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PREDICTING BIOCONCENTRATION AND RESIDUE-BASED EFFECTS OF XENOBIOTICS IN AND ON AQUATIC BIOTA

PART B – INTERNATIONAL RESEARCH AND TECHNICAL ASSISTANCE

COOPERATIVE AGREEMENT CR 995189
WITH
U.S. ENVIRONMENTAL PROTECTION AGENCY
REGION V, CHICAGO, ILLINOIS

COMPLETION REPORT
AUGUST 1994

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Region V, Chicago, Illinois

Completion Report
August 1994

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I. INTRODUCTION

This report describes research collaboration with, and technical assistance provided to, foreign governmental agencies and scientists through Cooperative Agreement CR 995189 between the U.S. Environmental Protection Agency (U.S. EPA) and Montana State University (MSU). Under this Cooperative Agreement, research has been conducted in the Republic of Lithuania, and technical assistance has been provided to scientists in Lithuania, Russia, and the People's Republic of China (P.R.C.).

The activities with Lithuania and Russia were conducted under long-standing environmental science exchange agreements initiated in accordance with the Memoranda of the Twelfth (1990) and Thirteenth (1991) Meetings of the U.S.-U.S.S.R. Joint Committee on Cooperation in the Field of Environmental Protection. One component of those agreements, Project 02.07-31, was initiated in 1988 between U.S. EPA and scientists from the Soviet Union Republic of Lithuania.

The exchange program with P.R.C. was initiated under a protocol between the U.S. and P.R.C. for Scientific and Technical Cooperation in the Field of Environmental Protection: Annex 3, Environmental Processes and Effects Research.

Activities conducted with scientists in Lithuania are described in Section II. Only one exchange with scientists from Russia was undertaken and this is described in Section III. Exchanges with scientists from China are described in Section IV.

II. EXCHANGE PROGRAM WITH LITHUANIA

Background

The agreement for environmental research and technical assistance between U.S. EPA and Lithuania was initiated in 1988 as "Project 02.07-31", before dissolution of the Soviet Union. Since the independence of Lithuania, this project has continued, although it is now referred to, among its participants, simply as "Project 31".

Research under Project 31 has developed in five technical areas: (1) identification of organic and inorganic chemical pollutants, and monitoring of chemical and biological parameters, in Lithuanian surface and ground waters; (2) ecological and water quality modeling, including evaluation of assumptions for modelling fate and effects of pollutants; (3) measurement of transformation and equilibrium constants for predicting fate and effects of pollutants; (4) investigation of microbial transformation processes; and (5) toxicity testing of identified pollutants on biological species indigenous to the Baltic Republics.

Cooperators in Project 31 on the U.S. side have been personnel from MSU Fisheries Bioassay Laboratory (MSU-FBL) and the U.S. EPA Environmental Research Laboratory, Athens, Georgia (ERL-Athens), and on the Lithuanian side from the Lithuania Environmental Protection Department (LEPD), Vilnius University, Kaunas Technical University, Klaipėda University, and certain institutes of the Lithuania Academy of Sciences.

The approach by the U.S. side has been directed toward assessing the nature of environmental data being collected in Lithuania, assisting in its collection while avoiding redundancy of effort, and assisting in upgrading capability of Lithuanian scientists to perform chemical analyses, toxicity testing, data processing, and modelling. These latter objectives have included providing scientific training, technical assistance, and, within our budgetary constraints, supplies and equipment.

Since the beginning of Project 31, personnel from MSU-FBL have worked in Lithuania on ten separate occasions, six of these during the tenure of the present Cooperative Agreement with U.S. EPA which began in 1991. These trips have involved collection of field data, exchange of technical information, and the providing of technical assistance for the operation and maintenance of laboratory instrumentation.

Water Quality Assessments in Lithuania

Under this Cooperative Agreement, we have completed two studies on the quality of surface waters in Lithuania, working jointly with scientists from ERL-Athens, LEPD, and the Ecological Spectroscopy Laboratory (ESL) of the Lithuania Institute of Physics. One of these studies involved identification and distribution of hazardous organic chemicals, and the other identification and distribution of trace metals. We have also provided technical assistance in the collection of data for two other closely related studies, one on identification of chemicals at two pesticide storage facilities, and the other involving an inter-laboratory comparison of analytical methods for trace metals in water samples.

In the first of these studies, water samples were collected from 45 locations throughout Lithuania between 1990 and 1993 to determine distribution and nature of hazardous chemicals in Lithuanian surface waters (Ellington et al. 1994b). Organic chemicals were extracted from the water samples at the time of, or soon after, collection in Lithuania, using solid phase extraction cartridges or disks. These cartridges and disks were then taken to the U.S. for elution and analysis at ERL-Athens by means of gas chromatography (GC) and infrared/mass spectrometry (IR/MS). In addition to the expected hydrocarbons and fatty acids detected in several samples, alachlor was detected in a sample from Klaipėda Harbor, and 2-(methylthio) benzothiazole and a dialkylthiophene were identified in a sample taken from the Nemunas River at Grigiškės. Five hydrological water variables (pH, temperature, dissolved oxygen, conductivity, and redox potential) were also measured at most of the sampling sites. Several of the water bodies sampled were identified as being in a state of decline. A copy of the paper describing this study is included as part of this completion report (Appendix A).

Water samples were also collected from two sites being used to store hazardous chemicals, and these samples were analyzed to determine what chemicals might migrate from these sites into the surrounding environment (Ellington et al. 1994a). One of these sites, at Zigmantiškės, is a controlled landfill for banned, unsuitable, and unknown pesticides. Water samples were collected from this site on five occasions during 1990-1993. The other storage site studied was at a warehouse near Utena being used as a temporary storage facility for toxic agrichemicals. An accidental fire occurred in April 1993 at the warehouse, and water used to extinguish the fire seeped into the immediate ground environment. Catchment and surface water samples were collected at the Utena site in June and October 1993, and atrazine and several chlorinated carboxylic acids, including 2,4-D, were detected in all of these samples. It is recommended that monitoring in the region of both sites be continued to detect underground water movements, and that an immunoassay test be used routinely to monitor the most mobile chemical detected, possibly atrazine because of its water solubility and low partitioning to organic carbon. A copy of the paper describing the work at these two sites is included as part of this completion report (Appendix B).

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During our field trips for collection of water samples for analysis of hazardous organic chemicals, samples were also collected at many of the same locations for determination of trace metals, and analyses were subsequently performed by atomic absorption spectroscopy at ESL (Kvietkus et al 1994b). Samples from the Kulpė River at Šiauliai and from the Baltic Sea near Klaipėda Harbor had high concentrations of trace metals. Except for a high chromium concentration detected in the Kulpė River at Šiauliai, and high copper and lead detected at Melnragė (Klaipėda Harbor), other metal concentrations ranged from below detection limits to factors of 2-3 times higher than surface water criteria recommended by U.S. EPA. It is recommended that future investigations focus on sites downstream from municipalities, and from industrial activities, such as electroplating, that involve metals. A copy of the paper describing the environmental research on trace metals is included as part of this completion report (Appendix C).

In conjunction with the trace metal distribution study, duplicate samples were collected at several of the sampling sites to enable inter-laboratory comparison of results (Kvietkus et al. 1994a). Analyses were performed at ERL-Athens on 19 samples which were duplicates of samples analyzed at ESL. Eight metals were measured for comparison (cadmium, cobalt, chromium, copper, lead, manganese, nickel, and zinc). A correlation equation was determined for only six of these, however, because there were considerable differences in the zinc analyses, indicating possible sample contamination, and concentrations of cobalt were lower than the detection limits of the analytical method used at ERL-Athens. Differences were also noted among the values obtained for trace metals in the seawater samples. The regression line plot calculated for six of the metals, omitting cobalt and zinc, had a slope of 0.957 and a coefficient of determination of 0.829, indicating the data are similar, although there was a bias toward slightly higher concentrations reported by ESL. This inter-laboratory comparison study identified six metals that can be analyzed in freshwater samples with confidence in results. The analyses for zinc present a problem that must be addressed, and additional work is also needed to determine reasons for differences in results in analyses of the metals in seawater. A copy of the paper describing this inter-laboratory comparison study is included as part of this completion report (Appendix D).

Environmental Chemistry Training Courses at MSU-FBL

During spring 1992 and spring 1993, especially designed courses on instrument analysis of chemicals in the environment were provided at MSU-FBL to Lithuanian scientists as part of the Cooperative Agreement. The instructor for these course was John Neuman, Senior Research Chemist at MSU-FBL. The outlines for these courses are included in this completion report as Appendices E and F.

The first of these training courses conducted at MSU-FBL took place during January-May 1992 for two scientists from Vilnius University, Anolda Četkauskaitė from the Department of Biochemistry, and Birutė Bielinytė from the Department of

Chemistry. The curriculum developed for this course emphasized analyses of environmental samples for organic xenobiotics, utilizing electron-capture gas chromatography (ECD-GC), (Appendix E). Emphasis was placed on generation of analytical results employing quality assurance/quality control (QA/QC) protocols found in the 600 and 8000 methods series of the Research Conservation and Recovery Act (RCRA) published by U.S. EPA. The trainees worked on the analysis of environmental samples for both chlorinated hydrocarbons and chlorinated phenols. These chemicals are found world-wide and have been reported to be present in Baltic and Scandinavian waters. Laboratory exercises began with preparation of standard solutions and proceeded through the various analytical steps of established procedures. QA/QC protocols were followed that included surrogate additions to samples analyzed. Quantitation of target compounds was achieved by both external and internal standard techniques. The course was essentially a laboratory exercise emphasizing "hands-on" operation each day.

In addition to the training sessions on sample analysis, each trainee was given an independent project. One of Dr. Četkauskaitė's undertakings at MSU resulted in elaboration of an experimental approach using solvent-filled dialysis bags to sample for xenobiotics in surface waters (Appendix G), and we have since pursued this with experimental field studies in Lithuania. Dr. Bielinytė undertook a project on the synthesis and purification of gas chromatographable derivatives of some of the chlorinated phenols currently being studied. As a consequence of her work during spring 1992, Dr. Bielinytė was offered a graduate stipend at MSU Department of Chemistry, and she returned to Bozeman in January 1993 where she is now pursuing advanced studies.

The second training course at MSU-FBL was conducted during March-April 1993 for two analytical chemists from LEPD, Svetlana Urbonienė and Nijolė Štriupkuvienė. At the time of their training at MSU-FBL, both were on the staff of the LEPD Water Analysis Laboratory, where Dr. Štriupkuvienė was the supervisor. During this training course (Appendix F), emphasis was placed on using U.S. EPA QA/QC protocols for the analyses of organics in water and animal tissue samples. Classes of compounds such as polychlorinated biphenyls (PCBs), chlorinated pesticides, and phenols were analyzed by electron-capture detection GC (ECD-GC). High pressure liquid chromatography (HPLC) analysis was also introduced using it as a cleanup tool for samples subsequently analyzed by GC. Reference standards used during these activities were subsequently taken to Lithuania to be utilized as required. Data generated by these analyses were manipulated using the GC data system to demonstrate and teach techniques routinely used in the U.S. during environmental monitoring. Topics such as preparation and handling of analytical standards, sample storage and chain-of-custody, linear regression analysis, response factors, surrogate and matrix spike protocols were all discussed in detail. U.S. EPA methods were provided to the trainees as guidelines.

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This course also included a tour of a private analytical laboratory in Bozeman specializing in environmental analysis using U.S. EPA methodology, and time was also spent in the MSU Chemistry Department mass spectrometer (MS) facility where demonstrations were arranged showing how MS is used to identify unknowns in environmental samples. Upon their return to Lithuania, Dr. Štriupkuvienė continued at Supervisor of the Water Analysis Laboratory, and Dr. Urbonienė became supervisor of the Air Analysis Laboratory.

At the beginning of the 1993 course, Dr. Judita Šukytė, Chief of Research at LEPD, also visited MSU-FBL for 10 days to meet with MSU-FBL staff to assist in setting up the schedule for the training course in light of the then-current needs at LEPD, and to review the facilities at MSU in anticipation of future scientific and educational exchanges. Dr. Jackson Ellington, co-researcher on Project 31 from ERL-Athens, participated in this meeting.

Technical Assistance in Lithuania on Environmental Chemistry

During the tenure of this Cooperative Agreement, John F. Neuman of MSU-FBL participated in four of the trips to Lithuania to provide technical assistance in instrument repair, methods of chemical analysis, and data processing. Each trip lasted 2 weeks.

During a trip in June 1993, assistance was provided to Jackson Ellington, ERL-Athens, who had been asked by U.S. EPA Office of International Activities (OIA) to prepare an evaluation of the overall analytical capabilities of LEPD. As part of this evaluation, instruments at LEPD were examined in some detail. Performance of GC equipment was evaluated using column packings brought from the U.S. Efforts were also devoted to the rejuvenation of a capillary GC at LEPD which was being used primarily to screen fuel oils, and for analysis of air samples. Time was also spent touring the various regional LEPD laboratories across Lithuania. Two days were devoted to the repair of an HPLC system at Vilnius University. This system, used for herbicide degradation studies, had been donated to Vilnius University by ERL-Athens.

Subsequent visits in October and December 1993, and in February 1994, involved additional work in the laboratories of LEPD and Vilnius University. Efforts included establishing gradient elution HPLC capabilities required for the analysis of polynuclear aromatic hydrocarbons (PAHs) using both ultra-violet (UV) and fluorescence detectors. Some repair work was involved in establishing this capability. Analytical standards used were provided by FBL. Water samples collected from two pesticide storage sites were extracted for bases, neutrals, and acids (BNAs) using U.S. EPA Method 624. Surrogates, also provided by MSU-FBL, were incorporated into the analyses of these samples. LEPD personnel were directly involved throughout the entire sample work-up that included standards preparation and dilution. Extracts were returned to ERL-Athens for GC-MS analysis. A new capillary column, especially designed for the analysis of air pollutants and fuel oil components, was provided to LEPD, installed and made

operational. During all of these trips in 1993 and 1994, training of LEPD personnel in HPLC operations continued, with time given to the analysis of phenols in water. Instructional assistance was also provided to chemists at LEPD in analysis of biological samples for chlorinated pesticides. Selected sample extracts were brought back to the U.S. for GC-MS confirmation of residues.

Computer Systems Training at MSU-FBL and in Lithuania

Because all laboratory-related data generated at MSU-FBL are compiled and analyzed using desktop computers (PCs), instruction was given on software routines during both the 1992 and 1993 training courses at FBL. All analytical data, calculations, tabulations, and reports generated by FBL are completed using a combination of software programs including QUATTRO PRO™ (spreadsheets), WORD PERFECT™ (word processing), INSTAT™ (statistics), and SLIDEWRITE PLUS™ (graphics). In preparation for the training session conducted in 1993, two second-hand PCs (8088s) were obtained, refurbished, and loaded with software described above so that each student had her own system. This part of the instruction program was so successful, and generated so much interest by the trainees, that we transported these PCs, including the software and files created during the training session, to Lithuania on our trip in June of 1993. These systems are now on indefinite loan to LEPD from MSU. During our next three visits, more systems were taken to Lithuania and distributed as needed to various groups of collaborating scientists.

To date, MSU-FBL has provided one new and ten refurbished PCs, and five new printers, to various Lithuanian research cooperators under Project 31; four PCs have been provided to LEPD, three to different groups within the Ecology Institute, one to Vilnius University, one to Kaunas Technical University, and two to Vytautas Magnus University. All of this equipment was purchased with MSU funds.

All of these systems have hard drives, and at least one floppy disk drive. Each was evaluated for condition, "tuned up", and repaired as needed at the MSU micro-computer service center before shipment. Voltage converters/surge protectors were provided with each unit, allowing operation at either 220 or 110 volts. As each system was unpacked and installed in Lithuania, it was re-checked by MSU-FBL personnel to ensure working condition, and in each case operation instruction was given to prospective users. Software documentation has been provided with the hope that a user group would develop among the various individuals working with the systems. Since our most recent trip to Lithuania in February 1994, seven additional PCs have been acquired and refurbished at MSU expense, and we await separate funding so these can eventually be taken to Lithuania for use by Project 31 cooperators.

Technical Assistance in Lithuania on Aquatic Toxicology Research

Beginning in 1991, we have provided assistance to Dr. Zita Vosylienė and Dr. Ginteras Svecevičius at the Aquatic Research Laboratory (ARL) of the Institute of Ecology. Pre-fabricated components of a flow-through proportional diluter (Mount and Brungs 1967) were taken to Lithuania from MSU and installed at ARL. Within the first year after installation, scientists at ARL used this diluter to conduct a study on the acute toxicity of copper to five indigenous species of fishes. Considering the many local supply problems to be overcome, this is a considerable accomplishment on the part of the Lithuanian scientists.

In 1993 we provided ARL with pre-fabricated components for a continuous flow mini-diluter (Benoit et al. 1982), and assisted in its installation. Although the Benoit-type mini-diluter is more prone to malfunction than the amazingly reliable Mount and Brungs-type diluter, it has the advantage that generally far less toxicant solution is used, resulting in less toxic waste for treatment prior to disposal. We are currently helping ARL personnel build up their collection of published documents on aquatic toxicity testing.

Technical Assistance in Lithuania on Radiology Studies

During April-May 1993, Dr. Janina Baršienė, geneticist at the Lithuania Institute of Ecology, was hosted at MSU-FBL through this Cooperative Agreement for 6 weeks to collect background information on effects on biota of chemical and radionuclide discharges into the environment. Of special interest in Lithuania are the impacts of the Chernobyl and Ignalina nuclear power facilities. While at MSU, Dr. Baršienė worked with Volney Steele, M.D., a retired pathologist from Bozeman, who has worked within Russia over the past three years examining victims of radiation poisoning, as part of a team of specialists sponsored by Project Hope. One of the tasks undertaken by Dr. Baršienė was to conduct computerized literature searches, with Dr. Steele's assistance, on effects of radionuclides. These searches included the BIOSIS™, MEDLINE™, and CHERNOLIT™ data files.

To follow up on this work, Dr. Steele visited Lithuania for 10 days in June 1993 after completing a 1-month assignment in Russia for Project Hope. Dr. Steele conferred with Lithuanian physicists and physicians from Vilnius and Kaunas about impacts on human health and on the environment as a consequence of the Chernobyl accident, and from radionuclide leakage from the Ignalina nuclear power facility. A report based on Dr. Steele's observations and interviews with Lithuanian specialists has resulted in a proposal for a clinical study to support or modify the limited available information on human health effects from the Ignalina nuclear power facility.

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Technical Assistance in Lithuania on Bioindicators of Pollution

In June 1993, Sharon Eversman, Associate Professor of Botany at MSU, visited Lithuania for 10 days under this Cooperative Agreement in response to a request by Dr. Kęstutis Kvietkus, a Project 31 research collaborator at the Institute of Physics. Dr. Eversman is a specialist on lichens which are frequently used as pollution indicators. Botanists in Finland, Norway, and Sweden have historically been among the world leaders in this application. Dr. Eversman participated in the 1991 U.S. Forest Service workshop on "Lichens as Bioindicators of Air Quality", and is co-author of chapters in the subsequent report (Huckaby, 1993). Copies of this report have been provided to LEPD by U.S. EPA.

While in Lithuania, Dr. Eversman was requested to meet with personnel from LEPD, the Botany Institute, and the municipality of Kaunas. In addition, she spent time collecting study specimens in the upper Nemunas River basin, including the Dzūkija Nature Preserve. A summary of Dr. Eversman's recommendations to LEPD about lichens as pollution biomonitors in Lithuania is attached (Appendix H).

Editorial Assistance on Environmental Studies in Lithuania

In spring 1993 I undertook a review of nine manuscripts on environmental topics, written in English, by Lithuanian authors who requested advice as to their suitability for publication in western scientific literature. Many of the manuscripts were of descriptive nature, and I felt these would be more of regional significance than of international scientific interest. After conference with U.S. EPA personnel, and subsequently with the authors, it was agreed that the manuscripts be prepared for publication as a U.S. EPA Ecological Research Series document. As a group, the papers could tell much about what is presently known regarding environmental conditions and environmental studies in Lithuania. Following a suggestion made by Lithuanian scientists, it was also agreed to include four manuscripts being prepared by U.S. and Lithuanian scientists working under Project 31.

During summer 1993 the manuscripts were retyped at MSU on a word processor, and during each of my next three visits to Lithuania (October and December 1993, February 1994), I met with at least one, and as many as four, authors of each manuscript and we re-wrote the manuscripts where necessary to clarify the meaning and the English presentation. During the October and December 1993 visits, two of the original manuscripts were withdrawn but six new manuscripts were added by Lithuanian scientists. Between visits to Lithuania, the manuscripts received peer review by specialists both at MSU and at ERL-Athens. The final document contained 17 papers by 51 Lithuanian and five U.S. authors, and a camera-ready copy for U.S. EPA publication has been sent to the EPA printing office; publication is anticipated during fall 1994. A preliminary version of this document was issued in June 1994 as a FBL Technical Report (MSU-FBL 1994).

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Šiauliai Conference

During May 1992 I travelled to Lithuania to participate in and contribute to the Baltic Republics Monitoring Workshop in Šiauliai (the "Šiauliai Conference") sponsored by U.S. EPA-OIA. A preliminary "draft" report on some parts of the proceedings of this workshop were released by U.S. EPA Region V.

III. EXCHANGE PROGRAM WITH RUSSIA

Several topic of collaborative research with different offices and experimental stations within the U.S.S.R., were proposed under this Cooperative Agreement. These involved studies on (1) water quality planning and management of river basins, lakes, and estuaries, (2) forms and mechanisms of transportation of chemicals, radionuclides, and trace gases, and (3) effect of pollutants on aquatic ecosystems and development of water quality criteria.

None of the studies proposed for work within the U.S.S.R. was funded, so none was undertaken. I did, however, travel to Russia, enroute Lithuania, in July 1992 to participate in a symposium marking the 20th anniversary of the U.S.-U.S.S.R. agreement for cooperation in research on environmental sciences. The symposium was held at the Freshwater Biological Institute of the Russian Academy of Sciences, in Borok, Jaraslavl. Such a symposium had been held every 5 years since the inception of the U.S.-U.S.S.R. environmental science agreement, and this was perhaps the last of its type. I presented a paper on recent research at FBL (Neuman et al. 1992) supported by this Cooperative Agreement, and this paper is included as part of this completion report as Appendix I.

IV. EXCHANGE PROGRAM WITH PEOPLE'S REPUBLIC OF CHINA

The proposal for this Cooperative Agreement contained provision both for field research within the People's Republic of China (P.R.C.) and for logistical support for P.R.C. scientists visiting the U.S. Because no funding was made available under this Cooperative Agreement for field research within P.R.C., none was undertaken. Limited funding was provided, however, for logistical support within the U.S. in two areas.

The first area involved assistance for a study entitled "Investigation of Metals Pollution in Lake Poyang", delineated under the Agreement between the U.S. and P.R.C. Groups participating in this investigation include the Research Center for Eco-Environmental Sciences (RCES) in Beijing, ERL-Athens, and MSU-FBL. The study included development of field validation for a metals speciation model (MINTEQA2), using data from Lake Poyang and an associated upstream copper smelter site, and involves assistance to P.R.C. scientists by FBL personnel in establishing routine "on-site" bioassay testing programs to provide data for modelling purposes. Although curtailed funding did not permit this assistance, assistance was provided to support the visit of Dr. Lin Yuhan, RCES, to work with modelling specialists at ERL-Athens for 5 weeks during February-March 1993.

The second area involved support for an on-going series of international symposia, sponsored separately in part by U.S. EPA, to exchange information on fish physiology, fish toxicology, and water quality management. The first of these symposia was held in September 1988 at Zhongshan University, Guangzhou, P.R.C., the second in September 1990 at the University of California-Davis, Sacramento, and the third in November 1992 at Nanjing University, Nanjing, P.R.C. Representing the U.S. side, I have helped with the organization of these three symposia, and at each I have co-chaired the sessions on fish toxicology. Under this Cooperative Agreement, I co-edited the proceedings of both the Sacramento Symposium (U.S. EPA 1993) and the Nanjing Symposium (U.S. EPA In Press).

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V. ACKNOWLEDGEMENTS

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APPENDIX A

HAZARDOUS CHEMICALS IN SURFACE WATERS OF LITHUANIA, 1990-1993

J.J. Ellington, R.V. Thurston,
J. Šukytė, and K. Kvietkus

From

ENVIRONMENTAL STUDIES
IN THE
NEMUNAS RIVER BASIN, LITHUANIA

In Press

Environmental Research Laboratory
U.S. Environmental Protection Agency
Athens, Georgia, USA

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HAZARDOUS CHEMICALS IN SURFACE WATERS OF LITHUANIA, 1990-1993

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J. Šukytė[3], and K. Kvietkus[4]

ABSTRACT

Water samples were collected from 45 locations throughout Lithuania between 1990 and 1993 as part of a study to determine the distribution and nature of hazardous chemicals in Lithuanian surface waters. Organic chemicals were extracted from the water samples by use of solid phase extraction cartridges or disks in Lithuania, and the cartridges and disks were taken to the United States for elution and analysis by means of gas chromatography and infrared/mass spectrometry. The herbicide, alachlor, was identified in a sample taken from Klaipėda Harbor, and 2-(methylthio)benzothiazole and a dialkylthiophene were identified in a sample taken from the Nemunas River at Grigiškės, in addition to the expected hydrocarbons and fatty acids detected at several locations. Five hydrological water quality variables (pH, temperature, dissolved oxygen, conductivity, and redox potential) were measured at most of the sampling sites. Several water bodies were identified as being in a state of decline.

INTRODUCTION

A field study on the quality of surface waters in the Republic of Lithuania was initiated in 1990 under the sponsorship of the risk assessment program of the United States Environmental Protection Agency (U.S. EPA), Environmental Research Laboratory, Athens, Georgia (ERL-Athens). During six separate visits in December 1990, July 1991, June and July 1992, and June and October 1993, water samples were collected from 45 locations and sediment samples from two locations throughout Lithuania. These visits were conducted in cooperation with scientists from the Lithuania Environmental Protection Department, the Lithuania Academy of Sciences, and Vilnius University. Sampling sites were located on the Nemunas River and five of its tributaries, Kaunas Reservoir, Kuršių Marios Lagoon, Klaipėda Harbor, the Baltic Sea coast, Lake Dusia, Lake Drūkšiai, and the Kulpė River (Figure 1, Appendix A). Organic chemicals were extracted from the water samples by use of solid phase extraction cartridges or disks and these cartridges and disks were taken back to ERL-Athens for elution and analysis by means of gas chromatography and infrared/mass spectrometry.

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During the 1990-1992 expeditions, hydrological water quality variables were also measured at each site using a remote sampling probe. Water samples were also collected from monitoring wells at a controlled landfill for pesticide wastes at Zigmantiškės and from runoff water at an agrochemicals storage facility at Utena; results of analysis of those samples are reported separately (Ellington *et al.* 1994).

