

EXPERIMENTAL PROCEDURES FOR HAZARD ASSESSMENT  
IN THE MARINE ENVIRONMENT

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L. LANDNER

The Esther Project  
The National Swedish Environment Protection Board  
Box 1302  
S-171 25 Solna, Sweden

**ABSTRACT**

The environmental hazard of a chemical is a function of two factors : (1) the potential for exposure to the chemical, and (2) the potential noxious effects of the chemical on living organisms or abiotic systems. In most hazard assessment protocols, the exposure and the effects analyses are carried out as two separate sequences of steps, before, at a preselected level of refinement, the "predicted environmental concentration" is compared to the "no adverse biological effect concentration".

A general discussion is presented on the theoretical aspects of hazard assessment, together with a few examples of some current approaches to notification procedures for new chemicals. Then, some of the recently used approaches to hazard assessment in the marine environment as applied to ocean-disposed wastes, either by direct dumping or through discharge by coastal outfall, will be reviewed and discussed.

Attention is drawn to a number of pitfalls and difficulties not generally considered in environmental hazard assessments based on predictive laboratory testing and mathematical modelling. These difficulties arise in obtaining good estimates of :

- the kinetics of biodegradation at low ambient concentrations of recalcitrant substances under natural conditions ;

- the fraction of xenobiotics in marine waters which are bioavailable to various organisms under different environmental conditions ;
- the type and scope of systemic effects in the marine ecosystem.

Particular emphasis is furthermore given to the occurrence, in the marine environment, of bacterial transformation of xenobiotics into metabolites having properties more hazardous than the parent compounds. For each of these points, suggestions are given for experimental procedures which might be expected to circumvent the difficulties.

### **KEYWORDS**

Marine ecotoxicology, Hazard assessment, Bioassays, Methods, Legislation, Exposure analysis, Biodegradation, Bioavailability, Model ecosystem, Review.

### **INTRODUCTION**

The environmental hazard of chemicals, or mixtures, contained for example in industrial effluents, can be described as a function of two factors : (1) the potential for exposure to the chemical (or mixture), and (2) the potential noxious effects on living organisms or abiotic systems (OECD Hazard Assessment Project, 1982). This implies that chemicals to which no exposure occurs do not present any hazard. A comprehensive analysis of the potential environmental exposure to a given chemical is therefore an indispensable part of the hazard assessment procedure.

The central role of OECD in the development of such procedures was pointed out by Bro-Rasmussen and Christiansen (1983) in their comprehensive review of hazard assessment, understood as an integrated evaluation of the potential exposure and the potential effects of chemicals in the environment. It seems therefore pertinent to base a discussion on principles of hazard assessment on OECD work in this area.

A general discussion will first be presented on the theoretical aspects of hazard assessment, together with a few examples of some current approaches to notification procedures for new chemicals ; some specific situations will then be discussed in more detail. In considering the hazard

to the marine ecosystem resulting from the release of chemicals or wastes, it is generally possible to obtain a certain amount of site-specific information. This allows a more detailed evaluation to be made of the direct environmental exposure as well as to the ecosystem being directly exposed. This information may then be incorporated into the overall assessment procedure which may involve application of methods differing significantly from those used at the screening stage. A few examples of recent studies will be presented.

Finally, attention is drawn to a number of pitfalls and aspects not generally considered in environmental hazard assessments based on predictive laboratory testing and mathematical modelling. These difficulties arise from obtaining good estimates of : (1) the kinetics of biodegradation at low ambient concentrations of recalcitrant substances under natural conditions ; (2) the concentrations of xenobiotics in marine waters which are bioavailable under various environmental conditions ; and (3) the type and scope of systemic effects at the highest organizational level in the marine ecosystem. Particular emphasis will furthermore be given to the occurrence, in the environment, of bacterial transformation of xenobiotics into metabolites having properties more hazardous than the parent compounds. For each of these points, suggestions will be given for experimental procedures which might be expected to circumvent the difficulties.

#### **PRINCIPLES OF HAZARD ASSESSMENT IN RELATION TO NOTIFICATION OF NEW CHEMICALS**

During the last decade, most industrialized countries have issued extensive legislation designed to control the use and spreading of chemicals. A prospective approach is used in most of these "Product Control Laws" or "Toxic Substances Laws". Before placing chemicals on the market, manufacturers and/or importers are requested to notify national authorities of the manufacture or marketing of new chemicals. These notifications generally contain a compendium of data on physicochemical properties and possible noxious effects of the chemicals. From such data bases, attempts have been made to develop preliminary hazard assessment schemes aiming at an integrated evaluation of the properties estimated from the results of the prospective testing of the chemical (e.g. Klein and Haberland, 1983 ; Schmidt-Bleek *et al.*, 1983).

The limited data base which ought to be available at the time of notification of new chemicals (the EEC Base Set of Data or the OECD Minimum Pre-marketing Set of Data, MPD) does not allow anything except quite general assessments to be made. In place of a true environmental hazard assessment, a "hazard ranking" or "hazard classification" of the new chemical could possibly be made in relation to well-known "bench-mark chemicals". The preliminary hazard assessment (or hazard classification) procedures which have been proposed for notification of new chemicals are usually based upon a generalized situation in which the total biosphere is subject to potential exposure. At this stage, it is difficult, if not impossible, to predict with any reasonable certainty whether any one sector of the environment, or any one ecosystem would be the primary target for the chemical.

It is thus obvious that, even if laws and regulations on production and use of chemicals are intranational, there are strong incentives for harmonizing test methodologies and assessment procedures in different countries. In this context, OECD has proven to be of primary importance through its achievement of a mutual acceptance of test data among member countries, through its compilations and evaluation of valid Guidelines for Testing of Chemicals (OECD, 1981), and through its development of provisional Data Interpretation Guides (DIGs) based on the testing guidelines.

