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UNITED NATIONS INDUSTRIAL DEVELOPMENT ORGANIZATION
Vienna International Centre, P.O. Box 300, 1400 Vienna, Austria
Tel: (+43-1) 26026-0 · www.unido.org · unido@unido.org

ICS-UNIDO WORKSHOP ON CONTAMINATION OF FOOD AND AGROPRODUCTS

28 September – 1 October 2000
Varaždin, Croatia

United Nations Industrial
Development Organization

International Centre for Science
and High Technology

Institute for Medical Research
and Occupational Health

Workshop on Contamination of Food and Agroproducts

Varaždin, Croatia
28 September – 1 October 2000

Reprints from the
ARCHIVES
OF INDUSTRIAL HYGIENE AND TOXICOLOGY
vol. 52, issues 1–2, year 2001

Organisers

United Nations Industrial Development
Organization, Vienna, Austria

International Centre for Science
and High Technology, Trieste, Italy

Institute for Medical Research
and Occupational Health, Zagreb, Croatia

ARHIV
ZA HIGIJENU RADA I TOKSIKOLOGIJU

ARCHIVES
OF INDUSTRIAL HYGIENE AND TOXICOLOGY

1

ISSN 0004-1254 UDC 613-6 CODEN: AHRTAN

VOLUME 52
2001

Arh Hig Rada Toksikol
Vol. 52. No.1.pp. 1-162
Zagreb, March 2001

INSTITUTE FOR MEDICAL RESEARCH
AND OCCUPATIONAL HEALTH
ZAGREB, CROATIA

ISSN 0004-1254

ARHIV ZA HIGIJENU RADA I TOKSIKOLOGIJU ◇ ARCHIVES OF INDUSTRIAL HYGIENE AND TOXICOLOGY

ARH HIG RADA TOKSIKOL ▫ VOL 52 NO 1 PP. 1–162 ▫ ZAGREB, CROATIA 2001

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Arh Hig Rada Toksikol 2001;52:1

FOREWORD OF THE GUEST EDITOR

ICS-UNIDO Workshop on Contamination of Food and Agroproducts

The first and the second issue of the 52nd volume of the *Archives* bring a selection of papers presented at the Workshop on Contamination of Food and Agroproducts that was held from 28 September to 1 October 2000 in Varaždin, Croatia. The organisers of the Workshop were International Centre for Science and High Technology of the United Nations Industrial Development Organization (ICS-UNIDO), Trieste, Italy and the Institute for Medical Research and Occupational Health, Zagreb, Croatia. The Workshop was organised under the auspices of the Ministry of Health, Ministry of Agriculture and Forestry, and Ministry of Science and Technology of the Republic of Croatia.

Croatia was invited to host the Workshop and the Institute for Medical Research and Occupational Health, Zagreb, was given the privilege to act as its organiser.

The Workshop covered topics related to food contamination such as chemical, microbiological, and biological contamination, as well as storage and prevention of food contamination with specific focus on problems of the Central and East European countries. The Workshop encompassed a number of analytes (metals, pesticides, mould, and bacteria) in various sample materials (water, food, samples of human and animal origin, samples from industrial processes). Invited speakers from seven countries (Belgium, Canada, Croatia, Italy, Slovak Republic, Slovenia, and the United Kingdom) gave eleven lectures. The programme included twelve country and institutional presentations. International participants (13) were from Albania (3), Bulgaria (1), Hungaria (3), Macedonia (1), Latvia (1), Romania (1), Slovak Republic (1) and Slovenia (2). Together with 41 Croatian participants the Workshop gathered 65 participants.

ICS-UNIDO and the Institute for Medical Research and Occupational Health have agreed to publish a selection of papers from the Workshop in a special issue of the *Archives of Industrial Hygiene and Toxicology*. According to the policy of the *Archives* all papers were reviewed. Eight papers are presented in this issue whereas the next issue will bring the rest. The two issues give the full coverage of the scientific programme.

Our thanks are due to all who supported the Workshop, above all the International Centre for Science and High Technology of the United Nations Industrial Development Organization, Trieste, Italy, which promoted and financed the Workshop, and to the Istituto Italiano di Cultura Zagabria, Zagreb which supported the publication of the Book of Abstracts. We hope that this endeavour will stimulate the adoption of current principles in control and standardisation, and in protection of food against contamination. We thank the authors and the participants of the Workshop for their contributions as well as all those who have helped their publication.

Guest Editor
Blanka Krauthacker
Local Organiser

Arh Hig Rada Toksikol 2001;52:3-9

ICS-UNIDO Programme in the Area of Pure and Applied Chemistry. Activities in the Subprogramme of Remediation and Focus on Food Contamination

STANISLAV MIERTUS

ICS-UNIDO, Trieste, Italy

The United Nations Industrial Development Organization (UNIDO) is a specialized agency of the United Nations dedicated to promoting sustainable industrial development in developing countries and countries with economies in transition. It harnesses the joint forces of the government and the private sector to foster competitive industrial production, to develop international industrial production and international industrial partnerships and to promote socially equitable and environmentally friendly industrial development.

UNIDO is the only worldwide organization dealing exclusively with industry from a development perspective. UNIDO's services are non-profit making, neutral and specialized. UNIDO acts as a catalyst to help generate national economic wealth and raise industrial capacity through its role of a worldwide forum for industrial development and by providing technical cooperation services. UNIDO's ultimate goal is to create a better life for people by laying industrial foundations for long term prosperity and economic strength.

The *International Centre for Science and High Technology (ICS)* is an institution within the legal framework of UNIDO with the headquarters located in Trieste, Italy in the AREA Science Park.

The Centre's mandate relates to the transfer of know-how and technology in favor of developing countries, and is justified by the perception that a competitive industrial technological capability cannot be built-up without adequate scientific knowledge and commitment to a sustainable development approach based on new and environmentally friendly technologies.

In the present Work Programme the ICS's activities focus on specific sectors within the areas of:

- Pure and Applied Chemistry,
- Earth, Environment and Marine Sciences

- New Materials and High Technology,
- Technology Management

The main tools for the implementation of the Work Programme of the ICS Area of Pure and Applied Chemistry, are the following:

- Organization of Training Events (EGMs, Training Courses and Workshops).

These training events organized either at ICS, in Italy, or in the various interested developing countries are the main tool for the spread of information, the technology transfer and the awareness building in the scientific fields developed in this area.

- Information Packages and Publication Activities.

Information and data collected are compiled and organized in information packages and in several publications (such as the proceedings of the books of proceedings) on the various scientific fields.

- Fellowships and on Job Training Schemes.

ICS grants fellowships (lasting from 6 months to 1 year) for junior researchers coming from developing countries, their job at ICS focuses on issues related to follow-up project proposals (or case studies), on the assistance in the preparation of data (survey of companies/technologies) for the information packages, and in the preparation of publications and contributions.

- Networking and participation of ICS in international projects and initiatives.
- Evaluation and development of Project Proposals

Project Proposals received by Institutions/Governmental Bodies of the various developing countries are received and evaluated by ICS. Feasibility Studies are carried out by the Centre, when applicable and the relevant search of suitable funding Institutions is performed.

In selecting the specific subprogrammes of each area and their related activities, special consideration was given to their relevance in relation to the scientific and technological development of developing countries and economies in transition.

Considering that sustainable development depends upon the harmonization of economic growth and environment conservation and protection, the ICS area of Pure and Applied Chemistry has identified as priority fields in its work programme the following themes, which are of key relevance to economic and industrial development as well as environmental protection:

- Catalysis & Sustainable Chemistry
- Environmentally Degradable Plastics
- Combinatorial Chemistry and Technologies
- Remediation

Catalysis & Sustainable Chemistry

It is an important scientific and technological area for the development of environmentally friendly chemical processes, which in turn form the basis for cleaner industrial development and are also the key elements for an industrial pollution prevention approach. New, less pollutant processes together with the optimisation of existing processes depend to a great extent upon the improvement of catalyst performance in the heavy and fine chemical production lines with a direct impact on the generated by-products or waste.

Environmentally Degradable Plastics

The expanding global production and consumption of polymeric materials coupled with increasing public awareness of environmental issues have created serious concern about the problems related to the disposal of plastic waste generated by various sectors of human activity. Besides recycling, re-use, incineration and composting, new technological developments of environmental degradable plastics contribute dramatically to the tackling of the environmental issue in specific sectors of plastics use.

Combinatorial Chemistry and Combinatorial Technologies

These technologies have a strong impact on the development of new chemicals (pharmaceutical industries, agro-chemicals, new materials). Developing countries need to get acquainted with and gain expertise in combinatorial technologies to help local enterprises remain competitive and economically viable in the coming decades. Combinatorial chemistry and combinatorial technology have a potential influence not only on industrial growth, but also on environment protection. In fact, by optimising industrial processes and production, with the lowering of relevant costs, less amounts of waste and by-products are created.

Remediation

One of the most urgent problems to be faced at a global level is the decontamination of soil and waters due to domestic and industrial activities. Large polluted areas beside having lost their eco-functionality, often represent a serious risk for human health. The policy for the restoration of natural resources is thus a priority in developing as well as in industrialized countries.

In the last years several remediation technologies have been developed for the decontamination of polluted sites and many of them have proved to be very promising to clean up contaminated water and soils.

Bio-remediation is a very effective and widely applied clean-up technology, which is able to degrade hazardous, toxic or merely offensive pollutants. *In situ* as well as *ex-situ* bio-remediation applications are proved to be able to clean-up sites which have been contaminated by a wide range of compounds that were once believed to be recalcitrant, such as chlorinated solvents, PCBs, chlorofluorocarbons and other stable compounds.

Other technologies, based on thermal and/or physico-chemical processes, have already been developed on an industrial basis and many emerging techniques seem to be very promising to handle different kinds of contamination as they have proved to reach significant results both in pilot scale and in full scale applications.

In conclusion, bio-remediation technologies together with physico-chemical and thermal methods represent an important way of facing the crucial problems of environmental recovery. Research and development efforts are extending their applicability and it is expected that there will be an increased use of these technologies leading, especially in developing countries, to a very promising industrial market development.

As for the other subprogrammes, the ICS Subprogramme on Remediation is implemented through the following activities:

- EGMs, Training Courses and Workshops,
- Fellowships
- Networking
- Follow-up projects

The training events in the Remediation subprogramme, develop the following main contents and aim at the following main objectives:

- To train on the fundamental concepts of remediation
- To introduce and describe new, modern remediation technologies
- To show practical applications of remediation technologies through site demonstrations
- To train in the solution of actual pollution problems through problem solving sessions
- To promote the exchange of experiences through open discussions among participants and gather specific information through their direct interaction with lecturers
- To discuss possible common initiative

Events

The following main training events were organized in the past years (from 1996)

1. Scientific Planning and Coordination Meeting (SPCM) on »Bioremediation«, November 1996, Trieste, Italy
2. Training Course (TC) on »Soil, Environmental Assessment and Bioremediation Technologies«, June 1997, Budapest, Hungary
3. TC on »Technological and Economic Aspects of Soil Bio/Phyto Remediation«, October 1997, Plovdiv, Bulgaria
4. Expert Group Meeting (EGM) on »Environmental Pollution and BATEV in Remediation«, March 1998, Trieste, Italy
5. Workshop (Ws) on »Waste Management and Remediaiton of Polluted Sites for Sustainable Development«, May 1998, Hanoi, Vietnam
6. TC on »Remediation Technologies: New Trends and Tools for Soil Decontamination«, December 1998, Katovice, Poland
7. Ws on »Remediation Technologies: Applicability and Economic Viability in Northern Africa and the Middle East«, October 1999, Cairo, Egypt

Moreover, within the Remediation Subprogramme the following products and projects (publications) are being developed and prepared at ICS:

Products

- Four National Reports (Brazil, Egypt, Russia, Vietnam) on »Pollution Problems, available remediation technologies and their economic viability«

- ☐ Compendium on Remediation »Survey of Remediation Technologies, applicability, economic viability and main players«

Developed in close cooperation with the United Nations Economic Commission for Europe (UN-ECE)

Projects

- ☐ *»Integrated info-package on remediation technologies and their application«*
  Counterparts: Germany, Poland, Slovak Rep.
  project submitted to the Leonardo da Vinci Programme of the European Commission
- ☐ *»Integrated remediation approach for soil decontamination: a tool for water resources protection«*
  Counterparts: Austria, Germany, Slovenia
  project to be re-submitted to the 5th Framework Programme of the European Commission
- ☐ *»Development and Application of Novel Technologies for Used Oil Treatment and Regeneration in CEE and NIS Countries«*
  Counterparts: CEE and NIS countries

and the following activities/initiatives have been planned for Year 2000

Training Events 2000

Title: EGM on *»Trends in Contaminants Monitoring and Remediation«*
Venue and dates: Trieste, Italy, May 2000

Title: Workshop on *»Environmental Pollution and Remediation Technologies in NIS Countries«*
Venue and dates: Uzbekistan, September 2000

Title: Seminar on *»Contamination of Food and Agroproducts«*
Venue and dates: Croatia, September 2000

Title: Workshop on *»Environmental Pollution and Remediation Technologies in Latin American Countries«*
Venue and dates: Colombia, November 2000

Products 2000

- ☐ A comprehensive publication on *»Methods, Technologies, and Applicability of Remediation«*
  Publication of selected materials collected in 1997–1998 ICS training events
- ☐ ICS Proceedings from Expert Group Meetings on »POPs and pesticides Contamination: Remediation Technologies« (Trieste 17–19 April 2000) and on »Clean Technologies for the Reduction and Elimination of POPs« (Trieste 4–5 May 2000)
  ICS Publication

- ☐ The triangle of Sustainable Chemistry. Innovation, Economy and Environment
  F. Gennari, F. Pizzio, S. Miertus
  La Chimica e l'Industria (June 2000) 5. 514–519
- ☐ An Overview of Soil Remediation Technologies
  T. Jakpa, E. Gonzalez, A. Lodolo, S. Miertus
  Genetic Engineering and Biotechnology – Emerging Technologies Series 3 and 4/1998
  UNIDO Publication (December 1999)
- ☐ *Impegno mondiale contro I POPs*
  S. Miertus
  La Chimica e l'Industria (March 2000) 5. 142–145

Projects 2000

- ☐ Development of a Decision Support System on remediation technologies
  Objectives: To develop new tools for technical evaluation, efficiency and economic analysis of remediation technologies
  To create a tool for decision in remediation initiatives,
  To apply DSS tools in ICS projects on remediation.
- ☐ *»Integrated Approach to Solve Urgent Contamination Problems Caused by Industrial Activities in CEE Countries«*
  Objectives: To survey industrially polluted sites and to select hot spots
  To carry out feasibility studies and evaluate suitable and viable remediation technologies
  To develop selected pilot projects
  To disseminate knowledge and information
  Counterparts: Albania, Hungary, Poland, Slovak Rep.

Food and Agroproducts Contamination

Within the subprogramme on Remediation, particular attention has been focused on problems relevant to food and agroproducts contamination monitoring and prevention.

The increasing demand of food production systems in recent years have led to large sections of the food chain becoming contaminated. Although some of the reported increase in contamination is due to better detection and recording, it is undoubtedly a real problem.

The aim is to assure the safety and integrity of the food supply by anticipating risks, tracing the sources of contaminants throughout the complete food chain and quantifying risk factors and optimising methods for detecting undesirable components

The introduction and implementation of new systems of contamination control for food and agroproducts, together with a proper planning and management of food production, storage and distribution will provide a substantial input to the reduction of risks related to contaminated food assumption. These issues are tightly related to remediation technologies which are the tools for food contamination prevention.

For these reasons, there is the need of enhancing activities in this field and of forming an indigenous class of professionals able to act as experts in their own countries and to embrace the responsibility of transmitting the acquired knowledge in the relevant sectors of their home countries.

With the international cooperation and the correspondent support, ICS is therefore promoting the development and application of food and agroproducts quality control methodologies in developing countries and contributing to strengthen national capabilities with adequate programmes and initiatives for local experts.

Because of the importance of the issue in Central and East European countries, it was agreed to organize a workshop on *»Contamination of Food and Agroproducts«, to be held in Croatia*, in Varaždin, from 28 September to 1 October 2000 in cooperation with the »Institute for Medical Research and Occupational Health« of Zagreb, under the auspices of the Ministry of Health of the Republic of Croatia, Ministry of Agriculture and Forestry of the Republic of Croatia and the Ministry of Science and Technology of the Republic of Croatia.

The workshop gave an updated review on the problems of contamination of food and agroproducts and on modern available food quality control methodologies. It can be useful for the Industry of the Region as it represented an opportunity for the regional and local industrialists to make contact with up-to-date techniques devoted to production, distribution and control of food quality.

The workshop collected updated information on activities and projects carried out in selected CEE countries, identifying the strategies adopted case by case and the possibility to link these initiatives and launch joint projects.

The first two issues of *Arhiv za higijenu rada i toksikologiju – Archives of Industrial Hygiene and Toxicology*, vol. 52, summarize the selected presented papers from the workshop.

Arh Hig Rada Toksikol 2001;52:11–21

CONFERENCE PAPER

QUALITY OF LIFE PROGRAMME – FOOD, NUTRITION, AND HEALTH – PROJECTS PROMOTION

ACHIM BOENKE

European Commission, Brussels, Belgium

Received September 2000

The EC Quality of Life Programme (QoL), Key Action 1 – Food, Nutrition & Health aims at providing a healthy, safe, and high-quality food supply leading to reinforced consumer's confidence in the safety of the European food. Key Action 1 is currently supporting several European projects investigating analytical methods for food control including sensors, risk analysis, and food safety standardisation. Their objectives range from the development and validation of prevention strategies for mycotoxin formation via the development of a communication platform for Genetically Modified Organisms (GMO), validation and standardisation of diagnostic Polymerase Chain Reaction (PCR) for food-borne pathogens, up to the evaluation of the potential cancer-preventing activity of pro-and prebiotic (»SYNBIOTIC«) combinations in human volunteers. This paper also informs on future research needs in food safety.

Key words:
analytical methods, European Research, food safety, legislation, quality management, risk analysis, standardisation

The general aim of Key Action 1 (KA1) within the EC Quality of Life Programme (QoL) is to better understand consumer's requirements and to provide a healthy, safe, and high quality food supply while improving the competitiveness of the European food industry (1). The overall objectives are: addressing consumer needs and enhancing the competitiveness of the European food industry; assuring the safety and integrity of the food supply; and understanding the role of nutrition in health and well

Presented at the ICS-UNIDO Workshop on Contamination of Food and Agroproducts, 28 September – 1 October 2000, Varaždin, Croatia

being. Furthermore, pre-and co-normative research objectives form specific and essential objectives of KA1.

These research objectives assume that the money being spent by every European household on food and drink, which is on average 20% of household's disposable income (2), is not leading to consumer's health damage, trade, economical and job losses. Namely, the European food and drink processing industries alone employ about 2.3 million people, and the 1996 consumption within the Community reached nearly Euro 500,000 million (2).

Furthermore, the »*problem solving research approach*« also aims at avoiding food-borne illnesses such as those reported in 1990 (120 cases per 100,000 population from 11 European countries) (3). Consequently, the Community research in the food domain grew in importance and budget during the nineties. This is shown by the fact that the budget allocated to food nearly doubled with subsequent programmes. The FAIR-programme had the budget of Euro 123 million for food alone. The »Quality of Life and Management of Living Resources« programme of the 5th Framework Programme (5FP) has been going on since 1999. Its Key Action 1 on »Food, Nutrition and Health« has been allocated Euro 290 million to support the European food research over the period 1999–2002, out of which about Euro 35 million was already allocated to new food safety research projects.

The 15 projects briefly described in this article are related to the research areas of food safety which were opened in the first call for proposals in 1999 and contribute to the answers on the research priorities of KA1. These 15 projects are based on »problem solving« and aim at providing scientifically validated, reliable, and cost-effective and time-effective solutions to the specific area of food safety research to ensure consumer health and safety, preparation and updating of sound, scientifically based EU regulations, and strengthening of the competitive position of European food and food processing industry. The projects also have a collaborative character as different partners from various sectors such as food industry, official control laboratories, private and official research organisations, universities, and consumer organisations work together.

The following text is a brief overview of the objectives and the duration of the 15 projects grouped by objectives and priorities specified in the KA1's work programme:

DEVELOPMENT OF TESTS AND PROCESSES TO DETECT AND ELIMINATE INFECTIOUS AND TOXIC AGENTS THROUGHOUT THE FOOD CHAIN

Validation and standardisation of diagnostic PCR (Contract No. QLK1–1999–00226)

The project is seeking to validate and standardise the use of polymerase chain reaction (PCR) for detection of food-borne pathogens. Additional specific project objectives include validating a simple method for purifying DNA from bacterial cultures, establishing a central collection of certified DNA sample materials, establishing a databank containing key food-pathogen DNA sequence, listing strains for specificity testing, developing standardised reagents, and validating pre-PCR sample treatment methods. To facilitate its routine use for diagnostic purposes, participants in this project are being asked to undertake a series of specific projects, such as to construct a DNA sample library and primer databank, validate the widely used thermocyclers and other automated equipment, and to develop uniform guidelines describing how tests should be conducted. Because much of the challenge in applying PCR to food-borne pathogens is technical, there is also a need to develop standardised training manuals and procedures for those who will conduct such tests.

Rapid detection of transnational food-borne viral infections and elucidation of transmission routes through molecular tracing and development of a common database (Contract No. QLK1–1999–00594)

The project team has set the following overall objectives: to study the importance of enteric viruses as causes of illness across Europe with a special focus on multinational outbreaks of infection with Norwalk-like viruses and hepatitis A virus; to develop novel, standardised, rapid methods for virus detection and typing to be used in all participating laboratories; to establish the framework for a rapid, prepublication exchange of epidemiological, virological, and molecular diagnostic data; to determine which are the high-risk foods and major transmission routes of food-borne viral infections in the different countries and between countries; to describe the pattern of diversity within and between countries, and identify potential pandemic strains at the onset; and to investigate the mechanisms of emergence of these strains, including the possibility of spillover from animal reservoirs.

Reliable, standardised, specific, and quantitative detection of genetically modified food (Contract No. QLK1–1999–01301)

The primary objectives include the following: to develop reliable and transformation-event-specific tests for qualitative and quantitative detection of genetic modifications in food; to develop reliable and transformation-event-specific multiplex tests for determination of the diversity of genetic modifications in food; and to investigate how improved methods for detection of genetically modified foods will influence consumer confidence in food security and trust in science and risk regulators. Its specific goals are the following:

- to define the domain of application of a standard DNA extraction procedure for complex and processed food (quality and yield);
- to design species-specific reference-gene primer-probe sets for qualitative and quantitative PCR amplification and detection;
- to characterise the DNA sequence of the genetically modified organisms (GMOs) junction regions (insertion site and insert) for each of at least twelve GMOs;
- to design GMO-transformation-specific primer-probe sets for qualitative and quantitative PCR amplification and detection for each of at least twelve GMOs;
- to select the best primer-probe sets for multiplex qualitative and specific quantitative detection of genetic modifications in food and validate them in ring-trials;
- to investigate how improved methods for detection of genetically modified foods will influence consumer confidence in food security and trust in science and risk regulators;

- ☐ to identify of the domain of application and matrix-limitations for a standard DNA extraction protocol;
- ☐ to identify and characterise suitable taxon-specific reference genes, and the development of reference gene specific primer-probe sets for qualitative and quantitative PCR amplification and detection (that will define the 100% of the ingredient);
- ☐ to characterise the sequence of transformation events (obtain DNA sequence of junction regions);
- ☐ to develop, validate through ring-trials, and propose European standards for PCR-based transformation event-specific qualitative detection of at least 12 GMOs in single and multiplex assays.

IMPROVED UNDERSTANDING AND CONTROL OF CONTAMINATION CONDITIONS

Prevention of ochratoxin A in cereals (Contract No. QLK1–1999–00433)

This project aims at establishing methods to prevent ochratoxin A in cereal products by applying the Hazard Analysis Critical Control Point (HACCP) approach. It includes the whole food chain from the field to the final product. The objectives and expected achievements are divided in four important steps of the HACCP managing programme for ochratoxin A in cereals: identification of the critical control points (CCP); establishment of critical limits for the CCPs; development of rapid monitoring methods; and establishment of corrective action in the event of deviation of a critical limit. The outcome of all tasks will serve as a pool of knowledge and recommendations for HACCP-based ochratoxin management programmes, which will increase food safety and the marketability for the producer.

Safe organic vegetables and vegetable products by reducing risk factors and sources of fungal contaminants throughout the production chain: the carrot – Alternaria model (Contract No. QLK1–1999–00986)

The overall objective of this project is to develop strategies to ensure a safe supply of organic food by developing detection methods for future standardisation and by linking to the biotoxin working group CEN TC275/WG5, by anticipating mycotoxin risks, by tracing the sources of contaminants in the food production chain, and by eliminating the risk factors. The research will be done with the model carrot – *Alternaria*, the fungus being a known producer of harmful mycotoxins.

Hazard analysis and control of food contamination: prevention of Fusarium mycotoxins entering the human and animal food chain (Control Mycotox Food) (Contract No. QLK1–1999–00996)

The overall objectives of this project are to examine systems of pre-harvest crop treatment, and post-harvest control to remove contaminants and prevent fungal development in food. It will provide biological and chemical means of detoxifying mycotoxins. The best combinations of treatments in the chain will be identified by the HACCP approach. The six key objectives to control and prevent contamination of food with Fusarium species and their mycotoxins are: development of critical control systems; preharvest biocontrol; post-harvest control; decontamination using microbial inoculants for prevention of entry into animal production systems; and decontamination using physical means.

Early detection of toxigenic Fusarium species and ochratoxigenic fungi in plant products (Contract No. QLK1–1999–01380)

The aim of this project is to prevent and reduce the consumer health risk from mycotoxin contamination in food and feed. This will be achieved through the development of molecular diagnostic methods for an early detection of toxigenic *Fusarium* species and ochratoxigenic fungi in plant products, new and more sensitive immunological tests for detection of mycotoxins in food, and biochemical and molecular studies to characterise genes responsible for ochratoxin A synthesis. In addition, this project aims at providing tools to reduce and control the risk of mycotoxins in plant products and in the food chain obtaining agro-industrial products of greater market value, which leads to enhanced economic development of rural areas (farmers, animal breeders, etc.).

NEW METHODOLOGIES FOR ASSESSING MICROBIAL, CHEMICAL, AND ALLERGENIC RISKS AND EXPOSURES

Development, validation, and application of stochastic modelling of human exposure to food chemicals and nutrients (Contract No. QLK1–1999–00155)

The objectives of this project are:

- to develop a comprehensive set of mathematical purpose-built algorithms to take account of all the necessary components for stochastic modelling of a variety of food chemicals and to develop appropriate computer software;
- to conduct a multi-centre study using existing national data and explore the influence of input distributions on the model output for the key components of the stochastic model of food chemical intake (i.e. food intake, chemical occurrence, chemical concentration, market share, brand loyalty, and correlated foods);
- to generate databases of true intakes of (i) food additives, based on brand level food consumption and ingredient composition, (ii) pesticide residues, based on duplicate diets, and (iii) nutrients, based on biomarker studies;
- to assess the validity of the developed stochastic modelling software against true intakes, to conduct a comprehensive sensitivity analysis of validated models, and to compare these intakes against those derived using current approaches to exposure assessment;
- to provide a comprehensive set of practical guidelines for the appropriate use of stochastic modelling of food chemical intake and to provide guidelines for correct interpretation of the output of stochastic modelling; and
- to actively communicate all research findings to national authorities and scientific bodies as well as standardisation bodies involved in food chemical exposure assessment at regular stages throughout the project and, furthermore, to incorporate their feedback in the development of the software and the guidelines.

