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EXECUTIVE SUMMARY

Detergents containing anionic surfactants represent 60% of the commercially used surfactants, cationic surfactants used as fabric softeners and disinfectants (quaternary ammonium cationics) represent 10%, while the rest is represented by non-ionic surfactants. Anionic detergents most widely used are LAS (linear-secondary alkylbenzenesulfonate), rapidly degraded by environmental microorganisms, derived from linear alkylbenzenes (LAB) by sulfonation of alkylbenzene with H_2SO_4 or SO_3 . Degradation of anionic detergents in sewage treatment plants before reaching natural waters is not always complete, and alkyl phenols which are highly toxic to fish and small water invertebrates may be formed during aerobic and especially during anaerobic treatment in anoxic conditions. It has been demonstrated that primary biodegradation of LAS in estuarine waters depends strongly upon several factors such as the origin of the bacterial culture, the temperature conditions and the structure of alkylbenzenes.

The most important sources of anionic detergents in the Mediterranean sea are land-based sources, and the detergents are introduced into the marine environment directly from outfalls discharging into the sea or through coastal dispersion and indirectly through rivers, canals or other watercourses, including underground watercourses, or through runoff. Information on the amount of anionic detergents reaching the Mediterranean sea is sparse. A pilot monitoring survey carried out in 1992 provided some more information on levels of anionic detergents. Levels in seawater ranged from 0.01 to 4.2 mg/L, in effluents from 0.11 to 34.07 mg/L and in rivers from 0.06 to 26.86 mg/L. This study, however, had to be restricted to a few coastal areas and the results cannot be interpreted as providing any indication of the situation prevailing in the Mediterranean as a whole. Overall assessment of the situation with regard to the Mediterranean was made on the basis of current knowledge at global level, taking into consideration the existing differences in sampling and analytical techniques. For the purpose of the study, the levels of concentration were determined by methylene blue active substances (MBAS) and LAS.

Considering that the ingestion of small quantities, even repeatedly, of seawater polluted by anionic surfactants may be regarded as free of risk, the poor percutaneous absorption and the low toxicity of anionic detergents suggest that there appears to be no risk to human health through contact with detergent-polluted seawater, provided that concentrations in any particular locality do not reach levels sufficient to produce visible foam on the surface. Nevertheless, it must be borne in mind that another effect of detergents on the skin barrier is that it allows the absorption of other compounds which are temporarily present.

Concentrations of anionic detergents measured in the Mediterranean sea may represent a risk for marine biota on all those occasions when they reach levels of effect. The danger to fish comes from exposure of the gill tissues to the detergent rather than from ingestion. Very important toxic effects are experienced by fish swimming in water containing LAS (or other detergents) at low (few mg/L) concentrations. It must also be taken into account that no data are available to support the hypothesis of a widespread risk for marine biota in the Mediterranean sea from anionic detergents, but only some which may lead to the belief that there are some risk situations where high amounts of non-degraded anionic detergents are present in seawater, especially in areas where there is a discharge of untreated sewage wastes.

In a number of Mediterranean countries, detergents are limited by legal restriction with regard to their discharge into the marine environment. Such restrictions generally refer to the use of a high percentage of anionic detergents and to permissible concentrations of detergents in the discharges as well as in the sea.

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 II to the Protocol includes, as one of its items, non-biodegradable detergents and other surface-active substances.

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.

The 1976 Barcelona Convention has been ratified, acceded to, or approved by all twenty one 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 non-biodegradable detergents and other surface-active substances, a Consultation meeting was jointly convened by WHO and UNEP in Athens from 27 to 29 February 1992. The meeting agreed that the first assessment should cover anionic detergents, and also agreed on the modalities of a pilot monitoring study in selected Mediterranean areas to be held during 1992. This study was carried out in selected coastal areas in Italy, Malta, Spain and Slovenia by the Institute of Hygiene and Preventive Medicine of the University of Genoa, the Toxicology Unit of the Department of Health, Malta, the Department of Inorganic Chemistry and Chemical Engineering of the University of Alicante and the Department of Chemistry and Chemical Technology of the University of Ljubljana.

The present document, overall technical responsibility for which was entrusted to the World Health Organization, has been mainly prepared by Professor S. Kanitz, Institute of Hygiene and Preventive Medicine, University of Genoa, Italy. It attempts to provide an assessment of the state of pollution of the Mediterranean Sea by anionic detergents, 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. Apart from the results of the pilot monitoring study carried out in 1992, other monitoring results from a number of Mediterranean countries have also been drawn upon.

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. GENERAL FACTS ABOUT DETERGENTS

2.1 Introduction

The replacement of soap by synthetic surfactants occurred around the year 1950 and took place because of the development of alkylbenzenesulfonates (ABS) which made it technically and economically feasible. ABS soon became the most used surfactants, first in the USA and very soon after also in almost all the industrialized countries. The reasons for this success were essentially their good deterative properties in hard water and the lower price of ABS with respect to soap.

The term surfactant is an abbreviation for surface active agent: this kind of molecule tends to concentrate at the surface and interfaces of an aqueous solution, altering surface properties. Detergent is a term for describing a product or formulation which contains usually about 10-30% of surfactant, a larger proportion of builders (Na tripoliphosphate and other chelants), and minor constituents.

Very soon the presence of synthetic surfactants was detected in raw wastewater, in treated sewage and in many receiving waters because of their foaming properties even at very low concentrations (1 mg/L). It became clear that, even though they were practically non toxic to humans, their presence was aesthetical undesirable. For these reasons alone the US Public Health Service set for them a limit of 0.5 mg/L in the drinking water quality standards (1962). The same value was proposed in the USSR and in South Africa, while WHO set a limit of 0.2 mg/L (Wells, 1978). The problem was resolved when ABS were substituted with LAS (linear alkylbenzenesulfonates) which are rapidly degraded by environmental microorganisms.

As surfactants constitute the active ingredient of detergents this term has been used to run this document.

2.2 Surfactants: nature, structure and Behavior

Surfactants are characterized by the presence of a strongly hydrophilic group and a strongly hydrophobic group linked together in the same molecule. These compounds have a number of physico-chemical properties in common. (Hutchinson and Shinoda, 1967; Lucassen-Reynders *et al.*, 1981).

2.2.1 Surface phenomena

The presence of a hydrophilic group together with a hydrophobic (water repellent) one leads to a higher concentration of surfactants at the surfaces and boundaries of the solution. At the air-water interface the molecules are oriented (hydrophilic groups in the water) forming a surface film which lowers the surface tension of the water and favors bubbling and foaming. In the presence of a mixture of water with an immiscible liquid such a layer will form at the liquid-liquid interface, favoring dispersion and emulsification. Similar phenomena occurs at the liquid-solid interface. If the solid surface is smooth and inert there is a very small adsorption effect: on the contrary in the case of activated carbon or ion exchange resins there is a strong attraction for both hydrophilic and hydrophobic groups.

2.2.2 Micelles

When surfactants molecules are in aqueous solution they aggregate into larger, oriented groups, known as micelles. This event takes place when a certain minimum concentration, called critical micelles concentration (CMC) is reached. Any added increment forms micelles instead of single dissolved molecules. When the solubility limit is reached further increments form a solid or liquid (hydrated) phase. Also in the micelles the surfactant molecules are oriented, with their hydrophobic portions clustered together and the hydrophilic ends extending outwards, as a consequence of the absence of attraction of the hydrophobic groups by the water molecules. Micelles are in equilibrium with the single molecules and have different shapes and sizes, which are related to many factors (chemical nature of the surfactant, salinity of the solution, temperature, etc.). The CMC varies accordingly, from 100 to 10000 mg/L. (Tanford, 1980).

Many organic substances may be solubilized in aqueous solution by the presence of surfactant micelles and they end up dispersed in the micelle. The micelles do not participate in the biodegradation of surfactants as this phenomena is peculiar to concentrations not superior to 10 mg/L while the CMC is 10 - 100 times higher.

2.2.3 Chemical structure

Hydrophobic and hydrophilic groups and their linking have been studied beginning 70 years ago, but only few combinations have reached widespread commercial use. (Lindner, 1964; Schick, 1967; Jungermann, 1970; Linfield, 1976).

2.2.3.1 Hydrophobic groups

The most common hydrophobic group is the hydrocarbon radical with 10-20 carbon atoms which may be supplied by agriculture and by the petroleum industry. From agriculture come oils and fats, above all triglyceride esters of fatty acids which are readily hydrolyzed. A plant or animal fatty acid usually contains an even number of carbon atoms, commonly 16 or 18, linked in a straight chain. They may be saturated or there may be one or more double bonds along the chain. Less common are hydroxyl or other groups on the chain.

Industry very often uses fatty acids from coconut oil and tallow, which are converted to soap by neutralizing them with alkali. Otherwise the carboxyl group of a fatty acid may be reduced to an alcohol group, giving a fatty alcohol which is an intermediate in the synthesis of other types of surfactants.

The petroleum industry supplies mainly hydrocarbons (from the paraffins of crude oil) as hydrophobic groups. The chain lengths are usually C_{10} - C_{20} and originate from cuts boiling higher than gasoline (kerosene and higher). As paraffins (chemically non-reactive) are difficult to convert to surfactants, olefins, alkylbenzenes or alcohols are preferred as intermediates.

Olefins with the desired chain length (C_{10} - C_{20}) are produced either by building them up from smaller molecules (polymerization of ethylene and propylene) or cracking paraffins down to -olefins. A third way is represented by the dehydrogenation of the paraffins of the same chain length.

Alkylbenzenes began to be used as surfactants intermediates via chlorination of kerosene and Friedel-Crafts alkylation of benzene. Very soon industry began to produce tetrapropylene alkylbenzene which derives from the addition of benzene at the double bond of the olefin using aluminum chloride or hydrogen fluoride as Friedel-Crafts catalysts. The commercial products would be better called polypropylenes than tetrapropylenes because alkyl groups other than C_{12} are present.

Polypropylene derivatives are no longer produced because many of their components have a very strong biological stability. For this reason since 1965 the US industry has shifted from TBS (tetrapropylene derived alkylbenzene sulfonate) to the well-known straight-chain products called LAS (linear-secondary-alkylbenzenesulfonate). The linear alkylbenzenes for LAS may be prepared from linear paraffins, via the intermediate chloroparaffins or from linear olefins. Their chain lengths range from C_{10} to C_{15} according to the properties desired in the final LAS.

Alkylation of benzene with a linear olefin ordinarily results in a mixture of all possible linear secondary alkylbenzenes of that chain length, even though the original olefin may have

been a single pure compound. Commercial linear alkylbenzene includes as its main component any or all of the phenylalkanes from C₁₀ to C₁₄ plus small amounts of impurities (from 5 to 20 %) such as nonlinear alkylbenzenes and linear phenylene alkanes as dialkyltetralins and dialkylindanes (DTI). However, it is known that DTI are converted to sulfonates as are the alkylbenzenes and are similar to these for what regards surface activity, biodegradability and toxicity.

Also long-chain alcohols (initially primary ones, more recently linear secondary ones) can be used as sources of hydrophobic groups, linear and more recently also branched ones, by reduction of the carboxyl group of a natural fatty acid.

Adding phenol to linear olefins, by acid-catalyzed (or thermal) alkylation, produces various linear alkylphenol hydrophobes, which are similar to linear alkylbenzenes.

Other hydrophobes are not derived from hydrocarbons, like for example, the polymers of propylene oxide. These compounds are characterized by the possibility of control of the hydrophobic character, which increases with the degree of polymerization. (Wulf *et al.*, 1967; Seifert *et al.*, 1976; Eganhouse *et al.*, 1983a and 1983b).

2.2.3.2 Hydrophilic groups

There are two classes of hydrophilic groups: those which ionize in aqueous solution and those which do not. The former give origin to highly ionizing salts after neutralization. The nonionic ones contain recurrent groups: increasing their number in the molecule increases the hydrophilicity of the aggregate. Common hydrophilic groups are sulfonate, sulfate, carboxylate, quaternary ammonium, polyoxyethylene, sucrose and polypeptide.

2.3 Types of Surfactants

There exists a very large number of surfactants which derive from the numerous combinations of hydrophobic groups, hydrophilic groups and their linking modes. They can be divided into three main groups:

- anionic surfactants, which give negatively charged ions in aqueous solution (sulfonate, sulphate and carboxylate groups). These compounds are produced by sulfonation or sulfation of a chosen hydrophobe. They represent the biggest fraction (about 60%) of commercially used surfactants.
- cationic surfactants, which give positively charged ions in aqueous solution, such as quaternary ammonium derivatives. This group represents a small fraction of all surfactants (about 10%) and their properties limit their use as fabric softeners and as disinfectants.
- nonionic surfactants, which do not ionize in aqueous solution. The most used nonionic surfactants contain a polyether hydrophile group derived from ethylene oxide.

2.3.1 Anionic surfactants

The most widely used anionic surfactants are LAS. Besides these there can be found representatives of the following other classes: alkanesulfonates, primary alkane

sulfonates, olefin sulfonates, ester and amide sulfonates, sulfo fatty acids, sulfosuccinate esters, primary alkylsulphates, secondary alkyl sulphates, sulphated nonionics.

2.3.2 Nonionic surfactants

The nonionic surfactants, which have been widely used commercially, have a polyoxyethylene hydrophilic group and can be attributed to the following four groups: alcohol ethoxylates, alkylphenol ethoxylates, polyethylene esters and polyoxyethylene-polyoxypropylene derivatives (Schick, 1967). Other types may have sugars or other polyols as the hydrophilic group and are mostly used as emulsifiers in baking and in other applications.

2.3.3 Cationic surfactants

The best known compounds are the quaternary ammonium cationics, of five types: alkyltrimethylammonium, alkylbenzyltrimethylammonium, dialkyldimethylammonium alkylpyridinium and alkylimidazolium. Some of these are also used as fabric softeners but all have few detergent properties. There are also some non quaternary cationic surfactants (alkyldimethylamine oxide, alkylamine, alkylamine ethoxylate) used because of their good detergent properties. It may be useful to remember that when cationic and anionic surfactants are contemporarily present in a solution they neutralize each other as oppositely charged surfactant ions join each other to form neutral ion pairs. According to the different conditions existing in the solution micellization and precipitation also occur. Despite the neutralization effect, adding one surfactant to a mixture where the opposite type is in excess gives as a result a remarkable decrease in surface tension and critical micelle concentration. (Hua and Chao, 1964; Schwuger, 1971; Lucassen-Reynders *et al.*, 1981). The aquatic toxicity is usually neutralized when both types are present and the same happens for the inhibiting action of anionic surfactants on anaerobic sludge digestion. A small number of surfactants (amphoteric surfactants) also exist, which do not fall into one of the three above mentioned groups and are known for their special properties.

Surfactants are used in detergents, household cleaning and personal care products and, to a lesser extent, in pesticides, herbicides, paints, plastics, and in the mining, oil, and textile industries. These compounds usually represent 10-18% of granular and liquid detergents and are the largest ingredient of the 20-25 compounds used in these products (Hoglund, 1976; Richtler and Knaut, 1988). Approximately 15 million tons of soap and synthetic surfactants were used worldwide in 1987 (Berth and Jeschke, 1989). Reported volumes of surfactants used annually vary. In the Proceedings of the Second World Surfactant Congress (Bryan, 1988; Richtler and Knaut, 1988; Roes and de Groot, 1988) it can be found that anionic surfactants are the most commonly used of the three major classes almost everywhere (1.8×10^6 MT in 1987) while during the same year figures for nonionic surfactants (alkyl ethoxylates and alkylphenol ethoxylates) were 4.7×10^5 and 3.9×10^6 MT, respectively and cationic surfactants 1.5×10^5 MT in Western Europe and 1.9×10^5 MT in the U.S. Overall, the annual rate of increase in surfactant use is greater for the cationic types (4-5%) than for the remaining classes (2-3%) (Boethling, 1984; Roes and de Groot, 1988).

Surfactants have been reported in natural waters that receive municipal wastes in the U.S., Japan, and Western Europe by, among others, Matulová (1964), Nyberg (1976), Margaritis and Creese (1979), Fischer (1980), Sivak *et al.* (1982), Kikuchi *et al.* (1986), and Tarazona and Nuñez (1987). Several surfactants and their breakdown products also have been measured to various degrees in drinking water (Crawthorne *et al.*, 1984; Ventura *et al.* 1989), sewage sludge, sludge-amended soils, and sediments (Waters and Topping, 1982; Takada and Ishiwatari, 1985; McEvoy and Giger, 1985; De Henau *et al.*, 1986; Brunner *et al.* 1988).

Surfactants usually enter U.S. rivers as part of treated municipal effluent; however, in other countries, surfactants enter waterways largely untreated (Yamane *et al.*, 1984). Some surfactants are largely removed by sewage treatment (Games *et al.*, 1982; Sullivan, 1983; Boethling, 1984; Brown *et al.*, 1986) and biodegradation occurs to various degrees (Sivak *et al.* 1982; Larson and Vashon, 1983; Larson, 1983; Boethling, 1984; Richtler and Knaut, 1988).

2.4 Natural Surfactants

It must be mentioned that there is a large variety of natural surfactants which appear to be indispensable: components of cell membranes, emulsifiers during digestion and assimilation of food, etc.

Natural sources for hydrophobe groups are fatty acids, steroids and terpenes. The hydrophiles are the same as those mentioned for man made compounds. The natural surfactant molecule structures are much more numerous than those produced by the chemical industry.

A restricted list of natural surfactants includes:

- saponins (strongly foaming glycosides of vegetal origin and not very toxic following oral ingestion), (Tschesche and Wulf, 1973; Voogt, 1977);
- sulfolipids and sulfatides (lipid hydrophobe with a sulfonate or sulphate hydrophile, also of vegetal origin: one of them is present in a non photosynthetic marine diatom, *Nitzschia alba*), (Anderson *et al.*, 1979; Tremblay and Kates, 1979; Harwood, 1980);
- lecithins and the lung surfactant (lecithins are phosphatidyl cholines, ubiquitous components of cell membranes; the lung surfactant comprises a lecithin, dipalmitoyl phosphatidylcholine, associated with one or more proteins and carbohydrates), (Colacicco *et al.*, 1977; Sawada *et al.*, 1977; Zanker *et al.*, 1978, Magoon *et al.*, 1983; Yu *et al.*, 1983; Lynn *et al.*, 1974; Ellsset *et al.*, 1976);
- bile acids and bile salts (bile is necessary for the emulsification and digestion of food), (Hoehn, 1964; Brooks, 1970);
- taurides (the hydrophile group taurine is a relative of cysteine and is ubiquitous in animal species), (Holwerda and Vonk, 1973);
- microbial surfactants (surfactants of varying chemical nature, frequently hydroxy fatty acid derivatives of polysaccharides, are produced during the biodegradation of hydrocarbons by microbial species) have emulsifying properties and may become industrially commercialized in the future. As an exception *B. subtilis* produces a carbohydrate free surfactant, surfactin or subtilisin, which is absent when hexadecane is added to the growth medium (usually growth on alkane promotes synthesis of surfactants from other microorganisms while glucose prevents it). (Gerson and Zajic, 1979; Cooper and Zajic, 1980; Ratledge, 1980; Wiken and Knox, 1980; Cooper *et al.*, 1981).

2.5 Solubilizing Effects of Surfactants

Solubilizing effects of surfactants have received considerable attention because of their potential action on relatively water insoluble compounds, such as DDT, 1,2,3-trichlorobenzene (McBain, 1942), chloromethanes, and polycyclic aromatic hydrocarbons (Klevens, 1949 and 1950).

Analogous to other surfactant applications (Hinze, 1987), this phenomenon is the basis for the suggested use of surfactants as solubilizing agents for enhancing biological remediation technologies, pump- and -treat operations, or soil-washing operations (Nash, 1987; Roy and Griffin, 1988; Mueller *et al.*, 1989; Vigon and Rubin, 1989).

There is also concern over the environmental fate of surfactants per se and their effects on other pollutants. The potential adverse effects of surfactants- that of facilitating the unwanted transport of other hazardous chemicals in the environment- have also been noted (Enfield and Bengtsson, 1988; Huling, 1989; Kile and Chiou, 1989; Valsaraj and Thibodeaux, 1989).

The extent to which surfactants influence pollutant distribution in environmental systems depends on the combined effects of pollutant sorption reactions with the sedimentary materials (Karickhoff, 1984), pollutant solubilization by micellar or monomer surfactant (Tomida *et al.*, 1978a and 1978b; Almgren *et al.*, 1979a and 1979b; Treiner, 1983; Kile and Chiou, 1989; Valsaraj and Thibodeaux, 1989) and surfactant interactions with sediment or soil (medium) components.

Properties of various surfactant solutions have been known for some time, including the magnitude of the micellar solubilization of various carcinogens (Hinze, 1987; Mueller *et al.*, 1989), estimates on the locus of solubilization within the micelles (Nash, 1987; Vigon and Rubin, 1989) and even the carcinogenesis of such micellar solutions (Mueller *et al.*, 1989; Roy and Griffin, 1988). For some types of surfactants and classes of compounds, the solubilization process is known to be concentration dependent, due to anomalous changes in the compounds micellar activity coefficients (Klevens, 1950; Enfield and Bengtsson, 1988; Kile and Chiou, 1989; Valsaraj and Thibodeaux, 1989) or to normal boiling points (Huling, 1989; Karickhoff, 1984).

2.6 Biodegradation of Surfactants

Biodegradation may be defined as destruction of chemical compounds by the biological action of living organisms. Surfactant biodegradation, is a process which takes place in the environment receiving wastewaters, for which the living organisms of main interest are bacteria. The degradation of surfactants occurs by oxidation.

According to the Biodegradability Subcommittee of the Water Pollution Control Federation (WPCF) (1967), one can define as primary biodegradation the event that has occurred when the molecules have been oxidized or otherwise altered by bacterial action to such an extent that its surfactancy properties are no longer evident or when they no longer respond to analytical procedures more or less specific for detecting the original surfactants.

Although primary biodegradation eliminates the problem of foamy waters. it is also necessary to consider the further steps of biodegradation until ultimate biodegradation is reached, i.e. the complete transformation of the molecule to carbon dioxide, water, inorganic salts, etc.

The conversion of organic matter to carbon dioxide and water is also referred to as mineralization, and ultimate biodegradation is often considered equivalent to complete mineralization. The latter term implies, however, that all constituent atoms of the original

compound have been converted to inorganic species. Between primary and ultimate biodegradation the WPCF (1967) considers the concept of "environmentally acceptable biodegradation" defined as "susceptibility to biodegradation yielding end-products which are totally acceptable in the receiving environment which includes air, soil, and water although principal interest may be in treatability in waste disposal facilities." Distinctions have also been drawn between ready biodegradability and inherent biodegradability by the OECD Group of Experts (Gerike and Fischer, 1981), according to whether one accepts that a biodegradation process begins immediately after exposure of a compound to environmental microorganism, or whether there is a first step represented by acclimation of the live microorganism, (lasting hours, days, or weeks) before real biodegradation starts.

Almost all surfactants are biodegradable, even the resistant components of TBS but each with a different rate of biodegradation. This fact offers no advantage if the time required exceeds that available in a particular sewage treatment process under the operating conditions, whether the removal takes place by biodegradation or by some other process. Adsorption, for example, takes place in these systems, concentrating dissolved substances onto the bacterial surface. Surfactants are liable to adsorption, and when solid content (i.e., bacteria or zooglycal flocs) is high there may be a satisfactory removal of the surfactant at first. However after a certain time the process stops because all adsorption sites are saturated by surfactant molecules. With a high rate biodegradable surfactant, this blocking does not occur because the adsorption sites are freed for further use as soon as the adsorbed molecules are oxidized or transported into the cell. The tendency of surfactant molecules to concentrate at the liquid-air interface favors the concentration of the surfactant in the foam while the main body of the solution contains a much lower surfactant concentration.

Removal of surfactants through chemical reactions is theoretically possible and follows three possible courses: precipitation, hydrolysis, and oxidation. Precipitation can occur via the use of a suitable adsorbent charcoal. Hydrolysis is used to destroy alkyl sulphates but it occurs in biological systems.

Most of the surfactant biodegradation is accomplished by bacteria, but algae can also intervene as described by Wurtz-Arlet (1964), Klein and McGauhey (1964) and Davis and Gloyna (1967). Lee (1970) found that nine strains of soil fungi, representing four genera, could grow on either anionic or nonionic surfactants derived from C₁₁₋₁₅ linear secondary alcohols, as the sole source of carbon.

In the usual biodegradation systems too, non bacterial forms of life, monocellular or multicellular, are commonly present, feeding on the bacteria and on each other if not on the chemical foods. Their presence does not seem necessary for the biodegradation but they are useful ecological indicators, particularly in activated sludge and trickling filter systems.

Bacterial species suitable for surfactant biodegradation are everywhere in the environment: in the soil, in natural waters, in sewage and in the air. Along with members of genus *Pseudomonas*, biodegradation of surfactants involves: *Achromobacter*, *Aerobacter*, *Alcaligenes*, *Citrobacter*, *Escherichia*, *Micrococcus*, *Mycobacterium*, *Neisseria*, *Proteus*, *Serratia*, *Vibrio*, *Zooglea*, etc. (De Ley, 1964).

At different temperatures the species distribution may be quite different in bacterial populations open to the outside environment. In real or simulated environmental systems (Guthrie *et al.*, 1974; Inniss and Mayfield, 1979) bacterial population seasonal changes can be observed or induced by artificial temperature changes.

Surfactants can have bacteriostatic and bactericidal effects. High concentrations of surfactants may inhibit or damage the bacteria, rendering them unable to accomplish a degradation which would have occurred at lower concentrations.

Although it is believed that adsorption is an important factor, most Authors who have examined the question have found that this process plays a rather insignificant role during removal of surfactants, when compared with that of biodegradation. In particular, Sweeney (1966) presented extensive data on LAS in activated sludge systems showing that 97-98% of the removal is by biodegradation, 2-3% by adsorption.

The first great generalization on surfactant biodegradation from the chemical structure point of view stated that alkylsulphates are biodegradable and ABS are not. Subsequently it became clear that the important factor is the linearity of the hydrophobic group while the chemical nature and mode of attachment are of only minor significance.

