BIOMONITORING OF ENVIRONMENTAL POLLUTION

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Summary

Environmental health is one of the most acute global problems. There are no corners of the globe where chemicals produced by humans cannot be found. The number of these chemicals is continuously increasing, as is their use. Some are quite persistent and their half-lives can be decades. At the same time, the number of endangered species is

increasing and people's health is at serious risk. Lipophilic pollutants accumulate in all organisms as they contain lipids. There are several methods of monitoring the effects of pollutant chemicals. In addition to feral species, caged animals and plants, as well as tissue and cell cultures, are used in studies of biomonitoring. The levels and activities of enzymes responsible for the biotransformation of pollutants reflect the level of pollution, if the organisms survive the pollutant load. For instance, the enzymes of the cytochrome P-450 family are often highly induced in exposed organisms. Because the metabolism of pollutants increases the production of oxygen-free and other radicals, the enzymes of the antioxidant defense and repair systems become induced. The increased transcription of respective genes can also be followed. Population studies indicate that pollutants having hormonal activity have already changed the sex ratios in, for example, the aquatic environment. However, the occurrence of diseases in humans is the ultimate source of information on ecosystem health.

1. Introduction and Background

Industry, energy production, transportation, and intensive agriculture and silviculture, in addition to municipal wastes, chemically load the environment. Rachel Carson was the first person who directed the public's attention to the pollution problem with her book *Silent Spring*—birds were not singing any more—published in 1962. In addition, carbon dioxide—very necessary for photosynthesis—is now considered one of the most problematic pollutants, due to its excessive release, with other chemicals and particles, from fossil carbon sources in energy production.

In Rio de Janeiro, in 1992, governments agreed on a Framework Convention on Climate Change (FCCC), with the ultimate aim of reducing the level of greenhouse gases in the atmosphere to safe levels. In Kyoto, in 1997, the world's industrialized countries agreed on reductions that they were committed to reach by 2008–2012. A number of countries have already ratified the reduction plan. Without the Kyoto agreement the level of, for example, carbon dioxide were predicted to increase by 20 µL L^{-1} between 1990 and 2010. However, at the same time it has been estimated that the level would increase by a further 15 µL L^{-1}, due to production in developing countries. The warming-up of the atmosphere, caused by the accumulation of greenhouse gases, would increase the melting of the polar ice caps and therefore raise the sea level, with widespread economic and social implications, not to mention the pollution by organic pyrosynthetic chemicals—some of which are carcinogenic.

When people use wood for cooking and heating, they cut down trees, destroy forests, and increase air pollution. When they make charcoal, they spoil the soil and ground water too. In addition, they clear forest land for agriculture. Forests contribute to the maintenance of a proper climate, clean the air, and also, by all these means, maintain and promote biodiversity.

Carbon dioxide is bound to calcium to form inorganic calcium carbonate in the seas, but this is a slow process. Carbon dioxide is used by plants and other carbon-fixing organisms to synthesize organic compounds. Trees form an especially valuable sink of carbon dioxide. One kilogram of wood removes about two kilograms of carbon dioxide from the air, because oxygen is released to the same time. The use of wood for purposes

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that keep the carbon bound for a long time will help in maintain the proper carbon dioxide balance in the atmosphere. It is important to design power plants that use rapidly growing wood and other biomass, as well as capturing carbon dioxide in addition to other pollutants in the burning process.

Air pollution is not only limited to carbon dioxide and other carbon compounds. The level of small particles in the air—as well as the concentration of sulfur dioxide and nitrogen oxides (NOX)—is exceeding the limits recommended by the WHO in many large cities. The levels measured in many areas affect the health of plants, animals, and human beings, especially children, and cancer incidences increase in affected areas. Ozone is a commonly occurring pollutant that has a large impact on the yield of agricultural crops. The dose response of crops in the field is complex, with influences on numerous biotic and abiotic factors, including microclimatic variables. Ozone is also one of the most important direct risk factors in human health.

Pollutants released into the air end up in rivers, lakes, and seas—the pollution of which, by organic and inorganic compounds, continues globally. Radionuclides form a special category of pollutants. There are sites where the safety limits are exceeded, due to natural radioactivity, and there are sites where the radioactivity is high due to nuclear experiments. Accidents in power plants and possible military or terrorist use represent special categories of danger. A new, potentially problematic agent is electromagnetic pollution. Telecommunication is increasingly widely used. The electromagnetic pollution of the world has gained little attention so far. There are, however, examples that indicate it also merits serious concern, because at least some organisms are sensitive to it.