Food Safety in Europe: Risk assessment of chemicals in food and diet (FOSIE) (Contract No. QLK1–1999–00156)

The project will focus on the following objectives:
- to carry out a detailed state-of-the-art appraisal of all stages involved in risk assessment and seek to integrate these in the most relevant manner for assessing risk using the matrix approach;
- to explore the means of improving the principles applied to, and the scientific basis of, risk assessment with respect to natural toxicants, food additives, and contaminants in the food chain, including possible interactions between individual chemicals and effects of the food matrix;
- to identify gaps in knowledge that might lead to differences in interpretation of toxicological and exposure data and the research need to reduce these;
- to determine the nature and the level of testing needed for safety evaluation relevant to the nature of the chemical, level of use/occurrence in the diet and human exposure (including novel foods and processes and nutritional supplements);
- to add a European contribution to international initiatives to harmonise principles, terminology and methodology for risk assessment;
- to move toward a scientifically transparent and justifiable consensus on risk assessment issues;
- to assist risk managers in developing appropriate defensible food standards that adequately protect the safety of the consumer whilst allowing for innovation in food production and processing.

Risk assessment of Cryptosporidium parvum, an emerging pathogen in the food and water chain in Europe (Contract No. QLK1–1999–00775)

This project will investigate *Cryptosporidium parvum*, an emerging pathogen and will establish the risk that *C. parvum* poses to the food and water industry. The objectives of the project are:
- development of routine procedures for the isolation and detection of oocysts from test samples (food and water);
- determination of the survival and infectivity of *C. parvum* in the food and water;
- development of procedures for control of *C. parvum* in the food and water industry;
- development of a risk assessment model for *C. parvum* for the food and water industry.

European network safety of genetically modified food (Contract No. QLK1–1999–01182)

The objectives of this network are:
- to identify key issues of the safety evaluation of genetically modified food crops, and to examine whether current research methods are adequate to characterise specific safety hazards;
- to co-ordinate ongoing research regarding safety testing of transgenic foods within the European research framework program 5 (FP5);
- to design new sufficiently sensitive and specific (*in vitro*) test methodologies for safety and nutritional evaluation of whole complex foods;

- to address the risks of gene transfer from genetically modified organisms to the gut micro-flora of humans and animals;
- to examine new strategies for the detection of genetically modified food (GMF), which enable detection at specific threshold levels for raw materials, processed products, and food ingredients;
- to examine the fate of genetically modified raw materials and processed products throughout food production chains (tracking and tracing);
- to develop criteria for quality assurance systems to guarantee »non-GMO-containing« materials throughout food chains;
- to develop a communication platform of GMO producers, scientists involved in research and safety evaluation of GMOs, retailers, regulatory authorities, and consumer groups with the scope to improve safety assessment procedures, risk management strategies, and risk communication.

New methods for the safety testing of transgenic food (Contract No. QLK1–1999–00651)

The overall objective of this project is to develop and validate the scientific methodology which is necessary for assessing the safety of food from genetically modified plants in accordance with the EU Regulation 258/97 of 27 January 1997 concerning novel foods and novel food ingredients. The specific objectives are:

- to improve the sensitivity and specificity of standard OECD guideline toxicity tests towards detection of specific chemical entities in the genetically modified food matrix by the measurement of additional biological endpoints based on prior knowledge;
- to improve knowledge through precise information regarding the gene construct, its site of insertion and the chemical and toxicological characteristics of the gene product based on chemical analytical studies and short-term *in vivo* and *in vitro* studies;
- to improve knowledge through precise information regarding unintended secondary changes in the genetically modified food item, which may alter the nutritional-toxicological properties of that food. The project will examine a two-step safety testing procedure for genetically modified plant food, having chemical analysis and short term *in vivo* and *in vitro* testing in the first step and the core 90-day rat study in the second step. The test design will be evaluated through the testing of 3 transgenic rice varieties (gene inserts: GNA lectin, PHA-E lectin, Bt-toxin) measured against the testing of spiked rice.

ROLE OF FOOD IN PROMOTING AND SUSTAINING HEALTH WITH RESPECT TO DIET AND NUTRITION, TOXICOLOGY, EPIDEMIOLOGY, ENVIRONMENTAL INTERACTION, CONSUMER CHOICE, AND PUBLIC HEALTH

Evaluation of the safety and efficacy of iron supplementation in pregnant women (Contract No. QLK1–1999–00337)

Iron deficiency during pregnancy is common and can have harmful effects on the mother and child. Supplementation with iron is desirable even if the mother's iron levels are only slightly lower than recommended. The amount prescribed, however, varies widely and there is a growing concern about the risks associated with excessive iron accumulation. This project will examine the effect of two doses of iron supplements, given within the clinical range, on a variety of indicators of health and well being in pregnant women. Concurrently, it will study the effects of supplementation directly in humans and will test the involved mechanisms using cell culture and animal models. In addition, it will identify the optimum levels of supplementation during pregnancy and will also determine the maximum safe level. Iron supplements have unpleasant side effects and our data will provide information why this should be the case. Supplementation will have effects on the metabolism of other nutrients in addition to iron, and our experiments will seek to show which nutrients are affected and what are the mechanisms of interaction. A further aim will be to provide rational guidelines for supplementation strategies and recommendations for safe levels of supplementation.

Synbiotics and cancer prevention in humans (Contract No. QLK1–1999–00346)

The overall aim of the proposal is to evaluate the potential cancer-preventing activity of pro-biotic and prebiotic (»SYNBIOTIC«) combinations in human volunteers. The strategy that will be followed to attain this target consists out of 5 major points:

- to identify synbiotic combinations offering the greatest competitive advantages in the colonic ecosystem, using *in vitro* fermentation techniques;
- to confirm the anti-carcinogenic effect of the most promising synbiotic combination in a well-established animal model for colon carcinogenesis;
- to refine the range of biomarkers suitable for use in the human intervention study on synbiotics and CRC risk and to establish conditions for sample storage and transport for the study;
- to gain insight into the underlying mechanisms involved in the anticancer effects in the rat model by the use of biomarkers reflecting different stages of the carcinogenic process (initiation, promotion, and metastasis) and immunological parameters;
- to evaluate the potential of a synbiotic combination in human subjects (adenoma patients) using the biomarkers as above to prevent colorectal cancer.

ESCODD – European Standards Committee on Oxidative DNA Damage (Contract No. QLK1–1999–00568)

The principal objectives of this Concerted Action are the following:

- to validate HPLC, GC-MS, and LC-MS-MS methods used for the measurement of 8-oxo-7,8-dihydroguanine (8-oxo-gua) and 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxo-dG) using reference standard DNA samples analysed in parallel in different laboratories, including heavy labelled standards for mass spectrometry;
- to increase the sensitivity and reliability of these »conventional« methods;
- to measure DNA oxidation in parallel on identical samples using »conventional« methods and repair endonuclease-based methods;
- to reach a consensus on the average level of oxidation in normal human DNA.

It is clear that oxidation of guanosine during sample preparation for GC-MS and HPLC is a serious problem. It is essential that it is eliminated, that the protocols are standardised, and that the variability and errors in different assays are reduced if we are to achieve a sound judgment of the amount of damage present – a prerequisite for assessing the importance of oxidative DNA damage in the aetiology of diseases such as cancer, Down's syndrome, cystic fibrosis and premature ageing syndromes such as the Werner's syndrome, as well as its role in normal ageing. This Concerted Action will provide for the systematic exchange of standards and samples between participants with regular meetings to discuss the results and to optimise methodology. In addition, scientists will take part in exchanges between laboratories to facilitate the adoption of improvements in technique.

IDENTIFIED FUTURE RESEARCH NEEDS IN THE FOOD SAFETY AREA BASED ON THE WORK PROGRAMME COVERAGE ANALYSIS AND ALL SUPPORTED PROJECTS UP TO NOW

Based on the currently supported projects a work programme coverage analysis has been made which revealed a number of research needs in the food safety area, most of which is covered by food safety and technology proposals submitted in the year 2000 such as economic impacts of HACCP on food industry, slaughterhouses, and so on; processes for inactivating non-conventional agents; safety assessment of convenience foods and catering systems including lifestyles; consolidation of databases taking into account risk groups, link nutrients and hazards, and regional differences; assessment of effects of long-term exposure and mixtures of known hazards; quantitative risk assessment including validation of methodologies; allergenic risk and exposure assessment; epidemiological studies and their links to food consumption data; microbial ecology of food-borne pathogens; transfer mechanisms of antibiotic resistance between animal, microbial, and human reservoirs via food ingestion; optimisation of sampling protocols, specifically retail sampling; rapid detection tests and multiresidue procedures in areas such as migration, prions, phytotoxins, and so on; determination of infectivity levels of non-conventional agents in animal tissues and products; risk communication including risk perception by increasing understanding based on breaking down barriers and networking; and performance written standards (i.e. criteria-based pre-normative research), not restricted only to descriptive written standards for analytical methods and tests.

CONCLUSIONS

In view of these broad implications and important roles of the 15 KA1 projects, it can be concluded that they substantially contribute to the objectives of KA1, to the Community food legislation (2), and to the following overall aims:

- ensuring a high level of protection of public health, safety, and the consumer as well as restoring consumer's confidence;
- supporting European legislation as well as pre-and co-normative research in the area of food safety and technology;
- ensuring the free movement of goods within the internal market by actively using the standardisation type approach;
- ensuring that the legislation is primarily based on scientific evidence, and science based risk analysis;
- ensuring the competitiveness of the European industry;
- providing support to the use of the HACCP approach.

Further information on the work programme, guides for proposers, evaluation approach and forms, additional details on supported QoL-KA1-research projects can be found on the internet: *http://www.cordis.lu/life*. The Web page also provides hyperlinks to other Web pages showing results of various supported EC-research projects in the food safety area under the previous EC-Framework Programme 4.

Acknowledgements The author gratefully acknowledges the collaboration and support of many scientists, coordinators, and European organisations who will make this work possible and on whose skill depends the reliability of the results.

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Sažetak

PROGRAM KVALITETE ŽIVLJENJA: HRANA, PREHRANA I ZDRAVLJE – PROMOCIJA PROJEKATA

Program kvalitete življenja (QoL) Europske zajednice, Ključna akcija 1 (KA1) - Hrana, prehrana i zdravlje ima svrhu pružanja zdrave, sigurne i visokokvalitetne opskrbe hranom sa svrhom jačanja povjerenja potrošača u sigurnost europske hrane. Ključna akcija trenutno podupire nekoliko različitih europskih projekata ispitivanjem analitičkih metoda za kontrolu hrane uključivši senzore, procjenu rizika i standardizaciju sigurnosti hrane. Njihov cilj polazi od razvoja i vrednovanja strategije prevencije za stvaranje mikotoksina preko razvoja komunikacijskih platforma za genetički modificirane organizme (GMO), validacije i standardizacije dijagnostičke metode lančane reakcije polimerazom (PCR) za patogene nastale u hrani, do evaluacije potencijalne aktivnosti sprečavanja karcinoma probiotskih i pre-biotskih ("sinbiotskih") kombinacija u humanih dobrovoljaca. U ovom radu navedene su također potrebe za daljnjim istraživanjima u području sigurnosti hrane.

Ključne riječi:
analitičke metode, europska istraživanja, sigurnost hrane, GMO, HACCP, zakonodavstvo, PCR, upravljanje kakvoćom, analiza rizika, standardizacija

Requests for reprints:

Achim Boenke,
European Commission: 1st Thematic Programme
Quality of Life and Management of Living Resources (QoL)
Key Action 1 – Food, Nutrition & Health
200 Rue de la Loi, B–1049 Brussels; Belgium
E-mail: *achim.boenke@cec.eu.int*

CONFERENCE PAPER

CONTAMINATION OF FOOD WITH MYCOTOXINS AND HUMAN HEALTH

MAJA PERAICA AND ANA-MARIJA DOMIJAN

Institute for Medical Research and Occupational Health, Zagreb, Croatia

Received November 2000

Mycotoxins are natural contaminants of cereals and other commodities throughout the world. They are produced by various strains of moulds, particularly in tropical countries. Due to significant trade of cereals, humans in temperate countries can also be exposed to mycotoxins. The most common route of exposure to mycotoxins is ingestion, but it may also involve dermal, respiratory, and parenteral routes, the last being associated with drug abuse. Apart from acute and chronic toxic effects on human health called mycotoxicosis, some mycotoxins are proved or suspected human carcinogens. This paper describes various human diseases caused by ergot, aflatoxin, ochratoxin A, 3-nitropropionic acid, trichothecene, zearalenone, and fumonisin. It also gives a quick review of human carcinogenicity evaluations of the International Agency for Research on Cancer and of regulatory limits of mycotoxin concentrations in various commodities.

Key words:
3-nitropropionic acid, aflatoxins, food, fumonisins, mycotoxicosis, ochratoxin A, trichothecenes, zearalenone

Mould and mycotoxin contamination of food is serious although usually neglected. According to Food and Agriculture Organization, 25% of the world crop is contaminated by moulds (1). Fungal invasion of agricultural commodities is common in the fields (*Fusarium spp.*, *Aspergillus spp.* and *Penicillium spp.*) with considerable seasonal variations. Mycotoxins are usually found in mixed form. The production of mycotoxins does not correlate directly with the growth of moulds, and while fungistatic and fungicidal compounds may affect the mould invasion, this does not necessarily entail the drop in the level of mycotoxins.

Presented at the ICS-UNIDO Workshop on Contamination of Food and Agroproducts, 28 September – 1 October 2000, Varaždin, Croatia

There is no doubt about the importance of mycotoxins in the human history. The first recognised acute intoxication was described in France in 945, when a large number of persons were ill of ergotism (2). This potentially fatal disease, caused by metabolites of ergot, has now almost disappeared due to the use of barley resistant to various *Claviceps* strains which produce the ergot alkaloids. The importance of application of hygienic measures in prevention of human exposure to mycotoxins was demonstrated in the eradication of so called »yellow rice disease« (*shosin-kake* in Japanese). This fatal cardiomiopathy similar to the lesions seen in beri-beri, was caused by citreoviridin, the metabolic product of *Penicillium citreonigrum* (3). The disease, common in lower social strata in Japan, disappeared immediately after the introduction of rigorous measures which excluded mouldy (or »yellow«) rice from the market. Another disease, that has not been seen in a severe form for decades and which involved a large number of persons, was the »alimentary toxic aleukia« common in the USSR (4). The population was exposed to trichothecenes from unharvested wheat contaminated by *Fusarium* moulds.

Some moulds and products of moulds are used in food industry in the production of cheese, sausages, beer, wine and in pharmaceutical industry as antibiotics. The classification of mould products as antibiotics or mycotoxins is arbitrary and depends on the toxicity of the compound.

The most common human exposure to mycotoxins is oral, that is, by ingestion of contaminated plant-based food or of residues and metabolites in animal products, such as aflatoxin M_1. Other routes of exposure are respiratory, dermal, and parenteral, though the last is associated with drug abuse. Respiratory exposure occurs during professional contact with large quantities of processed contaminated food (aflatoxins and ochratoxin A)(5–7) or the exposure occurs in highly contaminated households (8) and office buildings with artificial ventilation (9). Dermal exposure is limited to mycotoxins which can pass the dermal barrier (trichothecenes) (10). Heroin abusers in the

Table 1. *Postulated human mycotoxicoses*

Mycotoxin	Disease or syndrome
Aflatoxin	Liver lesions, cirrhosis, liver carcinoma, Kwashiorkor, Reye's syndrome
Cyclopiazonic acid	Kodua
Citreoviridin	Cardiomiopathy
Ergot	Ergotism
Fumonisin B_1	Oesophageal carcinoma
Fusarium metabolites	Akakabi-byo
F. equiseti metabolites	Kashin-beck disease
3-nitropropionic acid	Mouldy cane disease
Ochratoxin A	Endemic nephropathy, urothelial tumours
Trichothecenes	Alimentary toxic aleukia
Zearalenone	Premature telarche, cervical cancer

European countries are parenterally exposed to aflatoxin B_1 which contaminates heroin imported from tropical countries (11).

There are several diseases which are supposed to be mycotoxin-related (Table 1). Mycotoxins are suspected to cause a disease in instances when it appears in several persons with no obvious connection to a known etiologic agent such as micro-organisms. However, the causal relationship between exposure to mycotoxins and the disease development should be confirmed by epidemiological studies.

Mycotoxins cause acute and chronic intoxications (mycotoxicoses), allergies, and tumours. In experimental animals and in the experiments *in vitro* they may demonstrate genotoxic, mutagenic, cytotoxic, and teratogenic properties. Some of them, such as sporodesmine, are toxic for animals, but not for humans. Others, such as ochratoxin A or aflatoxins, have the same target organ in all experimental animals. For such mycotoxins it is possible to extrapolate the results from animals to humans. The target organs of other groups of mycotoxins are so different in different animal species that it is extremely hard to infer their effect on humans. For example, protean mycotoxins are fumonisins, which produce pulmonary oedema in pigs, leukoencephalomalacia in horses and are hepatotoxic in rats, mice, rabbits and swine and nephrotoxic in male swine, rats, and rabbits (12). Mycotoxicoses are more frequently noticed in animals, because a large number of animals are fed with specific feed. It was proposed that the following criteria should be met to link a mycotoxin to a specific human disease: occurrence of mycotoxin in food samples, human exposure and incidence, reproducibility of characteristic symptoms in experimental animals, and the similar mode of action in human and animal models (13).

The greatest human health concern related to mycotoxins is the cancer risk based on long-term, low-level exposure to carcinogenic toxins such as the aflatoxins, ochratoxin A, fumonisins, and zearalenone.

ERGOT

Ergot is the common name for sclerotia of fungi of the genus *Claviceps* which produce ergot alkaloids. The sclerotium is a dark coloured, hard fungal mass that replaces the seed or kernel of the plant. Ergot alkaloids are also secondary metabolites of some strains of *Penicillium, Aspergillus* and *Rhizopus* sp. (14). The source of ergot strongly influences the type of alkaloids present, as well as the clinical picture of ergotism (15).

Claviceps purpurea produces ergotamine-ergocristine alkaloids, which cause the gangrenous form of ergotism because of their vasoconstrictive activity. The initial symptoms are the leg oedema with severe pains. Paresthesias are followed by gangrene at the tendons with painless demarcation. In 1977–1978, Ethiopia saw the last recorded outbreak of gangrenous ergotism striking 140 persons of whom 34% died (16). It seems that the cause of this outbreak was the long wet season which favoured the growth of wild oat susceptible to *Claviceps purpurea*.

The other type of ergotism, related to intoxication with clavine alkaloids from *Claviceps fusiformis* is the convulsive form, last seen in India in 1975 and affecting

78 persons (17). It was characterised by gastrointestinal symptoms (nausea, vomiting, and giddiness) followed by neurological symptoms (drowsiness, prolonged sleepiness, twitching, convulsions, blindness, and paralysis). The onset of the symptoms ranged from one to 48 hours after ingestion of contaminated food. No fatalities were recorded.

Ergotism is extremely rare today, primarily because the standard cleaning and milling processes remove most of ergot, leaving very low levels of the alkaloids in flour. In addition, the alkaloids are relatively unstable and are usually destroyed by baking and cooking.

AFLATOXINS

Aflatoxins (B_1, B_2, G_1, and G_2) are highly toxic hepatotoxins produced by various strains of *Aspergillus* in tropical and subtropical regions. The first massive mycotoxicosis which attracted scientific attention occurred in England in 1961, when tons of groundnuts contaminated by *A. flavus* and *A. parasiticus* containing aflatoxin and cyclopiazonic acid were used for the production of formulated feed for turkeys. The most abundant and the most studied is aflatoxin B_1 (AFB_1) which together with other aflatoxins naturally contaminates maize, sorghum, nuts, cotton seed, sunflower seed, coffee, and other energy-rich products. When the feed consumed by cows contains AFB_1, the toxic metabolite aflatoxin M_1 with toxicity similar to AFB_1 is excreted in milk.

In several episodes of acute aflatoxicosis in Asia and Africa aflatoxins caused severe lesions of liver in malnourished adults, often with the fatal outcome (18–20). Due to seasonal appearance of childhood diseases such as kwashiorkor, Reye's syndrome, and neonatal jaundice in tropical countries which coincided with periodical high concentrations of aflatoxins in food, it was believed that aflatoxins were involved in the etiology of these diseases. In several studies AFB_1 and aflatoxicol (metabolic product of AFB_1) were found more frequently in the serum, liver, urine, and stool of children suffering from kwashiorkor than in controls (21–24). However, it is still not clear whether the finding of aflatoxins is the cause or the conequence of kwashiorkor. The role of aflatoxins in the development of Reye's syndrome (encephalopathy with severe lesions of kidney and liver following influenza or varicella) was never proved, regardless of frequent findings of aflatoxins in the liver of children who died of this syndrome (25–31). The syndrome was also connected with the use of salicylates and phenotiazines (31, 32). The last two decades saw a drop in the incidence of Reye's syndrome, which associated with the diminished use of salicylates in the treatment of fever in children (33). However, a large investigation performed in Nigeria on 327 babies with jaundice and 80 matching controls proved that serum glucose-6-phosphate dehydrogenase deficiency and aflatoxins were significant risk factors for the development of neonatal jaundice (34).

The DNA lesions in organs (in liver after oral and in lungs after respiratory exposure) of experimental animals with a high incidence of tumors werw caused by the formation of aflatoxin B_1-DNA adducts. A good correlation of aflatoxin B_1-DNA adducts production in target organs and aflatoxin B_1-DNA adducts on albumin and their excretion in urine was found. The production of aflatoxin B_1-DNA adducts depends on exposure, nourishment, the intake of antioxidants, viral infection, and genetic polymorphism (35). It was also found that AFB_1 triggers mutation of the p53 tumour suppressor gene.

The incidence of primary liver carcinoma is unusually high in some African and Asian regions. Exposure to aflatoxins and of the incidence of primary liver carcinoma were investigated in Thailand (36), Kenya (37), Mozambique (38), Swaziland (39), and Mozambique/Transkei (40). Although the populations of those countries were not serologically tested for HbsAg, the results pointed to the role of aflatoxins in the development of this disease. The role of aflatoxins in the development of the primary liver carcinoma, irrespective of the hepatitis B virus, was confirmed in investigations performed in China and Swaziland (41–43). Based on these data, the Working Group of the International Agency for Research on Cancer (44) declared in 1987 that there was enough evidence of the carcinogenicity of aflatoxin in humans. In 1992, another group of scientists relied on the epidemiological data to declare that there was enough evidence that the mixture of aflatoxins was hepatocarcinogen in humans and that the metabolite aflatoxin M_1 was a potential hepatocarcinogen in humans (45). A study in Taiwan showed that the risk of development of the primary liver carcinoma is considerably higher if the exposure to aflatoxin is combined with the presence of hepatitis B virus. The authors believe that the hepatitis B vaccination could be an effective prevention of tumours in countries with high aflatoxin contamination (35).

Aflatoxins are considered unavoidable contaminants of food, since they cannot be prevented or eliminated by the current agricultural practice. However, blanching and electronic eye colour sorting may reduce the concentration of total aflatoxins to 5 μg/kg of peanuts (46). Specific limits have already been set in a number of countries and they range from 0 to 30 μg/kg for aflatoxin B_1 in foodstuffs and from 0 to 50 μg/kg for total aflatoxins.

OCHRATOXIN A

Ochratoxins are a group of mycotoxins produced during grain storage by several *Penicillium* and *Aspergillus* strains all over the world (47). The most toxic and the most frequent is ochratoxin A (OTA), found in various commodities of plant and animal origin such as cereals, coffee, bread, wine, beer, pork meat, sausages, eggs, and milk.

OTA has been shown to be nephrotoxic, immunosupressive, carcinogenic, and teratogenic in all experimental animals tested so far. Owing to the similarity of morphological and functional kidney lesions in OTA-induced porcine nephropathy, this mycotoxin has been proposed as the causative agent of endemic nephropathy (48). Endemic nephropathy occurs in rural population of some regions of Croatia, Bosnia and Herzegovina, Yugoslavia, Bulgaria, and Romania. It has been estimated that about 20,000 people are either suffering from or are suspect of the disease (49). The main features of this fatal renal disease are bilateral, primarily chronic lesions of the renal cortex (tubular degeneration, interstitial fibrosis, and hyalinisation of the glomeruli) (50). Many samples of food and feed produced in the endemic area of Croatia contained ochratoxin A (51–53). It was recently shown that concentrations of OTA in maize samples collected in the endemic regions in years with favourable climate for the production of ochratoxin A were higher than in other regions of Croatia (54). Blood ochratoxin A is found more frequently and in higher concentrations in inhabitants from endemic regions than in controls (53, 55).

In Tunisia, ochratoxin A has been detected in high concentration in the blood and food of patients with kidney impairment of unknown etiology (56, 57). In Italy, significantly higher OTA concentration was found in patients treated with dialysis than in transplanted subjects, patients with chronic glomerulonephritis, renal calculus, cysts, chronic renal failure and healthy subjects (58). It is not clear weather the high concentration of OTA in blood of patients with serious impairment of kidney function is caused by this mycotoxin or it is only the consequence of reduced glomerular filtration rate.

Furthermore, OTA was found in food and feed in a number of countries where endemic nephropathy has not been recorded so far (47). Although it was frequently found in low concentration in human blood (59), the significance of such finding is not clear.

In endemic regions of Croatia, Bulgaria, and Yugoslavia, the respective incidence of otherwise rare urothelial tumours of the pelvis and urethra is 50, 90, and 100 times greater than in non-endemic regions (60–62). As the incidence, clinical course, and prognosis of those tumours differs from urothelial tumours found in non-endemic regions, it was suggested that OTA may be the causal agent for both endemic nephropathy and urothelial tumours (63).

International Agency for Research on Cancer classified ochratoxin A as a compound possibly carcinogenic to humans (Group 2B) (44). Regulatory limits for OTA in various food have been set in six, mostly Scandinavian countries and range between 1 and 300 μg/kg.

3-NITROPROPIONIC ACID

3-nitropropionic acid (3-NPA) is the secondary metabolite of *Arthrinium* sp. considered to cause acute food-poisoning called »Mouldy sugarcane poisoning« (MSP) (64). MSP occurs in winter (February and March) in 13 provinces of Northern China as a consequence of the ingestion of sugarcane stored for two months or longer and infested with *Arthrinium* sp. In the period 1972–88, 884 persons were affected by the outbreaks of MSP and 88 (10%) died (65). The main epidemiological feature is the small number of persons in one outbreak (one to five persons), victims being mostly children and young persons (65). Generally, the incubation period is 2–3 hours after ingestion of mouldy sugar cane, and the main clinical symptoms are vomiting, dystonia, stare to one side, convulsions, carpopedal spasm, and coma. Delayed dystonia develops in 10–50% of patients as the consequence of bilateral symmetric necrosis of the basal ganglia. The development of delayed symptoms may be predicted by abnormal basal ganglia visible on cranial computerised tomography scans (66). In adults 3-NPA causes gastrointestinal symptoms, whereas signs of severe encephalopathy are not common (67).