#### **ANALYSIS OF ENVIRONMENTAL EXPOSURE**

The types of information needed to estimate the environmental exposure of a chemical are presented in Fig. 1. The flux of a chemical from the technosphere to the biosphere cannot be determined only on the basis of results from laboratory tests. It has to be estimated on the basis of the quantities marketed, the patterns of usage, the types of application, and the nature of the sector of the environment which receives the discharge (Schmidt-Bleek *et al.*, 1983). At the time of notification of a new chemical, much of this information will not be known. However, the environmental fate of a chemical once it has entered the environment is a function of a number of factors :

1. its distribution between the major sectors of the environment (air, water, soil, sediment, suspended particles and biota) ;
2. its degradability in these sectors via biotic or abiotic processes;
3. its potential for accumulation in specific sectors.

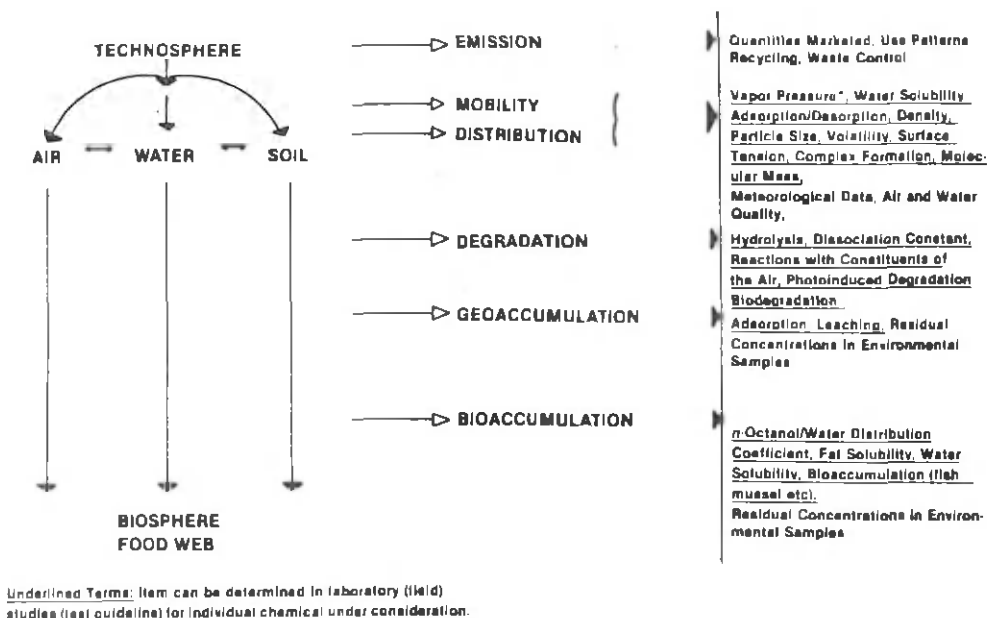


Fig. 1. Information useful for determining environmental exposure (from Schmidt-Bleek et al., 1983).

This general distribution can be calculated from the physicochemical properties of the chemical and some established environmental ("unit world") characteristics.

The most practical models currently used for estimating Potential Environmental Distribution (PED) are based on the Mackay fugacity principle (Klein and Schmidt-Bleek, 1982 ; Mackay and Paterson, 1982 ; Wood et al., 1982). In these models, the environment is hypothetically divided into five main compartments, with relative volumes selected according to the specific problem to be solved (Fig. 2). This approach has proven to be very useful in estimating the relative distribution of a chemical. In its simplest form, it is based on a number of explicit assumptions : (1) that a state of thermodynamic equilibrium exists between the various compartments ; (2) that there is a homogeneous distribution of the chemical within the individual compartments ; and (3) that transfer processes are more rapid than degradation. In more sophisticated versions of the model, more realistic assumptions are introduced. Knowledge about the PED helps to focus attention

on that compartment in the environment containing the highest potential mass or concentration of the chemical. It will, therefore, indicate which compartment particularly merits detailed studies of degradation or of effects on the biota.

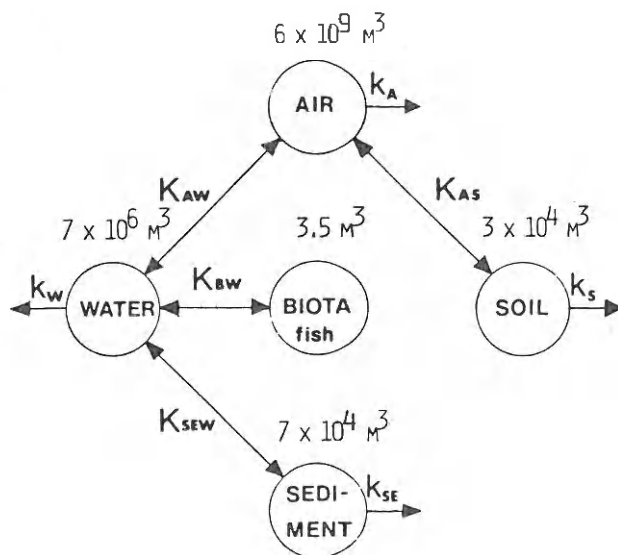


Fig. 2. Environmental compartments with volumes and pathways considered in calculations of PED. Constants are either equilibria (K) or rate constants (k) (from OECD, 1982).

## ANALYSIS OF ENVIRONMENTAL EFFECTS

An analysis of effects should, ideally, consist of the evaluation of effects at all ecologically relevant organization levels, and include studies of individuals, populations and communities and, finally, the complete ecosystem (Fig. 3). In an initial hazard classification, however, it has been found realistic to recommend application of only a few simple tests at the individual/population level (Bro-Rasmussen and Christiansen, 1983). For example, in the OECD Guidelines for Testing of Chemicals (1981), the environmental biota are represented by rats, fish, Daphnia, and algae.

ORGANIZATION LEVEL	MPD TEST LEVEL	HIGHER TEST LEVELS		
		LABORATORY	MODEL ECOSYSTEM	FIELD
ECOSYSTEMS				INSTABILITY PRODUCTIVITY DISTURBANCE
COMMUNITIES			STRUCTURAL DISORDERS DIVERSITY CHANGES	
POPULATIONS		POPULATION ALTERATIONS REPRODUCTION FAILURES		
INDIVIDUALS	ACUTE TOXICITY, FISH D:O , DAPHNIA D:O , ALGAE	BIOACCUMULATION, FISH, MUSSELS FISH, SUBACUTE TOXICITY EARTH-WORMS, ACUTE TOXICITY MAMMALS, ACUTE & SUBACUTE TOXICITY BIRDS, ACUTE & SUBACUTE TOXICITY PLANT DAMAGES		

Fig. 3. Ecotoxicological effects of chemicals at different levels of biological organization.

