Aquatic Ecotoxicology: From the Ecosystem to the Cellular and Molecular Levels

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This review of aquatic ecotoxicology is presented in three parts. First, we discuss the fundamental concepts and stress the importance of its ecological basis and the complexity and diversity of the field of investigation, which result from actions and interactions between the physicochemical characteristics of the biotopes, the structural and functional properties of the living organisms, and the contamination modalities. Ecotoxicological mechanisms, regardless of the level of biological complexity, primarily depend on the bioavailability of the toxic products. Numerous processes control the chemical fate of contaminants in the water column and/or sediment compartments; accessibility to the biological barriers that separate the organisms from their surrounding medium depends directly on bioavailability. Second, we review the principal methodologies of the field, from in situ studies at the ecosystem/ecomplex level to bioassays or single-species tests. Third, we focus on mercury, selected as a reference contaminant, in order to illustrate the main ecotoxicological concepts, the complementarity between field and laboratory studies, and the indispensable multidisciplinarity of the approaches. — Environ Health Perspect 106(Suppl 1):21–35 (1997)

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Introduction

Ecotoxicology has been defined in the literature in many ways over the past two decades. Depending on their research objectives, in particular their fundamental or applied training, many authors restrict themselves to a more or less limited area of investigation. For example, according to Butler (1), “Ecotoxicology is concerned with the toxic effects of chemical and physical agents on living organisms, especially on populations and communities within defined ecosystems; it includes the transfer pathways of these agents and their interactions with the environment.”

The main objective of ecotoxicology is to study structural and functional disturbances induced in the short, medium, and long-term by contamination factors on ecological systems. These factors, including all physical, chemical, and sometimes biological agents, result essentially from the direct and indirect effects of anthropogenic activities.

We present the fundamental concepts of aquatic ecotoxicology and stress the importance of the ecological basis and the complexity and diversity of the field of investigation. We also discuss the principal study methods and the relationship between reductionism/representativity in the processes that occur in the natural environment. Additionally, we focus on mercury as a reference contaminant in order to illustrate the main ecotoxicological concepts and the complementarity between field and laboratory studies in aquatic ecotoxicology.

Fundamental Concepts in Aquatic Ecotoxicology

Ecological Basis

Ecotoxicology is based on the fundamental concepts of ecology, which aims to understand relationships between living organisms and their surrounding environment through a systemic analysis using functional units of varying size and level of complexity. These units range from the ecosphere to terrestrial or aquatic microsystems. Ecological systems function on the basis of actions and interactions among abiotic factors, which characterize the physicochemistry of the biotopes, and biotic factors, which relate to the biological component. This component may be examined at many levels, from the cellular and molecular basis to the biocenosis (all the species at the ecosystem level), via the intermediary levels of organization and population (all the individuals of a single species). Transition to higher biological levels is based on the rule of additivity; each higher level is the sum of the basic structures that make up the level below and also is based on the emergence of new properties in relation to both structure and function. Under natural conditions, the abiotic and biotic factors are extremely diversified and vary constantly both in time and in space: “The complexity and the individual history of each ecosystem give them unique properties which are not duplicated at another place and in many cases not even at the same place at different times” [Seitz (2)].

Lake systems, considered to be prototypes of ecosystems, correspond to more or less closed units; they are well defined physically and are characterized by a certain functional autonomy. However, they are open systems with permanent exchanges with the atmosphere and particularly with their surrounding terrestrial basins: “They operate as retention zones for inorganic and organic components transported from the watershed, the soil/vegetation complex representing the major compartment of transfer between these two systems” [Capblanq (3)]. As in any ecological system, the normal functioning and natural evolution of lake systems rely on the dependence and interdependence of the various communities of living organisms and their interactions with the abiotic environment. A global approach to the metabolic activity of living organisms enables us to distinguish two main components: First, the photosynthetic production of organic material by autotrophic organisms (phytoplankton, macrophytes, periphyton, bacteria); second, the consumption and degradation of this material by heterotrophs (animal species, microbes), with the concomitant recycling of nutrients, by mineralization (Figure 1). Production of organic matter and biodegradation are regulated by a complex set of
morphodynamic and climatic factors (depth, residence time of water, solar and wind energy, etc.), which impose varying levels of constraint on the chemical and physical properties of the water column (temperature, transparency, gas concentrations, dissolved salts, etc.). These properties continuously change in response to seasonal variations and to feedback effects from the metabolic activity of biota. In contrast to terrestrial systems, primary production in lakes is mainly carried out by microphytes, the phytoplankton being rapidly consumed by herbivorous species, thus inducing a fast renewal of the biomasses and a rapid recycling of the nutrients (4,5). Stratification cycles in the water column, caused by thermic energy exchanges between the atmosphere and the surface water layers, act in combination with the water temperature/density relationship. This combined action, together with the progressive shift from oligotrophy to eutrophy, thus makes the medium richer in nutrients—nitrogen and phosphorus—and increases primary production. These processes lead to a perturbation in the overall system function and to the natural fate of shallow lakes—their ultimate disappearance via eutrophication and filling-in processes (5,6). Paralleling these natural processes, anthropogenic actions contribute to the rate of acceleration at which these systems evolve, which leads in turn to dysfunctions of varying severity (e.g., dystrophy). Agricultural activity on watersheds further increases, through fertilizer spreading (nitrates, phosphates, etc.), the amount of inorganic nutrients or introduces products toxic for numerous species (herbicides, insecticides, fungicides) into the aquatic biotopes, thus increasing disequilibrium within the systems.