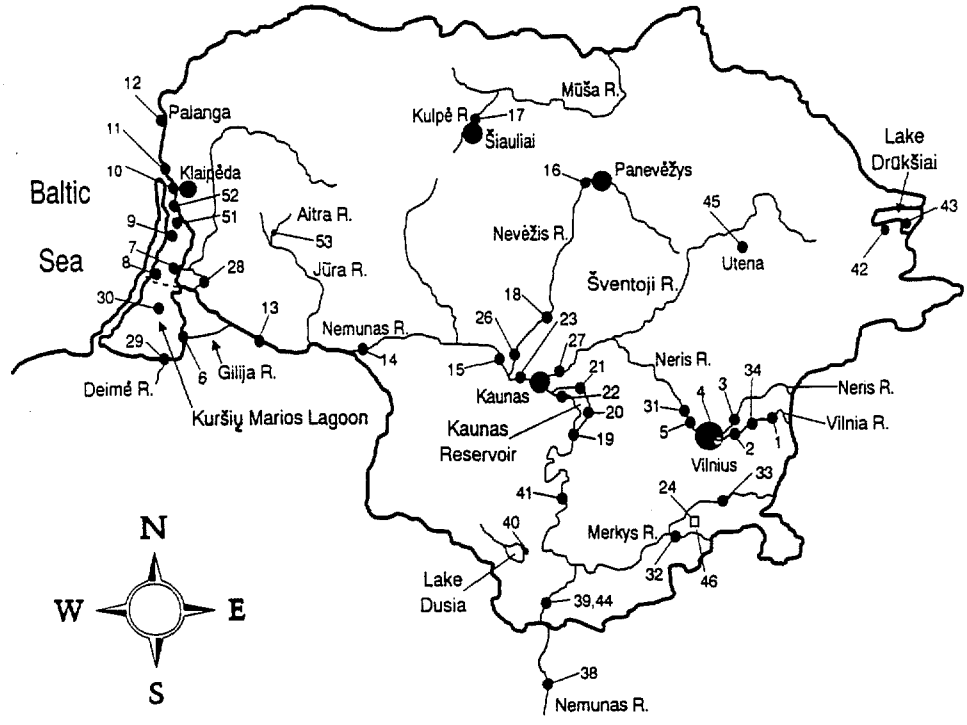

Figure 1. Field study water sampling stations.

METHODS

Hydrochemical and Physical Water Quality Variables

On-site water quality measurements in 1990 and 1991 were made with a Hydrolab Surveyor 2, and in 1992 with a Surveyor 3 (Hydrolab Corporation, Austin, Texas, USA), equipped with pH, temperature, dissolved oxygen (DO), conductance, redox potential, and depth sensors. The pH electrode (accuracy ± 0.2 units, resolution ± 0.01 unit) was calibrated with buffer solutions at pH 3, 7, and 10. The temperature sensor (accuracy $\pm 0.2°C$, resolution ± 0.01) was verified by simultaneous emersion in a flask of water with a thermometer certified by the U.S. National Institute of Standards and Technology.

The DO sensor (accuracy ±2%, resolution 0.01 mg/L) was calibrated by the saturated air method as described in the instrument manual. The conductance sensor (accuracy ±1% of range, resolution 4 digits) was calibrated with solutions of potassium chloride prepared with distilled, deionized water. The redox potential sensor (accuracy ±20 mV, resolution 1 mV) was calibrated with quinhydrone dissolved in pH 4 and pH 7 buffer solutions. The stated accuracies are the variations anticipated by the manufacturer if measurements are made under ideal conditions.

Blanks and Filter Preparation for Collection of Organic Chemicals

The adsorption media for concentration of organics from the water samples were of three types: Bakerbond™ SPE cartridges (6 ml) that contained 500 mg of octylsilane (C-8) or octadecylsilane (C-18), Burdick and Jackson cartridges (6 ml) that contained 500 or 1000 mg of C-8 or C-18, and Empore™ extraction disks (47 mm) consisting of 500 mg of C-8 or C-18 suspended in a Teflon fibril network. The Bakerbond™ cartridges were precleaned at ERL-Athens by removing lettering on the cartridges with a tissue soaked in hexane and extracting the cartridges in a Soxhlet apparatus overnight with hexane:acetone (9:1). The Burdick and Jackson cartridges and the Empore™ disks were precleaned by applying vacuum and pulling through the sorbent bed, in sequence, two 5-ml portions of the final extraction solvent, methanol, and organic-free distilled, deionized water. The Bakerbond™ and Burdick and Jackson cartridges were capped with solvent-cleaned aluminum foil and stored in screw-cap glass vials until sample collection. The Empore™ disks were wrapped flat in solvent-cleaned aluminum foil and stored and transferred in petri dishes. Two blanks of each solid phase sorbent were left refrigerated at ERL-Athens during each sampling trip and two were taken on the sampling trip where they were kept with the solid phase sorbent containers and returned to ERL-Athens for extraction and analysis together with the samples. Silicone tubing (Masterflex™) was used for pumping water during some sampling regimes, and this was checked for leachable organic contaminants by slicing a 2-cm portion of the tubing into cross sections approximately 3 mm thick, and extracting these by immersion under ethyl ether with frequent stirring for 6 hours. Organic chemicals in the ethyl ether extract were identified before and after treatment with diazomethane. Low levels of silica-containing compounds, hydrocarbons, and alkyl fatty acids were detected in the extract.

Sampling Techniques in the Field for Organic Chemicals

Water samples were filtered through borosilicate microfiber prefilters (Micro Filtration Systems), 47 mm diameter, to remove particles > 2.7 μm at time of collection, or within 24 hours thereafter. The extraction cartridges and disks were activated with methanol according to supplier specifications prior to concentration of the organic chemicals. The C-18 cartridges were used to concentrate nonpolar organic chemicals from the water samples. Carboxylic acids and phenols were extracted from water samples by adjusting the pH of the sample ≤ 2 to protonate the anions before passage of the water through the prefilter and the C-8 cartridge or disk.

A hand pump (Masterflex™) was used in the field to force the water samples through the prefilter contained in an in-line 47-mm filter holder, then through the SPE cartridges at the exit end of the silicone tubing. When laboratory facilities were available, water samples were pumped through the prefilters at the sampling site and then transported to the laboratory where they were pulled through the cartridges and disks by vacuum supplied by water aspiration or mechanical pump. The amount of water sampled at each location varied from 0.2 to 1.5 L, but generally water was pumped or pulled through the SPE cartridges or disks until flow decreased to a few ml/minute. Upon return to ERL-Athens, the SPE cartridges and disks were stored at 4°C until elution and analysis.

Extraction and Derivatization of Organic Chemicals

Prior to eluting the sorbed organics from the SPE cartridges with solvent, excess water was removed by applying vacuum. Carboxylic acids and phenols were eluted from the C-8 cartridges with 5 ml methanol/acetone 9:1. Neutrals were similarly eluted from the C-18 cartridges with 5 ml hexane/acetone 9:1. The disks were placed in a test tube with 5 ml of hexane/acetone 9:1 and sonicated for 2 minutes, and after standing for 5 minutes the solvent was transferred to another tube and the extraction repeated. In 1992 the extraction sequence for the disks was changed to a sequence of 5 ml of ethyl acetate, 5 ml of methylene chloride, and finally 5 ml of ethyl acetate/methylene chloride (1:1) (U.S. EPA 1991). Extracts were reduced to a final volume of 1 ml. Methyl esters of carboxylic acids were formed in the extract from the C-8 cartridges, which were those used for extraction of the pH 2 water samples, by bubbling gaseous diazomethane through the final volume.

The sediment samples collected for analysis of organic chemicals were extracted according to U.S. EPA Method 8151 for the extraction of herbicides and U.S. EPA Method 3550A for the extraction of base/neutrals (U.S. EPA 1992). The appropriate surrogate and matrix spikes and method blanks were performed as prescribed.

Gas Chromatography (GC) Analysis

High resolution GC analysis of sample extracts was performed with a Hewlett-Packard model 5890 GC equipped with both flame ionization (FID) and electron capture (ECD) detectors, splitless inlet, and autosampler (7673A). Separation of sample components was achieved using a 30 m x 0.32 mm i.d. x 0.25 μm film DB-5 capillary column operated initially at 40°C for 2 minutes, then temperature-programmed to 290°C at 8°/minute. Helium carrier gas was used at a flow rate of 30 cm/second. Detector temperatures were set at 300°C (FID) and 350°C (ECD). Injections of 1 μL were made at an inlet temperature of 250°C.

GC/Mass Spectrometry (GC/MS) and GC/Fourier Transform-Infrared Spectrometry/Mass Spectrometry (GC/FT-IR/MS) Analysis

Low resolution electron-impact (EI) GC/MS was performed on either a Finnigan 4500 (Finnigan MAT, San Jose, California), or a Hewlett-Packard (Palo Alto, California) mass selective detector (MSD). Chemical ionization (CI) mass spectrometry with methane gas was performed on the Finnigan 4500 system. High resolution EI GC/MS analyses of sample extracts were performed on a VG 70-SEQ high resolution hybrid mass spectrometer (VG Instruments, Manchester, United Kingdom). The GC/FT-IR/MS analyses were performed on a Hewlett-Packard system equipped with a 5965B infrared detector and a 5971 series mass selective detector. The gas chromatographs attached to the mass spectrometers were equipped with capillary columns similar in dimensions, liquid phases, and flows to the one described above; the temperature program was also the same.

SAMPLING PROGRAM, RESULTS, AND DISCUSSION

Hydrochemical and Physical Water Quality

In December 1990, the measured pH values ranged from a low of 7.5 in the Kulpė River at Šiauliai (LT-17) to a high of 8.7 at Nida (LT-08) (Appendix B). The high temperature, low DO, and high conductivity (1.55 mS/cm) at Šiauliai were due to the high volume of industrial effluent released into the Kulpė River as it flows through an industrial section of Šiauliai. The other values reported in Appendix B are within the ranges expected for the time of year and the high volumes of surface water caused by heavy rainfall.

In July 1991, the surface DO levels in Kaunas Reservoir were higher at the sampling sites closer to the dam and above deeper water (LT-19A, -19B, -20, -21, and -22) (Appendix C). The DO levels in the Nemunas River below Kaunas Reservoir and continuing into Kuršių Marios Lagoon were also lower than in other surface waters. The Kulpė River at Šiauliai (LT-17) again had a high conductivity value (2.14 mS/cm) for surface water and extremely low DO (0.53 mg/L).

In June 1992, the measured pH and DO values were higher than those measured in July 1991 at all but two sampling sites, Melnragė (LT-11) and Rasytė (LT-30) (Appendix D). The pH value of 9.5 and DO value of 17 mg/L recorded at Lake Drūkšiai (LT-43) are the result of extensive photosynthetic activity by the dense plant growth in the lake, both standing and planktonic. This photosynthetic activity, while raising the level of molecular oxygen, also increases the hydroxyl ion load with a corresponding increase in pH.

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Organic Chemicals in Water

In December 1990, water measurements were made and samples collected on SPE cartridges from 24 sites in Lithuania, including the Vilnia, Neris, Nevėžis, Kulpė, and Nemunas Rivers, Kaunas Reservoir, and Kuršių Marios Lagoon (Appendix B). Upon return to ERL-Athens, the sorbed organics were eluted and GC-FID and GC-ECD chromatograms were obtained. The retention times of all the major peaks in the chromatograms from the surface water samples were identical. GC/MS analysis of the 1990 surface water extracts identified the major peaks as several dialkyl phthalates, silica-containing components, saturated hydrocarbons, and some unidentified peaks with the same mass fragmentation pattern that appeared in every extract. Dialkyl phthalates are commonly found in environmental samples and stringent precautions are necessary to assure their presence in the collected sample is not due to accidental contamination. Hydrocarbons, phthalates, and fatty acids were found in a few of the method and field blanks but at lower levels than in the samples; the presence of phthalates in the samples may be real. Absence of man-made chemicals other than possibly phthalates in the surface water samples was surprising, but could have been caused by the ambient water temperature (4°C) at this time of year, and the high levels and flows of water from recent heavy rainfall. Both of these conditions would lower the concentration of organic chemicals in the surface water, possibly below the level of detection for the volume of water sampled. Organic chemicals identified in water samples collected during this and subsequent expeditions are listed in Table 1.

Table 1. Hazardous chemicals identified in surface water samples.

Chemical	Grigiškės (LT-31)	Kaunas Reservoir (LT-19-22)	Green Harbor (LT-52)
Alachlor[1]			x
Alkyl hydrocarbons[2]	x	x	
Aromatic hydrocarbons[2]	x		
Dialkylthiophene[2]	x		
2,4-Dichlorobenzoic acid[1]	x	x	
Fatty acids[2]	x	x	x
2-(Methylthio) benzothiazole[2]	x		
Phenanthrene carboxylic acid[2]	x	x	
Phthalates[1,3]	x	x	x
Silicon organics[2]	x	x	x
Tetrachlorinated unknowns[2]	x	x	

[1]Identified by GC/FT-IR/MS and confirmed with a standard
[2]Identified by GC/FT-IR/MS but not confirmed with standards
[3]Diisooctyl-, Butylbenzyl-, Di-n-octyl-, and Di-n-butylphthalates were observed the most frequently

In July 1991, 15 of the former sites were sampled, as were eight new locations including the Šalčia River (LT-32) downgradient from the long-term pesticide storage site at Zigmantiškes (LT-24) (Ellington *et al.* 1994), the Neris River (LT-31) downstream from Vilnius below a paper manufacturing plant, and the Merkys River (LT-33) downstream from a combined poultry growing and processing facility (Appendix C). Low resolution GC/MS indicated the presence of three tetrachlorinated isomers in the diazomethane-treated sample extracts from the Neris River and Kaunas Reservoir (LT-20) (Figure 2).

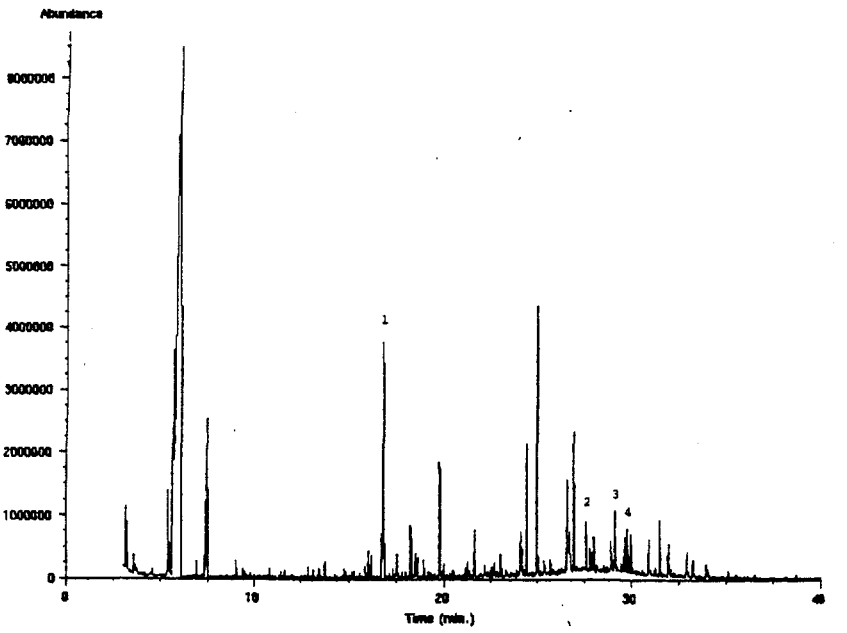

Figure 2. Total ion mass chromatogram of the solid phase extraction eluant from Kaunas Reservoir. GC/MS Peak 1 = 2,4-dichlorobenzoic acid methyl ester. GC/MS Peaks 2, 3, 4 = unknown tetrachlorinated isomers.

High resolution EI GC/MS of the three tetrachlorinated peaks in the Kaunas Reservoir water sample extract yielded best fit empirical formulae of either $C_7H_{13}Cl_4O_5P$ or $C_{14}H_8Cl_4O_2$. A tetrachlorinated trialkyl phosphate fits the former formula while tetrachlorinated aromatic esters and other structures containing carbonyl groups can be drawn for the latter. Both formulae are similar to those of compounds commonly used as electrical insulators and flame retardants. The compound containing phosphorus was eliminated as a possibility by further GC analysis of the sample using nitrogen and nitrogen-phosphorus specific detectors, respectively. The presence of the carbonyl functional group in the three unknowns was confirmed by GC/FT-IR. The loss of the m/z 31 ion (loss of $-OCH_3$) during electron impact GC/MS argued for the presence of a methoxy functional group, possibly a methyl ester. However, the absence of a strong peak in the 1250-1310 cm^{-1} region of the IR spectrum and the broad carbonyl peaks argued against the ester functionality. Detection of the unknowns only after treatment of the extract with diazomethane is evidence for a reactive hydrogen needed to form a methoxy group. A tetrachlorinated aromatic ring system containing a carbonyl group,

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methoxy group, and differing chlorine substitution is the most likely structure for the three unknown isomers. The surface water samples collected at Kaunas Reservoir and the Neris River at Grigiškės (LT-31) also contained 2,4-dichlorobenzoic acid in addition to the three tetrachlorinated unknowns. Fatty acids, identified in the Kaunas Reservoir and Grigiškės samples as their methyl esters, could be an indication of contamination by municipal sewage.

In June 1992, samples were collected at some of the sites previously sampled and new sampling sites were added at Lake Dusia, Lake Drūkšiai, and three locations on the Nemunas River, two of these upstream from Kaunas Reservoir, and one downriver from Gardinas in Belarus (Appendix D). Lake Drūkšai is both the source of and receiving body for the cooling water for the Ignalina nuclear power facility. Chemicals tentatively identified in surface water samples, but not confirmed with standards, included 2,2-dimethoxy-1,2-diphenyl ethanone, dialkylthiophene, and 2-(methylthio)thiophene.

During June 1993, water samples were collected at Juodkrantė, Klaipėda Harbor, and at three new sampling sites: Amber Cove (LT-51) and Green Harbor (LT-52) in the Klaipėda Harbor area of Kuršių Marios Lagoon, and the Aitra River (LT-53). Organics were extracted with Empore™ disks from the Klaipėda Harbor water samples, but only the Green Harbor sample was analyzed by GC/FT-IR/MS. This site is located at the mouth of a channel that is used for drydock-ship repair, and sewage from the Klaipėda municipal treatment facility is discharged into this channel. In addition to the expected fatty acids, hydrocarbons, and phthalates tentatively identified by interpretation of MS and IR spectra, an herbicide (alachlor) was identified and confirmed with a standard. The alachlor was present at approximately 3 ppb. The source of the alachlor remains unknown and it is uncertain if this concentration of alachlor poses a threat to aquatic organisms. The only compounds detected in the Aitra River sample were sulfur, trace amounts of phthalates and hydrocarbons, and low molecular weight carboxylic acids in the 10-40 ppb concentration range.

Organic Chemicals in Sediments

In June 1992, two sediment samples were collected for analysis for organic chemicals. One of these samples was collected off the mouth of the Gilija River (LT-06) as it flows into Kuršių Marios Lagoon, and the other from the Neris River at Grigiškės (LT-31), downstream from Vilnius. After extraction of the organics, the resulting acid/herbicide and base/neutral extracts were analyzed by GC/FT-IR/MS. Fatty acid methyl esters, hydrocarbons and the methyl ester of phenanthrene carboxylic acid were the only compounds identified with a high degree of confidence using both the spectral matching library and manual interpretation. The presence of multichlorine substitution was indicated in the mass spectra of several peaks in each extract but tentative identifications were not possible. The only compounds other than hydrocarbons identified in the base/neutral extracts were cholesterol in the Grigiškės sample and the octyl ester of phosphoric acid in the Gilija sample. Alkyl phosphate esters are commonly used in lubricating greases, and this is one possible source of the octyl ester.

CONCLUSIONS AND RECOMMENDATIONS

The majority of the population and industry of Lithuania are located in the Nemunas River basin which includes the Neris River. Anthropogenic chemicals were detected in only a few of the surface water samples, and we cannot explain the reasons for this. For the December 1990 samples, this might have been at least in part because of a combination of the low water temperatures and the dilution of surface waters from heavy rainfall during that season of the year. Failure to detect a larger number of chemicals in the samples collected during the summers of 1991-1993 might have been partially the result of a national economic recession, with commensurate decrease in industrial activity. The low levels of DO (<5 mg/L) in the Kaunas Reservoir in July 1991 is an indication of a water system in decline, as are the extremely high DO (17 mg/L) and pH (9.5) measurements in Lake Drūkšiai.

Other sites of concern are the Kulpė River in Šiauliai, outside the Nemunas River basin, which receives the majority of its total flow within the city limits from industrial and municipal sources, and the Deimė River flowing from Kaliningrad into the southern end of Kuršių Marios Lagoon. Analysis of sediment samples from rivers and lakes should be given a high priority, inasmuch as sediments contain the historical burden of pollution and are one of the eventual sinks for pollutants.

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Appendix A. Field study water sampling stations

Number	Name	Description
LT-01	Lavoriškės	Vilnia River, ca 40 km above Vilnius at hay field
LT-02	Pavilnys	Vilnia River, ca 8 km above Vilnius at dam
LT-03A	Pioneer Camp	Neris River, ca 16 km above Vilnius at Young Pioneer Camp
LT-03B	Turniškės	Neris River, ca 1-2 km below Pioneer Camp, and 102 km above Turniškės
LT-04	Gediminas Castle	Vilnia River, ca 200 m above confluence with Neris River
LT-05	Gariūnai	Neris River, ca 2 km below Vilnius wastewater treatment facility, near field with abandoned Soviet tanks
LT-06	Gilija	Kuršių Marios Lagoon off the mouth of the Gilija River (Matrosovo Kanalas)
LT-07	Uostadvaris	Kuršių Marios Lagoon, ca 10 m offshore at Uostadvaris
LT-08	Nida Station	Kuršių Marios Lagoon, ca 200 m offshore at Nida
LT-09	Juodkrantė	Kuršių Marios Lagoon, at Juodkrantė from the shore at boat beaching area
LT-10A-B	Klaipėda Harbor	Klaipėda Harbor, east side, from the dock, north side of Danė River mouth
LT-11A-D	Melnragė-A-D	Baltic Sea, at Melnragė, (A) from the end of breakwater, near grounded freighter, "Rudolph Breitscheid", (B) from boat 100 m west of "Rudolph Breitscheid", (C) from boat immediately inside mouth of breakwater, (D) from boat inside mouth of breakwater, opposite stone tower and ca 100 m downstream from museum
LT-12	Palanga	Baltic Sea, at Palanga from the pier 50 m from shore
LT-13-1,2	Sovetskas	Nemunas River, At Sovetskas, west of bridge, ca 3 km below one paper plant and ca 11 km below another, (1) from north bank, (2) from boat at river center
LT-14	Smalininkai	Nemunas River, at Smalininkai from the north bank near houses
LT-15A-C	Kulautuva-A-C	Nemunas River, ca 13 km below Nemunas-Nevėžis confluence, (A) from north bank, (B) south bank at Zapyškis, (C) north bank ca 1 km below Zapyškis
LT-16	Panevėžys	Nevėžis River, ca 1 km below discharge from Panevėžys wastewater treatment facility, south bank ca 200 m from abandoned church
LT-17	Šiauliai	Kulpė River, ca 100 m below abattoir and ca 1 km downriver from Šiauliai
LT-18	Kėdainiai	Nevėžis River, ca 1.5 km below wastewater treatment facility, from south bank at collective farm

(Continued)

Appendix A. Continued

Number	Name	Description
LT-19A	Nemunas, II Cut	Kaunas Reservoir, upper end inside Nemunas River, before discharging into the reservoir
LT-19B	Lapaine	Kaunas Reservoir, upper end even farther inside Nemunas River
LT-20	Kaišiadorių	Kaunas Reservoir at Hydroaccumulation Electric Power (HAEP), near middle, 200 m offshore
LT-21	Dambrava, IV Cut	Kaunas Reservoir, near sauna, 200 m offshore
LT-22	Pažaislis, VII Cut	Kaunas Reservoir, near dam, 400 m off Pažaislis Monastery
LT-23A	Sand Beach	Nemunas River, below mouth of Neris River and above mouth of Nevėžis River, on south bank across from heavy industry
LT-23B	Lampedžiai	Nemunas River, below mouth of Neris River and above mouth of Nevėžis River, on north bank ca 500 m below LT-23A
LT-24A-F	Pesticide Storage Site	Ministry of Agriculture Pesticide Storage Site southwest of Vilnius, ca 3 km north of Šalčia River; Monitoring Wells #1-6 inside the site
LT-26A-B	Raudondvaris	Nevėžis River, (A) ca 2.5 km above confluence with Nemunas, from west bank, (B) ca 1 km above confluence with Nemunas, from west bank
LT-27	Neris at Kaunas	Neris River at last bridge confluence with Nemunas River, from north bank
LT-28A	Rusnė-A	Nemunas River at bridge, ca 10 km before Kuršių Marios Lagoon
LT-28B	Rusnė-B	Nemunas River, ca 2 km above bridge
LT-29	Deimė	Deimė River, immediately inside mouth before flowing into southernmost section of Kuršių Marios Lagoon
LT-30	Rasytė	Kuršių Marios Lagoon, approximate center
LT-31	Grigiškės	Neris River, ca 17 km west of Vilnius, from south bank
LT-32	Zigmantiškės	Šalčia River, ca 3 km south of Pesticide Disposal Site
LT-33	Jašiūnai	Merkys River, ca 25 km south of Vilnius
LT-34	New Vilnia	Vilnia River, ca 20 km above Vilnius and just above New Vilnia
LT-38	Sredny Gonkolsky	Nemunas River, east bank ca 5 km below Gardinas city limits (in Belarus)
LT-39	Liškiava	Nemunas River, west bank, ca 10 km below Druskininkai
Lt-40	Lake Dusia	Lake Dusia, east side, ca 40 km northwest of Druskininkai
LT-41	Alytus	Nemunas River, ca 3 km below Alytus water treatment facility

(Continued)

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Appendix A. Continued

Number	Name	Description
LT-42	Visaginas	Wastewater treatment facility outflow at Visaginas
LT-43	Lake Drūkšiai	Lake Drūkšiai, south shore of west arm
LT-44	Crusader Creek	Mouth of creek before flowing into Nemunas River, ca 40 m below LT-39
LT-45A-E	Utena Storage	Utena warehouse toxic chemical storage location: A - access hole to drainage pipe from under warehouse and water catchment area B - water catchment under warehouse, east side C - storage tank inside door on right side D - storage tank inside door on left side E - storage tanks LT-45C and LT-45D combined and treated
LT-46A-D	Pesticide Storage Site	Adjacent to the Ministry of Agriculture Pesticide Storage Site southwest of Vilnius, Monitoring Wells #1-4 outside the fence
LT-51	Amber Cove	Kuršių Marios Lagoon, 1 km north of Juodkrantė, ca 400 m offshore
LT-52	Green Harbor	Klaipėda Harbor, ca 200 m offshore from discharge of Klaipėda municipal wastewater treatment facility
LT-53	Girėnai	Aitra River, east of Girėnai, ca 10 km above confluence with Jūra River

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Appendix B. Hydrochemical and physical water quality data, November-December 1990

