bw/day or 42 µg/person/day	WHO 1975
HCH	1.8 mg/person/day	EPA 1988
PCDD	No ADI but a figure of 60 µg daily intake considered tolerable by UK Government	HMSO 1989

* **Estimated intake - not necessarily regarded as tolerable (see text).**

According to IARC (1980) carcinogenicity tests in animals given Arsenic by a variety of routes did not reveal an increased incidence of tumours in any organ, but later studies claim that lung cancer in mice and a low incidence of respiratory tract cancers in hamsters had been induced by the intratracheal instillation of Arsenic trioxide (IARC, 1987).

There is both anedoctal and epidemiological evidence that Arsenic is carcinogenic to man, producing tumours of lung, skin and liver (IARC, 1980). Tumours of the lung occur from occupational exposure to Arsenic. According to Blejer and Wagner (1976), the "no effect level for lung tumours might lie in the very low microgramme range (1-40 µg/m^3 of respirable Arsenic)".

Epidemiological data collected from Taiwan afford a reliable guideline for assessing risk of cancer production by the oral route. The level of Arsenic found in the drinking water of the population exhibiting a high rate of skin cancer was 0.01 to 1.8 mg/litre (Tseng *et al.*, 1968). The level of Arsenic in control populations with the expected ("background") levels of skin cancer was approximately a tenth of the lowest dose causing skin cancer to man (0.001 to 0.002 mg/litre) (Tseng, 1977).

Liver tumours occurred in some vineyard workers exposed to high doses of pesticides containing Arsenic (IPCS, 1981), and in some patients treated with medicinal preparations containing Arsenic (IARC, 1987).

Both Arsenic (III) and Arsenic (V) are toxic to man. Acute effects include profound gastro-intestinal damage, resulting in severe vomiting and diarrhoea, muscular cramps and cardiac involvement (IPCS, 1981). The fatal dose of Arsenic (III) oxide has been reported to be in the range from 70 to 180 mg (Vallee *et al.*, 1960).

Arsenic is teratogenic in mouse, rat and hamster when given parenterally at high doses (IARC, 1980). It was embryotoxic but not teratogenic when given orally (IARC, 1980).

Two studies conducted by Nordstorm *et al.* (1978, 1979) claimed that there was an increased incidence of abortions and malformations in the offspring by women who either worked in smelters or lived close to a smelter. Emissions from these smelters contained other metallic components, so that it is impossible to say whether Arsenic played a causative role.

In unpolluted areas, intake by inhalation is 0.05 $\mu g/m^3$ or less; near power stations or smelters it is about 1 $\mu g/m^3$ (IPCS, 1981). Aerosols from sea-spray contribute to the intake of Arsenic in areas close to the seashore, but the amount absorbed from these sources is very small (De Renzi *et al.*, 1989).

In the USA, occupational exposure to an upper limit of 10 $\mu g/m^3$ (TWA - 8 hour period) is tolerated for inorganic Arsenic and 0.5 mg/m^3 for organic Arsenic (US, OSHA, 1979).

Levels in drinking water vary; concentrations up to 1.8 mg/litre have been recorded. Average levels are considerably less than this amount.

The WHO International Standard for inorganic Arsenic in drinking water is 0.05 mg/litre (WHO, 1971). In the European Community a limit of 0.1 mg/litre (max) is tolerated in drinking water.

In 1989, JECFA assigned a provisional tolerable weekly intake (PTWI) of 0.015 mg/kg bw for inorganic Arsenic, at the same time drawing attention to the fact that the safety margin is narrow since this intake is close to the levels which are known to cause skin disorders and cancer in man. JECFA also noted that exposure to inorganic Arsenic levels which do not cause arsenicism do not appear to carry a carcinogenic risk (WHO, 1989). Organic arsenicals do not appear to be a cause for concern (WHO, 1989).

According to GESAMP (1991) the PTWI is not exceeded by the consumption of 150 g/seafood/day/7 days/week even if the concentration of Arsenic in seafood is as high as 10 µg total Arsenic/g. In terms of wet weight, a number of fish samples analysed in the Mediterranean exceed this level. On the other hand, since most of the Arsenic in seafood is in the organic form, the risk to health is not as great as it would have been if the Arsenic were only in inorganic form.

5.2.2 Polycyclic Aromatic Hydrocarbons (PAHs)

PAHs are mainly formed by the incomplete combustion of organic materials, particularly fossil fuels. A small proportion appear to be formed by naturally decaying vegetation (Grasso, 1984). The carcinogenicity to humans of some PAHs and PAH-containing products is given in Table 12. All carcinogenic PAHs are also mutagenic in a variety of mutagenicity tests (IARC, 1983).

PAHs with four to seven fused rings have been shown to be carcinogenic to experimental animals particularly by skin painting. A few PAHs (3-methylcholanthrene, dimethylbenz(*a*)anthracene, benzo(*a*)pyrene and dibenz(*ah*)anthracene) have produced tumours when given in the diet. These include mammary adenocarcinoma, squamous cell carcinoma of the stomach, spindle cell sarcoma and leukaemia (Lo *et al.*, 1978).

Table 12

Carcinogenicity of Some PAHs and PAH-containing Products

1	2A	2B	3
Coal tar Mineral oils (unrefined)	Benzo(*a*)pyrene Dibenz(*ah*)anthracene	Benzofluoranthenes Dibenzaieridines Dibenzopyrenes	Anthracene Anthanthrene Benzofluorenes Benzo(*e*)pyrene Pyrene

Group 1 = carcinogenic in man
" 2A = probably carcinogenic in man
" 2B = possibly carcinogenic in man
" 3 = unclassifiable

From IARC (1987)

Although products containing PAHs have caused skin tumours in man from direct contact and lung cancer from the inhalation of fumes rich in PAH, there does not appear to be any epidemiological evidence that dietary PAHs make any appreciable contribution to the risk of human cancer (IARC, 1983). Attempts to link the high incidence of stomach cancer in Japan with PAH carcinogens in food are not convincing since other factors such as bracken, a delicacy in Japan, may be equally responsible (Grasso, 1984). Egually unconvincing is the suggestion that the high incidence of stomach cancer in Iceland may be caused by the high intake of smoked fish and meat (Lo *et al.*, 1978) since other areas of the world with a comparable intake of PAHs do not have a high incidence of stomach cancer. In addition, despite the apparently widespread distribution of PAHs in edible vegetables, there is no indication that vegetarians have a higher incidence of stomach cancer than the rest of the population.

The principal route of exposure to PAHs is from inhaling contaminated air either through urban pollution or cigarette smoking. The amounts inhaled vary considerably. Typical exposure levels of BaP (commonly regarded as a marker of total PAH) from urban air may vary from 0.05 $\mu g/m^3$ to 74 $\mu g/m^3$ (Hoffman and Wynder, 1976). From cigarette smoke it may vary from 25-252 µg/100 cigarettes in smokers and 10-1010 ng/m^3 in cigarette smoke polluted environment (IARC, 1983).

Food and drinking water are next in importance in contributing to human exposure to PAH. The total oral intake of PAH from these sources (based on BaP concentration) is estimated to be 1.6-16 µg/day/person in the USA (IARC, 1983). The contribution of seafood to this figure varies considerably depending on the amount of fish and other marine organisms in the diet and on the concentration of PAHs in their tissues.