TRICHOTHECENES

Trichothecenes are large group of 148 mycotoxins, produced mostly by *Fusarium* genus, although other genera (e.g. *Trichoderma*, *Trichotecium*, *Myrothecium*, and *Stachybotrys*) are also known to produce these compounds (68). Mycotoxins producing strains of *Fusarium* contaminate grain with mycotoxins in fields throughout the world (69) The most frequent contaminants are deoxynivalenol (DON), also known as vomitoxin, nivalenol (NIV), and diacetoxyscirpenol (DAS), while T-2 toxin is rarer (68).

The most severe manifestations of trichothecene toxicity with the fatal outcome in 60% of involved persons were seen in the USSR from 1932 to 1947 (4). This trichothecenes mycotoxicosis called »alimentary toxic aleukia« was the consequence of grain contamination with *Fusarium sporotrichoides*. The contamination of grain samples taken from the affected regions was 5–40%, whereas the regions not affected by the disease showed only a 2–8% contamination of samples with the fungus. The initial symptoms were gastritis, gastro-enteritis, abdominal and oesophageal pain, and diarrhoea. Longer consumption of contaminated grain (3–4 weeks) entailed generalised indisposition with vertigo, unpleasant taste in the mouth with progressive leukopenia, granulocytopenia, and lymphocytosis. In the terminal phase of the disease the patients had haemorrhagic diathesis and angina with petechial rash, catarrhal diphteric gangrenous laryngitis, aphonia, and asphyxia.

More recent outbreaks of trichothecenes mycotoxicoses were less severe and without the fatal outcome. Trichothecenes mycotoxicosis, called also »scabby grain toxicosis«, occurred in Japan (70), China (71, 72), and India (73, 74). The main symptoms were abdominal pain, nausea, vomiting, diarrhoea, dizziness, and headache. The symptoms usually appeared within hours after the ingestion of wheat, corn, or rice contaminated by DON, NIV, T-2 toxin, deoxynivalenol, and zearalenon.

The regulatory limit in wheat for deoxynivalenol is from 1000 to 4000 μg/kg in five countries, and for diacetoxyscirpenol 100 μg/kg in Israel. The limit for T-2 toxin is 100 μg/kg in two countries.

ZEARALENONE

Zearalenone (previously known as F-2 toxin) is a mycotoxin generated in the field or during storage of humid grain contaminated by various *Fusarium* spp. in the preharvest period. It is often found together with other *Fusarium* mycotoxins on maize, wheat, and other cereals. Contrary to the other *Fusarium* mycotoxins, zearalenone is more frequently found in ecologically than in conventionally produced wheat (75). Zearalenone and its metabolic products may be found in food of animal origin (meat, milk, and cheese) (76).

Zeranol, a growth-promoting derivative of zearalenone, has been approved by the USA Food and Drug Administration for use in animal husbandry instead of diethylstilbestrol. Zeranol is formed in cattle *in vivo* from feed contaminated by zearalenone (77).

In experimental and domestic animals zearalenone and zeranol have uterotrophic effect, and, being weak oestrogens, they cause inhibition of the hypothalamus and anterior pituitary and the atrophy of the ovaries, testes, prostate, and seminal vesicles (78–80). There are several reports on breast enlargement and precocious sexual development of children possibly connected with food-related exposure to oestrogens. In Puerto Rico, zearalenone was found in the blood of children with precocious sexual development (81). As maize is not produced in Puerto Rico, the authors believe that the affected children consumed chicken meet fed on the fodder imported from the USA. Since 1989, the south-east part of Hungary has been recording an ever increasing number of telarche and mastopathy in patients most of whom consumed »healthy« food (82). Zearalenone was found in 5 out of 36 serum samples.

The carcinogenicity of zearalenone for humans could not be evaluated because of the absence of epidemiological data (44).

FUMONISINS

Fumonisins are the most recent isolated group of mycotoxins. They are produced by *Fusarium verticillioides* (Sacc.), Nirenberg (=*Fusarium moniliforme* Sheldon), and related species on maize that has been affected by the moulds before the harvest. Maize and maize products may contain significant amounts of fumonisins, and fumonisin contamination of maize was found in many countries (83) including Croatia (54). Fumonisins are heat-stable (84), light-stable, and water soluble (45). Natural contamination of maize by fumonisin B_1 is more frequent than contamination with the other 14 fumonisins (12).

Thanks to poor absorption and rapid excretion in animals, fumonisins are not significantly transferred into pork, chicken meat, eggs, and milk (85, 86). Fumonisins disturb the sphingolipid metabolism, and the resulting increase in the sphinganine/sphingosine ratio is used as a biomarker of animal exposure. It was shown that the target organs of fumonisin toxicity are liver (mice, rats, equids, rabbits, pigs, and non-human primates), kidney (pigs, sheep, mice, and rats), brain (horses), and lungs (pigs) (12). The carcinogenicity of fumonisin B_1 in rodents varies between species, strains, and the sexes.

A single outbreak of acute human mycotoxicosis was reported in 27 villages of India, when the poorest social strata were exposed to fumonisin B_1 in mouldy maize and sorghum (87). Fumonisin B_1 was found in much higher concentrations in food consumed by the affected than by the control households. The main features of the disease were transient abdominal pain, borborygmus, and diarrhoea which started half an hour to one hour after the consumption of unleavened bread.

In some regions of Africa (Transkei), Asia (China), and Europe (Italy) the exposure to fumonisins from maize is connected with higher incidence of oesophageal cancer (45).

Fumonisins have a weak cancer-initiating and a strong cancer-promoting potential, which was proved on rats which developed hepatocellular carcinoma and cholangiocarcinoma. The promoting potential of fumonisins is suspected to be the causative agent of the primary liver carcinoma which is very frequent in the Chinese region of Haimen (88). Concentrations of aflatoxin B_1, deoxynivalenol and fumonisins B_1, B_2, and B_3 were measured in samples of maize from Haimen and Penlai, a region with low incidence of the primary carcinoma. The aflatoxin concentration was low and the incidence of aflatoxin positive findings was similar in both regions, but the concentrations of fumonisins and deoxynivalenol were much higher in Haimen.

An IARC working group classified the toxins from *F. moniliforme* as possibly carcinogen to humans (Group 2B) (44). Limits for human daily exposure, as well as the limits of fumonisins in maize are yet to be determined.

CONCLUSIONS

Mycotoxins are frequent food contaminants. Validated analytical methods for mycotoxins in various commodities should be accepted all over the world to enhance the control of food quality. International organizations (World Health Organization and Food and Agriculture Organization) have recommended regulatory levels and the tolerable daily intake for most mycotoxins, yet those do not oblige national legislations to introduce them. Regulatory levels of mycotoxins should follow the ALARA (as low as reasonably achievable) principles in order to reduce grain trade barriers. The main scope of all these efforts is to decrease the risk of mycotoxin exposure.

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Sažetak

MIKOTOKSINI U HRANI I NJIHOV UČINAK NA LJUDSKO ZDRAVLJE

Mikotoksini su metaboliti plijesni čija važnost za rast i razvoj plijesni nije razjašnjena. Ti spojevi vrlo različitih kemijskih struktura onečišćuju žitarice i druge namirnice osobito u tropskim krajevima, jer viša temperatura i vlažnost pogoduju rastu plijesni. Čovjek je najčešće izložen mikotoksinima putem hrane, no u nekim slučajevima može doći do njihova udisanja, prolaska kroz kožu ili parenteralne izloženosti. Akutno i kronično oštećenje zdravlja zbog djelovanja mikotoksina naziva se mikotoksikoza, a za neke se mikotoksine pretpostavlja ili je dokazano da su karcinogeni za ljude. U ovom radu opisani su različite bolesti ili sindromi u ljudi koje uzrokuju alkaloidi ergota, aflatoksini, okratoksin A, 3-nitropropionska kiselina, trihoteceni, zearalenon i fumonizini. Iznesene su i procjene Međunarodne agencije za istraživanje karcinoma o karcinogenosti pojedinih mikotoksina, kao i zakonski propisi o dopuštenim koncentracijama pojedinih mikotoksina u različitim namirnicama.

Ključne riječi:
3-nitropropionska kiselina, aflatoksini, fumonizini, hrana, ljudi, mikotoksikoze, mikotoksini, okratoksin A, trihoteceni, zearalenon

Requests for reprints:

Maja Peraica, M. D., Ph. D.
Institute for Medical Research and Occupational Health
P. O. Box 291, HR-10001 Zagreb, Croatia
E-mail: *Maja.Peraica@imi.hr*

Arh Hig Rada Toksikol 2001;52:37–41

CONFERENCE PAPER

SEASONAL VARIATIONS OF AFLATOXIN M_1 IN THE FARM MILK IN ALBANIA

EDMOND PANARITI

Institute of Veterinary Research, Tirana, Albania

Received November 2000

This paper presents monitoring data on levels of aflatoxin M_1 in the farm-gate milk in Albania. The monitoring included 120 evenly distributed samples collected in winter and summer from various farms all over the country. The levels of aflatoxin M_1 were determined using the quantitative thin layer chromatography. On average, the winter milk samples revealed higher concentrations of aflatoxin M_1 than the summer samples. Thirteen percent of the winter samples resulted above the 0.5 μg/kg level, as compared to 3% of the summer samples exceeding that level. Skimmed and semi-skimmed milk from the same source contained comparatively lower levels of aflatoxin M_1 than whole milk.

Key words:
animal feed, TLC determination of AFM_1

Poor agricultural practices in Albania and inappropriate storage of animal feedstuff constitute a favourable medium for the growth of toxic moulds. It has been indicated that the ensilaged maize mostly used for feeding lactating cows during winter is heavily contaminated with moulds (1). The mould contamination is related to inappropriate storage conditions and high humidity which is characteristic for this kind of feed (>17%).

Aspergillus and *Penicillium* species prevail over other present mycotic species. Literature data have pointed to the overwhelming presence of toxicogenic *strains Aspergillus flavus* and *Aspergillus parasiticus*, known for producing aflatoxins (2). Aflatoxins are a group of structurally related difuranocoumarin derivatives of which 18 types have been identified by now (3, 4). Aflatoxin B_1 (AFB_1) is the most frequent in food. This compound has been found at relatively high levels in animal feeds in Albania, sometimes exceeding the concentration of 400 μg/kg (5). Research of labora-

Presented at the ICS-UNIDO Workshop on Contamination of Food and Agroproducts, 28 September – 1 October 2000, Varaždin, Croatia

tory animals has shown that aflatoxins are liver carcinogens in animals and there is evidence to suggest that they are also human carcinogens, with AFB_1 being the most potent and the aflatoxin M_1 (AFM_1) less so (6, 7). As a matter of fact, lactating cows exposed to AFB_1-contaminated feeds convert the toxin into the hydroxylated version of the primary compound, the resulting metabolite AFM_1. Therefore the main concern in milk should be the AFM_1 levels.

The goal of the screening survey described below was to assess the levels of AFM_1 in the farm milk of Albania and investigate their seasonal fluctuations using the quantitative thin layer chromatography (TLC) method.

MATERIALS AND METHODS

Materials

The investigation involved 120 milk samples obtained from farms throughout Albania. Samples were obtained in equal numbers during summer (when cows were at pasture) and winter (when cows consumed more concentrated feedstuffs).

Method of Analysis

The screening for AFM_1 was performed at the Institute of Veterinary Research in Tirana, using the quantitative thin layer chromatography. The limit of detection was 0.05 μg/kg. The analysis used spiked samples to assess recoveries; the acceptable recovery range was 65–100% and all spiked milk samples fell within that range. The winter samples recovery range was 67–99%, whereas the summer samples range fell within 73–92%.

The coefficients of variation calculated for the recoveries were 9.7% and 5.8%, respectively. The average recovery value for AFM_1 in farm milk was 87%. All results were corrected using recovery values determined day by day.

Extraction of Samples

The extraction of milk samples involved the blending of a 20-ml milk sample with 80 ml of methanol and 50 ml of water. The mixture was additionally treated with 50 ml of petroleum ether in a 250-ml separator for removal of fat. After the layers were separated by vigorous shaking, the petroleum ether phase was discarded (8, 9). The mixture was then submitted to extraction with 100 ml chloroform and consequently evaporated to the residual volume of 0.5 ml.

Thin layer chromatography

Aflatoxin M_1(Sigma) standards were used at a concentration of 0.2 μg/ml. The procedure involved TLC plates Kieselgel 60 without the fluorescence indicator. The spiking of blank and fortified sample extracts (with 5 μl standard addition) as well as of the standards was performed by an automatic spiking sample device (Camag). One 20 μl aliquot of sample extract and 2, 4, 6, 8, and 10 μl aliquots of M$_1$ standards were spotted on each TLC plate. The plates were previously soaked in diethylether in a saturated chamber to avoid the interference of potential impurities. The Plates (20x20 cm) were developed at ca 12 cm into a chamber containing chloroform/trichlorethylene/n-amylalcohol/formic acid (80+15+4+1 v/v).

A TLC scanning analytical system Camag II, equipped with the data elaboration software Cats 3 was used for the quantitative determination of AFM$_1$ in milk samples. The detection was performed at the excitation wavelength of 365 nm and emission wavelength of 430 nm.

RESULTS AND DISCUSSION

Relatively higher concentrations of AFM$_1$ were determined in the winter milk samples than in the summer ones (Table 1). Almost 13% of the screened winter milk samples contained AFM$_1$ levels higher than 0.5 μg/kg, with the maximum reaching 0.85 μg/kg. The respective summer values were 3% and 0.65 μg/kg. Skimmed and semi-skimmed milk contained comparatively lower levels of AFM$_1$ than the whole milk, indicating a partial removal of AFM$_1$ residues through skimming. The relatively higher levels of AFM$_1$ levels in milk in winter should be attributed to the dominant diet of stored feedstuffs associated with the season. This is an indication of greater exposure risk for consumers during this period. On the other hand AFM$_1$ is rather heat-stable and once inside the product its levels remain virtually unaffected by heating such as pasteurisation and sterilisation (10, 11). It should be noted that the determined residual levels of AFM$_1$ in milk should be considered compatible with the reported relatively high

Table 1 *Aflatoxin M$_1$ levels in farm-gate milk in Albania*

Milk type	No. of samples	Number of contaminated samples				Maximum level (μg/kg)
		<0.05 μg/kg	0.05–0.1 μg/kg	0.1–0.5 μg/kg	>0.5 μg/kg	
Whole (summer)	30	8	8	12	2	0.65
Skimmed and semi-skimmed (summer)	30	21	7	2	–	0.2
Sub-total	60	29 (48)	15 (25)	14 (23)	2 (3)	
Full (winter)	30	4	14	4	8	0.85
Skimmed and semi-skimmed (winter)	30	17	13	–	–	0.08
Subtotal	60	21 (35)	27 (45)	4 (6)	8 (13)	
Total	120	50 (42)	42 (35)	18 (15)	10 (13)	

Percentages are given in parentheses
The results are corrected for recovery. Limit of determination=0.05 μg/kg

levels of aflatoxin B_1 in animal feedstuffs. The AFM_1 levels found in milk samples issue concern because of their potential impact on the consumer's health. As AFM_1 is not included in the Albanian regulations, the risk is assessed with reference to the EU regulatory limit of 0.05 μg/kg. For the aflatoxins in general, only a zero level would ensure absolute safety for consumers (12, 13). As thie zero level is not attainable in practice, the risk associated with the residual levels of AFM_1 in milk should be weighed and assessed by health authorities and become a matter of concern for the animal breeders.

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Sažetak

SEZONSKE VARIJACIJE AFLATOKSINA M_1 U MLIJEKU SKUPLJENOM NA FARMAMA U ALBANIJI

U ovom radu prikazani su rezultati praćenja razina aflatoksina M_1 u mlijeku skupljenom na farmama u Albaniji. Analizirano je 120 uzoraka skupljenih ravnomjerno tijekom zime i ljeta na različitim farmama diljem zemlje. Za određivanje aflatoksina M_1 primijenjena je kvantitativna tankoslojna kromatografija. U uzorcima skupljenim tijekom zime uglavnom su određene više razine aflatoksina M_1 nego u uzorcima skupljenim tijekom ljeta. U 13% uzoraka skupljenih tijekom zime nađeno je više od 0,5 μg/kg, dok je samo 3% uzoraka skupljenih tijekom ljeta prelazilo navedenu granicu. Obrano ili poluobrano mlijeko iz istih izvora sadržavalo je niže razine aflatoksina M_1 u usporedbi s punomasnim mlijekom.

Ključne riječi:
mikotoksini, mlijeko

Requests for reprints:

Edmond Panariti, Ph. D.
Institute of Veterinary Research
Tirana 10, Albania
E-mail: *panariti@icc.al.eu.org*

Arh Hig Rada Toksikol 2001;52:43–48

CONFERENCE PAPER

ALLERGENIC COMPONENTS OF STORED AGRO PRODUCTS

ZLATKO KORUNIĆ

Diatom Research and Consulting, Guelph, Ontario, Canada

Received November 2000

The increasing occurrence of allergies in the developed countries and greater interest in food safety has drawn attention to the presence of allergens in agro products during storage. Stored agro products can contain allergenic contaminants of plant, microbial, or animal origin. Several stored-product insect and mite species were confirmed as sources of allergens. The most important are storage mites because they cause occupational asthma by inhalation and anaphylactic reactions when ingested in high numbers. The urine of rats and mice can be a significant source of allergens that are mainly present as large airborne particles. Stored moulds are also confirmed as potential source of allergens. However, it is likely that the major problem resulting from the presence of moulds in food will be due to the toxic effects of mycotoxins, rather than allergenic effects. The adoption of good management and processing technique can help to minimise the risk.

Key words:
allergenic effects, contaminants, food, storage pests

Problems that occur in grain and grain products during storage include the whole spectrum of hygiene hazards: chemical treatment and chemical residues, mycotoxins, and potential allergy problems. The condition of stored grain is determined by a complex interaction between grain, macro- and micro-environment, and a variety of organisms including microorganisms, insects, mites, and rodents which may attack it. Grain provides an abundant source of nutrients, and the natural consequence of the type of ecosystem described above will normally be spoilage (bio-deterioration) of the grain. Reliable studies indicate that post-harvest losses of major food commodities in the developing countries are enormous. A conservative estimate is that they reach

Presented at the ICS-UNIDO Workshop on Contamination of Food and Agroproducts, 28 September – 1 October 2000, Varaždin, Croatia

tens of millions of tons a year valued billions of dollars. The usual and the readiest way is to measure the loss as a reduction in weight of food available for consumption – the so called quantitative loss. However, losses in quality are more difficult to define, but may be equally or even more important than quantitative losses, especially if the deterioration and the contamination of stored grain with organisms, their metabolites, allergens, and excrements renders the food unsuitable for consumption by humans and animals. In the developing countries the contamination of foods prior to consumption is a serious problem, especially where the need to eat may outweigh food safety.

The purpose of this paper is to give an overview of published data about the contamination of agro products with allergens during the storage.

Contaminants of agro products during storage

The presence of arthropod pests in the urban environment results in a variety of medical problems. It is well known that arthropods are renowned vectors of bacterial and viral infections, and a cause of food poisoning. Furthermore, arthropods secrete or excrete allergens causing atopic and insect-venom allergy (1, 2).

The increasing occurrence of allergies in the developed countries and greater interest in food safety have drawn attention to the presence of allergens in agro products during storage. The association between sensitivity to arthropod allergens and atopic diseases is strong. According to a review paper published by *Van Lynden-Van Nes and co-workers* (3), arthropods are partly responsible for up to 80% of disease cases (Table 1).

Table 1 *Prevalence of sensitisation (in %) for arthropod allergens in allergic patients in different area of Western Europe and North America (3)*

Arthropod allergens	United Kingdom	Netherlands	Scandinavia	Mediterranean	North America
House dust mites	80	80	2–27	15–49	24–78
Storage mites	>30	65–70	6–45	10	12
Cockroaches	10	10	10	10	7–69

During the last couple of years several researchers investigated the causes of respiratory symptoms in workers in grain and processing industry. The association between respiratory symptoms and occupational exposure was confirmed by various tests. Stored agricultural products can contain allergenic contaminants of plant (grain dust), microbial (moulds), or animal origin (insects, mites, and rodents) (4). The most important are storage mites because they cause occupational asthma by inhalation and anaphylactic reactions when ingested in great quantities. Unfortunately, there is still no data that allow estimation of average and maximal daily intake of allergenic mites or suggest what levels might be safe.

CONTAMINANTS OF PLANT ORIGIN

Pollen from oil seed rape has been recognized as a cause of allergic sensitisation (5). However, it appears that the problem can be even worse after this crop has been harvested, as *Suh and co-workers* (6) report that a case of IgE-mediated occupational asthma in the animal feed industry could be associated with oil seed rape dust.

Žuškin and co-workers (7) described workers exposed to cocoa dust extract and flour dust who ran a high risk for developing allergic sensitisation. *Gomoshinskii and co-workers,* (8) found out that the levels of IgE antibodies against rice, wheat gluten, and maize were elevated in several of 130 children suffering from food intolerance.

Respiratory problems in bakery workers are some of the most frequently reported occupational problems (9). A group of nearly 200 bakers showed a significant correlation between sensitisation to wheat flour, rye flour, and soybean flour and alpha-amylase, asthma case history, and inhalation challenge tests.

Contaminants of microbial origin

Epidemiological data showing the importance of allergic contaminants of microbial origin are still limited. *Sander and co-workers* (10) suggested *Aspergillus oryzae* as a cause of occupational allergy and a possible food allergen. *Heaney and co-workers* (11) attributed a case of asthma in a brewery worker to a grinding of malt contaminated with *Aspergillus niger.* It is likely that the main health problems resulting from the presence of moulds in food will be due to the toxic effect of mycotoxins, rather than allergenic effects (4).

Contaminants of animal origin

The significance of allergenic contaminants of animal origin is well recognised. There is an accumulating body of recent literature reports of severe allergic reactions, including anaphylaxis, attributed to the ingestion of storage mites. To date, anaphylactic reactions have been observed after ingestion of four species of mites: *Tyrophagus putrescentiae* (12), *Deramatophagoides farinae, Tyreophagus entomophagus* (13), and *Suidasia* spp. (14). *Armentia and co-workers* (15) reported that skin prick tests and radio-allergo-sorbent tests (RAST) showed sensitisation among 50 workers to house dust mites *D. pteronyssinus* and *D. farinae* (56%), storage insects *Tenebrio molitor* (50%), various species of storage mites including *Lepidoglyphus destructor* and *T. putrescentiae* (both 38%), and the oriental cockroach *Blatta orientalis* (36%). *Bernton and Brown* (16) implicated *Plodia interpunctella* as a source of allergens, *Yourkova and co-workers* (17) and *Herling and co-workers* (18) *Sitophilus granarius* (L.), *Moscato and Dellabianco* (19) and *Andrist and co-workers* (20) *Ephestia kuehniella,* and *Rijckaert and co-workers* (21) and *Turner and co-workers* (22) *Liposcelis bostrychophila. Towney and co-workers* (23) and *Hage-Hamsten and co-workers* (24) found out that not only body extracts, but also faeces can be an important source of allergens.

Macan and co-workers (25) analysed a medical history questionnaire, skin prick test (SPT) and specific immunoglobulin E (sIgE) to storage mites *L. destructor* and *T. putrescentiae* performed on 26 male paper mill workers and 36 postmen. They discovered that paper mill workers manifested a significantly higher frequency of positive test results to *L. destructor* and *T. putrescentiae* than did the postmen. Paper mill workers manifested significantly greater mean skin reactivity to *T. putrescentiae* than to *L. destructor* while the postmen did not manifest such difference. Respiratory symptoms were found in 40% of paper mill workers to *L. destructor*, and in 53.8% to *T. putrescentiae*. All postmen with sensitivity to *L. destructor* and 83.3% with sensitivity to *T. putrescentiae* had respiratory symptoms. The study results addressed the necessity to monitor sensitisation to mites and to establish methods for identification and quantification of mites in the working and general environment.

Gordon and co-workers (26) reported that rats were the source of allergens, especially their urine. They studied animal rearing facilities and demonstrated that rat and mouse urinary allergens were mainly present as large airborne particles.

CONCLUSIONS

Some cases of apparent sensitisation to agro products during storage are caused by the presence of allergenic contaminants. Contaminants include various species of storage mites, insects, rodent urine, and moulds.

Storage mites probably contribute the most to the overall load of allergens on workers and consumers. Unfortunately, there are no data which allow estimation of average and maximal daily intakes of allergenic mites or which can suggest a safe level of consumption.

Methods are urgently needed to measure allergenic contaminants in various agro products and to identify and eradicate the sources of contamination.

The presence of contamination of agro products and the possibility to avoid it must be properly addressed. The adoption of good management and processing can help to minimise the risk.

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Sažetak

ALERGENI U POLJOPRIVREDNIM PROIZVODIMA TIJEKOM USKLADIŠTENJA

Na poljoprivrednim proizvodima tijekom čuvanja u različitim skladišnim objektima pojavljuje se niz rizičnih situacija poput kontaminacije grinjama, insektima, glodavcima, plijesnima, nedostatne higijene, uporabe opasnih i toksičnih pesticida, razvoja mikotoksina i alergena. Problemi su osobito naglašeni u zemljama u razvoju gdje su zalihe hrane nedostatne i zbog potrebe za jelom zanemaruju se potencijalne i stvarne opasnosti koje takva hrana može imati za ljudsko zdravlje. Smatra se da dobar sustav integrirane zaštite robe poljoprivrednog podrijetla može znatno smanjiti potencijalnu i stvarnu opasnost nastalu onečišćenjem tijekom čuvanja u skladištima.

Porast pojava alergije u razvijenim zemljama i želja da se poveća sigurnost hrane usmjerila je pozornost znanstvenika na mogući razvoj alergena na uskladištenoj poljoprivrednoj robi i u njoj tijekom čuvanja. Istražujući moguće uzroke pojave respiratornih simptoma u radnika zaposlenih u žitarskoj i mlinarskoj industriji, brojnim testovima i u objavljenim radovima potvrđena je uska povezanost između profesionalnih bolesti i kontaminacije uskladištene robe i radnog prostora grinjama, insektima, glodavcima i plijesnima. Uskladišteni proizvodi mogu sadržavati alergijska onečišćenja biljnog, mikrobiološkog i životinjskog podrijetla. Utvrđeno je da su grinje najvažniji onečišćivači robe i proizvođači alergena. Uzrokuju astmu kad se unose u organizam disanjem. Osobito su opasne kad se u velikom broju unose u organizam s hranom jer izazivaju anafilaktičke reakcije. Premda su kao izvori alergena potvrđene i neke skladišne gljivice (*Aspergillus oryzae* i *A. niger*), smatra se da su njihovi mikotoksini znatno opasniji problem.

Kao izvori alergena navode se ove skladišne grinje i insekti: grinje *Dermatophagoides farinae, Lepidoglyphus destructor, Tyrophagus putrescentiae, Tyreophagus entomophagus* i *Suidasia spp.*, insekti *Tenebrio molitor, Sitophilus granarius, Plodia interpunctella, Ephestia kuehniella, Liposcellis bostrychophila* i *Blatta orientalis*. Izmet artropoda kao i mokraća štakora i miševa jednako su važni izvori alergena.