Station Number	Station Name	Depth[1] (m)	Temp.[2] (C)	Dissolved Oxygen[3] (mg/L)	pH[4]	Conductivity[5] (mS/cm)	Salinity (ppt)	Redox Potential[6] (mV)
				Kulpė River				
LT-17	Šiauliai	Sur	11.17	4.66	7.50	1.55	0.3	41
				Vilnia River				
LT-01	Lavoriškės	0.6	5.22	10.28	7.89	0.444	0.0	91
LT-02	Pavilnys	Sur	4.79	9.70	7.79	0.463	0.0	95
				Neris River				
LT-03A	Pioneer Camp-A	Sur	3.27	11.74	7.98	0.415	0.0	97
LT-04	Gediminas Castle	Sur	4.90	11.78	8.14	0.472	0.0	111
LT-05	Gariūnai	Sur	3.45	11.25	8.13	4.23	0.0	90
				Nevėžis River				
LT-16	Panevėžys	0.8	2.68	11.95	7.86	0.798	0.0	105
LT-18	Kėdainiai	0.6	2.62	12.41	8.04	0.876	0.0	127
				Kaunas Reservoir				
LT-19A	Nemunas-II	3.3	2.66	11.61	8.09	0.484	0.0	121
LT-20	Kaišiadorių	1.0	2.53	11.55	8.06	0.484	0.0	138
LT-21	Dambrava-IV	0.9	2.82	11.30	8.10	0.486	0.0	148
LT-22	Pažaislis-VII	0.9	2.98	11.15	8.11	0.487	0.0	148
				Nemunas River				
LT-23A	Sand Beach-A	0.7	3.14	10.97	8.12	0.512	0.0	145
LT-15A	Kulautuva-A	0.7	1.60	10.89	8.06	0.784	0.0	157
LT-14	Smalininkai	0.8	2.38	11.01	8.05	0.570	0.0	166
LT-13A-1	Sovetskas-A	0.7	2.62	7.40	7.62	0.635	0.0	120
				Kuršių Marios Lagoon				
LT-06	Gilija	Sur	3.77	10.43	7.89	0.546	0.0	97
LT-07	Uostadvaris	Sur	3.60	11.06	8.02	0.524	0.0	112
LT-08	Nida Station	Sur	3.95	11.77	8.27	0.467	0.0	106
LT-09	Juodkrantė	0.9	3.13	12.00	8.10	0.480	0.0	89
LT-10A	Klaipėda Harbor-A	0.7	5.24	11.34	7.58	6.44	3.2	95
				Baltic Sea Coast				
LT-11A	Melnragė-A	0.7	6.29	11.43	7.79	11.02	5.9	101
LT-12	Palanga	0.7	5.59	11.71	7.92	9.29	3.8	160

[1]Depth probe accuracy ±0.45 m, Sur = approximately 0.3-0.5 m below surface [2]Temperature accuracy ±0.2° [3]DO accuracy ± 2% [4]pH accuracy ±0.2 units [5]Conductivity accuracy ±1% of range, resolution 4 digits [6]Redox accuracy ±20 mV

Appendix C. Hydrochemical and physical water quality data, July 1991

Station Number	Station Name	Depth[1] (m)	Temp.[2] (C)	Dissolved Oxygen[3] (mg/L)	pH[4]	Conductivity[5] (mS/cm)	Salinity (ppt)	Redox Potential[6] (mV)
				Kulpė River				
LT-17	Šiauliai	Sur	21.80	0.53	7.69	2.14	0.6	- 95
				Neris River				
LT-31	Grigiškės	Sur	18.53	8.30	8.10	0.476	0.0	61
LT-27	Neris at Kaunas	Sur	21.06	14.51	8.58	0.453	0.0	83
				Šalčia River				
LT-32	Zigmantiškes	Sur	15.82	8.46	7.72	0.452	0.0	94
				Merkys River				
LT-33	Jašiūnai	Sur	14.73	9.32	7.91	0.458	0.0	102
				Nevėžis River				
LT-16	Panevėžys	Sur	19.14	6.85	7.82	0.728	0.0	76
LT-18	Kėdainiai	Sur	21.20	7.20	7.84	0.808	0.0	61
LT-26A	Raudondvaris	Sur	22.25	5.40	7.55	0.829	0.0	106
				Kaunas Reservoir				
LT-19A	Nemunas-II	0.5	22.65	1.45	7.56	0.478	0.0	92
"	"	2.0	22.71	1.42	7.62	0.479	0.0	83
"	"	4.0	22.54	0.58	7.58	0.478	0.0	85
"	"	6.0	22.58	0.26	7.60	0.477	0.0	76
"	"	8.0	22.58	0.19	7.62	0.477	0.0	70
LT-19B	Lapaine	0.5	22.74	2.58	7.83	0.486	0.0	53
"	"	1.0	22.71	2.54	7.83	0.486	0.0	51
"	"	2.0	22.61	2.21	7.81	0.487	0.0	50
"	"	3.0	22.60	2.16	7.80	0.486	0.0	49
"	"	4.0	22.60	2.11	7.80	0.486	0.0	47
"	"	5.0	22.59	2.16	7.81	0.485	0.0	45
"	"	6.0	22.58	2.09	7.80	0.485	0.0	43
"	"	7.0	22.54	1.73	7.78	0.486	0.0	41
"	"	8.0	22.51	1.66	7.77	0.486	0.0	39
"	"	9.0	22.5	1.49	7.74	0.487	0.0	37
"	"	10.0	22.5	1.22	7.72	0.487	0.0	33
"	"	11.0	22.5	1.42	7.70	0.489	0.0	30

(Continued)

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Appendix C. Continued

Station Number	Station Name	Depth[1] (m)	Temp.[2] (C)	Dissolved Oxygen[3] (mg/L)	pH[4]	Conductivity[5] (mS/cm)	Salinity (ppt)	Redox Potential[6] (mV)
				Kaunas Reservoir				
LT-20	Kaišiadorių	0.5	22.81	1.13	7.60	0.476	0.0	114
"	"	2.0	22.85	1.13	7.62	0.477	0.0	109
"	"	3.0	22.85	1.05	7.64	0.477	0.0	104
"	"	4.0	22.85	1.03	7.65	0.477	0.0	101
"	"	6.0	22.73	0.91	7.63	0.478	0.0	122
"	"	8.0	22.71	0.65	7.63	0.477	0.0	116
"	"	10.0	22.30	0.54	7.60	0.490	0.0	115
"	"	12.0	22.09	0.55	7.58	0.496	0.0	113
LT-21	Dambrava-IV	0.5	22.48	4.76	7.72	0.461	0.0	117
"	"	2.0	22.51	4.91	7.74	0.459	0.0	115
"	"	4.0	22.50	4.99	7.81	0.458	0.0	113
"	"	6.0	22.44	5.56	7.83	0.456	0.0	112
"	"	8.0	22.30	4.49	7.70	0.456	0.0	115
"	"	10.0	22.17	3.14	7.61	0.456	0.0	116
"	"	12.0	22.15	2.74	7.59	0.456	0.0	115
"	"	14.0	22.17	2.71	7.59	0.453	0.0	99
LT-22	Pažaislis-VII	0.5	22.22	8.44	7.93	0.443	0.0	141
"	"	1.0	22.05	5.22	7.81	0.446	0.0	140
"	"	2.0	21.59	3.45	7.62	0.447	0.0	146
"	"	4.0	21.45	3.25	7.55	0.488	0.0	145
"	"	6.0	21.37	2.98	7.53	0.448	0.0	145
"	"	8.0	21.28	2.84	7.52	0.449	0.0	144
"	"	10.0	21.18	2.54	7.50	0.447	0.0	142
"	"	12.0	21.12	2.31	7.49	0.449	0.0	141
"	"	14.0	20.75	1.45	7.46	0.450	0.0	142
"	"	16.0	20.66	1.21	7.44	0.450	0.0	141
"	"	18.0	20.67	1.31	7.45	0.449	0.0	139
"	"	20.0	20.65	1.23	7.45	0.450	0.0	138
				Nemunas River				
LT-23B	Lampedžiai-B	Sur	20.97	12.02	8.52	0.466	0.0	73
LT-15B	Zapyškis-B	Sur	20.56	5.14	7.47	0.464	0.0	93
LT-14	Smalininkai	Sur	20.25	11.88	8.42	0.462	0.0	74
LT-13-1	Sovetskas	Sur	20.55	10.28	7.88	0.516	0.0	75
LT-28A	Rusnė-A	Sur	20.72	11.70	8.21	0.484	0.0	82

(Continued)

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Appendix C. Continued

Station Number	Station Name	Depth[1] (m)	Temp.[2] (C)	Dissolved Oxygen[3] (mg/L)	pH[4]	Conductivity[5] (mS/cm)	Salinity (ppt)	Redox Potential[6] (mV)
				Kuršių Marios Lagoon				
LT-29	Deimė	0.5	21.40	3.88	7.48	0.593	0.0	86
"	"	2.0	21.21	3.76	7.50	0.593	0.0	83
"	"	4.0	20.90	2.89	7.47	0.592	0.0	83
"	"	6.0	20.85	2.75	7.46	0.590	0.0	81
"	"	8.0	20.84	2.77	7.44	0.590	0.0	75
"	"	10.0	20.84	2.66	7.43	0.590	0.0	75
LT-06	Gilija	0.5	20.93	8.68	7.75	0.495	0.0	105
"	"	1.5	20.29	9.35	8.01	0.481	0.0	97
LT-30	Rasytė	0.5	19.72	9.71	8.37	0.475	0.0	88
"	"	2.0	19.69	9.60	8.33	0.475	0.0	87
"	"	4.0	19.17	7.28	8.02	0.480	0.0	95
LT-07	Uostadvaris	0.5	20.18	11.03	8.47	0.467	0.0	57
"	"	2.0	20.14	10.96	8.45	0.468	0.0	53
"	"	4.0	20.12	10.75	8.40	0.467	0.0	52
"	"	6.0	20.13	10.77	8.36	0.467	0.0	50
LT-8	Nida Station	0.5	19.83	7.17	8.08	0.476	0.0	106
"	"	2.0	19.70	7.20	8.03	0.477	0.0	108
LT-10A	Klaipėda Harbor-A	Sur	19.27	9.22	8.34	5.30	2.4	115
				Baltic Sea Coast				
LT-11A	Melnragė-A	Sur	18.84	8.45	8.18	11.72	6.4	78
LT-12	Palanga	Sur	19.78	8.57	8.48	10.25	5.5	78
"	"	1.5	19.73	8.40	8.50	10.26	5.5	72

[1]Depth probe not operating - actual depth measurements reported. Sur = approximately 0.3-0.5 m below surface
[2]Temperature accuracy ±0.2° [3]DO accuracy ± 2% [4]pH accuracy ±0.2 units
[5]Conductivity accuracy ±1% of range, resolution 4 digits [6]Redox accuracy ±20 mV

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Appendix D. Hydrochemical and physical water quality data, June 1992

Station Number	Station Name	Depth[1] (m)	Temp.[2] (C)	Dissolved Oxygen[3] (mg/L)	pH[4]	Conductivity[5] (mS/cm)	Salinity (ppt)	Redox Potential[6] (mV)
				Lakes				
LT-40	Lake Dusia	Sur	19.81	9.32	8.46	0.327	0.2	302
LT-43	Lake Drūkšiai	Sur	22.14	17.15	9.49	0.320	0.2	264
				Vilnia River				
LT-34	New Vilnia	Sur	15.80	12.18	8.08	–	–	297
LT-04	Gediminas Castle	Sur	17.64	11.44	8.17	0.509	0.3	298
				Neris River				
LT-03B	Turniškės-B	Sur	19.23	13.59	8.62	–	–	279
LT-31	Grigiškės	Sur	19.29	13.20	8.55	–	–	276
				Nevėžis River				
LT-26B	Raudondvaris	Sur	19.41	10.52	8.52	0.421	0.2	333
				Nemunas River				
LT-38	Sredny Gonkolsky	Sur	21.64	9.78	8.51	0.377	0.2	284
LT-39	Liškiava	Sur	20.16	7.99	8.31	0.366	0.2	305
LT-44	Crusader Creek	Sur	11.71	9.73	8.10	0.460	0.2	311
LT-41	Alytus	Sur	21.11	9.76	8.55	0.399	0.2	303
LT-15C	Kulautuva-C	Sur	19.53	10.42	8.53	0.431	0.2	322
LT-13-2	Sovetskas	Sur	21.45	14.98	8.97	0.405	0.2	286
LT-28B-2	Rusnė-B	Sur	21.98	14.36	8.94	0.400	0.2	276
				Kurišių Marios Lagoon				
LT-29	Deimė	Sur	22.10	6.07	8.10	0.558	0.3	330
"	"	5	21.93	5.15	7.99	0.560	0.3	331
"	"	10	21.65	4.11	7.89	0.558	0.3	334
"	"	12	26.64	3.95	7.86	0.556	0.3	334
LT-06	Gilija	Sur	20.16	11.07	8.95	0.424	0.2	292
"	"	2.5	19.98	10.37	8.95	0.428	0.2	317
LT-30	Rasytė	0.7	20.46	8.79	8.69	0.441	0.2	319
"	"	2.2	20.47	8.83	8.75	0.442	0.2	314
LT-07	Uostadvaris	Sur	22.08	13.34	8.84	0.411	0.2	309
"	"	4.5	21.50	9.89	8.57	0.424	0.2	322
LT-08	Nida Station	Sur	20.50	7.99	8.57	0.429	0.2	310
				Baltic Sea Coast				
LT-11B	Melnragė-B	0.6	7.63	7.81	7.61	12.56	7.2	392
"	"	8	6.59	7.25	7.57	12.62	7.2	389
LT-11C-2	Melnragė-C	Sur	6.83	7.33	7.31	12.54	7.2	409
"	"	10	6.58	6.95	7.48	12.57	7.2	396
LT-11D-2	Melnrage-D	Sur	7.06	6.77	7.58	12.66	7.3	420
"	"	10	6.89	6.88	7.59	12.69	7.3	425

[1]Depth probe accuracy ±0.45 m, Sur = approximately 0.3-0.5 m below surface [2]Temperature accuracy ±0.2° [3]DO accuracy ± 2% [4]pH accuracy ±0.2 units [5]Conductivity accuracy ±1% of range, resolution 4 digits [6]Redox accuracy ±20 mV

APPENDIX B

CHEMICALS IDENTIFIED AT TWO PESTICIDE STORAGE SITES IN LITHUANIA, 1990-1993

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N. Štriupkuvienė, and J.F. Neuman

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CHEMICALS IDENTIFIED AT TWO PESTICIDE STORAGE SITES IN LITHUANIA, 1990-1993

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N. Štriupkuvienė[2], and J. F. Neuman[3]

ABSTRACT

Water samples were collected from two sites in Lithuania being used to store hazardous chemicals, and these samples were analyzed to determine what chemicals might migrate from these sites into the surrounding environment. One of these sites, at Zigmantiškės, is a controlled landfill for banned, unsuitable, and unknown pesticides. Water samples were collected from wells inside the boundary of this landfill on five occasions during the period 1990-1993, and on two occasions during 1993 immediately outside the landfill boundary. Chemicals considered to be mobile in underground water, *e.g.*, s-triazine herbicides, were identified in water samples from inside the boundary but were below the level of detection in samples from outside the boundary. The second site is a warehouse near Utena that is being used as a storage facility for toxic chemicals. A fire occurred at the warehouse in April 1993, and water used to extinguish the fire seeped into the warehouse basement and its drainage system. Water was subsequently pumped from the basement into temporary storage tanks to reduce the amount of contaminated water seeping into the local aquifer. In June and October 1993 water samples were collected for analysis from the basement, the drainage system, and the storage tanks. Atrazine and several chlorinated carboxylic acids, including 2,4-D, were identified in all of these samples.

INTRODUCTION

A field study on the quality of surface waters in the Republic of Lithuania was initiated in 1990 under the sponsorship of the risk assessment program of the United States Environmental Protection Agency (U.S. EPA), Environmental Research Laboratory, Athens, Georgia (ERL-Athens). During the course of this study, two sites that did not involve surface waters were also investigated, one at Zigmantiškės and the other at Utena.

The Zigmantiškės site is a controlled landfill established by the Ministry of Agriculture of Lithuania for the storage of banned, unsuitable, and unknown pesticides and related wastes; it is located 40 km southwest of Vilnius, between the Merkys and Šalčia Rivers. The perimeter of the landfill is enclosed by a fence and monitoring wells

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have been drilled both inside and outside the perimeter (Figure 1). The second site is a warehouse near Utena that is now being used as an agrochemical storage facility. It is located 115 km north of Vilnius, in the Šventoji River watershed. Approximately 32,000 kg of pesticides and unknown powders had been collected and placed in temporary storage at the warehouse when, in April 1993, a fire started somewhere within the mass of the stored pesticides. Water, used to extinguish the flames, drained into the basement of the warehouse, and although much of this runoff water was collected by pumping into holding tanks, some of it reached an underground drainage system that empties to a nearby stream.

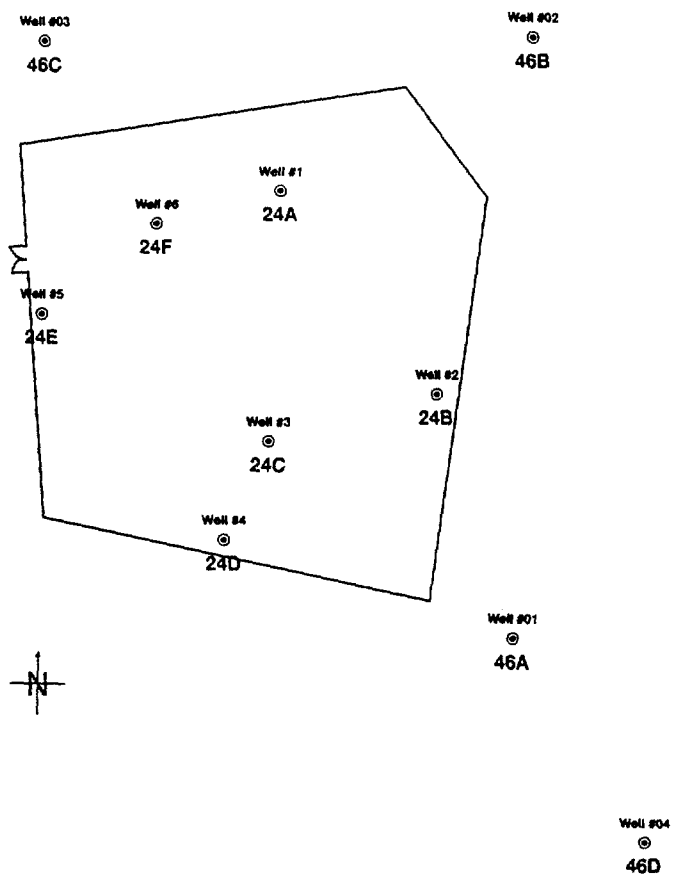

Figure 1. Zigmantiškės Pesticide Storage Site.

Water samples were collected from the Zigmantiškės site on five occasions between 1990 and 1993, and from the Utena site on two occasions in 1993. These water samples were passed through cartridges or disks containing sorbent media to extract the organic chemicals, and the cartridges and disks were taken to ERL-Athens for elution and identification of the sorbed organics. This manuscript presents the results of these analyses.

METHODS

Water samples were collected from monitoring wells inside the fenced boundary at the Zigmantiškės site in December 1990, July 1991, June 1992, June 1993, and October 1993. In May 1993, monitoring wells were installed outside the fenced boundary by direction of the Environmental Protection Department (EPD), and in June and October 1993 samples were also collected from these monitoring wells. Water samples were collected from three locations at the Utena site in June and October 1993.

The cartridges and disks used for extraction of organic chemicals from water samples in this study were pre-cleaned at ERL-Athens, and activated in Lithuania. After water samples were collected, those to be later analyzed for organic acids were adjusted to pH 2 with sulfuric acid before passage through a cartridge or disk. After return to ERL-Athens, the cartridges and disks were eluted with organic solvent. Those eluants to be analyzed for organic acids were treated with diazomethane before chromatographic/ spectral analysis. Gas chromatographs (GC) were used to separate the analytes in the extracts. These were then identified using low and high resolution mass spectrometers (MS) and Fourier transform-infrared spectrometers (FT-IR) interfaced to the GC. The methods used for the preparation and activation of the cartridges and disks, collection of samples, extraction of the organic chemicals, and identification of the extracted organics has been reported by Ellington *et al.* (1994).

RESULTS

Zigmantiškės Pesticide Storage Site

Organic chemicals identified from water samples collected at the Zigmantiškės storage site are listed in Table 1. Dialkyl phthalates are commonly found in environmental samples, so stringent precautions are necessary to assure their presence in the collected samples is not due to accidental contamination. Hydrocarbons, phthalates, and fatty acids were found in a few of the method and field blanks, although at lower levels than in the groundwater samples. At the present time, it is not known whether the phthalates identified in the groundwater samples are representative of the ground water from which the samples were taken, or whether they resulted from contamination during collection and/or sample preparation in the field prior to analysis.

Analysis of the extract of a groundwater sample taken from monitoring well #6 (LT-24F) during December 1990 revealed the presence of dimethyl sulfone, caprolactam, 2,4-dichlorobenzoic acid (derivatized to form the methyl ester), simazine, atrazine, and propazine. The latter three compounds are s-triazine herbicides that have been detected worldwide in ground and surface waters. Atrazine is one of the most heavily used herbicides worldwide, with global release in 1980 estimated at 90,000 tons (Rippen 1987). The identities of the s-triazines and the dichlorobenzoic acid were confirmed with standards. We believe the 2,4-dichlorobenzoic acid is most likely a product of degradation of a more complex chemical. The 2,4-dichlorobenzene fragment is part of

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the structure of several agricultural and/or industrial chemicals including chlorfenvinphos, propiconazole, and polychlorinated biphenyls, whose degradation in the environment would yield 2,4-dichlorobenzoic acid. Caprolactam is a monomer used in the synthesis of high molecular weight polymers including Nylon 6, and dimethyl sulfone is also a solvent for high temperature reactions.

Table 1. Anthropogenic chemicals identified in water samples from monitoring wells at the Ministry of Agriculture Pesticide Storage Site, Zigmantiškės (LT-24).

Alkyl hydrocarbons[1]	2,2-Dimethoxy-1,2-diphenyl ethanone[1]	Pristane[2]
Atrazine[2]	Dimethyl sulfone[1]	Prometryn[2]
Benzophenone[1]	Fatty acids[1]	Propazine[2]
Caprolactam[1]	Lenacil[2]	Pyridine[1]
Desmetryn[2]	Lindane[2]	Silicon organics[1]
Dialkylthiophene[1]	2-(Methylthio) benzothiazole[1]	Simazine[2]
2,4-Dichlorobenzoic acid[2]	Phthalates[2,3]	Tetrachlorinated unknowns[1]
Dichlorprop[1]	Phytane[2]	

[1]Identified by GC/FT-IR/MS but not confirmed with standards
[2]Identified by GC/FT-IR/MS and confirmed with a standard
[3]Diisooctyl-, Butylbenzyl-, Di-n-octyl-, and Di-n-butylphthalates were observed the most frequently

In July 1991, new compounds identified in water samples taken from monitoring well #6 were benzophenone, pyridine, pristane, and phytane. Three compounds that contained four chlorine atoms each were detected in the acidified sample from well #6 after elution of organics from a solid phase extraction (SPE) cartridge and treatment of the concentrated eluant with diazomethane. A molecular weight of 348 was determined by chemical ionization GC/MS for all three compounds, indicating they were isomers. These isomers were also found in samples from the Neris River and Kaunas Reservoir (Ellington *et al.* 1994). Best fit empirical formulae of $C_7H_{13}Cl_4O_5P$ and $C_{14}H_8Cl_4O_2$ were determined by high resolution GC/MS. A tetrachlorinated trialkyl phosphate fits the former formula while tetrachlorinated aromatic esters and other structures containing carbonyl groups fit the latter. Both formulae are similar to those of compounds commonly used as electrical insulators and flame retardants. The compound containing phosphorus was eliminated as a possibility by further GC analysis using nitrogen and nitrogen-phosphorus specific detectors. The presence of the carbonyl functional group in the three unknowns was confirmed by GC/FT-IR. The loss of the m/z 31 ion (loss of $-OCH_3$) during low resolution electron impact GC/MS argued for the presence of a methoxy functional group, possibly a methyl ester. However, the absence of a strong peak in the 1250-1310 cm^{-1} region of the IR spectrum and the broad carbonyl peaks argued against the ester functionality. Detection of the unknowns only after treatment of the extract with diazomethane is evidence that they contained a reactive hydrogen. A tetrachlorinated aromatic ring system containing a carbonyl group, methoxy group, and differing chlorine substitution is the most likely structure for the

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three unknown isomers. The presence of pristane and phytane could be an indication of contamination by crude oil, while benzophenone and pyridine are used in the synthesis of organics.

In June 1992, samples were also taken from wells #1, 2, 3, and 5 (LT-24A-C,E) which had not been sampled during previous trips. Chemicals in a sample from well #5 (LT-24E), first tentatively identified by GC/MS and/or GC/FT-IR/MS, and subsequently confirmed with standards, were: atrazine, desmetryn, lenacil, lindane, prometryn, propachlor, propazine, and simazine. Chemicals tentatively identified in this same sample, but not confirmed with standards, included 2,2-dimethoxy-1,2-diphenyl ethanone, dialkylthiophene, and 2-(methylthio)thiophene.

During June and October 1993, water samples were collected from three of the four monitoring wells (LT-46A-D) installed outside the boundary fence of the landfill in May 1993. Chemicals tentatively identified by GC/FT-IR/MS in extracts of samples from wells #01 and 02 (LT-46A and B), downgradient from the underground water flow from the landfill, were sulfur, dibutyl- and dioctylphthalates, hydrocarbons, and carboxylic acids. These chemicals were almost identical to those found in the water taken from well #4 (LT-24D) located 1.5 m inside the boundary and upgradient from well #01 outside the boundary. Except for phthalates and hydrocarbons, none of the chemicals identified in samples from wells #1, 2, 3, 5, or 6 inside the boundary appeared in samples from well #4 also inside the boundary, or wells #01 or 02 outside the boundary. Phthalates and hydrocarbons were detected in trace amounts in the majority of samples, and we believe their presence was due to contamination from equipment during sampling procedures. The absence in wells outside the boundary of other chemicals found in wells inside the boundary, especially the extremely mobile chemical atrazine, is evidence that chemicals at the Zigmantiškės site have not migrated beyond the landfill boundary at this time.

Utena Pesticide Storage Warehouse

The chemicals stored in the warehouse at Utena at the time of the fire in April 1993 had been inventoried by the Lithuania EPD and the Ministry of Agriculture. The inventory showed 31,586 kg of pesticides and unknown powders, with the unknown powders (23,730 kg) and unknown solids (1,610 kg) comprising the majority of that inventory. The remaining pesticides consisted of fentiuramas (3,458 kg), 2% ethylmercury chloride (190 kg), ziramas (970 kg), nitrophen (1,070 kg), and DNOC (2-methyl-4,6-dinitrophenol, 558 kg) (LEPD 1993). After the fire, runoff water was pumped from the basement of the warehouse into two 50,000-L tanks (LT-45C,D) to prevent environmental contamination from water seeping into the drainage system and into a nearby creek. The volume of water in both tanks was approximately 26,000 L. In June 1993 a 100 ml sample of water from LT-45D was extracted by EPA Method 505 (base/neutrals) and EPA method 515.1 (chlorinated acids), and the extracts were analyzed by GC/MS (U.S. EPA 1991). Compounds identified from this sample are listed in Table 2.

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Table 2. Anthropogenic chemicals[1] identified in water samples collected at the Toxic Chemical Storage Warehouse, Utena (LT-45). (Detected concentrations were in the ppb range.)