The limited information obtained from the simple biological effect data included in the MPD has, nonetheless, a real value since it can be used for potential hazard to organisms in the aquatic environment. So far, however, there are no rational or generally accepted rules for extrapolating results from these simple ecotoxicological tests to the establishment of safe and environmentally adequate protection levels.

#### INTEGRATION OF DATA

At the level of initial hazard assessment based on MPD data, the object of the exercise is a scientifically based indication of the potential hazard. It is useful for classification of different chemicals into hazard categories and for estimating the relative hazard of a chemical compared with that of well established "bench-mark" chemicals. The order in which the various elements are integrated to obtain a relation between the exposure and the effect parameters in the hazard assessment "equation" may vary considerably from case to case. When, however, a number of chemicals are

being assessed, and when the quantity and quality of data for each is relatively consistent, a predetermined order of integration, or a fixed assessment strategy may be applied.

For notification of new chemicals, several proposals have been presented recently (Schmidt-Bleek *et al.*, 1982 ; Jouany *et al.*, 1983 ; Klein and Haberland, 1983). Attempts have been made to find a uniform procedure for the integrated initial assessment of relative hazard of chemicals which would be acceptable to the OECD member countries. No consensus has, however, been reached so far. It is generally felt that, although fixed strategies for initial hazard assessment based on MPD data are valuable in providing a basis for labelling and the issue of cautionary warnings, the final phases of integration cannot generally follow predetermined rules (e.g. Bro-Rasmussen and Christiansen, 1983). The professional judgement of the experienced ecosystem analyst will determine both the nature of the final phases and the outcome of the integrated hazard assessment.

#### HAZARD ASSESSMENT IN THE MARINE ENVIRONMENT

In the subsequent discussion, the marine environment will be considered as including the Baltic sea ecosystem. Some of the consequences of the difference in salinities are briefly discussed later.

In cases where it is possible to restrict the assessment to a relatively well-defined sector of the total environment, such as the marine ecosystem, much more specific data are available than when the general case is considered. For example, in assessing the hazard resulting from release of chemicals or wastes into the marine environment, the following basic information is generally available :

1. the point of input (land-based point sources, dumping areas, seabottom industrial activities, etc.) ;
2. the rates of release ;
3. the hydraulic conditions (currents, tidal conditions, river outfalls, etc.) ;
4. the natural physicochemical conditions (salinity, turbidity, oxygen concentration, temperature regime, etc.)
5. the potential vulnerability of the target communities exposed to the chemical and among which the effects are ultimately to be evaluated.



The access to these and other specific data will considerably increase the data base to be used in the assessment and will, therefore, result in a correspondingly higher degree of confidence in the outcome of the analysis.

#### **ESTIMATION OF POTENTIAL ENVIRONMENTAL CONCENTRATIONS (PEC)**

In situations where a point source for release of wastes into the marine ecosystem can be defined, it is recommended that, instead of depending on PED calculations, the PEC of the chemical (or of a trace substance contained in the discharge) should be calculated. The same approach is possible in cases for which well-defined scenarios can be set up and which may additionally be able to simulate the effect of rare accidents or spills.

The precision with which the PEC may be estimated will, of course, depend upon a number of factors : (1) the quality of the data available for the physicochemical properties of the key component(s) of the discharge ; (2) the volume and disposal pattern of the discharge ; and (3) the sector of the environment which receives the discharge. In particular, the pattern of release must be sufficiently well understood to allow assumptions to be made about the receiving ecosystem close to the point of release (Landner, 1982).

Furthermore, the physical processes of advection (*i.e.* transport) and dispersion (*i.e.* mixing) in the marine system must be considered. Existing methods for estimating both homogeneous and heterogeneous (solution and solids) transport and mixing should be refined. Particular emphasis should be given to those areas of hydraulics and hydrodynamics that relate to developing concentration-time profiles of chemicals that remain in solution or are associated with particulate matter, or assume both forms.

A great number of more or less sophisticated advection-dispersion mathematical models have been developed during recent years ; these range from simple black-box models to highly elaborate three-dimensional hydrodynamic models. It is beyond the scope of this review to discuss this subject in detail. However, it is pertinent to emphasize that models used to simulate the environmental fate of chemicals should be as simple as possible, and should concentrate on obtaining maximally accurate estimates of PEC only for the most sensitive compartments or sectors of the ecosystem.

This leads us to some general considerations concerning the possibility of identifying "weak links", vulnerable functions or regulating factors in the ecosystem.

#### **EVALUATION OF EFFECTS ON THE MARINE ECOSYSTEM**

There is a plethora of questions concerning ecosystem structure and function. These are of cardinal importance both for a proper understanding of laboratory-derived data and for a meaningful interpretation of their consequences in the natural environment. Certain characteristics of populations and ecosystems are particularly vulnerable to the presence of a chemical, and should be considered in all environmental impact assessments. Pertinent characteristics of populations include changes in mortality, fecundity, growth rates, age distribution, and phenotypic variation. Most of these parameters can be estimated with satisfactory accuracy by means of current laboratory tests. However, there is a need for development of improved methods for measuring changes in parameters such as diversity, levels of productivity, degree of connectivity, resistance and resilience, interaction of species, taxonomic variability, flow of energy and essential nutrients, and compositions of functional groups (US National Research Council, 1981).

One of the main difficulties in ecotoxicology is to assess the ecological significance of effects observed in the laboratory. This problem becomes even more difficult because of the capacity of biotic systems to repair damage and to adapt to adverse conditions. Much attention has lately been paid to the stress tolerance, the stability and the recovery potential of an ecosystem after both single and repeated disturbances (Karlsson, 1983).

So far, professional ecologists have played only a modest role in the field of ecotoxicology (Koeman, 1982). There is obviously a real need in the future to find ways to improve communication between representatives from basic and theoretical ecological research on the one hand, and ecotoxicologists and those dealing with environmental hazard assessment on the other. The contribution of ecologists would be to identify key properties of relevant ecosystems, and to assess the potential vulnerabilities of various types of ecosystems to chemical exposure.