Ključne riječi:
alergijski učinci, onečišćenja uskladištene hrane, skladišni štetnici

Requests for reprints:

Zlatko Korunić, Ph. D.
Diatom Research and Consulting
14 Greenwich Drive, Guelph ON, Canada N1H 8B8
E-mail: *zkorunic@diatom-research.com*

Arh Hig Rada Toksikol 2001;52:49-59

CONFERENCE PAPER

ELECTROCHEMICAL BIOSENSORS FOR EVALUATION OF CONTAMINANTS IN FOOD

MARCO MASCINI AND ILARIA PALCHETTI

University of Florence, Department of Publich Health, Epidemiology, and Environmental Analytical Chemistry, Florence, Italy

Received November 2000

This paper describes the application of electrochemical disposable biosensors in food analysis, which have recently been developed in our laboratory. Disposable biosensors, based on acetylcholinesterase inhibition activity, were exploited for testing the presence of organophosphorus and carbamate pesticides in water, fruit, and vegetable samples. The paper further describes preliminary tests for the detection of genetically modified organisms and hybridisation by coupling the DNA biosensors with the polymerase chain reaction.

Keywords:
acetylcholinesterase, biosensors, DNA, genetically modified organism, pesticides, screen-printed electrodes

For the past couple of years, food industry has been using electrochemical biosensors to control food (1, 2). The biosensors combine the inherent specificity of biological recognition processes and the sensitivity and speed of electrochemical measurements (3–5). This paper brings the recent results of their application in detection of pesticides (6–9). Organophosphorus and carbamate pesticides have gradually replaced the organochlorines and, although they are characterised by low environmental persistence, they generally show higher acute toxicity. The toxicity has a direct impact on human health because it causes an irreversible inhibition of acetylcholinesterase which is an enzyme involved in the nerve-impulse transmission. The measurement of the anticholinesterase activity of these pesticides can be used as a screening test for the evaluation of the presence of these compounds in food matrices. The inhibition can be evaluated using screen-printed electrodes for amperometric or voltammetric measurements. Screen-printed electrodes are planar devices, based on different layers of ink printed on a plastic or ceramic substrate. The interest in these devices is due to the possibility of making them disposable. In electrochemistry a disposable sensor has the advantage of not suffering from the electrode fouling that can result in the loss of

Presented at the ICS-UNIDO Workshop on Contamination of Food and Agroproducts, 28 September – 1 October 2000, Varaždin, Croatia

sensitivity and reproducibility. Moreover, these devices can be miniaturised and made suitable for on-site measurement. This paper brings some results using a disposable screen-printed carbon electrode. The carbon surface was modified by deposition of a mediator, tetracyanoquinodimethane (TCNQ). Acetylcholinesterase cleaves the acetylthiocholine added to the sample to thiocholine. The amount of thiocholine produced is directly related to the enzyme activity and can be evaluated by differential pulse voltammetry (DPV). DPV is a faster technique than the earlier used chronoamperometry (8), especially in combination with screen-printed electrodes.

In this approach, the enzyme inhibition is referred to as total anticholinesterase activity (TAA) and is expressed as »equivalent μg/L« of a reference pesticide. The TAA index does not measure the concentration of a specific compound, but is related to a class of compounds and to their toxicity. Therefore, the TAA index indicates whether a sample should undergo further analysis (10).

Moreover, this paper reports results obtained with a DNA-based biosensor for transgenic organisms. There is an increasing need of screening methods to identify genetically modified organisms (GMOs) in food (11). Currently the most cultivated GMOs are soya and maize, and the control and detection of these foodstuffs are important issues (12). Our strategy has been to immobilise sequences of 25-mer single-stranded oligonucleotides (probe) on carbon screen-printed electrodes. The immobilised oligonucleotides complement the sequence of the most common inserts in the GMOs: the Promoter 35S and the TNOS terminator (11). The food samples were previously amplified by polymerase chain reaction (PCR). The ability of the probe to recognise the target sequences was first tested using synthetic full complementary target oligonucleotides. Non-complementary synthetic oligonucleotides were used as negative control. An electrochemical marker (daunomycin) was used to signal the probe-target interaction (13, 14) that intercalates the double-stranded DNA. The procedure consisted of four steps:

1) the synthetic oligonucleotide was immobilised onto the electrode surface by adsorption at controlled potential;
2) the obtained DNA biosensor was incubated with the amplified sample or with the solution containing the target;
3) the probe was then immersed in the daunomycin solution, which intercalates the hybrid formed on the electrode surface; and
4) finally, the daunomycin anodic peak was used to detect whether hybridisation took place.

MATERIALS AND METHODS

Pesticide detection

Acetylcholinesterase (AChE, EC 3.1.1.7) from the electric eel (530 U/mg protein), acetylthiocholine (ATCh chloride salt), and the mediator 7,7,8,8, tetracyanoquinodimethane (TCNQ) were purchased from Sigma (USA). Carbofuran was obtained from Polyscience Corporation (USA). Standard solutions were prepared daily by dissolving the pesticide in the acetonitrile purchased from Sigma (USA). All other reagents were of analytical grade and were obtained from Merck (Germany). The water was from a reagent grade ion-exchange system from Millipore Inc. (USA).

All electrochemical measurements were performed using a computer controlled polarographic analyser model 433 A (Amel, Italy). The reagents and the procedure for electrode screen-printing were reported elsewhere (6–8). The electrochemical cell consisted of a screen-printed carbon working electrode and silver reference and counter screen-printed electrodes.

In DPV, the potential was scanned in a defined range and the pulses were superimposed on the potential ramp. The current was measured immediately before pulse application and at the end of the pulse, and the difference between the two currents was registered. Since DPV is a differential technique, the response is similar to the first derivative of a conventional voltammogram, that is, a peak.

To perform the measurement, the carbon surface of the working electrode was modified with 2 μl of a suspension obtained by mixing 5 μl of 1×10^{-3} mol/L TCNQ solution in acetonitrile and 50 μl Nafion®.

In order to optimise the determination of the acetylcholinesterase activity we developed the following procedure; 20 μl of a stock solution of enzyme (50 U/μl) and 20 μl of a 5×10^{-2} mol/L acetylthiocholine were mixed in 1 ml of buffer phosphate 0.1 mol/L at pH 7.5; the reaction was allowed to proceed for 5 min at room temperature. Then 100 μl of the solution were deposited onto the planar surface of the TCNQ-Nafion® SPE and the DPV measurement was performed from +0.35 to +0.7 V vs. Ag pseudo-reference electrode; with the pulse amplitude of 50 mV, scan rate 25 mV/s and pulse width of 60 ms. The current peak obtained at + 0.5 V was set as the analytical signal. To measure the total anticholinesterase activity using the acetylthiocholine sensor, 50 μl of pesticide standard solutions or river water samples were added to 1 ml of 0.1 mol/L phosphate buffer solution pH 7.5 with acetylcholinesterase (1 U/ml) and incubated for 10 min. Then we added 20 μl of the 5×10^{-2} mol/L acetylthiocholine solution. After 5 min, 100 μl of this solution were directly dropped on the surface of the sensor. The oxidation current peak obtained by DPV was measured (I_2) and compared with the oxidation current value obtained without pesticide (I_1).

The percent of inhibition (I%) was obtained according to the following formula:

$$I\% = 100\{(I_1 - I_2)/I_1\}$$

The total anticholinesterase activity can be expressed as the amount of compounds which inhibits cholinesterase in a percentage equivalent to inhibition by a known amount of a pesticide (such as carbofuran, a carbamate pesticide) taken as the reference compound. Carbofuran was chosen as the reference pesticide because it is widely use for crop protection and is one of the most frequently found pesticides in water samples taken from the River Arno. The procedure for standard gas-chromatography of the river water samples was described earlier (9).

GMO detection

Synthetic oligonucleotides were from Pharmacia Biotech (Milan, Italy). The PCR reagents and the procedure were reported elsewhere (14). Saline-sodium citrate buffer (2xSSC, containing 300 mmol/L NaCl and 30 mmol/L $C_3H_5Na_3O_7$, pH 7.4), acetic acid, sodium acetate, daunomycin hydrochloride were from Sigma (Milan, Italy). Certified reference material (2% soya flour) was from Fluka, (Milan, Italy). The reagents and the procedure for electrode screen-printing were reported elsewhere (6, 7). The electrochemical cell consisted of a screen-printed carbon working electrode, a platinum counter electrode, and a saturated calomel reference electrode (SCE). The electrochemical experiments were performed with an Autolab PGSTA 10 electrochemical analysis system and a GPES4 software package (Ecochemie, the Netherlands). Chronopotentiometry records the variation of the potential (E) with time resulting from the application of a constant current to the electrode. In general, the derivative signal (dE/dt) is plotted vs. the potential, producing the analytical signal as a peak.

For the immobilisation of the probe onto the electrode surface, the specific oligonucleotides were diluted in an appropriate buffer solution (2xSSC) pH 7.4, reaching the final concentration of 4 μg/ml. The deposition was carried out applying the positive potential +0.5V vs. SCE, for 120 s. To check whether the immobilisation was successful, a chronopotentiogram was used to observe the guanine oxidation peak as confirmation of the deposition after the immobilisation.

The probe base sequences were:

GGCCATCGTTGAAGATGCCTCTGCC (P35S)
AATGATTAATTGCGGGACTCTAATC (TNOS)

The hybridisation reaction was first tested using synthetic oligonucleotides at different concentrations. The diluted oligonucleotide solution was prepared in 2xSSC buffer; 50 μl of the diluted solution was applied onto the working electrode surface for 4 min and then the sensor surface was washed with 2xSSC buffer; then the working electrode was immersed in a 10 μM daunomycin solution in 2xSSC buffer for 120 s. After the washing with a 2xSSC buffer, chronopotentiometry is carried out in 2xSSC buffer, applying a constant current of +1 μA in a potential range from +0.2V to +1.1V vs. SCE. The analytical signal is the daunomycin oxidation peak area around +0.45 V vs. SCE.

When the amplified samples from the certified material were analysed, the procedure continued with denaturation of the diluted PCR-amplified double-stranded DNA by heating at 95 °C for 5 min. and rapid cooling with ice for 2 min. The denaturated amplified sample (50 μl) was deposited onto the electrode surface to react with the immobilised complementary probe for 4 min. The steps for the synthetic oligonucleotides are the same as described above.

RESULTS AND DISCUSSION

Pesticide detection

The rate of thiocholine production in the presence of organophosphorus and carbamate pesticides, which bind to the enzyme acetylcholinesterase and inhibit it, drops with their increase. We tested different compounds using the described biosensors. Figure 1A shows the voltammograms obtained in the absence and in presence of two different

Figure 1 *Calibration curve of carbofuran A) DP voltammograms obtained in absence and in presence of two different concentrations of carbofuran and a scan obtained in the buffer without the enzyme; B) Inhibition curve of carbofuran obtained by plotting the inhibition value* $\{i\%=[(i_1-i_2)/i_1]x100\}$ *vs. concentration of inhibitor*

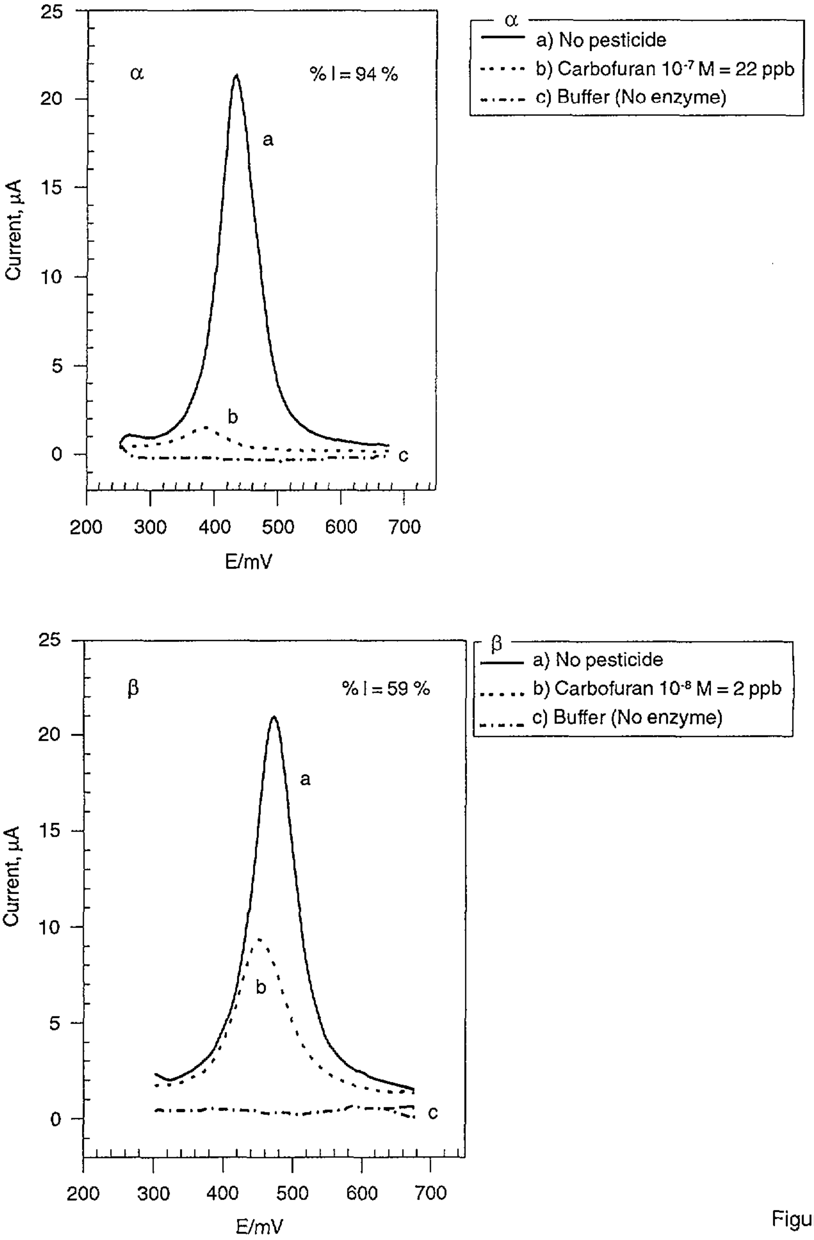

Figure 1a

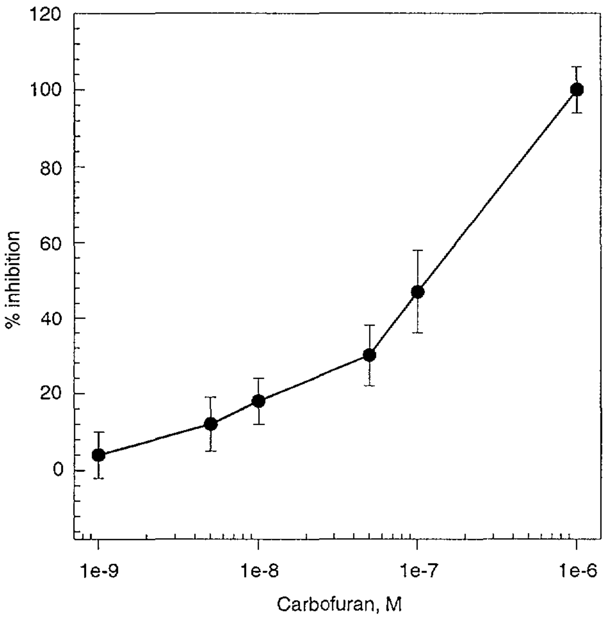

Figure 1b

concentrations of carbofuran, a carbamate pesticide considered as reference, since it is very common in agriculture. Figure 1B shows the calibration curve of this pesticide. Its limit of detection was $1x10^{-9}$ M.

Table 1 shows the results obtained with acetylthiocholine biosensor for the water analysis of the River Arno (9). Since the anticholinesterase pesticide concentration in the Arno water is very low, in many cases below 0.1 μg/L, we have pre-concentrated the samples using the solid-phase extraction (SPE) technique (9). The samples were pre-concentrated 100 times, shifting the detection limit to $9x10^{-11}$ M. Table 1 illustrates the results obtained using a standard method (gas chromatography) and the biosensor. There is a good correlation between the two sets of data. However, the results obtained by the biosensor were slightly greater than those obtained by the reference method, since the sensor determines a group of cholinesterase-inhibiting compounds. In fact, acetylcholinesterase activity may be inhibited by several compounds other than pesticides such as heavy metals and some toxins. The TAA is a general index combining these different contributions, although the effect of heavy metal on acetylcholinesterase activity is negligible compared to the effect of pesticides (8).

Table 2 shows the results obtained analysing fruit and vegetable samples; 5 g of a sample were ground and treated with 15 ml of ethylacetate; 5 ml were then dried

Table 1 *Comparison of the results obtained by gas chromatography (GC-ITDMS) and the acetylthiocholinesterase biosensor. The river water samples were 100 times concentrated by solid-phase extraction. Carbofuran was the reference pesticide. Samples were collected in different days during spring-summer 1999*

Gas Chromatography		Biosensor	
Samples	**Carbofuran M**	**% Inhibition**	**Carbofuran *Equivalent Conc.* M**
1 (17/4/99)	2×10^{-13}	–	$<9 \times 10^{-11}$
2 (21/4/99)	2×10^{-13}	–	$<9 \times 10^{-11}$
3 (30/4/99)	2×10^{-13}	–	$<9 \times 10^{-11}$
4 (24/5/99)	1.2×10^{-10}	40 ± 9	1×10^{-9}
5 (25/5/99)	1.1×10^{-10}	30 ± 7	4×10^{-10}
6 (26/5/99)	1.0×10^{-10}	30 ± 7	4×10^{-10}
7 (27/5/99)	6.0×10^{-11}	15 ± 6	1×10^{-10}
8 (7/6/99)	1.5×10^{-10}	40 ± 9	1×10^{-9}
9 (21/6/99)	1.1×10^{-10}	40 ± 9	1×10^{-9}
10 (3/7/99)	4×10^{-13}	–	$<9 \times 10^{-11}$
11 (9/7/99)	5×10^{-13}	–	$<9 \times 10^{-11}$
12 (13/7/99)	2×10^{-13}	–	$<9 \times 10^{-11}$
13 (16/7/99)	8×10^{-13}	–	$<9 \times 10^{-11}$

and dissolved in a 2 ml buffer. One millilitre was the sample volume used for the evaluation of pesticides.

Table 2 suggests that this simple procedure can be effective in analysing such samples, even *in situ*, that is, with a portable apparatus.

Table 2 *Inhibition values obtained by the analysis of fruit and vegetable samples*

Samples	Biosensor I%
Grape	93 ± 3
Grape	50 ± 2
Grape	0
Orange	40 ± 3
Pear	14 ± 1
Celery	30 ± 2
Melon	10 ± 1
Lettuce	31 ± 2

GMOs detection

Figure 2 shows calibration curves at different concentrations for complementary synthetic oligonucleotides using electrodes with P35S or TNOS probe immobilised on the surface of the working electrode. The daunomycin peak area is proportionally increasing with the concentration of complementary oligonucleotides either for P35S or TNOS probe. Figure 3 compares complementary with non-complementary signals. The difference is significant when the concentration is above 2 mg/kg.

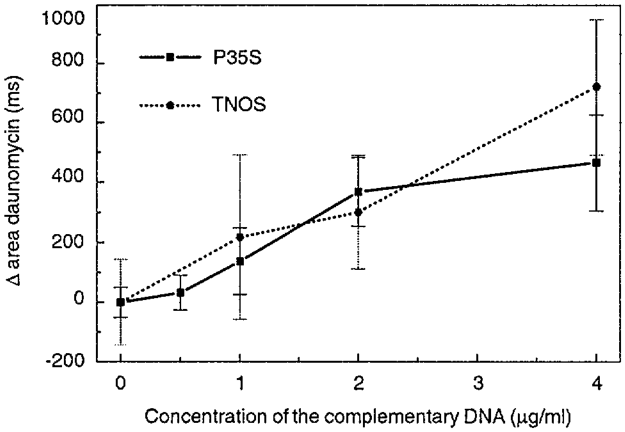

Figure 2 *Calibration curves for the sensors with immobilised P35S and TNOS using the relative complementary oligonucleotides*

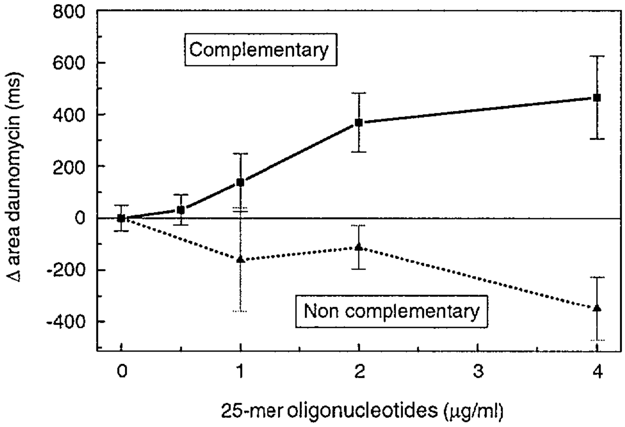

Figure 3 *P35S probe immobilised onto the electrode surface: response to the specific complementary and non-complementary 25-mer oligonucleotides*

The DNA probe was then applied to 2% transgenic soya flour (from certified reference material). In order to test a possible interfering signal in the matrix, a blank was tested with all PCR reagents, but without the target sequence and no interference was observed. Different dilutions of the amplified transgenic soya sample (2%) were assayed with both DNA biosensors with immobilised oligonucleotides (P35S or TNOS). Preliminary results are shown in Table 3 where the analytical signal is reported vs. the sample dilution from 1:20 to 1:160. When the TNOS probe was immobilised on the sensor surface, the dilution of the relative amplified sample giving the higher response was 1:80. When the P35S probe was immobilised, an increase in the daunomycin peak area was observed only at the 1:20 dilution of the relative amplified sample. These preliminary experiments show the applicability of such devices in rapid evaluation of GMOs.

Table 3 *Response of the electrode with P35S and TNOS probes in real samples amplified by PCR. Soya P35S-amplified samples were analysed with the P35S probe. Soya TNOS-amplified samples were analysed with the TNOS probe*

Dilutions	Soya TNOS	Soya P35S
1:20	0	+343 ± 380
1:40	+430 ± 240	-130 ± 130
1:80	+610 ± 350	-90 ± 180
1:160	+340 ± 250	0

CONCLUSION

We have demonstrated that biosensors can offer fast and reliable results for food analysis. Acetylcholinesterase activity inhibition can be used to detect organophosphorus and carbamate pesticides in different food samples with minimal pretreatment. Such measurement can be used as a screening test before performing longer and expensive analysis.

Moreover, we have shown that the DNA biosensor is able to recognise oligonucleotides and, through PCR amplification, the presence of transgenic soya in certified reference material. The sensor is specific for the complementary sequence.

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Sažetak

ELEKTROKEMIJSKI BIOSENZORI ZA PROCJENU ONEČIŠĆIVAČA RADI POBOLJŠANJA KAKVOĆE HRANE

U radu su opisane neke primjene jednokratnih elektrokemijskih biosenzora za analizu hrane koji su nedavno razvijeni u našem laboratoriju. Biosenzori za jednokratnu upotrebu, bazirani na inhibiciji aktivnosti kolinesteraze primijenjeni su za ispitivanje prisutnosti organofosfornih i karbamatnih pesticida u uzorcima voda, voća i povrća. Razvijena je skupina DNK-biosenzora, a u ovom radu prikazani su rezultati preliminarnih ispitivanja detekcije genetički modificiranih organizama i hibridizacije vezanjem PCR i DNK-biosenzora.

Ključne riječi:
acetilkolinesteraza, biosenzori, DNK, genetički modificirani organizmi, pesticidi

Requests for reprints:

Marco Mascini, Ph. D.
Università di Firenze, Dipartimento di Sanità Pubblica,
Epidemiologia e Chimica Analitica Ambientale,
Via Gino Capponi 9, 50121 Firenze, Italy
E-mail: *mascini@unifi.it*

Arh Hig Rada Toksikol 2001;52:61-67

CONFERENCE PAPER

MICROBIOLOGICAL EXAMINATION AND PROFICIENCY TESTING IN DAIRY LABORATORIES

SLAVICA GOLC TEGER,

University of Ljubljana, Biotechnical Faculty, Domžale, Slovenia

Received December 2000

This paper considers the main factors in the assessment of microbiological examination of food and discusses a few points related to validation of quantitative and qualitative microbiological methods. Within the scope of accredited methods, the author defines the terms such as *conform reference*, *equivalence of reference method*, and *in-house method*. The paper describes evaluation of a routine method with respect to the official method based on results obtained by automatic epifluorescent microscopy using the BactoScan 8000 instrument for determination of bacteriological quality of milk and provides general guidance for the establishment of a conversion relationship between the two methods. The paper gives an overview of the quality assurance aspects involved in the application of the routine method and concludes with an example of interlaboratory proficiency study for the epifluorescent microscopic method which is regularly applied in dairy laboratories.

Key words:
conform reference, dairy laboratories, epifluorescent miscroscopy, microbiological methods, proficiency testing, quality assurance

The most important aspects in the assessment of microbiological examination are the validation of microbiological methods, implementation of quality controls, use of the terms conform, equivalent of, and in-house method and arrangements regarding the scope of accredited methods. The paper will present the practical use of quality assurance aspects in the application of routine method using the BactoScan 8000

Presented at the ICS-UNIDO Workshop on Contamination of Food and Agroproducts, 28 September – 1 October 2000, Varaždin, Croatia

instrument for quantitative determination of the bacteriological quality of milk. Special emphasis will be placed on the importance of interlaboratory proficiency testing.

VALIDATION OF MICROBIOLOGICAL METHODS

The term validation is used for a process demonstrating that a particular method is suitable for the intended purpose. There are few sources in the field of microbiology that cover method evaluation. Nevertheless, methods submitted for accreditation must be validated. The validation of microbiological methods should not rely on the same principle as of chemical methods. This is why the Dutch Accreditation Council organises the preparation of the international Explanatory Document on Microbiology (RvA-T2) that would include a selection of performance characteristics dependent on the testing method.

In reality, only in a very limited number of cases will a laboratory fully develop and validate a new microbiological method. Often the laboratory itself applies limited validation. When a laboratory adopts a reference method or a method that has been developed and validated elsewhere, it has to demonstrate that it can apply the method properly in its own environment and obtain correct results. The verification of limited validation must be accomplished by the use of proper internal and external quality controls.

Microbiological methods are divided into either qualitative methods that demonstrate the presence or absence of the target microorganism, either directly or indirectly, in a defined quantity of test material or quantitative methods that determine the number of microorganisms present by direct enumeration (colony forming units) or indirectly (most probable number counts, colour absorbance, impedance) in a defined quantity of material. When a reference method is used, a laboratory should demonstrate its competence to meet the performance characteristics described in the national or international standard. For qualitative microbiological standard methods the performance characteristic is the limit of detection, while for quantitative microbiological standard methods these are trueness, repeatability, and reproducibility.

Microbiological investigations may be undertaken using alternative (rapid) methods such as immunological, molecular biological, or instrumental. The validation of these methods includes the assessment of their equivalence to the corresponding reference method (1).