Chemicals by class[2]	LT-45A	LT-45D	LT-45E
Carboxylic acids			
Chlorobutenoic acid		x	
Chloropropanoic acid		x	
Dichloroacetic acid		x	
Dichlorobutenoic acid		x	
Dichloropropanoic acid		x	
Trichloropropanoic acid		x	
Trichloroacetic acid	x	x	
Phenols			
Chloromethylphenol		x	
Dichlorophenol		x	
Dimethylphenol		x	
Methylphenol		x	
Trichlorophenol		x	x
Phenoxycarboxylic acids			
Chloromethylphenoxyacetic acid	x	x	
Chloromethylphenoxybutanoic acid		x	
Dichlorophenoxyacetic acid	x	x	x
Dichloromethylphenoxyacetic acid		x	x
Dichlorophenoxybutanoic acid		x	
Dimethylphenoxyacetic acid		x	
Dimethylchlorophenoxyacetic acid		x	
N,N-Dimethyl-2-(chloromethylphenoxy)-acetamide		x	
Methylphenoxyacetic acid		x	
Trichloromethylphenoxyacetic acid	x	x	x
s-Triazine/phosphate pesticides			
Atrazine	x	x	
Propazine	x	x	
Simazine	x	x	
Vapona		x	
Ureas and thiooxamides			
Lenacil		x	
Tetramethylurea	x	x	x
Tetramethylthiourea		x	
N,N,N',N'-Tetramethylthiooxamide		x	
N,N,N',N'-Tetramethyldithiooxamide		x	

[1]The EPA/NIH Mass Spectral Library was used for tentative identification of the chemicals identified by "x". Atrazine, simazine, propazine, and lenacil were confirmed with standards.

[2]High resolution GC/MS was used to determine the molecular ions and empirical formulas. Identical empirical formulas for some peaks indicated isomers but the structures of the individual isomers could not be determined from the spectral information.

Mercury was suspected to be in the water in the holding tanks, so in the summer of 1993 the Lithuania EPD attempted to precipitate this mercury by the addition of ferric chloride. In addition, calcium hypochlorite was added to degrade other pesticides. Water in LT-45C was transferred to LT-45D, and the combined contents were treated with $FeCl_3 \cdot 6H_2O$ and $Ca(OCl)_2$. In October 1993, samples of water from the ferric chloride-treated water (LT-45E) and from the underground drainage system (LT-45A) were extracted and the resulting base/neutral and acid fractions were analyzed by GC/MS. The compounds identified in these two samples are also listed in Table 2.

Three chemicals, namely, tetramethylurea, and dichloro- and trichloromethylphenoxyacetic acids, were detected in all three samples. The ferric chloride/calcium hypochlorite treatment had reduced the concentrations of many chemicals below their detection level, and the chemicals that were detected in that sample were factors of 2-8 below the levels previously determined in the untreated run-off water (LT-45D). The concentration of isomers of hexachlorocyclohexane (HCH), namely γ-HCH (lindane), and α-HCH, was reduced 50- to 200-fold by the ferric chloride/calcium hypochlorite treatment from initial concentrations down to concentrations of 0.06-0.11 ppb. The treatment also reduced the concentrations of dichlorophenoxyacetic acids from 250 down to 30 ppb, and tetramethylurea was reduced from 600 to 200 ppb. The corresponding concentrations for these two chemicals in the drainage system water (LT-45A) were 12 ppb and 30 ppb, respectively. The s-triazine herbicide concentrations ranged from 250 ppb in the holding tank water to 10 ppb in the drainage system water, and was not detected in the treated water.

CONCLUSIONS AND RECOMMENDATIONS

The Zigmantiškės Pesticide Storage Site is only one of several possible groundwater pollution sources in Lithuania. The ferric chloride/calcium hypochlorite treatment of the runoff water in the tanks at Utena substantially lowered the concentrations of many of the chemicals, some to below their detection limits, but the toxicity of the treated water remains unknown. Chemicals in groundwater have the potential to migrate and contaminate off-site water supplies, and for this reason monitoring should be continued in the area of the Zigmantiškės site and should be expanded to include other such facilities as they are identified. The monitoring at the Zigmantiškės site, now that several of the chemicals in the underground water have been identified, could be based on an immunoassay test for the most mobile chemical. Atrazine, because of its water solubility and low partitioning to organic carbon, is one of the more mobile chemicals identified in samples from both Zigmantiškės and Utena. Commercial immunoassay test kits are available for atrazine and could be used to monitor its movement in underground water at both sites. These kits are relatively inexpensive, their use does not require extensive technical training, and the results can be provided on location within minutes.

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APPENDIX C

TRACE METAL CONCENTRATIONS IN NATURAL WATERS IN LITHUANIA, 1991-1992

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TRACE METAL CONCENTRATIONS IN NATURAL WATERS IN LITHUANIA, 1991-1992

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ABSTRACT

The concentrations of ten metals in natural waters in Lithuania were measured in 1991 and 1992 to understand the influence of industrial wastes on the quality of those waters. The analyses were performed by atomic absorption spectroscopy. Samples from the Kulpė River at Šiauliai and from the Baltic Sea near Klaipėda Harbor had high trace metal concentrations. Hydrological parameters such as pH, dissolved oxygen, conductivity, redox potential, and salinity were also measured; these showed significant differences among the sampling sites.

INTRODUCTION

Low mineral levels and simple chemical composition are general characteristics of Lithuanian rivers (Garunkštis 1988). The anthropogenic chemical composition of small rivers in Lithuania is usually determined by the chemicals used in agriculture, and the composition of large rivers is influenced mostly by sewage from industrial enterprises and big cities. Lithuanian rivers are generally alkaline because dissolved calcium, magnesium, and carbonate ions comprise up to 85% of all dissolved substances. The abundance of these ions is caused by carbonated sediments and by climatic conditions that stimulate the leaching of carbonates. The lowest levels of carbonates are found in the small rivers, flowing from marsh waters (Garunkštis 1988).

Of the area of Lithuania, 70 percent (46,600 km^2) falls within the Nemunas River basin, so the water quality of most rivers in Lithuania influence the quality of the Nemunas River and Kuršių Marios Lagoon, into which the Nemunas River flows. Kuršių Marios Lagoon (1,610 km^2) is the largest reservoir of fresh water in Lithuania. In addition to Kuršių Marios Lagoon being the recipient of pollutants discharged from the Nemunas River, other major sources of pollution to the lagoon are the discharge of the Deimė River from Kaliningrad and several wastewater outfalls from the City of Klaipėda into Klaipėda Harbor, a major shipping port. Approximately 26 factories discharge their contaminated waters into the northern part of the lagoon (Griškevičius 1987).

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The number of analyses performed on river waters to measure trace metals has greatly increased in recent years due to improvements in analytical techniques used for geoexploration and environmental management (Salomons and Förstner 1984). There are very significant regional differences with respect to various processes occurring in rivers and other natural water basins, to concentrations of different elements, and to compounds and poisonous effects which they induce (Anikiev *et al.* 1990, Nimmo *et al.* 1989). Natural water, especially river water, has a direct influence on groundwater, which is used as drinking water (Pelig-Ba *et al.* 1991), with resultant effects on humans (McClain and Becker 1975). Until 1990, there were no detailed investigations of heavy metals in natural waters in Lithuania, mainly because of the lack of adequate instrumentation.

The present study was undertaken during 1991-1992 to assess the distribution of trace metals in Lithuanian surface waters. Water samples were collected and analyzed for 10 trace metals at 23 sites on five rivers, Kaunas Reservoir, Kuršių Marios Lagoon, and the Baltic Sea at the mouth of Klaipėda Harbor (Figure 1, Table 1). Twenty of these sites were sampled during July 1991, a representative climate year, and 15 sites were sampled during June 1992, following an especially dry spring. Eight of the sites were sampled both years, enabling comparison between the representative and dry periods.

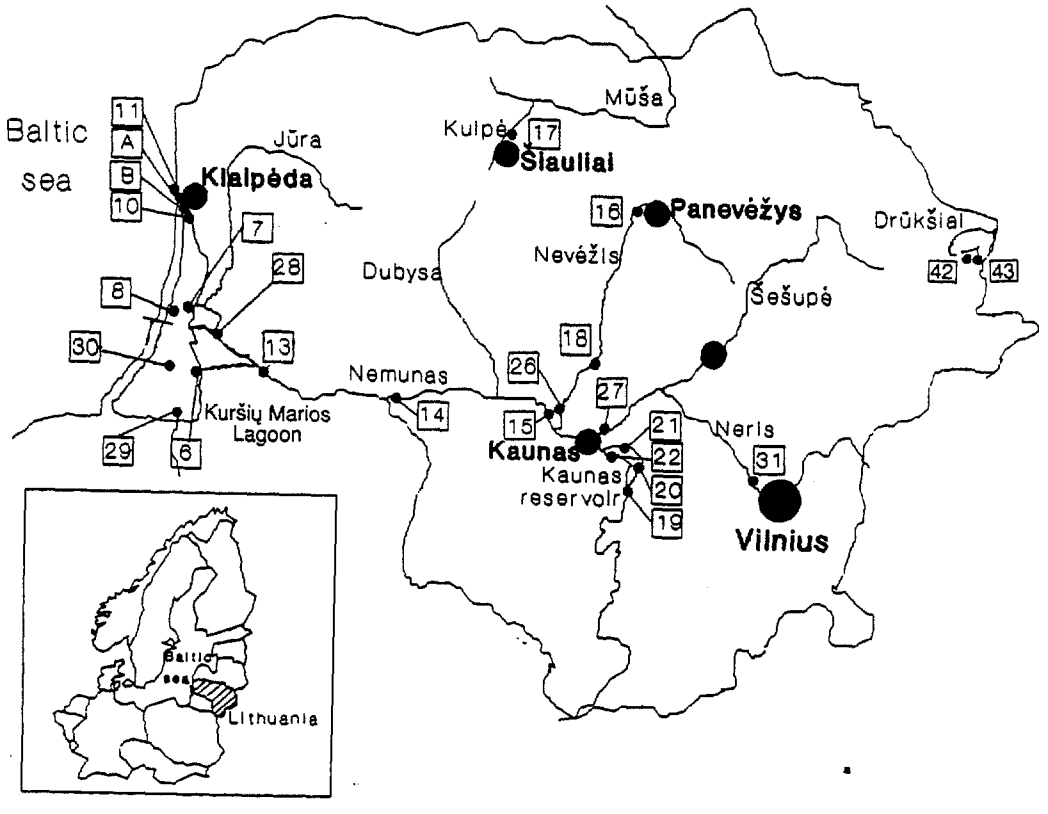

Figure 1. Map of surface water sampling sites.

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Table 1. Description of surface water sampling sites.

Number	Name	Description
LT-06	Gilija	Kuršių Marios Lagoon at mouth of Gilija River
LT-07	Uostadvaris	Kuršių Marios Lagoon from the Neringa Spit 17 km east of Nida
LT-08	Nida Station	Kuršių Marios Lagoon 1.5 km off the dock at Nida
LT-10A-B	Klaipėda Harbor	Klaipėda Harbor (A) north side of mouth of Dané River, (B) middle of channel off Dané River
LT-11A-B	Melnragė	Entrance to Klaipėda Harbor at the Baltic Sea, (A) east side of east breakwater, (B) west side of east breakwater
LT-13-1-3	Sovetskas	Nemunas River at Sovetskas, (1) south bank, (2) middle of channel, (3) north bank
LT-14	Smalininkai	Nemunas River north bank, ca 50 km upstream from Sovetskas
LT-15B-C	Kulautuva	Nemunas River north bank, 20 km downstream from Kaunas and 13 m downstream from Nevėžis River-Nemunas River confluence, (B) south bank at Zapyškis, (C) north bank ca 1 km below Zapyškis
LT-16	Panevėžys	Nevėžis River 11 km downstream from Panevėžys and 1 km below the discharge pipe of wastewater treatment facility
LT-17	Šiauliai	Kulpė River at Šiauliai
LT-18	Kėdainiai	Nevėžis River, east bank, 1.5 m below wastewater treatment facility
LT-19A	Nemunas, II Cut	Nemunas River just before emptying into Kaunas Reservoir
LT-20	Kaišiadorių	Kaunas Reservoir, 300 m offshore from HydroAccumulation Electric Power facility (HAEP)
LT-21	Dambrava, IV Cut	Kaunas Reservoir below HAEP
LT-22	Pažaislis, VII Cut	Kaunas Reservoir at dam, within sight of Pažaislis Monastery
LT-26B	Raudondvaris	Nevėžis River, west bank, 10 km west of Kaunas
LT-27	Neris at Kaunas	Neris River immediately above bridge before confluence with Nemunas River
LT-28A-B	Rusnė	Nemunas River, delta region near Rusnė, (A) 10 km upstream from Kuršių Marios Lagoon, (B) ca 12 km upstream from Kuršių Marios Lagoon
LT-29	Deimė	Kuršių Marios Lagoon, southernmost section in the mouth of Deimė River
LT-30	Rasytė	Kuršių Marios Lagoon, in the "center"
LT-31	Grigiškės	Neris River, 17 km west of Vilnius
LT-42	Visaginas	Sewer line from Ignalina NPP, near Lake Drūkšiai
LT-43	Drūkšiai	Lake Drūkšiai, south shore of west arm

METHODS

All samples were collected at a depth of approximately 0.5 m. In 1991, samples were stored in nitric acid-washed 50-ml plastic bottles to which one drop of metal-free nitric acid had been added, and then stored refrigerated until analyzed within 4 months of collection. Sampling and analysis in 1992 was identical to 1991, with the exception that glass bottles were used instead of plastic bottles. One sample from each location was analyzed by a graphite furnace atomic absorption spectrophotometer Zeeman/3030, Perkin-Elmer, USA. Ten trace metals--cadmium, chromium, cobalt, copper, iron, lead, manganese, nickel, vanadium, and zinc--were analyzed, and six hydrological parameters--pH, dissolved oxygen, conductivity, redox potential, salinity, and temperature--were measured. Detection limits and calibration concentration ranges of the instrument for different elements are reported in Table 2. These were determined by methods similar to those prescribed in the instrument operating manual. No matrix modifiers were used. Operational conditions are reported by Kvietkus *et al.* (1994).

Table 2. Detection limits and calibration concentration ranges of the instrument (AAS).

Metal	Calibration concentration ranges (μg/L)	Detection limit (μg/L)	Sampling quantity (μL)
Pb	10-50	0.5	50
Cu	10-50	0.5	40
Ni	10-50	0.8	80
Co	10-50	0.9	60
V	10-50	1.0	100
Cr	10-50	0.5	20
Zn	10-50	0.3	5
Fe	20-100	1.0	20
Cd	1-5	0.2	40
Mn	10-50	0.3	20

At the same time water samples were collected for trace metal analysis, on-site water quality measurements were made at most stations using a Hydrolab™ Surveyor 2 (Hydrolab Corporation, Austin, Texas, USA) equipped with pH, temperature, dissolved oxygen (DO), conductivity, redox potential, and depth sensors. The pH electrode (accuracy ±0.2 units, resolution ±0.1 unit) was calibrated with pH 3, 7, and 10 buffer

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solutions. The temperature sensor (accuracy ±0.2°C, resolution ±0.01) was verified by simultaneous immersion in a flask of water with a thermometer certified by the U.S. National Institute of Standards and Technology. The DO sensor (accuracy ±2%, resolution 0.01 mg/L) was calibrated by the air saturation method. The conductivity sensor (accuracy ±1% of range, resolution 4 digits) was calibrated with solutions of potassium chloride prepared with distilled, deionized water. The redox potential sensor (accuracy ±20 mV) was calibrated with aqueous solutions of quinhydrone.

RESULTS AND DISCUSSION

Trace Metals

With the exception of zinc, the measured concentrations of metals (Table 3) at stations where water samples were taken in both 1991 and 1992 varied by a factor of five or less. The concentrations of zinc in the 1992 samples were an order of magnitude or more greater than in samples collected at corresponding locations in 1991. We believe that in 1992 there was an unidentified error affecting the measurement of zinc, possibly contamination related to preservation and/or storage of samples prior to analysis. Accordingly, although we are reporting the zinc values obtained for 1992, they will not be considered further in the discussion.

Freshwater chronic toxicity criteria (FCT) values recommended by the U.S. EPA (1992) for six of the metals (cadmium, chromium, copper, lead, nickel, and zinc) are listed in Table 3 Footnote 1; the maximum contaminant levels (MCL) used in the former USSR (Bespamiatnov and Krotov 1985) for cobalt, iron, vanadium and manganese are listed in Footnote 2.

In 1991, out of 200 individual measurements, 18 of these exceeded either the FCT or MCL criteria, and of these, seven measurements exceeded these criteria by more than two-fold. In 1992, 150 measurements were made. Excluding zinc, out of the remaining 135 measurements none exceeded MCL criteria, and although 11 exceeded FCT criteria, only two of these, both for lead, exceeded the criteria more than two-fold. Although the number of values exceeding FCT and MCL criteria were fewer in 1992 than 1991, part of the reason for this is that in 1992 we did not resample some of the sites found to be most polluted based on the 1991 sampling.

Of all metals measured, iron most often exceeded the MCL criterion; this happened in 13 out of 35 samples. The only location at which iron was markedly higher than the criterion, however, was the Nemunas River at Rusnė (LT-28). We sampled the Kulpė River at Šiauliai (LT-17) in 1991 and obtained measurements for lead, copper, and chromium that exceeded FCT criteria values by factors of two, three, and 50 respectively. Cadmium, copper, and lead measurements at Melnragė (LT-11), also made in 1991, exceeded FCT criteria by two-, five-, and 50-fold. Klaipėda Harbor (LT-10) was sampled in both 1991 and 1992, and although the measurement for lead in the single sample collected in 1991 was below the detection limit, lead in samples collected in 1992 at two separate locations were five- and ten-fold the FCT criterion.

Table 3. Trace metal concentrations in natural waters in Lithuania (values expressed in μg/L).

Site number	Pb[1] 1991	Pb[1] 1992	Cu[1] 1991	Cu[1] 1992	Ni[1] 1991	Ni[1] 1992	Co[2] 1991	Co[2] 1992	V[2] 1991	V[2] 1992
LT-06	BD[3]	BD	2.7	5.7	1.8	0.8	0.9	BD	2.4	1.1
LT-07	BD	BD	1.7	3.6	1.2	2.0	BD	1.7	1.3	BD
LT-08	0.5	0.9	2.9	4.5	0.9	1.1	BD	BD	1.8	1.0
LT-10A	BD	59.0	2.7	2.9	1.2	5.5	3.0	61.0	BD	6.1
LT-10B	–[4]	16.0	–	3.0	–	3.5	–	160	–	5.1
LT-11A	152	–	66.0	–	29.5	–	29.0	–	10.9	–
LT-13-1	BD	BD	6.3	7.4	2.2	1.6	BD	0.9	1.5	1.5
LT-13-2	–	BD	–	2.6	–	0.8	–	1.0	–	1.0
LT-13-3	–	BD	–	2.4	–	1.0	–	1.7	–	1.4
LT-14	1.8	–	10.4	–	2.8	–	BD	–	1.2	–
LT-15B	BD	–	0.8	–	1.3	–	BD	–	BD	–
LT-15C	–	BD	–	6.3	–	2.1	–	1.3	–	1.9
LT-16	4.7	–	14.3	–	18.0	–	BD	–	1.2	–
LT-17	7.9	–	32.0	–	35.0	–	1.0	–	1.9	–
LT-18	BD	–	3.7	–	6.6	–	0.9	–	7.7	–
LT-19A	BD	–	19.6	–	2.8	–	BD	–	1.2	–
LT-20	BD	–	5.7	–	1.0	–	BD	–	BD	–
LT-21	BD	–	1.6	–	1.4	–	BD	–	1.0	–
LT-22	0.8	–	3.1	–	1.3	–	BD	–	1.1	–
LT-26B	–	0.5	–	3.2	–	BD	–	BD	–	BD
LT-27	BD	–	4.6	–	1.6	–	BD	–	BD	–
LT-28A	0.8	–	3.2	–	2.6	–	1.7	–	2.2	–
LT-28B	–	BD	–	4.9	–	1.4	–	1.7	–	1.8
LT-29	BD	0.6	0.7	3.8	0.8	0.8	0.8	0.9	1.1	0.8
LT-30	0.6	0.9	1.8	66	1.5	2.0	BD	BD	2.1	1.4
LT-31	0.6	–	6.0	–	2.3	–	BD	–	BD	–
LT-42	–	0.5	–	0.42	–	1.1	–	BD	–	BD
LT-43	–	BD	–	8.4	–	BD	–	BD	–	BD

[1]U.S. EPA FCT criteria values (see text) in μg/L are Pb 3.2, Cu 12*, Ni 160*, CrVI 11, Cd 1.1*, Zn 110* (* = hardness dependent criteria, 100 mg/L as $CaCO_3$)

[2]Former USSR standards (see text) in μg/L are Co 100, V 100, Mn 10,000, Fe 500

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Table 3. Continued

Site number	Cr[1] 1991	Cr[1] 1992	Mn[2] 1991	Mn[2] 1992	Fe[2] 1991	Fe[2] 1992	Cd[1] 1991	Cd[1] 1992	Zn[1] 1991	Zn[1] 1992
LT-06	0.6	1.1	120	163	730	276	BD	0.23	3.5	320
LT-07	0.5	1.0	128	206	450	191	BD	0.20	2.7	23.0
LT-08	0.6	1.5	148	170	750	781	BD	0.20	4.8	740
LT-10A	BD	BD	120	75	120	133	BD	2.0	152	360
LT-10B	–	BD	–	68	–	112	–	1.7	–	290
LT-11A	BD	–	33	–	250	–	2.4	–	4.0	–
LT-13-1	1.6	1.4	202	240	780	642	BD	BD	12.9	570
LT-13-2	–	1.2	–	350	–	426	–	2.0	–	250
LT-13-3	–	0.9	–	2120	–	380	–	BD	–	380
LT-14	1.2	–	158	–	450	–	BD	–	6.6	–
LT-15B	BD	–	157	–	310	–	BD	–	2.7	–
LT-15C	–	1.6	–	270	–	508	–	BD	–	17.7
LT-16	1.3	–	66	–	270	–	0.30	–	7.2	–
LT-17	520	–	81	–	820	–	0.45	–	43.0	–
LT-18	1.5	–	76	–	110	–	0.40	–	3.3	–
LT-19A	BD	–	175	–	350	–	BD	–	10.4	–
LT-20	BD	–	178	–	280	–	BD	–	5.0	–
LT=21	BD	–	120	–	300	–	BD	–	1.7	–
LT-22	BD	–	60	–	30	–	BD	–	2.9	–
LT-26B	–	0.9	–	131	–	170	–	0.24	–	13.5
LT-27	0.9	–	127	–	530	–	BD	–	6.7	–
LT-28A	2.7	–	207	–	2430	–	BD	–	7.2	–
LT-28B	–	1.7	–	306	–	707	–	0.22	–	60.4
LT-29	0.7	0.6	156	163	560	276	BD	0.21	3.3	13.7
LT-30	1.2	2.2	102	172	510	688	0.27	0.34	90	570
LT-31	3.7	–	103	–	290	–	0.80	–	14.5	–
LT-42	–	BD	–	34	–	140	–	BD	–	63.1
LT-43	–	BD	–	30	–	20	–	0.26	–	440

[3]BD = Below detection limit

[4]– = Not sampled

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Hydrological Parameters

The hydrological parameters measured in 1991 and 1992 at the stations at which water samples were taken for trace metal analyses are reported in Tables 4 and 5. Metal speciation in aquatic solution is dependent upon such factors as temperature, pH, redox, alkalinity, and concentration of the cations present.

Table 4. Hydrological parameters of natural waters in Lithuania, 1991.

Site No.		Temp.[1] °C	pH[2]	DO[3] mg/L	Conductivity[4] mS/cm	Redox[5] mV	Salinity ppt
LT-06	Gilija[6]	20.9	7.75	8.7	0.50	105	0.0
LT-07	Uostadvaris[6]	20.2	8.47	11.0	0.47	57	0.0
LT-08	Nida Station[6]	19.8	8.08	7.2	0.48	106	0.0
LT-10A	Klaipėda Harbor-A	19.3	8.34	9.2	5.30	115	2.4
LT-11A	Melnragė-A[6]	18.8	8.18	8.5	11.7	78	6.4
LT-13-1	Sovetskas-A[6]	20.6	7.88	10.3	0.52	75	0.0
LT-14	Smalininkai	20.3	8.42	11.9	0.46	74	0.0
LT-15B	Zapyškis[6]	20.6	7.47	5.1	0.46	93	0.0
LT-16	Panevėžys	19.1	7.82	6.9	0.73	76	0.0
LT-17	Šiauliai	21.8	7.69	0.5	2.14	−95	0.6
LT-18	Kėdainiai	21.2	7.84	7.2	0.81	61	0.0
LT-19A	Nemunas, II Cut	22.7	7.56	1.5	0.48	92	0.0
LT-20	Kaišiadorių	22.8	7.60	1.1	0.48	114	0.0
LT-21	Dambrava, IV Cut	22.5	7.72	4.8	0.46	117	0.0
LT-22	Pažaislis, VII Cut	22.2	7.93	8.4	0.44	141	0.0
LT-27	Neris at Kaunas	21.1	8.58	14.5	0.45	83	0.0
LT-28A	Rusnė-A[6]	20.7	8.21	11.7	0.48	82	0.0
LT-29	Deimė[6]	21.4	7.48	3.9	0.59	86	0.0
LT-30	Rasytė	19.7	8.37	9.7	0.48	88	0.0
LT-31	Grigiškės	18.5	8.10	8.3	0.48	61	0.0

[1]Temperature probe accurate to ±0.2°C
[2]pH probe accurate to ±0.2 units
[3]DO probe accurate to 2%
[4]Conductivity sensor ±1% of range, resolution 4 digits
[5]Redox probe accurate to ±20 mV
[6]Locations resampled in 1992

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Table 5. Hydrological parameters of natural waters in Lithuania, 1992.

Site number	Name	Temp.[1] °C	pH[2]	DO[3] mg/L	Conductivity[4] mS/cm	Redox[5] mV	Salinity ppt
LT-06	Gilija[6]	20.2	8.95	11.1	0.4	292	0.2
LT-07	Uostadvaris[6]	22.1	8.84	13.3	0.4	309	0.2
LT-08	Nida Station[6]	20.5	8.57	8.0	0.4	310	0.2
LT-11B	Melnragė-B[6]	7.63	7.61	7.8	12.6	392	7.2
LT-13-2	Sovetskas-A[6]	21.5	8.97	15.0	0.4	286	0.2
LT-15C	Kulautuva[6]	19.5	8.53	10.4	0.4	322	0.2
LT-26B	Raudondvaris	19.4	8.52	10.5	0.4	333	0.2
LT-28B-2	Rusnė-B[6]	22.0	8.94	14.4	0.4	276	0.2
LT-29	Deimė[6]	22.1	8.10	6.1	0.6	330	0.3
LT-30	Rasytė	20.5	8.69	8.8	0.4	319	0.2
LT-43	Drūkšiai	22.1	9.49	17.2	0.3	264	0.2

[1]Temperature probe accurate to ±0.2°C [2]pH probe accurate to ±0.2 units [3]DO probe accurate to 2%
[4]Conductivity sensor ±1% of range, resolution 4 digits [5]Redox probe accurate to ±20 mV
[6]Locations samples in 1991

The pH values of natural waters from which we collected samples for metal analyses in 1991 were found to be very similar throughout, and were within a range of 7.5-8.6 (Table 4). In 1992, a slightly lower value (7.3) was found in the Baltic Sea at Melnragė, at the mouth of Klaipėda Harbor (Table 5). According to theoretical calculations, it has been suggested that the heavy metals in Lithuanian natural waters are in a carbonated form (Turner *et al.* 1981). This is supported also by the fact that natural waters of Lithuania are generally alkaline (Garunkštis 1988).