Contemporary limnology has become a much more experimental and mechanistic science. Many parameters are quantified, including flux between compartments, and the processes responsible for the evolution of the overall systems are investigated (5). Recent systemic studies based on multidisciplinary approaches have led to the formulation of dynamic and functional models (7); experiments ranging from the laboratory level to manipulation of the overall ecosystem provide a better approach to fundamental mechanisms (5). Nevertheless, our knowledge of global processes remains incomplete and often is compartmentalized. Ecological uncertainties make it very difficult, if not impossible, to select levels and analysis criteria sufficiently representative of the ecosystem studied in order to produce a specific diagnosis on its function and state of development.

**Complexity and Diversity of the Ecotoxicological Factors**

Basic knowledge of the ecological processes is essential in any ecotoxicological study. These ecological processes are the frame of reference to define the degree of disturbance to the system, its reversibility, and ultimately the qualitative and quantitative estimates of the medium- and long-term risks. The variety of ecosystems and the diversity of the natural and anthropogenic perturbations to those systems are the reasons for the lack of adequate baseline data for comparison between disturbed and undisturbed ecosystems, i.e., the classic problem of distinguishing signal from noise (8). The stability and recovery capacity of ecosystems depend on the characteristics of the stress (duration, frequency, intensity, novelty) and the history of the system. Ecosystems are not under steady-state conditions, even in the absence of human interference.

At the aquatic ecosystem level, the contamination of living organisms, whatever their level of biological complexity and their position in the food webs, closely depend on the bioavailability of the toxic products present in the biotopes (9). This key concept results from the numerous mechanisms controlling the chemical fate of contaminants in the water column and/or sediment compartments. As shown in Figure 2, metal bioavailability is strongly linked to the direct and indirect effects of the abiotic factors that determine chemical speciation reactions in the medium (complexation with the different inorganic and organic ligands in the dissolved and particulate phases) and to the exposure regime (11). Accessibility to the biological barriers, which separate the organisms from their surrounding medium, cell membranes and epithelial structures (gills, gut wall, integument), and control the ad- and absorption processes, directly depend on bioavailability. Moreover, abiotic factors act simultaneously on living organisms; depending on their variation ranges and on the adaptive capacities of the individuals, they can induce structural and functional

![Figure 1. Schematic representation of a lake ecosystem showing the main physicochemical (left) and biological (right) processes operating in the production and transformation of dissolved and suspended matter in lake water. Modified from Capblancq (3).](image-url)
disturbances of varying severity. For example, a large increase or decrease in water temperature can lead rapidly, given the heterothermal nature of the aquatic species, to a modification in the internal temperature. This modification, depending on the degree of thermal stress, can induce adaptive responses at organism level (e.g., respiratory and circulatory functions) and at the cellular and molecular levels (enzymatic activities, membrane fluidity, etc.). The degree of exchange between individuals and their surrounding environment can be modified considerably, as can capacities of uptake, sequestration, or biotransformation of toxic products at tissue and cell levels.

Thus, contaminant transfers between biotopes and living organisms, together with the toxicological effects, result from actions and interactions between the three fundamental poles of ecotoxicology: abiotic, contamination, and biotic factors (9). Interindividual and interspecies differences account for the high diversity of responses between ecosystems. These responses are based on direct biological effects, including the mortality of different life stages and disruption of reproductive cycles, etc., and indirect biological effects, essentially based on species-to-species interactions, which play a fundamental role in the structure and function of the systems (trophic relationships via predator and prey interactions, disruption of habitats, etc.). Ecotoxicological impacts at the ecosystem level can lead to new functions at the community level, such as primary and secondary production and nutrient cycling, with a marked delay between the steps from organism to whole ecosystem (Figure 3)(12).

**Principal Study Methods in Aquatic Ecotoxicology**

The complexity of the mechanisms involved in ecotoxicology and the breadth and diversity of the field of investigation lead to the crucial issue of research methodologies and their representativity in relation to the processes that occur in the natural environment.

**In Situ Studies**

For many research scientists, the only realistic approach in aquatic ecotoxicology consists of studies carried out *in situ* on the ecosystem or ecocomplex (a set of connected and interactive ecosystems), such as a lake and its catchment basin (13). Information from such studies brings together all the processes mentioned above, which are highly representative and therefore provide invaluable direct studies of nature. They also form a frame of reference, both for the distribution and fate of contaminants within the different abiotic and biotic compartments of the systems studied and for certain effects produced in the structure and the function of these systems. Nevertheless, such studies inevitably confront the complexity of the phenomena involved and the reductionism in space (using a smaller number of sampling stations) and in time (limiting sampling periods) imposed by the constraints of these investigation methods. Hence it is often very difficult to move beyond the descriptive stage, as the diversity of factors and their variability make it difficult to formulate interpretive hypotheses regarding the mechanisms. "Capturing key signals that describe central processes or consequences will continue to be a major challenge in the rapidly changing field of aquatic ecotoxicology" (14). Induced
changes in the specific structure of communities and in their structural properties can be assessed at global levels, but studies of this kind require major resources to set up sampling strategies and collect and interpret data (inventories of flora and fauna, quantification of biomasses and productivities, etc.). Given these constraints, many methodological compromises have been developed in order to limit the area of investigation covered in field studies and to facilitate their establishment. For example, the determination of fairly simple indices, such as abundance, biomass, species richness, saprobic index, biotic score, etc., or the selection of indicator species make it possible to estimate the quality of the environments, to establish interstation or interecosystem comparisons, or to follow medium- or long-term developments (12,15,16).

Listed below are biological indicators of ecological effects selected to optimize the detection of potential or actual changes in selected ecological end points (modified from Kelly and Harwell (8)).