## CURRENT APPROACHES TO HAZARD ASSESSMENT IN THE MARINE ENVIRONMENT

The protocols presented in the US Ocean Dumping Regulations (USEPA, 1977) and associated guidelines (USEPA and Army Corps of Engineers, 1977) provide impetus to the use of "effects-based" rather than "technology-based" standards for controlling discharges of chemicals and wastes to marine systems. According to these protocols, hazard evaluations have to be performed for both water-column and benthic organisms (Fig. 4). Liquid and suspended particulate phases of a discharged waste are considered potentially hazardous to organisms in the water column, whereas the solid phase is regarded as a threat to the benthos (Rose and Ward, 1981).

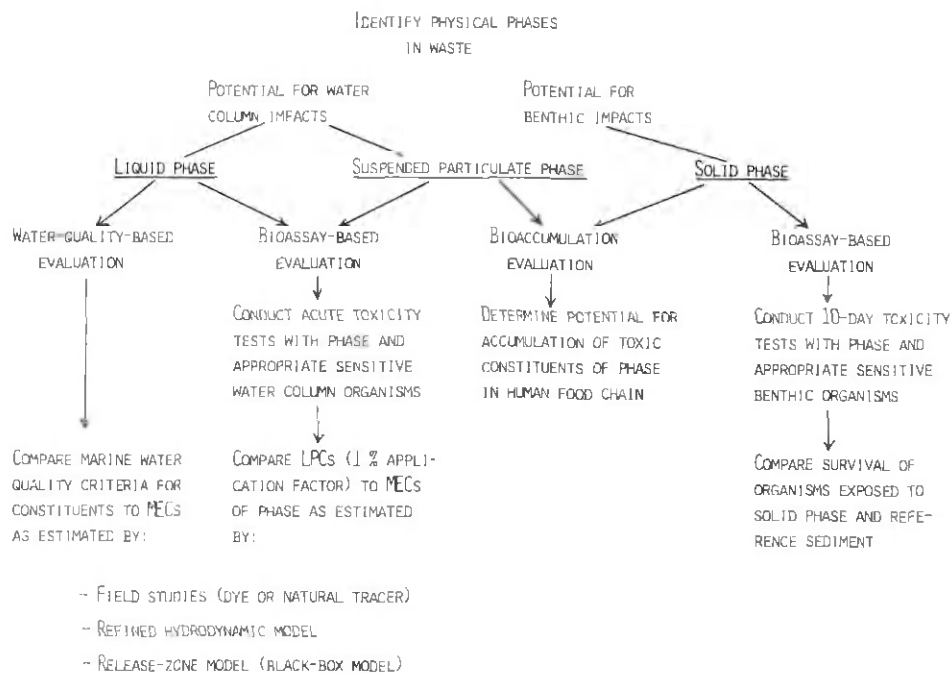


Fig. 4. Flow chart of hazard assessment procedure (modified after Rose and Ward, 1981).

The hazard of the liquid phase of a waste may be evaluated using either water-quality-based or bioassay-based techniques. The latter approach, in which water-column organisms are exposed for a short period of time to the phase, is considered more reliable than the former, since the synergistic and antagonistic effects of all constituents of the phase are assessed rather than the isolated effects of only those constituents for which marine water quality criteria have been established. The suspended phase of a waste, like the solid phase, must be evaluated by a twofold approach in which both the toxicity and the potential for bioaccumulation are considered (Rose and Ward, 1981). In evaluating both the liquid and the suspended particulate phases, "limiting permissible concentrations" (LPCs) for discharged materials are compared with "maximum environmental concentrations" (MECs) of materials. The latter are estimated by : (a) field (plume) studies during discharge operations ; (b) mathematical hydrodynamic models ; or (c) a black-box "release zone" model, which assumes that a discharged material is homogeneously mixed 4 h after discharge into a mass of water bounded on the surface by the release zone and extending to the ocean floor, the pycnocline, or a depth of 20 m, whichever is shallowest.

In the toxicological evaluation of the solid phase, benthic organisms are exposed for 10 days to the solid phase, and to a reference sediment collected just outside the disposal site. Data from the control (unpolluted) sediment are utilized to demonstrate the vitality of bioassay organisms.

A number of examples showing the practical application of these protocols to the evaluation of hazards from wastes dumped in the ocean or discharged to the ocean by outfall, are given in the paper by Rose and Ward (1981). Furthermore, a comprehensive discussion on the statistical analysis of bioassay data is presented, as well as a discussion of procedures for determining the presence or lack of unacceptable hazard.

A similar approach to the one reviewed above was used in a recent Swedish study of the environmental hazards of the current emissions to the Baltic sea from a large iron and steel plant (Walterson et al., 1983). Traditional field monitoring in the discharge zone was not considered appropriate for an assessment of the impact of the current discharges, because the total amounts of wastes emitted had been drastically reduced during the last five years. Predictive methods to obtain MECs and LPCs were therefore used in order to separate the environmental concentrations and effects elicited by the current discharges from the combined impact on the

coastal ecosystem from previously accumulated wastes and the current discharges. A three-dimensional hydrodynamic model, developed by T. Hellström, was used to obtain concentration-time profiles for a number of points in the receiving coastal waters ; bioassay results from tests with the combined effluents together with literature data on "maximum acceptable toxicant concentrations" (MATCs) for various constituents of the effluents were used to obtain LPCs (Fig. 5).