Equivalence can be applied to the use of alternative (rapid) confirmation techniques; use of a different range of colony count than stated in the relevant standard; use of a different method for counting colonies or for calculation and expression of results than those stated in the relevant standard; and use of alternative (rapid) methods validated according to the relevant standard.

The *in-house method* is applicable to the use of a non-standard methods or the use of a standard method on a matrix that differs from the one specified by the standard method. When an in-house method is adopted it is recommended to select a non-standard method that has already been validated by a national or international organisation, or an accepted method that has already been in use in particular professional branches (e.g. the International Dairy Federation, IDF).

APLICATION OF QUALITY CONTROLS

One requirement for competence of laboratories in the International standard (2) is that a laboratory should ensure the quality of its results by using and assessing different quality controls. In the field of microbiology the use of correct quality controls is of particular importance, because the translation of the performance characteristic to microbiological examination is not always possible and it depends on the test matrix.

The terms »trueness« and »precision«, for example, are more difficult to define for microbiological examination than for analytical chemistry investigations. However, it is possible to demonstrate the technical control of the microbiological methods through control samples. Good microbiological reference samples are not yet available for all types of microorganisms.

Quality control can be implemented in a number of ways: internal serial (also called first line control) i.e. internal control of serial analyses assessed by technicians (using blanks, positive controls, negative controls, multiple observations); internal process (also called second line control) i.e. internal process control conducted by technicians, but assessed by laboratory management staff, e.g. the quality officer (using reference materials and spiked samples); and external (also called third line control) i.e. participation in an externally organised proficiency testing scheme. The quality controls will be described in the international Explanatory Document on Microbiology (RvA-T2 organised by Dutch Accreditation Council).

ROUTINE METHODS FOR QUANTITATIVE DETERMINATION OF THE BACTERIOLOGICAL QUALITY OF MILK

Particular routine methods are used for quantitative determination of the bacteriological quality of milk for a number different reasons such as: speed of analysis and/or response, ease of execution and/or automation, analytical attributes (specificity, limit of detection, limit of determination, repeatability, reproducibility, accuracy), extent to which the parameter of interest is expressed, and reduction of costs. Adequate operation of a routine method involves the application of quality assurance principles.

The below example of the use of a rapid microbiological method for routine work (3) that has been in use in Slovenia since 1995 (4) illustrates the evaluation of a routine method with a reference method for the determination of the bacteriological quality of milk based on automatic epifluorescent microscopy using the BactoScan 8000 instrument.

When introducing a routine method in order to comply with national and international regulations or standards, a number of issues is to be addressed:

- ☐ which criteria are essential for a routine method;
- ☐ what influencing factors should be considered when evaluating a routine method;
- ☐ how should a routine method replace a reference method;
- ☐ which constructive measures should be taken to improve the relationship between a reference and a routine method.

In most regulations for determination of the bacteriological quality of milk the reference method is the colony count at 30 C (5). However, many routine methods are applied, often with different methodological principles. The difference between the reference and routine values is expected to be less pronounced when the two methods are based on the same test principle.

The routine method must be evaluated as an estimate of the reference method according to general principles (6), either in-house or elsewhere.

In case of an expected deviation from the method of evaluation (e.g. milk composition, milk of other species, sample preservation, sample pre-treatment), the routine method must be validated additionally. The results of additional validation must be archived.

The laboratory must follow the following sets of control procedures: daily operating protocols, middle term controls, and long term controls. The daily protocol includes:

- start-up procedure for the instrument/equipment;
- start-up controls including limits;
- sample identification;
- sample storage and pre-treatment;
- sample checks (temperature, visible condition);
- sample analysis;
- routine checks during measurement (instrument settings, visual checks, temperature control, blanks, pilot samples);
- cleaning in between samples;
- shut-down procedure;
- truncation of results at the lower and upper limit of detection;
- evaluation and authorisation of results

Middle-term controls include:

- instrument checks;
- volume controls;
- carry-over controls;
- repeatability checks;
- within-lab reproducibility.

Long-term controls include:

- reproducibility;
- interlaboratory comparisons.

The purpose of interlaboratory comparisons is to establish that the precision characteristics of the laboratory's procedure are still in line with those recognised as feasible and achieved elsewhere and that the measured level is in agreement with that of the other participants. Conversion characteristics must express results in units of another method.

The results of interlaboratory comparisons are statistically evaluated. Graphical interpretations are frequently used. Figure 1 shows an evaluation of performance of laboratories that participated in international proficiency testing using the BactoScan instruments. It is presented as the Euclidian distance between accuracy and repeatability, which gives information about the performance characteristics of the instruments (7). Figure 2 illustrates a deviation of laboratory results from the total average.

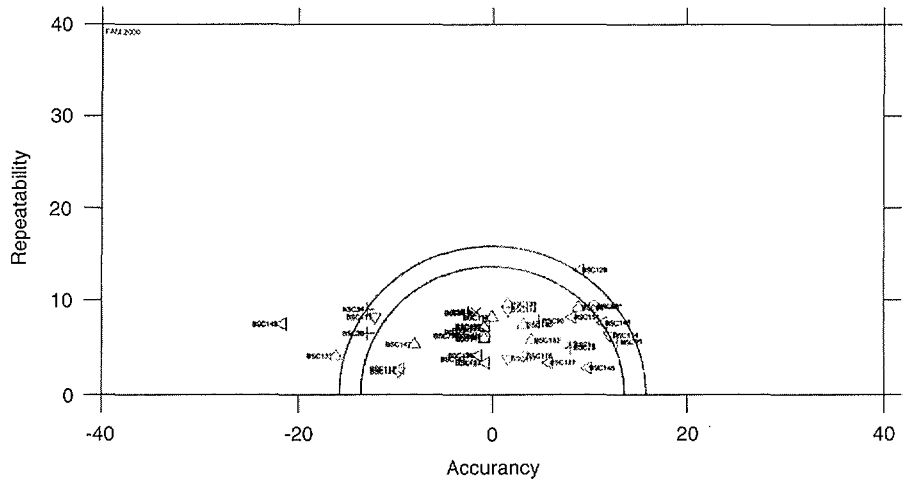

Figure 1 *Evaluation of performance of individual laboratories using the BactoScan instruments*

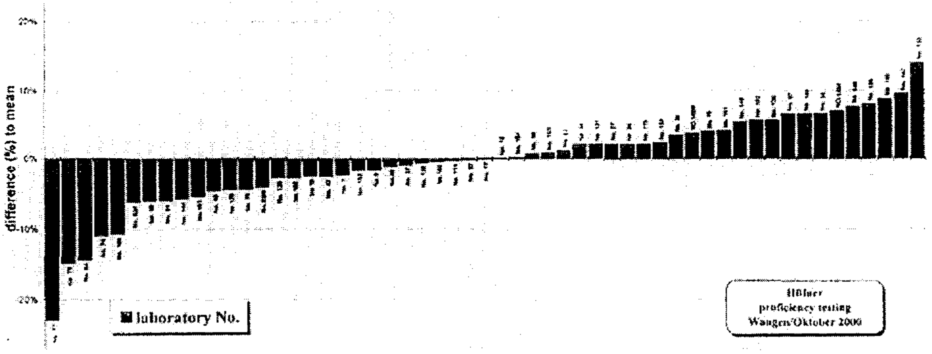

Figure 2 *Cell count proficiency testing – difference (%) from the mean of control milk samples*

The figure has been taken from the report on cell count collaborative study organised by Milk Standard Service, Wangen, Germany.

In order to make comparable the use of routine methods the International Standards Organization and AWI are preparing a new standard proposal »Milk-Quantitative determination of bacteriological quality – Protocol for establishing a conversion relationship between routine method result and anchor method results and its verification« (8).

CONCLUSIONS

In the assessment of microbiological examination the choice and the evaluation of a method, and the use of a quality assurance system are very important. This is why every laboratory should rely on good laboratory practice, protocols, and standards. Our experiences suggests that it is equally important to perform quality control of the reference plate count method as a prerequisite for a reliable evaluation of an alternative microbiological method. The performance of test methods is evaluated through participation in interlaboratory proficiency studies.

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Sažetak

MIKROBIOLOŠKO ISPITIVANJE I PROSUDBA VRSNOSTI LABORATORIJA ZA MLIJEČNE PROIZVODE

U ovome su članku prikazani glavni čimbenici u procjeni mikrobiološkog ispitivanja hrane. Prikazano je nekoliko točaka validacije kvantitativnih i kvalitativnih mikrobioloških metoda. Osim toga, definiraju se izrazi »sukladnost« i »istovjetnost« referentne metode s »internom« metodom u smislu akreditacija metoda. Članak opisuje kako evaluirati rutinsku metodu u odnosu na referentne/službene metode s obzirom na rezultate dobivene brojenjem automatskim epifluorescentnim mikroskopom s pomoću uređaja BactoScan 8000 namijenjenog za utvrđivanje bakteriološke kakvoće mlijeka te daje opće upute za usporedbu navedenih metoda. Tu je i pregled aspekata osiguranja kakvoće koji se uzimaju u obzir u primjeni rutinske metode te primjer međulaboratorijske prosudbe vrsnosti uz pomoć epifluorescentne mikroskopske metode koja je uobičajena u laboratorijima za mliječne proizvode.

Ključne riječi:
BactoScan 8000, kontrola mlijeka, osiguranje kakvoće

Requests for reprints:

Slavica Golc Teger, Ph. D.
University of Ljubljana, Biotechnical Faculty
Zootechnical Department, Institute of Dairying
Groblje 3, SI-1230 Domžale, Slovenia
E-mail: *slavica.golc@bfro.uni-lj.si*

Arh Hig Rada Toksikol 2001;52:69-73

COUNTRY REPORT

CONTAMINATION OF FOOD AND AGRO PRODUCTS IN THE REPUBLIC OF MACEDONIA

VLADIMIR KENDROVSKI, ELISAVETA STIKOVA, AND LENCE KOLEVSKA

Republic Institute for Health Protection, Skopje, Republic of Macedonia

Received October 2000

The Republic of Macedonia has 662,000 ha of cultivated land with well-organised agricultural production. It is a well known producer of agricultural products. From 1991 to 1999, authorised laboratories conducted systematic control of about 6,500 food samples in 1991 to 36,000 by 1999 which included a whole range of hygienic quality parameters including microbiological and chemical. Microbiological contamination of food was reported to have caused disease outbreaks in 1,578 people in 1995, which dropped to 126 people in 1999. The decreasing trend of samples unfit for human consumption was observed for all food groups. As for the chemical parameters, an increasing trend of contaminated samples was noted only for additives, with peaks reaching 6.9% in 1998 and 4.9% in 1999 of the total number of analysed samples.

Key words:
chemical parameters, food contamination, *microbiological parameters*

It is estimated that every year around 130 million Europeans are affected by food-borne diseases (1). Diarrhoea – the major cause of death and retarded growth in infants – is the most common symptom of food-borne illnesses (2). While biological hazards are of great concern because contaminated foods can cause widespread outbreaks of diseases, chemical hazards may also cause food-borne illnesses, although generally affecting fewer people. Chemical hazards can originate from many sources such as agricultural chemicals (pesticides, fertilizers, and animal drugs), natural toxicants (plant, animal, or microbial products), preservatives, food additives, sulfating agents, environmental contaminants (lead, cadmium, mercury, arsenic, polychlorinated biphenyls (PCBs), and so on (3).

Sources of contamination of food, both chemical (pesticides, heavy metals, and other contaminants) and biological, can be found at any stage of the food chain.

Presented at the ICS-UNIDO Workshop on Contamination of Food and Agroproducts, 28 September – 1 October 2000, Varaždin, Croatia

Food can be contaminated during at various stages of agricultural production, storage, transport, processing, packaging, and final preparation (4).

The Republic of Macedonia has 662,000 ha of cultivated agricultural land and a well-organised agricultural production. Beside intense, yet deficit-producing industrial export, Macedonia is a well known producer of agricultural products (5).

The existing food legislation in the Republic of Macedonia is not updated and properly coordinated, that is, the food control system is based on the common approach, generally involving inspection and control of final products, after which follows the sanctioning of detected nonconformities (reactive as opposed to proactive approach).

MATERIALS AND METHODS

Food samples taken between 1991 and 1999 included domestic and imported products and were analysed according to parameters described in Act on Food and Objects of Common Use (published in the *Official Journal of the Republic of Macedonia* 51/84) and a number of bylaws. Authorised laboratories in the Republic of Macedonia used standard methods such as flame atomic absorption spectrometry for heavy metals and gas chromatography for pesticides to examine the hygienic and sanitary quality of food samples and check microbiological and chemical parameters. The analysed data have been and published in the Yearbooks of the Republic Institute for Health Protection in Skopje, Macedonia (6)

RESULTS AND DISCUSSION

Microbiological contamination of food has been recognised as a health hazard all over the world, including Macedonia. It can be of plant or animal origin and can occur in primary production (ill or infected animals, contaminated plant products consumed fresh), processing, storage, or distribution. Biological contamination, which is mainly bacterial, can cause food-borne infections or intoxications. (7) The Republic Institute for Health Protection maintains the national surveillance system for monitoring incidents of food poisoning with enteric pathogens. A food-borne intoxication is caused by ingestion of already formed toxins such as staphylococcal enterotoxin produced by some bacteria which multiply in food (2). Table 1 shows the annual distribution of

Table 1 *Annual distribution of microbiological food-borne poisoning incidents and epidemic outbreaks reported for the period 1991–1999 in the Republic of Macedonia*

Microbiological food-borne diseases	1991	1992	1993	1994	1995	1996	1997	1998	1999
Total number of victims	1574	1319	1253	1365	1578	1456	237	305	126
Total number of outbreaks	4	3	3	8	4	4	9	7	5

incidents of food-borne microbiological poisoning and registered outbreaks in the Republic of Macedonia. The Table 2 shows the annual distribution percentage of samples found unsuitable for human consumption by food groups for the period 1991–1999. In that 10-year period, the number of food samples included in systematic control grew from about 6,500 samples in 1991 to about 36,000 in 1999.

Table 2 *Microbiological distribution of improper samples by food groups expressed in percentages*

Food groups	1991	1992	1993	1994	1995	1996	1997	1998	1999
Milk	8.58	10.5	4.5	6	5.7	6.2	7.8	9.05	4.17
Milk products	3.38	4.8	4.3	16.4	2.4	6.6	18.5	8.5	9.9
Meat	31.8	23.9	9.1	9.8	8.8	6.2	7.8	13.7	3.75
Meat products	8.25	5.6	6.3	13	2.4	3.5	7.6	5.98	3.01
Fruit products	3.3	2.5	2.9	1.4	4.8	5.6	2.3	1.6	0
Vegetable products	1.7	4	8.1	2.1	8.6	2.5	7.8	0.2	2.5
Nonalcoholic beverages	1.1	2.5	2.3	16.5	2.4	1.1	2.3	1.54	2.7

Chemical analysis for contaminants mainly concerned the imported products. Table 3 shows the distribution of samples by contaminants for the period 1991–1999 and the percentage of improper samples in the total number of the examined samples.

Table 3

Contaminants	1991	1992	1993	1994	1995	1996	1997	1998	1999
Pesticides-TN	1946	2990	4650	12334	10402	9871	10199	9218	9329
% of IS	0.51	0.2	0.84	0	0.02	0.01	0	0.01	0.05
Heavy metals-TN	2958	379	5484	12882	13713	14544	14503	13756	13499
% of IS	0.067	1.1	0.51	0.04	0.36	0.15	0.07	0	0.08
Additives-TN	575	1363	3145	3119	3594	7537	8507	2655	3944
% of IS	0	0.51	5.56	0.25	3.3	1.8	2.3	6.9	4.3
Mycotoxins-TN	344	1084	2084	1434	1823	1669	2245	1733	1477
% of IS	0	0	0	1.28	0.77	0	0	0.01	0
Antibiotics-TN	827	1241	1505	3955	4880	6923	4109	3065	597
% of IS	0.96	0.08	0.13	0.07	0	0	0	0	2.6
Hormones-TN	350	534	777	718	578	113	577	686	678
% of IS	0	0	0	0	0	0	0	0	0

Of pesticides, only organochlorine and organophosphoric insecticides are monitored in food. Carbamate insecticides and herbicides have been tested in a limited number of rice samples taken in the area of Kočani. Of 10,000 samples tested for the presence of insecticides a year, 0,01% were above the maximum permitted concentration (MPC). Of organochlorine insecticides, the presence of lindane and hexachlorocyclohexanes isomers (HCH) were detected. The tested rice samples from the area of Kočani showed concentrations 10–100 times and 2–20 times below the MPC for lindane and HCH, respectively. The organic chemical plant OHIS Skopje located near the village of Lisice poses a particular threat of food contamination. Namely, 10% of samples from that area showed HCH concentrations twice as high as the MPC. Organophosphoric pesticides were detected in none of 10–12,000 samples tested each year. Of herbicides, propanil was found in an average concentration of 0.01 mg/kg and molinate in concentrations of 0.1 mg/kg, which is significantly below the MPC, whereas carbaril was not detected at all.

Detected concentrations of lindane in plant products were very low, ranging between 0.2 and 2.6% of MPC. In milk, those concentrations were 17%, in milk products 24%, in meat 7% and meat products 4.8% of the MPC. Compared to the WHO-FAO results, our findings keep within the average ranges for individual products in Europe. Contamination of milk with HCH is 5 times lower than MPC, of milk products 6 times, of meat and meat products 20 times, of fruit 10 times, and of vegetables 3 times (5).

Annually, 4,000 samples of additives undergo testing, of which only 1,530 are added to domestic products. Two percent of imported products were prohibited for sale, as they contained prohibited additives or excessive quantities of additives. In domestic production, additive-related problems usually occur with small enterprises which lack professional approach to food processing. The most frequent violations concern excessive quantities of additives in refreshing beverages, meat products, and artificial sweeteners in confectionery products and ice creams in particular.

CONCLUSION

The large number of private farmers and small food processing enterprises and an even larger number of small trade and catering firms in the Republic of Macedonia make the legal control very difficult. Given the general, imprecise nature of legal stipulations, many of those entities work on improper premises, with inadequate equipment, or with unskilled staff, failing thus to secure standard hygienic conditions. Food is often sold on traditional marketplaces with poor hygienic conditions. The so called fast food restaurants seem to pose the greatest risk, and the most frequently reported violations by shops and catering services are associated with the use of groceries of unknown origin, inadequate thermal treatment, failure to observe prescribed storing temperatures, and low level of staff training.

However, when it comes to microbiological parameters, this trend has been decreasing trend in all food groups. As for the chemical parameters, an increase has been noted only for additives.

The Republic of Macedonia should establish a modern system of food control by adopting laws and other legal acts conforming with international recommendations, regulations, and standards. The national Food Agency established in 1999 and acting within the Ministry of Health should clearly define the responsibilities of relevant ministries and establish a common administration service for fast and effective control over health safety and inspection of food with full interdisciplinary staffing, purchase of missing equipment for diagnostic laboratories, and additional training of the existing and employment of new staff on the basis of sectional competencies defined by the Priorities and Action Plan.

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Sažetak

KONTAMINACIJA HRANE I AGROPROIZVODA U REPUBLICI MAKEDONIJI

Republika Makedonija ima 662.000 ha kultiviranih poljoprivrednih površina s dobro organiziranom poljoprivrednom proizvodnjom. Pored brojnog deficitarnog industrijskog izvoza, Makedonija je dobro poznati proizvođač ponajprije poljoprivrednih proizvoda. Tijekom proteklog 10 godišnjeg razdoblja (1991-1999) prehrambeni su proizvodi uključeni u sistematsku kontrolu u autoriziranim laboratorijima te je tako od 6500 proizvoda 1991. godine do 36000 uzoraka analizirano na zdravstvenu ispravnost, mikrobiološke i kemijske parametre.

Mikrobiološka kontaminacija hrane uzrokovala je nekoliko akcidenata a objavljeni broj žrtava kretao se od 1578 u 1995. god. do 126 osoba u 1995. god. Uočljiv je trend smanjenja uzoraka hrane koji nisu za ljudsku prehranu u svim vrstama hrane.

Od kemijskih parametara jedino je za aditive uočen trend porasta broja uzoraka koji ne zadovoljavaju kriterije za ljudsku prehranu. Najveći broj uzoraka koji nisu bili prihvatljivi, u odnosu na sve analizirane uzorke, zabilježen je 1998. god. (6,9%) i 1999. god. (4,9%).

Ključne riječi:
kemijski parametri, kontaminacija hrane, mikrobiološki parametri

Arh Hig Rada Toksikol 2001;52:75-80

COUNTRY REPORT

PUBLIC HEALTH SURVEILLANCE IN LATVIA

ANDIS GROSPIŅŠ[1], **IRINA SOLDATENKO**[2], **AND ARTŪRS ŠKUTE**[3]

Ministry of Welfare, Riga[1]*, Hygienic Laboratory Environmental Health Centre*[2]*, Daugavpils Pedagogical University*[3]*, Daugavpils, Latvia*

Received November 2000

This paper presents public health surveillance data in Latvia. This is the first time these data are being published since the restoration of the Second Republic. The paper describes the state-of-the-art monitoring system of food and water safety. It also contains epidemiological data for Latvia. The conclusion brings a success story about the implementation of the monitoring programme in the city of Daugavpils.

Key words:
epidemiological data, food, monitoring, water control

At the moment, Latvia has no systematic surveillance and monitoring programme for public health, food safety, and water quality assessment. The main reason lies in allocation of inadequate financial resources. Furthermore, continuous reorganisation of government administration responsible for food safety monitoring impedes the solution. It is expected that the following 5 years will see improvement in budget allocations and implementation of proper monitoring programme designs.

QUALITY OF FOOD

The quality of food has a significant influence on public health (1). Low quality food may cause acute contagious diseases. The main food infections are salmonella, shigellosis, and acute intestinal infections such as hepatitis A, typhoid, leptospirosis, yersiniosis, trichinosis, diphyillobothriosis, and botulism.

Presented at the ICS-UNIDO Workshop on Contamination of Food and Agroproducts, 28 September – 1 October 2000, Varaždin, Croatia

The incidence of acute intestinal diseases has grown for the past few years. In 1999, a large number of group morbidity with acute intestinal diseases, not characteristic of recent years, was registered in families and in public catering. The spread of contagious diseases was stimulated by the failure to comply with hygienic standards. Due to lack of information, people often purchase food products of doubtful quality, even for children. The exact information on food poisoning incidents is not available at the moment, as many people choose to treat themselves instead of seeking medical help. Thus many cases are not registered and sometimes people wait with help until it is difficult to establish the cause of the disease (Table1).

Table 1 *Epidemiological situation in Latvia (1994–1998)*

Name of disease	No. of cases	No. of cases per 100,000 people	Average no. of cases during 1994–1998	Comparison(%)	
				Average no. of cases in the last	No. of cases in 1998 5 years
Hepatitis	1652	67.72	2785	-41	-32
Acute A hepatitis	702	28.78	2168	-68	-56
Acute B hepatitis	453	18.57	432	+5	+14
Acute C hepatitis	247	10.13	98	+152	+47
Typhoid	3	0.12	2	+50	–
Salmonella	915	37.51	947	-3	-17
Shigelosis	439	18.00	657	-35	+35
Botulism	6	0.25	3	+100	+100
Diseases of known aetiology	1455	59.64	608	+139	+40
Diseases of unknown aetiology	1519	62.27	1319	+15	+31
Leptospirosis	43	1.76	54	-20	+34
Yersiniosis	96	3.94	168	-43	-6

Salmonella morbidity tends to decline but it is still very high – 915 cases in 1999. The highest salmonella morbidity level in 1999 was registered in the Aluksne district and the lowest morbidity level in the Saldus district (2.6–133.2 cases per 100,000 inhabitants).

Unfortunately, non-contagious diseases that might be caused by food of poor quality (such as dioxin-caused diseases, allergies, and ontological diseases) are not analysed in Latvia.

Public health is strongly influenced by products, processes, and services used by the population. They must be as safe and as harmless to health as possible. Therefore the European Union and Latvia have drafted safety requirements for products, processes, and services. The defining of requirements is one part of the problem while ensuring compliance is another. The system of control, supervision, and compliance assessment established in the country is intended for this purpose. It is prescribed by

Regulations on respective institutions, the Law On Supervision over the Circulation of Food, the Law On Consumer Protection, the Law On Compliance Assessment, and the regulations of the Cabinet of Ministers developed on its basis. In the Republic of Latvia, compliance assessment is performed by accredited testing and calibrating laboratories and certification and inspection agencies which bear full responsibility for their work.

In 1999 several scandals related to the quality of food proved that the food control system in Latvia needed reinforcement. The dioxin scandal revealed the strengths and weaknesses of our food control system. All agencies controlling food (the State Sanitary Inspectorate, the Sanitary Border Inspectorate, and the State Veterinary Service) reacted efficiently to information received and acted in a co-ordinated manner. However, it turned out that no maximum permissible dioxin limit in food had been established at the national level (nor had it been established at the international level); that there was no laboratory capacity for establishing the limit; and that the exchange of information among food control and monitoring agencies needed improvement. Thus the Latvia Food Centre was entrusted with the task of drafting the »Action Programme for Ensuring Food Safety in Emergency Cases« that would institute a procedure for providing information and mechanisms for operation.

The Regulations On Food Contamination of the Cabinet of Ministers (those have incorporated the European Union Directives 315193/EEC; 194 /97/EEC), were approved in 1999, establishing the maximum permissible contamination limits for food products and raw food, including the permissible dioxin levels.

The incident with contaminated Coca-Cola soft drinks found in the European trade networks highlighted another weak link in food control – what to do with food unfit for distribution. At present there are no uniform national regulations on how and when to destroy or put those food products to other use.

According to the media, the population of Latvia is very interested in consuming good quality and safe food and much attention is paid to the operation of the food control system. To evaluate the actual situation, it would be necessary to introduce a system of indicators that would show how well the food quality assurance system operates in the country and reveal its weaknesses. The information exchange network among institutions must be improved within the food control system. More effort should be invested in informing the public about matters and processes essential for the society and preventing ungrounded rumours. It is necessary to strengthen the material and technical basis of control agencies to ensure their speedy reaction.

QUALITY OF WATER AND THE QUALITY OF POTABLE WATER

According to Regulations of the Cabinet of Ministers No. 63 »Requirements for Compulsory Safety of Potable Water« (23 February 1999), as of 1999 water quality indicators were to comply with the European Union Directives. The National Environment Health Centre for monitoring potable water quality developed the national plan which was implemented in 1999 through monitoring of 14,300 water samples taken from regional, municipal, and household water supplies, as well as from water supply vehicles.

The samples were assessed according to chemical and microbiological indicators. Of 13,500 samples tested according to microbiological indicators, 17.6% did not comply with the requirements for potable water safety. Of 12,300 samples tested according to chemical indicators (iron, organoleptics - qualities of taste and of color), 52.4% did not comply with the requirements for potable water safety. These results are alarming. A considerable part of potable water in Latvia does not comply with requirements due to high iron content. It must be pointed out that iron contamination does not pose a serious threat to health, but rather the question of water quality. With that respect, the Environmental Health Action Plan identified the following priorities as early as 1997: quality improvement of potable water, renewal of pipelines in the water supply system, and the installation of equipment for removing iron.