Dissolved oxygen in natural waters is of great importance because it represents the vital capacity of water. Very low values were found in some parts of the Kaunas Reservoir (1.1-1.5 mg/L) and Kuršių Marios Lagoon (3.9 mg/L) in 1991, and an extremely low value of 0.53 mg/L was found in the Kulpė River near Šiauliai, where wastewater discharges from an industrial complex are discharged directly into the river. In 1992, DO values ranged from 6 to 17 mg/L (Table 5), and at all seven of the freshwater locations sampled both years the DO values ranged from 1 to 5 mg/L higher in 1992 than in 1991. Dissolved oxygen values measured at Melnragė, in the Baltic Sea at the mouth of Klaipėda Harbor, were as much as 1 mg/L lower. It has been suggested that these higher values in 1992 may be related to a decrease in industrial production because of the current economic crisis, with corresponding decreases in wastewater discharges into freshwater bodies.

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During 1991 and 1992, the conductivity of the water in the Neris and Nemunas Rivers, Kaunas Reservoir, and the freshwater area of Kuršių Marios Lagoon ranged from 0.44 to 0.59 mS/cm. Slightly higher values for conductivity, relative to other surface waters, were observed in the Nevėžis River at Panevėžys, Kėdainiai, and Raudondvaris (0.73-0.81 mS/cm), and an extremely high value for freshwater of 2.14 mS/cm was measured in the Kulpė River at Šiauliai. Elevated conductivity values were also found in other more polluted rivers, and inside the mouth of Klaipėda Harbor where high conductivity values are normal due to infusion of ions and salts from the Baltic Sea. Salinity at Melnragė on the Baltic Sea was approximately 6 ppt. Speciation of metals in seawater can be different from that in freshwater, where anions other than carbonates may prevail (Turner *et al.* 1981): toxicity to aquatic animals of heavy metals can depend upon that speciation. This has been detailed for copper by Pagenkopf *et al.* 1974 and Chakoumakos *et al.* 1979.

Temperature, salinity, and conductivity values at all sites were within ranges considered to be normal, except for the Kulpė River at Šiauliai. The negative redox value measured in 1991 in the Kulpė River at Šiauliai (Table 4) is indicative of reducing conditions and very low DO levels. The products of contaminant degradation under the reducing conditions at Šiauliai would differ from other surface waters where oxidative conditions exist and positive redox values were obtained.

CONCLUSIONS AND RECOMMENDATIONS

The number of sampling sites visited and samples analyzed for this study were restricted due to time constraints; but even so, information obtained was sufficient to confirm two "hot spots," namely Klaipėda Harbor and the Kulpė River at Šiauliai. Except for the chromium concentration in the Kulpė River sample and the high lead and copper concentrations measured at Melnragė (Klaipėda Harbor), the other metal concentrations ranged from below detection limits to a factor of 2-3 higher than criteria values recommended by the U.S. EPA (1992). Because only about one-third of wastewaters discharged in Lithuania are treated to meet any established standards, future metal analyses should be focused on the sites downstream from cities and industries involved in metal plating and other activities that use metals. Detailed investigations are needed on trace metal concentrations in the rivers flowing into Kuršių Marios Lagoon. In addition, a study is needed on pollution sources discharging into the Kulpė River at Šiauliai.

ACKNOWLEDGEMENTS

Transportation for our expeditions in Lithuania was provided by the Lithuania Department of Environmental Protection. This research was supported in part by U.S. EPA Cooperative Agreements CR816369 and CR995189 to Montana State University.

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APPENDIX D

INTER-LABORATORY COMPARISON OF METHODS FOR THE DETERMINATION OF HEAVY METALS IN NATURAL WATERS

K. Kvietkus, D. Čeburnis,
J.J. Ellington, and Y. Shane Yu

From

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IN THE
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U.S. Environmental Protection Agency
Athens, Georgia, USA

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INTER-LABORATORY COMPARISON OF METHODS FOR THE DETERMINATION OF HEAVY METALS IN NATURAL WATERS

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J.J. Ellington[2], and Y. Shane Yu[3]

ABSTRACT

An inter-laboratory study was conducted by the Ecological Spectroscopy Laboratory (ESL), Institute of Physics of the Lithuania Academy of Sciences, and the Environmental Research Laboratory, Athens, Georgia (ERL-A), of the U.S. Environmental Protection Agency, to compare the analytical methods used by each laboratory to measure trace metals in natural waters. Duplicate water samples were collected at 19 locations throughout Lithuania in July 1991, and each laboratory analyzed these samples for eight metals: cadmium, cobalt, chromium, copper, lead, manganese, nickel, and zinc. Analyses were performed by graphite furnace atomic absorption spectroscopy (AAS). Some samples required dilution before analysis in order to reduce absorbance to levels where accurate background correction and quantitation could be achieved using the Zeeman effect. Although eight metals were measured, a correlation equation was determined for only six of these because of considerable differences reported for zinc, and because of the failure to detect cobalt in any of the samples analyzed at ERL-A. The regression line plot that was calculated for the six metals had a slope of 0.957 and a coefficient of determination of 0.829.

INTRODUCTION

Until 1990, no detailed investigations of heavy metals in natural waters had been performed in Lithuania, mainly because of a lack of adequate instrumentation. Also, nitric acid with sub parts-per-billion metal content for sample preservation was not available, and usually only glass containers were available for sample collection and storage. Bottles made of glass often contain appreciable amounts of metals in their walls, and these metals can easily dissolve and contaminate the sample (Ross 1986). To avoid such contamination, bottles must be washed in strong acid to leach the metals from the container wall. Polyethylene or teflon bottles are preferred for storing samples that contain trace amounts of metals because, after acid washing, these containers are least likely to contaminate the water samples (Moody and Lindstrom 1977).

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An additional problem, associated with water analysis for metals, is that metal concentrations in the bulk aqueous phase can alter during storage due to sorption--commonly referred to as the "container wall effect." This effect is minimized by acidifying the samples with nitric acid. Another factor that affects the measured concentration of metals in water samples is the analytical method, e.g., the conditions of atomization of the sample and the use of matrix modifiers to minimize interferences and improve quantitation.

As part of a joint US/Lithuania cooperative research program, water samples were collected in duplicate for analysis of heavy metals at both ESL and ERL-A. This inter-laboratory comparison would be useful for two reasons: (1) as a background from which to discuss and compare the instrument variables and the techniques employed by each laboratory, (2) as a database through which trends or possible problems associated with the measurement of some of the metals could be revealed.

MATERIALS AND METHODS

Samples were collected in 50-ml plastic bottles, precleaned at ERL-A by washing with 3% nitric acid (Fisher Scientific Co., Trace Metal Grade) and rinsing with organic-free deionized water. Nitric acid (300 μL) was then added immediately after cleaning to lower the pH of the water to ≤ 2 to serve as a sample preservative and to minimize sorption to the wall of the container. The samples were stored under refrigeration and analyzed (within 4 months of collection) without filtration . All glass and plastic used in the analyses were acid-washed and rinsed with metals-free water prior to use. The metals for which analyses were performed were cadmium, cobalt, copper, chromium, lead, manganese, nickel, and zinc.

Analyses at ESL were performed on a Perkin-Elmer Zeeman 3030 atomic absorption spectrophotometer (AAS) equipped with an AS-60 autosampler, and a HGA-600 graphite furnace with a L'vov platform. The AAS system was controlled by an internal microcomputer using a keyboard and video monitor. The internal gas flow rate was 300 mL/min. ERL-A used a Perkin-Elmer Zeeman 5100 PC AAS, equipped with an AS-60 autosampler, an HGA-600 graphite furnace with a L'vov platform, and an IBM PS/2 50Z computer with graphics-based application software. The internal gas flow rate was 300 mL/min, except during atomization when it was 0 mL/min (Tables 1 and 2).

At each laboratory, calibration standards (1 to 40 μg/L) were prepared at the time of analysis by a series of dilutions using organics-free deionized water that was made 0.2% in nitric acid. ESL stock solutions (1,000 mg/L) were obtained from the Physics/Chemistry Institute of the Ukrainian Academy of Sciences, and ERL-A stock solutions of each metal (1,000 mg/L) were obtained from Fisher Scientific Co. At ESL, a programmed non-linear calibration method based on a two-coefficient equation, part of the 3030 software, was used. At ERL-A, non-linear calibration curves that satisfied correlation coefficients of at least 0.995 were used.

Table 1. Ramp and hold times for furnace condition (in seconds).

	Ramp		Hold	
Step	ESL	ERL-A	ESL	ERL-A
Dry	2	1	10-50	50
Char	2	1	10	30
Cool	–	1	–	15
Atomize	0	0	3	5
Clean	1	1	3	5

Table 2. Furnace temperature and instrument operating conditions.

	Temperature (°C)					Conditions (nm)	
Element	Dry	Char	Cool	Atomize	Clean	Wavelength	Slit
				ESL			
Cadmium	110	250	–	1600	2650	228.8	0.7
Chromium	110	1200	–	2300	2650	357.9	0.7
Cobalt	110	1000	–	2200	2650	242.5	0.2
Copper	110	900	–	2000	2650	324.8	0.7
Lead	110	500	–	1800	2650	283.3	0.7
Manganese	110	1000	–	1900	2650	279.5	0.2
Nickel	110	1300	–	2300	2650	232.0	0.2
Zinc	110	400	–	1600	2650	213.9	0.7
				ERL-A			
Cadmium	120	850	20	1650	2600	228.8	0.7
Chromium	120	1650	20	2500	2600	357.9	0.7
Cobalt	120	1400	20	2500	2600	242.5	0.7
Copper	120	1300	20	2500	2600	324.8	0.7
Lead	120	850	20	1800	2600	283.3	0.7
Manganese	120	1400	20	2200	2600	279.5	0.7
Nickel	120	1400	20	2500	2600	232.0	0.7
Zinc	120	700	20	1800	2600	213.9	0.7

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Nitric acid/deionized water blanks were prepared at both ESL and ERL-A by adding purified water (18 MΩ cm^{-1}) to 50-ml plastic bottles that contained nitric acid. Metal impurities were not detected in ESL and ERL-A blanks. Matrix modifiers were not available for use in the analysis of samples at ESL; magnesium nitrate was used as a matrix modifier at ERL-A for each metal except nickel, which was analyzed without the addition of a modifier.

By agreement, each laboratory analyzed samples using instrument conditions and sampling techniques that had been used historically at that laboratory. The operating conditions in Tables 1 and 2 are reflective of the use of matrix modifiers at ERL-A that allowed higher char temperature, faster ramps, and longer hold times. Higher temperatures enhance removal of compounds from the matrix that could interfere with the analysis. The modifiers also shorten the time required for the furnace wall and the vapor to attain a stable temperature, an essential characteristic of the L'vov platform technique. A modification of the method described in the instrument operating manual (Perkin-Elmer 1982) was used to determine the instrument detection limits for each metal (Table 3).

Table 3. Instrument detection limits.

Element	ESL		ERL-A	
	Detection Limit (μg/L)	Sample Amount (μL)	Detection Limit (μg/L)	Sample Amount (μL)
Cadmium	0.3	20	0.3	20
Chromium	0.5	20	0.5	20
Cobalt	0.9	60	0.7	20
Copper	0.5	40	0.6	20
Lead	0.5	50	0.9	20
Manganese	0.3	20	0.9	20
Nickel	0.8	80	1.2	20
Zinc	0.3	5	0.6	20

RESULTS AND DISCUSSION

Each laboratory analyzed for eight metals in water samples collected simultaneously from 19 sampling stations; this totalled 152 measurements at each laboratory, and a combined total of 304 measurements (Table 4). Of these 304 measurements, 119 were reported as below detection limits, 44 by ESL and 75 by ERL-A. Most of the measurements at ERL-A for cadmium, cobalt, lead, and nickel were below detection limits; cobalt was not found in any sample, and nickel in only three. However, at ESL nickel was found in all 19 samples, although cadmium, cobalt, and lead were detected in half or fewer. Both laboratories reported chromium, copper, manganese, and zinc in most or all of the samples.

There were two areas where the results at ESL and ERL-A differed significantly. Measurements at ERL-A for zinc in five of the samples were five to 35 times higher than at ESL, while measurements for zinc at ESL for two of the samples were five and 10 times higher than at ERL-A. In the case of the seawater samples (LT-11A), seven of the metals measured considerably higher at ESL than at ERL-A. Reasons for both of these large discrepancies will need further study. Elimination of the zinc data, the seawater sample data, and those data pairs where either of the two laboratories reported a concentration below detection, left 52 data pairs for six of the eight metals. A log-log regression plot of these 52 data pairs was calculated using Slide Write Plus™ software (Figure 1).

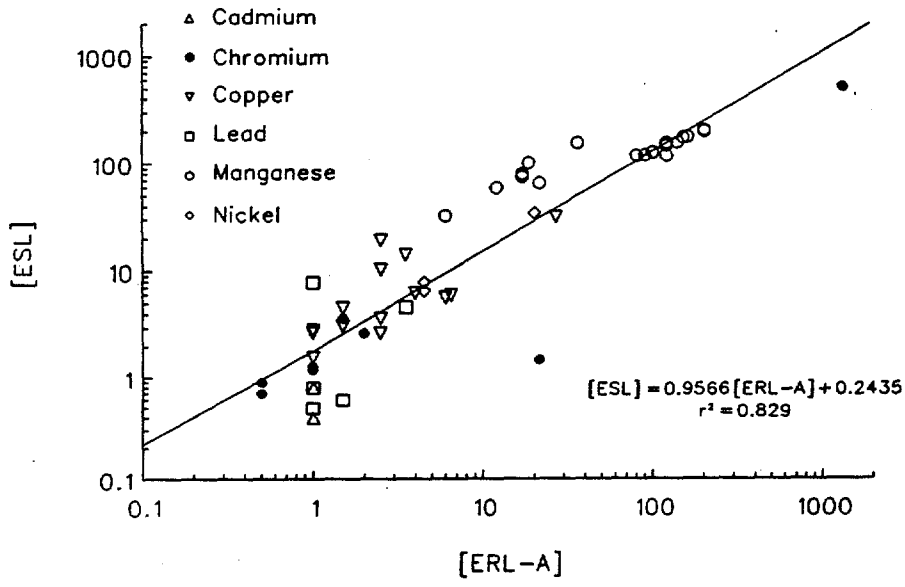

Figure 1. Regression line for metals analyses at Ecological Spectroscopy Laboratory and Environmental Research Laboratory-Athens.

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Table 4. Results of water analyses by element[1] (concentrations reported in $\mu g/L$).

	Cadmium		Chromium		Copper	
Sample	ESL	ERL-A	ESL	ERL-A	ESL	ERL-A
LT-06	BD[3]	BD	0.6±0.1	BD	2.7±0.1	2.5±0.5
LT-07	BD	BD	0.5±0.1	BD	1.7±0.1	BD
LT-08	BD	BD	0.6±0.1	BD	2.9±0.5	1.0±0.2
LT-10A	BD	BD	BD	19.0±2.0	2.7±0.1	1.0±0.2
LT-11A	2.4±1.0	BD	BD	BD	66.0±1.0	BD
LT-13-1	BD	BD	1.6±0.1	BD	6.3±0.1	4.0±1.0
LT-14	BD	BD	1.2±0.2	1.0±1.0	10.4±0.1	2.5±0.5
LT-15B	BD	BD	BD	BD	0.8±0.1	BD
LT-16	0.3±0.1	BD	1.3±0.3	1.0±0.5	14.3±0.1	3.5±0.5
LT-17	0.45±0.05	BD	520±60	1300±80	32.0±1.0	27.0±0.2
LT-18	0.4±0.1	1.0±0.3	1.5±0.3	21.5±3.5	3.7±0.1	2.5±0.5
LT-19A	BD	BD	BD	9.5±0.5	19.6±0.1	2.5±1.0
LT-20	BD	BD	BD	4.0±2.0	5.7±0.1	6.0±1.0
LT-21	BD	BD	BD	3.0±2.0	1.6±0.1	1.0±0.2
LT-22	BD	BD	BD	2.0±1.0	3.1±0.1	1.5±0.5
LT-27	BD	BD	0.9±0.1	0.5±0.5	4.6±0.1	1.5±0.5
LT-28A	BD	BD	2.7±0.2	2.0±1.0	3.2±0.2	1.5±0.5
LT-29	BD	BD	0.7±0.1	0.5±0.5	0.7±0.2	BD
LT-31	0.8±0.3	1.0±0.3	3.7±0.2	1.5±0.5	6.0±0.1	6.5±0.5

[1]Cobalt omitted from table because all measurements at ERL-A were below detection limit. Values for cobalt at ESL were: LT-06, 0.9±2; LT-10A, 3.0±1.5; LT-11A, 29.0±5.0; LT-17, 1.0±0.1; LT-18, 0.9±0.1; LT-28A, 1.7±0.3; LT-29, 0.8±0.1

Table 4. Continued.

Lead		Manganese		Nickel		Zinc[2]	
ESL	ERL-A	ESL	ERL-A	ESL	ERL-A	ESL	ERL-A
BD	BD	120±1.0	120±3.0	1.8±0.4	BD	3.5±0.1	9.0±1.0
BD	BD	128±2.0	100±3.0	1.2±0.1	BD	2.7±0.1	2.0±0.2
0.5±0.5	1.0±0.3	148±2.0	120±3.0	0.9±0.1	BD	4.8±0.4	3.0±0.2
BD	BD	120±1.0	80.0±4.5	1.2±0.4	BD	152±30.0	15.0±1.2
152±5.0	BD	33.0±1.0	6.0±0.9	29.5±3.0	BD	4.0±0.2	25.0±2.0
BD	BD	202±2.0	200±3.0	2.2±0.1	BD	12.9±0.4	460±20
1.8±0.3	BD	158±4.0	140±3.0	2.8±0.1	BD	6.6±0.1	150±10.0
BD	BD	157±2.0	120±3.0	1.3±0.1	BD	2.7±0.4	3.0±0.2
4.7±0.3	3.5±0.5	66.0±1.0	21.5±0.5	8.0±0.1	4.5±0.5	7.2±0.3	9.0±0.2
7.9±0.3	1.0±0.3	81.0±1.0	17.0±1.5	35.0±0.1	20.0±0.4	43.0±3.0	7.5±2.5
BD	BD	76.0±2.0	17.0±1.0	6.6±0.1	4.5±0.5	3.3±0.3	7.0±0.2
BD	BD	175±3.0	150±10	2.8±0.1	BD	10.4±1.3	20.0±2.0
BD	BD	178±4.0	160±3.0	1.0±0.1	BD	5.0±0.5	25.0±2.0
BD	BD	120±2.0	90.0±10	1.4±0.1	BD	1.7±0.2	2.0±0.2
0.8±0.5	BD	60.1±1.0	12.0±0.9	1.3±0.1	BD	2.9±0.4	3.5±0.5
BD	BD	127±2.0	100±9.0	1.6±0.1	BD	6.7±0.8	5.5±0.5
0.8±0.3	1.0±0.3	207±5.0	200±9.0	2.6±0.1	BD	7.2±0.1	6.0±0.2
BD	BD	156±1.0	36.0±1.0	0.8±0.2	BD	2.3±0.3	2.0±0.2
0.6±0.2	1.5±0.5	103±1.0	18.5±0.5	2.3±0.1	BD	14.5±0.1	200±6.0

[2]Zinc data were not used in calculating the regression line (Figure 1).

[3]BD Below detection limit of the instrument used.

The coefficient of determination (r^2) of 0.829 and slope of 0.957 are an indication that the results of the measurement processes in each laboratory are similar. Of the 52 data pairs, ESL reported higher values for 43, which is reflected in the +0.244 offset of the plot. We cannot determine from the data available whether the frequently higher values reported by ESL are the result of an unknown background source of metals or some other cause at either ESL or ERL-A.

CONCLUSION

This inter-laboratory comparison study provided the desired interaction between chemists at ESL and ERL-A and identified six metals that can be analyzed with confidence in results. The analysis for zinc presents a problem that must be addressed, and the analysis for cobalt will require a concentration step to attain easily quantifiable concentrations. Additional work will be needed to determine reasons for differences in the results of analyses for metals in seawater. The slope and correlation coefficient for data on six of the metals measured indicate the data are similar, with a bias toward frequently higher concentrations at ESL.

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APPENDIX E

ENVIRONMENTAL CHEMISTRY TRAINING SESSION FOR LITHUANIAN SCIENTISTS - 1992

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ENVIRONMENTAL CHEMISTRY TRAINING SESSION FOR LITHUANIAN SCIENTISTS - 1992

January 28 - May 22, 1992

OBJECTIVE

The goals of this training session were first to introduce the Lithuanian scientists to the approach the U.S. EPA uses in the analyses of environmental samples. Following discussions about the history of these procedures and techniques which also included a brief overview of the laws involved, this course became a totally "hands-on" exercise with emphasis on using EPA types of methodologies and quality assurance/quality control (QA/QC) criteria to do a variety of environmental analyses. Desktop computers were used extensively throughout the session to store, manipulate, display, and summarize data collected. Software used; all of which is commercially available, included word processing, spreadsheets, graphics and statistics.

Students: Anolda Četkauskaitė
Department of Biochemistry
Vilnius University
Vilnius

Birutė Bielinytė
Department of Chemistry
Vilnius University
Vilnius

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Week of	Curriculum
1/28	Laboratory tour and orientation - discussion of session goals
	Brief description of the various EPA environmental laws
	Analytical methods associated with these laws
	Overview of FBL research projects and QA/QC program
	Analytical standards - sources, purity, and storage
	Stock standard preparation 1,2,4,5-tetrachlorobenzene (TCB); 1,2,3,4-tetrachlorobenzene, a surrogate (SR); and pentachlorobenzene, the internal standard
	Dilution regimes and preparation of GC calibration standards
	GC introduction - Varian 6000 complete with 401 Vista data system (DS) and autosampler
	Ni^{63} electron capture detector (ECD) - theory and operation
	Injection of calibration standards
2/03	Data reduction - calibration standards chromatograms internal standard vs external standard techniques calibration curves and linear regression
	DOS primer and introduction to spreadsheets - QUATTRO PRO™
	Varian Associates - chromatography short course Montana State Department of Agriculture Laboratory Montana State University
	TCB analysis in environmental samples - fish tissue - storage stability study
	Soxhlet extraction - QA/QC - surrogate incorporation

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GC-DS training
peak integration - theory and operation
analytical method - preparation - options

TCB-fish extract purification - florisil column chromatography

GC analysis - TCB-fish extracts - internal standard addition - dilutions
manual data manipulation - calculations
spreadsheet data manipulation

2/10 GC-DS and spreadsheet training - calculations - continued

2/17 TCB analysis - water and fish - UBC depuration test # 238,
emphasis on use of QA/QC program and spreadsheets

2/24 Finish analyses started 2/17

Initiate method setup for the analysis of tetrachloroguaiacol (TCG)
standards preparation - TCG, the surrogate pentachlorophenol (PCP)
and the internal standard hexachlorobenzene (HCB)
calibration standards methylation (diazomethane), dilution
GC-DS method establishment - prepare calibration curve
spreadsheet preparation of TCG analysis

3/02 TCG analysis - water - UBC tests 234-237 - uptake study

3/09 TCG analysis - fish - UBC tests 234-237
continue homogenation of large fish
extraction of fish homogenates using POLYTRON™ homogenizer
QA/QC - surrogate and matrix spike sample preparation
methylation and cleanup of extracts

3/16 GC analysis samples prepared week of 3/9

3/23 Continue TCG - fish analysis

3/30 Spreadsheet preparation - external standard GC worksheet

Introduction - PC graphics software - SLIDEWRITE PLUS™
- Loading software into the PC
- Text, graph, draw (annotate), file, print
- Curve fit, error bars, equations, statistics

Introduction - PC basic statistics software - INSTAT™
- Descriptive statistics - mean, SD and SEM
- T-tests or nonparametric tests
- Linear regression and correlation

4/06 TCB analysis - fish and water samples
- UBC test #239 - depuration study

4/13 TCB analysis - continued

4/20 Individual projects

Solvent-filled dialysis membranes in environmental analysis
- Laboratory experiments to test solvent-filled dialysis membranes for environmental analysis as a precursor to using this method in Lithuania

Synthesis of both methyl ether and pentafluorobenzyl derivatives of TCG and PCP
- Literature search - Montana State University library
- Synthesis and purification
- Purification evaluation using the Department of Chemistry Mass Spectroscopy Center

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APPENDIX F

ENVIRONMENTAL CHEMISTRY TRAINING SESSION FOR LITHUANIAN SCIENTISTS - 1993

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ENVIRONMENTAL CHEMISTRY TRAINING SESSION FOR LITHUANIAN SCIENTISTS - 1993

March 19 - April 29,1993

OBJECTIVE

The goals of this training session were first to introduce the Lithuanian scientists to the approach the U.S. EPA uses in the analyses of environmental samples. Following discussions about the history of these procedures and techniques which also included a brief overview of the laws involved, this course became a totally "hands-on" exercise with emphasis on using EPA types of methodologies and quality assurance/quality control (QA/QC) criteria to do a variety of environmental analyses. Desktop computers (IBM 8088) were used extensively throughout the session to store, manipulate, display, and summarize data collected. Software used; all of which is commercially available, included word processing, spreadsheets, graphics and statistics. Upon completion of the course, the computers used by the students were shipped to their laboratories at the Lithuania Department of Environmental Protection (DEP) in Vilnius. These systems were then installed by Fisheries Bioassay Laboratory staff and remain at DEP on indefinite loan from Montana State University, Project 31.