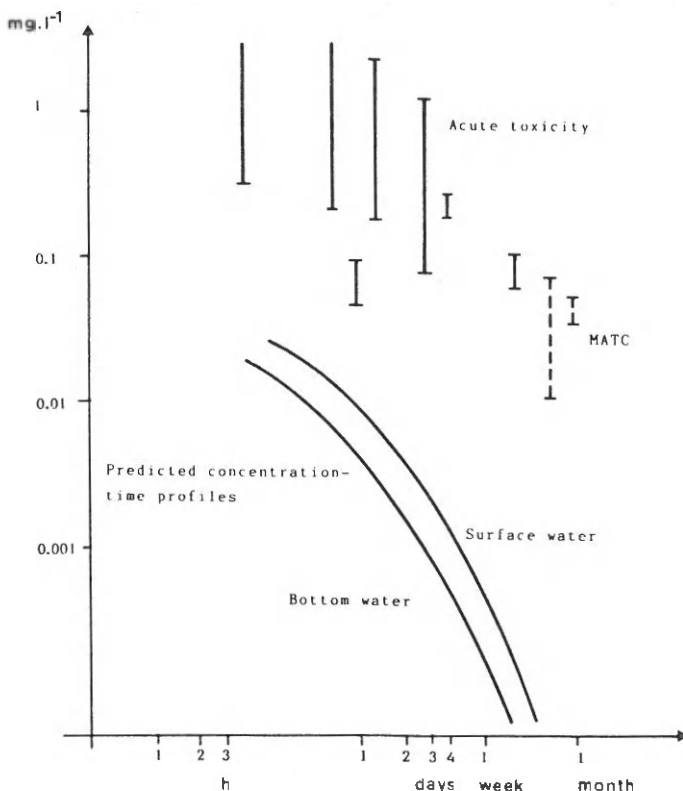


Fig. 5. Comparison of reported bioassay data for total free cyanide, a constituent of steel plant waste water, and potential environmental concentrations (concentration-time profiles) as estimated by a hydrodynamic model (from Walterson et al., 1983).

The aim of the study was to determine whether the pollution abatement programme at the steel plant, which was designed on a purely technological basis, was in fact appropriate when judged by effects-based criteria. If this was not the case, the study should then attempt to identify the component(s) whose concentrations should be reduced in order to comply with the requirement of "no adverse effects" on the coastal ecosystem.

### **BIODEGRADATION AND BIOTRANSFORMATION OF CHEMICALS IN THE MARINE ENVIRONMENT**

An important part of the exposure analysis, within both the initial and the more advanced hazard assessment, is the estimation of reaction rates which determine the persistence of organic chemicals. Plausible reactions include photolysis, chemical reactions such as hydrolysis, oxidation and reduction, together with processes of biodegradation and biotransformation. In the marine environment, it seems reasonable to assume that, at a certain distance and time from the point of discharge, the major degradation and transformation processes are biotic. The following discussion will, therefore, be restricted to transformation processes carried out by microorganisms, particularly bacteria.

### **ESTIMATION OF PERSISTENCE OF CHEMICALS**

In the present state of the art, it can reasonably be claimed that, for readily degraded compounds, data from simple tests for potential biodegradability carried out according to the OECD Test Guidelines (1981), are useful as input to an initial hazard assessment. For an advanced hazard assessment, however, current laboratory practices for routine testing of biotransformation are not sufficiently sophisticated to yield the data required. There is, for example, a real need for further development and validation of appropriate simulation tests (Gledhill *et al.*, 1980). The influence of important environmental parameters ( $P_{O_2}$ , pH, temperature, redox potential, etc.) on degradation rate constants has not so far been systematically examined either in the laboratory or in the field. Furthermore, rate constants are available only for a limited number of transformation pathways and for a restricted range of environmental conditions.

If rate constants which are system-independent can be obtained, it may be possible at least for certain types of transformations, to develop relationships between the rate constants and parameters dependent on molecular structure. Well-informed microbiologists disagree among themselves as to whether broad generalizations and structure-activity relationships can be developed for biodegradation of organic structures in the same way as chemists have done for certain abiotic chemical processes (Mill, 1981). The reason for disagreement arises from the fact that biodegradation, as opposed to biotransformation, involves a series of consecutive reactions of which only the first is determined by the structure of the parent substance. The Hansch approach (Hansch and Leo, 1979) has been valuable in situations where a series of structurally-related substances were evaluated (e.g. plant hormones, pharmaceuticals, etc.) so that this approach has certainly value for restricted problems in biotransformation. For example, Baughman *et al.* (1980) Wolfe *et al.* (1980) showed that good correlation existed between the rates of enzyme-mediated and hydroxide-catalysed hydrolysis for compounds of diverse chemical structures, notably for different groups of esters. Similar correlation that might be established between abiotic and biotic reaction rate constants for single transformations might provide valuable shortcuts in the generation of data for PED and PEC calculations.

One important conclusion from the above discussion is that we urgently need a much fuller understanding of the various mechanisms involved in the transformation of organic molecules by microbes. Besides a systematization of the great amount of information existing in the literature on various biotransformation mechanisms and their relative importance in the microbial world, there is a need for detailed studies of the degradative pathways of major environmental contaminants. Such studies should also include analysis of intermediary metabolites even when they have only transitory existence, since they may be toxic to other components of the ecosystem.

Using this approach, a highly schematic outline of patterns of biotransformation has been proposed by Neilson *et al.* (1983a), (Fig. 6). Such a simple classification might be helpful at the assessment stage since it draws attention to a number of sometimes neglected considerations : (1) that intermediate metabolites may be more resistant to degradation than the parent compound ; (2) that intermediates may be more toxic (Tyler and Finn, 1974) ; and (3) that intermediates may specifically inhibit further

degradation of the parent compound (Klecka and Gibson, 1981). The special case of the formation of lipophilic metabolites which are both persistent and toxic will be discussed later.

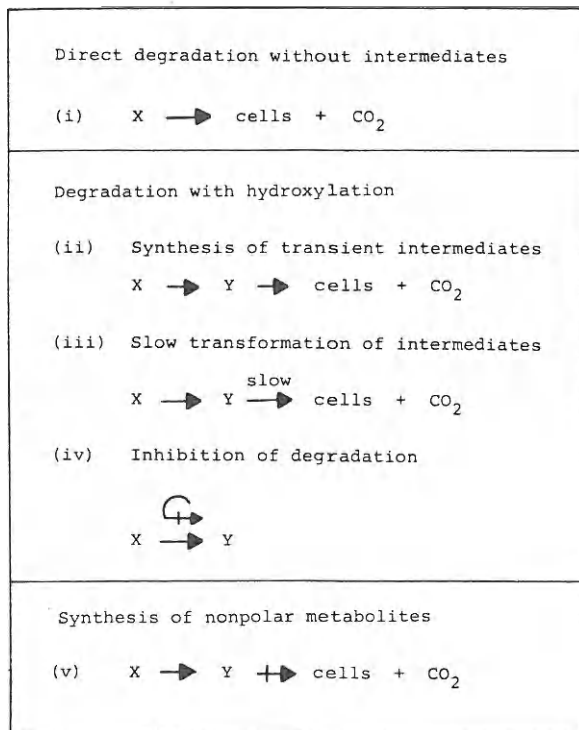


Fig. 6. Schematic outline of patterns of bio-degradation.