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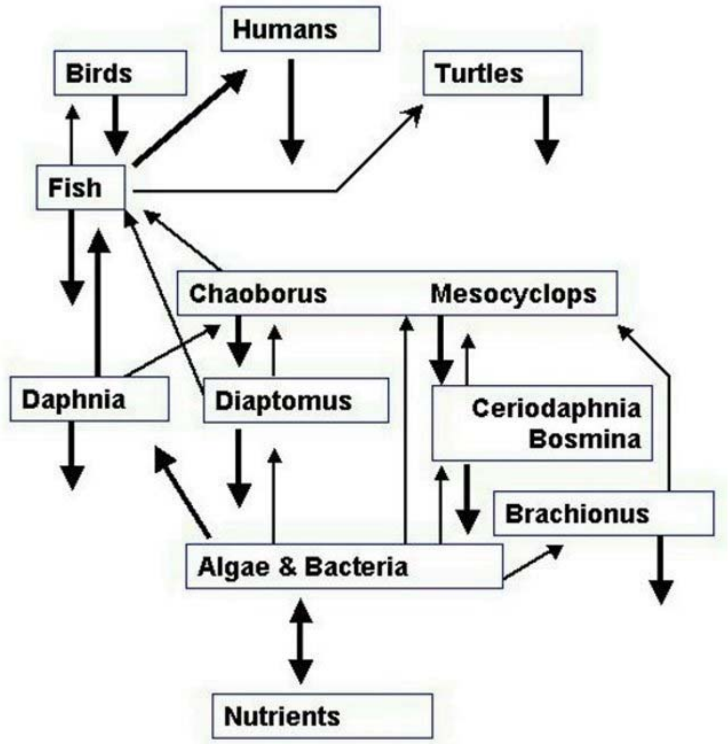

Figure 1. Schematic description of the food chain of a lake

Biomonitoring is a regular investigation (registration) of behavioral, functional, and morphological changes in organisms and their populations under the action of chemical, physical, and ecological factors. Organisms form food webs and interact with each other in many ways (see Figure 1).

The action of anthropogenic and nonanthropogenic factors should be studied. The main aim of biomonitoring is to find out the responses caused by pollution and other factors on living organisms in order to help decision making on ecosystem health problems.

Chemical pollution is usually divided into inorganic pollution (metals, acids, carbon dioxide, nitrogen and phosphorus compounds, and ozone) and organic pollution, which can further be divided into pollution due to persistent organochlorine compounds, polycyclic aromatic hydrocarbons, phenols, phthalates, organometallic compounds, and so on.

Lipophilic pollutants accumulate in all organisms as they contain lipids. There are several methods of monitoring the effects of pollutant chemicals. In addition to feral species, caged animals and plants as well as tissue and cell cultures are used in studies of biomonitoring. The levels and activities of enzymes responsible for the biotransformation of pollutants reflect the level of pollution, before morphological changes take place.

Genotoxicity—factors such as mutagenic compounds and radiation, including ultraviolet light—is perhaps the most important area of biomonitoring, because such toxicity can cause carcinoma of somatic cells and/or harmful mutations of ova and sperm cells, affecting the health of the next generation. The increasing content of mutagens in aquatic products such as fish used in human nutrition accounts for the necessity for monitoring genotoxic effects in coastal areas, especially in fisheries. Such substances accumulating in animals not only modify existing ecosystems, but represent a direct danger for man as well. Mutations are hereditary changes resulting in the increase or decrease of the genetic material or in changes of chromosome structure or DNA nucleotide sequences.

The increasing use of biomarkers in environmental biomonitoring programs has raised the problem of data management and intercomparison. A research project—the Pollution Effect Network (PEN)—has been proposed; it will entail the creation of an online warehouse for biomarker data (see <http://www.muf.unipmn.it/pen>). The web site will contain repository sections and expert system procedures able to integrate information from different biomarkers and provide a ranking of organism health status in terms of synthetic stress syndrome indexes.