In general, fish from unpolluted waters do not contain detectable amounts of PAHs (Lo, 1978) but, in areas polluted with PAH, it has been stated that they may contain 1,000-100,000 times that in fish in clean water (Weldre *et al.*, 1977; IARC, 1983). The situation in the Mediterranean areas covered by the recent pilot monitoring project is shown in Tables 4 and 7.

No ADI has been set for the total intake of PAH, but there are some acceptable levels of PAH in drinking water. The US Environmental Protection Agency has published an acceptable concentration for BaP in drinking water of 0.028 µg/litre (GESAMP, 1991) while the WHO (1984) guideline for BaP in drinking water is 0.01 µg/litre representing 0.1-0.3% of total PAHs ingested calculated to be 10-30 µg/person/day. In these calculations it is assumed that BaP is a marker for total PAHs. Rugen (1989) has proposed that acceptable concentrations of other PAHs should be based on the BaP concentration with a factor derived from the relative carcinogenic potency of BaP.

According to GESAMP (1991) consumption of 150 g/day of fish or shellfish from unpolluted water and containing about 1 µg/kg PAHs would not appreciably increase the dietary intake of PAH. Consumption of fish (particularly shellfish) from polluted waters would appreciably increase the PAH intake and could present an unacceptable risk. In this context, the sum-total of individual PAHs recorded simultaneously in the same specimens of mussels are well in excess of the above-quoted level in certain areas of the Mediterranean (*vide* Tables 4 and 7).

5.2.3 Polychlorinated Biphenyls (PCBs)

Polychlorinated biphenyls (PCBs) have been widely used in electrical equipment such as capacitors and transformers and, to a lesser extent, in hydraulic and heat exchange appliances. These are usually regarded as enclosed systems and while in service they are unlikely to lead to a significant contamination of the environment. Improper collection, storage, transportation and disposal of PCB-containing wastes may, however, result in an increased environmental contamination (WHO, 1988).

PCBs gave negative results in several *in vitro* mutagenicity tests, but 4-chlorobiphenyl is reported to have induced DNA repair in CHO cells (IARC, 1987). Lymphocytes from a group of 15 workers acutely exposed to PCBs showed no increased incidence of chromosomal aberrations or Sister Chromatid Exchange (SCE) (Elo *et al.*, 1985).

Carcinogenicity studies in animals revealed that mixtures of PCBs containing a significant amount of more highly chlorinated homologues produced a high incidence of hepatocellular carcinomas in rats when given at dietary levels in the range of 100-1,000 mg/kg. Hyperplastic nodules appeared in the liver at 25 mg/kg in rats and mice (NCI, 1978; Norback *et al.*, 1985). In one experiment, Aroclor 1254 produced few liver tumours in rats but increased the incidence of adenocarcinoma of the stomach and of intestinal metaplasia (Ward, 1985).

In man the principal effects of prolonged exposure to PCBs are chloracne, a disfiguring skin disease, neuropathy and liver toxicity. These effects were observed under conditions of occupational exposure to 0.1 mg/m^3 - 1.44 mg/m^3 for 5-14 months (Meigs *et al.*, 1954) and after the ingestion of rice oil accidentally contaminated with PCBs (total dose 633 mg over a period of a few weeks) and PCDFs (3.4 mg) and PCQ (596 mg) (Habayuchi *et al.*, 1979).

PCBs, like other chlorinated hydrocarbons, are readily stored in fat and are removed very slowly (WHO, 1988).

Several epidemiological studies have been carried out on groups of workers occupationally exposed to PCBs. Although in some studies a higher than expected incidence of various types of cancer was found, no firm conclusions could be drawn because important confounding factors, e.g., exposure to other chemicals, could not be excluded. In one study where exposure was principally or exclusively to PCBs, the result was negative (Brown, 1987; Shalat *et al.*, 1989; WHO, 1988). Claims that babies born from women exposed occupationally to PCBs had low birth weight or showed behavioural changes were unsubstantiated (Fein *et al.*, 1984).

It would appear that seafood is the principal source of PCBs for whole populations. Intake can be quite variable, depending particularly on the amounts and source of freshwater or marine fish consumed (WHO, 1988; GESAMP 1991). A review of reports of PCBs in seafood (GESAMP, 1991), indicates that the edible parts of vertebrate fish may contain several hundred µg/kg PCBs. Mediterranean data are shown in Tables 3 and 5. In the USA a tolerance level of 5,000 µg/kg PCB was set and later reduced to 2,000 µg/kg (Mearns, 1988). According to Bennett (1983) dietary intake of PCBs has been estimated to range from 5-100 µg/day with a mean intake of 24 µg/day. Acceptable mean daily intakes from food for PCBs are given below (WHO, 1985):

COUNTRY	µg/Day/Person
Netherlands	< 11.6
Germany	< 6.4
United Kingdom	< 40.0
Canada	< 0.1
Japan	< 15.0
U.S.A.	< 0.1 - 1.9

Intake of PCBs from air and water is comparatively much smaller than that from food. For example, the PCB content in air varied from < 36 ng/m^3 (Holland) to 1 ng/m^3 (Norway). Concentrations in drinking water are between 0.1-0.5 ng/litre.

An individual consuming 150 g of fish a day containing 1 µg/g of PCBs (which is higher than concentrations found in Mediterranean seafood) would ingest 150 µg of PCB (or 2 µg/kg bw approximately). This level is several orders of magnitude lower than the levels

which produced symptoms in man or adverse effects in experimental animals. To this, one could add that there is no epidemiological evidence of cancer in man produced by PCB exposure, and the liver cancer in animals are likely to have been produced by a non-genotoxic mechanism, thus giving some degree of reassurance that, at this level of intake, adverse effects are unlikely to occur in man.

Nevertheless, levels of about 2 µg/kg bw/day are much higher than acceptable mean levels of daily intake recommended by several countries (see above) so that some degree of caution has to be exercised before accepting levels of intake of this sort.

5.2.4 Polybrominated Biphenyls (PBBs)

PBBs like PCBs are extremely stable compounds and persist for a considerable time in the environment (e.g., soil, water) (Jacobs *et al.*, 1976) and in body fat (Stross *et al.*, 1979), and are encountered mainly in water and sediments close to the sites where they are manufactured. They are not mutagenic in *in vitro* and *in vivo* tests (IARC, 1986) but hepatocellular carcinomas were reported in mice and rats given 0.1-10 mg/kg bw of hexabromobiphenyl (Fire Master FF1). The increased tumour incidence occurred at the 3.0 and 10 mg/kg bw levels in rats and at the 10 mg/kg bw level in mice (Gupta *et al.*, 1983; NTP, 1983; Kimbrough *et al.*, 1981). A low, non-statistically significant increase in hepatocellular carcinomas was found in the offspring of female Sherman rats treated with 200 mg/kg bw with Fire Master FF1 during pregnancy (Groce and Kimbrough, 1984).

In experimental animals, PBBs produce liver enlargement and are potent inducers of mixed function oxidase activity. They have a low order of acute toxicity (LD_{50} > 17g/kg bw/rat) (IARC, 1986).

High doses of PBB close to those producing maternal toxicity are toxic to the rat foetus and induce foetal malformations. Low doses do not produce effects of this sort (IARC, 1986).

A high prevalence of abnormal liver function tests was found in Michigan farmers exposed accidentally to fairly high levels of PBB. In addition, the levels of T- and B-lymphocytes were lower than expected and higher rates of musculo-skeletal, dermatological and neurological disorders were reported (Anderson *et al.*, 1978a, 1978b). However, no mention of an increase in the incidence of tumours occurred in this group of Michigan farmers or in a small cohort of workers occupationally exposed for several years (IARC, 1986).