The chemical mechanisms used by bacteria for the biodegradation of surfactants are those which they already have, either fully developed or latent, for utilizing normal foods in their normal life processes. These reactions are catalyzed by enzymes. Biodegradable surfactants also serve as food, and their degradation involves the same reactions, even if with modifications because of the peculiar molecular combinations of hydrophobic and hydrophilic groups. The net overall reaction in bacteria is oxidation, as it is in animals. Three of the general oxidative mechanisms and pathways which appear to be particularly pertinent in the bacterial utilization of surfactants are:

- (1) terminal or ω -oxidation (which is often also initial oxidation), the first step in the degradation at the terminal of the hydrophobic group,
- (2) β -oxidation, the process whereby the aliphatic portion of the hydrophobic group is degraded,
- (3) aromatic oxidation, which is pertinent when the hydrophobic group contains a benzene ring.

2.6.1 The biodegradation process in LAS

When primary biodegradation of LAS occurs the surfactant loses its two environmental presence indicators, its foaming properties and its methylene blue response. Tests on LAS biodegradation effluents have shown that they are not toxic to mammals and aquatic life.

Some researchers believe that ultimate biodegradation of the LAS to carbon dioxide and water does not accompany these favorable signs and that the biodegradation process might stop, leaving resistant intermediates undetectable by toxicity, foaming properties, or MBAS response. Laboratory results indicates that ultimate biodegradation is readily accomplished by LAS and it is probable that this should also happen in the environment. The main metabolic pathway begins with an attack on the end of the alkyl chain to form a carboxyl group, followed by rapid β -oxidation of the chain, then more slowly by oxidation of the ring with simultaneous conversion of the sulfonate group to inorganic sulphate. Evidence for the completeness of the mineralization is available from biodegradation parameters: oxygen uptake, organic carbon removal, and carbon dioxide formation.

It is interesting that Ryckman (1956) was able to operate an activated sludge unit using the LAS as the sole food for bacteria. A closer approach to natural systems was accomplished by Larson and Perry (1981) using Ohio river water and its bacteria with 5 ppm commercial LAS. Rapid oxygen uptake with first order kinetics began after a lag of about 3 days, reaching about

70% of TOD (Total Oxygen Demand) in another 8 days and extrapolating to an asymptote of about 75%.

The ultimate biodegradation of LAS must, by definition, dispose of the sulfonate group either by complete scission from the rest of the LAS molecule, breaking the C-S bond, or by breaking the molecule down to an S-containing fragment small enough for direct incorporation by the cell for synthesis of normal protoplasm; the latter shortcut has not yet been reported.

During aerobic biodegradation of LAS, inorganic sulphate is the most frequent metabolite and it approaches the theoretical amount. Formation of inorganic sulfite has not yet been demonstrated but it might be possible for some organisms, just as it is with short-chain ABS.

Fractions of intermediates in LAS biodegradation have a composition consistent with a pathway of chain oxidation, ring oxidation and sulphate liberation. Kruger (1964) and Wickbold (1964) found in the effluent from the official German CAS test (3-hr retention time, fed with commercial LAS) fractions characterized as follows:

5-10% undegraded LAS, in accord with the distance principle,
35-40% alkyl aromatic hydroxy carboxylate sulfonates,
10-20% aliphatic carboxylate sulfonates,
35-50% inorganic sulphate.

The last three fractions were subject to further degradation.

Degradation of surfactants in sewage treatment plants before reaching natural waters (Willems, 1973; Kravetz, 1981; Steber and Wierich, 1985) is not always complete, and alkyl phenols highly toxic to fish and small water invertebrates may be formed during aerobic and especially during anaerobic treatment in anoxic sediments (Giger *et al.*, 1981; Stephanou and Giger, 1982).

The various types of surfactants have quite different effects on fermentation processes in anoxic sludge. Anionic surfactants always inhibit methane formation in incubation experiments with sediment and sludge samples with accumulation of acetate. Since acetate cleavage usually accounts for 60-70% of the total methane formation in neutral anoxic environments (Lawrence and McCarty, 1969; Schink, 1985; Kaspar and Wuhmann, 1978) both acetate accumulation and inhibition of methanogenesis can be explained by a direct toxic effect on the acetate-cleaving methanogens (Hanaki *et al.*, 1981). However, it could also be that hydrogen-oxidizing methanogens are inhibited and that acetogenic anaerobes take (Phelps and Zeikus, 1984) their place.

In incubation experiments with sodium dodecylsulphate (SDS), a marked increase in sulfide formation was always observed and (Postgate, 1983) it was assumed that sulphate is first released from the SDS molecule by hydrolytic cleavage. An alkylsulfatase enzyme able to cleave SDS has so far been described only for an aerobic *Pseudomonas* strain (Humphreys *et al.*, 1986).

These and other results suggest that anionic surfactants are not degraded at all, under anaerobic conditions. Desulfonation of alkylsulfonates has as yet only been observed with aerobic bacteria, and probably requires molecular oxygen as a reactant in an oxygenase reaction (Payne and Feisal, 1963; Cain and Farr, 1968).

2.7 Biodegradation of Surfactants in Seawater

Coastal waters, and estuaries in particular, are acknowledged as being not only the most productive but also very sensitive areas of biological activity which could be seriously endangered if the self-depuration capacity of the environment is not high enough to take care of anthropogenic pollutants.

While many observations of the LAS biodegradation (Painter and Zabel, 1988) in wastewater treatment plants and in freshwater systems (Swisher, 1963 and 1987; Hrsak *et al.*, 1981; Larson and Payne, 1981; Larson, 1990) are available, reports dealing with their fate in estuarine and coastal waters which represent the ultimate recipients of domestic and industrial wastewaters are relatively scarce (Hon-Nami and Hanya, 1980; Devescovi *et al.*, 1986; Vives-Rego *et al.*, 1987; Sales *et al.*, 1987; Quiroga *et al.*, 1989; Quiroga and Sales, 1990).

The majority of the biodegradation studies were performed using a relatively non-specific analytical MBAS-method (Abbot, 1962) for the determination of LAS (Sales *et al.*, 1987; Vives-Rego *et al.*, 1987; Quiroga *et al.*, 1989). Therefore their results give only a figure of overall biodegradation of LAS and it has been shown that the environmental behavior of the individual homologues and/or isomers could be significantly different (Hon-Nami and Hanya, 1980).

It has been demonstrated (Terzic *et al.*, 1992) that primary biodegradation of LAS in estuarine waters depends strongly upon several factors such as the origin of the bacterial culture, the temperature conditions and the structure of the alkylbenzenes. The mixed bacterial culture originating from the freshwater layer of a highly stratified estuary reveals a much greater ability to degrade LAS than that from the underlying saline water, while the mixed bacterial culture from the reference coastal station was less efficient. This suggests that the bacteria responsible for the observed biodegradation might have been mainly of terrestrial origin, while marine bacteria need to be adapted to LAS for longer time periods. The biodegradation rate was significantly higher in summer (23EC) than in winter (14EC) temperature conditions.

The most readily biodegradable homologues present in the commercial LAS mixture are those with the longest alkyl chains. For different isomers of the same homologue, the biodegradation rate increases with increasing distance of the sulphophenyl group from the terminal methyl group. Consequently, estuarine and coastal waters are expected to be relatively enriched in lower homologues, as compared to the original LAS composition. This fact underlines the positive ecotoxicological effects of biodegradation, as it is known that the lower homologues are less toxic to aquatic organisms.

When surfactants reach the sea, the high ionic strength of the medium causes a fall in their critical micelle concentration (Corrin and Harkins, 1947; Shinoda, 1967) while their solubility is greatly reduced. As a result, these compounds accumulate in the sediments close to waste outlets. This represents a danger to benthic communities, which are of great importance in the marine food-chains.

Quiroga *et al.* (1989) studied the degradation of surfactants (especially LAS) in the Bay of Cadiz, one of the areas of the European coast with the greatest potential for the development of marine aquaculture, where the renovation of seawater, due to flushing, is rather small. It has been shown that the pollution levels being produced there by urban and industrial waste are becoming extremely serious. (Sales *et al.*, 1983; Establier *et al.*, 1985).

The local environmental conditions (above all temperature and salinity) were found to be particularly sensitive to climatic and meteorological variations. In fact temperature was found to have a decisive effect on the degradation rate. At temperatures of 20-25°C, degradation exceeded 90% within less than 10 days of assay whereas at temperatures under 10°C, degradation scarcely takes place (the percentage of degradation did not exceed 5% after 21 days of assay). Variation in salinity was particularly marked during wet seasons and disturbed the activity of bacterial flora, while slowing down biodegradation of surfactant. All this means that the pollution potential of urban waste containing detergents undergoes significant seasonal variations.

Quiroga *et al.* also (1989) gave particular attention the effect of sediments, known to have a high bacterial content, on its biodegradation process. The rate of surfactant degradation was remarkably accelerated in the presence of sediments, except in those tests where anoxic conditions were established, as probably the period of acclimatization of the bacterial flora was substantially shortened. Also the type of sediment is important (granulometry, % of sand component, of clay component and of organic matter). Some inhibition of bacterial growth may be due to the toxicity of some of the heavy metals when present at very high concentrations in the sediment (Pb, Cu).

Another aspect regarding the persistence of surfactants in seawater was examined by Takada and Ishiwatari (1990) who considered the fact that linear alkylbenzenes (LAB) with alkyl carbon numbers of 10-14 are used as raw materials of LAS surfactants from which LAS are synthesized by sulfonation of alkylbenzene with H₂SO₄ or SO₃. The yield of the sulfonation is generally high, but some residual unsulfonated LAB persist and are carried with LAS into detergents. Takada found LAB pollution in sediments of Tokyo Bay (Ishiwatari *et al.*, 1983; Takada *et al.*, 1984) and as an ubiquitous constituent in riverain environments (treated and untreated domestic wastewater, river water, and sediments) in Tokyo (Takada and Ishiwatari, 1987). Eganhouse *et al.* (1983 a) showed that LABs are present in municipal wastes in southern California and in nearby coastal sediments. Albaiges *et al.* (1987) also found LAB in sediments and tissues of fish in the western Mediterranean.

The wide distribution of LAB in riverain environments (Takada and Ishiwatari, 1987), estuarine sediments (Ishiwatari *et al.*, 1983; Takada *et al.*, 1984; Eganhouse *et al.*, 1983 a; Eganhouse and Kaplan, 1988; Vallset *et al.*, 1989), and pelagic fishes (Albaiges *et al.*, 1987) shows their high persistency and for this reason LAB have been proposed as a good indicator of domestic waste derived pollutants (Takada and Ishiwatari, 1987; Eganhouse *et al.*, 1983 a; Eganhouse *et al.* 1988; Vivian 1986). LAB consist of isomers that differ in the position of the phenyl group on the alkyl side chain. In the aquatic environment changes in the isomeric composition of LAB have been observed (Takada and Ishiwatari, 1987; Eganhouse *et al.*, 1983 a; Albaiges *et al.*, 1987) even if in untreated domestic wastes, the relative abundance of isomers with a given alkyl chain length is nearly equal. For river and coastal sediments, however, internal isomers (those having the phenyl attachment toward the middle of the alkyl chain; e.g., 6-C₁₂) dominate over external isomers (those having the phenyl attachment near the end of the alkyl chain).

This difference in isomeric composition may be caused by selective biodegradation of external isomers relative to internal isomers (Takada and Ishiwatari, 1987; Eganhouse *et al.*, 1983 a; Albaiges *et al.*, 1987). If this is true, the isomeric composition of LAB may provide information on the degree of its biodegradation. Tests carried out using untreated domestic waste showed that the isomeric composition of LAB (potential molecular tracers of domestic waste), changes systematically due to microbial degradation.

It was also proved that external LAB isomers are more rapidly biodegraded than internal LAB isomers. The degree of LAB degradation was found to be quantitatively related to the change in their isomeric composition. The isomeric composition represented by the Internal/External ratio may be used as an indicator of LAB degradation. When applied to estimating the persistence of LAB around Tokyo, the results indicated that the degree of LAB degradation in estuarine and bay sediments is ~45%.

3. SOURCES AND INPUTS OF DETERGENTS INTO THE MEDITERRANEAN SEA

3.1 Introduction

The most important sources of detergents in the Mediterranean Sea are the land-based sources which introduce them into the marine environment:

a) directly, from outfalls discharging into the sea or through coastal dispersion, b) indirectly through rivers, canals or other watercourses, including underground watercourses, or through run off.

Another source of detergents is related to the use of chemical dispersants (mixtures of solvents and surface-active agents which reduce oil/water interfacial tension and hence stabilize small oil droplets dispersed in the water column) and other agents for control of oil spills. In fact laboratory studies of dispersants currently in use have shown that their acute toxicity is usually lower than that of crude oils and refined oil products (Wells and Harris, 1980; Wells, 1984).

In coastal areas it is possible that aerosol generation from the sea surface transfers surfactants to the atmosphere, together with other pollutants. This is particularly true for surfactants which tend to concentrate at the air-water boundary due to their surface activity. In this way inhabitants of coastal areas may inhale various concentrations of surfactants. (Fontana, 1976).

Surfactants enter rivers principally as a component of untreated municipal effluents (Yamane *et al.*, 1984). These compounds are usually removed by sewage treatment (Games *et al.*, 1982; Sullivan, 1983; Boethling, 1984; Brown *et al.*, 1986). During the sewage treatment the dissolved fraction of surfactants increases substantially from 38% to 82% of the total amount which is present in the effluent. The removal of surfactants varies from 93% after a biological treatment to 41% after a primary treatment (sedimentation).

The importance of sewage treatment is evidenced by the observation of Chiou *et al.* (1991) that the small amount of neutral oils in commercial LAS (1.7%) contributes significantly to the enhanced solubility of organic compounds (such as DDT, PCB, etc.) which are present in the effluents, probably owing to the formation of oil-surfactant emulsions. Therefore the discharge into rivers and into the sea of untreated wastewater, which contains a significant level of oils and surface-active agents, could lead to the potential mobilization of organic pollutants and LABs in aquatic environments. This circumstance may become of great concern where large amounts of surfactants are being discharged and no adequate facilities are available for wastewater treatment, a case more likely to be found in some developing countries. Also the benefit of using sewage sludge containing LAS and other surfactants as soil amendment may

need to be reevaluated in the light of its potential impact on the mobility of organic pollutants under certain conditions.

3.2 Inputs of Detergents Into Sea

Levels of surfactants in rivers in Europe, in the U.S.A., Japan, etc., have been reported by, among others, Matulova (1964), Nyberg (1976), Margaritis and Creese (1979), Fischer (1980), Sivak *et al.* (1982), Kikuchi *et al.* (1986), and Tarazona and Nuñez (1987).

Many data on the presence of surfactant in sewage treatment plants, river water-sediment sites and sludge amended soil sites have been presented by Rapaport and Eckhoff (1990) who summarized monitoring data collected in Europe and North America from 1973 to 1986.

Linear alkyl benzene sulfonate (LAS) average concentrations in sewage entering treatment plants were found to be 3.5 ± 1.2 mg/L (average chain length of 12.0). These levels agree with those reported for Swiss influent samples (2.4 ± 0.9 mg/L) and for a Japanese treatment plant (2-3 mg/L). As far as the USA is concerned, average concentrations measured in several plants (3.7 ± 1.1 mg/L) correspond to predicted levels.

Surfactant concentrations in effluent sewage vary from mean values of 0.06 (range 0.01-0.13) to 2.1 (range 1.7-2.5) mg/L respectively for activated sludge and primary treatment. In the USA plants it was calculated that the breakdown, as a function of effluent flow discharged into fresh waters, is minus 75% for activated sludge, minus 21% for trickling filters and minus 4% for primary sedimentation (Rapaport 1988). Swiss treatment plants are rated with a median removal rate of 99.5% with an effluent concentration of 0.01-0.33 (mean value: 0.09) while for a Japanese plant a concentration of 0.13-0.56 mg/L is given.

Average effluent chain length was calculated to be $C_{11.8}$ (for activated sludge), $C_{12.0}$ (for trickling filter) and $C_{11.8}$ (for primary treatment). It is probable that during activated sludge treatment, selective removal of longer chain homologues occurs (by sorption/settling and biodegradation).

The average concentration of LAS measured in anaerobic sludge was $4,680 \pm 1,460$ mg/kg (dry weight) with a chain length of 12.5 ± 0.2 . This supports the preferential removal of the higher molecular weight homologues by sorption.

In Austria LAS in sewage sludge is reported to be on average $4,200 \pm 1,200$ mg/kg (Giger *et al.* 1987). Previous data from Swiss, Finnish and US plants ranged from 2,900 to 11,900 mg/kg (McEvoy and Giger, 1986).

Apparently LAS do not degrade in anaerobic conditions. Their presence in anaerobic digested sludge should result from adsorption to primary sludge while they degrade quickly during secondary aerobic treatment.

River water levels, downcurrent from sewage treatment plants outfalls, in different Countries (USA, Canada, Germany) are reported to be 0.1 ± 0.09 mg/L (average chain length of 12 ± 0.2). In a study concerning 8 activated sludge plants in the USA in the year 1986 LAS ranged from 0.01 to 0.04 mg/L.

Instream concentrations in four English rivers (Waters and Carrigan, 1983) were equal to 0.008 - 0.019 mg/L above sewage outfall, 0.011 - 0.173 mg/L immediately below and

0.007 - 0.095 mg/L at a distance superior of 5 km below outfalls. Analogous data in four German rivers was found to be between 0.03 to 0.04 mg/L (Gilbert and Kleiser, 1988).

In Japan, where most domestic sewage is not treated, Kobuke (1985) reported ranges from 0.004 to 2.5 mg/L and a geometric mean of 0.13 in river-waters.

Eganhouse *et al.* (1983) demonstrated that the long-chain linear alkylbenzenes (LABs) in the waste effluents of Southern California survive treatment when discharged to the ocean and are preserved in nearby marine sediments. They derive from common LAS-containing detergents and, when exposed to the marine environment, there is a depletion of the external relative to the internal isomers, very probably due to microbial action as was observed in biodegradation studies of the sulfonated analogues. The vertical distribution of LABs in the near-shore sediments located 6 km away from the waste outfall system proved to be explainable with knowledge of waste effluent chemical parameters and background historical data. According to Eganhouse *et al.* (1983) LAB persistence may provide an organic geochemical means of marking time in a sediment column, something which should be studied more thoroughly.

In 1986 Eganhouse confirmed that the observation of LABs in detergents indicates that a major route for their introduction into effluents derives from their incomplete sulfonation. He tested this hypothesis by attempting to account for the LAB mass emissions in Los Angeles County treatment plants, analyzing the LABs in household laundry detergents. He proved that only 13% of the LAB emissions could be attributed to direct introduction of detergent residues. This may be due to a significant industrial and non laundry input, lack of representativeness of the detergent samples and generation of LABs during or after use by desulfonation of LAS. Anyway LABs offer a unique possibility of determining the contribution made by municipal wastes. They have physico-chemical properties intermediate between aliphatic and polynuclear aromatic hydrocarbons and should be good model compounds for analyzing the fate and transport of these pollutants in the environment. It is interesting to note that Ishiwatari *et al.* (1983) found that LABs represented only ca. 1,3% by weight while in the marine sediments (Tokyo Bay) they were present in comparable amounts to their sulfonated analogues. It was concluded that this was due to their higher water solubility.

Analyzing marine sediment at a site 6 km down current from the LA County outfall system Eganhouse (1986) found that where LAB concentrations begin to increase, the TAB (indicators of non degradable ABS mostly used before 1963-65 when there was a changeover to LAS) concentration profile shows a maximum. Other analytical data (% of organic C, N, total DDT and total hydrocarbons) permitted him to establish the age of sediments at other depth.

Anomalous data were found in the surface sediments, which may be explained by mixing due to storm events and/or biological activity besides the different biodegradability of TABs and LABs.

The conclusion of Eganhouse are that LABs which are present in municipal wastewaters, as a result of the use of domestic and industrial detergents, are potentially waste-specific molecular tracers. Changes in the isomer composition of the LABs with depth in the sediments are suggestive of microbial alteration while the vertical distribution of the TABs and LABs can be used as a geochronological tool to reconstruct waste depositional histories.

Even though it is known that biodegradation occurs to various degrees (Sivak *et al.* 1982; Larson and Vashon, 1983; Larson, 1983; Boethling, 1984; Richtler and Knaut, 1988) and that recently Rapaport and Eckhoff (1990) related that LAS should not accumulate in the

environment because of their rapid biodegradation ($t_{1/2}$ in river waters: <2d; $t_{1/2}$ in soil: <1-3 months), very few studies have been done on the biodegradation of surfactants in the marine environment. When (Quiroga *et al.*, 1989) surfactants reach the sea, the great dilution that occurs makes the effects in the sea (i.e. foaming) less spectacular than in inland aquatic ecosystems. But the high ionic strength of the medium causes a fall in their critical micelle concentration and their solubility is greatly reduced. Therefore these substances may accumulate in the sediments close to waste outlets.

Another aspect which was studied by Hon-Nami and Hanya (1980) was that regarding the percentage composition of individual components of LAS among various aquatic environments (rivers, estuaries and bay-water) which is influenced by the speed of biodegradation of LAS mixtures. These Authors related that while the LAS composition in estuaries was similar to that in river water, the situation in bay water was different from that predicted by previous results: the proportions of C₁₀ and C₁₁ LAS slightly decreased, and those of C₁₂ and C₁₃ LAS increased as compared with the river and the estuary waters. The biodegradation of LAS is considered by the Authors as one possible explanation for the above mentioned results. In an aquatic environment, however, remaining concentration of LAS will be influenced by adsorption, flocculation and other phenomena together with biodegradation.

In order to estimate the impact of the pollutants, via riverain transport, on a coastal zone, it is necessary to know the quantity and the mode of the input: seasonal variation, form of entry (dissolved or suspended form), timing (continuous or intermittent) are fundamental considerations for predicting the behavior of the pollutants in a coastal environment. Takada *et al.* (1992) report the results of a two-year biweekly monitoring of LAS and LABs in the Tokyo bay. As is known the properties of LABs are different from those of LAS, although both are discharged into the aquatic environment from the same sources. LABs are hydrophobic and resistant to biodegradation, while LAS are hydrophilic and easily biodegraded. A comparison of their behavior should be useful for predicting the behavior of pollutants with a wide range of physicochemical properties in coastal zones. In Tokyo bay the annual flux of LAS (almost all supplied in the dissolved form) creates an accumulation of 0.4 ton/year in the sediment indicating that the annual flux which is estimated at 71 ton/year is degraded more than 99.9%. LABs are present in dissolved form from 10 to 80%.

Valls *et al.* (1989) identified a widespread occurrence in coastal areas of trialkylamines (TAMs), as trace impurities in quaternary ammonium salts used as fabric softeners in household laundry detergents. They correlate in the sedimentary record with other surfactant markers such as the long-chain alkylbenzenes (LABs) and may serve as indicators of sewage contamination of coastal areas.

3.3 Inputs of Detergents into the Mediterranean Sea

3.3.1 Monitoring programs

The data available to the W.H.O.-Regional Office for Europe in Athens for an assessment of the pollution of the Mediterranean Sea by anionic detergents at this date are contained in the following official documents:

- a) Monitoring results from Spain (1989-1991) and former Yugoslavia (1983-1986 and 1991);
- b) Final Reports on a pilot monitoring project on detergents in the Mediterranean Sea (1992), promoted by UNEP-WHO within the framework of MED-POL Phase II, to which Institutions from various Countries participated (Spain, Malta, Greece, Slovenia and Italy);
- c) Annual Reports of the Italian Ministry of Health (Sistema informativo sanitario-Ministero della Sanità, Direzione generale servizi igiene pubblica-Istituto Superiore di Sanità) entitled: "Quality of bathing waters".

Hereafter the essential information about the above mentioned monitoring programs will be reported and subsequently the results of these programs will be summarized.

3.3.1.1 Reports on monitoring results from Spain (1989-1991) and former Yugoslavia (1983-1986 and 1991)

Monitoring results from Spain

Monitoring data from Spain are reported in papers where one can find the analytical results for several parameters in domestic and industrial effluents and in river waters:

- a) Data for the year 1989: "Archivo MEDPOL, Aportes Fluviales and Vertidos Urbanos e Industriales".
- b) Data for the year 1990: "Contenido del Fichero VERMP90.DBF (Vertidos).
- c) Data for the year 1991: "Contenido del Fichero VERMP91.DBF (Vertidos).

The above mentioned data are not provided with any detailed information on features of sampling points, topography and pollution sources.

During the first period (1989), effluents and river waters were sampled and analyses for many parameters besides detergents (hydrocarbons, chlorinated pesticides, PCB and phenols).

Monitoring of the riverain input was carried out in the following 18 localities: Rio Ter-Desemb., Rio Llobregat-Des., Rio Ebro-Amposta, Rio Jucar Cullera, Rio Segura-Des., Portman.L.Roberto, Cartagena.Algameca, Cartagena.Asur, Cartagena.Enfersa, Cartagena.Ert, Cartagena.E.De Zinc., Cartagena.Repsol P., Almeria, Malaga.Guadal.Dcha., Malaga.Misericordia, Marbella.Est.Bombeo, La Linea.Atunara, Algeciras.Varadero. The total number of collected samples was 99.

Monitoring of the domestic and industrial wastewaters was carried out in the following 26 localities: Safa-Blanes, Mataro, Barcelona, El Prat De Llobregat, Basf-Tarragona,

C.Messer-Tarragona, Eiasa-Tarragona, Ert-Tarragona, Repsol-Tarragona, Tarragona, Castellon, Petromed-Castellon, Carraixet-Valencia, Rascana-Valencia, Valencia, Alicante, Palma De Mallorca, La Manga.H.Entremar., La Manga.H.Galua, Cartagena.Algameca, Almeria, Malaga.Guadal.Dcha., Malaga. Misericordia, Marbella.Est.Bombeo, La Linea.Atunara, Algeciras.Varadero. The total number of collected samples was 128.

Also, during the second and third period of sampling, (1990 and 1991), many parameters besides detergents were examined in the effluents: hourly flow, suspended solid, chemical oxygen demand, total phosphorus, total nitrogen, total chromium, total cadmium, halogenated hydrocarbons, PCB and phenols.