**Purposes for indicators**
- **Intrinsic importance**: indicator is end point
  - economic species
- **Early warning indicator**: rapid indication of potential effect
  - use when end point is slow or delayed in response
  - minimal time lag in response to stress; rapid response rate
  - signal-to-noise ratio low; discrimination low
  - screening tool; accept false positives
- **Sensitive indicator**: reliability in predicting actual response
  - use when end point is relatively insensitive
  - stress specificity
  - signal-to-noise ratio high
  - minimize false positives
- **Process/functional indicator**: end point is process
  - monitoring other than biota, e.g., decomposition rates
  - complement structural indicators

**Criteria for selecting indicators**
- **Signal-to-noise ratio**
  - sensitivity to stress
  - intrinsic stochasticity
- **Rapid response**
  - early exposure, e.g., low trophic level
  - quick dynamics, e.g., short life span, short life cycle phase
- **Reliability/specificity of response**
- ease/economy of monitoring
  - field sampling
  - lab identification
  - preexisting database, e.g., fisheries catch data
  - easy process test, e.g., decomposition, chlorophyll
- relevance to end point
  - addresses "so what" question
- monitoring of feedback to regulation
  - adaptive management

Detection of biochemical responses to pollutants from selected species collected in the field—the biomarker concept—has received considerable attention during the last decade in the assessment of functional effects in contaminated ecosystems. Biomarkers represent an organism's attempt to compensate for or tolerate stressors in the environment (17). For example, mixed-function oxygenase (MFO) reactions in the whole organism or in organs (liver, gills) may indicate accumulation of organic compounds such as oils or some halogenated biphenyls (18). Increased levels of metallothioneins (low molecular weight, metal-binding proteins rich in cysteine) appear to be characteristic of exposure to several metals (Cd, Cu, Zn). Their induction has been proposed as tissular or cellular indicators in aquatic animals despite the large number of other agents able to induce their biosynthesis, such as hormones and second messengers, growth factors, vitamins, cytotoxic agents, stress-producing conditions, etc. (19,20). Bioprobes or biosensors with potential application at field level were recently developed with two essential monitoring applications: the detection of specific pollutants, using enzyme- or antibody-based devices; and the detection of unexpected changes in environmental chemistry, using broad-spectrum whole cell biosensors (21).

**Single-Species Approaches at the Laboratory Level**

Experimental studies using monospecific models at the laboratory level are at the other end of the methodology complexity scale (Figure 4). These studies fulfill two main objectives. The first is linked to mechanistic approaches that, because they use lower biological levels of organization and can monitor a large number of parameters, provide a better understanding of ecotoxicological mechanisms. This research generally complements toxicological studies, especially those based on more advanced investigation methods, such as sophisticated biophysical and biochemical techniques (22). It enables us to investigate, for example, fundamental links between chemical speciation reactions of heavy metals in the medium and biouptake at the biological barrier level or structural and functional toxic effects at cellular or molecular levels through the use of isolated organs (e.g., perfused gills) or culture cells (23–25). The second objective, from a more applied viewpoint, is to set up standardized toxicity tests based on one species, or a battery of single-species tests to predict the toxicity of pure chemicals or effluents on the laboratory level and under carefully controlled conditions (quality assurance/quality control requirements under standard operating procedures). Bioassays are widely used in aquatic ecotoxicology. Most regulatory studies, established to estimate the risk involved in marketing a new chemical product or in assessing the quality of an environment, are based on these methodologies (26).
aquatic species selected for such methodologies, apart from the universal cladoceran *Daphnia magna* and a few phytoplanktonic algae (*Chlorella, Scenedesmus*), differ from country to country but the biological models are similar in position within the freshwater communities: primary producers, zooplanktonic species, mollusks, and fish (27). Data from standardized tests are reliable, repeatable, and rapidly collected; they are generally used as screening tools to provide information on acute and chronic effects from a very large set of criteria: death, immobility, reproduction, growth, physiological functions (e.g., respiration and hematological parameters), behavior, genotoxicity, etc. Standardization necessitates rigorous experimental designs with a high degree of control of the abiotic and biotic factors. Such control can only be obtained by simplification—sometimes drastically—of the experimental conditions. This leads to major problems with the representativeness of results and extrapolation in relation to occurrences in the natural environment (Table 1). Accurate predictions of ecotoxic effects from bioassays are extremely difficult, if not impossible (28). For example, almost all nonspecific tests in aquatic ecotoxicology are carried out in artificial environments (synthetic river water) in order to avoid variations in the main physicochemical factors and in their direct or indirect effects on the bioavailability of the contaminants studied and on the organisms. Similarly, only the direct contamination route via the water column is considered in the majority of freshwater bioassays, whereas for many chemical products, trophic transfers represent the chief contamination route. However, despite uncertainties in prediction or risk estimation, these methods are essential tools and they provide indispensable information; it is important to be aware of the limitations of these data and to apply them with care whenever it is necessary to extrapolate to a complex, natural ecosystem (Figure 5) (29–31). According to Chapman (32) "the field does not necessarily validate the laboratory. Each provides a different viewpoint. Different views, in the form of tools such as toxicity tests and field studies, together provide the best overall perspective."

**Multispecies Models**

Major research programs have been developed to devise ecotoxicological models that occupy an intermediate place between field studies and the nonspecific approach (Figure 4). These models are intended to facilitate predictions of community level responses and to reduce uncertainties when extrapolating from indoor bioassays to field conditions.