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2. Biomarker Molecules

2.1. Genomic and mRNA Analyses

Mutations arise both spontaneously and under the action of various factors such as chemical substances, ultraviolet light (UV-B), or ionizing radiation. Aggressive chemicals and the reactive metabolites produced from any chemical can act on the nucleic acids. DNA strand breaks occur. The reactive compounds also form DNA adducts.

Chromosome mutations cause chromosome reorganization, including changes in linkage groups (translocation), a sequence of changes within a chromosome (inversion), fragmentation of chromosomes, and the loss of chromosome parts (deletion), as well as a doubling of chromosome parts (duplication) or the inclusion of other genetic elements into chromosomes (insertion). Point mutations are changes of DNA sequences at the level of single nucleotides.

Genotoxicity tests can be classified according to the information they provide as follows:

- *Chromosomal aberration tests*: Eucaryotic *in vivo* and *in vitro* tests have been developed. Aberration can often be detected by using a light microscope. A cytogenetic analysis of structural disturbances of chromosomes in metaphase and anaphase of mitosis, and a micronucleus test, are widely used.
- *Recombination assays*: *In vivo* and *in vitro* sister chromatid exchanges in eucaryotic systems, as well as for reciprocal mitotic crossing-over and mitotic gene conversion in yeast, are used in biomonitoring.
- Detection for dominant lethals can be made (e.g., on mice) and recessive lethals on Drosophila.
- Single cell gel electrophoresis (SCCE) or comet assay provides valuable information on the environmental pollutants affecting the genetic material in individual cells.
- *The micro array techniques*: Polymerase chain reaction (PSR) helps in getting small amounts of DNA multiplied for analyses, while differential display techniques can be used to detect changes in the gene expression.
- *The specific messenger ribonucleic acids (mRNA)*: The levels of mRNA increase faster than the respective proteins in the cells. This method is not sensitive to the possible inhibitory action of chemicals on the protein synthesis.
- Modified nucleic acid metabolites can be found even in urine.
- *Gene (point) mutation analyses on microorganisms or cultured animal cells*: Assays for both direct and indirect mutations are used. The most common is the Ames test, based on reversion to histidine prototrophy in *Salmonella typhimurium* strains.

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2.2. Proteomics: Enzymes and Other Proteins

One might expect the earliest responses to chemical or physical pollution would be seen in the membrane functions of the cells, where many receptors and transporters are located. Unfortunately, the measurement of these functions is technically difficult. The membrane functions cannot change very much without lethal effects.

Pollutants are usually metabolized in organisms. These reactions are catalyzed by a large group of enzymes (see *Biotransformation of Xenobiotics and Hormones*). The measurement of the biotransformation enzymes, like that of the 7-ethoxyresorufin O-deethylase (EROD), is rather easy. The amount of the enzymes can also be measured with the aid of antibodies. This also helps to quantify the enzymes that have been inhibited by the pollutants.

Synthesis of the biotransformation enzymes is quickly induced by the exposure to many pollutants (see Figure 2). The cytochrome P-450 and UDPglucuronosyltransferase enzymes are good examples (see *Biotransformation of Xenobiotics and Hormones*).