Students:

Nijole Štriupkuvienė
Department of Environmental Protection
Central Laboratory
Vilnius

Svetlana Urbonienė
Department of Environmental Protection
Central Laboratory
Vilnius

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Week of	Curriculum
3/19	Introduction and overview
	Laboratory tour
	Instrumentation descriptions and capabilities
	Brief discussion about origination of EPA analytical methods i.e., from environmental laws: CERCLA, RCRA, SDWA, SUPERFUND (CLP)
	Personnel computer assignments and DOS primer
	Spreadsheet introduction - QUATTRO™ PRO
3/22	Standard solution preparation (stock) and protocol using tetrachloroquaiacol (TCG), the surrogate (SR) pentachlorophenol (PCP), and internal standard hexachlorobenzene (HCB)
	Calibration curve standard solution preparation including methylation
	GC Data System (DS) introduction
	Preparation of a analytical method for internal standard calibration using the autosampler
	Preparation of a spreadsheet for GC calibration data
	FBL QA/QC program philosophy - standards, samples and GC analyses
	GC-ECD analyses of calibration standards prepared 3/22 and establishment of calibration curves and linear regression parameters using both hand held calculators and spreadsheets
	Spreadsheet development incorporating standard curve regressions and calculations for analyses of samples
	Spreadsheet development incorporating surrogate (SR) addition and recovery information
	Spreadsheet development incorporating results to report

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3/29 GC spreadsheet for IS quantitation completed and tested

Analysis of fish samples for TCG - storage stability study
method outline - EPA method reference # 515, 604, CLP

QA/QC protocol - surrogate addition - Matrix Spike addition

Sample preparation and surrogate and or matrix spike addition

Sample extraction, aliquoting, solvent removal, methylation

Florisil column cleanup of methylated extract

Internal standard (HCB) addition to purified extract

GC-ECD DS analytical method creation - internal standard method

Quantitation of TCG and SR using both the DS (peak areas) and
a strip chart recorder (peak height) - data comparison
discussion of calculation formulas and how the DS calculates results
using Response Factors (RFs)

Dilutions of extracts analyzed 3/31
spreadsheet used to calculate results generated 3/31 and 4/01 using
linear regression parameters from the calibration runs done earlier

Use of mid-point calibration curve standard response factors (Rfs) to
insure integrity of calibration curve on a daily basis (ie. CV=$\pm$10%)

Results tabulation and comparisons

DS peak integration - theory and operation
Timed events; peak reject (PR), signal-to-noise ratio (SN), tangent
percent, Peak width (WI), horizontal forward and backward baseline (HF,
HB), Inhibit integration (II), Valley baseline (VB)

Data reintegration and recalculation from disk stored raw data

TCG results comparison; peak height (strip chart recorder) vs peak
area (DS)

TCG results comparison; Response factors (DS) vs calibration curve
linear regression (spreadsheet)

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4/05 TCG data comparison continued - conclusions

MSU library tour
computer search options
journals available
government documents

TCG-water analysis
method outline - EPA method reference # 515, 604
extraction - Method Blank, SR addition, Matrix Spike
methylation of extract aliquot, dilution with IS

TCG-water analysis continued
GC method creation and GC analysis
results - discussion

Spreadsheet calculations - discussion - QA/QC requirements
conclusions

Spreadsheet modification - single injection per standard/sample

HPLC installation (Waters 600E/441)
discussion - theory and operation

GC columns (packed) - preparation and conditioning mixed phase column for chlorinated pesticides including chlordane, toxaphene, and PCBs

EPA method reference # 608, CLP

4/12 HPLC analysis of chlorinated phenols - normal phase (silica column)

GC-ECD DS setup with new mixed phase column

Preparation of chlorinated pesticide and PCB GC standards

GC analysis each standard - establish optimum instrument conditions

Build GC DS methods for all standards chromatographed 3/13 from disk-stored raw data using external standard quantitation method

Finish building DS methods - check methods by injection of diluted GC standards

Build GC spreadsheet for chlorinated pesticides and PCBs using data generated earlier - single injection - external standard - surrogate recovery

Initiate HPLC cleanup of TCG-fish extracts from 3/30

Interface HPLC detector signal to DS and build HPLC DS method

Establish HPLC conditions separating TCG from PCP

4/19 Spreadsheet development for pesticide/PCB calculations - continued

Spreadsheet development continued - completed

Spreadsheet as applied to any chromatography analyses

HPLC cleanup of TCG-fish extracts
extract aliquot preparation and injection,
collection of TCG eluant from column,
solvent removal and methylation

MSU Chemistry Department Mass Spectrometry Laboratory tour
observe GC-MS analysis of pesticide standard mixtures using capillary column chromatography - Theory and operation
quadrapole vs magnetic sector

GC analyses of HPLC purified extracts from 4/21 - data comparison
troubleshoot low recovery as compared to earlier results

FBL QA/QC Program - detailed discussion that included:
SOP documentation
tabulations of calibration curve linear regression parameters
tabulations of daily mid-point calibration standard RFs
tabulations of SR and Matrix Spike sample percent recoveries
sample activity tracking log
laboratory notebooks
instrument logs

Inter-Mountain Laboratories Inc., Bozeman, - tour
this laboratory does all types of environmental analysis (organics and metals) using EPA protocols and methods

4/26 Analyses of volatile organics (VOCs) - discussion
various EPA methods
sampling techniques

Selective GC detectors

GPC cleanup of soil/sediment samples for phenols and pesticides/PCB's
discussion of EPA methodology - CLP methods

GC retention time data - All pesticides, herbicides, PCBs and phthalates
source - U.S. Food and Drug Administration (FDA) Pesticide Manual

Evaluation - Millipore EnviroGard™ Triazine Test Kit used for screening water samples in the field for triazine herbicides at the sub-ppb levels

Prepare atrazine standard solutions and fortify laboratory water at various concentrations

Evaluation - Triazine test kit - continued
estimate atrazine in fortified water samples using test kit instructions
measure absorbance of standards and samples using spectrophotometer
establish calibration curve and calculate concentration in water
use of graphics software display data
compare results to theoretical fortification level
conclusions

Training Program - Review of accomplishments

APPENDIX G

SOLVENT-FILLED DIALYSIS MEMBRANES IN ENVIRONMENTAL ANALYSIS: EFFICIENCY AND APPLICATIONS

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May 1992

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SOLVENT-FILLED DIALYSIS MEMBRANES IN ENVIRONMENTAL ANALYSIS: EFFICIENCY AND APPLICATIONS

A. Četkauskaitė

INTRODUCTION

Application of Dialysis Membrane Chambers in Environmental Analysis and Biotechnology.

The introduction of ultrafiltration in 1969-1970 in biotechnology caused the spread of membrane filtration and dialysis methods to other fields of research such as environmental analysis (Figure 1) [1-5,7-10]. In 1972, membrane filter chambers were used to evaluate bacteria injury by organic and inorganic pollutants in surface waters [3]. In 1980, Bryne and Aylott [6] patented a semipermeable membrane device, filled with nonpolar solvent, based on the process of passive membrane diffusion of small nonpolar organic molecules from water as an alternative to classical extraction methods. Parallely, starting in 1980, extraction methods in biotechnology were applied on a large scale using membrane (dialysis) bioreactors, or membrane-assisted extractive (organic solvent-extractant/water) bioreactor systems, in various fermentation processes [5,7]. Application of semipermeable membranes, especially in biotechnology, led to solving problems characteristic of all classical extraction methods: appreciable amounts of organic substances can be lost as micro-emulsified droplets during extraction processes [7]. Another important problem that was widely investigated in fermentation biotechnology, and more recently in environmental pollution monitoring, is the rejection of organic compounds or selectivity of the membrane material used under various conditions [2,5,8]. The selectivity of membrane separations of the organic azeotrope (e.g., dimethyl carbonate/methanol) is well analyzed in various solvent recovery processes in chemical engineering [9].

The goal of this brief introduction is to emphasize that new data on application and mechanisms of function of dialysis membranes in water/solvent systems are coming from the fields of biotechnology and chemical engineering, though there are no citations [1,10] or citation is minimal [2] from the research data in those fields in the articles, analyzing environmental monitoring of waters using dialysis membranes filled with solvents or lipids [1,10]. Two-phase extraction systems in environmental analysis methods for organic pollutants in surface waters have been standardized for over a decade [11]. So, it is useful to review briefly extraction procedures recommended by the U.S. Environmental Protection Agency (U.S. EPA) "Test Methods for Analysis of Organic Compounds in Wastewaters and Surface Waters" (Series 600), because of the possibility of various alternative extraction methods such as APHA et al. [12].

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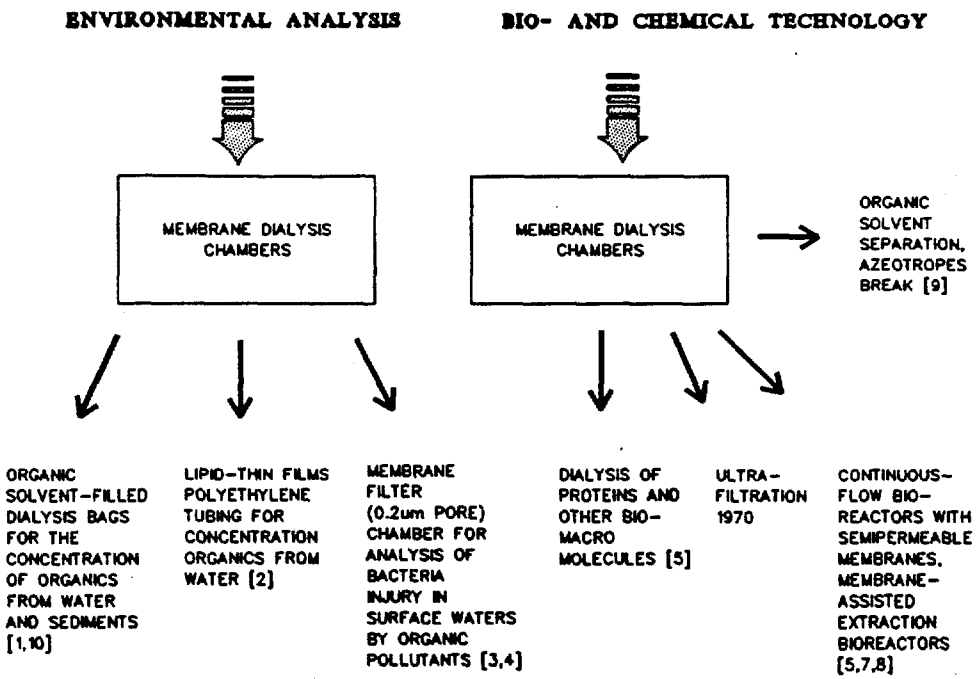

Figure 1. Application of dialysis membrane chambers in environmental analysis and biotechnology.

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Application of Classical Two-Phase Solvent Extraction Systems in EPA Methods (600 Series) for Organic Chemical Analysis in Water and Wastewater.

According to the U.S. EPA Test Methods 600 series for wastewaters [13], extraction procedures for the following set of methods are essentially the same: 606 (phthalate esters), 608 (organochlorine pesticides and polychlorinated biphenyls), 609 (nitroaromatics and isophorone), 610 (polynuclear aromatic hydrocarbons), 611 (haloethers), 612 (chlorinated hydrocarbons). Methylene chloride is the solvent most often recommended in water sample extraction even though gas chromatography (GC) analysis is performed with an electron capture detector (ECD) (Table 1) [13]. Extract concentration is usually performed with a Kuderna-Danish (KD) concentrator and evaporation in the presence of a Snyder column is carried out at 60 – 65°C, although there is possible loss of compounds during concentration because of their thermal decomposition (hexachlorocyclopentadiene), oxidation (benzidine), or evaporation depending on high volatility (dichlorobenzenes) [12,13].

Extraction procedures for organochlorine pesticides and polychlorinated biphenyls (PCBs) (Method 608) can be performed in several different ways that reflect Methods 6410B and 6630B for gas chromatography/mass spectroscopy (GC/MS) [12]. "Standard Methods" 14th and 17th editions published extraction procedures based on use of various mixtures of ethyl ether (EE) with petroleum ether (PE) [11,12]. According to Standard Methods, florisil fractination with 6% and 15% ethyl ether in petroleum ether will separate Aldrin, DDT, Heptachlor, Lindane, pentachlorobenzene, PCBs, etc. from endosulfan I, endrin, dieldrin, dichloran, and phthalate esters [11,12].

So, for organochlorine pesticides (Method 608) we have one extraction method version in U.S. EPA (1982) and the Federal Register (1984), and others published in Standard Methods [11-14]. A chloroform extraction procedure for benziolines (Method 605, HPLC analysis and 625-GC/MS analysis) was announced by U.S. EPA [13], but does not appear in Standard Methods (6410B) [12]. This gives more freedom to the investigator to select an extraction solvent system more suitable for compounds being analyzed or for the detector used; such a situation we have with hexane extraction in case of further GC analysis with ECD, instead of conventional methylene chloride extraction. Use of organic solvent hydrocarbons, or alcohols of higher molecular weight and hydrophobicity, for extraction is limited by their lower volatility that can cause the loss of extractable compounds at higher temperatures (>65°C) in standard KD concentrators. In case of non-conventional concentration methods, it is advantageous to use hydrocarbons of higher molecular weight.

Table 1. Comparison of U.S. EPA "Test Methods" and APHA et al. "Standard Methods".

Chemical	EPA Test Method Number (1982) for GC	for GC/MS	for HPLC	Standard Methods (17-th Ed.,1989) No, for GC/MS	Extraction Procedure Solvent	Volume	Conditions	Method
Chlorinated Hydrocarbons								
1,2,4-Trichlorobenzene	612	625			Methylene chloride	60 ml*2	2 min.*2	Kuderna-Danish
Hexachlorobenzene	612	625		6410 B (GC and HPLC)	- " -	- " -	- " -	concentrator, Snyder column, + 60C,+65C
Polynuclear Aromatic Hydrocarbons								
Benzo(a)anthracene		625	610	6410 B	Methylene chloride	250-100 ml	2 min.*2	- " -
Benzo(b)fluoranthene		625	610	(Base/neutral	- " -	3 steps	pH>11,	
Benzo(a)pyrene,etc.		625	610	extractables)	- " -	- " -	pH=7	
Phthalate Esters								
Benzyl Butyl Phthalate	606	625		6410 B	Methylene chloride	- " -	- " -	- " -
Pesticides and Polichlorinated Biphenyls								
DDT-group	608	625		6431C - 6410B	- " -	- " -	- " -	- " -
Endrin	608	625		6431C - 6410B	- " -	- " -	- " -	- " -
Aldrin	608	625		6431C - 6410B	- " -	- " -	- " -	- " -
Heptachlor	608	625		6431C - 6410B	- " -	- " -	- " -	- " -
Chlordane	608	625		6431C - 6410B	- " -	- " -	- " -	- " -
BHC-group	608	625		6431C - 6410B	- " -	- " -	- " -	- " -
PCBs-group	608	625		6431C - 6410B	- " -	- " -	- " -	- " -
(Hexachlorbenzene & oth.)	608	625		6431C - 6410B	- " -	- " -	- " -	- " -
Pesticides and Polychlorinated Biphenyls								
DDT-group	608	625		6630 B	6%Ethyl Ether(EE)	60 ml*3	2 min.*3	- " -
Endrin	608	625		6630 B	in Petroleum Ether	- " -	- " -	- " -
Aldrin	608	625		6630 B	(PE): DDD,DDE,DDT	- " -	- " -	- " -
Heptachlor	608	625		6630 B	Aldrin,Chlordane,	- " -	- " -	- " -
Chlordane	608	625		6630 B	BHC,PCBs,Hepta-	- " -	- " -	- " -
BHC-group	608	625		6630 B	chlor, etc.	- " -	- " -	- " -
PCBs-group	608	625		6630 B	15% EE in PE:	- " -	- " -	- " -
(Hexachlorbenzene & oth.)	608	625		6630 B	EndosulfanI,Endrin Dieldrin,Phthalate Esters 50% EE in PE EndosulfanII, Captan	- " -	- " -	- " -

(Continued)

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Table 1. Continued.

Chemical	EPA Test Method Number (1982) for GC	for GC/MS	for HPLC	Standard Methods (17-th Ed.,1989) No, for GC/MS	Extraction Procedure Solvent	Volume	Conditions	Method
Phenols								
2,4-Dichlorophenol	604	625		6420	Methylene chloride	60 ml*2	2 min.*2	Kuderna-Danish
2,4,6-Trichlorophenol	604	625		6420	- " -	- " -	pH=1-2	concentrator,
Pentachlorophenol	604	625		6420	- " -	- " -		Snyder column,
2,4-Dinitrophenol	604	625		6420	- " -	- " -		+ 60C,+65C
Benzidines								
		625	605	6410 B	Chloroform (EPA) Methylene chloride (Std.Meth.)	100 ml +20 ml*2 addit.extr.	1)pH low, 2)pH basic	- " -
Volatile Organics (Purgable Halocarbons)								
Chlorometane	601	624		6210 B	Purge	and	Trap	25 cm long Meth
Chloroethane	601	624		6210 B				-Silicone-coate
Chloroform	601	624		6210 B				packing,includi
Chlorobenzene	601	624		6210 B				15 cm of 2,6-di
Methylene chloride	601	624		6210 B				phenylene oxide
Tetrachloroethene	601	624		6210 B				polymer and 8 c
Trichloroethene	601	624		6210 B				of silica gel
Purgable Aromatics								
Toluene	602	624		6220 A-E	Purge	and	Trap	25 cm long Trap
Ethylbenzene	602	624		(Drinking Water)				packed with 1 c
Benzene	602	624		- " -	(For 5 ml water samples)			Methyl Silicone
1,2-Dichlorobenzene	602	625		6210 B				coated packing
1,3-Dichlorobenzene	602	625		6220C-Drink.Water				and 23 cm of 2,
1,4-Dichlorobenzene	602	625		- " -				diphenylene oxi
								de polymer

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Relationship Between Bioconcentration Factors, Water Solubility, Octanol–Water Partition Coefficients, and Solvent Concentration Factors for Some Organic Pollutants.

The publication of Hansen et al. [20] on PCB content in various fish tissues is an example of a data base in toxicology showing that residual contents of organic pollutants vary considerably in different tissues of fishes. In 1985, Chiou [15] determined partition coefficients between lipid triolein and water (K_{TW}) for 38, mostly aromatic, chlorinated compounds and compared these to fish bioconcentration factors for the chlorobenzene derivatives, obtained by other investigators. The data of these investigations are presented in Table 2. It is obvious, that when published bioconcentration factor values [log(BCF)] of organic compounds in fishes are based on the lipid content, rather than on total mass of fish, they are approximately equal to the K_{TW} which suggests at least near equilibrium for solute partitioning between water and fish lipid. The close correlation between K_{TW} and K_{OW} suggests that K_{OW} is also a good predictor for lipid–water partition coefficients and BCF.

Bioconcentration factors of chlorobenzenes in guppies [16] and rainbow trout [17] were determined using the following flow-through systems: (1) guppies -- temperature 21°C, water flow rate 2 liters/hour, fish weight ≈ 0.65 g; and (2) rainbow trout -- temperature 15°C, water flow rate 360 liters/hour, fish weight ≈ 250 g. Rate of bioconcentration is primarily dependent upon flow rate, rather than other environmental conditions. Also from data presented by Chiou [15] it is possible to suggest that a linear relationship between log K_{OW} and log K_{TW} (or $K_{LIP/W}$) will be expected for structurally related chemicals but not for different types of hydrophobic chemicals. This was confirmed by thermodynamic studies of fish/water and octanol/water partitioning of some chlorinated benzenes in static water experiments [19]. Comparing the data presented in Table 2 and Table 3, we can observe that for chlorinated benzenes and PCBs, the rate of bioaccumulation by fish in flowing waters is lower by a factor 2 than corresponding K_{OW}. In static waters, the rate of bioconcentration for whole fish is approximately 10 times less than the rate of bioconcentration in flowing waters [18]. Although enhancement of metabolic/transport processes of organic pollutants in fishes takes place in flowing systems, the comparison of the time needed to reach equilibrium (steady-state) in static and flow-through systems is of value in bioassays, and in passive concentration systems such as solvent-filled dialysis membrane bags used for water pollution monitoring.

In 1970, Hartung and Klingler [21] compared p,p′–DDT solubilities in various petroleum oils and showed that theoretical partition coefficients $K_{OIL/W}$ are extremely high for DDT (see Table 4). Comparison of partition coefficients for DDT $K_{OIL/W}$ with $K_{HEX/W}$ shows that for hexane $K_{HEX/W}$ of DDT is lower by a factor of 3 [21,22]. Those authors emphasized that oil concentrations in sediments may reach levels as high as 17.8% but more commonly average approximately 0.5% in areas with an extended history of oil pollution [21]. This work is an example that the accumulation of organic pollutants in sediments follows first-order kinetics. Another conclusion is that oils, as an example of higher molecular weight hydrocarbons, concentrate pollutants more efficiently than do lipids.

Table 2. Comparison of lipid-based bioconcentration factor (BCF), octanol-water (K_{OW}), and triolein-water (K_{TW}) partition coefficients of selected organic compounds [15].

Chemical	log Kow	log Ktw	Lipid-based log BCF (1)	
			Guppies [16]	Rainbow Trout [17]
1,4-Dichlorobenzene	3.39	3.55	3.26	3.64 - 3.96
1,3,5,-Trichlorobenzene	4.31	4.36	4.15	4.34 - 4.67
1,2,3,5-Tetrachlorobenzene	4.59	4.69	4.86	n.d.
1,2,4,5-Tetrachlorobenzene	4.70	4.70		4.80 - 5.17
Pentachlorobenzene	5.20	5.27	5.42	5.19 - 5.36
Hexachlorobenzene	5.50	5.50	5.46	5.16 - 5.37
Biphenyl	4.09	4.37		
2,2',5,5'-PCB	5.81	5.62		
2,2',4,5,5'-PCB	6.11	5.81		
p,p'-DDT	6.36	5.90		

(1) - Experiment conditions see text

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Table 3. Water solubility, octanol-water partition coefficient (K_{OW}), and BCF data of some organic pollutants.

Chemical	Water	Octanol-Water	Bioconcentration Factor	
	Solubility ppm	Partition Coefficient Kow / log Kow	Flowing Water BCF / log BCF	Static Water BCF / log BCF
A. Substituted Benzenes and Halobenzenes				
1,2,4,5-Tetrachlorobenzene	6.00	47,000 / 4.67	4,500 / 3.65	
Pentachlorobenzene	0.135	154,000 / 5.18	5,000 / 3.70	
Hexachlorobenzene	0.035	168,000 / 5.23	8,600 / 3.93	290 / 2.46
B. Halogenated Biphenyls				
Arochlor 1248	0.017	1,288,000 / 6.11	72,950 / 4.86	11,860 / 4.07
Arochlor 1254	0.01	2,017,000 / 6.30	45,600 / 4.66	12,150 / 4.08
2,2',4,4',5,5'-Hexachlorobiphenyl	0.001	3,700,000 / 6.57	46,000 / 4.66	
Biphenyl	7.5	7,540 / 3.88	340 / 2.53	
C. Symmetrical Triazines				
Atrazine	33.00	476 / 2.68	0.00	11 / 1.04
Cyanazine	171.00	150 / 2.18		0.00
Simazine	3.50	155 / 2.19	1 / 0	
Prometryn	48.00			
D. Miscellaneous				
Pentachlorophenol	14.00	102,000 / 5.01	16 / 1.20	130 / 2.11
Dichlobenyl (benzoic acid herbicide)	18.00	235 / 2.37		55 / 1.74
Diuron	42.00	94 / 1.97		
Linuron	75.00	154 / 2.18		
Fenuron	3850.00	10 / 1		

Table 4. Oil-water ($K_{OIL/W}$) and hexane water (K_{HW}) partition coefficients of p,p'-DDT and chlorinated pesticides [21,22].

Solvent (s)	Partition Coefficients Ksw (1) and Logarithms of Ksw (Ksw / log Ksw)				
	DDT	Heptachlor	Aldrin	Dieldrin	Lindane
Hexane	91,000 / 4.96	111,000 / 5.05	100,000 / 5.00	36,000 / 4.56	1,730 / 3.24
Transformer Oil	53,250,000 / 7.73	n.d.	n.d.	n.d.	n.d.
Spindel Oil	69,800,000 / 7.84	n.d.	n.d.	n.d.	n.d.
Diesel Fuel	54,333,333 / 7.74	n.d.	n.d.	n.d.	n.d.
Techn. White Oil	40,833,333 / 7.61	n.d.	n.d.	n.d.	n.d.
Medic. Mineral Oil	41,666,666 / 7.62	n.d.	n.d.	n.d.	n.d.
20W Lubr. Oil	28,416,666 / 7.45	n.d.	n.d.	n.d.	n.d.

(1) - For DDT solubility in water 1.2 ug/L [21].

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For our purpose, data presented in Tables 2-4 allow us to calculate an approximately equilibrium concentration for DDT in different solvents, based on their partition coefficients and solubility in water (K_{HW}, K_{OW}, K_{TW}). Let us assume that solubility of p,p'-DDT in water is 1.2 μg/liter [21], and partition coefficients for p,p'-DDT $K_{HEX/W}$ = 91,000 (logK=4.96), $K_{OCT/W}$ = 2,290,867 (log K=6.36), $K_{TRIOL/W}$ = 794,328 (log K=5.90). From this, we can calculate the following concentrations of p,p'-DDT in those solvents: C_{HEX} = 109,200 μg/L (109 μg/ml), C_{OCT} = 2,290,867 μg/L (229 μg/ml), C_{TRIOL} = 661,940 μg/L (662 μg/ml). This means that for DDT even though its concentration in hexane is less than that in lipids by 6-10 times, we still can ensure a good recovery using hexane.

Concentration of Lipophilic Compounds from Water using Dialysis Membranes filled with Organic Solvents.

According to Fish and coauthors [25], when the sampler is at steady state, flux of organic compounds through the membrane (J_m) can be expressed, according to Fick's first law, as:

$$J_m = \frac{D \cdot A}{L}\left(K_W \cdot C_W - K_S \cdot C_S\right)$$

where:

D = the diffusion coefficient;
A = the surface area of the membrane;
L = the membrane thickness;
K_W and K_S = the membrane-water and membrane-solvent partition coefficients for the diffusing compound;
C_W and C_S = the concentrations of the compound in water and solvent.

Before 1991, there were four groups of investigators that had already published results on monitoring of pollution using solvent-filled dialysis membrane devices in surface waters [1,2,10,23-25]. The main data from these studies are summarized in Table 5.

Johnson [10] reported that for PCBs, steady state was not reached in 32 days as concentration of Arochlor 1248 slowly increased, although at that time concentrations were 110 μg/liter in hexane-filled dialysis membranes and 0.43-0.56 μg/liter in spiked water. The same results for PCBs (2,2',5,6'-tetrachlorobiphenyls, Clophen A40) was reported by Sodergren in 1990 [23], although in 1987 his data, obtained in static and flow-through systems, showed that for Clophen A50, hexachlorobenzene, and p,p'-DDT, equilibrium was apparently established after 48 hours in a concentration range of 1-1,000 μg/g of solvent [1]. Detailed analysis of those data, presented in graphic form, led to the conclusion that at 48 hours true equilibrium is not reached, especially in a continuous-flow system, but equilibrium between water and hexane inside dialysis bags does not occur until 140 hours (≈6 days). Data on accumulation of ^{14}C labeled

pesticides in lipid-filled polyethylene tubing show that steady state was reached for mirex in approximately 4 days; for 3,3′,4,4′-tetrachlorobiphenyl in 1-6 days; and for tenvalerate in 2 days [2]. Hassett et al. [24] reported that PCBs could be detected in hexane-filled polyethylene membrane samplers after as little as 8 hours exposure, and in all cases after 24 hours exposure; they also demonstrated that in distilled water the sampling rate of PCBs depends upon temperature.

In 1991, Johnson [10] concluded that solvent-filled dialysis membranes can be valuable only for qualitative monitoring of organic pollutants, but further research is required in quantifying the method before concentrations in water, sediment, or tissue can be estimated reliably. The conclusions of Sodergren [23] are more detailed and precise: the dialysis concentration factor (DCF) was smaller than the BCF; e.g., for the PCB Clophan A50, log DCF=2.4 and log BCF=3.0. The log DCF for Clophan A50 was also about 3-4 orders smaller than log P(K_{OW}). The main conclusion is that solvent-filled membranes can be exposed in the field for a considerable length of time and their function can be checked with internal standards [23]. Huckins et al. [2] demonstrated a good correlation between PCB accumulation (K_{LW}) in lipid-filled semipermeable membranes (SPM) and the K_{OW} of the PCBs. They also demonstrated a linear relationship between the bioconcentration of PCBs in goldfish and the concentration of PCBs in SPMs filled with grass carp lipid or Triolein. The range of concentration tested was from 0 to 11.1^5, and ^{14}C-labeled PCBs were used.

Several investigators reported that no degradation or periphyton growth occurred on solvent-filled dialysis bags [1,2,10,23-25]. Solvent loss, however, was observed from 67.5% of hexane-filled tetrachlorobiphenyl polyethylene membranes tested after a 3-week exposure period in field water or distilled water. A slower diffusing solvent, such as octane, was recommended [24]. New dialytic or pre-evaporation techniques have been developed that allow for quantitative isolation and concentration of organochlorine components from aqueous environments [2,25]. These systems use polyethylene or hollow-fiber SPMs containing lipid extracts, and are referred to as analytical sampler/extractor/concentrators.