In November 1974, the US FDA adopted acceptable upper limits of 0.3 mg/kg in the fat of milk, meat and poultry and 0.05 mg/kg in whole eggs (about 20 µg daily/man assuming a 60 g protein intake) (Di Carlo *et al.*, 1978; NTP, 1982).

It would appear that PBBs are, principally, a hazard close to areas where they are manufactured, used or stored. Like PCBs, they are not mutagenic, and are carcinogenic to experimental animals at high dose levels, hence intakes of the order of 20 µg daily are unlikely to cause adverse effects.

There is relatively little information on concentration of PBBs in seafood. Because of the similarity of toxic effects between PBBs and PCBs, it is desirable to keep levels of daily intake close to the figure regarded as acceptable by the FDA for terrestrial food. In this

context, levels recorded in mussels in certain Mediterranean areas were quite low (Albaiges and Bayona, 1991).

5.2.5 Toxaphene

Toxaphene is an insecticide chiefly used in North America against major cotton pests, but also used in other areas. It is present principally in river water receiving effluents from plants where it is manufactured or receiving runoff water from crops sprayed with toxaphene (IARC, 1979b). It is only slowly biodegradable (Nash *et al.*, 1973) and accumulates in body fat. It was found to be mutagenic to *S. typhimurium* without metabolic activation. The dominant lethal test, carried out orally and intra-peritoneally was negative.

Toxaphene is moderately toxic. The LD_{50} is 90 mg/kg bw in rats (Gaines, 1960). It induces various hepatic microsomal enzymes (Kinoshita *et al.*, 1966) and centrilobular hypertrophy of the liver (Ortega *et al.*, 1957). Toxaphene is not embryotoxic and is teratogenic at dose levels which induce maternal toxicity (IARC, 1979b).

Carcinogenicity of Toxaphene was studied in both rats and mice. In mice, time weighted average doses by the oral route of 99 mg/kg (low dose) and 198 mg/kg (high dose) for approximately 90-91 weeks, resulted in the production of a high incidence of heptocellular carcinomas in both low and high dose groups. In rats, time weighted average doses by the oral route of 1080 or 1112 mg/kg (female and male respectively) and 540 or 556 mg/kg (female and male respectively) produced a high incidence of tumours of the thyroid in the highest dose groups in both sexes. No hepatic tumours were produced (NCI, 1979).

In man, the acute lethal dose has been estimated to be between 2-7 g/person. No increase in tumour incidence was found in 199 employees who were or had been employed in the manufacture of Toxaphene between 1949 and 1977 for periods ranging from 6 months to 26 years (Ottoboni, 1977).

Tolerance levels of 0.1-7 mg/kg of Toxaphene have been established on fruit and vegetables and of 5 mg/litre in navigable water. Catfish from commercial ponds was found to contain an average concentration of 1.98 mg/kg (Hawthorne *et al.*, 1974).

According to EPA guidelines, maximum limits of 0.005 ppm, 6 ppm and 0.0007 ppm are tolerated in water, crude soya bean oil, raw agricultural products and meat, fish and poultry (EPA, 1976, 1987). Assuming a consumption of 500 g mixed food, then the tolerated intake of Toxaphene is about 100 pg daily.

The liver tumours induced by Toxaphene in the mouse are probably a consequence induced in the activity of mixed function oxidases (Grasso and Hinton, 1991). The thyroid tumours in the rate are probably due to a disturbance of thyroid hormone balance of the type induced by inducers of m.f.o. activity (Grasso and Hinton, 1991). In fact, Toxaphene was given a 2B category (i.e., probably carcinogenic in man) by IARC (1987). Nevertheless, because of the positive mutagenicity test in *S. typhimurium* one cannot exclude entirely some direct effect on the genome.

Taking into account the limited geographical areas in which it is used, and the limited agricultural application, it is unlikely that it will present a hazard to populations in general, but may do so to fishing communities close to areas of its commercial use.

5.2.6 Mirex

Mirex has been used as a pesticide and fire-retardant principally in the U.S.A. (IARC, 1979d), but also in other areas, including the Mediterranean. In areas close to where it has been sprayed, it enters into most items of food and persists for several months.

Tolerances for residues of Mirex in food products in the U.S.A. are as follows: 0.1 mg/kg in the fat of meat and 0.01 mg/kg in or on all agricultural raw material (U.S. EPA, 1976). On a mixed diet, this would be about 7 µg/person/day.

Mutagenicity tests in *Salmonella* were negative. A dominant lethal test was also negative.

Mirex was tested for its carcinogenicity in 2 strains of mice at a single dose level (10 mg rising to 26 mg/kg bw). A much higher incidence of hepatocellular carcinoma was found in treated compared with control mice (Innes *et al.*, 1969). Mirex was also fed to rats at levels of 50 or 100 ppm in the diet for 18 months. A dose related incidence of hepatocellular tumours was observed in the treated groups. No such tumours occurred in controls (Ulland *et al.*, 1977). In a more recent study, Mirex induced a statistically significant increase in tumours of the liver, adrenal medulla and transitional cell epithelium of the kidney (NTP, 1987).

Since Mirex is a powerful inducer of m.f.o. activity, it is likely that the liver tumours in rats and mice were induced by a non-genotoxic mechanism. In fact, IARC (1987) gave it a low category of carcinogenic risk to humans (2B).

Mirex is relatively non-toxic. The LD_{50} in rats is from 600-700 mg/kg bw. It causes liver enlargement at levels of 1 to 10 mg/kg, increased mixed function oxidase activity and fatty change. At high doses it induced hepatocellular necrosis (Kendall, 1974; Villeneuve *et al.*, 1977). At doses which produce maternal toxicity there was a reduction of foetuses and some foetal abnormalities. Lower doses were without effect (IARC, 1979d).

The highest residue levels recorded in the Mediterranean during the recent pilot monitoring study (*vide* Table 3) were 26.49 µg/kg in fish and 12.04 µg/kg in mussels (both in terms of fresh weight) (Kanitz *et al.*, 1990), which are lower than the amount allowed in the fat of animal meat in the U.S.A. Assuming a daily intake of 150 g of seafood containing 30 µg/kg of Mirex, then the daily intake would be approximately 4.5 µg/person which is lower than the tolerated intake from food, but which does not take other sources into consideration.

5.2.7 Dichlorodiphenyltrichloroethane (DDT)

DDT is a broad sprectrum insecticide, effective against a variety of insect pests. It is stable under most environmental conditions and is resistant to complete breakdown by the enzymes present in soil microorganisms and higher organisms. Its persistence in the environment is mainly due to the fact that it is soluble in fat and virtually insoluble in water (WHO, 1979).

DDT was not mutagenic in the *S. typhimurium* test or in fungi. It induced chromosomal aberrations but not micronuclei in bone marrow cells and spermatocytes of mice. No such effects were observed in the bone marrow cells or rats. It caused dominant lethal mutations in *Drosophila* but was negative in tests for chromosomal abnormalities, mutations or unscheduled DNA synthesis in human cells *in vitro* (IARC, 1987).

Epidemiological studies by Laws (1973) and WHO (1979) on heavily exposed workers did not reveal any evidence of carcinogenicity in man by DDT. Despite the small number of cases in these studies, the period of observation lasted several years, granting some credibility to the negative findings. 19 epidemiological studies on various population groups exposed to DDT were reviewed by IARC (1987). IARC came to the conclusion that the evidence for the carcinogenicity of DDT in man was inadequate (IARC, 1987).