There are two main types of multispecies models. The first type is based on experimental studies in outdoor conditions in order to emulate the structural and functional properties of ecosystems while still retaining some of the advantages of the experimental approach. Some of these advantages include monitoring certain abiotic or biotic factors, possibly setting up experimental controls and replicates, and testing the effects of scale. These methodologies "attempt to bridge the gap between laboratory experiments and field observations" (33). The design of field test studies in aquatic ecotoxicology depends on their objectives. The objectives may be to develop and validate predictive models for chemical fate and/or effects, to evaluate

| Source of uncertainty | Source of insensitivity |
|-----------------------|-------------------------|
| Choice of species      | Unrepresented           |
|                       | Highly sensitive species|
|                       | Ecological keystone species|
|                       | Long-lived upper trophic levels|
| Exposure time          | Underestimated           |
|                       | Effects of lifelong exposure|
| Exposure route         | Rarely considered        |
|                       | Additive pathways        |
| Multigenerational life cycle | Unrepresented              |
|                       | Progressive accumulation of toxic effects through generations|
| Higher order secondary effects | Changed biological interactions|
|                       | Effects on community structure when keystone species are lost|
| Interaction with natural disturbances | Cost of metal stress in tolerance to natural perturbation: individuals and populations|

From Luoma (28), with permission.

![Figure 5](https://example.com/f5.png)
environmental quality standards derived from laboratory toxicity data through extrapolation, to study resilience of ecosystems in terms of time required for restoration after physicochemical disturbance, or to obtain data required for regulatory purposes in order to assess fate and/or effects in natural ecosystems (34). Many outdoor ecotoxicological models have been developed over the past few decades, with marked differences according to their scale (from liters to millions of liters) (Figure 6), their biological component complexity (from two selected species to complex natural assemblages), their study environment (ponds, lakes, streams), their dependence on natural reference systems, and also their operational time scales (from hours to years). The terms mesocosm, outdoor microcosm, pond, enclosure, and artificial stream have been used to describe such test systems (36,37); most of these studies have been carried out on pesticides (insecticides, herbicides) (38). Since the early 1980s, freshwater field tests have been required by several countries (including the United States and the United Kingdom) to support the registration of some pesticides; these tests are requested only if laboratory data together with environmental exposure estimations suggested that aquatic ecosystems might be at an unacceptable level of risk. In 1987, the U.S. Environmental Protection Agency (U.S. EPA) published a technical guidance document for the conduct of aquatic mesocosm tests (39). Several international workshops were organized in the United States and Europe, including Society of Environmental Toxicology and Chemistry-RESOLVE in Wintergreen, Virginia, in 1991, and the European Workshop on Freshwater Field Tests (EWOFFT) in Potsdam, Germany, in 1992, which resulted in the publication of proposals for the conduct of such field tests (40,41). A growing number of reports indicate that outdoor mesocosms and artificial streams are powerful tools for developing predictions of the indirect effects of toxicants or mixtures of toxicants on whole-community assemblages. Nevertheless, the U.S. EPA recently decided to discontinue mesocosm studies on pesticide risk assessment on the basis that “they do not provide substantial information for making risk decisions beyond that already revealed by lower tiered studies” (42). In the European Community, no real strategy is currently defined to integrate this type of methodology in the regulations governing authorizations to market new chemical products.

The second type of multispecies model is laboratory-based and aims to produce more complex, and therefore more representative, ecotoxicological models than single species tests, in relation both to biotic factors (multispecies systems) and abiotic factors (indoor microcosms with a mixed biotope: for example, natural sediment and water column). Models vary according to the purpose of the experiments and the level of control and artificiality of the systems. The first well-defined experimental system was the Metcalf model for the evaluation of pesticide biodegradability and ecological biomagnification (43). More recently, the standardized aquatic microcosm set up by Taub (44) made it possible to demonstrate significant effects of toxicants on ecological interactions within and between two trophic levels—alternative food chains were based on 10 species of primary producers and 5 species of grazers (Figure 7).

As shown in Figure 4, the approaches to aquatic ecotoxicology, varying from the field to fairly simple laboratory tests, are complemented by theoretical approaches based on mathematical models. Many models are currently available, with differences according to their objectives and to the complexity and diversity of the ecotoxicological criteria. For example, quantitative structure–activity relationships (QSARs), essentially based on the physicochemical properties of the contaminants

![Figure 6](image-url)  
**Figure 6.** Size distribution of lentic outdoor freshwater micro-/mesocosms in which structural responses under pesticide treatments have been studied (n = 85). Modified from Brock and Budde (35).

![Figure 7](image-url)  
**Figure 7.** Principal components of a standardized aquatic indoor microcosm, based on an artificial medium with 10 species of primary producers and 5 species of grazers. From Taub (44), with permission.
(hydrophilic/hydrophobic properties, $K_{ow}$, Henry's constant, molar volume, electronic parameters, etc.), are frequently used for the prediction of the fate of chemicals in the environment, their bioaccumulation capacities, and sometimes their toxicological effects (45). At the other end of the complexity scale, deterministic simulation models incorporate the major processes of transport and chemical fate of contaminants in aquatic systems and enable quantification of their bioaccumulation and trophic transfers. Certain models, such as the mercury cycling model (46), take into account the influence of key limnological parameters on the biogeochemical cycles at ecosystem level.

**Mercury Contamination of Freshwater Systems**

We have selected mercury as an example to illustrate the fundamental links and complementarity between field and indoor studies in freshwater ecotoxicology. Mercury is a natural component of the ecosphere with a low relative abundance (ranking 62nd), although for geochemical reasons it tends to be concentrated in vast mercuric belts (47). Mercury occurs in various physical and chemical forms in the environment: minerals (cinnabar, HgS), metallic Hg\(^0\), inorganic forms (mercuric, Hg\(^+\), and mercuric, Hg\(^2\)), and organomercurials (methylmercury, MeHg; dimethylmercury, Me\(_2\)Hg, etc.). These different forms and their associations with complex ligands regulate transport pathways, residence times within the different compartments of the biogeochemical cycle (atmosphere, geosphere, hydrosphere, biosphere), bioaccumulation and trophic transfers along the food webs, and toxic effects on living organisms. Humans use large amounts of mercury—world production is estimated at 8,000 to 10,000 tons/year (48). Mercury's different chemical forms are used in diverse processes (catalysis, amalgamation, electrical/battery, chloralkali, instruments, laboratory, etc.), though some processes no longer use mercury because of the risk associated with its use (fungicides in agriculture, for example). Numerous secondary sources contribute a large amount of mercury into the environment: coal combustion, waste incineration, metal smelters, etc. (49). Current estimates for anthropogenic interference with the natural cycle range from approximately 50 to 75% of total annual Hg emissions to the atmosphere; recent modeling suggests that the present atmospheric Hg burden has increased by a factor of 3 during the last 100 years, with a current rate of increase of about 0.6% year\(^{-1}\) (about 0.01 mg m\(^{-2}\) year\(^{-1}\)) (50, 51).