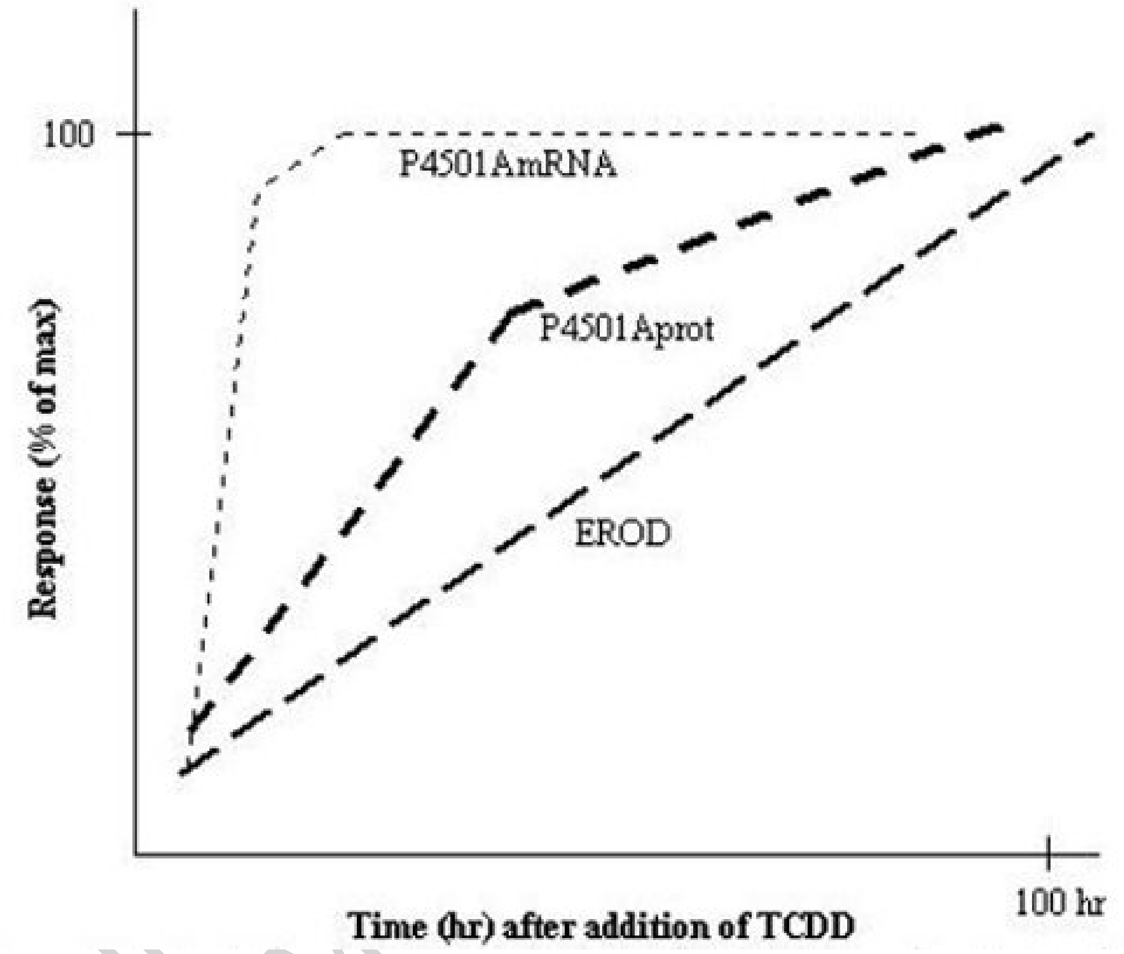

Figure 2. The level of messenger RNA increases faster than that of the biotransformation enzyme protein concentration and the 7-ethoxyresorufin O-deethylase (EROD) activity in liver cell culture in the presence of inducer TCDD. The pollutant may also cause an inhibition of the enzyme activity, and only the level of mRNA and the enzyme protein amount reveals the increased enzyme synthesis. So, for instance, the induction caused by the chemicals released by paper and pulp mills can be only partially detected if enzyme activity alone is measured.
Source: modified from Pesonen et al. (1992).

In parallel with the oxidation of the pollutants, reactive metabolites and reactive oxygen species are also generated. They interact with the proteins, lipids, and nucleic acids. The proteins are carbonylated. The peroxidation of lipids also takes place. The unsaturated fatty acids are split with the release of malone dialdehyde, which can quite easily be measured, for example, in the blood (see Figure 3).

The increased production of antioxidant enzymes, such as superoxide dismutase, catalase, glutathione peroxidase, and glutathione S-transferase, can take place in cells exposed to pollutants. Furthermore, the consumption of antioxidant cofactors, such as the oxidation of glutathione, can be measured (see Figure 3).