DDT is a compound of moderately acute toxicity. The oral LD_{50} in the rat is 500-2,500 mg/kg bw and in the mouse it is 300-1,600 mg/kg bw in aqueous suspension. When dissolved in oil the LD_{50} is approximately 1/3-1/2 of the LD_{50} of DDT in aqueous suspension (WHO, 1979).

At dosage levels close to the lethal dose, adverse effects appear in the CNS and in the liver. The CNS effects consist of hyperirritability, tremors and convulsions. The hepatic effects consist of focal hepatocellular necrosis (WHO, 1979). At lower dosage levels, liver enlargement and stimulation of m.f.o. activity occurs in a dose-related fashion (Conney, 1967).

No ill effects in man have been reported from DDT exposure by the inhalation and dermal routes even under conditions of high level exposure such as spraying the interior of houses in anti-malarial campaigns or its liberal application in mass de-lousing of troops and civilians during World War II (WHO, 1979).

The only demonstrable effects of DDT in the general population are storage of the compound and some of its derivatives in the tissues and their excretion in urine and milk (Laug *et al.*, 1951; Denes, 1962). In the more heavily exposed an increased ability to metabolize DDT itself or a test drug (e.g., phenylbutazone) more rapidly than average was also demonstrable (Kolmodin *et al.*, 1969; Poland *et al.*, 1970).

Very high doses of DDT such as may be ingested by accident or with suicidal intent induce CNS changes characterized by incoordination, tremor, anaesthesia and convulsion. The lethal dose in man is not known but it would appear that doses in excess of 300 mg may cause toxic symptoms and loss of life (WHO, 1979).

Food represents the major source of intake of DDT in the general population. At the peak of DDT usage this intake was 0.04 mg/man/day, water contributed 1/1000 of this amount.

DDT exposure from food varies from one country to another. This variation is about three-fold but may be as high as ten-fold. The concentration of unmetabolized DDT in muscle tissue (meat) is, in many parts of the world, below level of detection (Villeneuve, 1987) but in heavily polluted areas may average 50 µg/kg. The concentrations in fish liver and liver oil are generally about 10 times those in muscle -the highest value found is 7000 µg/kg (Magos, 1989). Most concentrations of DDT products in shellfish appear to be below 100 µg/kg Magos (1989). Recent Mediterranean data is shown in Tables 3 and 6.

The allowable daily intake was set at 0.02 mg/kg bw (FAO/WHO, 1984).

DDT induced hepatic tumours in rats and mice at very high levels of administration. The mutagenicity studies were inconsistent suggesting that mutagenicity of DDT is either weak or equivocal. These results, together with the absence of any epidemiological evidence of carcinogenicity in man, would suggest that the carcinogenic hazard to man is very low

indeed. Calculations of daily intake provide some further reassurance. Thus assuming a consumption of 150 g/day of fish in a fishing community and an average of 50 µg/kg of DDT in fish (which approxi-mately corresponds with Mediterranean concentrations) the daily intake would be 0.125 µg/kg bw. This would constitute 1/160 of the acceptable daily intake.

5.2.8 Hexachlorobenzene (HCB)

HCB is used principally in the control of fungi which infect the seeds of onions, sorghum and wheat and as a wood preservative. The content of HCB in commercial grades varies from about 12-80% the remainder being impurities (IARC, 1979d).

In mutagenicity studies HCB was negative in the dominant lethal test in mice and *in vitro* tests with *Saccharomyes cerevisiae* and other organisms (Gorski *et al.*, 1985; Guerzoni *et al.*, 1976). HCB was not mutagenic in a recently conducted study in several systems (Siekel *et al.*, 1991). In carcinogenicity studies a statistically significant increase in liver tumours occurred in rats fed 100 or 200 mg HCB/kg body weight. No tumours were observed in rats treated with 50 mg HCB/kg body weight or in controls (Cabral *et al.*, 1979). Hepatic tumours (*hepatomas* and *haemangiosarcomas*) were increased statistically and in a dose-related manner in hamsters treated with 50, 100 or 200 mg HCB/kg body weight (Cabral *et al.*, 1977).

HCB was minimally teratogenic when given at high doses (Khers, 1974; IARC, 1979).

Although there seems to be an association between naturally occurring porphyria and human hepatocellular carcinoma (Kordac, 1972; Axelson, 1986), no excess of liver cancer was observed 25 years after an epidemic of porphyria from accidental consumption of grain heavily contaminated with HCB (Peters, 1982). In this epidemic the estimated daily intake was 50-200 mg/kg HCB over a relatively long period (months) before symptoms appeared. Follow-up studies of 32 of the patients have shown that abnormal porphyrin metabolism and active symptomatology persisted for 20 years after the epidemic (Peters *et al.*, 1978; Peters, 1976) suggesting that the course of HCB-induced porphyria may be different from the naturally occurring disease insofar as its association with liver cancer is concerned.

According to IARC (1987) there are no available reports of a direct association between HCB and human cancer.

The LD_{50} in rats is between 3,500-10,000 mg/kg bw. Death is due to neurotoxic effects (Booth and McDowell, 1975).

Repeated administration of HCB at fairly high dose levels, (about 500 mg HCB/kg body weight) to rats results in porphyria, immunosuppression, hepatomegaly and induction of m.f.o. activity (den Tonkelaar *et al.*, 1978; Loose *et al.*, 1977; Kimbrough and Linder, 1974; Grant *et al.*, 1974; Stonard, 1975).

Various estimates of dietary intake of HCB from food, in which amounts are generally low, have been made. In the U.S.A. this estimate ranged from 0.4 to 0.08 µg/day/man (U.S. F.D.A.). In Italy, it was 4.11 µg/man/day. In Japan it can be estimated to be 18 µg/kg (Leoni and D'Arca, 1976, from figures provided by Morita *et al.*, 1975).

A dose of 0.6 µg has been established as a conditional acceptable daily intake in man (36 µg for a 60 kg/man) (WHO, 1975). According to EPA guidelines, the intake from

water over a long-term period (several months) should not exceed 50-175 µg/litre (U.S. P.H.S. 1990).

Consumption of 150 g of fish containing 3.26 µg/kg (fresh weight) of HCB (the highest amount recorded during the 1989-90 pilot monitoring project) would lead to an intake of just below 0.5 µg which is a fraction of the conditional acceptable daily intake. At these low levels it is unlikely that HCB will present a carcinogenic risk for man. The negative epidemiological data provide a further degree of reassurance.

5.2.9 Hexachlorocyclohexane (HCH)

8 isomers of HCH are recognized. The ones most commonly found in commercial preparations are the *a-*, *g-* and *d-* isomers. The *g*-isomer (lindane) is the most active isomer as an insecticide and most technical preparations of HCH contain a substantial proportion (up to 99.9%) of this isomer. The name lindane is restricted to essentially pure preparations of the *g*-isomer. HCH is only slowly degradable in soil (IARC, 1979c).

Lindane, like other chlorinated hydrocarbon insecticides, is stored in human body fat (Solly and Shanks, 1974).

a- and *b*-HCH and lindane were not mutagenic in bacteria, yeast or *Drosophila*. Lindane induced chromosome aberrations in plant cells and chromatid breaks in human lymphocytes *in vitro* (IARC, 1979c).