As mercury has been used since ancient times (Hg was the seventh metal of antiquity and has been known and used for more than 3500 years), cases of poisoning also have a long history: the first recorded case was a mercury miner in the 15th century (52). Ecotoxicologically, the poisoning outbreak in Japan in 1953 to 1960 (Minamata disease) gave rise to an international awareness of the risks linked with the emission of Hg into aquatic systems and of the complex mechanisms that led to the poisoning of huge numbers of the population (899 cases were officially recognized, with 143 deaths and numerous congenital and infant cases) (53). A factory producing acetaldehyde and vinyl chloride had used mercury as a catalyst and considerable amounts of metal had been directly discharged into the enclosed marine bay of Minamata. After lengthy epidemiological studies, the cause of the disease was identified as high metal concentrations in fish and shellfish, which were the staple diet for the families of fishermen living on the banks of Minamata Bay (54). Complex new phenomena were discovered on the global ecosystem scale, including the concept of biomagnification, whereby Hg concentrations increase considerably in successive levels of the marine food webs, from primary producers to terminal carnivorous consumers (fish, marine mammals).

Since the beginning of the 1980s, ecotoxicological problems linked to the contamination of aquatic systems by mercury have entered a new phase. High mercury levels were found in carnivorous fish living in thousands of forest lakes in the Northern Hemisphere—in areas remote from human activity—and in the hydroelectric reservoirs constructed in northern Quebec, Canada, close to James Bay. These field studies demonstrate a new pattern of Hg pollution, with the food webs becoming a vital element linking the metal chemical fate in the environment and human health risks. For example, boreal forest lakes in Sweden are characterized by very low human population densities in their catchment areas and most of the lakes have never been affected by Hg discharge into their watersheds. In more than half of these 83,000 lakes, it was estimated that total mercury concentrations in 1-kg pikes (Esox lucius) exceeded the health advisory limit for safe human consumption (1 mg Hg/kg, fresh weight); on the other hand, total mercury levels in the water column were lower than 10 ng/liter (48).

**Recent Field Studies at Ecosystem Level**

Results from the Mercury in Temperate Lakes project (MTL), connected with the National Acidic Precipitation Assessment project (NAPAP) in north-central Wisconsin in the United States, clearly demonstrate the progress made in field studies in aquatic ecotoxicology. This progress is mainly linked to the multidisciplinary nature of the research, which is able to benefit from all the new forms of technology established by the various branches of research, such as limnology, analytical chemistry, geochemistry, and microbiology, as well as new analytical procedures. By using ultraclean techniques during the sampling steps and determining total Hg by cold vapor atomic fluorescence spectrometry (CVAF) after a dual preconcentration step (Hg amalgamation on gold traps), it is now possible to reach detection limits (DL) of 0.02 ng Hg/liter or 10\(^{-13}\) mol/liter (55). These procedures now challenge virtually all measurements carried out on earlier water samples (precipitation, surface and groundwater, porewater in sediments), revealing overestimations as high as, or greater than, a factor of 500 (56). Moreover, using new specific analytical procedures based on the combination of an ethylation phase (NaBEt\(_4\)), separation of the different volatile Hg chemical forms by isothermal gas chromatography, electrothermal atomization, and Hg\(^{2+}\) determination by CVAF, it is now possible to simultaneously determine the principal chemical forms of the metal (Hg\(^0\), Hg\(_{2+}\), MeHg, Me\(_2\)Hg) in the different matrices (water, sediment, biota), with a DL close to the picogram level (57).

Over a 3-year period, total Hg and MeHg mass balance were analyzed at the ecosystem level in Little Rock Lake, Wisconsin (58). Figure 8 shows mercury distribution in the principal abiotic and biotic compartments and metal fluxes between atmosphere, water column, and sediments. The results indicate that atmospheric deposition was the major source of mercury in the lake, but MeHg accounted for only 1% of the input. About half of the total Hg burden estimated in the water column compartment was in the biota; the MeHg pool in the fish corresponded to 73% of the total amount in the water column, including seston (mostly phytoplankton).
and zooplankton. Most of the net atmospheric Hg inputs into the lake (1 g Hg/year) accumulated in sediments, which were the largest Hg reservoir in the Little Rock Lake system. The percentage of MeHg versus total Hg in the water column was close to 10%. Mercury release as Hg⁰ from the lake into the atmosphere was about 5% of the deposited input; this average value was lower than in results obtained from the other seven Wisconsin lakes studied under the MTL project (10–50%). The mass balance showed that almost all MeHg formed within the lake system; endogenous methylation reactions were a key process in metal bioaccumulation at the food web level.

MeHg biomagnification occurred in the lake—the organic compound was bioconcentrated by a factor of 3 million times in the whole fish (bioconcentration factor (BCF) = [Hg] organism/[Hg] water, estimated in 1-year-old yellow perch, *Perca flavescens*); BCF values increased by about 0.5 log units per trophic level (59). Data obtained on fish showed that almost all accumulated Hg was in the monomethylated form. Average percentages of MeHg in the phytoplankton and zooplankton were close to 15 and 30%, respectively (60). Comparative analysis of Hg concentrations in fish from several lakes shows marked variability between individuals of the same species as well as between different species. For example, the lowest and highest values within individuals of the same species and age can differ by a factor of 10 or more (48).