The monitoring was carried out in the following 21 localities (5 rivers and 16 effluents): Rio Ter, Mataro, Barcelona, Prat, Rio Llobregat, Tarragona, Rio Ebro, Castellon, Barranco Carraixet, Acequia Rascana, Rio Jucar, Alicante Rio Segura, Palma De Mallorca, Cartagena.Algameca, Almeria, Malaga, Marbella, La Linea, Algeciras. The total number of the collected samples was 482.

No comments about the results are given in the above mentioned papers.

Monitoring results from former Yugoslavia

There are two Reports available from former Yugoslavia which contain data relative to a National Monitoring Programme. The periods taken into account were respectively: 1983-1986 and 1991.

a) National Monitoring Programme of former Yugoslavia
Report for the period 1983-1986

The Report for the period 1983-1986 covers the first four years of implementation of the Monitoring Programme of former Yugoslavia. Twelve Institutes participated in the programme and the coordinator of the activities in former Yugoslavia was The Committee for Building, Housing, Public Works and Environmental Protection of the Socialist Republic of Croatia. The Report is based on the Annual National Reports for the years 1983 to 1986.

Twelve Institutes of former Yugoslavia were involved in the realization of the MED POL (PHASE II) activities, consisting of research and monitoring programmes. The design and discussion of the National Programme of the Monitoring of Pollution of former Yugoslavia in the Adriatic sea started in 1982, while it actually came into operation at the beginning of 1983.

During the first year of the programme only four Institutes, one from Slovenia and three from Croatia, participated in the monitoring. The following year (1984) five other Institutes joined the programme, so that in 1985 and 1986 twelve Institutes, two from Slovenia, eight from Croatia and two from Montenegro worked on the programme. In this way, pollution was monitored in almost all the most important areas of the Adriatic coast of former Yugoslavia.

The Report contains all necessary information on the organization of the programme (participating Institutes, methodology, geographic coverage, measurement schedules) and the results obtained in the period 1983-1986 are discussed thoroughly. Extensive concluding remarks and recommendations for future work are also given.

The pollution monitoring programme of the Adriatic Sea has been subdivided into Pollution source monitoring, Coastal waters monitoring (including estuaries) and Reference area monitoring.

Pollution source monitoring and coastal water monitoring were carried out in the following areas:

- Slovenian coastal area (from Koper to Savudrija),
- Rovinj area,
- Pula area,
- Rijeka area,
- Sibenik area,
- Split area,
- Dubrovnik area,
- Montenegrin coastal area.

Reference areas were monitored at the:

- Rovinj-Po profile,
- Kornati area,
- island Vis area,
- coastal area of Montenegro.

Parameters monitored in effluents of all the above mentioned areas were, besides anionic surfactants: mercury, cadmium, high molecular weight halogenated hydrocarbons, petroleum hydrocarbons, suspended solid, COD, BOD₅, phosphorus, nitrogen, faecal coliforms, phenols, lead, chromium and zinc.

In coastal zone water, suspended matter, sediments and biota were examined for the presence of: mercury, cadmium, high molecular weight halogenated hydrocarbons, COD, BOD₅ and phosphorus; nitrogen and faecal coliforms (in estuarine water); faecal coliforms and dissolved petroleum hydrocarbons (in coastal water); mercury and cadmium (in suspended matter); mercury, high molecular weight halogenated hydrocarbons and petroleum hydrocarbons (in sediments); mercury, cadmium, high molecular weight halogenated hydrocarbons, petroleum hydrocarbons and faecal coliforms (in edible bivalves).

Anionic detergents were determined with a methylene blue standard method. A preconcentration step through a nitrogen stream was also performed by some laboratories. Other laboratories used either direct chloroform extraction with certain modifications or the procedure reported in USA Standard Methods or a method for anionic detergent determination described in the German literature (Hill, 1970).

b) National Monitoring Programme of former Yugoslavia Report for 1991

This program belongs to the above mentioned activities regarding the Long-term Programme for Pollution Monitoring and Research in the Mediterranean Sea (MED POL- Phase II).

The twelve Institutes of former Yugoslavia that had taken part in the National Programme of Monitoring of Pollution in the Adriatic Sea started in 1982, other joined the programme, so that in 1988 14 Institutions participated in the monitoring: two from Slovenia, ten from Croatia and two from Montenegro.

The pollution monitoring programme of the Adriatic Sea has been subdivided into Pollution source monitoring, Coastal waters monitoring (including estuaries) and Reference area monitoring.

Pollution source monitoring and coastal waters monitoring were carried out in the following areas:

- Slovenian coastal area (from Koper to Savudrija),
- Rovinj area,
- Pula area,
- Rijeka Bay area,
- Zadar area,
- Krka Estuary area,
- Klek-Neum area,
- Split area,
- Dubrovnik area,
- Montenegrin coastal area.

Reference areas were monitored at the:

- Rovinj-Po profile,
- Kornati Archipelago area,
- Vis Island area.

Besides anionic detergents the parameters monitored in effluents for the National Programme of Monitoring of 1991, were: mercury, cadmium, petroleum hydrocarbons, suspended solid, COD, BOD₅, phosphorus, nitrogen, faecal coliforms, phenols, lead, chromium, zinc.

In coastal zones water, suspended matter, sediments and biota were examined for the presence of: mercury, phosphorus, nitrogen and faecal coliforms (in estuarine water); oil slick observations and faecal coliforms (in coastal water); mercury and cadmium (in suspended matter); mercury, high molecular weight halogenated hydrocarbons and petroleum hydrocarbons (in sediments); total mercury, organic mercury, cadmium, high molecular weight halogenated hydrocarbons, petroleum hydrocarbons and faecal coliforms (in edible bivalves).

At reference areas the study regarded following parameters: specific physical and chemical parameters and mercury (in water); mercury and high molecular weight halogenated hydrocarbons (in sediments); mercury, cadmium, and high molecular weight halogenated hydrocarbons (in zooplankton).

The programme for 1990 included for the first time observations of atmospheric pollution transport: measurement of pH, conductivity, acidity, alkalinity, SO₄-S, NH₄, NO₃-N, Na, K, Mg, Ca, Cl, Pb, Cu, Zn, and total suspended matter. Moreover measurements were made in aerosols (Cd, Pb, and total suspended matter) while standard meteorological parameters were recorded.

3.3.1.2 Final Reports on a pilot monitoring project on detergents in the Mediterranean Sea (1992)

This monitoring project was promoted by UNEP-WHO within the framework of MED-POL Phase II, in which Institutes from various Countries participated (Spain, Malta, Greece, Slovenia and Italy).

This pilot project had the scope of obtaining data on pollution levels of non biodegradable detergents in various matrices of the marine environment in selected localities in the Mediterranean.

The activities of the pilot project were conducted over a period of one year (1 January-31 December 1992), within the framework of the Long-term Programme for Pollution Monitoring and Research in the Mediterranean Sea (MED POL- Phase II).

The objectives of the consultations of experts, convened jointly by WHO and UNEP, also within the framework of the MED POL-Phase II Programme, in collaboration with the Environmental Pollution Research Project, Ministry of the Environment, Physical Planning and Public Works, Greece, included the following:

- to finalize agreement on a protocol for implementation of the pilot project, including areas to be monitored, frequency and methodology;
- to review the draft outline content of the document on assessment of the state of pollution in the Mediterranean Sea by anionic detergents;
- to make appropriate recommendations.

The following five Institutes participated in the Pilot Project:

- Environmental Pollution Control Project- Ministry of Environment, Physical Planning and Public Works, Athens;
- Institute of Hygiene and Preventive Medicine, University of Genoa;
- Toxicology Unit - Department of Health, Valletta;
- Department of Inorganic Chemistry and Chemical Engineering, University of Alicante;
- Department of Chemistry and Chemical Technology of University of Ljubljana.

The pollution monitoring programme of the Mediterranean Sea has been subdivided in pollution sources monitoring (effluents or rivers discharging into the marine environment), marine waters monitoring (recreational areas in the vicinity of, or likely to be affected by, effluents) and reference area monitoring.

It was agreed that the pollution source monitoring and coastal waters monitoring would be located in the following areas:

- Saronik Gulf, near Athens (Greece),
- Pineos river, between Larissa and the sea (Greece),
- Ligurian sea, between La Spezia and Genoa (Italy),
- Albufereta Beach and Babel Bay, Alicante (Spain),
- Upper Adriatic in the areas of Koper and Piran (Slovenia),
- along the NE coast of Malta Island, near to Wied Ghammieg, Xghajra and Salina Bay (Qalet Marku).

For each monitored area, the following data would be collected:

- population served by outfall structure from which the effluents are discharged and/or equivalent information in cases of river effluents;
- identification of any rivers or streams flowing into the sea in the vicinity of the outfall;
- identification of all marine areas of recreational and/or other use likely to be affected by the effluents or rivers;
- seasonal fluctuations of currents in the monitored areas.

a) Spain final report on the "Pilot Monitoring Project on detergent in the Mediterranean (WHO/UNEP JOINT PROJECT. MED POL PHASE II)"

The study was carried out along the coast of Alicante in marine areas located near to Albufereta Beach, Babel Bay and the town of Santa Pola (Playa Lisa, Santiago Bernabeu Avenue). Levels of concentration of methylene blue active substances (MBAS) and linear alkylbenzene sulfonate (LAS) were determined for each sample.

To determine the level of concentration of MBAS the method proposed by "Standard Methods for Examination of Water and Wastewater" (APHA, 17th Edition 1989) was combined with a method of surfactant separation by sublation. This allowed the elimination of part of the interference due to other anions such as chlorides and sulphates which are found in high concentrations in marine matrices, and also the extraction of the large volume of water needed for the analysis.

b) Malta final report on the "Pilot Monitoring Project on detergent in the Mediterranean (WHO/UNEP JOINT PROJECT. MED POL PHASE II)"

The study was carried out along the NE coast of Malta Island in marine areas located near Wied Ghammieg, Xghajra and Salina Bay (Qalet Marku).

Monitoring of coastal waters pollution was carried out in: WIEDGHAMMIEQ, XGHAJRA and SALINABAY (QALET MARKU). Estimates of anionic detergents (such as MBAS) were made according to the Standard Methods for Estimation of Water and Wastewater (APHA, 17th Edition 1989) using sodium lauryl sulphate as standard.

c) Slovenia final report on the "Pilot Monitoring Project on detergent in the Mediterranean (WHO/UNEP JOINT PROJECT. MED POL PHASE II)"

The state of pollution by non-biodegradable anionic detergents in the upper Adriatic was assessed in the region of the Bay of Koper and the Bay of Piran in four localities.

All samples were analysed for concentrations of anionic detergents with the MBAS method, as described in "Standard Methods for Examination of Water and Wastewater" (17th Edition, 1989).

d) Italy final report on the "Pilot Monitoring Project on detergent in the Mediterranean (WHO/UNEP JOINT PROJECT. MED POL PHASE II)"

Monitoring was carried out along the Ligurian coast between La Spezia and Genoa in three areas:

- a bathing area of the town of Genoa
- a bathing area of the towns of Chiavari and Lavagna
- an area in front of the Portofino promontory (reference area).

e) No results were available from Greece

Monitoring of coastal waters pollution was carried out in front of the three above mentioned areas. In the same areas samples were also collected directly from the effluents of nearby sewage-treatment plants and from rivers. All samples were analysed for concentrations of anionic detergents with the MBAS method, as described in "Standard Methods for Examination of Water and Wastewater" (17th Edition, 1989).

3.3.2 Results of the monitoring programs

The monitoring results presented in the above mentioned "Reports on monitoring results from Spain and former Yugoslavia" and "Final Reports on a pilot monitoring project on detergents in the Mediterranean Sea promoted by UNEP-WHO within the framework of MED-POL Phase II (Spain, Malta, Slovenia and Italy)" are hereby summarized.

The results will be subdivided as follows:

- a) Effluents from urban and industrial areas
- b) Rivers

Reference values regarding not only the Mediterranean Sea but also other Seas and Oceans are given when available.

3.3.2.1 Effluents from urban and industrial areas

a) Monitoring results from Spain

For this Country the following data are available:

a.1) The monitoring data of several urban and industrial effluents and of river waters, over a period of three years (1989-1991).

The average values (\pm standard deviation) and the range of concentrations for all single effluents are reported in table 1. In table 1a detergent levels are reported for sampling points available only for the year 1989. Unfortunately these data are not accompanied by detailed information on sampling points and pollution sources.

During the first period of sampling (1989), the monitoring was carried out in 18 rivers (riverain inputs) and in 26 domestic and industrial wastewaters.

During the second and third period of sampling (1990 and 1991) the monitoring was carried out in 21 localities (5 rivers and 16 effluents).

As far as effluents are concerned, the mean concentrations of anionic detergents in wastewaters during the year 1989 ranged from 0.31 (mg/L) in Valencia to 34.07 (mg/L) in Malaga Misericordia. The median level was 3.01 (mg/L). During the year 1990 the mean concentrations varied from 0.11 (mg/L) in Valencia to 23.33 (mg/L) in Barcelona. The median level was 7.52

(mg/L). During the year 1991 the mean concentrations varied from 0.23 (mg/L) in Valencia to 16.43 (mg/L) in Cartagena Algameca. The median level was 5.58 (mg/L).

a.2) Monitoring data of the Spanish Final Report on the "Pilot Monitoring Project on detergent in the Mediterranean (WHO/UNEP JOINT PROJECT, MED POL PHASE II)"

This report contains the results of the detergent monitoring of effluents in two areas: Albufereta Beach and the Babel area. In Albufereta, the effluent (after passing through an activated sludge plant) flows into an underwater outfall with a 20 m diffuser, whose outlet is at a distance of 1336 m from the shore. The discharge is approximately 10,000 m³/d but it is not continuous and there are important fluctuations during the year, due to seasonal variations of the population and to the recycling of sewage for agricultural use.

In the Babel area wastewater is discharged through an outlet which is very close to the shoreline. The average volume of effluent is 48,000 m³/d, (range: from 30,000 m³/d to 60,000 m³/d). Of the total discharge approximately 30,000 m³/d is represented by untreated urban and industrial wastewater (20% industrial contribution), while the rest of the same wastewater, arrives at the outfall after passing through a treatment plant (activated sludge system).

Levels of concentration of anionic surfactants and LAS and minimum and maximum flow rates of wastewater discharging in the two marine areas are shown in table 2.

Concentrations of anionic detergents in Albufereta wastewaters were 0.40 as MBAS (mg/L) and 0.21 as LAS (mg/L) with a maximum flow rate of 4.00 Kg/d and 2.1 Kg/d respectively (see table 2).

Concentrations of anionic detergents in Babel Bay wastewaters were 28.4 as MBAS (mg/L) and 15.1 as LAS (mg/L) with a maximum flow rate of 1704 Kg/d and 906 Kg/d respectively (see table 2).

b) Monitoring results from former Yugoslavia

For this Country the following data are available:

b.1) National Monitoring Programme of former Yugoslavia Report for the period 1983-1986

Effluent monitoring was done during the period 1984-1986 in the following four areas:

- Pula area
- Rijeka area
- Sibenik area
- Split area.

Average values and relative ranges of anionic detergent levels (mg/L) measured in the industrial and urban wastewaters along the former Yugoslavia coast are reported in table 3: in brief, mean levels of anionic detergents in Pula, Rijeka, Sebenik and Split industrial and domestic effluents (1984 - 1986) vary from 0.056 to 3.43 mg/L (maximum value: 16.5 mg/L).

b.2) The Report for 1991 on the National Monitoring Programme of former Yugoslavia

This report contains the results of detergents monitoring in domestic and industrial wastewaters discharged into the sea in the following coastal areas:

- Slovenian coastal area
- Rijeka area
- Zadar area
- Split area.

In table 4 the results (mean value and standard deviation) of anionic detergent analysis in sewage effluents (1991) are presented. Mean values were in the 0.481 to 7.412 mg/L range.

c) Monitoring results from Italy

For this Country the following data are available:

c.1) The final report on the "Pilot Monitoring Project on detergent in the Mediterranean (WHO/UNEP JOINT PROJECT. MED POL PHASE II)"

This report contains the results of detergent monitoring in the effluents of two municipal sewage treatment plants which flow into the Ligurian Sea, located respectively in Genoa and in Chiavari. The average volume of sewage treated by the Genoa treatment plant (activated sludge) is 72,000 m³/d for a population of about 285,000 inhabitants. The average volume of sewage treated by the Chiavari treatment plant (activated sludge) ranges from 9,600 m³/d to 14,400 m³/d, corresponding to 40,000-60,000 inhabitants.

In table 5 the average concentrations and ranges for anionic detergents in the two treated effluents are given. Mean values ranged from 0.19 to 13.84 mg/L (as MBAS). This very high value was due to the fact that one of the treatment plants under observation was damaged by a violent flood.

3.3.2.2 Rivers

a) Monitoring results from Spain

For this Country the following data are available:

a.1) The monitoring data for river waters, over a period of three years (1989-1991)

The average values (\pm standard deviation) and the range of concentrations for single rivers are reported in table 7. In table 7a values are given for sampling points available only for the year 1989. During the year 1989 riverain input mean levels ranged from 0.12 (mg/L) in Rio Ebro to 26.86 (mg/L) in Malaga Misericordia. The median level was 1.33 (mg/L).

During the year 1990 the mean concentrations varied from 0.06 (mg/L) in Rio Ebro to 5.28 (mg/L) in Rio Llobregat. The median level was 0.17 (mg/L). During the year 1991 the mean concentrations varied from 0.09 (mg/L) in Rio Jucar e Rio Ter to 2.56 (mg/L) in Rio Llobregat. The median level was 0.26 (mg/L).

b) Monitoring results from former Yugoslavia

For this Country the following data are available:

b.1) The Report for the 1983-1986 National Monitoring Programme of former Yugoslavia, MAP Technical Report Series no. 23. UNEP, Athens, 1988

In this document one can find the results of detergent monitoring in rivers discharging into the sea in the following coastal areas:

- Slovenian coastal area
- Montenegrin coastal area.

As regards the Slovenian coastal area, monitoring was carried in the Rizana and Badasevica rivers (polluted with industrial and agricultural effluents), in the Drnica stream (polluted with agricultural effluents) and in the Dragonia stream (Reference point relatively unpolluted).

For the Montenegrin coastal area, monitoring was carried out at the mouth of the Rikavac stream (polluted by hospital and food industry) and of the Bojana river (polluted by a large agroindustrial complex).

The average values (\pm standard deviation) and the range of concentrations are presented in table 8: average range from 0.043 to 0.063 mg/L (maximum value 0.226 mg/L).

b.2) Pilot Monitoring Project on detergent in the Mediterranean (WHO/UNEP JOINT PROJECT, MED POL PHASE II). Final Report from Slovenia

The monitoring involved only the Rizana river. The results of the analysis (all values <0.01 mg/L) are reported in table 17.

c) Monitoring results from Italy

For this Country the following data are available:

c.1) The final report on the "Pilot Monitoring Project on detergent in the Mediterranean (WHO/UNEP JOINT PROJECT, MED POL PHASE II)"

In this report the results of detergents monitoring in two rivers discharging into the Ligurian Sea (the Bisagno river and the Entella river) are presented.

In table 9 one can see the average concentrations and ranges for anionic detergents. At the mouth of these two rivers the mean values of anionic detergents (as MBAS) ranged between 0.054 and 0.11 mg/L.

3.3.3 Other data available from the literature

There are not many data or studies on surfactants inputs from European nations and particularly for those flowing into the Mediterranean Sea besides those already referred to in the preceding section.

3.3.3.1 Effluents

Regarding the anionic detergent content of effluents among the results for former Yugoslavia, available from the literature (see table 6), are those relative to the presence of anionic detergents in specific types of industrial and urban effluents: 36.72 mg/L (plastic industry, 1979-80), 6.2 mg/L (textile factories, 1980), 2.1 mg/L (urban waste, 1984-86).

In France (at Toulon and Morlaix) average levels of anionic surfactants in urban untreated waste effluents were found to range between 8.1 and 25.6 mg/L (Marchand *et al.*, 1989). In the two above mentioned towns it was estimated that each inhabitant was responsible for an average emission of anionic detergents into the sewage of 2.2 g/die in Morlaix and 6.6 g/die in Toulon.

Levels of between 1.0 and 22.8 mg/L were reported for four other French urban effluents by Pierson *et al.* (1980).

Some data (1956 - 1966) are available for Italy regarding raw sewage, with concentrations of anionic detergents in the range of 0.22 to 31.12 mg/L (see table 6).

In the same table one can find levels of anionic detergents in a number of European industrial and urban wastewaters from different countries (Italy, France, Germany, former Yugoslavia and United Kingdom). These data, available from the literature, reveal average levels of between 0.07 and 36.72 mg/L.

In Spain Sanchez Lea *et al.* (1991) calculated the theoretical concentration of surfactants (measured by the MBAS method) to be found in the discharging point of sewers by considering the LAS consumption for domestic use in Madrid area (3.28 g/die/inh) and found it to be 16.44 mg/L, roughly corresponding to the MBAS content due to the high proportion of LAS in finished detergent products. Experimental data showed that influent MBAS values differ greatly from one treatment plant to the other and from one day to another. Determining LAS by the HPLC technique it was found that their level in comparison to MBAS was on average equal to 85%. The same ratio was found by analyzing river waters. Moreover it was observed that LAS concentrations determined in the influents of wastewater treatment plants were always much lower than the theoretical ones calculated at the discharging point, a removal probably due to adsorption/precipitation in the sewers.

An interesting observation concerning the Mediterranean Sea was made by Valls *et al.* (1989) who identified a widespread occurrence in coastal areas of trialkylamines (TAMs), as trace impurities in quaternary ammonium salts used as fabric softeners in household laundry detergents. They correlate in the sedimentary record with other surfactant markers such as the long-chain alkylbenzenes (LABs) and may serve as indicators of sewage contamination of coastal areas. Valls relates that the distribution of TAMs in surface sediments from the Mediterranean (and the North Sea) shows maximum concentrations in the vicinity of urban areas (Barcelona, Marseille, Terragona and Malaga). The vertical profile obtained in a sediment sample offshore from Barcelona indicates a more recent input than that of the LABs, use of which was introduced in the mid-1960s in developed countries. As before said the risk associated with TAMs in the marine environment is still unknown.

3.3.3.2 Rivers

With regard to rivers which flow into the Mediterranean sea, there are some data about levels of anionic detergents in 9 Italian rivers (1967 - 1977) with average concentrations (see table 10) from 0.22 to 3.4 mg/L (maximum concentration of 13.8 mg/L).

In table 10 one can find levels of anionic detergents found in rivers from different countries (Italy, Spain, Germany, United Kingdom, USA, Japan, etc.) available from the literature, with mean values between 0.008 and 2.6 mg/L (maximum concentration 3.5 mg/L).

4. LEVELS OF DETERGENTS IN THE MEDITERRANEAN SEA

The most important sources of detergents in the Mediterranean Sea are land-based sources: anionic surfactants are introduced into the marine environment via outfall discharges, from rivers, coastal establishments or outfalls, and any other source within the territory of Countries which are located around this Sea.

The purpose of this part of the present report is to describe what is known about the distribution of anionic surfactants in the Mediterranean Sea. The transport processes of LAS and ABS in the aquatic environment (e.g. removal by biodegradation and/or adsorption) have often been examined (see part 2 of this report). However, the flux of anionic detergents from freshwater to the coastal environment has not been studied extensively.

4.1 Monitoring Programs

4.1.1 Seawater

The data available to the W.H.O.-Regional Office for Europe in Athens for an assessment of the pollution of the Mediterranean Sea by anionic detergents at this date are contained in the following official documents:

- a) Monitoring results from former Yugoslavia (1983-1986).
- b) Final Reports on a pilot monitoring project on detergents in the Mediterranean Sea (1992), promoted by UNEP-WHO within the framework of MED-POL Phase II, to which Institutions from various Countries participated (Spain, Malta, Slovenia and Italy).
- c) Annual Reports of the Italian Ministry of Health (Sistema informativo sanitario-Ministero della Sanità, Direzione generale servizi igiene pubblica-Istituto Superiore di Sanità) entitled: "Quality of bathing waters". In the present report consideration will be given to the last two documents available, containing data on sampling and analysis carried out in the year 1992 and 1993.

In part 3 of this report one can find the essential information about the above mentioned monitoring programs and the results regarding effluents and rivers which flow into the Mediterranean sea. We will limit ourselves here to presenting data regarding measurements of anionic detergent levels in the Mediterranean seawater and in the marine biota.

Monitoring results from Spain

Data on coastal waters relative to this Country are available in the Final Report on the "Pilot Monitoring Project on detergent in the Mediterranean (WHO/UNEP JOINT PROJECT, MED POL PHASE II)".

In this report one can find the results of detergent monitoring in seawater along the coast of Alicante in the area of Albufereta Beach and Babel Bay. Reference seawater was sampled at five miles from the coast (at a depth of 60 meters). Sediment samples were collected not only in the area of Albufereta Beach and Babel Bay but also in the area of Santa Pola.

Data relating to seawater are summarized in tables 11 and 12, while those regarding the sediment are reported in table 13. The analysis of seawater from Albufereta Beach showed levels of MBAS (mg/L) between <0.01 and 0.04 and of LAS (mg/L) between <0.01 and 0.03. The analysis of seawater from Babel Bay which is known to be heavily polluted by effluents, showed levels of MBAS (mg/L) between <0.01 and 4.2 and of LAS (mg/L) between <0.01 and 1.94.

LAS concentration in sediments were very low, ranging from 0.01 to 0.02 mg/Kg and therefore LAS seems not to accumulate in the marine environment. MBAS range in the same points proved to be between 1.25 and 4.29 mg/Kg.

Monitoring results from former Yugoslavia

For this Country data are contained in the following documents:

- a) The Report for 1983-1986 of the National Monitoring Programme of former Yugoslavia

Here one can find the results of detergent monitoring which was carried out off the two coastal areas of Sibenik and Montenegro.

The averages and ranges of anionic detergent levels in seawater collected in this areas are shown in table 16. It can be observed that mean levels were respectively 0.004 and 0.045 mg/L (maximum value: 0.122 mg/L).

- b) The Final Report from Slovenia of the Pilot Monitoring Project on detergent in the Mediterranean (WHO/UNEP JOINT PROJECT, MED POL PHASE II)

In this report there are data on anionic detergent levels in seawater between Koper and Piran, near Izola, Piran, Valdoltra and Zusterma. The samples were collected during May, October and December 1992.