#### EXTRAPOLATION TO THE NATURAL ENVIRONMENT

In any given ecosystem, the types of degradative organisms present will depend on a number of environmental parameters ; of these, salinity may play an important role. While, for example, bacteria isolated from Baltic sea water and sediment samples do not have a salt requirement, bacteria originating from samples of the Swedish west coast have an obligate salt requirement and would be considered truly marine organisms (Neilson, 1980ab). The question of importance in the present context is the metabolic versatility of these groups of organisms. Compared with the number of



investigations of freshwater systems, surprisingly few seem to have been devoted to the marine environment. Specialized groups such as barophiles and psychrophiles (references in Tabor *et al.*, 1982) have attracted moderate attention, and *in situ* studies have examined the metabolism of readily degraded substrates such as acetate, glutamate, and glucose. Of greater significance in the present context, however, are extensive studies with pure strains of marine bacteria (Baumann and Baumann, 1981), which have shown that these organisms have a metabolic versatility in no way inferior to that of well-established freshwater taxa.

A number of other less widely appreciated factors, which affect the design of experiments on biodegradation should be taken into consideration :

1. In order to be able to utilize laboratory-derived degradation rates for extrapolation to the natural environment, it is necessary to study not only the dependence of rate constants on major environmental variables such as  $PO_2$ , pH, salinity, and temperature, but also to gain a better understanding of the patterns of induction of enzymes involved in the catabolism of substrates. The importance of this lies in the fact that, in natural ecosystems, there is virtually never only one potential source of carbon. The effect of cosubstrates on the induction pattern and functioning on enzymes therefore becomes of cardinal importance. Attention is drawn to some recent reviews (Dalton and Stirling, 1982 ; Slater and Bull, 1982).
2. The question of the capability of microorganisms to degrade and utilize organic substrates occurring at the very low concentrations prevailing in natural seawater is of great significance. It has been suggested that chemicals may have a greater degree of persistence in the environment than would have been predicted from the results of short-term laboratory experiments, employing high concentrations of the substrate. On the other hand, it seems plausible that, in natural situations, oligotrophic bacteria which are able to scavenge extremely low concentrations of nutrients are widely distributed (Kuznetsov *et al.*, 1979 ; Poindexter, 1981). The central issue is the efficiency with which low concentrations of substrate may be transported into the cells.

3. Catabolic plasmids have been shown to play an increasingly wider role in the degradation of unusual substances (references in Neilson et al., 1983a). Few systematic studies on the natural occurrence of degradative plasmids seem to have been carried out so that the frequency of their occurrence and their catabolic significance are largely unknown.
4. In spite of the wide spectrum of metabolic potential which has been revealed in anaerobic bacteria, surprisingly little interest has centered on the anaerobic metabolism of complex synthetic chemicals. Anaerobic degradative processes may play an important role in determining the persistence of compounds that tend to accumulate in sediments. Their role in the transformation of organic chemicals in sediment-water systems should therefore be given increased attention.

#### **BACTERIAL TRANSFORMATION OF XENOBIOTICS INTO METABOLITES HAVING MORE HAZARDOUS PROPERTIES THAN THE PARENT COMPOUNDS**

Possibly the most alarming aspect of biotransformation of chemicals in the marine environment is the synthesis of metabolites with properties presenting an increased hazard to the ecosystem compared with the original compound. In situations for which no reliable data on biodegradability exist, it is generally assumed that the worst case is represented by a substance which is totally resistant to biodegradation. This, however, may not be true if non-polar metabolites, or those with enhanced toxicity, are formed as a result of bacterial activity. This may, in fact, be a much more frequent situation than has hitherto been realized. Some illustrative examples are given below.

Bacterial methylation of inorganic mercury in marine sediments which results in the formation of methylated forms of mercury which are both highly toxic and display a high bioconcentration potential, is well-known even among the general public. Indeed, the first demonstration of its occurrence (Jensen and Jerneløv, 1969) has played a significant role in raising a general consciousness of the consequences of environmental pollution. Methylation of mercury and certain other elements is discussed in several reviews (e.g. Summers and Silver, 1978) and will not be discussed further here.

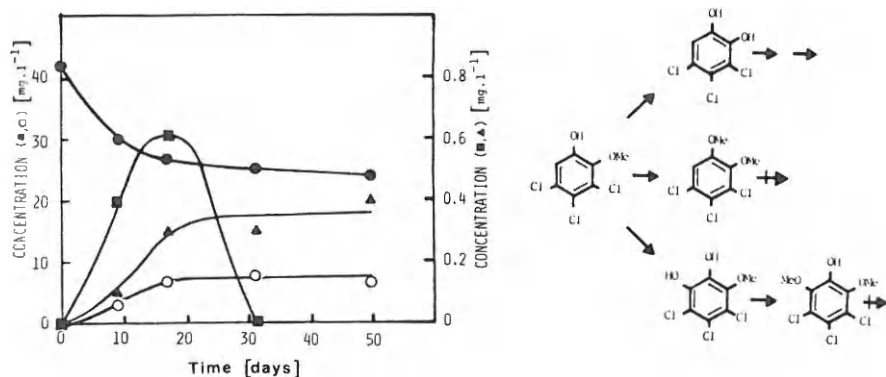


Fig. 7. Kinetics of biodegradation of 3,4,5-trichloroguaiacol (●) and simultaneous synthesis of 3,4,5-trichloroveratrole (▲), -syringol (○), and -catechol (■) (from Neilson *et al.*, 1983a).