The four common isomers, as well as technical grade of HCH, were tested for carcinogenicity in rats and mice. The *a*-isomer induced hepatic tumours in mice and rats, the *b*-isomer, lindane and HCH (technical) in mice only. The tumours induced in the mouse by these isomers developed at doses greater than 100 mg HCB/kg body weight in life-time studies. In the rats, dose levels of *a*-HCH that were effective in producing hepatocellular tumours were of the order of 300 mg HCB/kg body weight or greater (IARC, 1979c). Lymphoreticular neoplasms were also induced in in-bred mice by technical grade HCH (Kashyap *et al.*, 1979).

A questionable increase in thyroid tumours was found in male rats treated with lindane at dose levels of 236 and 472 and in female rats at doses of 135 and 270 mg HCB/kg body weight (IARC, 1979c).

Carcinogenicity studies were also conducted in dogs and hamsters but they were considered to be inadequate for evaluation of human risk (IARC, 1987).

An increased incidence of lung cancer was reported in 285 workers who had applied various pesticides, including HCH, in an agricultural setting. Although the incidence of tumours was greater than that expected from smoking, the presence of other chemicals (including solvents) does not allow the increased incidence of tumours to be attributed to lindane (Barthel, 1981). Later epidemiological studies claimed an association between occupational exposure to HCH and leukaemia, soft-tissue sarcomas and lymphomas. These studies were considered to be inadequate for assessing the carcinogenic risk for man from HCH exposure (IARC, 1987).

The LC_{50} of *a-*, *b-*, *d*-isomers and of technical HCH is over 500 mg/kg in rats and mice. That of the *g*-isomer is about 86 mg/kg in mice and about 100 mg/kg in rats (WHO, 1969).

Doses of lindane which are acutely toxic in rats induce diarrhoea, convulsions and respiratory failure (Chen and Boyd, 1968). Lower doses induce liver enlargement, induction of m.f.o. activity and fatty change or focal necrosis of the liver (Schulte-Hermann, 1974; Fitzhugh *et al.*, 1950).

Lindane is embryotoxic but not teratogenic (IARC, 1979).

The concentration of lindane or HCH on foodstuffs varied considerably from country to country in the 60s and 70s when it was extensively used as a pesticide, (*e.g.*, average daily intake, U.S. 3 µg/day, Spain 11.52 µg/day, Japan 3.5 µg/day). Restriction of its use has resulted in considerable diminution of residues in food and hence of its daily intake (IARC, 1979c).

A maximum acceptable daily intake of lindane for humans was established at 0-0.01 mg/kg bw/day (WHO, 1976). The current EPA guideline for oral intake is 1.8 mg/day (EPA, 1988).

Assuming a daily intake of 150 g of fish containing 1.20 mg/kg lindane (representing an approximate average of levels recorded in the Mediterranean) the amount ingested would be 180 µg or approximately 3 µg/kg bw in a 60 kg man, which is a third of the maximum acceptable intake in 1976. At this dose level it is unlikely that HCH would present a carcinogenic hazard to man. Further reassurance can be gained from the fact that despite prolonged exposure under occupational conditions there is no evidence for an increase in tumour incidence in the workers exposed. In addition, few tumours have been induced in animals despite the high doses administered.

5.2.10 Nitrilotriacetic Acid and its Salts (NTA)

NTA is an aminocarboxylic acid that can sequester metal ions as water-soluble complexes (Anderson *et al.*, 1985).

A large number of mutagenicity and clastogenicity tests have been carried out on NTA and its salts. Some *in vitro* results, primarily in tests for chromosomal anomalies, were positive. One test in *Drosophila* was positive but a second test was negative. Two *in vitro* tests in mice were negative. All the positive results were observed at very high, near lethal, concentrations (IARC, 1990).

In mice, administration of NTA at 7,500 or 15,000 ppm in the diet for 18 months resulted in a statistically significant and dose-related increase of renal adenocarcinoma in males. A very low incidence of the same type of tumour was observed in female mice at the highest dose only. The experiment was terminated at 21 months (NCI, 1977). In rats fed the same dietary concentrations of NTA for 18 months and killed at 24 months there was a dose-related increase in tubular-cell adenomas and adenocarcinomas in males and in transitional and squamous cell carcinomas of the urinary bladder in females. In both sexes, the tumour incidence at the highest dose was statistically significant (NCI, 1977).

The trisodium salt of NTA (monohydrate) was also tested for carcinogenicity in mice at 2,500 and 5,000 ppm for 18 months in the diet. The mice were killed at 21 months. No urinary tract tumours were observed but a few animals in the treated groups developed haematopoietic tumours. The same salt was tested in rats at 0, 200, 2,000 or 20,000 ppm in the drinking waster for 104 weeks. Tubular cell adenomas and adenocarcinomas as well as transitional cell tumours were observed in both males and females of the treated groups.

The incidence of both the transitional cell tumours and tubular cell tumours was dose-related and statistically significant.

A statistically significant increase in the incidence of tubular tumours was observed when NTA was administered in drinking water to rats at 1,000 ppm in drinking water (Goyer *et al.*, 1981) but the disodium salt administered at 5,000 ppm to mice or rats in conventional life-time studies failed to elicit any tumours (Greenblatt and Lijinski, 1974; Lijinski *et al.*, 1973). There are no epidemiological data on the carcinogenicity of NTA in humans.

NTA and its salts are not metabolized in the mammalian organism and are excreted unchanged in the urine. The renal content of NTA is higher than that in any other tissue. Evidence of renal tubular cell damage and ulceration and hyperplasia in the urinary tract epithelium were observed in short-term studies in rats and mice treated with high levels (comparable to those that produced tumours in carcinogenicity studies in rodents) of NTA or of its sodium salts (Anderson *et al.*, 1985).

The principal route of human exposure is from drinking water. The US Food and Drug Administration has approved the use of NTA (trisodium salt) as an additive to boiler water used in the preparation of steam that will come into contact with food. It may not exceed 5 mg/litre (ppm) in boiler feedwater and may not be used when steam comes into contact with milk and milk products (IARC, 1990).

Occupational exposure is limited to 1 mg/m^3/8 hour TWA or 2 mg/m^3 STEL (Monsanto Co, 1985).

Studies on the effects of NTA on reproduction and prenatal toxicity were carried out in mice, rats and rabbits. No teratogenic effects were observed but some bladder defects were found in rat foetuses in one study (IARC, 1990). Furthermore, there is no evidence that NTA or its salts enhance the reproductive toxicity of heavy metals in experimental animals (MacClain and Siekeirka, 1975).

The data suggest that renal cancer in rats and mice was induced by dose levels which were clearly nephrotoxic in short-term tests. This would suggest that the tumours arose as a consequence of cell damage and consequent repair. The questionable mutagenicity studies support the view that the renal tumours were induced by a non-genotoxic mechanism. At the low level present in water it is unlikely to present a carcinogenic hazard for man.

5.2.11 Low Molecular Weight Helogenated Hydrocarbons (Solvents)

A very large number of such compounds are used in industry and many of these find their way in the drinking water and eventually in the sea.

Because of the many compounds involved, it is difficult to make a selection for special discussion and appraisal. An attempt has been made by IARC to identify those which appear to have caused the greatest concern (Table 13). This brief evaluation is based on data derived from IARC (1979a).

The only solvent in this group that has given unequivocally positive results in mutagenicity test is 1,2-dichloroethane. It was positive in more than one test system and the results were clearly linear over a wide range of concentrations in at least one system (Drosophila). The other solvents which are listed as positive in the table were tested only in prokaryotes (IARC, 1979a).