Field studies have shown that not only age but also size and weight may influence Hg bioaccumulation in fish either directly or indirectly. The physicochemical characteristics of the biotopes also play a fundamental role. Numerous data from lake surveys in Scandinavia, the United States, and Canada indicate that Hg concentrations in fish are negatively correlated to lake pH or alkalinity (61,62); moreover, Hg concentrations in fish from low alkalinity lakes without anthropogenic metal sources often exceed those from Hg-contaminated waters with high alkalinites. When a lake is acidified, complex changes occur in the turnover of substances and in the biotic structure of the ecosystem (48,63). High levels of Hg in biota from acidic forest lakes in Sweden may be the result of low productivity rather than a direct influence of pH on the metal biogeochemical cycle (48,64). After the first 2-year stage of experimental acidification of the treatment basin (pH 5.6) of Little Rock Lake, total Hg determinations in yellow perch differed from the unacidified reference basin (pH 6.1) by about 20%. These results were linked to differences in within-basin processes that influenced the bioavailability of the metal—perhaps a greater methylation of Hg at the lower pH (59). Recent data from acidified lakes in Ontario, Canada, show that Hg concentrations in fish are positively correlated with dissolved organic carbon concentrations (DOC) in the water (65). Acidification may also modify the abiotic and biotic processes controlling transformations of the different chemical forms of the metal (66). An increased reduction of HgII in high-pH lake waters may be an important mechanism for decreasing the supply of substrate for in-lake methylation (67). However, increases in the rates of methylation relative to demethylation at low pH may also contribute to the increased MeHg concentration in fish from acidified lakes (68). Interesting results were recently obtained from planktonic samples collected in the
two basins of the Little Rock Lake (60): Comparison of Hg burdens in individual zooplankton from the acidified and reference basins shows that changes in total Hg concentrations as a result of acidification were small and not statistically significant. However, MeHg constitutes more than 90% of the total Hg concentrations in cladocerans from the acidified basin, compared to less than 30% in the reference basin.

It is not possible to determine the respective proportions of the two contamination routes through field studies: the direct route (DR), via Hg in the water, and the trophic route (TR), via the ingestion of contaminated prey. This is because of their superposition and the highly complex interspecies relationships involved. Nevertheless, empirical evidence indicates that diet is the primary uptake route of MeHg by fish in natural conditions. According to Spry and Wiener (61), the TR appears to represent more than 90% of Hg bioaccumulated in carnivorous fish. Several field studies show that changes in the diet of carnivorous fish, because of the depletion of prey or the increase in predator size, have a marked effect on metal bioaccumulation, even when the contamination pressure from the DR remains identical (48). Moreover, given the low concentrations of total Hg and MeHg in the water column in lakes or hydroelectric reservoirs, it is difficult to account for the levels of Hg bioaccumulation measured in fish after several months of direct exposure.

Field studies also have revealed that sediments play an important role in the biogeochemistry of mercury within aquatic systems (69). The sediments represent a major storage compartment: about 99% of the Hg in a typical forest lake is present in this compartment (70). The sediments are also a favorable site for transformations of Hg chemical forms, notably methylation reactions. Isotopic methods, wet chemistry, and mass–balance approach have recently confirmed the important role played by sediments or, more exactly, by layers within a few centimeters of the sediment surface in MeHg production. Sulfate-reducing bacteria are described as important Hg methylators in anoxic sediments; Hg methylation may also take place in sediments with a high decomposition of organic matter via extracellular enzymes (71,72). Among the benthic species, insect larvae may have an important influence on Hg release from the sediment compartments (73) and studies on hydroelectric reservoirs and lakes in northern Quebec, Canada, show that these species constitute up to 90% of the diet of many fish.

A recent review by Rudd (72) of the sources of MeHg in freshwater systems highlights some marked differences between lakes. For example, atmospheric MeHg deposition in southern Sweden are ten times higher than the average rates estimated in northwestern Ontario in the Experimental Lakes Area Reservoir project; similarly, terrestrial catchment areas that contain wetlands have been identified as important sources of MeHg. It has long been recognized that the in-lake production of MeHg was essentially based on methylating bacteria in the superficial sediment layers. Today, many studies have shown that internal MeHg production also occurs in the water column via biotic and abiotic methylation (74,75). More recently, flooded terrestrial surfaces, as in the hydroelectric reservoirs in northern Quebec, have been shown to be important sources of MeHg (76). Contradictory study results have been published on fish and Hg methylation in the gut (72). Given the predominance of the methylated form of Hg in terminal consumers in lake systems, it is important to define the mechanisms responsible for methylation in the natural environment, to localize them in the different compartments, and to quantify MeHg production when confronted with the antagonistic reactions of demethylation. Our present knowledge of these fundamental processes is still very limited: “The reason that our understanding of internal MeHg production is so vague is that there are no methods for the measurement of natural rates of Hg methylation or demethylation in the field” (72). Isotopic methods based on 203HgII and 14CH3HgX do not give quantitative rate information; they are useful to determine methylation sites in the water column or sediment compartments or to analyze factors influencing rates of methylation or demethylation (72).

Although considerable progress has been made in the quantification of Hg compound distribution and flux between the different abiotic and biotic compartments in freshwater systems, a very limited amount of data has been published on the toxic effects induced under such exposure conditions in living organisms. According to Wiener and Spry (77), neurotoxicity seems to be the most probable chronic response of wild adult fish to dietary MeHg. Nevertheless, the primary effects, if any, under these exposure conditions would be reduced reproductive success in fish populations as a result of the toxicity of maternally derived MeHg to the embryonic and larval stages.