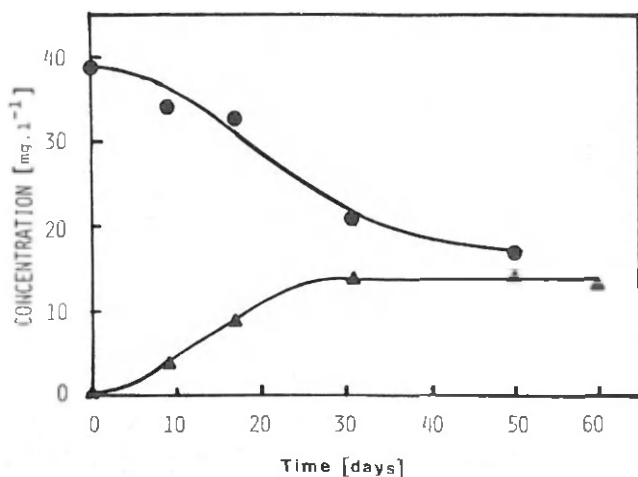


Fig. 8. Kinetics of biodegradation of 4,5,6-trichloroguaiacol (●) and bacterial synthesis of 3,4,5-trichloroveratrole (▲). (Neilson *et al.*, 1983a)

Recently, a comparable phenomenon with possibly even more disturbing consequences for the Baltic ecosystem was established, when Neilson *et al.* (1983b) demonstrated that bacteria isolated from marine sediments had the capability of methylating a range of chlorinated phenols and guaiacols with the formation of highly lipophilic and persistent chloroanisoles and chloroveratroles. Although in some cases, the chloroveratrole was only a minor component (Fig. 7), in others, this was the dominant reaction (Fig. 8). The yield of pentachloroanisole from pentachlorophenol was relatively low (Fig. 9); however, the reaction may be of considerable environmental significance since the metabolite has an extremely high bioconcentration potential (Neilson *et al.*, 1984) and has been shown to occur in field samples (Miyazaki *et al.*, 1981; Renberg *et al.*, 1983).

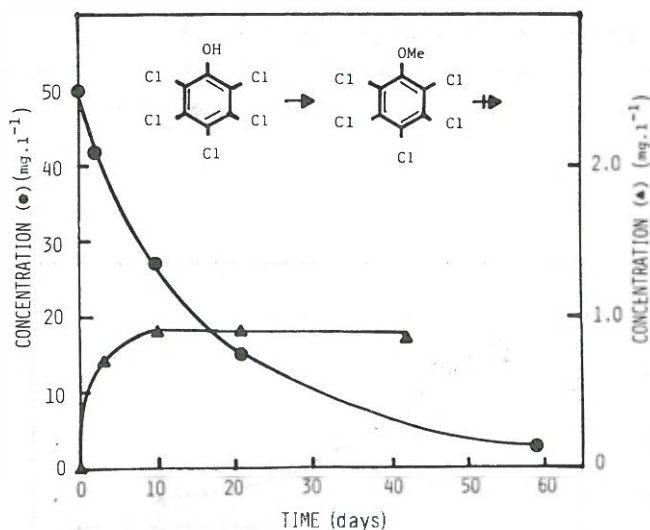


Fig. 9. Bacterial transformation of pentachlorophenol (●) into pentachloroanisole (▲) (from Neilson *et al.*, 1983a).

Chlorinated guaiacols are ubiquitous constituents of effluents from pulp-mill bleach plants using conventional chlorine bleaching, and the total release to the Baltic sea of such compounds is in the order of 100 metric tons per year. By comparison, discharge of high molecular weight chlorinated lignin to the same environment is at least three orders of magnitude higher.

It was, therefore, particularly disturbing when it was shown (Neilson *et al.*, 1983b) that this polymeric material, previously supposed to be biologically inert, also yielded significant quantities of tri- and tetrachloroveratrole after incubation with bacteria isolated from the Baltic sea (Fig. 10). The possibility that this biotransformation was a laboratory artefact and did not take place in the natural environment, was ruled out by the finding of elevated concentrations of chlorinated veratroles in liver fat and muscle tissue of various species of Baltic sea fish (Neilson *et al.*, 1984). Since no other source of the polychlorinated veratroles is known, it must be concluded that these substances have originated from bacterial methylation of the corresponding guaiacols.

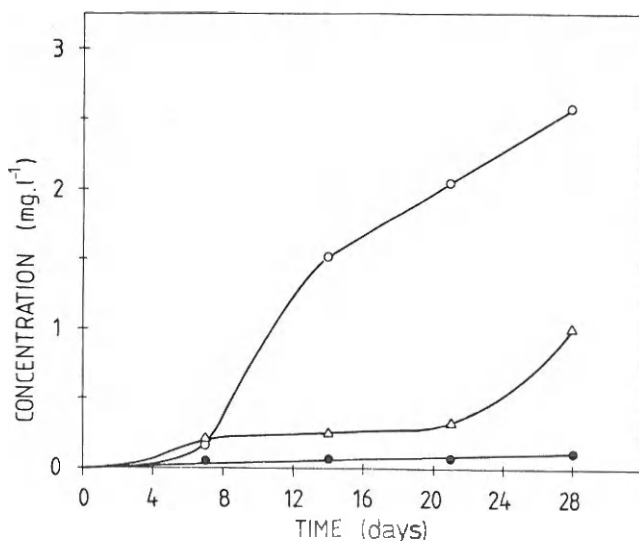


Fig. 10. Bacterial formation of 3,4,5,-trichloroveratrole (O) and tetrachloroveratrole (Δ) from high molecular weight chlorinated lignin from a pulp mill bleachery (from Neilson *et al.*, 1983a).

A series of toxicity tests has shown that the neutral O-methylated metabolites have a greater toxicity to fish larvae than the original substances (phenol and guaiacols) and that, in contrast to the phenolic compounds, these biotransformation products have the capability of inducing deformation in fish larvae (Neilson *et al.*, 1984). The last mentioned observation is particularly challenging, since elevated frequencies of

occurrence of spinal deformation in fish have been reported from areas in the Baltic sea in the neighbourhood of pulp mill discharges (Bengtsson, 1982). It has, however, not, been possible so far to induce spinal deformities in the test fish (Brachydanio rerio) during experiments in which the fish were exposed to raw bleach-plant effluents or to components known to occur in these effluents. Deformation of larvae was observed only after exposure to the neutral, methylated products of bacterial transformation of these components.