Table 13

Toxicological profile of some low molecular weight halogenated hydrocarbons (Solvents)

(Based on data derived from IARC, 1979a)

	LD_{50} mg/kg bw	Liver Necrosis	Mut	Terat	Cancer	Organ
Carbon tetrachloride	2.92-12.1	+ve	-ve	Fetotoxic	+ve	Liver (R and M) ?mammary gland (R)
Chloroform	120-1188	+ve	-ve	*	+ve	Liver (M) Kidney (R)
1,2-Dichloroethane	up to 700	+ve*	+ve	ND	+ve	Lung, haematopoietic tissue (M, R) Forestomach (R) Mammary (R)
Dichloromethane	up to 2000	+ve	+ve	-ve	?+ve	Lung (M)
Hexachloroethane	4.5	+ve	ND	ND	+ve	Liver (M) Kidney (R)
1,1,2,2-Tetrachloroethane	250-820	+ve	+ve	+ve	+ve	Liver (M, R)
Tetrachloroethylene	>5000	+ve	-ve	-ve	+ve	Liver (M, R)
1,1,1-Trichloroethane**	>5000	+ve	+ve	-ve	+ve	Liver (M)
1,1,2-Trichloroethane	up to 835	+ve*	-ve	ND	+ve	Liver (m, R) Adrenals (M)
Trichlorethylene	189-7200	+ve	+ve	-ve	+ve	Liver (M) Lung

CNS depression (**) Stimulates m.f.o. activity (*) Kidney necrosis also ND = No information available

M = Mouse R = Rat

The LD_{50} figures indicate that the compounds reviewed possess a wide range of toxic potential. Carbon tetrachloride would appear to be the most toxic - tetrachloroethylene, the least toxic. Concentrations in drinking water are normally several orders of magnitude below the LD_{50} and one could, therefore, assume that they present little or no toxic hazard to man. If one assumes that the concentration in seawater will not exceed those in drinking water by a significant amount, then the risk of toxicity from compounds of this sort in seawater is likely to be minimal, if present at all.

All the solvents listed in the table are carcinogenic. Most of them produce liver cancer in rats or mice or in both species. Since many of the solvents are either non-mutagenic or of doubtful mutagenicity the cancers must have arisen by some non-genotoxic mechanism. This mechanism is the most likely for carbon tetrachloride, chloroform, tetrachlorothylene and 1,1,2-trichloroethane, since these compounds are unequivocally non-mutagenic. This supposition is supported by the fact that liver necrosis was observed in short-term tests at levels close to those used in carcinogenicity studies and it is now generally accepted that repeated episodes of necrosis and regeneration are likely to lead to tumour formation (Grasso *et al.*, 1991).

The same comments can also be applied to dichloroethane, 1,1,1,1-tetrachloroethane, 1,1,1-trichloroethane and trichloroethylene although one cannot entirely dismiss the possibility that a genotixic mechanism may be partially responsible for the development of these tumours.

1,2-Dichloroethane would appear to be in a different category from the other solvents. It produced tumours in several organs and is unequivocally mutagenic. It is likely that at least some of the tumours (e.g., haemangiosarcomas) may have been produced by a genotoxic mechanism. It is not certain to what extent these carcinogenic and mutagenic results can be attributed to the parent compound or to the impurities that might be present. Nevertheless, commercial preparation of this solvent would appear to present a greater carcinogenic hazard to man than any of the other solvents.

5.2.12 Polychlorinated Dibenzodioxin (PCDD) and Polychlorinated Dibenzofurans (PCDF)

About 75 PCDDs and 135 PCDFs have been identified in the environment but data of sufficiently good quality to assess human risk exist only for 2,3,7,8-tetra CDD (TCDD). For the other congeners and isomers, toxicological data are limited to acute or short-term exposure or to *in vitro* studies. Most of these isomers and congeners have been detected by isomer-specific analyses of divers environmental samples such as industrial wastes, soil, municipal wastes and human adipose tissue (IPCS, 1989, WHO, 1988a).

In the absence of adequate data to evaluate the hazard from these chemicals, several models have been proposed to relate the toxicity of PCDFs and PCDDs in the environment to that of TCDD. The results of these models are called "TCDD Toxic Equivalent" (TTE).

The TTE values for the various compounds differ from model to model but they are consistently a fraction of the values for TCDD, indicating that the various congeners and isomers studied are less toxic than TCDD.

If each compound were to be considered as toxic as TCDD one would overestimate the toxic hazard from this group of compounds. An overestimate of this sort would mean

that any calculation of a tolerated dose for this group of compounds would have a built-in "safety factor".

In this section attention will be principally directed to the mutagenicity and carcinogenicity of TCDD but data from other isomers and congeners will be included as necessary to assist in evaluating the carcinogenic and toxic hazard from this group of compounds.

TCDD was found to be mutagenic in *S. typhimurium* TA 1532 and *E. coli* Sol 4 by one investigator. Negative results were obtained by other investigators using 4 strains of *S. typhimurium* (TA 1532, 1535, 1537 and 1538). A cell transformation test using kidney hamster cells was positive but a dominant lethal test in mice and a cytogenetic study in rat bone marrow were negative (Giri, 1986; WHO, 1988a).

Studies in rats demonstrate that covalent binding to DNA is 4-6 orders of magnitude lower than that of most chemical carcinogens and the binding with DNA is equivalent to one molecule of TCDD per DNA of 35 cells (Poland and Glover, 1975).

Liver, nasal turbinate and hard palate tumours showed a statistically significant increase in both sexes of Sprague-Dawley rats given 0.1 µg/kg bw (top dose). In addition, liver tumours showed a statistically significant increase in females given 0.01 and 0.1 µg/kg bw. Lung tumours were significantly increased at the highest dose in females. No increase in tumours was observed at the lowest dose (0.001) in either sex (Kociba *et al.*, 1978). In subsequent studies, tumours of liver and thyroid were observed at the highest dose level in rats given TCDD in amounts comparable to those of the Kociba experiment (NIH, 1982a). No tumours of lung of hard palate were found in the NIH experiment. In mice, liver and thyroid tumours were observed at doses of 2 µg/kg/week (highest). Lower doses did not increase tumour incidence in any organ (NIH, 1982b; WHO, 1989a).

There is no reliable evidence that TCDD is carcinogenic or teratogenic to man (Fara and del Corno, 1985; WHO, 1989a, IARC, 1987).

TCDD is foetotoxic and teratogenic in mice and rats at dose levels ranging from 0.5 µg/kg to 3 µg/kg. Adverse effects on reproduction (reduced fertility) were observed on diets of 18 mg/kg bw/day. The no effect level in monkeys is about 200 mg/kg bw (WHO, 1989a).

TCDD is extremely toxic to all mammalian species tested. The oral LD_{50} is in the µg/kg to low mg/kg range. It is 2 µg/kg bw in the guinea pig, one of the most sensitive species to TCDD toxicity. TCDD is a powerful inducer of m.f.o. activity and causes marked liver enlargement (WHO, 1989a).

The principal type of exposure to TCDDs occurs during the manufacture of 2,4,5 trichlorophenols, in which it occurs as an impurity. Less commonly, exposure may also occur from leakage or explosion of reaction vessels. Under these conditions of high level exposure adverse effects occurred in skin (chloracne, hyperkeratosis or hyperpigmentation) and in the liver (mild fibrosis, elevated serum transaminase) and other organs such as CNS (Crow, 1970; Kimbrough, 1974; WHO, 1989a).