Even in light of the mass of data collected from field studies, many questions remain about the mechanisms involved and the direct and indirect roles of the various ecotoxicological factors. It is at this level that experimental approaches can contribute arguments to assist interpretation. We have selected examples from laboratory studies into bioaccumulation mechanisms from the trophic chain level to cellular and molecular levels in order to illustrate how the two approaches can be complementary.

**Complementarity of Experimental Approaches: Analysis of Inorganic Mercury and Methylmercury Bioaccumulation and Trophic Transfer Mechanisms**

A detailed comparative approach to inorganic Hg and MeHg transfers was set up on an experimental four-link trophic chain (Figure 9). Phytoplanktonic algae were the primary producers and carnivorous fish the third-level consumers. Results for the primary producer *Chlorella vulgaris* showed very rapid and high bioaccumulation capacities for the two Hg chemical forms, leading to similar BCF after 24-hr exposure (Figure 9A) (78). For each consumer species, the two contamination routes were analyzed: direct uptake from the water and trophic uptake from living prey previously exposed to the two Hg compounds. Trophic transfers between contaminated algae and the zooplanktonic herbivorous species (*Daphnia magna*) revealed marked differences between the two compounds: at 18°C, the estimated transfer rates were close to 6% for inorganic Hg and 58% for the methylated form (Figure 9B). Optimized transfers between algae and daphnia, without over-abundant food supply, were greater than 90% for MeHg (79). Recent data obtained by Mason et al. (80) were in agreement with these values: transfer rates for HgII and MeHg between diatoms (*Thalassiosira weissflogii*) and calanoid copepods (*Acartia tonsa, Temora longicornis*) were 15 and 62%, respectively. Comparative studies of MeHg bioaccumulation in the third level of the experimental trophic chain, the mosquito fish (*Gambusia affinis*), from the DR and the global route (direct and trophic), showed marked differences: the estimated average BCFs after 30 days of exposure were 2500 and 27,000, respectively (Figure 9C) (81). A similar experimental approach with rainbow trout alevins (*Onchorhynchus
mykiss), fed for 10 days with daphnia previously contaminated with HgII or MeHg, showed average transfer rates of 41 and 92%, respectively (82). The upper link of the trophic chain was the adult rainbow trout. Fish were fed living prey (3 alevins/day per trout) for 30 days (Figure 9D). The estimated trophic transfer rates were 54% for inorganic Hg and 95% for MeHg; these were in agreement with assimilation efficiencies for dietary uptake in several fish species (77).

Results from trophic transfers at the experimental food chain level, from primary producer to terminal consumer, show a
marked difference overall between the two chemical forms of the metal: 1.3% for HgII and 51 or 87% for MeHg, depending on the transfer rate retained between the first two levels (82–84). These results provide a basis for the interpretation of field study data, especially the predominance of the methylated form of the metal in the terminal consumers and the empirical evidence that diet is the primary route of MeHg uptake by fish (48, 61, 64, 77).

Through a detailed experimental approach to Hg bioaccumulation in fish based on a separate analysis of the four components of this global process—direct contamination, trophic contamination, and decontamination after direct or trophic contamination—in the whole organism and in the main organs (liver, brain, gills, skeletal muscle, posterior intestine, kidneys, spleen, blood), we were able to contribute further to the interpretive analysis of results from in situ studies. After 30 days of direct exposure via the surrounding water, with precise control of the contamination pressure for the two Hg chemical forms, the ratio between the average concentrations measured in the whole organisms was close to 6.0, in favor of methylmercury. For all the organs, higher values were obtained after contamination with MeHg (Figure 10) (83, 85). After 250 days of depuration, decontamination rates at the whole-fish level were 28 and 37% for inorganic Hg and MeHg, respectively. During this period, the average weight of the organisms increased by a factor of 4, leading to marked growth dilution effects on the concentration criteria. Several organs, such as the gills and liver, showed a rapid and marked decrease in their Hg burdens. Other organs or tissues, on the other hand, such as the skeletal muscle after exposure to MeHg and the kidneys after exposure to HgII, showed a large increase in their Hg burdens during the decontamination phase. These fundamental processes were the result of metal transfers between donor and receiver compartments (86). The most important conclusion was in relation to the skeletal muscle: although the fish were no longer subjected to Hg contamination, this tissue received large amounts of Hg during the first 20 days of the decontamination phase, leading to a 200% increase in the average metal burden, with no further significant decrease up to the end of the 250-day period. These processes are important as the skeletal muscle represents about 55% of the fresh weight of the fish and constitutes virtually all of the flesh eaten by humans (83).

Trophic contamination of the trout was characterized by a marked difference in Hg concentrations in the whole fish after 30-day exposure: the average ratio between the two Hg chemical forms was about 3, in favor of MeHg (87). Metal distribution in the eight organs revealed considerable divergence (Figure 10): For inorganic Hg, there were higher concentrations in the posterior intestine than in the other organs; for MeHg, the distribution was more homogeneous (87). During the depuration phase after trophic contamination, inorganic Hg accumulated in the intestine disappeared rapidly and almost totally during the first 2 weeks, with no significant increase of the Hg burdens in the other organs, notably the blood and kidney; inorganic Hg was eliminated through the feces (82).