Within the framework of the programme for assessing potable water, specialists at health centres inspected 30 wells in each administrative territory of Latvia (a total of 2784 wells). Samples were analysed according to microbiological, organoleptic, and chemical indicators. The analysis showed that the concentration of microbiological contaminants in those wells was comparatively high. In locations where high microbiological contamination in wells and parts of the water supply pipelines has been determined, water should be boiled before use to prevent the risk to human health. The chemical content of potable water is influenced by the geological structure of the area. In the water from wells in Latvia, the average concentration of ammonia ions, nitrates and nitrites is lower than the permissible level for potable water. However, the average iron content (0.51 mg/l) is above the permissible level for potable water. The main problems are the contamination of shallow wells with nitrogen compounds from ground water or underground water as a result of improper management or disregard of protective zones around the wells.

Quality of bathing waters

During the first quarter of 1999, specialists of the National Environment Health Centre drafted and approved the »Procedure for Monitoring Bathing Places« that introduced uniform documentation and established the procedure for choosing the place and time for taking samples. It stipulates that samples are to be taken only in official or potential bathing places and that the laboratory analyses are to be performed according to methodologies recognized by the European Union. This would allow comparison of data for this country with the data of foreign countries.

During the bathing season of 1999, the bathing water quality studies were conducted in 162 inland bathing sites in Latvia with the purpose to establish the degree of compliance with the requirements for bathing places. According to chemical indicators, 28.5% of the samples did not meet the requirements. The cause of non-compliance was mostly the excessive content of active ingredients and oil products on the surface, colour, the low oxygen saturation level in separate bodies of water, and mechanical admixtures. Car-washing in the close vicinity of recreation areas may have caused the excessive content of active ingredients and oil products on the surface. The high oxygen consumption points to contamination by organic substances which stimulate the overgrowth of water bodies and the appearance of micro-organisms that result in the deterioration of colour, smell, taste, and overall appearance of water. Further, 18.2% of samples did not comply with the microbiological requirements. However, no pathogenic microflora was found.

During the bathing season 1999, 1018 samples of bathing water were taken from 36 fixed sampling sites on the Latvian coast in the Gulf of Riga. The chemical content was established for 500 samples of which 12 (2.4%) did not meet the standard. The microbiological testing involved 518 samples of which 54 (10.6%) failed to meet the standard (Table 2).

Table 2 *Water quality in bathing places in Latvia (samples not meeting the standards are expressed in percentages)*

Inland bathing places	1997	1998	1999
Chemical indicators	40.0	25.7	28.5
Microbiological indicators	35.5	13.9	18.2

In comparison with 1998, the quality of water has not undergone any substantial change. It must be emphasised that pathogenic microflora was found in none of the samples collected throughout the bathing season (1 June – 1 September).

An example of food and water control

The best situation from both organizational and operational points of view is found in the city of Daugavpils. The city Centre for Public Health is responsible for monitoring and analysis operations in the field of public health. The Laboratory for Hygienic Research provides a wide spectrum of water and food quality analyses and implements an ever growing programme for environmental risk assessment (Table 3). Food safety monitoring includes samples tests for radiological safety, heavy metal contamination, and the presence of pesticides.

Table 3 *Analytical research programme in Daugavpils (number of samples analysed by the Hygienic Research Laboratory)*

	1997	1998	1999
Drinking water samples	6,751	8,517	10,662
Food safety samples	2,882	3,039	2,849
Water quality analyses	6,523	8,258	9,247
Physical conditions	1,015	2,577	2,180
Air in the working zone	1,756	1,704	5,104
Food samples	2,147	2,165	723
Radiological analysis	507	615	711
Heavy metals	228	259	1,415
Pesticides	409	757	1,202
Total	20,318	27,891	41,093

As an example, Table 4 presents data on nitrate contamination of potatoes in Latvia. Compared to the results from Belgium (2), the highest acceptable concentrations in Latvia are taken as normal (they vary from 140 to 166 mg/kg). The nitrates content in potatoes in Latvia changes from year to year (44.8–74.4mg/kg). Drinking water samples are taken for routine analysis and are tested for heavy metals.

The reorganisation of national administration and the partial delegation of food safety analysis to the State Veterinary Service explain the drop in that activity. The monitoring is mostly financed through the national budget. Only a minor part is covered by private persons.

Table 4 *Average nitrate contamination in potatoes, mg/kg (data by Daugavpils Hygienic Laboratory)*

1995	1996	1997	1998	1999
54.3	44.8	47.4	74.4	52.0

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Sažetak

PRAĆENJE STANJA ZDRAVLJA LJUDI U LATVIJI

Članak analizira podatke o javnom zdravstvu u Latviji. Ovo je prva objava takovih rezultata od ponovnoga uspostavljanja Druge Republike. Članak predstavlja najnoviji sustav praćenja sigurnosti hrane i vode i epidemiološke podatke o Latviji. Osim toga, opisana je i uspješna primjena monitorinškog programa za grad Daugavpils.

Ključne riječi:
epidemiološki rezultati, kontrola hrane, kontrola voda, monitoring

Requests for reprints:

Artūrs Škute, Ph. D.
1 Parades Street, 120, Daugavpils, LV 5401, Latvia
E-mail: *artur@dpu.lv*

ARHIV
ZA HIGIJENU RADA I TOKSIKOLOGIJU

ARCHIVES
OF INDUSTRIAL HYGIENE AND TOXICOLOGY

2

ISSN 0004-1254 UDC 613-6 CODEN: AHRTAN

VOLUME 52
2001

Arh Hig Rada Toksikol
Vol. 52. No.2.pp. 163-290
Zagreb, June 2001

INSTITUTE FOR MEDICAL RESEARCH
AND OCCUPATIONAL HEALTH
ZAGREB, CROATIA

ISSN 0004–1254

ARHIV ZA HIGIJENU RADA I TOKSIKOLOGIJU ◇ ARCHIVES OF INDUSTRIAL HYGIENE AND TOXICOLOGY

ARH HIG RADA TOKSIKOL ▫ VOL 52 NO 2 PP. 163–290 ▫ ZAGREB, CROATIA 2001

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Arh Hig Rada Toksikol 2001;52:163–167

COUNTRY REPORT

CONTAMINATION OF FOOD AND AGRO PRODUCTS IN HUNGARY

JUDIT SOHÁR AND JÁNOS DOMOKI

Fodor József National Center for Public Health, National Institute of Food Hygiene and Nutrition, Budapest, Hungary

Received November 2000

This report gives a short survey of the situation concerning chemical contamination of foods in Hungary. It starts with a legal background and lists the institutions partaking in control and monitoring of food contamination. The report gives the most important food contaminants analysed in Hungary, and in some cases provides information about the actual levels. It concludes by stressing the importance and the need to unify the national monitoring system.

Key words:
heavy metals, mycotoxins, PAH, PCB, pesticide residues, polychlorinated compounds, residues

The aim of this paper is to give a short review of chemical food contamination, trends, and levels found, and the handling of this problem in Hungary.

REVIEW OF FOOD CONTAMINATION FINDINGS IN THE COUNTRY

The data on food contamination reviewed herein have mainly been collected from unpublished sources by institutions involved in food investigation. The sampling procedures and analytical methods used by these institutions are Hungarian standard methods, mainly based on the standards of the European Committee for Standardization, or in a few cases, other validated methods.

There are several sources of chemical contamination of foods and agro products (Figure 1). All these sources can result in contamination with remarkable risk to human health and they arouse questions of chemical safety of foods. The undesirable effect of some of them can be eliminated or decreased by applying systems like Hazard Analysis Critical Control Points (HACCP) or Good Manufacturing Practice (GMP), but not of all.

Presented at the ICS-UNIDO Workshop on Contamination of Food and Agroproducts, 28 September – 1 October 2000, Varaždin, Croatia

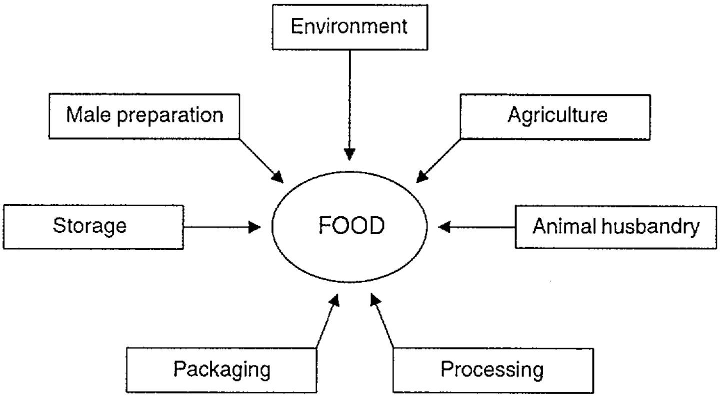

Figure 1 *Sources of chemical contamination of foods and agro products*

The basis of chemical safety of foods should be an up-to-date system of regulations which would bring enough safety for the consumers and could be fully observed by food producers. The regulations should include an appropriate food law, fair authorisation processes, Maximum Limits (ML), and Maximum Residue Limits (MRL), and other limitations and restrictions. The Hungarian regulations related to food safety have been adjusted to the international requirements for decades (Codex Alimentarius, West European food laws), as the country's food export was always remarkable and had to meet the requirements of the West European countries and the USA. Therefore the legal harmonization with the EU, which is a prerequisite of joining it, is proceeding smoothly, and the greater portion of it has already been completed.

In Hungary it is the right of the Minister of Health to establish maximum limits for food contaminants. There are three orders that cover the issue. Order No. 17/1999. (IV. 16.) of the Minister of Health (1) regulates the MLs of several chemical contaminants in foods, while order No. 2/1999. (II. 5.) (2) does it to the MLs of the veterinarian drug residues. Both integrate the relevant EU regulations and extend the list of MLs to more contaminants and more food items. The scope of the two orders is rather broad. It contains limitations for the following type of contaminants:

- residues of pesticides presently allowed and previously banned in foods of animal origin and residues of fumigants in fumigated foods;
- contaminants of technological origin (metals from utensils, benzo(a)pyren, polar compounds in frying oils, residues of cleaning/disinfecting agents;
- contaminants of environmental origin (PCBs, metals from all sources, some banned pesticides);
- contaminants of biological origin (mycotoxins, histamine);
- harmful substances of natural origin (HCN, methanol, morphine, narcotin, tebain, codein, nitrate, nitrite).

The third order which regulates the maximum residue limits of pesticides in foods of plant origin is under revision and subject to harmonization with relevant EU regulations.

Hungary has adopted all EU regulations published until now which are related to materials and articles coming into contact with food. Issues which have not yet been regulated by the EU are handled according to German regulations.

Rules are as good as they are observed, which means that they need control. In Hungary, institutes of three ministries are involved in food control. These are the National Institute of Food Hygiene and Nutrition, institutes of the National Service of Public Health and Medical Officers (Ministry of Health), National Food Investigation Institute, Veterinarian and Food Control Stations, Plant Health and Soil Protection Stations (Ministry of Agriculture), and Inspectorate of Consumer Protection (Ministry of Industry and Commerce).

The distribution of contaminants in approximately 30,000 food samples tested by these institutes every year is: metals 37%, pesticides 23%, food additives 20%, mycotoxins 12%, other toxins of natural origin 2.5%, veterinarian drug residues 1.5%, PCBs 1.5%, and PAHs 0.5%.

These investigations are mainly control tests for legal limits; they do not give the exact levels of the contaminants, but the ratio of nonconforming v. conforming samples. The general participation of nonconforming samples is below 4%.

Only a smaller portion of investigations give information about the actual levels of contaminants. These findings have been used as a basis for the general overview of the situation and tendencies in chemical food contamination in Hungary.

OVERVIEW OF CHEMICAL FOOD CONTAMINATION

The lead content in foodstuffs has continuously been decreasing over the past ten years and has substantially reached the European values. According to the 1997 data the calculated average daily intake of lead from food was 131 μg, that is 52% of the Tolerable Daily Intake (TDI) (with the assumed average body mass of 70 kg) established by the Joint FAO/WHO Expert Committee on Food Additives and Contaminants (JECFA).

In the case of cadmium the situation is less advantageous; the measured concentrations are stagnating or are slightly increasing.

The mercury content found in the domestic food is rather low, while in fish it was 35 μg/kg.

The analysis of metals also includes As, Cu, Zn, Ni, and Al. The average levels are well under the MLs. Exceptions are spirits with a higher copper content which were distilled in small distilleries using old copper apparatus.

The concentrations of PAHs or benzo(a)pyrene alone are mainly controlled in smoked foodstuffs. Concerning environmental contamination of vegetables and cereals only a small number of investigations are done annually.

Thirty years after the ban of chlorinated pesticides in Hungary, their residues are being detected only in trace levels in domestic foods. However, they were quite often found in imported foodstuffs, which led to import ban in several cases.

PCBs and/or PCB congeners are investigated in food of animal origin and human milk. The results of a 1997 survey on the PCB content in human milk (Table 1) suggest that PCB contamination in Hungary is not heavy (3).

Table 1 *PCBs in human milk (1997)(μg/kg, calculated for fat content)*

	PCB-138	PCB-153	PCB-180
Area with average pollution (41 samples)			
Range	<0.1 – 53.6	<0.1 – 115.54	<0.1 – 23.70
Median	2.71	38.61	2.86
90%	37.18	84.33	15.55
Area with higher pollution (29 samples)			
Range	10.33 – 35.65	30.14 – 110.0	6.97 – 24.64
Median	17.47	53.53	11.38
90%	24.72	91.92	17.22

The control of natural toxic compounds includes methanol in spirits, cyanide, solanin, histamine, NO_2, NO_3, and – perhaps uniquely – morphine content in poppy seed. In few cases did the control establish excessive histamine content in fish and cheese samples.

Pesticide residues were detected in less than half of the tested samples. The annual average percent of samples exceeding the MRL is 2–3%. An exception are early spring vegetables (lettuce, paprika, tomato, radish, and cucumber) grown in greenhouses with 6–8% of non-comforming samples. It is remarkable that generally the reason of the increase is not the excessive use, but the use of a forbidden agent.

Aflatoxins and ochratoxin A are given the greatest attention amongst mycotoxins. The control involves great quantities of samples of imported and domestic foods. The ratio of nonconforming and conforming samples is 1-2%, but the detectable amount (1–2 μg/kg) of the contaminants was found in most samples of coffee, cocoa, beans, muesli, flour, rice, bran, and red wine).

Generally, zearalenon (F-2 toxin) and T-2 toxin were not detected in the investigated foods. They were observed around the detection limit in 4–8% of the samples.

CONCLUSIONS

Hungarian regulations concerning food contaminants practically conform to those of the European Union. The difference is that the former are even more detailed, specifying maximum limits for more contaminants and more food items. Most laboratories authorised for food control have been accredited, whereas the rest are in the process of obtaining accreditation.

We believe that the hot spot, the burning issue related to food contaminants are not the levels found or the lack of the controlling capacities, but the absence of a unified national monitoring system in Hungary. The technical and intellectual potential of the workforce is rembarkable, but its effectiveness should be improved. The hazard identification, risk assessment, and risk management systems should be built to the benefit of all involved, but first and foremost of the consumers. The most urgent task of the Hungarian administration is to organise a unified and coordinated leadership of what is now several partial monitoring systems run by different ministries.

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Sažetak

ONEČIŠĆENJE HRANE I POLJOPRIVREDNIH PROIZVODA U MAĐARSKOJ

Autori u ovome kratkom pregledu opisuju kemijsko onečišćenje hrane u Mađarskoj. Nakon prikaza zakonske regulative autori daju popis ustanova koje u Mađarskoj nadziru i prate onečišćenje hrane. Članak navodi najvažnija onečišćivala hrane u toj zemlji te stvarne razine nekih od njih. Autori na kraju naglašavaju da je potrebno sjediniti sustav praćenja na državnoj razini.

Ključne riječi:
mikotoksini, organoklorovi spojevi, ostaci pesticida, PAU, PCB, teški metali

Requests for reprints:

János Domoki, Ph. D.
Fodor József National Center for Public Health
National Institute of Food Hygiene and Nutrition
Gyali út 3/a, H-1097 Budapest, Hungary
E-mail: *h5650dom@ella.hu*

Arh Hig Rada Toksikol 2001;52:169–175

COUNTRY REPORT

FOOD CONTAMINATION MONITORING IN CROATIA

KRUNOSLAV CAPAK, MARIJAN KATALENIĆ, AND ANDREJA BARIŠIN

Croatian National Institute of Public Health, Zagreb, Croatia

Received December 2000

The Croatian National Institute of Public Health implements the statistical food safety monitoring programme for foods marketed in Croatia in accordance with effective laws and regulations. Laboratories for food safety control, certified by the Ministry of Health, report their findings in quarterly notifications, using the standard forms and issue statements of compliance or non-compliance with current regulations, specifying the cause in case of the latter. This paper brings the results for the period 1993–99 as an illustration of the monitoring programme.

Key words:
laboratory analysis, legislation, monitoring, food safety

Microbiological and chemical food contamination as the common cause of consumer infections and poisonings is currently one of the world's leading public health problems. Due to changes in human lifestyle, dietary habits, and the globalisation of food supply it has become an increasing problem in the developed Western countries. One of the country's top priorities is to secure a sufficient food supply and prevent human health hazards related to food through a program of monitoring and control measures. To be effective, the Croatian sanitary surveillance system requires an efficient food production and a proper food control legislation, a well-organised, efficient and modern inspectorate, and a well-equipped laboratory service.

FOOD CONTROL METHODS

Food production and of the marketing system are regulated by the Infectious Diseases Act (1), Food and Object of Common Use Safety and Sanitary Surveillance Act (2), and a number of bylaws.

Presented at the ICS-UNIDO Workshop on Contamination of Food and Agroproducts, 28 September – 1 October 2000, Varaždin, Croatia

The Croatian Ministry of Health has appointed an interministerial commission to prepare new food legislation (Food Safety bill) with the main aim to harmonise the Croatian legislation with the EU's and to resolve overlapping responsibilities in the control and monitoring system between ministries. Medical surveillance of food handlers, laboratory control of raw food materials and final food products prior to marketing, and the inspection of manufacturing, marketing conditions, and of food safety at all stages follow the above regulations.

Today there are 23 licensed laboratories all over Croatia. Seven laboratories are licensed for special assays (these encompass special microbiological assays for the identification of species, some special physicochemical analyses, such as pesticide, metal, metalloid, biogenic amine, additive, vitamin, mineral and mycotoxin determinations; special estimations of bioresidue levels in animal samples, etc.) and the other fourteen for basic food analyses. The basic analysis covers sensory tests, basic microbiological assays, purity appraisals of food and of objects of common use, basic physicochemical analyses, freshwater quality assays according to the purpose of the water, physicochemical and microbiological water analyses, and so on (3). In their reports to the Croatian National Institute of Public Health (CNIPH), all certified laboratories have to specify the number and the type of analyses.

Subject to the above regulations, food samples are examined for a whole range of health safety, sensory, microbiological, and chemical parameters. Under the 1993 Health Act (4), CNIPH in Zagreb coordinates the activity of all certified laboratories with regional institutions. Furthermore, major, better equipped, and more experienced food control and environmental protection laboratories are located in Zagreb (Zagreb Public Health Institute) and in Osijek, Rijeka, Split, and Pula (regional public health institutes).

The surveillance of food production and distribution is carried out by the Sanitary Inspectorate which consists of the County Sanitary Inspection Service, Border Sanitary Inspection, and State Sanitary Inspection. While most food control laboratories are located in public health institutes, some are in other certified institutions such as at the Faculty of Nutrition and Biotechnology, Faculty of Veterinary Medicine, and the Veterinary Institute. The safety of foods of animal origin is also monitored by the Veterinary Inspectorate and enforced by the Market Inspectorate through the national food legislation.

In the inspection system, five food samples per 1,000 inhabitants are assayed under the »Minimum Annual Food and Object of Common Use Sampling Schedule« prepared annually in accordance with legal stipulations. As local county budgets often fail to provide sufficient money for the implementation of the plan, the means necessary for the effective sanitary food monitoring should be secured through the national budget. Certified laboratories are required to submit food-test reports showing the number of analysed food samples and their findings.

INFECTIOUS DISEASES, MICROBIOLOGICAL AND CHEMICAL CONTAMINATION

In 1999, the CNIPH Epidemiological Service (5), acting within the surveillance system for infectious diseases, was notified of 8,244 cases of food poisoning (bacterial infections or intoxications with bacterial toxins through food) from across the country. The corresponding 1997 and 1988 figures were 8,241 and 8,320, respectively. In the past few years, notified outbreaks varied between 47 and 66 (average 56) a year, affecting 581 to 1,492 people in each outbreak (average 1,036) (Table 1). A 5-year case-trend analysis for the period 1973–97 showed these diseases to be on the continuous rise (Table 2, Figure 1).

Table 1 *Foodborne disease outbreaks and cases, Croatia 1993–99 (4–11)*

Year	No. of outbreaks	Cases per outbreak	Single cases	Total number of cases	Cases of salmonellosis
1993	56	2,028	8,954	10,982	7,087
1994	67	1,811	7,237	9,048	4,931
1995	47	1,433	6,294	7,727	3,642
1996	47	928	5,553	6,481	2,899
1997	56	581	7,660	8,241	4,204
1998	65	1,492	6,828	8,320	4,288
1999	66	1,223	7,021	8,244	4,120

Table 2 *Cases of food intoxication in 5-year periods (3, 9)*

Period	Cases
1973–1977	17,035
1978–1982	20,844
1983–1987	39,959
1988–1992	41,111
1993–1997	42,479

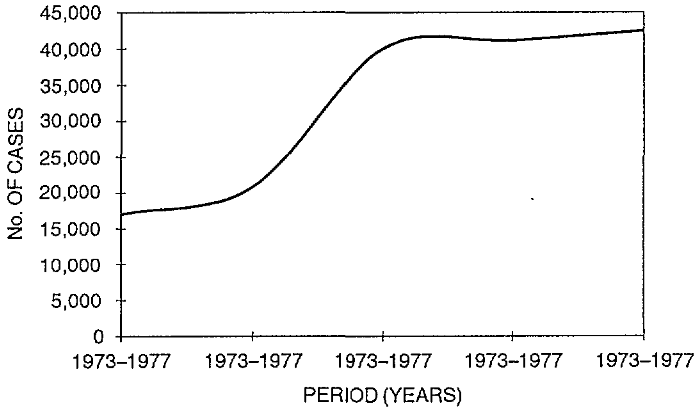

Figure 1 *Trends in the number of food intoxication cases in 5-year periods (3, 9)*

Microbiological food contamination is a frequent cause of epidemics. While salmonella and staphylococcal toxin were the most common bacterial causes, *Trichinella spiralis* took the lead among parasites. Conditions leading to these epidemics differed from one causative agent to another. The absence of veterinary monitoring of swine slaughters in households or small manufacturing facilities accounted for the outbreaks of trichinosis. Thermally underprocessed and originally contaminated foodstuffs were usually associated with salmonella outbreaks. Particularly noted was the outbreak of *Campylobacter jejuni* in a Zadar kindergarten in 1998 which affected 388 persons (Table 3).

According to the CNIPH Health Ecology Service (data from all certified laboratories), of 35,092–46,823 samples analysed annually for microbiological parameters

Table 3 *Foodborne disease-associated cases in outbreaks by causative agent, Croatia 1993–99 (4–11)*

Causative agent	Year						
	1993	1994	1995	1996	1997	1998	1999
	No. of cases						
Salmonella	1,374	1,240	900	403	378	723	614
Trichinella spiralis	25	63	115	156	49	298	291
Staphylococcus aureus	73	14	7	0	84	0	30
Shigella	192	7	0	0	0	4	4
Clostridium perfringens	0	0	50	65	0	16	57
Clostridium botulinum	0	3	0	5	3	9	12
Bacillus cereus	0	10	0	0	0	0	0
Mycetismus	35	0	0	0	6	0	0
Campylobacter jejuni	0	0	14	0	0	388	0
Escherichia coli	0	0	0	19	15	0	0
HAV	0	0	0	0	0	0	7
Histamine	0	17	0	0	0	0	0
Total known	1,699	1,354	1,086	648	535	1,438	1,015
Unknown	329	457	347	280	46	54	208
Total	2,028	1,811	1,433	928	581	1,492	1,223

Table 4 *Food samples found to be unsafe by microbiological assay (6–11)*

Year	Domestic			Imported			Total		
	Samples analysed	Unsafe		Samples analysed	Unsafe		Samples analysed	Unsafe	
		No.	%		No.	%		No.	%
1994	29,199	2,583	8.80	5,893	151	2.60	35,092	2,734	7.80
1995	32,902	3,520	10.70	7,509	174	2.30	40,411	3,694	9.10
1996	34,556	3,944	11.40	8,889	233	2.60	43,455	4,177	9.60
1997	33,624	3,113	9.26	12,402	314	2.53	46,026	3,427	7.44
1998	34,389	3,669	10.67	11,136	296	2.66	45,525	3,965	8.71
1999	35,405	2,830	7.99	11,417	186	1.63	46,823	3,260	6.96

2,734–4,177, or 6.96–9.60% are found unsafe (Table 4). As for chemical parameters, of 22,037–28,588 analysed food samples, 1,013–2,223 or 3.90–10.0% are found unsafe (Table 5).

Table 5 *Food samples found to be unsafe by chemical assay (6–11)*

Year	Domestic			Imported			Total		
	Samples analysed	Unsafe		Samples analysed	Unsafe		Samples analysed	Unsafe	
		No.	%		No.	%		No.	%
1994	9,320	874	9.40	12,717	697	5.34	22,037	1,553	10
1995	16,485	1,503	9.10	12,968	720	5.60	29,453	2,223	7.00
1996	12,958	827	6.38	13,048	603	4.62	26,006	1,430	5.50
1997	12,382	755	6.10	16,206	645	4.00	28,588	1,400	4.90
1998	13,763	651	4.73	14,667	525	3.58	28,430	1,176	4.14
1999	11,636	512	4.40	14,351	501	3.49	25,987	1,013	3.90

Increased counts of microorganisms and enterobacteria, coagulase-positive staphylococci, E. coli, and salmonella were the leading reasons for the unfitness of food for human consumption. As regards chemical parameters, the leading nonconformities were the inappropriate food composition, sensory properties, and the use of additives not allowed for particular types of food. There were some individual cases of excessive food additive levels.

PESTICIDES AND HEAVY METALS

A comparison of results (Table 6) from the 1989 food pesticide monitoring with the 1999 figures shows a significant decrease in the number of samples with excessive pesticide residue levels, confirming thus that proper practice was followed in the treatment of crops and the use of land.

Table 6 *Pesticide residues in food (3, 11)*

	Year	HCB	HCH	Lindane	DDT and metabolites	Organophosphorous
% with residues:	1989	27.8	32.7	71.0	76.7	33.0
	1999	19.5	51.7	68.5	83.6	13.8
N above MRL*:	1989	10	5	52	79	5
	1999	0	0	0	1	0

* MRL - Maximum residue limit
Number of analysed samples: 1989=2922; 1999=474

The fact that increased pesticide, heavy metal, cadmium, lead, and arsenic levels (Table 7) in food, which may indicate greater contamination of agricultural soil, were extremely rare suggests that such soils have been preserved from these types of contamination.