Another example of "undesired" biotransformation in the environment could be exemplified by the environmental fate of the flame retardant decabromobiphenylether. This compound has been classified as "completely safe for use as flame retardant" due to the failure to detect any toxic or carcinogenic effects during a two year study with rats (Mischutin, 1977). Furthermore, as determined from laboratory tests (Norris et al., 1973 ; Kociba et al., 1975), the bioaccumulation potential and membrane penetration of the molecule seems to be insignificant. Nonetheless, decabromobiphenylether has been detected in human hair samples from Arkansas, tetra- to decabromobiphenylethers have been found in sludge samples from the environment (DeCarlo, 1979), and tetra- to hexabromobiphenylethers have been identified in pike liver from an area exposed to discharges of decabromobiphenylether in Sweden (Andersson and Blomkvist, 1981).

The last observation suggests that the decabromo compound may, after release into the environment, undergo partial photodebromination (Norris et al., 1973) or reductive anaerobic biodegradation to tetra-, penta-, or hexabromo compounds. In either case, metabolites with greater potential for uptake and concentration in the biota may be produced. Anaerobic dehalogenation appears the more plausible in view of the wide spectrum of dehalogenation potential found in anaerobic bacteria (Jagnow et al., 1977 ; Chisa et al., 1982 ; Suflita et al., 1982). We may again be facing a situation, where the original compound, on the basis of its properties, is classified as environmentally safe, but where its transformation in the environment could yield degradation products which are both more readily available to the biota, and display a variety of toxic effects (cf. Koster et al., 1980).

The significance of the findings reported above is obvious : a traditional hazard assessment based solely on predictive testing of the effluents, or of the original compounds, would have given misleading

results. One possible way to circumvent this problem would be to place greater emphasis on studies of biotransformation than is usually done. As knowledge of biotransformation pathways and mechanisms accumulates, it would be successively easier to predict the possible occurrence of "exceptions" to the normal pathways.

Furthermore, long-term fate studies in microcosms designed to simulate the relevant sectors of the ecosystem may provide valuable information. This is also true for field monitoring of "unexpected metabolites", when we are dealing with chemicals or processes that have already been in use for some time.

### **BIOAVAILABILITY OF CHEMICALS IN THE MARINE ENVIRONMENT**

The problem of estimating the actual availability of various xenobiotics to aquatic organisms has already been addressed in the previous discussion. It is well established that a number of abiotic environmental factors determine the speciation and consequently the bioavailability of metals and other inorganic compounds in water (Bryan, 1980). Particularly important factors are the amount and type of suspended solids, redox potential, pH, and salinity ; all of these may affect the availability of potential ligands for the formation of metal complexes. For instance, when dealing with the assessment of the environmental hazard of complex industrial effluents containing various inorganic components, a thorough understanding of the precipitation and flocculation reactions taking place in the receiving body of water is of paramount importance, together with a knowledge of how these are influenced by temperature, salinity gradients, etc. (Lehtinen et al., 1984).

For an organic compound, its availability to aquatic biota is, on one hand determined by the size, lipophilicity, polarity and charge of the molecule, and on the other, by the physical and chemical properties of the membranes in the epithelia it must penetrate. Furthermore, the availability depends on the trophic level of the organism, on its developmental stage and on its feeding habit (filter feeding, detritus feeding, predation). A further complication arises from the ease with which aquatic organisms adapt to different environmental conditions (hardness, salinity, pH). This involves primarily changes in the properties of the membranes in the epithelia directly in contact with water. In fish, the effects of such

changes on the ion and water permeabilities are relatively well-known. In contrast, almost nothing is known about how these factors affect the permeability to various organic xenobiotics (Pärt, pers. commun.). Therefore, there is an obvious need to develop a simple membrane permeability test, which could be employed not only in the advanced hazard assessment, but also as a screening test in the initial assessment.

In several studies, attempts have been made to estimate the bio-availability of organic compounds to fish from the results of acute toxicity tests (Bruggeman et al., 1981 ; Küneman, 1981 ; Saarikoski and Viluksela, 1982). In these studies, excellent correlations have been obtained, for a range of compounds, between  $\log (1/LC50)$  and  $\log P_{ow}$  ( $P_{ow}$  = partitioning coefficient octanol/water). The toxicity of a substance, however, does not necessarily accurately reflect its availability due to possible reactions between the substance and a target molecule in the organism, or due to irregularities in the excretion patterns (Pärt, pers. commun.). The only way to obtain good estimates of the relative availabilities of chemicals to fish is to make direct measurements of the uptake rates in the gills. Methods for direct measurements of the transfer rates of chemicals through the gills have been presented by Payan and Matty (1975), Pärt and Svanberg (1981), McKim and Goeden (1982), and Pärt et al. (1983).

Such studies may be particularly important in order to delineate the consequences of alterations in the permeability of respiratory membranes resulting from previous accumulation of organochlorine compounds, on the bioavailability of other xenobiotics or on the regulation of serum osmolality. It is well-documented that several organochlorine compounds cause an alteration of membrane permeability (Vernberg et al., 1977 ; Neufeld and Pritchard, 1979).

#### USE OF MODEL ECOSYSTEMS IN ECOTOXICOLOGICAL RESEARCH

Not many words need to be used on this topic, since it is the subject of an excellent review presented during this symposium (Kuiper, 1984). For the sake of completeness, however, it should be emphasized that several of the difficulties discussed in previous sections of this review might be overcome reasonably well by experiments in well-designed model ecosystems.



Furthermore, such experiments constitute a valuable link between laboratory tests and the natural ecosystem, where the fate and effects of chemicals ultimately have to be evaluated.

If the purpose of the experiments is to provide adequate information to predict long-term effects of a chemical (or mixture) at low concentration, on the structure and function of natural ecosystems, and to predict the fate of the chemical in the system, highly realistic model ecosystems must be used. The system should primarily be used for identifying key mechanisms and weak links in the ecosystem, and as a whole to get a better understanding of the functioning and dynamics of communities.

Finally, attention is drawn to two recent papers : one from Lundgren (1983) which gives a comprehensive review of the possibilities of using model ecosystems as a tool in aquatic ecotoxicology, and one from Smies (1983) which presents a critical discussion of the same topic.

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