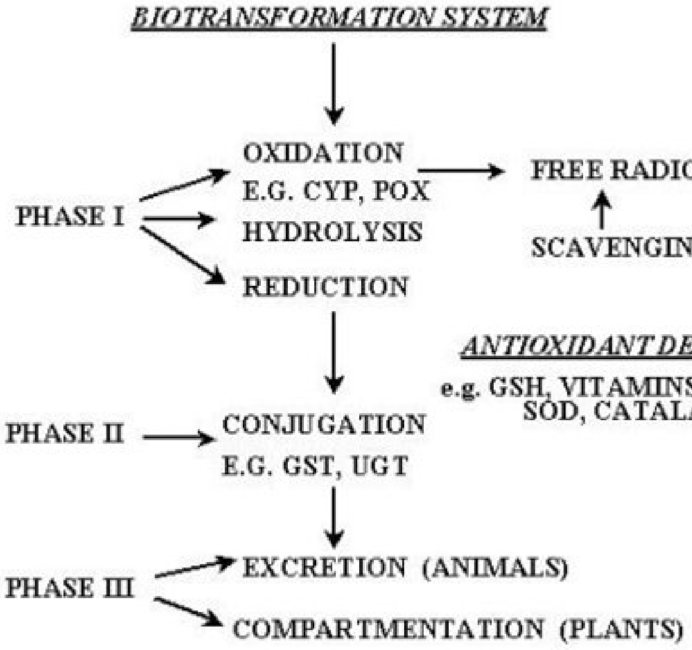

Figure 3. The pollutants can induce a biotransformation system (e.g., CYP = cytochrome P-450, POX = peroxidase (plants), GST = glutathione S-transferase, UGT = uridinediphosphate glucuronosyltransferase), antioxidant defense (e.g., GSH = glutathione, SOD = superoxide dismutase), and repair systems (e.g., Hsps = heat shock proteins or chaperons).

The cells have stress-proteins (also referred to as heat-shock proteins or Hsp—also called chaperons), which can repair the destroyed functional conformation of the proteins. Their induction occurs rapidly after an exposure to certain pollutants. Stress-proteins have recently been recognized as being one of the primary defense mechanisms activated by the occurrence of denatured proteins in the cell. The four major stress-protein families of 90, 70, 60, and 16–24 kDa are the most prominent and are frequently referred to as Hsp90, Hsp70, Hsp60, and low molecular weight stress-proteins. They are highly conserved in all organisms from bacteria to plants and humans. However, more knowledge on the kinetics and persistence of the stress response to complex environmental mixtures, on the influence of both physiological and environmental parameters (pH, eutrophication), on the constitutive levels of stress-proteins, and on the acquisition of tolerance, is required before one could safely use stress-proteins to assess on-site pollution. Nevertheless, as part of a test battery of complementary bioassays, stress proteins may be very valuable as broad response biomarkers for preliminary screening of environmental health (see Figure 3).

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The synthesis of metallothioneins and/or metallothionein-like proteins, which bind the toxic heavy metal pollutants, is also induced in exposed cells. Vitellogenin proteins are synthesized in the liver under hormonal control (e.g., in fish). If the pollutants interfere with the synthesis, this can be detected with specific antibodies.

Usually a set of several proteins and other parameters are recommended for use in biochemical biomonitoring of pollutant contamination problems. The next challenge in method development is analysis of the levels of all the responding proteins in the cells (i.e., the proteomics) when the cells are exposed to pollutants. This might also help in the bioidentification of the most important pollutants.

3. Models Used in Biomonitoring

Many different models have been developed to help biomonitoring, both to increase sensitivity and to save time and resources. Although much of the practical biomonitoring has been transferred into laboratories, this does not make field studies unnecessary. Sampling must always be guided by observations in the locations where the pollution occurs.

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Biographical Sketches

Dr. Sergei Vasilievich Kotelevtsev was born in 1948 in Moscow, USSR. He studied at the Biophysics Department, Moscow State University, School of Biology, Moscow State University, from 1966 to 1971, obtaining his Ph.D. (Biophysics) with a thesis on *Interrelation of Membrane Phospholipid Peroxidation and Drug Metabolism in Livermicrocosms*, in 1975. He was awarded his Doctorate of Science (Ecotoxicology) for work on *Response of the Biological Membrane to the Environmental Factors*, at Moscow State University in 1996.

He served at the Department of Biology, Moscow State University as: junior researcher, 1971–1979; senior researcher, 1979–1992; and Deputy Chief of Laboratory of Physical Chemistry of Biomembranes, since 1992. He has been Lecturer at the Department of Ecology Russian Peoples Friendship University, Moscow, since 1992. He swerved as a Fellow of the European Science Foundation (Toxicology and Environmental toxicology), Finland, in 1993.

His main fields of specialization include: metabolism of xenobiotics, radiobiology; other fields, biomonitoring of drugs, and environmental health. He is currently researching: biochemical monitoring of genotoxicity in water ecosystems; and mechanisms of xenobiotic biotransformation and the adaptation to the chemical and radioactive contamination.