Table 7 *The number of food samples assayed for mercury, arsenic, lead, cadmium, and the number and percentage of samples exceeding the maximum allowances (7–9)*

	Mercury			Arsenic			Lead			Cadmium		
Year	Total assays	Unsafe		Total assays	Unsafe		Total assays	Unsafe		Total assays	Unsafe	
		No.	%		No.	%		No.	%		No.	%
1995	4,795	5	0.1	6,995	2	0.03	7,445	8	0.11	5,684	9	0.16
1996	4,777	11	0.23	6,513	0	0	7,288	7	0.1	5,110	2	0.04
1997	5,415	3	0.06	7,459	18	0.24	8,189	12	0.15	6,171	3	0.03

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Sažetak

PRAĆENJE ISPRAVNOSTI NAMIRNICA U HRVATSKOJ

U skladu sa zakonom Hrvatski zavod za javno zdravstvo organizira i provodi statistički program praćenja zdravstvene ispravnosti namirnica koje su u prometu u Hrvatskoj. Laboratoriji koje je Ministarstvo zdravstva Republike Hrvatske ovlastilo za kontrolu ispravnosti namirnica izvješćuju na propisanim obrascima o analizama namirnica prema broju i vrsti. Osim toga, oni naznačuju odgovara li namirnica vrijedećim domaćim propisima ili ne, a u slučaju ustanovljenih prekršaja zakona navode uzrok prekršaja. Ovaj članak ilustrira neke rezultate praćenja u razdoblju 1993.-99. U tom periodu, od ukupno pregledanih uzoraka namirnica zdravstveno neispravno bilo je 6,96–9,60% zbog mikrobioloških parametara, a 3,90–10,0% zbog kemijskih parametara.

Kao najčešći uzroci neprikladnosti namirnica za ljudsku uporabu zapaženi su povećani broj mikroorganizama i enterobakterija, koagulaza-pozitivnih stafilokoka, *E.coli* te prisutnost salmonela. Kod kemijskih parametara najčešći uzroci zdravstvene neispravnosti bili su neprikladni sastav hrane i organoleptička svojstva, kao i upotreba aditiva nedopuštenih u određenoj vrsti namirnice. Otkriveno je nekoliko pojedinačnih slučajeva povećane količine aditiva za namirnice.

Ključne riječi:
laboratorijska analiza, trovanje hranom, zakonski propisi, zdravstvena ispravnost

Requests for reprints:

Krunoslav Capak, M. D., Ph. D.
Hrvatski zavod za javno zdravstvo
P. O. Box 684, HR-10000 Zagreb, Croatia
E-mail: *marijan.katalenic@zg.tel.hr*

Arh Hig Rada Toksikol 2001;52:177–180

COUNTRY REPORT

CONTROL OF FOOD AND AGRO PRODUCT CONTAMINATION IN BULGARIA

NIKOLAY RIZOV

National Center of Hygiene, Medical Ecology and Nutrition, Sofia, Bulgaria

Received December 2000

The ban of organochlorine pesticides in Bulgaria (1969) has led to their significant drop below the maximum residue limits. The climate and the imperfect storage facilities benefit the production of *Fusarium* toxins desoxynivalenol (folytoxin), zearalenon (F-2), T-2, ochratoxin A and aflatoxins in grain products, M_1 in milk products, and patulin in apple juices and nectars. Heavy metals are found in smaller concentrations than in industrially developed countries with the exception of lead, which may be due to the wide use of leaded gasoline. Bulgaria has 5 »hot spots« related to sources of heavy metals. An elaborated inventory PCB, PCDD, and PCDF sources suggests that there are no toxicologically significant environmental and food pollutions with these substances. Sanitary control is enforced by several laws and subordinate regulations on the national level, which, however, have failed to coordinate it with the relevant activities of the Ministries of Health and of Agriculture and Forestry.

Key words:
heavy metals, legislation, monitoring, mycotoxins, organochlorine pesticides, PCB, PCDD, PCDF, sanitary control

Bulgaria with its 8-million population and an area of 111,000 sq km is mainly an agricultural country with well developed food industry. Until 1989, the land was state-owned and integrated in cooperative and state farms. This kind of organisation of agricultural production and the economic system as a whole were the reasons for the frequent use of pesticides above the recommended doses, which resulted in exceeding the maximum residue limits (MRL).

Presented at the ICS-UNIDO Workshop on Contamination of Food and Agroproducts, 28 September – 1 October 2000, Varaždin, Croatia

Planned monitoring is performed by the Central Veterinary Medical Laboratory for foods of animal origin and by the Central Laboratory for Plant Protection – for plant products. The plans of both bodies conform with the EU requirements. The National Center of Hygiene, Medical Ecology and Nutrition conducts topical surveys such as dietary intake of heavy metals and nitrates in children from full-board kindergartens and schools.

Since 1969, the application of organochlorine pesticides in Bulgaria has been banned with the exception of lindane (γ-hexachlorocyclohexane) for technical cultures and domestic use and of heptachlor for disinsection of seed material. Some years ago their application was also practically suspended. The levels of organochlorine pesticides in foods are very low, significantly below MRL. This type of analyses is very rarely performed for the purposes of the State Sanitary Control (SSC), mainly for some products imported from countries bearing such risk. In single cases, in the export of cheese it is necessary to determine hexachlorocyclohexane isomers and even these analyses show concentrations well below MRL.

At present the entire agricultural production is private and is not financed by the state. The use of pesticides (organophosphorous, pyrethroids, herbicides, and fungicides) has decreased dramatically due to their high price and the progressive application of good agricultural practice. The State Sanitary Control focuses mainly on certain organophosphorous pesticides, herbicides based on 2,4-D, and some fungicides. Concentrations exceeding MRL are rarely found. Grain cultures, such as wheat, corn, barley, and sunflower constitute a significant part of agricultural production. The climate and the imperfect storage facilities benefit the production of *Fusarium* toxins desoxynivalenol (folytoxin), zearalenon (F-2), T-2 as well as ochratoxin A and aflatoxins in grain products, M_1 in milk products, and patulin in apple juices and nectars. The percentage of positive samples is a little greater than that for pesticides. Practically, they are found in input materials rather than in foods ready for consumption.

Heavy metals are found in smaller concentrations than in industrially developed countries. Lead, of course, is an exception, due to the wide use of leaded gasoline. In fact Bulgaria has 5 »hot spots« connected with sources of heavy metals: the metallurgy plant »Kremikovtsi« in Sofia (Pb, Mn, Cd), a smelter plant in Plovdiv (Pb, Cd) and in Kardzhali, and two copper plants: »Eliseina« (Cu, As) and »Srednogorie« (Cu, As).

Compared to other chemical pollutants, practically the greatest number of analyses for SSC and export purposes are conducted to determine the presence of those elements, mainly lead and cadmium, and in certain cases arsenic, copper, manganese, mercury, and zinc. Few are the samples showing excessive concentrations. They concern products mainly originating from the above mentioned »hot spots«. The determined concentrations are the real findings of food contamination. It is difficult to classify them uniquely as positive or negative as the same level of contamination may be considered positive by the EU standards and negative by the FAO, Russian, or other standards and vice versa.

Safety of food and agricultural products, and in particular of their contamination with chemical pollutants is controlled by the control bodies of two ministries – the Ministry of Health (MoH) and the Ministry of Agriculture and Forestry (MAF) – with fully overlapping authorities.

The State Sanitary Control is enforced by several laws and subordinate regulations. The duties of the hygiene and epidemiology network of MoH are regulated by the

Health Act, Food Act and some 10–15 subordinate regulations. The control bodies of MAF, the State Veterinary Sanitary Control (SVSC) and the National Service for Plant Protection, Quarantine, and Agrochemistry, work according to the Veterinary Medical Practice Act and Plant Protection Act and their subordinate regulations. The laws and the relevant SSC activities of MoH and MAF are not coordinated. Each of the 28 large cities (regions) in Bulgaria has its Hygiene & Epidemiology Inspectorate (HEI) and bodies of the State Veterinary Sanitary Control and plant protection services.

Each body comprises an inspectorate and a laboratory unit. As a rule, the laboratories are poorly equipped, particularly for determination of major chemical pollutants in food. All pollutants are determined at the National Center of Hygiene, Medical Ecology and Nutrition, the Central Veterinary Medical Laboratory, the Central Laboratory for Plant Protection, HEI – Sofia, HEI – Plovdiv, and HEI – Varna. Other bodies are also engaged in determining certain pesticides and mycotoxins, mainly using the TLC. Besides the SSC bodies, there are several well-equipped and accredited laboratories mainly for determination of heavy metal concentrations in foods.

Beside determination of chemical pollutants through the current sanitary control, until 1985 Bulgaria was almost fully monitoring pesticides, heavy metals, mycotoxins, and nitrates. That huge amount of analytical results was not entirely adequate, as some were obtained through invalidated methods which were often guided by secretive and illogical socialist government policies at the time. The number of samples analysed for the presence of chemical pollutants is now much smaller. These analyses are performed for the needs of the SSC and for export, if required by the importing country. Planned monitoring is performed by the Central Veterinary Medical Laboratory for foods of animal origin and by the Central Laboratory for Plant Protection – for plant products. The plans of both bodies conform with the EU requirements. The National Center of Hygiene, Medical Ecology and Nutrition conducts topical surveys such as dietary intake of heavy metals and nitrates in children from full-board kindergartens and schools.

The elaborated inventory of sources of PCBs, PCDDs, and PCDFs suggests that there are no toxicologically significant environmental and food pollutions with these substances.

Nevertheless, we have joined the WHO Programme for the determination of those compounds in human milk, and a team from the National Center of Hygiene, Medical Ecology and Nutrition is developing methods for determination of PCBs and PAHs in environmental samples and foods.

Sažetak

KONTROLA ONEČIŠĆENJA HRANE I POLJOPRIVREDNIH PROIZVODA U BUGARSKOJ

Zabrana uporabe organoklorovih pesticida u Bugarskoj 1969. godine dovela je do njihova značajnog smanjenja ispod maksimalno dopuštene razine. Klima i neprimjereni uvjeti skladištenja pogoduju nastanku toksina dezoksinivalenola (folitoksin), zearalenona (F-2), T-2 te okratoksina A i aflatoksina u žitarica, M_1 u mlijeku te patulina u jabučnim sokovima i nektarima.

Teški metali prisutni su u koncentracijama manjim nego u razvijenim zemljama, što ne vrijedi za olovo čiju široku rasprostranjenost možemo zahvaliti uporabi olovnog benzina. U Bugarskoj postoji pet onečišćenih područja koja su izvori onečišćenja teškim metalima. Praćenje onečišćenja osmišljavaju i provode Središnji veterinarski laboratorij (za hranu životinjskog podrijetla) te Središnji laboratorij za zaštitu bilja (za proizvode biljnoga podrijetla). Planovi praćenja koje izrađuju obje ustanove u skladu su sa zahtjevima EU. Državni centar za higijenu, zdravstvenu ekologiju i prehranu provodi istraživanja unosa teških metala i nitrata putem hrane u djece u vrtićima i školama. Detaljan popis izvora, PCB-a, PCDD-a i PCDF-a upućuje na to da u Bugarskoj nema toksikološki značajnoga onečišćenja okoliša ovim tvarima. Državna sanitarna kontrola regulirana je putem više zakona i propisa koji međutim nisu usklađeni sa sanitarnom kontrolom koju provode ministarstva zdravstva te poljoprivrede i šumarstva.

Ključne riječi:
organoklorovi pesticidi, teški metali, mikotoksini, monitoring, legislativa, PCB, PCDD, PCDF, državna sanitarna kontrola

Requests for reprints:

Nikolay Rizov, Ph. D.
National Center of Hygiene, Medical Ecology and Nutrition
15 D. Nestorov Str., 1431 Sofia, Bulgaria
E-mail: *N.Rizov@NCH.Aster.Net*

Arh Hig Rada Toksikol 2001;52:181–215

CONFERENCE PAPER

PERSISTENT, BIOACCUMULATIVE, AND TOXIC COMPOUNDS IN THE CENTRAL AND EASTERN EUROPEAN COUNTRIES – THE-STATE-OF-THE-ART REPORT – HUMAN EXPOSURE

IVAN HOLOUBEK, ANTON KOČAN, IRENA HOLOUBKOVÁ, KLARA HILSCHEROVÁ, JIŘI KOHOUTEK, JERZY FALANDYSZ, AND OTT ROOTS

Recetox-Tocoen & Associates, Brno, Czech Republic

Received November 2000

This review describes problems with persistent and bioaccumulative organic substances which posses toxic characteristics likely to cause adverse human health or environmental effects in countries of Central and Eastern Europe as far as human exposure is concerned. This paper is a part of a more detailed report on the subject.

Key words:
exposure, persistent organic pollutants

Organic substances that are persistent, bioaccumulative, and posses toxic characteristics likely to cause adverse human health or environmental effects are called PBTs (persistent, bioaccumulative, and toxic substances). In this context, »substance« means a single chemical species, or a number of chemical species, which form a specific group by virtue of (a) having similar properties and being emitted together into the environment or (b) forming a mixture normally marketed as a single product. Depending on their mobility in the environment, PBTs could be of local, regional or global concern (1).

The region of the Central and Eastern Europe is in this Report located in the area from the Baltic Sea to the south of Adriatic Sea and from the Czech Republic to Baltic

Presented at the ICS-UNIDO Workshop on Contamination of Food and Agroproducts, 28 September – 1 October 2000, Varaždin, Croatia

countries. All other countries from former Soviet Union are not included in the report for the lack of information.

In general, there is little data on levels of PBT compounds in many countries of the Central and Eastern Europe (CEE). The situation is better with data on the industrial and pesticide chemicals from Croatia, Czech Republic, Poland, and Slovakia. Other countries such as Bulgaria, Hungary, and Slovenia produce satisfactory information about pesticide contamination. The rest of the CEE countries is able to produce only limited data on PBT sources and levels (2).

Elevated levels of organochlorines compared to other European countries indicated high exposure of young women in the former Czechoslovakia. The first survey in the field of PBT compounds carried out in the former Czechoslovakia in the early 60s was initiated by concerns over serious contamination of humans caused by the widespread use of DDT. Besides the measurement of DDTs in the human adipose tissue, later studies also paid attention to hexachlorobenzene (HCB), hexachlorocyclohexanes isomers (HCHs), and polychlorinated biphenyls (PCBs) in the 80s. A gradual decrease in concentrations of DDT, HCHs, and HCB in human adipose tissue was observed upon their ban.

However, considerable differences in residue levels determined in the human fat from different part of the Czech Republic have been frequently found. Moreover, in some regions, the otherwise general decrease is not so pronounced due to serious contamination of the locally produced and distributed foodstuffs. A very similar situation has been found in other CEE countries.

This region has very specific problems related to environmental pollution, which are the results of the recent wars. The destruction of industrial facilities and spilling of chemicals have had the worst effect for the environment in Bosnia and Herzegovina, Croatia, Serbia, and Montenegro. A potential result of food, water, and environment pollution is the dramatic increase in the digestive system carcinoma, particularly of the large intestine which has been observed in Bosnia and Herzegovina for last two years. Below, we will describe levels of various PBTs in human samples from CEE countries.

HUMAN EXPOSURE TO CHLORINATED PESTICIDES AND POLYCHLORINATED BIPHENYLS

Former Czechoslovakia, Czech Republic

Serious contamination of food chains by PCBs occurred in the past in the Czech and Slovak Republics. The main source of local dietary exposure were meat, milk, dairy products, and eggs produced by farms, silos, sheds, and other agricultural facilities using paint with the PCB content. The composition of a commercial technical mixture Delor 106 that was contained in these paints corresponds to Aroclor 1254 (3).

The monitoring programme in the former Czechoslovakia established in mid 80s revealed remarkably high levels of PCBs in human milk. However, the content of contaminants used to be expressed at that time as »total PCBs«. It should be noted that the comparability of generated data was rather poor, reflecting different »quantification strategies« applied by individual laboratories. The implementation of the congener-specific method at the beginning of the 90s made it possible to get more information from various programmes about the PCB patterns (4).

Kočan and co-workers published a comparison between levels of PCBs and HCB found in human adipose tissue and blood samples from the former Czechoslovakia and other countries (5, 6). Unfortunately, the comparison between the present analytical results and older data is questionable because of different analytical performance, such as total PCB v. congener-specific analysis, packed column v. capillary column separation, and lower v. higher precision and accuracy. The importance of PCB analysis has increased after reports of dioxin-like toxicity of planar PCB congeners.

In many papers published in the last 15 years levels of lipophilic analytes in human blood samples are reported on a whole blood or a blood serum basis, but not on a lipid basis. This may be due, in part, to problems encountered in the quantitative isolation of blood serum lipids. However, many published procedures for the analysis of halogenated aromatics giving the results on a blood basis are actually also based on lipid isolation, but the isolated lipids are not weighed. Unfortunately, this approach makes the comparison between the levels of lipids from adipose tissue, blood, and milk impossible, although a high correlation has been observed between them.

PCBs and other organochlorinated compounds (OCCs) are most often monitored in human fat and human adipose tissue, but human serum or other tissues (placenta, liver, and heart) can also be analysed (7). The results concerning human biomonitoring of PCBs in the former Czechoslovakia and in the Czech and Slovak Republics published over the last two decades were summarised and reviewed by *Černá and Bencko* (7).

PCBs levels in human milk have been analysed extensively. The first data concerning the analysis of PCBs in body fluids and tissues of Czech population appeared in literature in 1985. The mean concentration of sum PCBs (related to the standard commercial mixture Delor 106) in 63 human milk specimens collected in Northern Bohemia was 2.83 mg/kg fat (i.e. 0.105 mg/kg milk with substantial inter-individual differences) (8).

In the 90s, such presentation of the PCB results was replaced by the analysis of individual congeners. In a WHO/EURO study of the region, the Czech and Slovak Republics showed higher levels of PCBs in districts producing technical mixtures of PCBs (district Michalovce, Eastern Slovakia) or using them intensively (district Uherské Hradiště, Eastern Czech Republic) (Table 1).

Table 1 *The mean concentration of indicator congeners 138, 153, and 180 in human milk of the Czech and Slovak populations (μg/kg fat); 1 sample pooled from 11 (Czech) and 10 (Slovak) individual samples (48, 49)*

Locality	Year	PCB 138	PCB 153	PCB 180	ΣPCBs[1]
Uherské Hradiště	1991–3	341.5	424.8	294.0	1,802.5[1]
Kladno		171.9	215	137.4	897.4[1]
Michalovce	1993–4	279.2	434.9	284.8	1,698[1]
Nitra		139.8	207.7	137.9	825.2[1]

[1] ΣPCBs = (PCB 138+PCB 153+PCB 180) x 1.70
1.70 = correction factor

Since 1994, the levels of indicator congeners in human milk have been systematically monitored within the System of Monitoring the Environmental Impact on Population Health of the Czech Republic. The results obtained in years 1994–1996 confirm the tendency toward a decrease (see Table 2) (9).

Table 2 *The mean and median concentrations of indicator PCB congeners 138, 153, and 180 in human milk of the Czech populations (μg/kg fat) (9)*

Year	N	PCB 138	PCB 153	PCB 180	ΣPCBs[1]
1994	282	236 (190)	398 (352)	308 (255)	1,601 (1,355)
1995	395	212 (178)	350 (323)	272 (248)	1,418 (1,273)
1996	285	184 (163)	230 (201)	150 (125)	959 (831)

[1] ΣPCBs = (PCB 138+PCB 153+PCB 180) x 1.70
1.70 = correction factor

Hajšlová and co-workers determined the concentrations of PCBs and OCCs in human milk samples from three regions in the Czech Republic (4). The results were generated by the congener-specific analyses and compared with similar studies from other European countries.

Table 3 compares the levels of indicator PCBs in human milk between the Czech Republic (Prague subset) and similar studies from Norway, UK, the Netherlands, and Germany (4).

The sum of indicator PCBs was higher in Czech samples than in those of foreign origin while the levels of lower chlorinated PCBs (congeners no. 28 and 52) and pentachlorobiphenyl no. 101 were below the quantification limit of 5 ng/g fat. This fact may be attributed to different contamination pattern of the Czech diet and, consequently, different dietary exposure. Technical mixtures with the prevailing content of hexachloro- and heptachlorobiphenyls were mostly the primary sources of environmental pollution in the former Czechoslovakia. PCB 153 was the dominant congener in all the samples.

Regarding the composition of the market basket of countries listed in Table 3, fish and fish products are now consumed in higher rates. The annual consumption statistics in Norway, UK, the Netherlands, and the former Czechoslovakia in the end 80s were 41.1 kg, 19.9 kg, 9.2 kg, and 6.8 kg of fish and fish products per capita, respectively (10). A relatively high content of PCBs was often reported in fish from the Baltic and the North Sea. As the biodegradation of PCBs in fish is very limited, the higher intake of lower chlorinated PCBs (that are typically accumulated in these biota) via this commodity may be reflected in elevated levels in human milk (4).

The comparison of PCB levels in human milk between three Czech regions – Prague standing for industrial urban agglomeration, Kladno, a small industrial city, and Uherské Hradiště, a locality characterised by high environmental burden of PCBs from paint industry and a wide use of these paints in local agricultural facilities –

Table 3 *Comparison of levels of indicator PCBs in human milk between the Czech Republic (Prague subset) and Norway, UK, the Netherlands, and Germany (ng/g in fat) (4)*

Country	**Norway[1] N=28**		**UK[2] N=32**		**The Netherlands[3] N=19**		**Germany[4] 1984/5 N=69**		**Germany[4] 1990/91 N=68**		**Czech Republic[5] 1993/4 N=17**	
Congener PCB No.:	Average (range)	Median	Average (range)	Median	Average (range)	Median	Average (range)	Median	Average (range)	Median	Average (range)	Median
28	7.8 (nd–24.2)	22.1	31.5 (nd–188)	17.0	12.1 (0.2–188.6)	5.8	0.5 (0.5–40)	5	17 (9–46)	18	nq[7]	–
52	nq[6] (nd–154)	–	26.2 (nd–32.7)	6.4	2.6 (0.5–60)	1.5	0.5 (6–44)	2	13	15	nq[7]	–
101	1.1 (nd–4.7)	nd	15.0 (nd–82)	4.9	1.5 (0.2–10.0)	1.1	15 (0.5–19)	21	14 (0.5–55)	17	nq[7]	–
118	26.2 (9.6–56.7)	23.7	28.6 (5–197)	19.4	35.5 (9.7–94.0)	32.7	na[7] (7–59)	–	na[7]	–	28.5	25
138	86.8 (74.6–185.9)	91.0	68.1 (18–261)	56.4	129.9 (43.8–314.3)	124.2	250 (60–660)	254	168 (65–669)	184	289[7] (100–558)	260
153	114.4 (49.6–259.4)	9.9	85.9 (27–275)	71.8	186.3 (59.9–475.7)	174.7	325 (70–750)	324	240 (108–968)	264	379 (142–702)	331
180	50.6 (9.7–108.3)	46.0	74.9 (1–210)	70.1	76.9 (2.5–418.8)	71.3	160 (40–400)	162	173 (75–1,023)	194	240 (91–447)	226
ΣPCBs	286.9	–	330.2	–	444.8	–	751	–	625	–	937	–

[1] (50)
[2] (51)
[3] (52)
[4] (53)
[5] (4)
[6] not quantified due to the interference with an unknown peak
[7] below the limit of quantification, <5 ng/g in fat
[8] PCB 138 was coeluted with PCB 163 (expressed as PCB 138)

clearly showed that the levels of PCBs were the highest in the latter locality (Table 4). This region is more contaminated than other regions and therefore the PCB levels in crops grown there are higher, resulting in higher body burden as evident from the human milk analysis. The levels of PCBs in Prague and Kladno were lower and did not distinctly differ from one another. As to the chlorinated pesticides, the higher content of DDT (and its metabolite DDE) found in Uherské Hradiště may be attributed to its wide agricultural use in the past. Levels of HCB, which may be formed in various combustion processes, were slightly higher in the industrial area such as Prague and Kladno. The contents of β-HCH was similar in all sets of examined samples (4).

Table 4 *Content of PCBs and OCCs in human milk – interregional comparison in the Czech Republic (ng/g in fat) (4)*

Region	ΣPCBs	β-HCH	DDTs	HCB	Number of samples
Prague	1,096	71	998	639	17
Kladno	860	79	832	570	17
Uherské Hradiště	1,529	80	1,283	482	12

ΣPCBs = sum of congeners nos.: 28, 66, 70, 74, 105, 118, 138, 153, 156, 170, and 180.
DDT = p,p'-DDE+p,p'-DDT

A newer study (11) was focused on distribution of PCBs and OCCs in various human tissue samples from selected regions of the Czech and Slovak Republics and on comparison with the results from other foreign and local studies.

The analysis included sets of samples consisting of various human tissues originating from three regions, two representing very high environmental contamination (Uherské Hradiště, Czech Republic and Michalovce, Slovakia) and one (Prague) representing industrial urban agglomeration with intensive anthropogenic activities and many potential sources of pollution. The results were used to assess PCB and OCC body burden of the Czech and Slovak population. The biotic samples were collected during post-mortem of people killed in car accidents (Michalovce and Prague) or who died of natural causes (Uherské Hradiště). The analysis included abdominal adipose tissue (from subcutaneous area), liver, kidney, heart muscle (in the case of region Uherské Hradiště only), and the adipose tissue from the mesenteric area (only in the sets from Prague and Michalovce).

These measurements (11) confirmed again that organochlorine residues in the tissues of Czech and Slovak origin were often higher (especially for PCBs and DDTs) than levels in humans from other countries. The sum of indicator PCBs in samples from Michalovce and Uherské Hradiště was substantially higher than for example in Finland, UK, or the Netherlands.

With exception of the Slovak samples, in all other studies tri- and tetrachlorobiphenyls (PCB 28 and 52) were present in trace concentrations (up to 10 μg/kg). Surprisingly high amounts of these congeners were found in the specimens collected in Michalovce. The elevated levels of lower chlorinated congeners in human tissues indicate actual exposure to PCB technical mixtures with the low chlorine content (12).

On the other hand, the high levels of PCB 28 and 74 only (the most persistent congeners among low chlorinated PCBs) suggest long-term occupational exposure to such technical mixtures (11). The occurrence of both types of exposures in the locality Michalovce are highly probable, but the data on the profession of donors were not available. The study showed that vegetables played an important part in the intake of lower chlorinated PCBs due to the ambient air contamination in the area. However, relatively high concentrations of PCB 28 found in Michalovce seem unlikely to be associated with the high consumption of vegetables.

Biological monitoring of different human tissues indicated that concentrations of PCBs in blood, adipose, and muscle tissues were about the same as when calculated on a lipid basis. In this study (11) higher PCB levels were found in tissue richer in fat such as adipose. The distribution patterns of PCBs in other analysed tissues revealed similarity to those found for the DDT group. HCH isomers predominantly accumulated in the liver and kidney, reaching concentrations several times higher (2.5–5 and 2–3 times more, respectively) than in the adipose tissue. Similarly, HCB tended to accumulate in the soft tissue.