Food is the main source of TCDD intake in man. It has been found to occur in a variety of foods from animal or vegetable sources. The data is fragmentary (WHO, 1988a) but it is estimated that a 70 kg/man has an average daily intake of 0.8 pg/kg bw/day of

TCDD from animal sources and 0.4 pg/kg bw/day from fish food (assuming a 70 g intake of animal fat and 10 g of fish food), making a total of 1.2 pg/kg bw/day.

Exposure via inhalation is expected to yield an intake of 0.1 pg/kg bw/day. Exposure via drinking water and other non-occupational sources is considered to be negligible (WHO, 1988a).

PCDFs were present in the PCDDs mixtures tested for carcinogenicity in animals. The biochemical changes produced by PCDFs in short-term tests are very similar to those produced by PCDDs consisting of liver enlargement and increased m.f.o. activity.

PCDFs occur as contaminants in the manufacture of chlorinated aromatic compounds and may also be formed as a result of combustion (IPCS, 1989). Their rate of degradation is very slow so that they persist in the environment for some considerable time. They are very sparingly soluble in water but readily soluble in lipids. They tend to concentrate in the food and in the body fat of man and animals (IPCS, 1989). No mutagenic activity was found when TCDF was tested in *S. typhimurium* strains TA98 and TA100 and in *S. cerevisiae* (IPCS, 1989). No data on the carcinogenicity of PCDFs are available on either animals or man.

The oral LD_{50} of TCDF is about 1 mg/kg in rats and greater than 6 mg/kg in mice. In guinea-pigs the LD_{50} over 80 days was 1-2 µg. In monkeys the LD_{50} is between 1000 and 1500 µg/kg (IPCS, 1989; WHO, 1988).

Short-term studies in rats at levels of 1-10 µg PCDFs and in the same concentration as that found in the contaminated rice oil causing "Yusho" produced an elevation of hepatic serum enzymes, hepatomegaly and induction of m.f.o. activity (Oishi, 1977 and Hori *et al.*, 1986). Thymic atrophy was also observed in these animals and was seen in mice fed 100 µg/TCDF/kg diet or higher doses. Other toxic effects in addition to thymic atrophy occurred at higher dose levels. Deaths occurred in guinea-pigs during an 88-day period of feeding TCDFs in diet at 1-2 µg/kg bw (Ioannou *et al.*, 1983).

PCDFs were reported to produce acne in man (Braun, 1955) and Vos and Kolman (1970) expressed the opinion that PCDFs account for the acnegenic properties of some commercial PCB mixtures.

In the absence of any carcinogenicity or epidemiological studies on PCDF, no direct assessment can be made on the carcinogenicity risk for man they represent. However, on the basis of the comments made earlier in this section some idea of the hazard from both PCDFs and PCDDs can be obtained from a consideration of the hazard presented by TCDD.

TCDD was not given an acceptable or tolerated level by WHO. However, the U.K. Government Committee on Toxicity have recommended a guideline of 60 pg per adult (HMSO, 1989). They stress that the guideline incorporates large safety factors and they note that the average dietary intake has been close to or above this guideline for many years without apparent harm. Similar guidelines have been arrived at by other chemicals.

5.3 Conclusions

The compounds considered in Section 5 are acknowledged to occur widely in the environment and have been the cause of much concern with regard to the health hazard they might present.

Arsenic, particularly in its inorganic form, is acknowledged to be a human carcinogen and has caused skin cancer in some communities whose drinking water is derived from geological formations rich in arsenic. Seafood would appear to make a significant contribution to the amount of arsenic in the diet if it forms a major source of protein. Fortunately, most of the arsenic in seafood is in the organic form which is less toxic and carcinogenic than the inorganic form thus providing some reassurance of safety. Nevertheless, every effort should be made to restrict intake to the levels recommended by various authorities.

PAHs containing a number of 4-6 ring compounds are strongly carcinogenic to animals when applied percutaneously and are present in products which are strongly associated with human skin cancer (e.g., soot, crude mineral oil) and lung cancer (Coke oven fumes). In animals, PAH administered by the oral route are carcinogenic to the fore-stomach, an anatomical region absent in man (Nagayo, 1973) but there is no evidence that food containing PAHs has produced stomach or other cancer in man. Nevertheless, it would be prudent to keep the level of contamination as low as possible in the environment in order to reduce its uptake in the human food-chain.

The other compounds considered in this section fall within the broad category of chlorinated hydrocarbons. They are all hepatocarcinogenic to rats and mice but epidemiological studies conducted on the ones most prevalent in the environment (e.g., DDT, HCB, TCDD) has failed to reveal any suggestion of human cancer even when exposure has been heavy and prolonged.

There is good scientific evidence that, in rodents, cancer of the liver often results from prolonged hepatocellular damage (Grasso and Hinton, 1991). At levels close to the doses employed in carcinogenicity studies some adverse effect ranging from liver enlargement to hepatocellular necrosis were observed suggesting that the liver tumours may have occurred as a consequence of these pathological findings. This view is supported by the fact that mutagenicity tests on these compounds in several systems both *in vitro* and *in vivo* were either negative or equivocal. On this basis the very small amount of the chemical that finds its way in seafood is most unlikely to present a risk of cancer.

There are some exceptions to this general view. Dioxin is extremely toxic to laboratory animals and although little adverse effects were observed in people exposed to fairly large doses through industrial accidents, some caution should be exercised when considering long-term exposure in man since under such conditions of exposure the human reaction may be different from the reaction to a single large exposure.

Dichloromethane is another exception to this rule. It is genotoxic in a number of systems and has produced tumours in more than one organ suggesting that the carcinogenicity of the compound may, to some extent, be due to its genotoxicity.

Despite these reservations, it can be concluded that provided the intake from seafood of the substances considered in this document is within the guidelines set by authoritative bodies and that this intake does not significantly add to the burden from

terrestrial food, there is little likelihood of adverse effects such as cancer developing in communities dependent on marine products for their subsistence.

6. CONTROL MEASURES

6.1 Existing national and international control measures

The rationale for legislative control of chemical pollution of the environment, including the marine, is based on overall toxicity of the chemical substance or substances in question to man or other living organisms, and carcinogenic, teratogenic and/or mutagenic hazards are taken into account within such an overall toxicological framework.

In the case of organohalogen compounds, a number of which have been considered in the present document, the current legal provisions for control are summarized on the basis of available information in Table 14. The situation regarding other chemicals is less clear although, in many countries, complete or partial coverage is effected by means of blanket legislation and/or administrative provisions referring to water pollution and/or to dangerous chemicals in general. The Spanish 1985 Law on Waters does not include an explicit provision for coastal waters, but regulates discharges into rivers which could pollute the sea. Regulations enacted in 1986 under this law includes limit values for a large number of substances (including Arsenic and pesticides) in effluents. The Italian 1976 Law on the protection of waters from pollution (which specifically includes marine waters) sets limit values for several substances in effluents, including Arsenic. French legislation also sets limit values on the concentrations of pollutants in effluents, but does not specifically mention the substances considered in this document. All the above-mentioned legislation is complemented in each country by other provisions in compliance with EEC Directives on organohalogen compounds.

A number of Mediterranean countries have recently promulgated legislation on the prevention and control of marine pollution from land-based sources. This legislation is mainly designed to provide for compliance with the provisions of the 1980 Athens Protocol. In the majority of cases, no specific regulations concerning limit values for concentrations in effluents, water or seafood appear to have been issued other than those listed in Table 14. In the case of Mediterranean EEC Member States, national legislation is based on the relevant EEC Directives.