He is a member of the International Society for the Study of Xenobiotics, USA, and the International Aquatic Ecosystem Health and Management Society. He was invited to lecture and participate at the NATO Advanced Study Institute and Research Workshop “Molecular Aspects of Oxidative Drug Metabolising Enzymes: Their Significance in Environmental Toxicology, Chemicalcarcinogenesis, and Health,” Turkey, 1993; and at “Biomarkers: a Pragmatic Basic for Remediation of Severe Pollution in Eastern Europe,” Poland, 1997.

He has produced 81 papers in refereed journals and 327 communications in scientific meeting, and is a member of the Editorial Advisory Board of the *Journal Aquatic Ecosystem Health and Management*.

Dr. Valerii Tonkopii was born in 1939 in Donetsk, Ukraine, USSR. He studied at the Donetsk Medical Institute, Russian Academy of Sciences and received his M.Sc. in1962. In 1968 he achieved his Ph.D. (Hyperbaric oxygenation at different acute poisoning), and in 1981 his D.M. (the study of anticholinesterase compounds toxic action), Doctor of Medicine.

In 1981 he became State USSR Prize Laureate (for the elaboration of organophosphorus poisoning antidotes), and Professor of Toxicology in 1983. He served as a military-medical officer in the Military-Medical Academy, Russian Academy of Sciences, 1962–1969; Senior Scientist Researcher: Head, Laboratory of Toxicology, Military-Medical Academy (MMA), Leningrad 1969–1990; and has been Head of Laboratory of Water Ecotoxicology, Institute for Lake Research, Russian Academy of Sciences, St. Petersburg since 1990. His research fields are: indication of highly toxic compounds and the elaboration of anticholinesterase inhibitor antidotes, and the development of nontraditional methods for xenobiotic bioidentification on the basis of toxic action mechanisms.

His teaching career includes work as: visiting lecturer in Military-Medical Academy and Institute of Toxicology, Leningrad; lecturer at the University of Helsinki (June 1993) in the Biological Institute of the University of Oslo (May 1994), and the US Army Medical Research Institute of Chemical Defense (Edgewood, USA, November 1994).

He has produced more than 300 publications and has participated in 50 international conferences and workshops.

Dr. Osmo Otto Päiviö Hänninen D.M.S. Ph.D., is Professor of Physiology, and Department Chairman at the University of Kuopio, Finland. He was born in 1939 in Lahti, Finland. He studied at the University of Helsinki and the University of Turku, Finland, where he received his M.Sc. (Biochemistry) in 1962; Licentiate of Medicine (MD), 1964; and Doctorate of Medical Sciences (DMS), 1966; and passed his dissertation in biochemistry for his Ph.D. in 1968. He has also studied genetics. He has been a specialist in sports medicine since 1986. He served as the Research Assistant of Professor K. Hartiala, 1962–1964; Assistant of Physiology, 1964–1965; Laborator of Physiology, 1966–1967; Docent of Physiology, from 1967, and Associate Professor of Biochemistry, 1969–1971, at the University of Turku; Acting Professor in the Planning Office, 1971–1972; and from 1972, Professor of Physiology and Chairman of the Department of Physiology, University of Kuopio; Vice-President of the University of Kuopio, 1972–1979; and President, University of Kuopio, 1981–1984. In addition, he served as Visiting Professor of Physiology at Shanghai Medical University, China, 1991–1992, and at Sun Yat Sen Medical University, Guangzhou, China, 1998–1999; as Foreign Member of the Russian Academy of Natural Sciences, from 1994; and as Secretary General, International Council for Laboratory Animal Science, 1988–1995. He was the President of Societas Physiologica Finlandiae, 1990–1999, and has been President of the International Society for Pathophysiology and a Member of the Executive Committee since 1994, and the Treasurer of the International Union of Biological Sciences since 1997.

His special interests in research are: biotransformation and adaptation to chemical loading, biomonitoring of toxicants, and comparative biochemical toxicology; muscle metabolism and function; ergonomics.

He has contributed 266 papers in refereed journals and 72 in proceedings, and written 55 reviews and 30 books or book chapters. He serves on the editorial board of four international journals and is at present the European Journal Editor of *Pathophysiology*.

Of his postgraduate students (32 in biotransformation, 27 in muscle metabolism and physiology, and five others), 12 serve as professors in China, Finland, Greece, Sweden, and the United States.

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