The levels of anionic detergents (surfactants as MBAS in mg/L) are shown in table 17 and are very low: in most cases they were <0.01 and 0.01 mg/L while only in some occasions values were between 0.01 and 0.06 mg/L.

Monitoring results from Malta

For this Country data can be found in the Final Report on the Pilot Monitoring Project on detergent in the Mediterranean (WHO/UNEP JOINT PROJECT, MED POL PHASE II) where there are the results of detergents monitoring in coastal seawaters around this isle.

Samples were collected from three areas: Wied Ghammieg, Xghajra and Salina Bay (Qalet Marku). This last was considered as a reference area. Samples were collected in May and October 1992.

The levels of anionic detergents (see table 14) were between 0.0099 and 0.024 mg/L. The concentration found near to a sewage effluent was of the same order as that in the reference area. This could either indicate some unknown source of anionic detergents in the reference area or that some naturally occurring methylene blue absorbing substances in the seawater round Malta could be masking the anthropogenic input of such substances.

Monitoring results from Italy

Data from this Country are present in the following reports:

- a) The final report on the "Pilot Monitoring Project on detergent in the Mediterranean (WHO/UNEP JOINT PROJECT. MED POL PHASE II)"

This report contains the results of anionic detergent monitoring along the coast of the Ligurian Sea, between La Spezia and Genoa. The first zone was chosen in front of a bathing area of the town of Genoa, while the second one in front of a bathing area of the adjoining towns of Chiavari and Lavagna. A third zone, at a distance between 80 and 200 meters from the coast, in front of the Portofino promontory, was chosen as a reference area. The average results and ranges for anionic detergents are presented in table 15.

The analysis of seawater during two periods of the year 1992 gave results contained within the range of 0.013 to 0.031 mg/L (MBAS). No substantial differences were noted between "polluted" areas and the reference area.

Some other data is available from the literature regarding the northern Adriatic and Tyrrhenian sea: here, various Authors (1968 - 1985) have measured anionic detergents (MBAS) along the coast, at average levels of 0.05 - 0.06 mg/L (see table 18).

- b) Annual Reports on bathing water analysis (1992 and 1993)

As regards bathing water quality, in addition to anionic detergents, the Italian law requires the determination of the following parameters: total coliforms, faecal coliforms, faecal streptococcus, salmonella, enteroviruses, pH, colour, transparency, mineral oils, phenols and dissolved oxygen.

The monitoring is carried out in two sampling campaigns every month from April to September. The monitoring does not take place in areas which are forbidden for bathing, like at the mouth of rivers and streams, or near discharge outlets or for other reasons such as the presence of harbours, military zones, etc.

Seawater monitoring is carried out along the whole Italian coast (4,703 km, including islands). In the year 1992 4036 sampling points were utilized and 43595 water samples were examined. In 1993 the sampling points were 4319 and the collected water samples were 46035.

The results are expressed as percentage of acceptable samples, according to the limiting concentrations for each parameter under the Italian law (D.P.R. June 8th 1982 n.470). The permitted level for anionic detergents is equal to 0.5 mg/L. Table 15a shows the percentage

of samples, for each Italian Province, in which anionic detergent levels were superior to the above mentioned limit.

Non acceptable samples during 1992 and 1993 were limited to 10 Provinces (out of a total of 53 Provinces) and in only 4 cases (Napoli, Catania, Messina and Palermo) did this happen during both years.

Reference values for coastal seawater

In table 18 one can find levels of anionic detergents found in seawater along the coast of various countries (Italy, former Yugoslavia, France, Spain, Romania, Japan and Canada), available from the literature.

4.1.2 Marine Biota

There appeared to be very few data available to UNEP-WHO on levels of anionic detergents in the Mediterranean Biota.

It was possible to find some data (see table 19) for different species of mussels and fish sampled at the Catania (Italy) fish market (1973), where they were present in a range from not detectable levels to a maximum of 2.1 (mg/Kg, wet basis) in *Mugil cephalus*. In the gulf of Naples (Italy) concentrations between 2.20 - 10.70 (mg/Kg, wet basis) were present in *Mytilus galloprovincialis*.

Reference values for biota

The levels of anionic detergent in biota samples taken along the Italian coast and in a Japanese bay are reported in table 19.

5. EFFECTS OF DETERGENTS ON MARINE BIOTA AND ON HUMAN HEALTH

5.1 Introduction

Surfactants are not chemically pure compounds but mixtures of individual chemical compounds, the composition of which may vary depending on both the production process and the starting materials used. Therefore, the toxicological data are theoretically only valid for the mixture studied even if they can also be used for all products which are chemically more or less closely related.

The widespread use of surfactants requires that the highest priority be given to human protection. Contact with surfactants is becoming more frequent and almost unavoidable. Acute toxicity is of interest particularly in cases of ingestion, (e.g. of cleaning products) by children. Chronic toxicity is of interest not only with regard to the intake of trace amounts which occurs as a consequence of the application of cleaning and rinsing agents as well as other household care products but also with food, drinking water and by contact with recreational waters (fresh- and salt-water).

The toxicity for living organisms found in rivers and seawaters (marine biota) is another very important consideration, due to the water pollution deriving from the presence of surfactants in municipal liquid wastes, above all when these are not treated.

In this part of the present report the available information on the effects of anionic surfactants on man and marine biota will be summarized. The order of the subjects discussed will be:

1. Effects on human health
 - a) exposure routes for man (oral and contact)
 - b) metabolic pathways of detergents following ingestion and parenteral intake
 - c) skin and mucous membrane exposure
 - d) acute, subacute and chronic toxicity data
 - e) effects on human skin and mucous membranes
 - f) carcinogenic, mutagenic and teratogenic effects

2. Effects on marine biota
 - a) exposure routes and acute toxicity data for aquatic animals
 - b) chronic and sublethal toxicity of surfactants to aquatic life
 - c) influence of environmental factors on detergent toxicity for marine biota
 - d) chronic toxicities of surfactants to algae
 - e) conclusions.

5.2 Effects on Human Health

5.2.1 Exposure routes for man (oral and contact)

Man may come into contact with detergents (containing anionic surfactants and many other compounds needed in the commercial products) in various situations. In the present report both ingestion and contact will be considered. Ingestion can take place as an accidental ingestion of concentrated detergents, especially in children, or as a continuous intake of small quantities of surfactants and related substances which are present in commercial detergents, through contaminated drinking water, food and the domestic use of household and cleaning products.

Contact (and sometimes also inhalation) can happen continuously during the domestic use of household and cleaning products and occasionally when man comes into contact with polluted recreational waters (fresh- and seawater).

5.2.2 Metabolic pathways of detergents following ingestion and parenteral intake

Ingested LAS are absorbed in the intestine and transported by the blood. When they reach the liver, they undergo β -oxidation; the more hydrophilic products are returned to the departing blood, while those less hydrophilic such as longer and intact LAS, are transported in the bile via the bile duct back to the intestine. There they are again absorbed into the blood and thus subject to more oxidation in the liver. The short sulphophenylalkanoates pass into the urine and are excreted with other hydrophilic salts. Any compounds failing to be absorbed during passage and recycling through the intestine are excreted with the faeces. Any carbon dioxide

formed in the oxidation is mostly exhaled through the lungs, transported to there by the blood (Michael, 1968).

Available data on tetrapropylene derived alkylbenzene sulfonate (TBS) and other types of anionic surfactants, show an overall pattern quite consistent with that exhibited by LAS.

Alkyl sulphates too (e.g. sodium dodecyl sulphate) are absorbed extensively from the intestine and only a small amount of the surfactant is degraded to free sulphate. There are not very many data on these compounds which include alkane and olefine sulfonates. More than 90% of the orally dose is found in the urine and the remainder in the faeces, indicating an efficient absorption from the intestine (Taylor, 1975).

In summary, anionic surfactants are readily absorbed from the intestinal tract and are transported to the liver probably via the hepatic portal blood supply. Involvement of the lymphatic system is minimal. Metabolism in the liver is rapid, as evidenced by the short duration of surfactants and their metabolites in the body, and complete, since the parent surfactant is rarely recovered in the urine. For most anionic surfactants, the urine is the main route of excretion of the metabolites, although the biliary route is an important one for some isomers of the ABS type. The data also indicate that surfactants do not become localized in any of the tissues of the body other than those associated with the detoxification mechanism and excretory process.

The data available on the fate of anionic surfactants administered to animals by parenteral routes suggest that they are rapidly metabolized and excreted via the same routes as orally administered surfactants. The findings by Taylor (1975), Knaak *et al.* (1966), and Merits (1975), that traces of unchanged surfactant were present in the urine of rats after i.v. administration may indicate that the metabolic system can be overloaded, resulting in a breakthrough of unchanged surfactant into the urine. Metabolites that have been identified suggest a common path of catabolism. The efficiency and rapidity with which the surfactants are metabolized by rats suggest that the enzyme systems involved require no induction as might be expected for systems normally employed in endogenous lipid metabolism.

5.2.3 Skin and mucous membrane exposure

5.2.3.1 Percutaneous absorption

The increasing availability of radioactively labeled detergents and a supply of human skin from autopsies has favoured laboratory studies of the penetration of detergents into skin. By measuring radioactivity levels in tape strippings, Blank and Gould (1959) showed that most of the anionic surfactants in the skin were recovered in the first two strippings of the corneum.

The epidermis appeared damaged when the pH was 11 or higher and great amounts of surfactants were found in the dermis. Blank and Gould (1961) also investigated the amount of penetration into excised human skin of sodium dodecanoate from dilute, buffered solutions of pH 7.5-12 and compared the data with the degree of erythema that developed in human skin *in situ*. They found that penetration into the dermis was minimal between pH 8.5 and 10.5 but increased below pH 8.5 and above pH 10.5. Thus the observed high penetration in the pH range of 7.5-8.5 was believed to be due to an increased lipid solubility.

Application of 0.005 M buffered solutions of sodium dodecanoate to human skin *in situ* showed that during 6 hr contact erythema and pruritus developed with solutions of pH 7.5, in agreement with the high penetration observed *in vitro*, but no erythema or pruritus was recorded when the pH of the applied solution was 9.5, at which pH virtually no penetration had

been found *in vitro*. Soon it was found that *in vitro* measurements of absorption of substances such as anionic detergents, which bind strongly to skin, have some shortcomings. In fact, the results of a comparison of the penetration of sodium dodecyl [³⁵S]sulphate into living and excised guinea pig skin (Choman, 1960) showed that *in vivo* skin absorption occurred to a depth of some 800_μm whereas in the non living skin absorption was recorded to only some 250_μm.

The first direct study of percutaneous absorption of alkyl sulphates *in vivo* was reported by Sprott (1965). The results showed that approximately 40% of the applied radioactivity was recovered from the rats treated with sodium dodecyl sulphate, whereas some 20% was recovered in the urine from the rats treated with sodium hexadecyl sulphate. In both cases the rate of excretion was rapid, with approximately half of the total recovery being excreted in the first day. When the duration of contact of sodium hexadecyl sulphate was increased from 15 to 30 min, the excretion of radioactivity in the urine was doubled and, significantly, absorption could be demonstrated in the short time of 10 min by the presence of radioactivity in the blood. Other types of surfactants were able to be absorbed through skin although experimental data were not provided.

A comparative study of the absorption of pure ¹⁴C-labeled detergents through guinea pig skin was reported by Prottey and Ferguson (1975). Different head groups were substituted on the hydrophobe, which remained constant at 12 carbon atoms. The nature of the head group was found to be very important in determining the extent of percutaneous absorption. Of the anionics examined sodium laurate was the best penetrant. Sodium dodecyl triethoxysulphate and sodium dodecanoyl isethionate were intermediary with an absorption of 1.16 and 0.63_Fg/cm², respectively whereas sodium dodecyl sulphate and sodium dodecyl sulfonate gave the least absorption of 0.13 and 0.09_Fg/cm², respectively.

Another study by Black and Howes (1979) showed that penetration was low for the alcohol sulphates and alcohol ether sulphates, but greater for the alcohol ethoxylates. For each anionic surfactant, penetration was proportional to the applied concentration and the number of applications, but increased more gradually with extended duration of contact.

5.2.3.2 Effect on absorption of water

Scheuplein and Ross (1970) investigated the effect of soap and detergent on the permeability of tritiated water through isolated human abdominal epidermis. Sodium dodecanoate at 1% (w/v) caused a large increase in the permeability to tritiated water after an initial lag period of approximately 5 hr. The permeability curve increased exponentially indicating that damage by the soap to the epidermis was continuous. When the concentration of sodium dodecanoate was increased to 5% (w/v), the permeability of the isolated epidermis to tritiated water rose more quickly after a reduced lag phase. These investigators also observed that the epidermis regained some of its barrier properties when the soap solutions were removed, but that use of the more concentrated solutions caused permanent damage. At 5% (w/v), Scheuplein and Ross (1970) found that sodium dodecanoate had a greater and earlier effect on the permeability of isolated human epidermis to tritiated water than had sodium dodecyl sulphate. Raising the pH of the sodium dodecyl sulphate to that of sodium dodecanoate (pH 10) had little effect on increasing the permeability of tritiated water by sodium dodecyl sulphate. Scheuplein and Ross (1970) believe that the mechanism of the effect of sodium dodecanoate on the stratum corneum is due to a reversible_α-β-conversion of keratin resulting from a denaturation by the soap, which facilitates the diffusion of water through the more porous membranes.

Using tritiated water, Sprott (1965) observed that groups of rats washed with solutions of two types of soap bars showed a significantly increased tritium content in the blood, but the differences between the conventional soap bar and the toilet bar containing synthetic detergents was not statistically significant. There was a tendency for the urine of the rats washed with solutions of the toilet bars to contain greater amounts of radioactivity than the control group, but only the effect of the conventional soap was statistically greater.

5.2.3.3 Effect on absorption of electrolytes

There have been many reports on the way in which surface-active agents enhance *in vivo* the absorption of electrolytes, particularly those known to elicit a contact dermatitis or delayed hypersensitization, such as nickel and chromate salts. Nilzen and Wikstrom (1955) showed that, while neither nickel sulphate or sodium dodecyl sulphate alone would cause an eczematous response, the combination did produce a contact dermatitis. Similarly, Kvornung and Svendsen (1956) reported that secondary alkyl sulphate (Teepol) facilitated the sensitization response to nickel chloride and potassium dichromate in susceptible patients. In human patch tests, these authors were able to show a skin response to the allergen in a more dilute solution when the challenge solution contained Teepol.

Vinson and Choman (1960) observed that sodium dodecyl sulphate (SDS) and sodium dodecyl benzene sulfonate applied to the skin of guinea pigs in a two-stage multiple-application test produced an eczematous response to nickel sulphate and they observed the deep penetration of nickel into the skin. Lindberg *et al.* (1989) found in the SDS treated skin a greater nickel penetration which they believed might be due to an increase in the "water channel" volume of the stratum corneum. Friberg *et al.* (1961) investigated the effect of soap and alkylaryl sulfonate on the penetration of mercuric chloride and methyl mercury dicyandiamide, both radioactively labeled, through guinea pig abdominal skin by disappearance measurements. Pretreatment of the skin by painting with a soft brush for 2 min twice daily with 1 % aqueous solution of soap did not affect the absorption of either of the two mercury compounds. However, a 1% aqueous solution of the alkylaryl sulfonate did show a tendency to increase the absorption of the methyl mercury dicyandiamide. Skog and Wahlberg (1962) confirmed the results reported by Friberg *et al.* (1961) and observed that there was no difference between the absorption through guinea pig dorsal or abdominal skin.

5.2.3.4 Effect on absorption of organic compounds

Bettley (1965) studied the penetration of sodium salicylate through isolated abdominal skin. The average penetration in the control cell was 82.5_Fg or approximately 21_Fg/cm²/day and at the end of the second week penetration was essentially the same increasing in the presence of soap from the control level of 82.5 to 675_Fg at the end of the first week, to 1200_Fg after 2 weeks, and to 1430_Fg after 3 weeks. Thus the action of the soap was to increase progressively the penetration of the salicylate.

Aguiar and Weiner (1969) observed that the effect of sodium dodecyl sulphate on the absorption of chloramphenicol through isolated hairless mouse skin was time-dependent and that there was no lag time, probably due to the thinness of the stratum corneum. Absorption was proportional to temperature. Addition of 0.2% sodium dodecyl sulphate caused the permeability of the skin to chloramphenicol to double, whereas addition of 0.4% sodium dodecyl sulphate had only a slightly greater effect than the 0.2%. At a concentration of sodium dodecyl sulphate (0.02%) below the CMC, the effect of the surfactant was to reduce the penetration of chloramphenicol through isolated hairless mouse skin. The authors suggest that below the

CMC the surfactant may complex with the drug and that at the high concentrations the surfactant may damage the stratum corneum.

Skog (1958) reported that in humans and guinea pigs, preexposure to the surfactants tended to increase the response to 2,4-dinitrochlorobenzene (DNCB), a known irritant and sensitizer, while in rabbits no gross differences in the response to DNCB were observed between pretreated and nonpretreated skin.

The treatment of guinea pigs with aqueous solutions of soap (5% w/v) or alkyl ether sulphate (0.045 N) for at least 5 min had a non reversible effect on the permeability to SARIN, an organophosphorus cholinesterase inhibitor (Fredricksson, 1969a). Only a slow recovery of the barrier properties was observed (Fredricksson, 1969b), which might be related to the regeneration of nondenatured proteins by the epidermis. Also Black *et al.* (1975) showed that anionic surfactants have different effects on the skin barrier properties using pyridine-2-thione oxide, also called zinc pyrithione (ZnPT).

The effect of sodium lauryl sulphate on the percutaneous absorption of butanol as a microemulsion through guinea pig skin was studied by Boman *et al.* (1989), who observed that there were marked increases in the blood concentration when compared with the levels after application of neat solvent, probably due to denaturation of keratin and hydration of the stratum corneum by sodium dodecyl sulphate.

From all the above mentioned studies it is clear that absorption of anionic detergents through skin is low and that *in vitro* and *in vivo* results are not directly comparable, probably due mainly to the strong binding of detergents to protein. The effects on the skin barrier are also progressive, so that increased duration of exposure will increase the absorption of the detergent. Higher concentrations will also promote absorption. While synthetic anionic detergents in aqueous solution have a pH near to neutrality, the alkalinity per se of aqueous soap solutions or of detergent products, can reduce the barrier properties of skin. The effect of detergents on the skin barrier also allows better absorption of other compounds. The greater impermeability of human skin (Bartek *et al.*, 1972, Howes and Black, 1976) means that only small amounts of anionic detergent are likely to be absorbed by humans, even when large skin surfaces are exposed. The poor percutaneous absorption and the low oral toxicity of anionic detergents (Gloxhuber, 1974) related to the low intestinal intake, suggest that the possibility of systemic toxic effects in humans is extremely unlikely.

5.2.4 Acute, subacute and chronic toxicity data

5.2.4.1 Acute toxicity data

Very little has been reported concerning systemic short-term studies attempting to correlate anionic surfactant structure with toxicity. Furthermore, the study protocols and approaches reported for the determination of toxicity have varied considerably between different laboratories, and many experimental details are not available. In most cases the surfactant was applied orally, in view of the likelihood of oral intake. In numerous cases more than one animal species was used in these studies; in most cases LD₅₀ values above 1 g/kg have been obtained, which shows that the acute toxicity is generally low. This generalization holds for anionic surfactants of widely different nature. There are only few acute toxicity studies relating to parenteral application. The data obtained show much higher acute toxicities than the ones found upon oral administration. It may be concluded that there is either no significant absorption of the surfactants from the intestinal tract or there is a rapid conversion to less toxic metabolites.

Hemolysis is a most characteristic consequence of the presence of the unchanged substances in the blood vessels.

While the group of anionic surfactants shows little toxicity upon oral administration, a certain dependency of the toxicity on the chain length of the alkyl group has been observed in the homologous series of alkyl sulphates and alkyl benzene sulfonates. The finding that compounds with 10-12 carbon atoms in the alkyl chain are less well tolerated than compounds with shorter or longer chains is also reflected in studies relating to local irritancy.

When linear alkyl benzene sulfonate replaced the branched chain tetrapropylene benzene sulfonate it was found that the acute oral toxicities of the linear and the branched chain product hardly differ.

The vast majority (about 98%) of human exposures to high doses of surfactants is accidental and most cases involve children under 6 years of age. The majority (about 70%) of all cases reported to poison control centers show none or only minor symptoms.

Because the most common washing and cleaning products used in households contain not only anionic surfactants but usually a mixture of these substances with other ingredients of differing concentrations, we can only state clinical and toxicological experience obtained with these mixtures. The degree of systemic toxicity caused by anionic surfactants is considered by different authors to be relatively small. Velvart (1989a) has stated that for adults, the oral ingestion of anionic surfactants up to 1.0 g/kg of body weight is relatively harmless, and Dreisbach and Robertson (1987) estimates the maximum safe amount for children to be 0.1-1.0 g/kg.

The most common symptoms after ingestion are nausea or vomiting shortly after the ingestion; salivation is sometimes described. Further symptoms are diarrhea, occasional abdominal discomfort, and intestinal distension. Aspiration or inhalation of powders or liquids is feared mostly because of possible pulmonary complications.

Serious symptoms caused by detergents reported in the literature were due principally to aspiration or when the substances were ingested with suicidal intention.

In conclusion ingestions of anionic surfactants are generally harmless if small quantities are swallowed. On the other hand, these substances should not be considered to be totally without risk, particularly as there is the possibility of their aspiration into the respiratory tract.

5.2.4.2 Subacute and chronic toxicity data

Many animal studies were carried out in order to assess the subacute and chronic toxicity of anionic surfactants. A few substances have even been tested with humans, generally in the context of therapeutic measures. The doses administered ranged from a few milligrams to toxic and lethal amounts. In most cases, the surfactant was applied with food; however, dosing in gavage or as admixture to the drinking water was occasionally used. The duration varied between a few weeks and 2 years.

From the data available none of the substances under observation gives rise to severe and specific toxic symptoms or severe organ injury. In most cases, the low-grade effects are related to a reduced increase in body weight during the experiment, which presumably has to do with reduced food intake or utilization. These effects may be related to the finding of certain

irritation in the mucous membrane of the intestinal tract, which has been observed in animals which had received high doses of surfactants. In some cases, low-grade changes were found in liver and kidneys, depending on the surfactant dose received.

The amounts of surfactants which are unavoidably ingested over long periods of time are well below the doses required to cause organic changes, as shown by animal experiments. The presence of 8% sodium lauryl sulphate in the food is lethal within 2 weeks as shown by Fitzhugh and Nelson (1948) with rats as test animals. Dissection showed intestinal inflammation and distention of the digestive system.

The influence of sodium dodecyl sulphate and sodium tetradecyl sulphate on the epithelial cells of the small intestine of mice at concentrations of 3×10^{-2} and 3×10^{-3} mol/L has been studied by Ruska (1961). Both substances behaved similarly. Cell elongation, swelling of mitochondria, and a slight widening of the interior of the endoplasmic reticulum in the Golgi complexes and a fine granulation of the nuclei were observed. Nucleus and cytoplasm structures were retained in finest dispersion. Also, the endoplasmic reticulum remained unchanged. These findings may be attributed to an increased solubility of the surfactants at interfaces, to the removal of an impermeable membrane, or to increased permeability.

Yoneyama *et al.* (1976) have studied the influence of ABS on liver and kidney function adding it to the drinking water (0.07, 0.02, and 0.6%) and to the diet (0.6 and 1.8%) of mice and rats. During the period of observation (9 months) not only a decrease in liver weights was observed but there were also reductions in the activities of several liver and kidney enzymes (glucose-6-phosphate dehydrogenase, lactate dehydrogenase, and glutamate pyruvate transaminase, Na⁺, K-ATPase).

Diarhea, reduced weight increases, and damage to internal organs such as cecum, liver, and kidneys were observed in the test animals fed with 1.8% LAS or ABS in the diet (Yoneyama *et al.*, 1973). The administration of 0.6% of the same substances in the diet led to a slight growth retardation, increased activity of alkaline phosphatase, decreases in the total amount of protein in the blood, and histological kidney changes. Slight kidney damage (histological changes) were also observed when LAS and ABS were administered at a level of 0.2% in the diet.

After inhalation exposure to 3.59 mg/m³ of dodecyl benzene sulfonate in the workplace atmosphere, blood changes were apparently induced in humans (Sawinsky *et al.*, 1984). Intravenous application of 50 mg keryl benzene sulfonate resulted in minimal respiratory trouble and certain minor changes in blood pressure in the case of cats as the test animals. Major amounts, e.g., 100 mg/kg lead to slight bradycardia, relaxation of the smooth musculature of the uterus and respiratory and circulatory problems. Paralysis of the smooth musculature of the gastrointestinal tract was achieved at a concentration of 0.047% keryl benzene sulfonate (Chrusciel *et al.*, 1966).

Contact of the esophageal and intestinal mucosa with 10% solution of sodium lauryl sulphate using a catheter or direct operative introduction into the stomach (Berenson and Temple, 1975) caused edema of the esophageal mucosa as well as congestion and loss of the superficial epithelial cells a few minutes after the application of the preparation. In the stomach, this treatment led to hydropic degeneration, loss of superficial mucosal cells, vascular congestion, edema of the submucosa, as well as occasional focal ulceration. More pronounced changes were observed after the application of the 20% solution of sodium lauryl sulphate.

The dermal application of very high doses (580 mg/kg) of sodium lauryl sulphate to rabbits led to irritation and tremor, tonic-clonic spasma, and respiratory collapse (Carson and Oser, 1964), phenomena probably caused by a critical concentration in the blood. Death was observed within 4 weeks upon repeated and daily oral applications of 0.02-1 g alkyl benzene sulfonate to white mice (Sanz Ibanez and Jiminez-Castellanos, 1964). In the dead animals a reactive hyperplasia of the capillary endothelium in various organs could be observed, with histological lesions such as necrotic lesions and histiocyte proliferation.