At international level, with respect to organohalogen compounds, EEC Council Directive 76/769/EEC of 27 July 1976 (EEC, 1976a) restricts the use of PCB and PCT to closed-system electrical equipment, hydraulic fluids, condensers, etc. Council Directive 76/403/EEC of 6 April 1976 (EEC, 1976b) regulates the disposal of these substances. The Community has set limits of discharge for certain organohalogen compounds (Table 15) and quality objectives (Table 16) for those countries wishing to apply this option.

From the more general aspect, EEC Council Directive 76/464/EEC of 4 May 1976 (EEC, 1976c) on pollution caused by certain dangerous substances into the aquatic environment of the Community includes "Substances in respect of which it has been proved that they possess carcinogenic properties in or via the aquatic environment". As in the case of the Protocol for the Protection of the Mediterranean Sea against Pollution from Land-based Sources, this provides double coverage for carcinogenic substances falling within chemical groups itemized separately in the same annex, and at the same time caters for other substances with the same properties not already covered. A communication to the Council

from the Commission dated 22 June 1982 (EEC, 1982) contains a list of 129 chemical substances, drawn from various groups, which are considered as potential candidates for eventual inclusion in List 1 to the 1976 Directive. Out of 21 substances selected as priority items within this list, Arsenic and its mineral compounds, Benzidine and PAHs (in particular 3,4-Benzopyrene and 3,4-Benzofluoranthene) are placed under the specific heading of carcinogens.

Table 14

Legal provisions for organohalogens in Mediterranean countries

COUNTRY	PROVISIONS
Algeria	Total ban on PCB, DDT and lindane
Cyprus	Control use of pesticides, ban on use of aldrin/dieldrin, preservative. DDT in fish should not exceed 5 mg kg^{-1}
Egypt	*
France	EEC directives apply. Control on production. Ban use of drins, DDT, HCH, HCB, toxaphene and DBCP in agriculture
Greece	EEC directives apply
Israel	Industrial effluent standard of 0.02 mg ℓ^{-1} for sewage
Italy	EEC directives apply. Indicative figure for PCB in fish of 5 mg kg^{-1}. Effluent standard of 0.05 mg ℓ^{-1} for organohalogen pesticides
Lebanon	*
Libya	Control on import and manufacture of all organohalogens
Malta	PCBs, PCTs and chlorinated pesticides are not manufactured and their importation and use is prohibited
Monaco	EEC directives apply
Morocco	*
Spain	EEC directives apply
Syria	*
Tunisia	*
Turkey	*
Yugoslavia	Limits for all organohalogens varying from 0.001-0-1 mg ℓ^{-1} depending on the category of water. For seafood, PCB 3 mg kg^{-1}, DDT 1.0 mg kg^{-1}, HCH (alpha, beta, gamma) 0.1 mg kg^{-1}, HCH (gamma) 0.5 mg kg^{-1} (pesticides on a fat wt basis)

* No information supplied

Table 15

EEC limits set for industrial discharges (mg ℓ^{-1}, monthly averages

INDUSTRIAL SECTOR	LIMIT	DATE OF APPLICATION	DIRECTIVE
1. Production of HCH	2	01.10.1988	84/491/EEC
2. Extraction of lindane	2	"	"
3. Production of HCH and extraction of lindane	2	"	"
4. Production of CCl_4	1.5	01.01.1988	86/280/EEC
5. Production of chloromethanes	1.5	01.01.1988	"
6. Production of CFCs	No limit set		"
7. Production of DDT	0.7 0.2	01.01.1988 01.01.1991	" "
8. Production of PCP	1	01.01.1988	"
9. Production of drins	2	01.01.1988	88/347/EEC
10. HCB production and processing	1	01.01.1990	"

Table 16

EEC quality objectives for certain organohalogens

COMPOUND	QUALITY OBJECTIVES
HCH (total)	20 ng ℓ^{-1} in territorial waters close to discharge points. 100 ng ℓ^{-1} in inland surface waters affected by discharges
CCl_4	12 µg ℓ^{-1} in all types of waters
DDT	10 µg ℓ^{-1} for pp DDT and 25 µg ℓ^{-1} for total DDT applies from 1.1.88 for all types of waters
PCP	2 µg ℓ^{-1} from 1.1.88 for all types of waters
Drins	30 ng ℓ^{-1} for all with a maximum of 5 ng for endrin
HCB	0.03 µg ℓ^{-1} from 1.1.90 for all waters

Note: The phrase "The concentration of (organohalogen) in sediments and/or mulluscs and/or shellfish and/or fish must not increase significantly with time" accompanies the objectives.

6.2 Action proposed for the Mediterranean

The present document is concerned solely with the carcinogenic, teratogenic and mutagenic hazards associated with the chemical substances dealt with. The actions proposed are limited to these aspects, and do not in any way preclude any remedial or control action which might be necessary either on overall toxicity and/or hazard grounds or

because of any hazard other than carcinogenic, teratogenic or mutagenic presented by the substances in question.

From the point of view of risks to marine organisms, the impossibility of arriving at any reliable estimate of such risks has been explained in detail in Section 4.4. For this reason, the main long-term action which is clearly indicated is the acquisition of more data as specified in sub-paragraphs (a) to (h) of the above-mentioned section. Apart from data of global as opposed to purely Mediterranean interest, which should be contributed to, but not viewed as a primary aim, specific requirements for the region include field surveys to determine levels and (to the extent possible) effects, supported by relevant research projects geared to ecological conditions specific to the Mediterranean.

In view of the relative sophistication of the work involved, arrangements could be made for some aspects to be tackled as joint interlaboratory projects on a bilateral or multilateral basis.

From the viewpoint of risks to human health, the general indication is that the carcinogenic risk as such appears to be low, in relation to overall risks arising from the general properties of the substances in question. One other important factor to consider is that, in practically every case, seafood consumption is not the only source of human intake and, more often than not, does not constitute a major proportion of the overall intake. Any legal or other statutory measure contemplated for seafood would therefore first have to take into account the global dimensions of the problem, if any, the significance of the contribution of marine pollution effects through seafood consumption to the total exposure, on the basis of levels encountered in the various environmental matrices serving as exposure sources, together with food consumption patterns of population groups.

On this basis, and on consideration of the relevant data as presented in the various sections of this document, it does not appear that, for most of the chemical substances reviewed, the imposition of legal or other statutory measures in the form of emission standards for effluent discharge into the marine environment or upper limits for concentrations in seafood on a common regional Mediterranean basis would be justified solely on the basis of carcinogenic risks. It should be stressed that this does not, by any means, preclude that such measures may not eventually be necessary either because of non-carcinogenic or combination effects, or because of circumstances (continuous or sporadic) prevailing in specific localities.

The only major point of concern is the levels of PAHs found in shellfish in certain areas of the Mediterranean, which could present an unacceptable cancer risk, at least to high consumers of seafood, on their own account, let alone in combination with other non-marine sources of intake. This may represent a localized, rather than a general problem. However, immediate action on the part of national and local authorities is required to determine levels of PAHs in seafood, particularly mussels, in areas where there is reason to suspect the presence of high PAH levels. In this context, considering the sources and the routes of transportation to the marine environment of these substances, such an exercise could be relatively complex.

Apart from providing a more accurate picture of the situation to national authorities, and enabling them to take any individual measure dictated by their own circumstances, comprehensive information on PAH levels in seafood throughout the region would determine the need or otherwise of developing common control measures at a later stage, as well as the exact form such measures should take.

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