It is, therefore, generally agreed by several authors that solvent-filled dialysis membranes can be utilized in monitoring lipophillic pollutants in aquatic environments. They also conclude that there is a correlation between concentration of pollutants in these solvent-filled dialysis membranes, and concentration in aquatic organisms. Use of solvent-filled dialysis membranes are especially valuable in zones of sublethal pollutant concentrations, where special extraction procedures have previously been required.

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Table 5. Literature summary – Concentration of lipophilic compounds from water using hexane-filled dialysis membranes.

Compounds tested	Compound concentr. in water	Organic solvent used	Volume (ml)	Temp., C	Dialysis time	Degree of concent-ration	Object of analysis	Remarks	Literature References
Arochlor1248 Arochlor1260	5 ug/L -"-	n-Hexane pesticide grade	40 ml -"-	17-23 C -"-	5 days control 32 days exp.	100 fold	a)cntrl-deionized charcoal-filtered water b) spring water c) hypertrophic pond water	Dialysis membr-anes:SpectraPor6 MW cutoff 1000, 8 mm flat width	Johnson G.D. Hexane-Filled Dialysis Bags for Monitoring Orga-nic Contaminants in Water.//Env.Sci.&Tech-nol.1991,v.25,N.11,
p,p'-DDE p,p'-DDT Clophen A50 (mixture of PCBs) Hexachlor-benzene	5-1065 ug/L in static system 0.03-10 ug/L in continu-ous-flow system	Hexane	3 ml	22 + 1 C static 11 + 1 C dynamic	7 days 8 - 10 days	uptake of: DDT= 9.1 ng/ml/day PCB=18.6 ng/ml/day	Laboratory expe-riment of static & continuous--flow system	Dialysis membr-anes:SpectraPor6 MW cutoff 1000. In static system the concentration declined: compou-nds adsorbed to the walls of dia-lysis bags & the container	Sodergren A. Solvent -Filled Dialysis Memb-ranes Simulate Uptake of Pollutants by Aqua-tic Organisms.//Env. Sci.&Technol.1987,v. 21,N. 9, p. 855-859.
Clophen A50 2,2',5,6'-Tetrachloro-biphenyl (UPAC No53)	0.4mg/kg dry wt of sediments	Hexane	4 ml	mostly field T-re	a) from 1 to se veral weeks b)month c)year	8.2 ng/g of PCBs after 8 days from sediments Amphipods G.pulex accumula-ted 5000 times more PCBs.	Membranes were buried in: a) PCB-contamina-ted sediment from receiving waters of paper mill b) in water of paper mill c) in sewage sludge	Dialysis membr-anes:SpectraPor3 &6,cutoff 1000-3500. The area of all membranes was held constant at about 1200 sq mm.	Sodergren A. Monito-ring of Persistent Lipophilic Pollu-tants in Water and Sediment by Solvent -Filled Dialysis Mem-branes.//Ecotoxicol. & Environm. Safety 1990,v.19,N.2, p.143 -149

(1) - The same principle of diffusion chambers (fitted with 0.2 um pore size filters: Nucleopore No 111506) was used at first about 20 years ago (Mc Feters G.A. & Stuart D.G. Appl.Microbiol., 1972,v.24,p.804-811) for the evaluation of survival of coliform bacteria in natural waters at field and laboratory studies and still is used now for various pathogenic bacteri-al strains in various surface and wastewaters (Terzieva S.I.,McFeters G.A. Can.J.Microbiol., 1991,v.37,p.785-790).

(Continued)

Table 5. Continued.

Compounds tested	Compound concentr. in water	Organic solvent used	Volume (ml)	Temp., C	Dialysis time	Degree of concent-ration	Object of analysis	Remarks	Literature References
Arochlor1242 2,4"-dichlo-robiphenyl 2,4,4"-trich-lorobiphenyl 2,3,3",4,6-pentachloro-biphenyl		n-Hexane		+25 C + 5 C	3 weeks 4 weeks	Sampling Rate (L/day/cm2) =0.0015T+ +0.025, where T-temperatu-re, C	in field studies: Hudzon River wa-ter in areas, contaminated with PCBs	Polyethylene membranes. Field samplers 23 sq.cm surface area, 0.025 mm thick, 3.8 cm DI	Hassett J.P., Force M. and Song H. A Passive In-Situ Sampler for Organic Compounds in Water.//Preprint Exten-ded Abstract".Natl.Me-eting of ACS, Division of Environm.Chem.,1989, v.29,p.496-497,Sept.10-15,Miami,FL
2,2",5,5"-te-trachlorobi-phenyl, 3,3",4,4"-te-trachlorobi-phenyl, Mirex Fenvalerate Asana 2(Du Pont). All chemicals - 14C-labeled	4.4 ug/L 2.2 ug/L 2.1 ug/L 2.l ug/L	Triolein or carp lipid	1 ml	+ 25 C 12-20 C	21 day in laboratory 9 days in pond; 644 c.m volume; outdoors	From 79.1% for Mirex to 94.9% for 2,2",5,5"-TCB	a) well water in laboratory b) pond water	Polyethylene "layflat" tubing (2.6 cm width and 76.5 um film thick-ness). Surface area of SPMDs, exposed to water was about 360 sq.cm and 86.4 cm long	Huckins J.N.,Tubergen M and Manuweera G.K.Semi-permeable Membrane Devi ces Containing Model Li pid.A New Approach to Monitoring the Bioavail ability of Lipophilic Contaminants and Esti-mating Their'Bioconcen-tration Potential.// Chemosphere 1990,v.20, No 5, p.533-552.
Clorinated benzenes PCBs Phthalate esters		Hexane Toluene				2-5 x 10 E3 or greater		Hollow fiber membranes: low density polyethylene (5 mil. thick)	Fish C.L., Mc Eachran I S. and Hassett J.P.Con-tinuous Flow Extraction and Concentration of Trace Organic Compounds from Water Using Hollow Fiber Membranes. Exten-ded Abstract. Natl.Mee-ting of ACS, Division of Environm. Chem.1989 Sept.10-15, Miami FL

Effect of Dissolved Organic Carbon on Extraction Efficiencies of Organic Compounds.

In aquatic environments, interactions of organic compounds with dissolved organic matter influence chemical transformations of the former, and biological transformations of the latter. Humic materials bind and increase apparent water solubility of organic compounds [30]; e.g., hydrophilic and hydrophobic amino acids [26], DDT, anthracene, biphenyl and 2,5,2′,5′–tetrachlorobiphenyl [27], and trichloroethylene and toluene [28]. Fish et al. [29] demonstrated that conventional hexane extraction of chlorinated hydrophobic compounds from Niagara River water can be enhanced by using special digestion techniques (Table 6). Relative recoveries of 23 compounds (chlorinated benzenes, polychlorinated biphenyls, DDT, DDE, mirex and others) revealed that in most cases extraction with hexane in the presence of chromic acid (90-96% H_2SO_4, 1% CrO_3) is more effective than traditional solvent-extraction methods. Separately, Kile and Chiou [30] have discussed water solubility enhancement of p,p′-DDT.

A variety of organic materials such as colloids and biocolloids, as well as inorganic suspended mineral precipitates found in both ground and surface waters, bind and transport organic pollutants [31-33]. In surface waters we can consider "migration forms" of appropriate organic pollutants the same as "migration forms" of metals, the existence of which depends upon pH, E_h, hardness, and photochemical (mainly light-induced redox) reactions. For example, cadmium increases the solubility of 2,2′,4,4′,6,6′–hexachlorobiphenyl in the water phase in the same way as dissolved organic carbon (DOC), and enhances the desorption process of this organic pollutant from columns filled with sandy, siliceous aquifer material [32]. The volatile organic compound, 1,2-dibromoethane (EDB), was found retained on hydrophylic soils much more strongly than would be predicted from its physico-chemical properties. The standard U.S. EPA methods for the extraction and determination of volatile organic compounds proved to be inadequate for EDB [33].

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Table 6. Extraction efficiencies of organochlorine compounds from Niagara River water determined by two methods [29].

Compound	log Kow	Extraction Efficiency (1) (Relative Recovery)	Mass Spiked (ng/L)
1,2,4-Trichlorobenzene	3.98	1.008	145.4
1,2,3,4-Tetrachlorobenzene	4.55	0.926	26.6
Pentachlorobenzene	5.03	0.960	39.3
Hexachlorobenzene	5.47	0.924	41.2
2-Chlorobiphenyl	4.50	0.577	984.0
4,4-Dichlorobiphenyl	5.36	0.947	249.0
2,2',5,5'-Tetrachlorobiphenyl	6.26	0.938	107.0
2,2',4,5,5'-Pentachlorobiphenyl	6.85	0.896	99.1
2,2',4,4',5,5'-Hexachlorobiphenyl	7.75	0.830	117.0
p,p'-DDT	6.36	0.830	139.0
p,p'-DDE	-	0.792	34.7
2,2',3,4,4',5,5'-Heptachlorobiphenyl	7.20	0.686	96.6
Mirex	6.89	0.640	63.4

(1) - Relative Recovery is the undigested fraction recovery per digested fraction recovery, where undigested fraction was recovered by conventional liquid-liquid extraction with hexane and digested fraction recovered after the break down of dissolved organic carbon combined with hexane extraction (sample treating with chromic acid and hexane).

MATERIALS AND METHODS

Reagents

1,2,4,5-tetrachlorobenzene (1234-TCB) - Chemical Service, Westchester, PA 19380, 95% pure; 1,2,3,4-tetrachlorobenzene (1234-TCB) - Aldrich Chemical Co., 98% pure; pentachlorobenzene (PCB) - Chemical Service; hexachlorobenzene (HCB) - Chemical Service, 99% pure; Hexane - Fisher Scientific, HPLC grade; Spectra/Por6 molecular porous membrane MWCO:1000, 7,6 mm diameter, vol/length 0.46 mL/cm, Fisher Scientific.

Equipment

Gas chromatograph (GC): Varian Model 6000 equipped with a ^{63}Ni ECD, autosampler, and VISTA 402 data system.

Preparation of Chlorobenzene GC Calibration Standards

Stock standard solutions of each chlorobenzene (CB) were prepared in hexane (100 ml volume) at the following concentrations: 1245-TCB - 999 μg/ml, 1234-TCB - 1003 μg/ml, PCB - 104 μg/ml, and HCB - 8.8 μg/ml. Aliquots of these standards were mixed and diluted with hexane resulting in a concentrated GC standard solution containing 1245-TCB at 200 μg/liter, 1234-TCB at 150 μg/liter, PCB - 100 μg/liter, and HCB - 132 μg/liter. Aliquots of this standard (0.5, 1, 3, 5, 8, and 10 ml) were added to a series of 100-ml volumetric flasks. Each flask was diluted to volume with hexane and the contents mixed. The resulting solutions were used as GC calibration standards and aliquots of each were injected into the GC operated as detailed below. Calibration curves were built plotting peak areas vs standard concentrations (μg/liter) .

Water Fortification (Spiking) Standards

Two fortification standard solutions containing all the CBs were prepared using acetone. The first standard (high standard) had 1245-TCB at 200 μg/liter, 1234-TCB at 150 μg/liter, PCB at 100 μg/liter, and HCB at 132 μg/liter. The low fortification standard contained all the same CBs at concentrations 100 times less. Aliquots of each standard (1.0 ml) were added to each of two glass bottles containing 20 liters of organics free double-distilled water. The contents of each were thoroughly mixed. CB concentrations were 100 and 10 parts-per-trillion (ppt) in each bottle respectively. A third bottle containing 20 liters of water was included to serve as a control.

Preparation of Dialysis Membranes for the Concentration of Chlorobenzenes from Water, and Experimental Conditions.

Spectra/Por6 membranes were soaked in dechlorinated tap water and rinsed for 3-4 hours to remove sodium benzoate, EDTA, and other preservative substances. The membranes were cut in 14-cm sections so that each segment had the same volume and surface area. Six dialysis membrane segments were each filled with 6 ml of hexane and sealed. Two of each were placed in each of the two 20-liter test bottles, and two were placed in the control bottle. These 20-liter bottles were placed in a water reservoir where the water temperature was maintained at 9°C. After a 10-day period, the membranes were removed and the concentration of each CB in both the hexane and the water, was determined.

Extraction of Chlorobenzene Compounds from Water by Standard Procedure according to EPA Method 612 for Chlorinated Hydrocarbons.

Extraction procedures were carried out according to EPA Method 612 except that hexane rather than methylene chloride was used as the extraction solvent. A 300-ml water sample was removed from each test bottle and the contents extracted with 60 ml of hexane using a 500-ml capacity separatory funnel. Each funnel was shaken for a 2-minute time period. Layers were allowed to separate for at least 10 minutes and then the hexane layer passed through ca. 25 g anhydrous sodium sulfate into a Kuderna Danish (KD) concentrator. The extraction procedure was repeated and the extracts combined in the concentrator. The combined extract volume was concentrated to ca. 10 ml using the KD system and then the volume was reduced to 2.0 ml using a gentle stream of purified nitrogen. Aliquots of this concentrated extract were subjected to GC analysis using the following instrument conditions: Inlet temperature - 200°C; Detector temperature - 300°C; Column - 180 m x 2 mm ID glass packed with 3% Carbowax-20M on 100/120 Supelcoport™; Carrier gas - nitrogen at 22 ml/min.; Retention time (minutes) - 1245-TCB 2.19, 1234-TCB 2.38, PCB 4.58, HCB 9.16; Autosampler injection volume - 3.5 μl, Plotter speed 1.0 cm/minute; Plotter attenuation - x128.

Statistical Data Calculation from External Standard Calibration Curve for Chlorinated Benzenes.

Tables for calibration curves and response factors for external standards of chlorinated benzenes were developed and calculated using the program QUATTRO PRO™. Linear regression parameters (slope, intercept, and correlation coefficient) were calculated using the same program. An external standard calibration curve was plotted for each CB using the graphics software program SlideWritePlus™.

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RESULTS AND DISCUSSION

The GC/ECD response of the GC standards for all the CBs was linear over the concentration range tested (Figure 2), and correlation coefficients (r) were all >0.99. Chlorinated benzenes (CB) were found in the hexane from every dialysis membrane exposed to fortified water, whereas no CBs were detected in the hexane from membranes placed in the control system (Figure 3). In situations where there were no noticeable leakages, the CB-hexane concentrations for similarly treated membranes agreed with one another fairly well (Table 7). A hexane-water enhancement of about 50 times was observed in the 100 ppt system. This enhancement value is the ratio of the CB-hexane concentration to the initial water fortification level. The enhancement in the 10 ppt system appears to be higher, but unfortunately, one of the two membranes placed in this system had a great amount of leakage and the data reported are of limited value. Enhancement was calculated in this manner because it was not possible to achieve accurate CB water levels from the water samples analyzed due to the high GC background (Figure 4). Even though these compounds can be observed in the chromatograms of these water sample extracts, quantitation was not attempted. Since the objective of this work was to become familiar with manipulating these membranes and to observe whether or not organics could be detected in the solvent, the fact that quantitative CB levels in the treated water could not be achieved was not of great concern for this preliminary work.

These initial results have demonstrated that chlorinated benzenes can be partitioned from water into hexane-filled dialysis membranes. All the xenobiotics tested were detected in the solvent even when the water concentration was extremely low (10 ppt). After a 10-day exposure period, these compounds were concentrated in the hexane contained in these membranes by factors ranging from 50 to 160. This technique needs to be further evaluated, and additional efforts should include the introduction of an appropriate surrogate or surrogates into the hexane to monitor the dynamics of the membrane during exposure. The GC/ECD analysis precision would be improved by the incorporation of an internal standard. Also the use of a chlorine-specific GC detector, rather than the ECD, would greatly facilitate the quantitation of these CBs. Solvents other than hexane and of higher molecular weights, such as octane or even decane, should be evaluated relative to respective abilities to enhance concentrations and prevent solvent leakage.

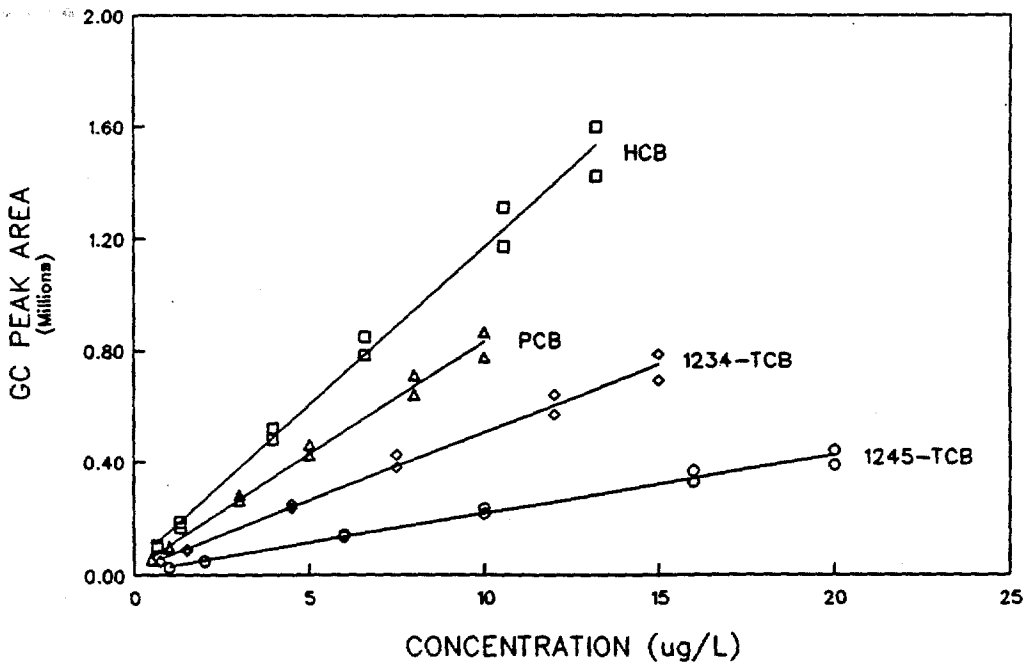

Figure 2. Calibration curves for chlorinated benzenes.

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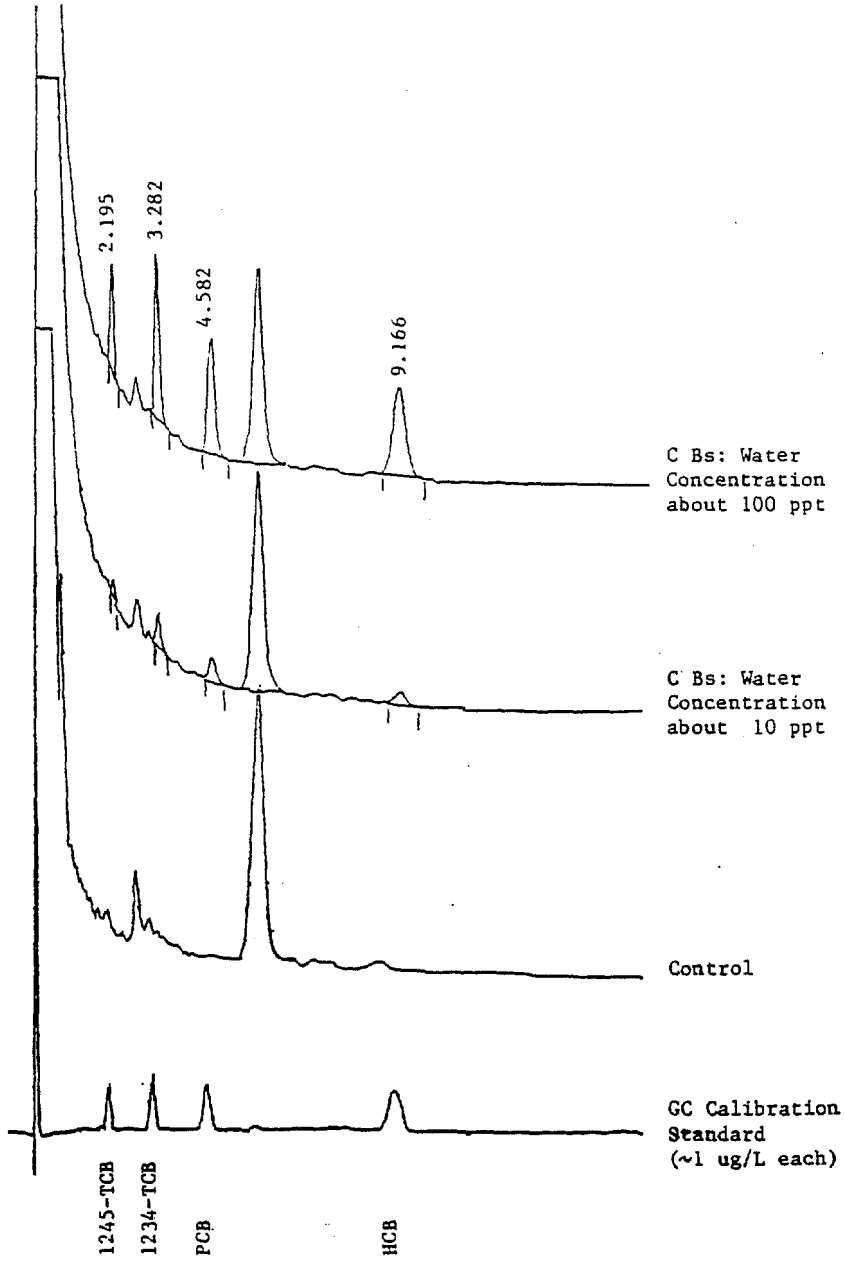

Figure 3. Chlorinated benzenes in hexane removed from dialysis membranes submerged in water 10 days at 9°C.

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Table 7. Concentration of chlorinated benzenes from water using hexane-filled dialysis membranes.

Compound	Water Fortification µg/L	Concentration in Hexane Inside Dialysis Membranes µg/L		Enhancement Factor (1)	
		I(2)	II(3)	I(2)	II(3)
		10 ppt environment			
1245-TCB	0.0100	0.723	1.62	72	162
1234-TCB	0.0075	1.065	2.75	142	370
PCB	0.0050	0.567	0.763	113	220
HCB	0.0066	0.497	0.763	75	120
		100 ppt environment			
1,2,4,5-TCB	0.100	4.46	5.12	45	51
1,2,3,4-TCB	0.075	4.63	5.46	62	55
PCB	0.050	2.74	2.96	55	59
HCB	0.066	3.31	3.19	50	48

(1)-Enhancement Factor = concentration in hexane/initial water fortification level.
(2)-Represents the average of 2 GC measurements. Hexane volume in membrane I was 5.5 ml for 10 ppt system and 6.0 ml for 100 ppt system; volume in membrane II (100 ppt) was 5 ml.
(3)-The dialysis membrane bag was leaking; initial 5.0 ml volume reduced to 2.5 ml.

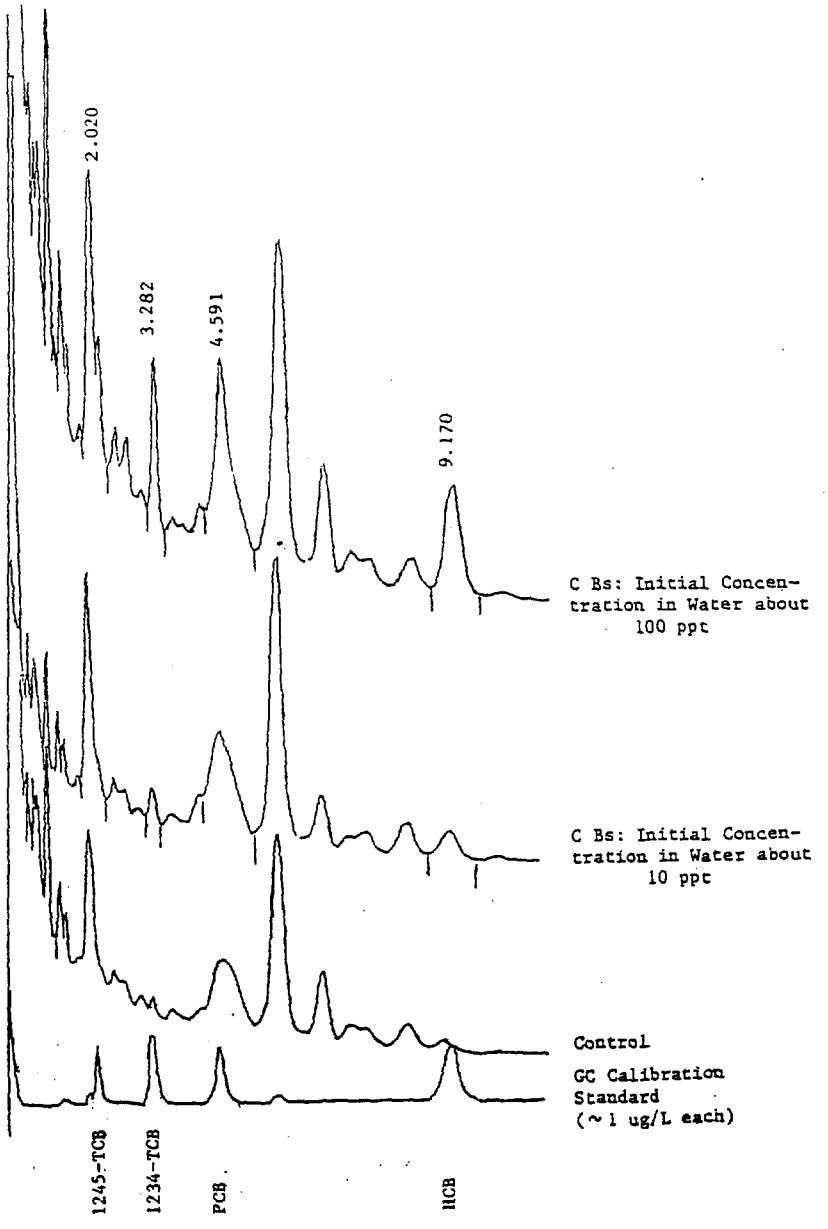

Figure 4. Chlorinated benzenes in fortified water after 10 days at 9°C.

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APPENDIX H

LICHENS AS POLLUTION BIOMONITORS IN LITHUANIA

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LICHENS AS POLLUTION BIOMONITORS IN LITHUANIA

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BACKGROUND

Lichens react in one of two ways when exposed to atmospheric pollutants: (1) sensitive species lose their healthy appearance and die in regions of heavy pollution, especially gaseous pollutants such as SO_2, O_3, and NO_x; and (2) less sensitive species can live for many years and accumulate materials such as metals and radionuclides absorbed from the atmosphere. These latter species can be sampled repeatedly for many years to indicate movement of atmospheric chemicals into living systems.

Many countries near Lithuania -- Norway, Sweden, Finland, Poland, Latvia, Estonia -- are compiling data files using biomonitors that include conditions of lichens near pollution sources, chemical analyses of lichens from various areas on the countries, and listing of lichen flora. Some of these same types of lichen studies have been started by individual botanists in Lithuania. Although no known analyses of lichen tissue have been published, eight metals have been quantitatively analyzed in two species of mosses, *Hylocomium splenden* and *Pleurozium schreberi*, from several locations (Augustaitis et al. 1991). Motiejunaitė (1989) completed a register of lichens in the northwest third of Lithuania, and plans to complete this inventory of the lichen flora of the country. This is a very valuable baseline step in giving present distributions of species. Unpublished data about lichen distribution and tissue chemistry are available from the Lithuanian Agricultural Academy, Kaunas.