Hall (1950) studied the danger of repeated inhalation of sodium lauryl sulphate, sodium alkylaryl sulfonate, sodium-2-ethylhexyl sulphate, and dialkyl sulfosuccinate using guinea pigs as test animals. Using 0.1, 0.5, and 1% concentrations and an exposure length of 8 hr/day on 6 consecutive days, all substances gave rise to a dose-dependent dyspnea of the test animals with the most severe effects in the animals treated with the highest concentrations. Slight to moderate respiratory symptoms were observed in the 0.5% groups, while the 0.1% aerosols were tolerated without toxic effects. None of the test animals died upon inhalation of the anionic surfactants applied at the 0.1 and 0.5% levels. The onset of the respiratory symptoms was rapid and they persisted for 1-3 hr after exposure. With the exception of a harder consistency of the lungs upon inhalation of surfactants at the 1% level, there were no findings in the dissections. However, dose-dependent changes in the lung tissue could be observed histologically. There was a pronounced segregation of polymorphonuclear cells and macrophages, as well as interstitial fibroblast proliferation, degeneration and desquamation of the pulmonary epithelium, and a proliferation of the alveolar epithelium, which could be observed in the lungs exposed at the 1% level. Large alveolar regions were filled with inflammatory material. The guinea pigs exposed to the 0.5% solutions showed only weak histological reactions, while 0.1% solutions were tolerated without histological findings.

5.2.4.3 Conclusive remarks on acute, subacute and chronic toxicity data

In conclusion, although anionic surfactants can be absorbed through the gastrointestinal tract, following ingestion, the results of systemic acute toxicity studies on a number of important substances indicate a low order of systemic toxicity.

These findings agree with clinical observations. The major dose-dependent effect of anionic surfactants is restricted to local damage to mucous membranes of the gastrointestinal tract. High doses lead to vomitus and diarrhea. The possibility of chronic toxicity has been studied by intense investigations in different species. Results obtained from animal experiments and exposures of up to 2 years revealed, even at high-dose levels, no risks attributable to possible intake under user conditions. No serious side effects have been observed and significant accumulation of this compound class in the organism has never been detected. On the basis of the available data and taking into account possible oral intake, anionic surfactants can be regarded as nonhazardous for human health as currently used in household products.

5.2.5 Effects on human skin and mucous membranes

Looking for evidence of the effects of anionic surfactants on the skin and mucous membranes of humans and for any evidence of allergic reaction to them, one can find only few studies dealing with specific types of organic detergents (Treuhooff, 1966) in the earlier dermatological literature.

The most important function of the skin is the maintenance of a permeability barrier between inner and outer milieu and its stratum corneum is its most important protective

component. To survive as an aqueous body in a terrestrial environment, mammals, but also plants, arthropods, reptiles, and birds have been forced to develop cellular mechanisms to regulate integumental water loss (Hadley, 1980 and 1989) based on lipids. The human epidermis consists principally of two components: protein-rich keratinocytes in different stages of differentiation and a lipid-rich intercellular substance.

The knowledge of the localization of different lipids as parts of the intercellular substance within the intercellular space of several cell layers, helps to understand the complex mechanism of blockade of the transcellular and paracellular pathway. According to Elias and co-workers (Elias, 1983; Elias *et al.*, 1981; Grubauer *et al.*, 1989; Lampe *et al.*, 1983) multilamellar-directed lipids play the major role in the permeability barrier function. These lipids derive from a specific organelle of the keratinocyte, the "lamellar body" or "Odland body." They are synthesized in the stratum spinosum and excreted by exocytosis in the stratum granulosum (Proksch, 1989; Elias, 1986). While the intercellular substance of the deeper layer also contains phospholipids, cholesterol sulphate, and sugars, the composition changes to minimal portion of ionic lipids and high content of neutral lipids in the upper layers of the stratum corneum. This variation in the composition of the intercellular substance explains the phenomenon of selective permeability and shows the prominent role of the stratum corneum as a hydrophobic barrier.

The different lipid contents (Proksch, 1989; Lampe *et al.*, 1983) in the stratum corneum of different parts of the body (e.g.: face 7-10%, abdomen 6-8%, legs 2-4%, palms and soles 1-2%) and the various thickness of the stratum corneum causes the variation of the barrier effect against hydrophilic substances (Elias, 1983; Elias, 1986). Since the water content of the stratum corneum is an important factor in maintaining its viscoelastic properties (Blank, 1952), the corneum has to keep a certain amount of water in order to guarantee extensibility and flexibility for the movements of the skin. Three main factors are responsible for this purpose (Leveque *et al.*, 1987):

1. The binding state of water molecules with the keratin components.
2. Epidermal lipids, mainly situated in the intercellular space.
3. The "natural moisturizing factors" (NMF), water-soluble substances (e.g., lactic acid, urea, pyrrolidone carbonic acid), which strongly influence the equilibrium rate of the stratum corneum with the environment.

The influence and importance of sebum as a probable fourth factor for skin function remains unclear; indeed sebum consists of different fractions of lipids that are excreted by sebaceous glands covering most of the skin surface (Nicolaidis, 1974). This led to an earlier interpretation as "water-lipid coat," or "acid coat" due to its slightly acidic pH.

Nevertheless it is known that sebum does not build a closed film on the skin (Gloor *et al.*, 1972; Gloor *et al.*, 1974), children before puberty possess a fully intact function of the skin barrier without a significant production of sebum (Yamamoto *et al.*, 1987) and areas on the body surface with higher sebum production do not have a better barrier function (Proksch, 1989).

In summary, the prominent factors of skin function, which anionic surfactants may act on, are surface lipids from the sebaceous glands, horny material from keratinocytes, intercellular lipids of different fractions and types, "natural moisturizing factors," and cellular membranes of living cells. Affecting diverse items of this system anionic surfactants may provoke changes in the barrier function, in the water content, in the surface milieu, etc.

With regard to the pathological consequences deriving from prolonged contact of the skin and the mucous membranes with surfactants, one can find in the literature many

investigations concerning the appearance of irritant contact dermatitis ascribed to soaps and synthetic detergents.

The information that an anionic detergent could cause allergic contact dermatitis first came from Sweden in 1954, with the report of a serious outbreak of dermatitis that was considered correlated with a particular detergent (Nilzen, 1958). which contained 6% lauryl sulphate and 20% alkylaryl sulfonate.

Detergents may also induce irritation on mucous membranes, as there is no protective series of layers of corneal squames on top of mucous membrane nor are they protected from excessive loss of moisture. This fact regards three different organs that may come in contact with anionic surfactants: the eye, the mouth and throat, and the urogenital tract (especially the female one).

In summary the contact of mucous membranes with anionic surfactants and products containing synthetic detergents should not give toxicity problems as long as the use conditions are "normal" (Velvart, 1989b; Temple, 1978). Ingestion's and/or misuse of products resulting in closer contact with concentrated detergents will lead to mucous membrane irritation of different degrees depending on several factors (the length of time of contact, the active concentration of surfactant, etc.). The only serious side effects that have been reported resulted from ingestion by children or babies who developed bronchitis and pneumonitis due to aspiration of the product (Velvart, 1989b).

5.2.6 Carcinogenic, mutagenic and teratogenic effects

5.2.6.1 Carcinogenicity

In common with other chemical products, surfactants are not pure materials; commonly found impurities include raw materials, byproducts, unreacted substances, etc. These facts must be considered when designing studies to evaluate potential toxicity or carcinogenicity. In evaluating the potential carcinogenicity of alkyl benzene sulfonate (ABS) and α -olefin sulfonate (AOS) Saffiotti *et al.* (1962) and Hunter and Benson (1976), paid particular attention to the impurities in the test samples. Their data indicate that surfactants themselves present no particular hazard to animals. The possibility that surfactants might influence solubility, dispersion mobility, or penetration of other potential carcinogens, or that they may engage in synergistic or antagonistic reactions with carcinogens, has also been investigated (Swisher, 1966). Although it is possible that carcinogens could be solubilized and eluted from soils by surfactants, there is no experimental evidence to substantiate this; solubilization of such substances to any appreciable extent will only occur at concentrations above the critical micelle formation (Lange and Raay, 1963; Bohm-Gossel and Kruger, 1965).

Tumors have been observed during the course of prolonged chronic toxicity tests and some researchers have examined the generation of tumors in their chronic toxicity tests on anionic surfactants (Neumann, 1966; Swisher, 1966; Gloxhuber, 1967; Swisher, 1968; Gloxhuber, 1974).

In all trials the tumor-producing rate in treated groups of animals was nearly the same as in control groups, and no carcinogenic potential was found either in LAS or ABS. (Saffiotti *et al.*, 1962; Bommann and Loeser, 1962, Bommann *et al.*, 1963; Paynter and Weir, 1960). Even after prolonged percutaneous exposure of rats to LAS there was no indication of carcinogenic activity (Taniguchi *et al.*, 1978). The same results were obtained evaluating detergent and surfactants mixtures for carcinogenicity.

5.2.6.2 Cocarcinogenic action

Gastric carcinogenesis using a carcinogen combined with a surfactant has been attempted by many investigators. In this case the surfactant would be acting as a cocarcinogen which has no carcinogenic action per se but has the ability to increase tumor incidence when supplied with a carcinogen (Saffiotti *et al.*, 1962).

Takahashi and al. reached the conclusion there may be a possibility of surfactants increasing the activity of harmful chemical substances in the living environment, although there have never been reports indicating that surfactants themselves cause gastric carcinogenesis (Takahashi 1970, Takahashi and Sato, 1967, 1968, 1969; Takahashi *et al.* 1969, 1970 a,b, 1973, 1975; Fukushima 1973; Fukushima *et al.*, 1972, 1974; Sugiura *et al.*, 1971, 1973; Shirai *et al.*, 1973).

Borneff (1960, 1963) believed that surfactants might increase the concentration of carcinogenic materials dissolved in water supplies but he concluded that the use of household detergents presents no hazard, either from enhancement of the carcinogenic action of other materials in the diet or from toxicity on the part of the detergent itself (Borneff, 1959, 1960, 1963, 1964; Borneff and Knerr 1959a,b, 1960; Borneff and Fischer 1961a,b,c, 1962a,b,c,d 1963; Borneff and Kunte 1964).

5.2.6.3 Solubilization of carcinogens in the environment

Janicke (1962) pointed out the possibilities of incorporating toxic and/or carcinogenic materials into living organisms, or eluting harmful materials from the soil into ground water by the physicochemical properties of detergents. As mentioned earlier, Borneff (1960, 1963) also conducted studies on ABS for cocarcinogenic potential, considering the possible interaction of surfactants with the carcinogenic hydrocarbons potentially present in our environment. Although this is possible in theory, experimental evidence has been presented indicating that this should not take place at the surfactant concentrations found in drinking water, and river water, etc. The solubilization of polycyclic aromatic compounds by various kinds of surfactants can occur only at surfactant concentrations above their own CMC (Lange and Raay, 1963; Böhm-Gössl and Kruger, 1965; Satälä, 1954) while it is well known that surfactant concentrations encountered in our environment are far below the CMC. From a practical viewpoint the possible hazards from this source are negligible.

5.2.6.4 Mutagenic/genetic properties

No mutagenic activity has been attributed to the main types of anionic surfactants when tested by classical reverse mutation assays. Some single positive response may be due to materials unrelated to the surfactants. Also commercial products based on anionic surfactants (Oda *et al.*, 1977) gave negative results for non-diluted and variously diluted products.

It can be assumed that the principal anionic surfactants now in use are not able to cause gene mutation and chromosomal damage.

Surfactants were also tested to investigate their ability to produce chromosome damage in the whole mammal, by *in vitro* and *in vivo* procedures (OECD, 1980-1982). Overall, no positive evidence was found of any mutagenic activity of the tested substances.

5.2.6.5 Teratogenic properties

No danger of teratogenicity is indicated by the results of the many studies performed using the major anionic surfactants, ABS and LAS in particular, performed in rats, mice, and rabbits. In addition, single and multigeneration studies in rats revealed no effects on the general reproductive process attributable to the chronic intake of these compounds.

However since 1969 a group of researchers, known as the Mikami group, (Mikami *et al.*, 1969, 1973a,b,c, 1976a,b, 1977a,b,c,d, 1981; Nagai, 1970; Ishimori, 1971; Iseki, 1972; Mikami and Sakai, 1973, 1974a,b; Sakai *et al.*, 1975; Tani, 1975; Wakai, 1975; Mikami, 1976, 1977a,b, 1980, 1981) has reported that commercial light-duty liquid detergents and surfactants caused significantly increased malformations in mice and rats following oral, dermal, and subcutaneous administration. According to the Mikami group the administration (in ICR/JCL mice) of 0.5 ml of 1% LAS solution to an area 3x4 cm of clipped skin daily from day 0 to day 11 of pregnancy resulted in incidences in the foetus of 100% subcutaneous congestion, 49.5% cleft palate, 100% bleeding and 9.9% club foot or similar anomalies (Wakai 1975). Similarly the same group reported a definite congestion in Wistar rat foetuses following the subcutaneous injection of a 1.0% LAS solution at 10 ml/kg to the dam (Mikami *et al.*, 1977b). No other laboratory, even in Japan, has been able to confirm these observations. The adoption of higher dosages during organogenesis, aiming particularly at teratogenic induction, has failed to substantiate such findings in mice, rats, or rabbits, although dosages causing maternal toxicity also induced some marginally significant reduction in intrauterine growth and increased rates of resorption. As a result of the innovatory nature of the Mikami group's reports, much public attention was attracted to the possibility that dermal applications of commercial detergents might be teratogenic. The Environmental Sanitation Bureau of the Japanese Ministry of Health and Welfare planned and promoted a series of group studies during 1973-1976. The results of the final joint project studies of the teratogenicity of LAS, in which investigators from four universities, including Mikami, followed precisely the same protocol, failed to support the original results of the Mikami group (Charlesworth, 1975; A. D. Little, 1977, 1981; Wilson and Fraser, 1977; Hiraga, 1981; Nishimura, 1981; Yoshida 1981a,b).

5.3 Effects on Marine Biota

5.3.1 Exposure routes and acute toxicity data for aquatic animals

The effects of surfactants on aquatic life have been studied intensively during the past 30 years (Marchetti, 1965; Abel, 1974; Gledhill, 1974; A. D. Little Co., 1977; Margaritis and Creese, 1979; Koskova and Kozlovskaya, 1979; Sivak *et al.* 1982; Gilbert and Pettigrew, 1984; Kimerle, 1989; Lewis, 1990). Toxicity of surfactants was reported for a broad variety of marine organisms, including fish, algae, bacteria and virus (Yamane *et al.*, 1984; Kimerle and Swisher, 1977; Florence *et al.*, 1984; Ward R.L. and Ashley, 1980).

Aquatic animal species (fish, invertebrates, and others) are generally sensitive to the presence of surfactants in their medium. Ôba and Takita (1984) showed that fish can sense the presence of a surfactant and avoid regions where its content exceeds the toxic threshold. They exhibit avoidance of other toxicants as well, chlorine for example (Brungs 1976).

The effects of surfactants on living cells (Wagener and Schink, 1987) are very complex and include penetration of the membrane and its fluidization, with partial solubilization of structural components (Florence *et al.*, 1984).

LAS differ widely (from 1 ppm to 100 ppm) in LC_{50} (median lethal concentration within 2-4 days) from one homologue to another. Also the aquatic toxicity of the isomers of a given homologue is different and increases as the phenyl attachment is moved from the centre of the chain toward the end. General and specific data on the LAS homologues and isomers have been published by Hirsch (1963), Borstlap (1964), Divo (1976), Kimerle and Swisher (1977), and Schöberl and Kunkel (1977).

The increase of the chain length is parallel to an increase of toxicity to aquatic life and of biodegradation rate and also of adsorption and partition into octanol. This may be related to the increased hydrophobicity as a consequence of the longer chain. Enrichment in the higher homologues and outer isomers in LAS adsorbed in river sediments has been observed by Honnami and Hanya (1980) while Yoshimura *et al.* (1984) found the opposite situation in the LAS remaining in solution in fresh-water, probably as a result of biodegradation.

Kimerle *et al.* (1975) showed that partition coefficients of LAS from aqueous solution into n-octanol and into fish tissues also, follows the same pattern as adsorption, increasing by about two orders of magnitude from C_9 -LAS to C_{15} -LAS, starting at around 10 for the C_9 and approximately doubling for each additional carbon. The same Author also observed that the toxicity to *Daphnia magna* increased in precisely the same logarithmic fashion for each increment of chain length.

Divo's (1976) toxicity data (for each LAS homologue from C_{10} through C_{14} , and also for each isomer of each homologue from C_{10} through C_{13} , along with biodegradation rate constants for all the LAS components) for goldfish (*Carassius auratus*) show that for the set of isomers of a given homologue the toxicity increases logarithmically with increased biodegradation rate; sets of homologues with a given phenyl position show an approximately logarithmic relationship also, but the toxicity increases faster, by a factor of about 2-3 for each added carbon in the chain and about 1.2-1.6 for each step toward the end of the chain in phenyl position. On the contrary, the biodegradation rate constant increases only linearly instead of geometrically with such changes in structure.

Aquatic organism have very different reactions toward surfactants. These compounds cause destruction of gill epithelium, impairment of chemoreceptor organs and damage to epidermis and pharyngeal walls (Bardach *et al.*, 1965; Brown *et al.*, 1968). High levels of surfactants cause hepatomas on the gill and consequently a diminution of oxygen uptake and impairment of electrolyte balance (Schmid and Mann, 1961). Prolonged exposure of carp fingerlings (*Cyprinus carpio*) to sublethal concentrations of ABS (Misra *et al.* 1991) may cause metabolic disturbances (e.g. impairment of glycogen synthesis).

Regarding the effects of surfactants on fish histology, Zaccone *et al.* (1985) described histological and histochemical changes in the epidermis of the catfish (*Heteropneustes fossilis*) after contact with these compounds.

It is of interest that the danger to fish comes from exposure of the gill tissues to the surfactant rather than from ingestion. For this reason very important toxic effects may be experienced by a fish swimming in water containing LAS (or other surfactant) at low ppm concentrations, while no ill effects resulted when Bock (1966) orally fed fish food with 20% LAS to fish (*Idus melanotus*) at about 1000 mg LAS per kg body weight. Similarly, Gloxhuber and Fisher (1968) found that 1-hexadecanesulfonate was tolerated by goldfish (*Carassius auratus*) upon intraperitoneal injection at 250 mg/kg with only transient disorientation for about an hour.

The mechanism for fish death due to detergent poisoning is not fully understood. It is even questionable whether detergents enter the fish body and to what extent they exert their action. Asphyxiation as a result of gill damage is, no doubt, one of the reasons for acute poisoning, but whether loss of gill function is the primary cause of death or is a secondary one has not been determined (Roy, 1988).

Roy (1990) also described a continuous profuse mucous secretion as a result of detergent exposure of a fish (*Rita rita*), which he correlated, through a loss of proteins, to the death of the animal.

Putnam (1948), in a review of the interaction of synthetic detergents and proteins, indicated that detergents may produce diverse effects by combining with the proteins, primarily through electrostatic forces.

The aquatic toxicity of LAS remaining after partial biodegradation is significantly lower because the more toxic longer homologues and outer isomers are quicker to degrade, in accord with the distance principle and is markedly reduced by formation of a carboxyl group at the end of the LAS alkyl chain. Swisher *et al.* (1976) confirmed this by exact quantitative data.

When fish live in water containing surfactant they absorb the surfactant, such as LAS, by partition from the water into their fatty tissues even when the concentration is well below the LC₅₀ and well below the no-effect level. The absorbed LAS is metabolized, possibly via the same initial pathways found in mammals and microbes, i.e., by oxidation of the chain to give shorter metabolites. Comotto *et al.* (1979) found LAS intermediates at relatively high concentration (and little intact LAS) in the fish gall bladder (where bile is stored). On the contrary the minicrustacean *Daphnia magna* absorbs LAS but apparently does not metabolize it.

Sodium dodecyl sulphate cause *rotavirus* inactivation after 1 hour at low concentrations (0.01-0.1%). Also other anionic and cationic surfactants have a virucidal effect which depends on the hydrophobic regions of these compounds. (Ward and Ashley, 1980).

5.3.2 Chronic and sublethal toxicity of surfactants to aquatic life

The literature on the toxicities of surfactants to aquatic life (Abel, 1974; A. D. Little Co., 1977, 1981; Koskova and Kozlovskaya, 1979; Margaritis and Creese, 1979; Sivak *et al.*, 1982; Lewis and Suprenant, 1983; Lewis and Wee, 1983; Cooper, 1988) is limited to acute effect levels, primarily for a few anionic and, to a lesser extent, for some nonionic compounds.

However a comprehensive knowledge of the chronic and sublethal effects of surfactants on aquatic animals is needed, since environmental safety assessments are based on chronic toxicity information and recently the role of sublethal effects in the safety assessment process has been identified as one of the key future research priorities in the environmental risk assessment process (Society of Environmental Toxicology and Chemistry, 1987).

5.3.2.1 Chronic toxicity for aquatic animals

Chronic toxicity tests usually include life cycle, partial life cycle and early life stage tests (Stephen *et al.*, 1985). In many cases the studies do not represent chronic toxicity data as commonly accepted by the scientific community. However, for simplicity, data generated in tests exceeding normal acute test duration of 48 h for invertebrates and 96 h for fish can be considered as "chronic toxicity" data.

5.3.2.2 Invertebrates

Daphnia magna has been the most common test species tested for chronic toxicity. LAS (linear alkylbenzene sulfonate), effect concentrations have ranged for this species from 0.005 to >10.0 mg/L. Values of less than 0.1 mg/L are few and the 0.005 mg/L effect value for *D. magna* reported by Lal *et al.* (1984) should be considered an exception. The no observed effect concentrations range from 1.3 to 3.3 mg/L, and the 21-d LC₅₀ values, 2.2 to 4.7 mg/L (Taylor, 1985). Kimerle (1989) reported NOEC (no observed effect concentration) values for *D. magna* that ranged from 0.1 mg/L (C₁₄ homologue) to 9.8 mg/L (C₁₀ homologue) while the NOEC value for a C_{11.7} LAS mixture and *Ceriodaphnia* was 3.0 mg/L. Masters *et al.* (1991) observed that the first effect concentrations for *Ceriodaphnia* and C_{11.8} LAS were <0.32 and 0.89 mg/L.

The effect concentrations for other invertebrate species using LAS as test compound are similar to those observed for daphnids: for instance they are between 0.2 and 0.4 mg/L for *Gammarus* (Arthur, 1970). Pittinger *et al.* (1989) reported that the NOEC for the midge was 319_Fg/g and the LOEC (lowest observed effect concentration) was 993_Fg/g based on sediment-adsorbed LAS concentrations. Bressan *et al.* (1989), reported that sediment-adsorbed LAS is scarcely toxic for other fresh water and marine benthic organisms.

Other anionic surfactants appear to be similar to LAS, although there are only limited data available. For example, first-effect concentrations for alkyl sulphate (AS) compounds were reported between 0.25 and 1.46 mg/L for flatworms and oyster and clam larvae (Hidu, 1965; Patzner and Adam, 1979) and a NOEC of 0.27 mg/L was reported for *D. magna* and an alkyl ethoxy sulphate (Maki, 1979a).

Chronic effect concentrations for surfactants and marine organisms have been reported for clams, oysters and mussels. Effects of LAS on oysters and mussels (changes in fertilization, egg development and larval growth) have occurred at concentrations exceeding 0.025 mg/L (Calabrese and Davis 1967; Granmo and Jorgensen, 1975). Threshold values of 0.010 and 0.050 mg/L LAS have been reported for oysters and sponges (Berth *et al.*, 1988). The NOEC values for the mysid shrimp and two LAS blends (C_{11.4} and C_{13.1}) were 0.4 and 0.04 mg/L, respectively (Kimerle, 1989).

5.3.2.3 Fish

The reported chronic toxicities of surfactants for fish are based largely on the response of fathead minnows to various blends and homologues of the anionic LAS. The first effect levels for LAS exceed 0.1 mg/L in most cases for the fathead minnow (Macek and Sleight, 1977; Holman and Macek, 1980), and for other fish species (Vailati *et al.*, 1975; Canton and Slooff 1982; Mckim *et al.*, 1975; Chattopadhyay and Konar, 1986a). Holman and Macek (1980) for example, reported NOEC values of 0.11-5.1 mg/L and LOEC values of 0.25-8.4 mg/L for fathead minnows in life cycle and early life stage tests using several LAS blends. The NOEC values for C₁₃ LAS and C_{11.8} LAS and the fathead minnow were 0.15 and 0.90 mg/L, respectively (Maki, 1979a). The greater toxicity of the higher alkyl chain length LAS blends observed by Maki (1979a) has been reported elsewhere (Kimerle and Swisher, 1977; Macek and Sleight, 1977; Holman and Macek, 1980). The first effect concentration of a C₁₄ LAS homologue was between 0.05 and 0.10 mg/L for the fathead minnow relative to 14.0-28.0 mg/L for a C₁₀ LAS homologue (Macek and Sleight, 1977).

Relatively few chronic toxicity values have been reported for nonionic and cationic surfactants and fish (Maki, 1979a; Yasunaga, 1976; Chattopadhyay and Konar 1986b).

5.3.2.4 Sublethal Toxicity for aquatic animals

Many studies describe the effects of anionic surfactants on several physiological processes of fish during exposures lasting from 15 min to 30 days. Effects on olfaction, respiration and gill physiology were more frequently monitored than other parameters and effects occurred in most cases at concentrations that exceed 0.1 mg/L. For example, changes in adrenergic control mechanisms and vasodilatation in salmon gills were noted at LAS concentrations of 0.6 mg/L or greater (Bolis and Rankin, 1978, 1980). The respiratory rate of bluegills was first altered at concentrations ranging from 0.39 to 2.20 mg/L for several anionic surfactants (Maki, 1979b). The low effect concentrations of 0.005 and 0.015 mg/L reported for LAS were based on changes in gill and skin morphology after 30 days of exposure (Misra *et al.*, 1985, 1987).