**Figure 10.** Bioaccumulation of inorganic mercury and methylmercury in the rainbow trout—whole fish and organ—after 30 days of exposure via the direct route (DR) (Hg added to the water) or the trophic route (TR) (ingestion of living contaminated prey). Evolution of Hg burdens in the gills (A) and the skeletal muscle (B) during the 250-day decontamination phase, after contamination by the DR. (C) Evolution of Hg burdens in the posterior intestine during the 60-day decontamination phase, after contamination by the trophic route. From Boudou et al. (23). Abbreviations: fw, fresh water; Bl, blood; Br, brain; G, gills; Int, posterior intestine; K, kidneys; L, liver; Mu, skeletal muscle; Sp, spleen; WF, whole fish.
These experimental studies clearly revealed the fundamental role played by the biological barriers involved in Hg uptake by fish. Particularly significant was the high specificity of the intestine wall to MeHg absorption in contrast to inorganic Hg adsorption at the microvilli interface on the apical part of the enterocytes with a very low uptake rate. Interactions between Hg chemical forms and species and biological barriers are of major interest in understanding bioaccumulation mechanisms and combined toxicological effects (23). Whatever the level of complexity of living organisms, these barriers control the uptake and accessibility of contaminants to the internal compartments: the cytosol at the cell level, the hemolymph or blood at the organism level. Although composed of a complex arrangement of epithelial cells, each barrier is in fact based on the same fundamental structure: the plasma membrane and its phospholipidic bilayer, including proteins (88). Basic Hg uptake by the cells is by passive diffusion through the membranes; facilitated transport mechanisms and uptake by carrier systems have also been described for a limited number of cell types, such as Hg-resistant bacteria or neuronal cells (75,89). A high MeHg bioaccumulation capacity is frequently attributed to the lipophilic character of this organic compound. Indeed, the predominance of MeHg burdens in the skeletal muscle of fish rather than in the fatty tissue clearly shows that bioaccumulation is not governed solely by the liposolubility of this chemical form. In fact, neutral chemical species of inorganic Hg have octanol/water partition coefficients \( K_{ow} \) similar to, indeed higher than, those of MeHg: HgCl\(_2\), 3.3; HgOHCl, 1.2; Hg(OH)\(_2\), 0.05; CH\(_3\)HgCl, 1.7; CH\(_3\)HgOH, 0.07 (80,90,91).

Biophysical studies based on lipidic model membranes (biomolecular lipid membranes, liposomes) and several complementary techniques (nuclear magnetic resonance, fluorescence polarization, \(^{203}\)Hg flux) have demonstrated the fundamental role of HgII chemical speciation reactions in the external medium in the metal’s accessibility to the cell interfaces by binding to proteins and phospholipid polar heads and in transport by diffusion through the hydrophobic core of the membranes (Figure 11) (90,92,93). For example, the neutral HgCl\(_2\) species binds specifically to the primary amine groups on serine and ethanolamine polar heads, inducing a strong rigidification of the phospholipidic bilayers because of the formation of bridges by Hg atoms between the adjacent polar heads. These effects were not significantly influenced by the electrical charges at the membrane interface; they differed greatly from results obtained with other divalent cations—Ca\(^{2+}\), Cd\(^{2+}\), Al\(^{3+}\)—and also with MeHg. Interactions of the organic compound with the phospholipidic bilayers were essentially based on the CH\(_3\)Hg\(^+\) species (94).

Recent experimental studies on mercury bioaccumulation in unicellular marine diatoms showed that passive uptake of uncharged chloride chemical species—HgCl\(_2\) and CH\(_3\)HgCl—was the principal bioaccumulation route of both inorganic and organic Hg. Analysis of mercury distribution at the subcellular level showed that inorganic Hg was essentially sequestered within the membranes (91%) because of its rate of reaction with thiol ligands, whereas there was a preferential accumulation of the methylated form in the cytoplasm. Differences in Hg assimilation by the herbivorous copepods described above appeared to be essentially caused by these specificities in metal distribution at the algal cell level: several zooplanktonic species digest the dissolved cytoplasmic content of the algae but simply eliminate the membrane material (80).

Experimental studies into the toxic effects of MeHg on the nervous system have shown that this compound can cross the cell membranes of the blood–brain barrier by forming a complex that mimics the structure of amino acids. Thus, MeHg reacts with the thiol-containing cysteine in plasma; this complex is also formed in the hydrolysis of glutathione. The molecular structure of the MeHg-cysteine complex is very similar to that of the large neutral amino acid methionine. MeHg transport across the membranes thus takes the indirect route via the methionine carrier. In mammals, the fetal brain is more vulnerable to the neurotoxic effects of MeHg. In fact, amino acid transport is two or more times faster than in the adult, and induces high levels of metal bioaccumulation and severe structural and functional disturbances (89). Results from Ballatori (95) also show that the glutathione transport system may act as an Hg carrier through the canalicular membrane in rat liver cells.

**Conclusion**

Aquatic ecotoxicology covers a vast and diverse field of investigation. In most cases, situations encountered in the natural environment are, for the most part, unique and ever changing. Clearly, current research methodologies used in this field are of primary importance; only a close degree of complementarity between in situ studies and experimental approaches in the

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**Figure 11.** Chemical speciation diagrams of inorganic mercury and methylmercury in solution. (A) varying pH with a constant pCl of 7.0; (B) varying pH with a constant pCl of 4.0; and (C) fundamental links with metal accessibility to the cellular membrane interface, binding to proteins and phospholipid polar heads and transport through the hydrophobic bilayer. From Boudou and Ribeyre (10).
laboratory will provide a better knowledge of the mechanisms involved and, at the same time, enable us to make a better assessment of the short-, medium- and long-term risks. A multidisciplinary approach is likewise essential. Finally, let us emphasize how important it is that greater effort be put into fundamental studies, with the aim of analyzing the ecotoxicological mechanisms involved in the various stages from the chemical fate of contaminants within biotopes, their bioavailability, their uptake across biological barriers, and their ultimate bioaccumulation and toxicity.

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