OBJECTIVES

Objectives of this proposal are: (1) to encourage botanists to coordinate and consolidate lichen information; (2) to establish a nationwide system of sites from which lichen species can be collected periodically for elemental analysis; (3) to facilitate cooperation between chemists and lichen biologists to ensure high quality tissue analyses. It appears there are several very capable botanists in Lithuania who could achieve such a set of objectives.

METHODS

Publications from two agencies, the Finland National Board of Waters and the Environment (Pylvanainen 1993) and the U.S. Forest Service (Huckaby 1993), outline different approaches to using lichens in monitoring air pollution. Lithuanian scientists should decide if they want a general baseline study from polluted and relatively non-

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polluted areas, or definite monitoring in the vicinity of known pollution sites (Smith et al. 1993) including sites for baselines in non-polluted areas, or both. In each case it may be desirable to include forest stands that are near current Lithuania Environmental Protection Department and U.S. Environmental Protection Agency "Project 31" water quality monitoring sites, locations where mosses have been sampled for element content (Augustaitis et al. 1991), and sites where forest tree data (including pine needle data) have been recorded. This would help coordinate lichen data with other available data.

The Scandinavian countries use a very abundant species, *Hypogymnia physodes*, as a visual estimation of air quality (Pylvanainen 1993); because this species is also common and widespread in Lithuania, it could be used in a similar manner. Two or three additional lichen species with wide distributions should also be collected from as many test sites as possible for tissue analysis for metals.

The metals measured in mosses reported by Augustaitis et al. (1991) were cadmium, chromium, copper, iron, lead, nickel, vanadium, and zinc; Lithuanian scientists can decide if measuring for these metals is adequate or if others are needed. Samples must be replicated for valid statistical analysis, and handled carefully during and after collection to prevent contamination (Jackson et al. 1993).

EXPERTISE REQUIRED

It is necessary to have a lichen specialist involved in a project so that species are collected and identified correctly, and to train the persons who will collect specimens. It also takes practice by a person acquainted with lichens to estimate viability of a species, as in the Scandinavian studies with *H. physodes*. While far less training is required for those persons who may be collectors of specimens in the field, the involvement of a lichen specialist is essential for coordinating activities related to lichen biomonitoring.

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APPENDIX I

BIOACCUMULATION OF WATERBORNE 1,2,4,5-TETRACHLOROBENZENE IN TISSUES OF RAINBOW TROUT

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APPENDIX I

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INTRODUCTION

During the past 3 years we have been studying the rates of uptake by fishes of selected synthetic organic chemicals, foreign to natural systems, known as xenobiotics. We have conducted several tests using an automated respirometer in which we have exposed fish to 1,2,4,5-tetrachlorobenzene (TCB). Our principal test animal has been the rainbow trout (*Oncorhynchus mykiss*), although we have also tested other fish species to compare results. One of our long-range objectives is to determine to what extent uptake and depuration rates of xenobiotics by fishes may be related to their respiration rates, in the hope of developing a simple predictive model describing the rates of uptake and depuration of xenobiotics as a function of the oxygen uptake rate and chemical properties of the chemical in question. In this presentation we will describe our approach to this research and discuss some of our results.

METHODS

Test Conditions

Many of our preliminary tests were conducted at Fisheries Bioassay Laboratory, Montana State University (MSU), and most of our respirometry experiments were conducted in a modified Brett-type respirometer at the Zoology Department, University of British Columbia (UBC). All fish tissue and water analyses were conducted at MSU. The UBC respirometer was designed and built specifically for our studies, and has been described in some detail by Gehrke et al. (1990). The respirometer can test fishes up to 2 kg in size and at swimming speeds as great as 2.5 m/second. Experiments can be conducted over several days with water velocity, temperature, pH, dissolved oxygen, and carbon dioxide all computer-controlled at predetermined levels, and data monitored continuously by the computer as well. For the tests at UBC described here, fish were sacrificed immediately after removal from the respirometer, and whole fish or fish tissues, and test water samples, were frozen and stored at -20°C until shipment to MSU for chemical analysis; shipment was by overnight air carrier with samples packed in dry ice. Fish tested at MSU were prepared for analysis the same as those tested at UBC. All samples were stored at -20°C at MSU prior to analysis.

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TCB Analysis

After receipt at MSU, small whole fish (<10 g), subsamples of homogenates of larger fish, and individual organ tissues, were blended to a fine powder with the aid of dry ice and anhydrous Na_2SO_4 while still frozen. At least two separate samples were prepared from homogenates of larger fish. Only single samples were prepared from smaller fish and fish tissues because of limitations in sample quantity. TCB was soxhlet extracted ($\geq$8 hours) from each sample using hexane. Surrogate (1,2,3,4-TCB) in amount comparable to the amount anticipated for TCB results (usually 100 μg) was added at the initiation of the extraction step. Lipids were removed from portions of this extract using florisil column chromatography; TCB and surrogate were eluted with 5% methyl-*t*-butyl ether/hexane. Pentachlorobenzene (PCB) internal standard (0.25 μg) was added to the purified extract and component concentrations were determined by electron capture gas chromatography (ECD-GC). Packed GC columns (1.8 m x 2 mm i.d.) containing 3% SE-30 or 3% Carbowax on 100/120 Supelcoport™ were used. A multipoint calibration curve (0.5 - 10 μg/l) was employed during quantitation. Both standards and sample extracts were injected in duplicate. When plasma samples were required, the caudal peduncle was severed immediately after the fish was sacrificed and blood was collected from the caudal artery into heparinized capillary tubes. Plasma was separated by centrifugation and 5.0 μl of plasma was removed by means of a 10 μl glass syringe and transferred to a 10 ml volumetric flask containing 0.05 μg internal standard (PCB). This solution was made to volume with hexane, and the contents mixed for approximately 1 minute using a vortex mixer. TCB was extracted from test water samples using hexane, and internal standard was added to the diluted extracts. Both plasma and water samples were analyzed for TCB as described above.

Lipid Analysis

Total lipid content in whole small fish (<10 g) or samples from homogenized larger fish (>30 g) was determined by a modification of a method described by Folch et al. (1957). While still frozen, small whole fish or subsamples of tissue homogenates from larger fish were ground to a powder using dry ice and anhydrous Na_2SO_4. Lipids were extracted by blending this mixture twice for 3 minutes with 2:1 chloroform:methanol. Both extracts and rinsings were vacuum filtered and combined into a 500 ml separatory funnel. Methanol was partitioned from the chloroform into aqueous KCl solution. The chloroform layer was vacuum filtered using a 0.2 μ Nylon-66 filter and the volume adjusted to 250 ml. A measured aliquot was transferred to a tared beaker and chloroform was removed using low heat and a gentle stream of dry N_2. The residue was dried ($\geq$12 hours) in a vacuum desiccator and weighed.

TESTS CONDUCTED, RESULTS, AND DISCUSSION

In several of our initial experiments rainbow trout were exposed to TCB for different time periods up to 6 hours under either static test conditions at MSU or forced swimming conditions in the respirometer at UBC. After test, fish were sacrificed and different tissues analyzed to determine any apparent sequence among them in increase of toxicant concentration. Tissues analyzed were blood plasma, gills, liver, kidney, brain, spleen, heart, upper gut, lower gut, white muscle, pink muscle, and adipose (Table 1).

Table 1. Fish tissue concentrations after exposure to TCB.

Exposure (hours)	n	Plasma	Gills	Liver	Kidney	Spleen	U. Gut	L. Gut	W. Mscl	P. Mscl	Heart	Brain	Adipose
					TEST 118	500 - 25 µg/l TCB		0 BL/s					
0.5	2	5.73	4.68	8.70	8.10	<1.5	2.50	3.44	4.03	11.85	10.40	10.60	2.99
1.0	2	10.34	8.65	36.35	13.15	9.84	4.23	10.41	6.82	31.30	15.45	25.90	1.17
3.0	3	10.09	8.58	39.73	17.85	10.55	5.86	16.18	9.49	83.00	19.97	42.00*	20.97
6.0	3	8.39	5.20	14.87	14.20	7.64	7.28	6.19	7.26	63.33	16.00	15.43	23.83
					TEST 105	400 - 50 µg/l TCB		1.25 BL/s					
1.0	2	3.74	4.13	7.85	5.38	2.78	1.93	7.68	3.54	18.50	5.33	11.15	20.59
2.0	2	6.85	4.24	11.25	6.43	2.59	3.32	7.34**	5.20	18.00	6.13	11.29	8.22
6.0	2	3.17	2.96	10.78	9.29	2.21	4.22	no data	7.09	38.55	5.19	8.99	59.75

Plasma µg/ml, Tissue µg/g * = two values, ** = one value

We also reviewed the data to determine if the rate of toxicant concentration increase in any one tissue might be representative of that in the fish. Because TCB is lipophilic, this presupposes one tissue might have a fat content representative of the fish as a whole, or at least with a fairly constant ratio to that of the fish as a whole. Standard deviations from the mean concentrations of TCB among the tissues analyzed were so great that we were unable to detect any sequential build-up among the tissues. Indeed, concentrations were not appreciably different between 1 and 6 hours except in the case of adipose tissue and possibly muscle. We also looked at the tissue/blood plasma ratio of the test fish, and once again did not see any consistent ratio (Table 2).

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Table 2. Ratio of fish tissue concentrations to blood plasma after exposure to TCB.

Exposure (hours)	n	Plasma µg/ml	Gills/ Plasma	Liver/ Plasma	Kidney/ Plasma	Spleen/ Plasma	U.Gut/ Plasma	L.Gut/ Plasma	W.Mscl/ Plasma	P.Muscl/ Plasma	Heart/ Plasma	Brain/ Plasma	Adipose/ Plasma
					TEST 118	500 - 25 µg/l TCB		0 BL/s					
0.5	2	5.73	0.816	1.52	1.41	0.0421	0.437	0.600	0.703	2.07	1.82	1.85	0.523
1.0	2	10.3	0.839	3.53	1.28	0.956	0.411	1.01	0.663	3.04	1.50	2.51	0.114
3.0	3	10.1	0.849	3.93	1.77	1.04	0.580	1.60	0.940	8.22	1.98	3.40	2.08
6.0	3	8.39	0.619	1.77	1.69	0.911	0.860	0.737	0.866	7.55	1.91	1.84	2.84
					TEST 105	400 - 50 µg/l TCB		1.25 BL/s					
1.0	2	3.87	1.07	2.03	1.39	0.720	0.499	1.98	0.915	4.78	1.38	2.88	5.32
2.0	2	6.85	0.619	1.64	0.939	0.378	0.484	1.05	0.759	2.63	0.896	1.65	1.20
6.0	2	3.17	0.934	3.40	2.93	0.699	1.33	no data	2.24	12.2	1.64	2.83	18.8

It was apparent we would need to measure TCB concentrations in whole fish, and our next tests were designed to compare blood plasma TCB concentration against that of the whole body over time. In our first experiment the concentrations of TCB in blood plasma and whole body were very similar after 2 hours exposure, but blood plasma concentration at 6 hours had not increased, whereas whole body concentration had more than doubled (Figure 1).

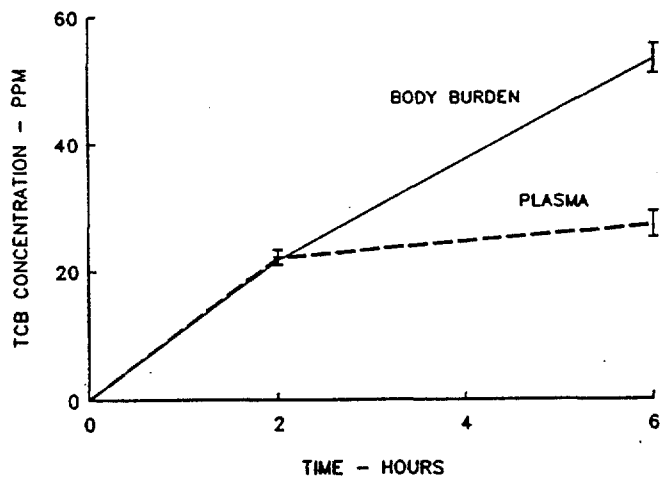

Figure 1. TCB plasma concentration vs whole body burden in rainbow trout.

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The obvious conclusion was that TCB equilibrium between exposure water and blood plasma was achieved in 2 hours or less, and after reaching saturation the blood was acting as a conduit to pass TCB from gills to body tissues. Thus, blood plasma could not be used to indicate either total body content or uptake rate of the toxicant.

In our next experiment 5-g rainbow trout were exposed to nominal TCB concentrations of 10, 50, and 100 μg/liter in holding tanks for several days, during which they were transferred to a fresh solution every 24 hours. Four fish were removed from each tank at 1, 2, 4, and 8 days, sacrificed, and blood plasma and whole body TCB concentrations measured. A tight pattern of TCB increase over time emerged for both blood plasma and whole body, and these patterns were also correlated with exposure concentrations (Figure 2), but once again equilibrium between TCB in exposure water and whole body lagged behind TCB equilibrium reached in blood plasma.

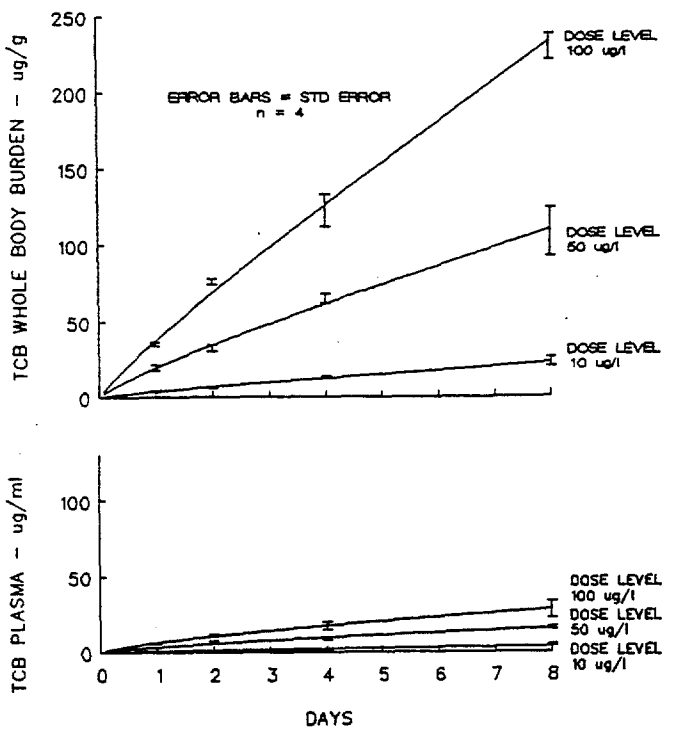

Figure 2. TCB plasma vs whole body burden in rainbow trout.

Earlier studies at MSU had shown the rate of uptake of some of the chemicals we would be studying can be extremely rapid, with equilibrium between whole body and water sometimes reached in just a few hours. Tischmak (1984) demonstrated that equilibrium for 2,4,6-trichloroaniline between fathead minnows (*Pimephales promelas*) and their environment was achieved in less than a day (Figure 3), and although equilibrium for TCB required 6-8 days, it was also apparent that the rate of uptake of TCB decreased considerably between initial exposure and 24 hours into test (Figure 4).

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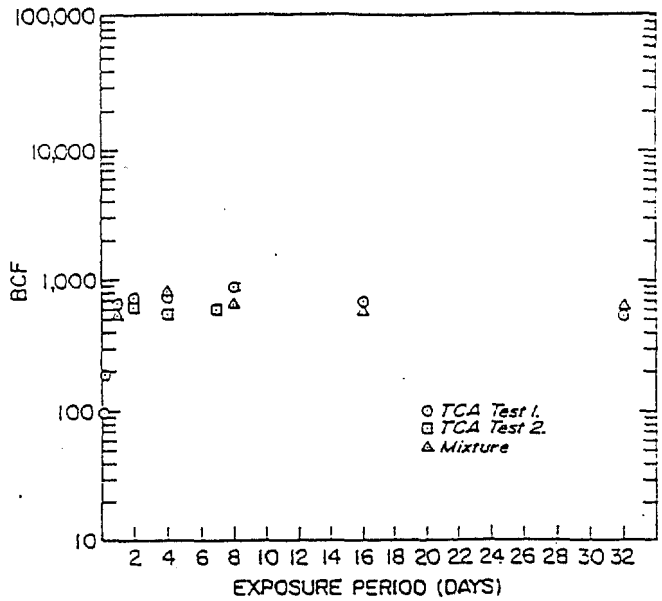

Figure 3. TCA bioconcentration profiles (from Tischmak 1984).

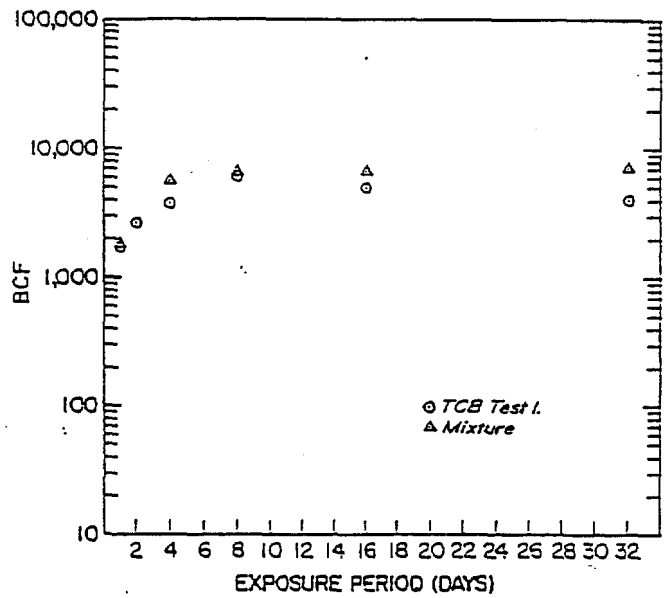

Figure 4. TCB bioconcentration profiles (from Tischmak 1984).

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Our next step was to conduct a series of short-term tests in which we exposed rainbow trout to TCB in the UBC respirometer to measure TCB uptake vs. oxygen consumed. We chose a 2-hour test period for several reasons, but principally because this was long enough to measure oxygen differences in the exposure water for as little as 100 g of test fish, and short enough so that rate of toxicant uptake by the test fish would not measurably vary between start and finish of the test period. The uptake of TCB by rainbow trout per gram of fish for three different weight classes is shown in Figure 5. Because of the lipophilicity of TCB, we also measured lipid content in whole fish samples from each weight class, and the data were replotted as uptake of TCB against total lipid (Figure 6).

Finally, we looked at uptake of TCB in relation to oxygen consumption rate for each of the three different weight classes of rainbow trout, each tested at two different swimming speeds (Figure 7). This latter curve demonstrates that there was a striking correlation between oxygen uptake and TCB uptake by these rainbow trout under our test conditions.

Separate from these chemical uptake studies, we have compiled a file of data from the published literature on respiratory oxygen requirements of fishes (Thurston and Gehrke, 1992). This file, called OXYREF, contains data from over 6800 individual laboratory tests in which oxygen consumption was measured. The data in our file include fish species, fish weight, certain test water conditions, measured respiratory oxygen requirements, and mode at which each fish was tested: "standard" (resting), "routine" (moving about), or "active" (measured swimming rate).

From OXYREF, we plotted oxygen consumption vs. weight for fishes in an active swimming mode, which is that mode under which we tested our fish at UBC in these TCB uptake experiments. If we now superimpose the data from our rainbow trout TCB experiments on the active swimming mode curve from OXYREF, one sees an excellent fit; the bulk of those data points from the higher swimming speed tests are slightly above the OXYREF curve, and the data points from the slower swimming speed tests are slightly below the curve (Figure 8).

There is a clear correlation between the rate of oxygen uptake and the rate of uptake of TCB among the freshwater fish species we tested. If this correlation holds for other representative fish species, and if the oxygen uptake rate and the water concentration of a chemical to which a fish is exposed are known, it may be possible to predict uptake rates of that chemical during exposure. We are continuing to expand OXYREF as more data become available in the literature, but in the present laboratory study if we can establish correlations between oxygen consumption and toxicant uptake and depuration, then OXYREF can provide a powerful tool for the prediction of bioaccumulation by fishes of xenobiotics.

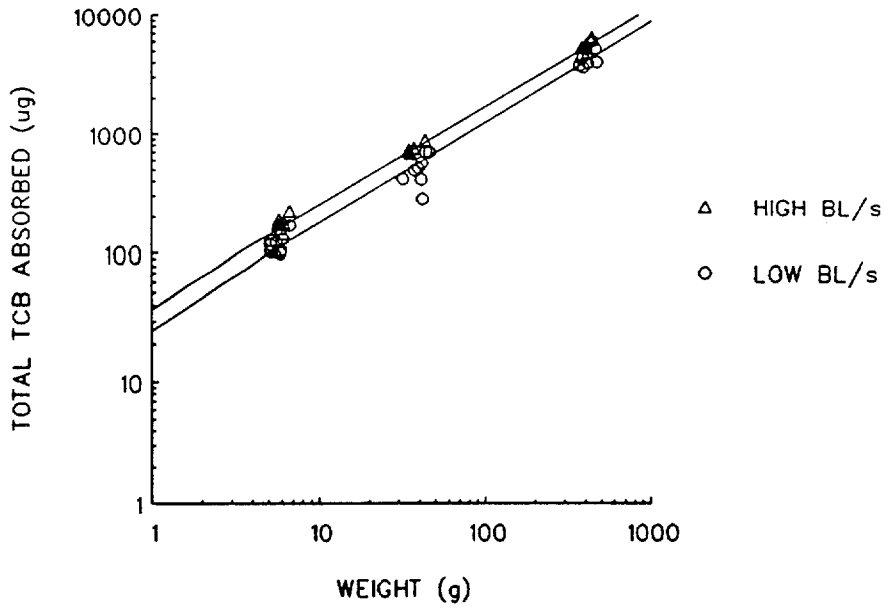

Figure 5. Rainbow trout at two swimming speeds--
TCB concentration vs fish weight.

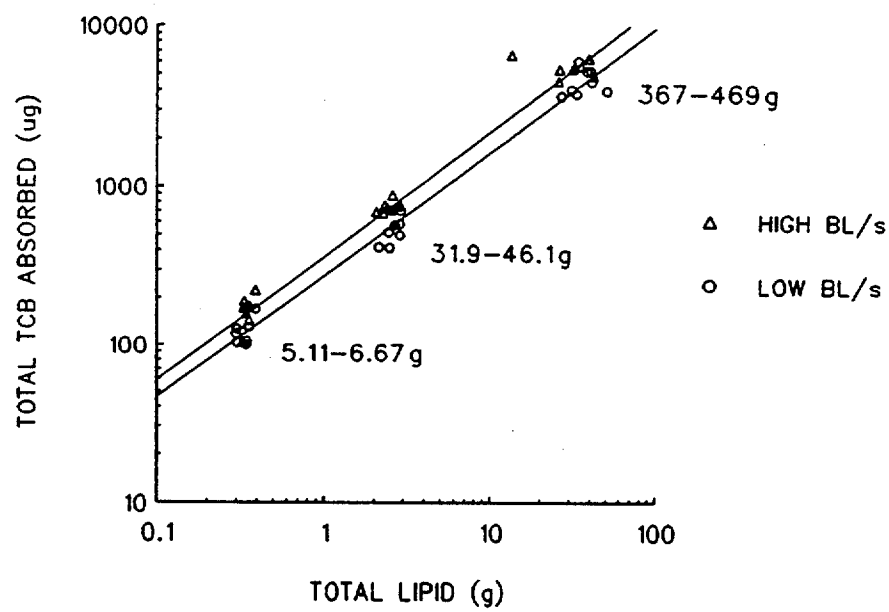

Figure 6. Rainbow trout at three weight classes--
TCB concentration vs lipid concentrations.

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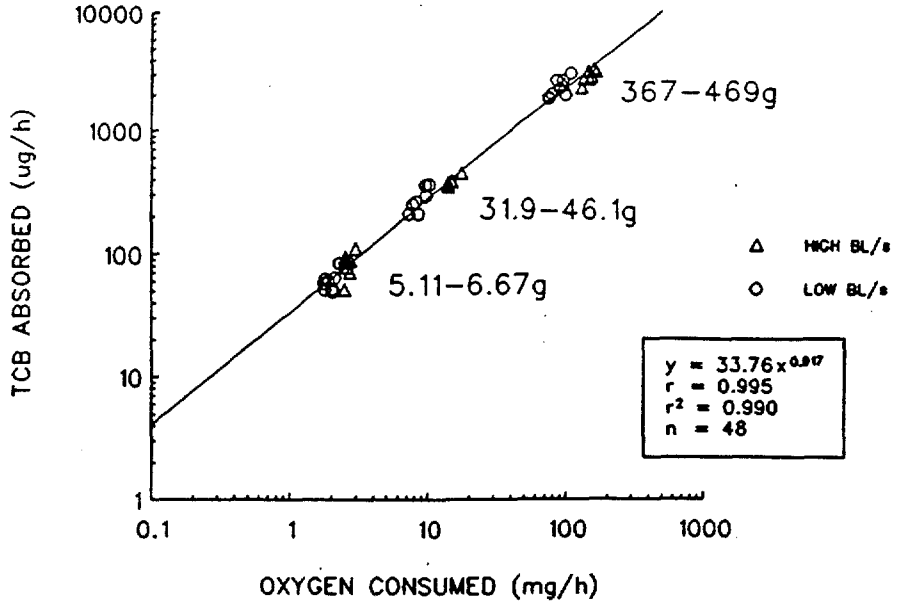

Figure 7. Rainbow trout at three weight classes--
TCB concentration vs oxygen consumed.

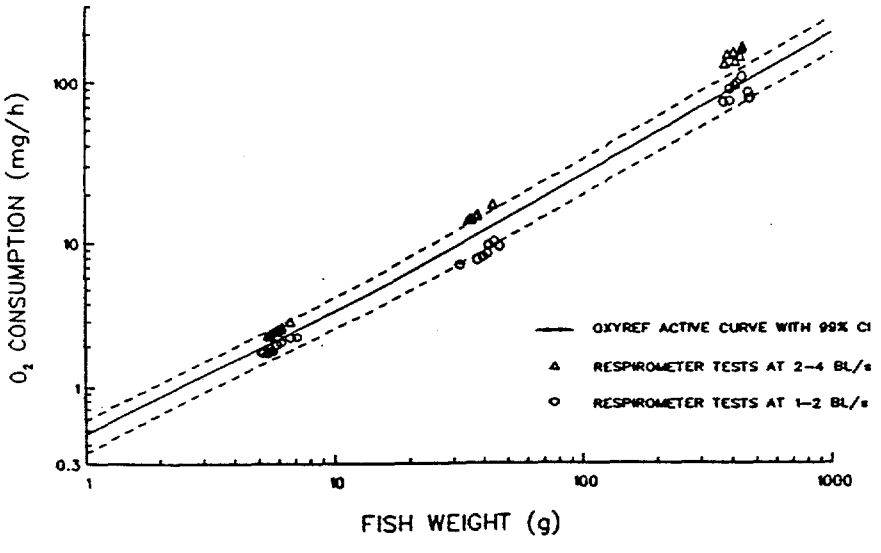

Figure 8. *OXYREF* data file active-swimming regression curve vs rainbow trout oxygen consumption during respirometer tests.

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