Sutterlin *et al.* (1971), in a comprehensive study, tested many surfactants for their stimulatory and blocking effectiveness on the olfactory epithelium of Atlantic salmon. Blocking effects were noted at 1 mg/L for several of the cationic surfactants and the anionic alkylbenzene sulfonate. No blocking effect was noted for the nonionic surfactants. Overall, the effects were reversible in many cases. Maciorowski *et al.* (1977) also reported that the effects of an anionic surfactant on intestinal damage to clams was reversible. The NOEC based on the respiratory rate of bluegill were 0.54 and >1.56 mg/L for two alkyl ethoxylates (Maki, 1979b). The physiological effect concentrations of anionic surfactants on species other than fish range from 0.015 to 3.0 mg/L. Moffett and Grosch (1967), for example, reported that 1-3 mg/L LAS caused developmental abnormalities in several marine invertebrates whereas 0.015 mg/L ABS reduced calcium uptake in a snail after 72 h exposure (Misra *et al.*, 1984).

The avoidance reaction by fish has been one of the more commonly monitored effect parameters in behavioural studies with surfactants. Avoidance of several anionic surfactants by a variety of fish species has been observed at concentrations ranging from 0.002 to 0.40 mg/L. The concentration resulting in a 65 % avoidance ratio by the Ayu for several anionic surfactants was 0.002-0.11 mg/L (Tatsukawa and Hidaka, 1978) whereas avoidance reactions of another fish species, the Medaka, for similar compounds ranged from 0.007 to 0.027 mg/L (Hidaka *et al.*, 1984). Other responses such as changes in swimming activity and in feeding behavior are expected at higher concentrations. The effects of LAS on these characteristics for trout, goldfish, cod and carp have occurred at concentrations between 0.2 and 5.0 mg/L (Marchetti, 1968; Swedmark *et al.*, 1976; Saboureau and Lesel, 1977; Walczak *et al.*, 1983). The avoidance responses, however, were erratic in many cases. Swedmark *et al.* (1971), in a comprehensive study, reported the effects of a variety of surfactants, including several nonionic compounds, on several characteristics of marine fish and invertebrates. Effect levels exceeded 0.5 mg/L in all cases based on changes in swimming activity, shell closures, byssal activity, locomotion and burrowing. Byssal activity and growth of mussels were affected by 0.056 mg/L of a nonylphenolic compound (Granmo *et al.*, 1989). The behavioral effects of cationic surfactants on aquatic life have not been reported.

In conclusion, the chronic and sublethal toxicity data base available is limited to a few commercially important surfactants, primarily various blends of LAS and to a lesser extent the nonionic and the cationic compounds. Toxicity data for anionic surfactants other than LAS such as the alkyl sulphates and the alkyl ethoxysulphates are fewer. Effects of most surfactants on structural and functional aspects of natural animal communities are unknown. Only a few studies have been reported describing the "long-term" effects of surfactants on natural zooplankton and invertebrate communities and these studies were conducted with LAS. Chattopadhyay and Konar (1985) reported that ostracods, rotifers and chironomids, in outdoor vats were adversely affected after 90 d exposure to 0.38-1.10 mg/L LAS based on the active

ingredient. Zooplankton were reduced significantly at 0.51 and 1.10 mg/L. Huber *et al.* (1987) reported that 5 mg/L LAS adversely affected cyclopod egg production and developmental stages after 8 weeks exposure in model pond ecosystems. *Cladocera* and phytoplankton were affected only after exposure to 10.0 mg/L. Ladle *et al.* (1989) found that sediment-bound LAS at concentrations of 1-40 µg/g had no impact on the invertebrate diversity in a stream survey conducted above and below a municipal discharge. The effects of LAS in combination with a petroleum refinery effluent were investigated on phytoplankton, zooplankton and benthic organisms in outdoor ponds (Panigrahi and Konar, 1986). Combinations of 1 mg/L LAS with 0.4-13% effluent were toxic to zooplankton.

The range of reported chronic toxicity values for surfactants and aquatic animals is wide. It is obvious that the toxicities of surfactants vary widely even within the same surfactant class. Furthermore, toxicities of surfactants can vary with the chemical structure such as for LAS where the toxicity varies with the length of the alkyl chain length (Kimerle and Swisher, 1977) and for the nonionic ethoxylated surfactants where toxicity varies with the length of the ethoxylate chain length (Sivak *et al.*, 1982; Hall *et al.*, 1989). The range of effect and no effect concentrations based on the studies appear to be 0.05-28.0 mg/L (anionic surfactants), 0.05-50.0 mg/L (nonionic) and 0.05-0.46 mg/L (cationic). The range for invertebrates is 0.04->10.0 mg/L (anionic), <0.1-20.0 mg/L (nonionic) and 0.009-1.27 mg/L (cationic).

Sublethal effect data predominate for LAS and, with the exception of fish avoidance responses, the effect levels typically exceed 0.1 mg/L. The effect concentrations for nonionic surfactants, with a few exceptions, exceed 0.5 mg/L. Reported sublethal responses for cationic surfactants are too few to indicate a data trend.

5.3.2.5 Influence of environmental factors on detergent toxicity for marine biota

The toxicity of contaminants like detergents on aquatic ecosystems can be affected by many environmental characteristics (Sprague, 1985) such as biotic factors (such as species type, life stage and nutritional status), and abiotic factors that change seasonally (temperature, water hardness and dissolved oxygen).

Several studies which discuss the effects of environmental modifying factors on toxicity (Abel, 1974; Sivak *et al.*, 1982) are at least 10 years old and the summarized data are for surfactants that in many cases are no longer used commercially. More recently Lewis has prepared a review paper on this subject (Lewis, 1992).

From this study it appears that the effects of mixtures containing surfactants, hardness and temperature have been investigated more frequently than other parameters.

5.3.2.5.1 Influence of mixtures on toxicity of surfactants

The toxicity of mixtures containing surfactants has received considerable attention since they are used as oil dispersants, adjuvants in pesticide formulations and are present in several combinations in commercial detergent and household cleaning products. (Singer *et al.*, 1990; Laws, 1981; Linden *et al.*, 1988) The toxicity of oil dispersants can be attributed in some cases to the surfactant component (Swedmark *et al.*, 1973; Nagell *et al.*, 1974) and this may also be so for pesticides (Caux *et al.*, 1986, 1988).

It is thought that the toxicity of detergents is attributable primarily to the surfactant component which can comprise up to 30% of the product volume.

Several types of toxicity tests have been used to determine the environmental impact of the commercial mixture above mentioned.

Most mixture studies with surfactants have been carried out in the laboratory, monitoring acute toxicity, but effects on physiological functions and bioconcentration potential have also been reported (Hille, 1970; Swedmark and Granmo, 1981; Topcuoglu and Birol, 1982; Lavie *et al.*, 1984; Pärt *et al.*, 1985). Most studies concern only single species of cultured invertebrates and fish but others have been extended to natural biotic communities in ponds, lakes and rivers (Tateme *et al.*, 1976; Azov *et al.*, 1982; Scott and Glooschenko, 1984; Panigrahi and Konar, 1986; Lewis *et al.*, 1986b; Lewis, 1991).

The surfactant most often combined in toxicity tests with pesticides, metals and oil has been LAS. Fresh-water fish have been the usual test species (such as rout, bluegill and fathead minnows) with exposure periods between 4 and 7 days and sometimes longer than a month (Caims and Scheier 1964; Dugan, 1967; Panigrahi and Konar, 1986). The exposures to the mixture constituents have been simultaneous and sequential (Caims and Scheier 1964; Dugan, 1967; Brown *et al.*, 1968). Dugan (1967), for example, exposed goldfish for 2 months to surfactants prior to their exposure to pesticides. Brown *et al.* (1968) exposed trout to 0.8 mg/L zinc for 100 days before exposing them to an anionic surfactant. Several mixture toxicity studies have been conducted by combining a surfactant with either a municipal effluent (Lewis *et al.*, 1986b) or an industrial effluent (Panigrahi and Konar, 1986) after the wastewater treatment process. The presence of less than 1% of an oil refining plant effluent increased the toxicity of LAS to zooplankton and phytoplankton in outdoor mesocosms. Turner *et al.* (1985), Versteeg and Woltering (1990) and Patoczka and Pulliam (1990) have utilized laboratory scale wastewater treatment systems to determine the treatment effect on the toxicity of combinations of surfactants and manufacturing plant effluent added to wastewater influent. These tests have been conducted with a freshwater invertebrate and alga (Versteeg and Woltering, 1990) as well as a marine mysid shrimp (Patoczka and Pulliam, 1990). Versteeg and Woltering (1990) reported that the addition of a detergent manufacturing plant effluent to municipal influent did not increase the toxicity of the resulting effluent after activated sludge treatment. This lack of effect was also observed for rainbow trout exposed to an effluent that had received additions of nonionic surfactant prior to treatment (Turner *et al.*, 1985).

Mixture toxicity effects can be synergistic (the mixture effect is greater than that expected on the basis of the effects of the single components tested alone), antagonistic (if the effect is less than the expected) or additive (if the mixture toxicity is equivalent to the combined toxicities of the individual mixture components). Therefore, it is difficult to generalize or predict the toxicities of mixtures containing these components. For example, combinations of copper and anionic surfactants were synergistic to trout but not copper-nonionic surfactant mixtures (Calamari and Marchetti, 1973). Some pesticide-LAS combinations are synergistic while others

are not (Solon and Nair, 1970). In contrast to these unpredictable trends, mixtures containing oil and surfactants have been consistently synergistic or more toxic than expected based on the toxicities of the separate mixture components (Hokanson and Smith, 1971 ; Rehwoldt *et al.*, 1974; Lavie *et al.*, 1984).

5.3.2.5.2 Influence of hardness on toxicity of surfactants

Water hardness is an abiotic factor that has been shown to affect surfactant toxicity in an unpredictable manner. The toxicity of several anionic surfactants (LAS, alkyl sulphates and alky-benzene sulfonate) for goldfish, bluegill and fathead minnows increases as the water hardness increases (Henderson *et al.*, 1959; Hokanson and Smith, 1971; Gafa, 1974; Eyaner *et al.*, 1985). These observations are not consistent with other results reported for the same surfactants (Henderson *et al.*, 1959; Holman and Macek, 1980; Lewis and Perry, 1981). It seems that the effect of hardness on surfactant toxicity depends upon whether a commercial product or single compounds were tested. Henderson *et al.* (1959) reported that detergent products were approximately two times more toxic in water of 400 mg/L hardness than in 20 mg/L hardness but this effect was not observed for single surfactants contained in the products. The toxicity of surfactants is also a function of both the culture and test water hardness (Maki and Bishop, 1979). LAS was significantly more toxic to a daphnid in soft water (25-30 mg/L CaCO₃) than in hard water (325-350 mg/L CaCO₃).

5.3.2.5.3 Influence of temperature and other seasonal factors on toxicity of surfactants

The effect of water temperature on the toxicity of most surfactants is poorly understood. In all studies investigating the effects of water temperatures ranging from 6 to 25EC on the toxicity of LAS it was demonstrated that increasing temperature increased the toxicity of this surfactant (Marchetti, 1968; Hokanson and Smith, 1971). Swedmark *et al.* (1971) reported that the toxicity of two surfactants to marine species increased passing from 6EC to 17EC. Likewise, the toxicity of LAS to goldfish and bluegill was greater at higher temperatures. In contrast to this trend, a diatom was more sensitive to three surfactants at 15EC than at 25EC (Nyberg, 1976).

The effects of several surfactants on phytoplankton photosynthesis in multiple toxicity tests (Lewis and Hamm, 1986) evaluated as 3 h EC₅₀ values, varied as much as 80-fold during a 5-month period. The differences in effect levels were attributed to seasonal changes in water temperature and phytoplankton dynamics. Tatem *et al.* (1976) reported that the sensitivity of grass shrimp to the anionic dodecyl sodium sulphate differed depending upon the season when the test species were collected in the field: the surfactant was more toxic to organisms collected during the winter months probably because their nutritional state was reduced.

5.3.2.5.4 Influence of suspended solids and dissolved organic substances on toxicity of surfactants

Several studies have investigated the effects of suspended solids and naturally occurring dissolved organic substances such as humic acids on surfactant toxicity. Maki and Bishop (1979) reported about a slight effect, with the addition of 50 mg/L of bentonite, on the toxicity of C₁₈LAS and C₁₄LAS to *D. magna* and very little for C₁₁LAS and a nonionic alkyethoxylate. Likewise, the acute toxicity of LAS to bluegill was not affected by the addition of 200 mg/l bentonite (Hokanson and Smith, 1971).

5.3.2.5.5 Influence of dissolved oxygen and salinity on toxicity of surfactants

The effects of dissolved oxygen and salinity on surfactant toxicity have been reported in only a few cases. Salinity increased the toxicity of a granular detergent containing 30% ABS to juvenile eels and mummichogs (Eisler, 1965). Likewise, an anionic surfactant was more toxic to a marine copepoda at higher salinities. Decreasing dissolved oxygen increased the toxicity of LAS to bluegills (Hokanson and Smith, 1971).

5.3.2.5.6 Conclusions on the influence of environmental factors on detergent toxicity for marine biota

In conclusion, the toxicity of surfactants in the marine environment is affected by a variety of interacting physical, chemical and biological factors and the reasons are not well known. More data are available for LAS than for other commercially-important anionic surfactants such as the alkyl sulphates and alkyl ethoxy sulphates while they are limited for cationic surfactants.

The available information is largely for acute effects on single species of freshwater fish. On the contrary effects on chronic toxicity and on natural assemblages of freshwater and marine animal life are almost unknown.

The environmental toxicity of these compounds should be studied under the most realistic conditions, i.e. in environments where the greatest exposure is likely to occur due to municipal wastewater discharges (Dourson and Stara, 1983; USEPA, 1984; Okkerman *et al.*, 1991).

5.3.3 Chronic toxicities of surfactants to algae

The chronic toxicity of surfactants to algae is not well known despite the unique ecological importance of this food chain component and the existence of a relatively abundant though scattered literature (Envirocontrol, 1981; Huber, 1984; Gilbert and Pettigrew, 1984; Cooper 1988). The subject is also (Anonymous, 1982; Richtler and Knaut, 1988; Roes and de Groot, 1988) missing from environmental legislation (Fischer, 1980; Dinkloh, 1983; Noll 1987; Fed. Regist. 1988). However experts (Lewis, 1990) believe that there is a need to evaluate precisely the algal toxicity for surfactants and detergent builders and to assess their potential impact in the environment.

The different effect levels reported for algae are due in part to differences in algal physiology. The toxicity of surfactants on algae has been reported and discussed in detail by, among others, Hicks and Neuhold, (1966); Nyberg, (1976, 1985, 1988); Ernst *et al.*, (1983); Roederer, (1987); Chawla *et al.*, (1986, 1987, 1988).

Generally, surfactants denature and bind proteins in the cell wall and consequently alter membrane permeability to nutrients and chemicals. Algal cell walls differ between species and cell wall thickness and chemical composition influence the severity of the effects. The thicker the cell wall the less likely the impact. High lipid and protein content in the cell wall allows penetration of hydrophobic surfactants. Charged surfactants (anionic and cationic) have been reported to have a greater denaturing effect than neutral compounds (Nyberg, 1976) and it has been reported that the toxic dosage of surfactants is inversely proportional to their ability to reduce surface tension (Bock, 1965).

Algal toxicity is usually rated with EC₅₀ values (the concentration reducing the effect parameter, e.g. growth, to 50% relative to the control algal population), with the algistatic value or EC₁₀₀ (the concentration that inhibits algal growth but allows the growth to continue when the algae are recultured in fresh medium that does not contain the test chemical) and with the algicidal concentration (the concentration which causes cell death; the test species will not grow when subcultured into a fresh medium that does not contain the test chemical).

Studies on the toxicity of surfactants on algae may be carried out by testing single species, more than one (comparative studies) species or by testing contemporarily one or more algal plus some animal and/or aquatic vegetation species.

The reported effect concentrations on algal species in the laboratory studies for anionic surfactants range from 0.003 to 4000 mg/L, most effect levels exceeding 1.0 mg/L. The exposure periods range from 30 min. to 21 days. The effect levels are dependent upon the test species, effect parameter, study duration, and surfactant type. The anionic surfactants most frequently used in algal toxicity tests have been the various blends of linear alkylbenzene sulfonate (LAS). Laboratory derived EC₅₀ values (96 hr) for LAS, even when determined under very similar experimental conditions, ranged from 1.4 to 116 mg/L for three freshwater species (Lewis and Hamm, 1986) and 72 h EC₅₀ values were between 10 and 100 mg/L for similar species (Yamane *et al.*, 1984).

A few reports are available for marine species whose sensitivity to several anionic surfactants appears to be particularly low with effect levels between 2 and 54 mg/L (Ukeles, 1965). Unusually sensitive is *Gymnodinium breve*, a red tide alga, with reported effect levels of 0.003 to 0.025 mg/L (Kutt and Martin, 1974; Hitchcock and Martin, 1977).

There are very few laboratory-derived reports on toxicities of nonionic surfactants (e.g. alkyl ethoxylates (AE) and Triton X-compounds) for algae, ranging from 0.003 to 17.784 mg/L.

The effects of cationic surfactants which have been determined on 21 freshwater and 14 saltwater species are between 0.1 and 1.0 mg/L, which are considerably less than those reported for the anionic and nonionic surfactants.

The effects of detergent builders (nitrilotriacetic acid) on a variety of freshwater algae species range from 1 to 1000 mg/L (Anderson *et al.* 1985; Maki and Macek, 1978).

Some Authors have studied the toxic effects on algae of surfactants in an effluent matrix after biological treatment. Neufahrt *et al.* (1976) and Camp (1974) observed that activated sludge treatment lowered the effluent's toxicity for a green alga, due to the removal (adsorption, biodegradation) of the surfactant.

The effects of surfactants on natural algal assemblages have been reported and in most cases toxicities were less than those demonstrated under laboratory conditions with the same test compound. Five mg/L of ABS was found stimulatory (approximately 5%) to river algal photosynthesis relative to concentrations of 25-50 mg/L which reduced photosynthesis (Hicks and Neuhold, 1966). Chattopadhyay and Konar, (1985) reported that LAS concentrations of 0.25 to 1.10 mg/L reduced photosynthesis in phytoplankton (40% after 90 days).

According to Huber *et al.* (1987) 5.0 mg/L LAS adversely affected (in a 2 years study) the community composition of phytoplankton and macrophytes in model ponds.

From numerous references it can be assumed, for different freshwater and for several marine forms (Ukeles 1965), that the response of algae exposed to the same surfactant is species-specific (Palmer and Maloney, 1955; Bringmann and Kuhn, 1978; Blanck *et al.*, 1984; Wängberg and Blanck, 1988). Algae vary considerably in their response to LAS (Gledhill, 1974, Yamane *et al.*, 1984; Lewis and Hamm, 1986; Chawla *et al.*, 1988). The difference in response of *M. aeruginosa* and *S. capricornutum*, for example, was three orders of magnitude (Lewis and Hamm, 1986). The green alga, *S. capricornutum* appeared to be the least sensitive of the test species in this study as well as in those reported by Yamane *et al.*, (1984). Nyberg (1985) reported that marine red alga, *Porphyridium purpureum*, was more sensitive to anionic and cationic surfactants than was *Nitzschia actinastroides*. In contrast, *P. purpureum* was less sensitive than *Nitzschia actinastroides* to the nonionic Triton X.

In several studies the sensitivities of algae to surfactants appears to be greater than or comparable to those for animal test species. For example, the 96h EC₅₀ values for an alkyl ethoxylate and three freshwater algal species were between 0.09 and 0.60 mg/L (Lewis and Hamm, 1986) while NOEC values (no observed effect concentrations) for a fish and invertebrate exposed to the same compound (Maki, 1979c) were 0.18 and 0.24 mg/L.

Patrick *et al.* (1968) found a diatom more sensitive to the anionic alkylbenzene sulfonate (120 hr TLm = 10 mg/L) than either an invertebrate (96 hr TLm = 34.2 mg/L) or fish (96 hr TLm = 17.4 mg/L). In other reports, fish were found more sensitive to LAS than algae were (Maki and Macek, 1978; Canton and Slooff, 1982; Lewis and Hamm, 1986), but daphnids were less sensitive than algae in one case (Canton and Slooff, 1982).

Effects of detergents on other aquatic vegetation have been determined: photosynthesis in the macrophytes *Elodea canadensis*, a *Chara sp.*, and *Myriophyllum spicatum*, was reduced by 50% after exposure to 1.0 mg/L LAS (Huber *et al.* 1987). Degens *et al.* (1950) reported that effect levels for several anionic surfactants on *Valisneria spiralis* and *Elodea canadensis* exceeded 40 mg/L. The 7d EC₅₀ values for duckweed, *Lemna minor*, and three surfactants ranged from 0.08 mg/L for the cationic cetyltrimethyl ammonium chloride to 2.7 mg/L for C_{11,8} LAS (Bishop and Perry, 1981). The sensitivity of *L. minor* was less than that for three freshwater algae exposed to a similar nonionic surfactant but it was comparable to the sensitivity of the algae to the anionic and cationic surfactants (Lewis and Hamm, 1986).

The toxicities of different surfactants representing either the same or different classes to the same alga test species vary by over four orders of magnitude (Lewis and Hamm, 1986). Cationic surfactants seem to have a greater effect on algae than non ionic or anionic surfactants.

The phytotoxic properties of cationic surfactants are well-known (Walker and Evans, 1978; Emst *et al.*, 1983; Boethling, 1984; Nyberg, 1988) but LAS was found to be more toxic than a cationic surfactant to the marine red tide organism, *G. breve* (Kutt and Martin, 1974).

Algal stimulation has been observed at surfactant concentrations usually greater than 2.0 mg/L (Palmer and Maloney, 1955; Matulova, 1964; Hicks and Neuhold, 1966; Camp, 1974; Nyberg, 1976; 1988; Karpinska-Smulikowska, 1984; Lewis *et al.* 1986a; Chawla *et al.* 1986). Growth, photosynthesis, biomass, and chlorophyll (a) of natural algal communities and various single species such as *Nitzschia holsatica*, *Chlamydomonas gelatinosa*, and *Scenedesmus quadricauda* increased after exposure to various anionic and nonionic surfactants.

Toxicities of surfactants were also compared to those of other chemicals, under the same experimental conditions. Bishop and Perry (1981) reported that duckweed is more sensitive to a cationic surfactant than to copper, however, copper is more toxic than the nonionic

and anionic surfactants, and diquat is more toxic than all the surfactants. Roederer (1987) found several anionic, nonionic, and cationic surfactants to be more toxic than lead to a freshwater diatom. Zinc is more toxic (0.1 mg/L) to *Selenastrum* than anionic and nonionic compounds (1.0-10.0 mg/L) (Camp, 1974). Sodium lauryl sulphate was reported less toxic than a variety of metals and non surfactant compounds (Whitton 1967; Blanck *et al.*, 1984; Wängberg and Blanck, 1988).

Few generalizations can be made concerning the toxicity of surfactants to algae. Experimental variables such as the surfactant type, test species, and effect parameters used in the studies vary considerably. In addition, analytical verification of the exposure concentrations was not usually carried out, primarily due to the lack of precise analytical methods such as those recently reported by Osburn, (1982, 1986), Simm *et al.*, (1988) and Ventura *et al.* (1989). Overall, differences in experimental approach have in part resulted in the wide range of reported effect levels for algae, 0.003 to 17.784 mg/L. This range of toxic effects encompasses those reported for surfactants and fish, 0.4 to 40.0 mg/L (Abel, 1974), and for vertebrates and invertebrates, 0.1 mg/L (Sivak *et al.*, 1982) and 0.005 to 50.0 mg/L (Lewis 1990).

In conclusion, it must be kept in mind that the current understanding of the effects of commercially important surfactants on laboratory cultured algae is based largely on the results for a few freshwater species. Moreover the toxicity of most surfactants is less when determined for natural algal communities under natural conditions. The toxicities of cationic surfactants are also greater than those of the other surfactants and detergent builders on the basis of laboratory and field-derived toxicity results.

5.3.4 Conclusions

The effects of the anionic detergents (ABS and LAS) have been the primary focus of scientific investigations concerning surfactant environmental safety. The importance of the nonionic and cationic surfactants to the detergent industry has increased during the past 15 years and consequently their use in toxicity tests has increased. However, the use of alkyl sulphates and alkyl ethoxylate sulphate in commercial products is substantial and increasing, and selected chronic toxicity determinations with these surfactants would determine if their toxicity is comparable to LAS. The key scientific need for nonionic surfactants is to determine the environmental concentrations in freshwater and saltwater environments of the major alkyl ethoxylates and compare these to the available toxicity data base. The reported toxicity data base for surfactants is dominated by data for fresh-water species. This reflects, in part, the availability of standard test methods and suitable culture techniques for fresh-water, while standard methods and culture techniques for marine species were until recently lacking. Consequently, the toxicities of surfactants on saltwater life have been and continue to be largely estimated from effects on freshwater life. Since this practice is not technically sound, in most cases, toxicity data for saltwater species are needed.

According to Lewis (1991) the chronic toxicities of surfactants for fresh-water aquatic plants are better understood, particularly on natural communities, than are those for animal life. With the exception of cationic surfactants, algae are not sensitive to surfactants. It even appears that the toxicity of the cationic surfactants observed in the laboratory for single algal species is less for natural phytoplankton communities, although additional supporting data for periphyton communities are needed. All in all, future investigations concerning the environmental safety of surfactants should center on understanding their chronic toxicities to animal life. Animal test species have exhibited a moderate degree of sensitivity to several major anionic and nonionic surfactants but at concentrations typically exceeding measured environmental concentrations. The few available effect and no effect levels for cationic surfactants appear to occur at lower concentrations than those for many anionic and nonionic surfactants but a definitive trend cannot be identified at this time due to the limited nature of the data base.

Commonly used surfactants for which a limited chronic toxicity data base exists include the alkyl sulphates (anionic), alkyl ethoxylate sulphates (anionic) and several of the monoalkyl and dialkyl quaternary ammonium salts (cationic). Of these compounds it would be expected, based on the basis of the available information for aquatic animal and plant life, that the toxicities of the anionic compounds would likely parallel those for LAS and be relatively harmless. However, the use of alkyl sulphates and alkyl ethoxylate sulphate in commercial products is substantial and increasing, and selected chronic toxicity determinations with these surfactants would determine if their toxicities are comparable to LAS. The key scientific need for nonionic surfactants is to determine the environmental concentrations in freshwater and saltwater environments of the major alkyl ethoxylates and compare these to the available toxicity data base.

This need for a realistic exposure analysis includes most other surfactants as well. The use of the non-ionic alkylphenol ethoxylates, particularly several of the nonylphenol ethoxylates is decreasing, due in part to their environmental toxicity and legislative action in Western Europe to ban their use (Richtler and Knaut, 1988). Therefore, they should be of a low priority from a research perspective. The toxicity data base for cationic surfactants needs to be expanded. The chronic toxicity tests needed for these surfactants are chemical-specific but would include baseline toxicity studies with saltwater and freshwater fish and invertebrates, tests investigating physiological and behavioral effects, and of greater priority, those determining effect levels for natural freshwater and saltwater animal assemblages.

6. ASSESSMENT OF RISKS TO MARINE BIOTA AND HUMAN HEALTH IN THE MEDITERRANEAN

6.1 Introductory Remarks

Risk assessments can be derived from data in the literature about environmental levels of pollutants, levels of effect concentrations (in the laboratory or in natural conditions) and by considering exposure-toxicity relationship. In the particular case of anionic surfactants, risk assessment should not be limited to surfactants but also to all substances which are present in detergent formulations. For instance Maki and Macek (1978) proposing zeolites as alternatives for the phosphates contained in detergent formulations observed that this substitution permits one to obtain compounds which are non toxic at environmental levels for freshwater and marine aquatic communities while also not contributing to the eutrophication potential of surface waters. Zeolites are also efficiently removed from wastewater through treatment processes.

As far as the subject of this report is concerned, the situation appears to be different for regarding the risk to human health and that to marine biota. In fact risks to human health deriving from the presence of anionic detergents in sea water, in the opinions of different Authors, appear to be practically negligible. As has already been discussed in part 5 of this report, the toxicity for man deriving from ingestion or contact with anionic surfactants is very low at the levels reported to be present in sea water. Moreover there are limiting concentrations for surfactants in recreational waters, well below those which could be harmful for the human organism.

Indirect effects to human health may derive from a mobilization by anionic surfactants of other pollutants contemporarily present. This subject was discussed in part 2 of this report.

According to Duthie (1977), Kimerle (1989) and Stern and Walker (1978) a quantitative safety assessment should be based on the ratio (safety margin) of the highest observed no-effect level for the most sensitive aquatic species and the aqueous environmental

exposure concentration. When this ratio is great, it may be concluded that surfactants will not have adverse effects on the aquatic environment. Other considerations, such as the ecological impact of impurities, potential bioaccumulation to higher life forms and long-term environmental sinks, should also be included.

6.2 Environmental Levels Measured in Seawater (and in Marine Biota), in Effluents and in Rivers Discharging into the Mediterranean Sea

A summary will be given here of what is known about environmental levels for the water of the Mediterranean sea water and for effluents and rivers discharging into this sea (see part 3 and part 4 of this report for a detailed description of the data available from studies and research carried out, included those within the UNEP-WHO programs).

Briefly, there are data available for only a few Mediterranean countries (Spain, Malta, Italy, former Yugoslavia and France). Obviously there exist data on this subject for other seas and oceans.

6.2.1 Anionic detergent levels in Mediterranean seawater

6.2.1.1 Data from Spain

In seawater (see tables 11-12) from Albufereta (Alicante coast) levels of MBAS were between <0.01 and 0.04 (mg/L) and those of LAS between <0.01 and 0.03(mg/L). The seawater from Babel Bay (in the same area), known to be heavily polluted by effluents, showed levels of MBAS between <0.01 and 4.2 (mg/L) and of LAS between <0.01 and 1.94 (mg/L).

In the available literature there are also some other values regarding Spanish sea waters reported from Martinez et al (1989) who measured levels of LAS in the range of 0.0 to 0.26 mg/L (see table 18).

6.2.1.2 Data from Malta

The analysis of seawater in three localities from the Maltese coast (Wied Ghammieg, Xghajra and Salina Bay) showed levels of anionic detergents (see table 14) between 0.0099 and 0.024 (mg/L). The concentration found near to a sewage effluent was of the same order as that in the reference area.

6.2.1.3 Data from Italy

The analysis of seawater in three localities of the Ligurian Gulf during two periods of the year 1992 gave results (see table 15) in the range between 0.013 and 0.031 mg/L (MBAS). No substantial differences were noted between "polluted" areas and the reference area.

Other data are available for Italian coastal waters and derive from bathing-water monitoring carried out according to a national law (DPR 8 June 1982, n.470). Non acceptable samples (anionic levels superior to 0.5 mg/L) for the last two years (1992 and 1993) were limited to 10 Provinces (out of a total of 53 Provinces) and only in 4 cases (Napoli, Catania, Messina and Palermo) did this happened during both years (see table 15a).

Some other data are available from the literature and they regard the Adriatic and the Tyrrhenian sea (see table 18): in both cases (data are limited to the northern part) various

Authors (1968 - 1985) measured anionic detergents (MBAS) at average concentrations of 0.05-0.06 mg/L.

6.2.1.4 Data from Eastern Adriatic

Data for different areas of the Eastern Adriatic coast (former Yugoslavia up to 1991 and Slovenia from 1992) are available (see tables 16-17-18). The average level for Rijeka Bay (1974-78) was 0.008 mg/L, while in other areas of the Adriatic Sea (1983-86) it was 0.021 mg/L. In the Sibenik area anionic detergents averaged 0.004 mg/L (1984) and in the Montenegrin coastal area (1985-86) 0.045 mg/L. More recent data, relative to the Bay of Koper and of Piran (1992), are very low: <0.01 - 0.01 mg/L. Only slightly higher are some data from Valdoltra (1992): 0.01 - 0.06 mg/L.

As can be seen, data are missing for the coastal waters of France, Greece, Turkey and all Countries whose coasts delimitate the Mediterranean sea to the South.

6.2.2 Anionic detergents levels in rivers and effluents which flow into the Mediterranean sea

6.2.2.1 Data from Spain

Anionic detergents in Spanish rivers were measured (1989, 1990 and 1991) as already reported in part 3 of this report. During 1989 mean values ranged from 0.12 to 26.86 mg/L, while during the following two years the range of mean values was 0.06 - 5.28 mg/L and 0.09 - 2.56 mg/L respectively (see tables 7 and 7a).

Concentrations of anionic detergents in Albufereta Beach wastewaters were found to be 0.40 mg/L (MBAS) and 0.21 mg/L (LAS) with a maximum flow rate of 4.00 Kg/d and 2.1 Kg/d respectively. Babel Bay wastewaters (in the same area) contained 28.4 mg/L (MBAS) and 15.1 (mg/L, LAS) with a maximum flow rate of 1704 Kg/d and 906 Kg/d respectively (see table 2).

MBAS concentration levels in sea water near to the point of discharge of the effluent in Babel Bay were found to be around 4 mg/L at 20 m from the discharge outlet while concentration levels of LAS were 1.25 and 1.94 mg/L in the same point. At a distance of about 100 m from the discharge site, these concentrations fell to levels equal or lower than 1 mg/L, reaching 0.03 mg/L and less at 700 m from the site. Higher concentrations were always found near the surface as opposed to those existing below the surface (see table 11 and 12).

The fast decrease of MBAS and LAS concentration at a short distance from the discharge point can be interpreted as a positive aspect which makes the emission of anionic surfactants in the seawater environmentally acceptable.

As far as other areas are concerned (see tables 1 and 1a), the mean concentrations of anionic detergents in effluents during the year 1989 ranged from 0.31 (mg/L) in Valencia to 34.07 (mg/L) in Malaga Misericordia, while the median level was 3.01 (mg/L). During the year 1990 the mean concentrations varied from 0.11 (mg/L) in Valencia to 23.33 (mg/L) in Barcelona and the median level was 7.52 (mg/L). During the year 1991 the mean concentrations varied from 0.23 (mg/L) in Valencia to 16.43 (mg/L) in Cartagena Algameca while the median level was 5.58 (mg/L).

6.2.2.2 Data from Malta

No data are available from Malta about effluents and riverine inputs.

6.2.2.3 Data from Italy

At the mouth of two Ligurian rivers mean values of anionic detergents were found in the range between 0.054 and 0.11 mg/L (see table 9).

There are also some data about levels of anionic detergents in 9 Italian rivers (1967-1977) where their average (see table 10) was found in the range of 0.22 - 3.4 mg/L (maximum concentration of 13.8 mg/L).

In the effluents of two Ligurian sewage treatment plants (see table 5) anionic detergents were found in the range of 0.19 - 13.84 mg/L (as MBAS). The high value was due to the fact that during one sampling period one of the treatment plants was damaged by a violent flood.

There are also some data (1956 - 1966) from Italy for raw sewage (see table 6) with levels from 0.22 to 31.12 mg/L.

6.2.2.4 Data from Eastern Adriatic

Data about river levels of anionic detergents are reported for Slovenia (1984) and Montenegro (1984-1986) where mean levels of 0.063 mg/L and 0.043 mg/L were found respectively (see table 8). More recent data are limited to the Rizana river (1992) with concentrations of <0.01 mg/L (see table 17).

More numerous are the results of investigations on anionic detergent concentrations in various types of effluents (industrial and domestic, 1984-86) in the Pula, Rijeka, Sibenik and Split areas (see table 3): mean levels vary from 0.056 to 3.43 mg/L (maximum value: 16.5). Results of some studies relate mean concentrations in different types of industrial and urban effluents (see table 6): 36.72 mg/L (plastic industry, 1979-80), 6.2 mg/L (textile factories, 1980), 2.1 mg/L (urban waste, 1984-86).

More recent data are those from the Slovenian coast, Rijeka, Zadar and Split (1991) with mean anionic detergent levels between 0.481 and 7.412 mg/L (see table 4).

6.3 Toxicity of Anionic Detergents for Human Health

An assessment of the risk to human health deriving from the presence of anionic detergents in the Mediterranean sea has to be limited to eventual problems from effects due to repeated contact with polluted recreational waters. No acute toxicity problems need be considered, taking into account what is known about levels of effect by ingestion.

The relative impermeability of human skin (Bartek *et al.*, 1972, Howes and Black, 1976) allows only small amounts of anionic detergent to be absorbed, even when large skin surfaces are exposed. The poor percutaneous absorption and the low toxicity of anionic detergents (ingestion of anionic surfactants up to 1.0 g/kg of body weight is relatively harmless) suggests that the possibility of systemic toxic effects in humans is extremely unlikely. Nevertheless it must not be forgotten that another effect of detergents on the skin barrier is to allow better absorption of other compounds, when contemporarily present.

In conclusion the ingestion of small quantities, even if repeatedly, of seawater polluted by anionic surfactants may be considered riskless.

As regards direct effects on the skin, anionic surfactants may cause changes of the barrier function, of the water content, of the surface milieu, etc. The clinically predominant observation of "irritation" after intense use or experimentally induced prolonged contact, which was formerly the only approach to characterizing skin compatibility and reaction with surfactants, consequently subsumes different aspects of skin-surfactant interaction. In our case, the repeated contact of the skin with sea water containing small concentrations of anionic detergents should not give toxicity problems as long as the use conditions are "normal".

6.4 Toxicity of Anionic Detergents for Marine Biota

A risk assessment for anionic detergents in the aquatic environment needs to include data for several test organisms (algae, invertebrate, fish) representing the trophic levels contained in the planktonic and benthic habitats of the environment (freshwater and saltwater) where the compound is present. In addition, current measured environmental concentrations of the specific compound are necessary.

6.4.1 Exposure routes and acute toxicity data for aquatic animals

Aquatic animal species (fish, invertebrates, and others) are generally sensitive to the presence of surfactants in their medium. Ôba and Takita (1984) showed that fish can sense the presence of surfactants and avoid regions where their content exceeds the toxic threshold.

Aquatic organisms have very different reactions towards surfactants. It is of interest that the danger to fish comes from exposure of the gill tissues to the surfactant rather than from ingestion. For this reason very important toxic effects are experienced by a fish swimming in water containing LAS (or other surfactant) at low (few ppm) concentrations, while no ill effects resulted when Bock (1966) orally fed fish food with 20% LAS to fish (*Idus melanotus*) at about 1000 mg LAS per kg body weight.

The mechanism of fish death due to detergent poisoning is not fully understood. It is even questionable whether detergents enter the fish body and to what extent they exert action internally. Asphyxiation as a result of gill damage is, no doubt, one of the reasons for acute poisoning, but whether loss of gill function is the primary cause of death or is a secondary one has not been determined (Roy, 1988a). The same Author (1988b) noted that fish exposed to low detergent concentrations did not show much change in behavior, whereas those placed in higher concentrations showed erratic movements, muscle spasms, and body torsion caused by the toxic effects of the detergent.

The aquatic toxicity of LAS remaining after partial biodegradation is significantly lower because the more toxic longer homologues and outer isomers are quicker to degrade, in accordance with the distance principle, and it is markedly reduced by formation of a carboxyl group at the end of the LAS alkyl chain. Swisher *et al.* (1976) confirmed this by exact quantitative data.

When fish live in water polluted by surfactants (e.g. LAS), they absorb it, by partition from the water into their fatty tissues, even when the concentration is well below the LC₅₀ and well below the no-effect level. The absorbed LAS is metabolized, possibly via the same initial pathways found in mammals and microbes, i.e., by oxidation of the chain to give shorter metabolites. Comotto *et al.* (1979) found LAS intermediates at relatively high concentration

(and little intact LAS) in the fish gall bladder (where bile is stored). On the contrary the micr crustacean *Daphnia magna* absorbs LAS but apparently does not metabolize it.

Sodium dodecyl sulphate cause *rotavirus* inactivation after 1 hour at low concentrations (0.01-0.1%). Also other anionic and cationic surfactants have a virucidal effect which depends on the hydrophobic regions of these compounds. (Ward and Ashley, 1980).

6.4.2 Exposure routes and chronic toxicity data for aquatic animals

Reported measured concentrations of specific surfactants in the environment have not been common until recently. Recent use of FAB mass spectrometry (Ventura *et al.*, 1989) and other analytical methodologies (Kikuchi *et al.*, 1986) allows confirmation that actual reported values for LAS, with few exceptions, are based on chemical-specific methodologies.

In most cases from the comparison of the more commonly observed toxicity values (>0.1 mg/L) and exposure data for LAS reveals that LAS is relatively non toxic to natural animal communities (Chattopadhyay and Konar, 1985; Huber *et al.*, 1987). The environmental data base for LAS is the most extensive of any surfactant (Kikuchi *et al.*, 1986; Huber, 1989) and the data for planktonic and benthic animal life and for aquatic vegetation (Lewis, 1991) supports the aquatic safety of this compound more so than for any other surfactant. Therefore, safety evaluations in freshwater and in saltwater environments should be considered limited and preliminary in nature since they are based largely on toxicity and exposure predictions which need validation.

As far as algae are concerned, several of the effect concentrations are less than some of the measured environmental levels. However the effect concentrations based on single species tests with algae are questionable and the tests are considered to have a low predictive value relative to tests conducted under natural conditions (Macek *et al.* 1978; Blanck *et al.*, 1984; Wångberg and Blanck, 1988). The latter are considered better indicators of environmental impact. In the case of LAS the reported field-derived effect levels exceed the currently measured environmental levels and this indicates the unlikelihood of an effect of these surfactants on freshwater algae, particularly phytoplankton. This type of data is either unavailable or unreported for saltwater algal communities.

Levels of sensitivity of the freshwater and of the few marine species observed in the laboratory toxicity tests seem to indicate the likelihood of scarce effects in the marine environment but field toxicity studies are needed to validate this prediction.

Toxicity studies with mixtures containing two or more surfactants have limited practical application for an environmental risk assessment because aquatic organisms are exposed continuously to numerous potential toxic substances originating from a variety of sources. The possible combinations of these chemicals are very large and the duration of the various exposures is practically unknown or unpredictable. Tests with sewage treatment plant effluents or using small scale treatment systems are realistic but limited in their general applicability since they are short-term and "site-specific". On the contrary, mixture studies, like those conducted by Spehar and Fiandt (1986), are valuable for determining the effectiveness of water quality values for surfactants. Many risk assessments for surfactants are based on no effect values (NOEC) derived from mixture studies. Although NOECs have been reported in the literature for several surfactants, their combined effect on aquatic life is still unknown.

6.4.3 Risk assessment of anionic detergents in the Mediterranean sea for marine biota

Concentrations of anionic detergents measured in the Mediterranean sea may represent a risk for marine biota on all those occasions when they reach levels of effect.

Taking into account the relatively few data available it appears that the possibility of toxic effects for marine biota exists, even if limited to specific areas, for example those near to river mouths and points of effluent discharge into the Mediterranean sea. In fact levels of anionic detergents above no effect levels were found in the coastal sea waters of all the nations where monitoring was carried out (Spain, Italy, former Yugoslavia).

A problem may also arise from the higher content of anionic detergents in marine sediments near points of effluent discharge and river mouths, as it has been observed in many localities. It must be recalled that in the same situations many other pollutants, with higher degrees of toxicity, are contemporarily present.

To summarize, no data are available to support the hypothesis of a widespread risk for marine biota in the Mediterranean sea from anionic detergents but only some which might lead to the belief that there are some risk situations where polluted freshwater and effluents reach the sea. It may be useful to integrate the data already available (covering only some coastal situations) with others relative to other countries extending observations to open sea water as well.

7. CONTROL MEASURES

7.1 Existing National and International Control Measures

Detergents found in the marine environment represent a chemical parameter which has to be monitored even when considering the aesthetic aspect of their presence. Some countries in the Mediterranean have set permissible concentrations of anionic, cationic and non-ionic detergents according to the category of the seawater. In Croatia the permissible concentration of anionic detergents for the I and II category of seawater is 0.1 mg/L, while for the III and IV category it is 0.5 mg/L.

Other countries have set maximum concentration limits for wastewater discharges into the sea. Turkey, in line with the above, has set a limit value of 10 mg/L for discharges into the deep sea containing surface active substances, while the discharge of substances which do not comply with Turkish standards of substances composition is not allowed. Greece, taking into consideration the biodegradability of anionic detergents requires that at least 80% of the discharged detergents is composed of biodegradable detergents, and the limit value of the detergents in such discharges is 5 mg/L. In other countries, although the requirements for detergents do not exist in their national legislation, in recognizing the need to control these substances, they are planning to include appropriate effluent standards and water quality objectives in their future legislation (Slovenia, Albania).

At international level, in the member states of the European Union, legislative measures for detergents in seawater are included in EEC Council Directive 76/160/EEC of 8 December 1975 concerning the quality of bathing water, as well as in the new proposed one. The parameter to monitor, is that of surface active substances reacting with methylene blue where the concentration as a guide value must be less than 0.3 mg/L, while the mandatory value is given according to visual inspection that there will be no lasting foam. There is also another EEC Council Directive (73/404/EEC) on the "approximation of the laws of the Member States

relating to detergents". This Directive prohibits the placing on the market and use of detergents where the average level of biodegradability of anionic, cationic and non-ionic surfactants is less than 90%. It also forbids member states from prohibiting or restricting the marketing of detergents which comply with these criteria.

7.2 Action Proposed for the Mediterranean

As compared with other pollutants, relatively sparse data exist on levels of anionic detergents in the Mediterranean. Although the results of the pilot project could perhaps be considered fairly representative of the situation existing generally in the countries in question, they cannot be extrapolated to overall Mediterranean level.

Although a preliminary assessment could be made from data available in terms of immediate practical action necessary, it is important to conduct monitoring on a wider level within the region. It is therefore recommended that monitoring of detergents in effluents be made a mandatory component of pollution source monitoring in MED POL Phase II and continued when MED POL Phase III becomes operational in 1996. The few results currently available from routine MED POL monitoring cannot be evaluated owing to considerable differences in sampling and analytical techniques, and this will have to be remedied as early as possible.

Monitoring of detergents in coastal recreational areas should also be performed within the framework of MED POL. As a routine measure, this could be done visually, with analysis only resorted to when any particular circumstance so demand.

Performance of the above work should provide a much wider data base at overall Mediterranean level within 2 to 3 years.

From the conclusions in Part 6 of this document, the general situation indicates (1) that there appears to be no risk to human health through contact with detergent-polluted seawater, provided that concentrations in any particular locality do not reach levels sufficient to produce visible foam on the surface, and (2) that risks to marine biota would appear to be confined to localities near sewage effluents and river mouth. The extent of this latter problem can only be estimated on the basis of more comprehensive monitoring.

As a first step, to be taken immediately, it is proposed that those Mediterranean States which have not already done so pass legislation restricting the use of detergents to those which are reasonably (e.g. 90%) biodegradable. Apart from helping greatly to reduce problems at source, this action would also be in keeping with internationally-recognized trends over the past two decades.

As a long-term measure, following the performance and evaluation of the monitoring programme proposed, it would be up to individual governments to take appropriate action to reduce the impact of detergent pollution in identified hot-spot areas. Such action would have to be individually tailored to suit the conditions prevailing in each particular locality so identified.

Table 1

Anionic detergent concentrations (mg/L) in urban and industrial effluents in Spain
(mean \pm s.d.; range)

SAMPLING POINT	n	YEAR	1989	n	YEAR	1990	n	YEAR	1991
		m \pm s.d.	range		m \pm s.d.	range		m \pm s.d.	range
Mataro (Spa 1-05)	6	9.00 \pm 3.97	3.25-15.00	12	20.19 \pm 10.37	8.44-37.20	12	10.68 \pm 6.05	1.66-19.10
Barcelona (Spa 1-07)	6	6.59 \pm 4.12	3.20-14.30	10	23.33 \pm 44.66	4.94-150.0	12	9.05 \pm 6.00	1.00-23.59
El Prat de Llobregat (Spa 1-08)	5	5.15 \pm 3.00	2.35-9.75	12	2.50 \pm 2.16 ^{***}	0.07-8.47 ^{***}	12	5.49 \pm 8.80 ^{***}	0.07-32.40 ^{***}
Tarragona (Spa 1-09)	6	8.57 \pm 5.40	1.60-14.50	11	6.22 \pm 6.33	0.44-18.10	11	3.94 \pm 5.70	0.08-14.80
Castellon (Spa 2-13)	6	2.56 \pm 1.15	1.10-4.35	12	7.53 \pm 3.80	0.27-11.30	12	5.58 \pm 3.90	0.36-12.80
Barranco Carraixet (Spa 2-15)	6	1.01 \pm 0.69 [*]	0.24-1.97 [*]	12	0.40 \pm 0.24	0.15-1.03	12	0.61 \pm 0.39	0.12-1.53
Acequia Rascana (Spa 2-15)	6	1.62 \pm 0.88 ^{**}	0.65-2.54 ^{**}	12	1.89 \pm 1.15	0.30-3.65	12	3.38 \pm 1.39	1.14-5.86
Valencia (Spa 2-15)	6	0.31 \pm 0.17	0.20-0.64	12	0.11 \pm 0.07	0.05-0.31	12	0.23 \pm 0.27	0.08-1.03
Alicante (Spa 2-18)	7	0.52 \pm 0.33	0.20-1.05	12	0.81 \pm 0.48	0.07-1.61	12	3.67 \pm 3.94	0.16-14.30
Palma de Mallorca (Spa 3-20)	4	3.01 \pm 3.86	0.55-8.75	12	5.06 \pm 3.07	0.80-12.70	12	1.75 \pm 3.65	0.17-13.30
Cartagena Algameca (Spa 4-30)	5	19.06 \pm 13.86	8.70-41.3	12	15.14 \pm 5.48	6.58-25.80	10	16.43 \pm 12.83	1.38-46.20
Almeria (Spa 5-35)	4	13.10 \pm 9.11	0.70-21.5	12	9.88 \pm 5.67	1.70-20.10	12	16.22 \pm 5.98	7.59-26.0
Malaga (Spa 5-40)		--	--	12	16.03 \pm 8.44	4.36-30.90	12	11.22 \pm 7.83	0.26-24.40
Marbella (Spa 5-42)		--	--	12	7.93 \pm 4.76	2.59-18.40	12	9.50 \pm 3.00	4.40-14.70
La Linea (Spa 5-43)		--	--	12	9.45 \pm 5.55	2.08-21.00	12	12.43 \pm 10.78	0.53-34.80
Algeciras (Spa 5-44)		--	--	12	7.52 \pm 4.07	1.18-14.40	12	9.64 \pm 5.34	

(From: Archivo MED POL, Vertidos urbanos e industriales. Data for the year 1989)

(From: Contenido del Fichero VERMP 90.DBF (Vertidos) Data for the year 1990)

(From: Contenido del Fichero VERMP 91.DBF (Vertidos) Data for the year 1991)

NOTES:

n = number of samples
m = mean
s.d. = standard deviation

* = sampling point Carraixet-Valencia
** = sampling point Rascana Valencia
*** = sampling point Pratt

Table 1a

Anionic detergent concentrations (mg/L) in urban and industrial effluents in Spain
(mean \pm s.d.; range)

SAMPLING POINT	n	YEAR 1989	
		m \pm s.d.	range
Safa-Blanes (Spa 1-4)	6	1.59 \pm 2.04	0.08 - 5.20
Bsf. Tarragona (Spa 1-9)	5	1.01 \pm 0.36	0.56 - 1.50
C. Messer-Tarragona (Spa 1-9)	5	1.04 \pm 0.70	0.40 - 1.93
Eisa-Tarragona (Spa 1-9)	6	0.54 \pm 2.20	0.32 - 0.90
Ert-Tarragona (Spa 1-9)	5	0.32 \pm 0.33	0.10 - 0.90
Repsol-Tarragona (Spa 1-9)	8	0.67 \pm 0.77	0.10 - 2.20
Petromed-Castellon (Spa 2-13)	4	0.92 \pm 0.60	0.29 - 1.55
La Manga H. Entremar (Spa 4-27)	1	6.8	-
La Manga H. Galua (Spa 4-27)	1	18	-
Malaga Guadal.dcha (Spa 5-40)	4	21.52 \pm 9.48	7.70 - 28.0
Malaga Misericordia (Spa 5-40)	4	34.07 \pm 14.03	14.10 - 44.2
Marbella Est.Bombeo (Spa 5-42)	4	6.25 \pm 7.54	0.20 - 16.20
La Linea Atunara (Spa 5-43)	4	13.55 \pm 8.02	8.40 - 25.40
Algeciras Varadero (Spa 5-44)	4	6.62 \pm 5.00	1.9 - 12.50

(From: Archivo MED POL, Vertidos urbanos e industriales. Data for the year 1989)

NOTES:

n = number of samples
m = mean
s.d. = standard deviation

Table 2

Anionic detergent concentrations in wastewaters
of the Alicante coast (Albufereta and Babel bay)

	ALBUFERTA		BABEL BAY	
	(MBAS)	(LAS)	(MBAS)	(LAS)
Concentration (mg/L)	0.40	0.21	28.4	15.1
Maximum flow rate (Kg/d)	4.00	2.1	1704	906
Minimum flow rate (Kg/d)	--	--	852	453

From: WHO (1992): Pilot monitoring project on anionic detergents in the Mediterranean (WHO/UNEP Joint Project, MED POL Phase II) Final Report from Alicante, Spain (January 1993)

Table 3

Anionic detergent concentrations (mg/L) in various effluents
along the Eastern Adriatic

Investigated area	Investigated period	Type of effluent	n	Mean	Range
Pula area	1984-86	Industrial wastewaters	12	1.527	0.14-3.6
Pula area	1984-86	Domestic wastewater	12	2.44	0.07-9
Rijeka area	1984-86	Industrial wastewaters	20	2.2	0.023-16.5
Sibenik area	1984-86	Industrial wastewaters	(^{**})	0.056	0.019-0.085
Split area	1984-86	Domestic wastewater	24	3.43	0.168-10.9

From: UNEP: National Monitoring Programme of Yugoslavia, Report for 1983-1986. MAP Technical Reports Series No. 23. UNEP, Athens, 1988)

NOTES:

n = number of samples

(^{**}) = value not available

Table 4

Anionic detergent concentrations (mg/L) in effluents
along the Eastern Adriatic coast
(investigated period: 1991)

Investigated area	n	m ± s.d.
Slovenian coast	18	1.39 ± 1.778
Rijeka	13	7.412 ± 12.391
Zadar	9	0.48 ± 1.357
Split	28	1.474 ± 1.145

From: National Monitoring Programme of Yugoslavia, Report for 1991. Long-Term Programme for Pollution Monitoring and Research in the Mediterranean Sea (MED POL - PHASE II). Zagreb, 1992

NOTES:

n = number of samples
m = mean
s.d. = standard deviation

Table 5

Anionic detergent concentrations (mg/L) in effluents of two sewage
treatment plants in Liguria (Italy)
(mean ± s.d.; range)

	n	May-July	Sept.Nov.	YEAR 1992
AREA "a" Sewage treatment plant (unfiltered samples)	6	0.19 ± 0.14 (0.07 - 0.39)	13.84 ± 0.99 (12.99 - 14.92)	6.04 ± 7.32 (0.07 - 14.92)
AREA "b" Sewage treatment plant (unfiltered samples)	6	3.47 ± 0.010 (3.38 - 3.57)	1.59 ± 0.38 (1.21 - 1.96)	2.53 ± 1.06 (1.21 - 3.57)

From: WHO (1992): Pilot monitoring project on anionic detergents in the Mediterranean. (WHO/UNEP Joint Project, MED POL Phase II) Final Report from Genoa, Italy (1993)

NOTE:

n = number of samples

Table 6

Anionic detergent concentrations (mg/L) in wastewater samples as reported in selected literature

Sampling point	Compounds	m ± s.d.	Range	References
Grosseto sewage, Italy	Anionic detergents*		1.6-2.5	Buonomini <i>et al.</i> , 1956
S. Marcellino Pistoiese sewage, Italy	Anionic detergents*	4		Buonomini <i>et al.</i> , 1956
Palermo sewage, Italy (1957)	Anionic detergents*		3.3-12.0	Comes, 1957
Torino sewage, Italy (1957)	Anionic detergents*		0.45-8.00	Luria and Dall'Acqua,
Modena sewage, Italy (1965-66)	Anionic detergents*		0.22-31.12	Vivoli and Provvionato,
Laundry effluent, Yugoslavia	Anionic detergents		70-112	Kozarac <i>et al.</i> , 1976
Plastic industry Yugoslavia (1979-80)	Anionic detergents	36.72	0.095-246	Vojvodic <i>et al.</i> , 1981
Textile factories, Yugoslavia (1980)	Anionic detergents	6.2	0.07-14.28	Vojvodic <i>et al.</i> , 1981
Yugoslav Adriatic towns, wastewaters	Anionic detergents	2.1	0.02-16.3	Anonymous, 1987
Four urban effluents after secondary	Anionic detergents		0.1-1.4	Pierson <i>et al.</i> , 1980
Effluent after primary treatment	Anionic detergents	17.0 ± 5.5		Marchand <i>et al.</i> , 1989
Effluent after secondary treatment	Anionic detergents	0.56 ± 0.41		Marchand <i>et al.</i> , 1989
Effluent sewage after activated sludge	LAS (UV-HPLC)	0.07	0.05-0.11	Rapaport and Eckhoff,
Effluents after primary and secondary	Anionic detergents	0.58	0.75-2.5	Eden and Truesdale 1967

NOTES:

* = anionic detergents determined with the method of Longwell and Maniece.

s.d. = standard deviation

Table 6 (continued)

Anionic detergent concentrations (mg/L) in wastewater samples as reported in selected literature

Sampling point	Compounds	m ± s.d.	References
Wastewater treatment plant oxidation pond, Haifa, Israel.	Anionic detergents	8.5	Manka and Rebhun 1982
Lime clarifier, Haifa, Israel.	Anionic detergents	6.5	Manka and Rebhun 1982
Effluent sewage after activated sludge treatment, from 3 Canadian Plants (1976-86)	LAS (GC)	0.09 ± 0.05	Rapaport and Eckhoff, 1990
Raw water nonfiltered, Nassau County, N.Y. USA (1965)	Detergent	0.77	Stevens and Peters 1966
Effluent sewage after activated sludge treatment, from 12 U.S. plants (1976-86)	LAS (GC)	0.05 ± 0.04	Rapaport and Eckhoff, 1990
Sewage from individual home, USA	Detergent	6.2	Watson <i>et al.</i> 1967
Raw wastewater, South Nevada, USA	MBAS	7	Culp and Culp 1971
Raw water nonfiltered, Nassau County-N. Y., USA	Detergent	0.77	Stevens and Peters, 1966
Tertiary effluent non filtered, USA	Detergent	<0.02	Stevens and Peters, 1966
Primary effluent Windhoek, South Africa	ABS	16.2	Stander and van Vuuren, 1970

NOTES:

m = mean

s.d. = standard deviation

Table 7

Anionic detergent concentrations (mg/L) in Spanish rivers
(mean \pm s.d.; range)

SAMPLING POINT	YEAR 1989			YEAR 1990			YEAR 1991		
	n	m \pm s.d.	range	n	m \pm s.d.	range	n	m \pm s.d.	range
Rio Ter (Spa 1-02)	5	0.19 \pm 0.07*	0.1-0.28 [†]	1	0.14 \pm 0.07	0.05-0.30	12	0.09 \pm 0.04	0.05-0.17
Rio Llobregat (Spa 1-08)	6	2.67 \pm 1.20**	1.20-4.20**	1	5.28 \pm 2.07	2.23-10.4	12	2.56 \pm 1.78	0.38-6.32
Rio Ebro (Spa 2-11)	6	0.12 \pm 0.07***	0.04-0.22***	8	0.06 \pm 0.02	0.05-0.11	4	0.68 \pm 1.03	0.08-2.22
Rio Jucar (Spa 2-16)	6	0.85 \pm 0.51E	0.40-1.80E	1	0.17 \pm 0.12	0.06-0.50	9	0.09 \pm 0.05	0.05-0.22
Rio Segura (Spa 2-19)	8	1.33 \pm 0.66EE	0.51-2.60EE	1	0.71 \pm 0.27	0.08-0.97	12	0.26 \pm 0.24	0.06-0.84

From: Archivo MEDPOL: Aportes fluviales (Spain). Data for the year 1989.

From: Contenido del Fichero VERMP 90.DBF, Vertidos (Spain). Data for the year 1990.

From: Contenido del Fichero VERMP 91.DBF, Vertidos (Spain). Data for the year 1991.

NOTES:

n = number of samples

m = mean

s.d. = standard deviation

* = sampling point Rio Ter-Desemb

** = sampling point Rio Llobergat-Des

*** = sampling point Rio Ebro-Amposta

E = sampling point Rio Jucar-Cullera

EE = sampling point Rio Segura-Des

Table 7a

Anionic detergent concentrations (mg/L) in Spanish rivers
(mean \pm s.d.; range)

SAMPLING POINT	YEAR 1989		
	n	m \pm s.d.	range
Portman L. Roberto (Spa 4-28)	1	0.1	
Cartagena Algameca (Spa 4-30)	8	14.51 \pm 12.45	3.8 - 41.3
Cartagena Asur (Spa 4-30)	3	0.37 \pm 0.15	0.2 - 0.5
Cartagena Enfersa (Spa 4-30)	3	1.27 \pm 1.07	0.1 - 2.2
Cartagena Ert (Spa 4-30)	3	8.80 \pm 9.34	0.3 - 18.8
Cartagena E. De Zinc (Spa 4-30)	3	1.20 \pm 0.46	0.8 - 1.7
Cartagena Repsol P. (Spa 4-30)	3	0.40 \pm 0.352	0.2 - 0.8
Almeria (Spa 5-35)	7	12.6 \pm 6.61	0.7 - 21.5
Malaga Guadal. dcha (Spa 5-40)	7	21.37 \pm 8.01	7.7 - 29.7
Malaga Misericordia (Spa 5-40)	7	26.86 \pm 14.38	9.8 - 44.2
Marbella Est. Bombeo (Spa 5-42)	7	7.67 \pm 5.82	0.2 - 16.2
la Linea Atunara (Spa 5-43)	7	13.0 \pm 6.03	8.4 - 25.4
Algeciras Varadero (Spa 5-44)	7	6.09 \pm 3.89	1.9 - 12.5

From: Archivo MED POL: Aportes fluviales (Spain). Data for the year 1989

NOTES:

n = number of samples
m = mean
s.d. = standard deviation

Table 8

Anionic detergent concentrations (mg/L) in river samples
from the Eastern Adriatic

Investigated area	Investigated period	Number of samples	Mean	Range
Slovenian coastal area	1984-86	20	0.063	0.005 - 0.226
Montenegrin coastal area	1986	4	0.043	0.022 - 0.065

From: UNEP: National Monitoring Programme of Yugoslavia, Report for 1983-1986.
MAP Technical Reports Series No. 23. UNEP, Athens, 1988

Table 9

Anionic detergent concentrations (mg/L) in two Ligurian rivers
(Italy)
(mean \pm s.d.; range)

	n	May-July	Sept.-Nov.	YEAR 1992
		m \pm s.d. (range)	m \pm s.d. (range)	m \pm s.d. (range)
AREA "a" Sewage treatment plant (unfiltered samples)	6	0.19 \pm 0.14 (0.07 - 0.39)	13.84 \pm 0.99 (12.99 - 14.92)	6.04 \pm 7.32 (0.07 - 14.92)
AREA "b" Sewage treatment plant (unfiltered samples)	6	3.47 \pm 0.010 (3.38 - 3.57)	1.59 \pm 0.38 (1.21 - 1.96)	2.53 \pm 1.06 (1.21 - 3.57)

From: WHO (1992): Pilot monitoring project on anionic detergents in the Mediterranean.
(WHO/UNEP Joint Project, MED POL Phase II) Final Report from Genoa, Italy (February, 1993)

NOTE:

n = number of samples
m = mean
s.d. = standard deviation

Table 10

Anionic detergent concentrations (mg/L) in river samples
as reported in selected literature

Sampling point	Compounds	Mean	Range	References
Tevere River (Roma, Italy)	MBAS	--	0.462-0.555	Muzzi and Borgioli, 1967
Arno River (Pisa, Italy)	MBAS	--	0.11-0.86	Lapucci <i>et al.</i> , 1967
Piave River (Eraclea-mouth, Italy)	ABS	--	0.05-1.58	Perin <i>et al.</i> , 1968
Po River (Chivasso, Italy)	ABS	0.7	0.35-1.4	Meucci and Verde, 1972
Adige River (Trento-South, Italy)	MBAS	0.22	0.15-0.28	Lauro and Maistri, 1968
Leiro Stream (Genoa-mouth, Italy)	MABS	0.59	0.18-1.90	La Rocca, <i>et al.</i> , 1977
Polcevera Stream (Genoa-mouth, Italy)	MABS	2.29	0.20-7.20	La Rocca, <i>et al.</i> , 1977
Bisagno River (Genoa-mouth, Italy)	MABS	3.4	0.1-13.8	La Rocca, <i>et al.</i> , 1977
Varenna Stream (Genoa-mouth, Italy)	MABS	1.26	0.01-3.90	La Rocca, <i>et al.</i> , 1977
Manzanares River (Madrid, Spain)	MBAS	2.6	1.50-3.50	Sanchez <i>et al.</i> , 1991
Manzanares River (Madrid, Spain)	LAS (HPLC)	2.35	1.25-3.50	Sanchez <i>et al.</i> , 1991
Ebro River (Spain)	LAS	--	0-0.34	Martinez <i>et al.</i> , 1989
Eleven European rivers	LAS	--	0.01-0.09	Hennes and Rapaport, 1989
Germans rivers	LAS	0.04	0.01-0.09	Matthijs and De Henau, 1987
Germans rivers	LAS	0.28	0.08-0.61	Topping and Waters, 1982
U.K. rivers	LAS	0.04	0.008-0.17	Gilbert and Kleiser, 1986
Town river, Mass. (USA)	LAS	--	0.04-0.59	Lewis and Wee, 1983
Ohio River (U.S.A.)	AES	0.008	--	Woltering <i>et al.</i> , 1987
Major U.S. rivers	LAS	--	0.01-0.04	Hennes and Rapaport, 1989
Eight U.S. rivers	LAS	--	0.01-3.3	A.D. Little Co., 1977
Unnamed U.S. river	LAS	--	0.01-0.27	Osburn, 1986
Tama River (Japan)	LAS (GLC-MS)	--	0.108-0.511	Hon-Nami and Hanya, 1980
Tama River (Japan)	MBAS	--	0.24-1.24	Hon-Nami and Hanya, 1980
Tamagawa River (Japan 1983-1986)	LAS (HPLC)	0.18	--	Takada <i>et al.</i> , 1992
Malaysia rivers and estuaries	MBAS	--	0-0.57	Ludwig and Sekaran, 1988

Table 11

Anionic detergent concentrations (mg/L) in seawater
from the Alicante Coast (Albufereta)

SAMPLING POINT	(MBAS)		(LAS)	
	SAMPLING I (3-6-92)	SAMPLING II (2-11-92)	SAMPLING I (3-6-92)	SAMPLING II (2-11-92)
Boil (surface)	0.01	0.04	0.01	0.01
Boil (deep)	<0.01	<0.01	<0.01	0.01
100 m East (surface)	0.01	0.03	<0.01	0.03
100 m East (deep)	<0.01	0.01	<0.01	0.01
100 m South-East (surface)	0.01	0.04	<0.01	0.03
100 m South-East (deep)	<0.01	0.02	<0.01	0.02
Beach (surface)	0.02	0.03	0.01	0.03
Beach (deep)	<0.01	<0.01	<0.01	0.01

From: WHO (1992): Pilot monitoring project on anionic detergents in the Mediterranean.

(WHO/UNEP Joint Project, MED POL Phase II) Final Report from Alicante, Spain (January, 1993).

Table 12

Anionic detergent concentrations (mg/L) in seawater
from the Alicante Coast (Babel bay)

SAMPLING POINT	(MBAS)		(LAS)	
	SAMPLING I (30-6-92)	SAMPLING II (27-10-92)	SAMPLING I (30-6-92)	SAMPLING II (27-10-92)
20 m (surface)	4.2	3.9	1.25	1.94
20 m (deep)	0.81	1.32	0.25	0.78
100 m East (surface)	1.30	0.77	0.88	0.44
100 m East (deep)	0.45	0.21	0.27	0.15
100 m West (surface)	0.08	0.04	0.04	0.03
100 m West (deep)	0.04	<0.01	<0.01	0.02
Beach (surface)	0.03	0.02	0.01	0.02
Beach (deep)	0.03	0.01	<0.01	0.01

From: WHO (1992): Pilot monitoring project on anionic detergents in the Mediterranean.

(WHO/UNEP Joint Project, MED POL Phase II) Final Report from Alicante, Spain (January, 1993).

Table 13

Anionic detergent concentrations (mg/Kg) in sediments
from the Alicante coast

SAMPLING POINT	(MBAS)	(LAS)
Albufereta Beach	1.68	0.02
Babel Bay	3.95	0.01
Santiago Bernabeu Avenue (Santa Pola)	1.25	0.01
Playa Lisa (Santa Pola)	4.29	0.02

From: WHO (1992): Pilot monitoring project on anionic detergents in the Mediterranean. (WHO/UNEP Joint Project, MED POL Phase II). Final report from Alicante, Spain (January, 1993)

Table 14

Anionic detergent concentrations (mg/L) in seawater
from the Malta coast
(mean \pm s.d.; range)

SAMPLING POINT	MAY 1992 m \pm s.d.	OCTOBER 1992 m \pm s.d.
S1 WIED CHAMMIEQ	0.0153 \pm 0.0012	0.024 \pm 0.0016
S2 XGHAJRA	0.0117 \pm 0.0009	0.021 \pm 0.0012
S3 SALINA BAY	0.0099 \pm 0.0008	0.024 \pm 0.0015

From: WHO (1992): Pilot monitoring project on anionic detergents in the Mediterranean. (WHO/UNEP Joint Project, MED POL Phase II). Final report from Malta (November, 1992)

NOTES:

m = mean
s.d. = standard deviation

Table 15

Anionic detergent concentrations (mg/L) in seawater of
the Ligurian sea (Italy)
(mean \pm s.d.; range)

		MAY - JULY	SEPT. - NOV.	YEAR 1992
	n	m \pm s.d. (range)	m \pm s.d. (range)	m \pm s.d. (range)
AREA "a "	24	0.019 \pm 0.015 (<0.01 - 0.047)	0.031 \pm 0.043 (<0.01- 0.121)	0.025 \pm 0.032 (<0.01 - 0.121)
AREA "b"	24	0.020 \pm 0.022 (<0.01- 0.076)	0.016 \pm 0.017 (<0.01 - 0.050)	0.018 \pm 0.019 (<0.01- 0.076)
AREA "c"	6	0.018 \pm 0.015 (<0.01 - 0.035)	0.013 \pm 0.008 (<0.01 - 0.021)	0.015 \pm 0.011 (<0.01 - 0.035)

From: WHO (1992): Pilot monitoring project on anionic detergents in the Mediterranean.
(WHO/UNEP Joint Project, MED POL PHASE II) Final Report from Genoa, Italy (February, 1993).

NOTES:

n = number of samples

m = mean

s.d. = standard deviation

Table 15a

Bathing water monitoring results according to the Italian law
D.P.R. 8-6-1982 n.470)

Provinces	Coastal length (Km)	Number of sampling points	Detergent pollution: % of unacceptable (**) samples	
			1992	1993
Ascoli-Piceno	48.0	57	0.0	0.9
Napoli	200.0	158	6.0	4.4
Salerno	256.0	148	0.5	0.0
Bari	220.0	134	0.0	1.3
Palermo	180.0	121	0.2	0.4
Messina	350.0	198	2.4	1.8
Catania	51.0	32	9.0	8.9
Ragusa	82.5	34	1.4	0.0
Livorno	282.4	177	0.6	0.0
Cosenza	242.0	277	1.1	0.0

From: Qualità delle acque di balneazione. Rapporto numerico. Anno 1992 (1993): Sistema Informati Sanitario-Ministero della Sanità. Direzione Generale Servizi Igiene Pubblica. Istituto Superiore di Sar Centro stampa Sistema Informativo Sanitario. Roma.

From: Qualità delle acque di balneazione. Rapporto numerico. Anno 1993 (1994): Sistema Informati Sanitario-Ministero della Sanità. Direzione Generale Servizi Igiene Pubblica. Istituto Superiore di Sar Centro stampa Sistema Informativo Sanitario. Roma.

NOTE:

(**) = concentration (as MBAS) superior to 0.5 mg/L

Table 16

Anionic detergent concentrations (mg/L) in seawater samples
from the Eastern Adriatic

Investigated area	Investigated period	Number of samples	Mean	Range
Sibenik area	1984	9	0.004	0.002 - 0.014
Montenegrin coastal area	1985-86	31	0.045	<0.01 - 0.122

From: UNEP: National Monitoring Programme of Yugoslavia, Report for 1983-1986. MAP Technical Reports Series No. 23. UNEP, Athens, 1988.

Table 17

Anionic detergent concentrations (mg/L) in marine and
river samples from the Slovenian coast

Sampling Station	Depth	May 1992	October 1992	December 1992
<u>Bay of Koper and Bay of Piran</u>				
A	0 m	<0.01	0.01	0.01
A	5 m	<0.01	0.01	
A	10 m	<0.01	<0.01	
A	15 m	<0.01	<0.01	
A	bottom	<0.01	0.01	
B	0 m	0.01	<0.01	0.01
B	5 m	<0.01	<0.01	
B	10 m	<0.01	<0.01	
B	15 m	<0.01	<0.01	
B	bottom	<0.01	<0.01	
C	0 m	<0.01	< 0.01	<0.01
D	0 m	0.01	0.01	<0.01
D	5 m	<0.01	<0.01	
D	10 m	<0.01	<0.01	
D	15 m	<0.01	<0.01	
D	bottom	<0.01	0.02	
E	0 m	0.02	0.01	<0.01
E	5 m	0.01	0.01	
E	10 m	0.01	0.01	
E	15 m	<0.01	0.01	
E	bottom	<0.01	<0.01	
<u>Near shellfish growing area</u>				
F	0 m	0.03	<0.01	<0.01
<u>Coastal marine points:</u>				
Izola		0.02	0.01	
Piran		0.02	0.01	
Valdoltra (1)		0.01	0.06	
(2)		0.04	0.01	
Zusterna		0.02	0.01	
<u>River sampling point:</u>				
Rizana River		<0.01	<0.01	

From: WHO (1992): Pilot monitoring project on anionic detergents in the Mediterranean. (WHO/UI Joint Project, MED POL Phase II) Final Report from Ljubljana, Slovenia (December, 1992).

Table 18

Anionic detergent concentrations (mg/L) in seawater samples
as reported in selected literature

Sampling point	Compounds	Mean	Range	References
Adriatic Sea (Rimini, Italy)	MBAS	-- --	b.d. - 0.80E b.d. - 1.78EE	Provisionato and Malavasi, 1968
Adriatic Sea (Gulf of Trieste, Italy)	--	0.06	b.d.- 0.23	Majori <i>et al.</i> , 1981
Adriatic Sea (North Italian coastal waters)	--	0.054	--	Blundo <i>et al.</i> , 1985
Tyrrhenian Sea (Tirrenia Marina-Pisa, Italy)	MBAS	--	0.005 - 0.012	Lapucci, 1968
Tyrrhenian Sea (coastal waters from La Spezia to Piombino, Italy)		0.058	0.009 - 0.17	De Renzi <i>et al.</i> , 1978
Adriatic Sea (Rijeka Bay- Yugoslavia, 1974-78)	--	0.008	0.002-0.029	Cosovic and Zutic, 1981
Adriatic Sea (Estuarine and coastal waters, Yugoslavia, 1983-1986)	--	0.021	0.002-0.122	Anonymous, 1987
French coastal waters, 1979-1980	--	0.01*	--	Boisson <i>et al.</i> , 1981
Spanish sea water	LAS	--	0-0.26	Martinez <i>et al.</i> , 1989
Rumanian coastal waters, (1978-1979)	--	0.14	0.07-0.35	Serbanescu <i>et al.</i> , 1981
Tokyo Bay (Japan)	LAS (GLC-MS)	--	<0.003-0.014	Hon-Nami and Hanya, 1980
Tokyo Bay (Japan)	MBAS	--	0.03-0.07	Hon-Nami and Hanya, 1980
Tokyo Bay (Japan)	LAS (HPLC-FP)	--	0.0008-0.03	Kikuchi <i>et al.</i> , 1986
Canadian coasts (polluted areas)	MBAS		0.001-0.2	Gagnon, 1983

NOTES:

b.d. = below detection

E = sampling points located in the open sea.

EE = sampling points located along the harbour- channel

* = sensitivity limit

Table 19

Anionic detergent concentrations (mg/kg) in biota samples
as reported in available literature (range)

SAMPLING AREAS	Catania coast-Italy* (Bellassai <i>et al.</i> , 1973)	Gulf of Naples-Italy (Sansone <i>et al.</i> , 1979)	Tokyo Bay-Japan (Kikuchi <i>et al.</i> , 1986)
SPECIES:	ABS (mg/Kg, wet basis)	ABS (mg/Kg, wet basis)	LAS (mg/Kg, wet basis)
<i>Mytilus edulis</i>	0.50 - 120	--	--
<i>Mytilus galloprovincialis</i>	--	2.20 - 10.70	--
<i>Konosirus punctatus</i>	--	--	<0.3
<i>Sardina pilchardus</i>	0	--	--
<i>Mugil cephalus</i>	1.4 - 2.1	--	--
<i>Mullus surmuletus</i>	0	--	--
<i>Labrus viridis</i>	0.22 - 0.80	--	--
<i>Crenilabrus mediterraneus</i>	0	--	--
<i>Pagellus mormyrus</i>	0	--	--
<i>Boops boops</i>	0.4 - 2.2	--	--
<i>Scomber scombrus</i>	0.02 - 0.25	--	--
<i>Trachurus trachurus</i>	0.01 - 0.02	--	--
<i>Scorpena scrofa</i>	0	--	--
<i>Scorpena porcus</i>	0	--	--
<i>Solea vulgaris</i>	0.01 - 0.03	--	--
<i>Merluccius vulgaris</i>	0	--	--
<i>Blennius ocellaris</i>	0.75 - 1.5	--	--
<i>Xiphias gladius</i>	0	--	--
<i>Donax trunculus</i>	0	--	--

NOTE:

* = samples taken from local fish market

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