The sample subset from the region Uherské Hradiště was analysed for 38 individual chlorobiphenyl congeners. The contribution of individual chlorobiphenyls to adipose tissue slightly differed from contribution to other tissues (liver, kidney and heart muscle) typically containing higher amounts of more polar lipids (phospholipids). The PCB pattern was almost equal in the latter matrices. The levels of relatively more polar lower chlorinated congeners were higher in these tissues than in the adipose tissue. Nevertheless, highly chlorinated PCBs, particularly those with 2,4,5-chlorines in one ring and at least one chlorine in 4 position in the second one (congeners nos. 138, 153, 170, and 180) prevailed in all analysed samples. This finding indicates long-term environmental exposure in the analysed region. The contribution of highly chlorinated PCBs to the total amount of PCBs exceeded 70%.

For general population, food is the main route of environmental exposure to polychlorinated biphenyls, dibenzo-p-dioxins, and dibenzofurans (PCBs/PCDDs/Fs) (12, 13). According to the System of Monitoring the Environmental Impact on Population Health of the Czech Republic, the intake of PCBs (expressed as the sum of 7 indicator PCB congeners) for the average Czech population in 1996 was 86 ng/kg body weight. The population exposure to 11 toxic PCB congeners in 1998 was estimated to 12.3 pg of international toxic equivalent of 2,3,7,8-tetrachlorodibenzo-p-dioxins (I-TEQ) per kg body weight. The levels of indicator PCB congeners in human milk show a declining trend (7, 13). The levels of PCDDs/PCDFs in the pooled human milk samples collected in 1998 were about 10 pg TEQ/g fat. In the recent years, the monitoring system also included the analysis of PCDDs/PCDFs in the human adipose tissue to determine the background exposure to these compounds.

In the years 1996–1999, adipose tissue samples were collected during postmortem according to a specific protocol in four districts of the Czech Republic. Two were industrial (Plzeň and Ústí nad Labem) and two rural and recreational (Beroun and Žd'ár nad Sázavou). The basic characteristics of sampled persons are summarised in Table 5.

Table 5 *Characteristics of analysed samples by sex, age, and region (13)*

Locality	Regions with lower pollution		Regions with higher pollution		Overall
	Beroun	Žd'ár nad Sázavou	Plzeň town	Ústí nad Labem	
N	12.0	14.0	12.0	23.0	61.0
Men	4.0	12.0	7.0	8.0	31.0
Women	8.0	2.0	5.0	15.0	30.0
Avg age	52.8	45.4	60.1	62.0	56.2
Range	40–62	27–60	45–74	42–84	27–84
Average age – men					51.3
Average age – women					61.0*

* P<0.01

Polychlorinated dibenzo-p-dioxin (PCDD) and polychlorinated dibenzofuran (PCDF) levels, selected PCB congeners with dioxin toxicity (77, 126, 169, 123, 14, 105, 167, 156, 157, and 189), as well as the indicator PCB congeners (28, 52, 101, 118, 138, 153, 180) were determined in the fat melted off from homogenised samples of human adipose tissue. The toxicity equivalency factors (TEFs) proposed by *Van den Berg and co-workers* (14) were used to calculate the dioxin toxic equivalency (TEQ). Table 6 shows the overall mean total TEQ values as well as their distribution between sexes and age groups. Because of the log-normal distribution, median values were used in descriptive statistics. The sum of all PCDDs was about twice as high as the sum of all PCDFs. The concentration of PCDDs/Fs/PCBs correlated with age (r=0.47, P<0.01). Differences between sexes in concentrations and TEQ values, though women showed slightly higher values, were not significant despite the fact that the mean

Table 6 *Concentrations of PCDDs/Fs/PCBs in human adipose tissue expressed as TEQ (pg/g fat) (13)*

Group	N		2,3,7,8-TCDD	PCDDs*	PCDFs	PCBs	Total
Overall study	61	Median	2.4	9.4	16.9	69.2	98.0
		Ranges	0.7–6.7	2.3–29.0	4.4–142	20.8–309	28.0–480
Men	31	Median	1.9	8.1	16.3	66.0	92.0
		Ranges	0.7–5.5	2.3–16.6	4.4–41.1	20.5–163	28.0–192
Women	30	Median	2.7	10.6	19.6	72.1	100.0
		Ranges	1.2–6.7	4.3–28.7	8.6–142	36.3–301	54.0–480
Overall, age >50	39	Median	2.6	10.4	19.1	80.4	102.0
		Ranges	0.7–6.7	3.3–29.0	6.7–142	30.0–309	40.0–480
Overall, age <50	22	Median	2.0	8.4	16.3	61.0	86.0
		Ranges	0.9–4.9	2.3–17.7	4.0–41.1	21.0–164	28.0–188

* PCDDs including 2,3,7,8 TCDD

age of women was almost 10 years greater than that of men and that the difference between the age groups was significant ($P<0.01$).

Table 7 shows the distribution of the total dioxin toxicity (TEQ) between PCDD, PCDF, and PCB groups. PCDDs (median value) contributed with about 9.4 pg TEQ/g fat, PCDFs with 16.9, and PCBs with 69.2 pg/g fat to the total TEQ value. Compared with levels in human milk, these levels were distinctly higher (13).

Table 7 *Dioxin toxic equivalents (TEQ) and the percentage of contribution to total TEQ (pg/g fat) in human adipose tissue (13)*

Group	PCDDs (%)	PCDFs (%)	PCBs (%)
Overall	9.8	17.7	69.2
Men	9.0	18.0	73.0
Women	10.4	19.2	70.5

In agreement with the results published by *Schechter and co-workers* (54), 2,3,4,7,8-PCDF was the dominant congener among the PCDD/F compounds in our group. This compound contributed to more than 80% of the dioxin toxic equivalents of PCDF congeners. OCDD as the congener found in the largest amount (its median concentration was 114 pg/g fat), participated with about 53% of the total PCDDs/Fs concentration, but its contribution to the dioxin toxicity was negligible. PCBs 156 and 126 accounted for 84% in men and 95% in women of TEQ portion derived from the PCB congeners with dioxin toxic activity.

Regional differences were observed in concentrations of individual congeners as well as in TEQ values. Samples from industrialised districts (N=35) showed slightly higher levels in the fat tissue than rural districts (N=26). However, the differences were not significant, probably because the mean age of persons in industrialised areas was significantly higher that in the rural areas. No significant regional or sex differences were observed in the contribution of individual congeners to the total TEQ.

Among the indicator PCB congeners, PCB 138, 153, and 180 contributed substantially to the total PCB level in human adipose tissue. The results are given in Table 8 as the sum of those congeners. The level of PCBs correlated significantly with age ($r=0.3$,

Table 8 *PCB levels expressed as the sum of indicator PCB congeners 138, 153, and 180 (median) (pg/g fat) (13)*

Group	Median	Ranges
Overall	1,753	786–8,167
Men	1,822	786–3,536
Women	1,695	836–8,167
Age >50	2,082*	1,147–8,178
Age <50	1,611	786–3,362

* $P<0.05$

P<0.01). Significantly higher values were obtained in the age group above 50 years (P<0.05) than in the group below 50 years of age. No significant sex or regional differences in concentration levels were found.

To conclude, the presented values bring the first human data characterising the background exposure of the Czech population. It is evident that the Czech population ran a higher exposure risk in the past. PCBs contributed more dioxin-like toxicity in human tissues than did dioxins and dibenzofurans. However, data concerning the level of PCDDs/Fs have been insufficient to delineate the time-related trends of the body burden of the Czech population, which stresses the importance of further monitoring activities.

Slovakia

The incidence of carcinogenic diseases in Slovakia is among the highest in the world. It is possible that increasing environmental pollution by toxic chemicals, including persistent chloroaromatic compounds, may contribute to this undesirable trend (5, 6). The potential exposure of human population in Slovakia has been studied systematically for many years. The researchers from the Institute of Preventive and Clinical Medicine in Bratislava have published many research papers and reports in the last decade.

The growing attention to the study of contamination of total diet, mother's milk and adapted cow's milk, dairy products, and other food commodities (15). The contamination levels of organochlorine insecticides and indicator congeners of PCBs in milk and butter from Bratislava markets were studied in 1994. The obtained results for organochlorine insecticides showed that the highest levels were measured for p,p'-DDE and p,p'-DDT. Those findings may be attributed to the use of non-standard imported feed in the farms. This was particularly true in the months of early spring when the domestic feed for animals was lacking. In spite of measures taken in 1973, lindane (γ-HCH) preparations (e. g. Lindan®, Hermal®, and Sanigran®) were applied. The findings of total DDT in Slovakia (0.059 mg/kg on fat basis) can be compared with those in butter in the Czech Republic, for example by *Strnad* (16), with mean of 0.053 mg/kg on fat basis and *Bartonicek and Rob* (17) with 0.033 mg/kg on fat basis. In Poland, the total mean DDT level in butter was 0.096 mg/kg on fat basis and the maximum 0.272 mg/kg on fat basis (v. the Slovak maximum of 0.208) (18).

Although the mean levels of HCB were relatively low, the maximum levels could be influenced by environmental contamination with this compound. Agriculture in the 90s was not the main source of HCB environmental inputs in the CEE countries. Many HCB residues came from the deposits of chemical wastes. A case in point are the outskirts of the Slovak capital Bratislava, considered a »hot spot« of HCB contamination (18). The HCB levels found (mean value 0.004 mg/kg on fat basis) were in a good correlation with those in the butter samples from the area of Schwerin (Germany) in 1990 with the mean content of 0.007 mg/kg on fat basis (17) and with the 1991 Czech report by *Strnad* (18) with a mean level of 0.014 mg/kg on fat basis. One year earlier (1990), *Bartonicek* (16) found the mean HCB level in butter at 0.044 mg/kg on fat basis.

As regards the conditions of the CEE milk farming at that time, one sample of butter represents a mean obtained from the milk coming from a number of farms. From this point of view, the contamination levels measured in the butter can be regarded as the measure of environmental contamination with persistent organochlorine compounds (17).

The results concerning PCBs (15) in fifty samples of mother's milk and 20 samples of adapted cow's milk for bottle feeding supplied from the lactarium of the Pediatric Hospital in Bratislava were examined for the presence of 6 indicator congeners of PCBs. Similar to the Czech study by *Schoula and co-workers* congeners nos. 138, 153, and 180 were predominant in all samples (4, 11). The highest mean as well as the maximum level was found for PCB 138 for both types of samples.

The average daily intake based on the sum of indicator congeners was 2.56 μg/kg bw for mother milk, and 0.85 μg/kg bw for adapted cow's milk. The calculated average daily intake from mother milk exceeded the value of Acceptable Daily Intake which is 1 μg/kg bw, but this was also observed in other countries. From this point of view bottle feeding would seem more advantageous, since the PCB levels are significantly lower in adapted cow's milk. Furthermore, the content of all congeners found in adapted cow's milk was substantially below the permissible level (0.04 mg/kg for 28, 52, 101, 180 and 0.05 mg/kg for 138 and 153 in fat).

Samples of subcutaneous abdominal adipose tissues were obtained from the Slovak population through post-mortem and analysed for the level of PCBs (20). The concentrations found in Bratislava (men, N=32, women, N=14), were in the ranges 0.8 to 10.4 and 0.6 to 7.7 mg/kg fat, in Trenčín (men, N=18, women, N=11) 2.1 to 5.5 and 0.4 to 6.1 and in Martin (men, N=17, women, N=13) 0.4 to 4.7 and 0.4 to 4.8, respectively.

Fifty samples of human blood collected in 1992 from the general human population living in five selected areas of the Slovak Republic (the Michalovce, Veľký Krtiš and Nitra Districts, Myjava area, and Bratislava) were analysed for 18 PCB congeners and some organochlorine pesticides (HCB, lindane, p,p'-DDE and p,p'-DDT). Table 9 shows the levels of these pollutants in serum lipids averaged for all the samples analysed.

Table 9 *Levels in serum lipids averaged from five selected areas in Slovakia in 1992 (μg/kg) (20)*

Pollutant	Mean	Median	Range
PCBs (18 congeners)	1.79	1.33	0.53– 9.20
HCB	5.38	4.27	0.16– 23.20
γ-HCH	0.012	–	<0.01– 0.18
p,p'-DDE	6.05	4.39	1.30– 34.80
p,p'-DDT	0.27	0.23	<0.01– 0.79

About three times higher levels of PCBs were found in the samples from the Michalovce District where PCB formulations had been produced. PCB levels in the Slovak population in 1992 were similar to earlier findings, but they substantially exceeded human PCB levels found in other countries. The difference was even more evident with HCB the levels of which were about hundred times higher than the corresponding levels in the USA, Japan, Finland, or Canada. The paper also described the arithmetic mean values of PCBs, p,p'-DDE, p,p'-DDT and HCB found in the serum lipids of men and women. The mean levels of p,p'-DDT and PCBs were lower in women.

The data reviewed herein suggest that the levels of PCBs and related compounds in humans from the former Czechoslovakia are higher than reported by studies of other countries. Higher exposure was found in the vicinity of PCB mixture manufacturing or using. Since PCB production was banned in 1984, the total environmental PCB contamination and consequent body burden in the general population of the Czech and Slovak Republics have decreased. However, human exposure has not been excluded entirely (7).

The other part of this project was focused on the investigation of levels of PCBs and selected organochlorine pesticides in human milk in the same five model areas (21). Based on the WHO protocol, the human milk samples were collected from breast-feeding mothers (primiparae, milk sampling 2 weeks-2 months after delivery, unchanged residence at least 5 years before pregnancy).

HCB and p,p'-DDE were present in the human milk samples at higher concentrations than individual PCB congeners and p,p'-DDT. HCB levels are noteworthy, as they are one or two orders of magnitude higher than reported from other countries (see Table 10). *Surprisingly high concentrations of HCB found in human samples* from Slovakia were probably caused by its use in agriculture and its formation during industrial manufacture of some chlorinated solvents.

Table 10 *PCB and OCP levels found in human milk lipids from various countries (21)*

Year of collection	No. of samples	Country	Average concentration (µg/kg, fat basis)				
			6 PCBs[1]	PCB-118	HCB	p,p'-DDE	p,p'-DDT
1986	412	Canada[2]	1,042	20	–	–	–
1991	16	Canada, Quebec[2]	109	17	–	–	–
1993	107	Canada, Quebec, (Inuit)	706	–	136	1,212	–
1991	32	UK	302	29	–	–	–
1990–91	57	UK, Wales	232	18	–	–	–
1991	28	Norway, Oslo[2]	261	26	–	–	–
1990–92	195	Netherlands, Rotterdam	439	35.5	–	–	–
1993	24	Jordan, Amman	351	–	–	–	–
1190–91	68	Germany, Middle Hesse	692	–	–	–	–
1991	113	Germany, N. Rhine Westphalia[2]	581	–	177	504	27
1989–90	59	Jordan, Amman	–	–	290	2,040	450
1991	51	Spain, Madrid	–	–	0.8	604	12.5
1985	100	Israel, Jerusalem	–	–	80	2,440	290
1979–80	54	USA, Hawaii	–	–	46	1,989	162
1981–82	50	Finland	–	–	64	850	36
1987	64	Italy	271	41	192	2,050	154
1990	25	Czech Rep., Jihlava	–	–	1,644	–	–
1993–4	50	Slovakia[3]	785	39	829	1,667	126

[1] The sum of IUPAC Nos. 28, 52, 101, 138, 153, and 180
[2] Not all 6 congeners were measured (mostly 138, 153, and 180)
[3] (25)

The levels of all pollutants determined in the human milk lipids were substantially lower than those in the blood and adipose tissue samples collected from the same areas. The differences in PCBs levels between milk samples from Michalovce District (location of a former PCB producer) and other districts were not as high as the differences in the adipose tissue and blood serum. This reason may be sought in the lower age of breast-feeding mothers than the mean ages of the adipose-tissue and blood donors and in the decreasing environmental OCP and PCB contamination after the ban.

The average ratios of p,p'-DDE to p,p'-DDT in the milk samples from Bratislava, Myjava, Nitra, Michalovce, and Velký Krtiš were 14.7, 12.6, 17.5, 9.8, and 13.9, respectively. This is less than was observed in the adipose tissue and blood serum samples (5, 6). PCB congeners nos. 153, 180, and 138 were dominant in all samples.

Having in mind that an average 5-kg infant suckling in the Michalovce District consumed 800 g of mother's milk, the calculated average intake of the sum of six PCB congeners was 7.5 μg/kg bw per day. This intake ranged from 2.8 to 4.4 μg/kg bw in the other four model areas, which is substantially higher than the acceptable or tolerable daily intake of 1 μg/kg bw per day (involves all PCB congeners) established in some countries. The calculated daily intake of HCB and p,p'-DDE+p,p'-DDT, ranged in all areas from 2.0 to 5.1 μg/kg bw and from 5.6 to 11.5 μg/kg bw, respectively. The intake of DDE+DDT was lower than the acceptable daily intake (ADI) value established by WHO (20 μg/kg bw per day), but the intake of HCB was two orders of magnitude higher than the tolerable daily intake of 0.08 μg/kg bw issued by the Health Protection Branch of Health and Welfare Canada.

The presented results from the pilot study involved a relatively small number of specimens and it was questionable to evaluate any statistical parameters. However, a very unique study was based on these results in 1997–1998 and its results are presented below.

Kočan and co-workers also studied the PCDDs/Fs and coplanar PCBs levels in blood serum samples collected from the Slovak human general population (5 districts) and occupationally exposed workers (22). These pollutants were also determined in blood samples taken from a limited number of workers employed for a long time at a municipal waste incinerator or a PCB production plant.

Table 11 summarises the levels of PCDDs/Fs and of two of the most toxic coplanar PCBs (PCB-126 and PCB-169). The mean total lipids in the serum were 6.79 g/L. The mean TEQ_{PCDDs}, TEQ_{PCDFs}, and $TEQ_{planPCBs}$ for each sampling location were similar, including the MWI samples, whereas TEQ_{PCDFs} and $TEQ_{planPCBs}$ values were several times higher in samples from Chemko Strážské (former producer of PCBs mixtures). Increased TEQ levels in the Chemko samples were caused mainly by higher concentrations of 2,3,4,7,8-PeCDF and 3,3',4,4', 5-pentaCB (126). 2,3,4,7,8-PeCDF contributed 53% to the total mean TEQ found in the Slovak general population, 60% in the case of the MWI workers, and 80% in the case of the occupationally exposed workers in PCB production. There were no substantial differences between the PCDDs, PCDFs, and planPCBs levels in the general population samples from the sampling districts.

In spite of long-term exposure at the old-type waste incinerator, PCDDs/Fs and coplanar PCBs levels in the workers blood kept within the general population levels (Table 7). This is in agreement with published data, although some studies report a slight increase in the concentrations of higher chlorinated congeners.

Table 11 *PCDD/F and coplanar levels (expressed as toxic equivalents, lipid-adjusted basis) in blood serum samples taken from the human general population of 5 model districts of Slovakia and occupationally exposed persons (MWI, former producer of PCBs). Only average values are described, for details see ref. (22)*

Area	Sex	Age	BMI (kg/m²)	Content (ng/kg, lipid adjusted)				
				I-TEQ PCDDs	I-TEQ PCDFs	I-TEQ PCDDs/Fs	WHO-TEQ planPCBs	ΣTEQs
Bratislava	3M/3F	37.0	26.3	5.6	18.2	24.1	9.6	33.7
Michalovce	3M/3F	35.3	26.9	6.5	13	19.5	12.9	32.4
Myjava	3M/3F	35.5	24.9	6.9	15.3	22.2	11.6	34.7
Nitra	3M/3F	33.3	25.7	6.8	12.7	19.4	7.6	27
Veľký Krtiš	3M/3F	40.0	27.0	6.5	8.8	15.4	7.9	23.3
Average (men)		36.9	26.3	6.6	15.3	22	10	32.1
Average (women)		35.5	26.0	6.3	12	15.3	9.6	28
Average (together)		36.2	26.1	6.5	13.5	20	9.9	30
MWI	4M	41.3	28.3	6	16.6	22.6	9.5	31.9
Chemko Co.	2M	42.0	30.8	7	68.3	75.3	63.1	138.4

It is noteworthy that the ratio between I-TEQ$_{PCDDs}$ and I-TEQ$_{PCDFs}$ found in the Slovak general population was less than 1 (about 0.5), whereas reports from other countries showed much higher ratio – from about 1 for Germany and Spain to 2.7 for the USA. This was due to relatively high levels of 2,3,4,7,8-PeCDF (I-TEQQ=0.5) in the Slovak human samples in proportion to the total TEQ's. On the other hand, the levels of 1,2,3,7,8-PeCDD (a congener with the same I-TEF) were found to be very low. Authors assumed that the increased 2,3,4,7,8-PeCDF levels observed in the Slovak samples were due to increased exposure to PCBs. This assumption was supported by findings of a high 2,3,4,7,8-PeCDF content in the blood lipids of the Chemko workers occupationally exposed to PCBs. The ratio between the mean 2,3,4,7,8-PeCDF level in the Chemko and the mean level in general population samples was 6 (121.2:21.1), which was also the ratio of the total PCB levels found in the same samples.

The aim of the above study was to investigate PCB and OCC levels in samples of human population (from the District Michalovce, location of former producer of PCBs and the District Stropkov). The basic topic of that project was environmental and human population load in the area contaminated with PCBs (23). Table 12 brings the first summary of results from measurements of the PCB content in blood samples of human population from these two regions.

Higher content of PCBs determined in various kinds of foods from the District Michalovce led to higher levels of PCBs in human population from this district. The average concentration of PCBs in fat samples from the blood serum of general population of that region (107 men and 108 women) was 4.2 μg/g, whereas in the District Stropkov (101 men and 104 women) it was 1.2 μg/g. The PCB level of 8.6 μg/g was found in occupationally exposed workers in the former PCB producer Chemko Strážské (27 men and 11 women). It is important to note that the exposure was 20–30 years before those measurements took place. A part of the general population was a group of 11 fishermen who consumed fish from contaminated waters of the Laborec River

Table 12 *Levels (means) of PCBs (sum of 9 congeners), HCB,* g*-HCH, p,p'-DDE and p,p'-DDT in samples of blood serum from population groups of Districts Michalovce (MI) and Stropkov (SP) (23)*

	Contents (µg/g fat of blood serum)					
	ΣPCB[1]	HCB	HCH	DDE	DDT	% of DDT in ΣDDTs
Professional exposure with PCBs (N=38)	8,567	1,699	22	3,867	130	3.3
– men (N=27)	10,115	1,674	21	4,262	145	3.3
– women (N=11)	4,767	1,761	22	2,899	94	2.4
Fishermen (MI, N=11)	12,205	1,453	33	3,733	260	6.5
General population MI (not occupationally exposed, N=215)	4,166	1,921	30	3,890	131	3.3
– with the occupationally exposed (N=253)	4,827	1,888	29	3,865	141	3.5
– with the occupationally exposed, randomly selected PCB (N=225)	4,222	1,912	30	3,873	141	3.5
– without fishermen (N=204)	3,753	1,945	30	3,874	136	3.4
General population SP (N=205)	1,206	1,622	38	2,571	98	3.7
Men MI (N=107); general population	4,802	1,419	33	3,528	152	4.1
Women MI (N=108); general population	3,536	2,418	27	4,199	134	3.1
Men SP (N=101); general population	1,385	1,217	36	2,940	98	3.2
Women SP (N=104); general population	1,033	2,015	37	2,503	97	3.7

[1] Sum of the following congeners: 28, 52, 101, 138, 153, 180, 118, 156, and 170.

and Zemplínská Šírava Dam. This small and not very representative group showed very high (58.7 mg/kg) and relatively low (1.6–2.9 mg/kg) contamination levels. Women showed higher levels of PCB contamination in all evaluated groups from both districts, but not significantly. However, the study confirmed the association between consumption of contaminated homemade food such as eggs or chicken meat, and higher contamination levels in humans and the results were statistically significant. The levels of PCBs, HCB, and p,p'-DDE, (including the Stropkov District) were higher in Slovakia than in the EU countries, the USA, or Canada.

Placental contamination with xenobiotics may act as a biological marker of mother's or the foetus' exposure via placenta (24). In Slovakia, PCB congeners were detected in the human food chain including human breast milk, where the PCB's levels were higher than in cow's milk. The aim of that study was to compare the contamination of human placentas with organic xenobiotics (selected organochlorine compounds) in five environmentally different Slovak regions.

Samples of placental tissue were taken from mothers in five environmentally different regions in the Slovak Republic. The samples were analysed for the concentrations of 21 selected organochlorine compounds – polychlorinated biphenyls (PCBs – PCB-28, PCB-52, PCB-101, PCB-118, PCB-138, PCB-153, PCB-180), chlorinated benzenes (1,4+1,3-DCBz, 1,2-DCBz, 1,3,5-TCBz, 1,2,4-TCBz, 1,2,3-TrCBz, TeCBz, PeCBz, HCB), and organochlorine insecticides (α-HCH, β-HCH, γ-HCH, δ-HCH, p,p'-DDT, p,p'-DDE).

The statistical analysis of the placental organochlorine compounds concentrations revealed that the mean contents of 12 (1,4+1,3-DCBz, 1,3,5-TrCBz, 1,2,3-TrCBz, TeCBz, PeCBz, HCB, α-HCH, β-HCH, γ-HCH, δ-HCH, p,p'-DDT, and PCB-101) out of 21 organochlorine compounds analysed were significantly higher in the Region 1

Table 13 *Concentrations of organochlorine compounds (mg/kg x 10^{-3}) in human placental samples in 5 regions of Slovakia related to predominant industrial sources of environmental pollution (24)*

	Compounds	Region 1 $\bar{X}\pm SD$	Region 2 $\bar{X}\pm SD$	Region 3 $\bar{X}\pm SD$	Region 4 $\bar{X}\pm SD$	Region 5 $\bar{X}\pm SD$
A	1,4+1,3-DCBz	2.7±3.6	1.5±2.3	4.10±8.80	11.40±21.00	2.6±5.4
	1,2-DCBz	6.3±9.0	0.2±0.3	0.01±0.04	0.03±0.10	12.1±14.0
	1,3,5-TrCBz	25.9±9.9	0.0±0.1	0.08±0.40	0.01±0.08	2.7±5.2
	1,2,4-TrCBz	4.9±10.1	0.1±0.3	0.20±0.30	0.30±0.50	6.8±9.9
	1,2,3-TrCBz	0.8±0.8	0.1±0.2	0.10±0.20	0.20±0.30	0.7±0.8
	TeCBz	0.7±2.0	0.6±2.7	0.20±0.80	0.09±0.50	0.3±1.4
	PeCBz	4.5±14.0	0.0±0.1	0.01±0.02	0.02±0.07	1.0±1.6
	HCB	2.0±9.5	0.3±0.3	0.40±1.10	0.40±0.50	0.5±0.5
B	α-HCH	0.3±0.6	0.0±0.0	0.20±0.30	0.10±0.20	0.2±0.7
	β-HCH	0.7±1.6	0.0±0.1	0.20±0.30	0.30±0.30	0.4±1.4
	γ-HCH	1.5±2.8	0.0±0.1	0.60±0.40	0.50±0.50	1.1±4.4
	δ-HCH	0.6±1.1	0.0±0.0	0.01±0.05	0.00±0.00	0.2±0.4
	p,p'-DDT	0.4±0.8	0.4±1.4	0.01±0.04	0.04±0.20	0.2±0.9
	p,p'-DDE	0.4±0.5	0.5±0.5	0.40±0.40	0.30±0.50	0.2±0.3
C	PCB-28	0.2±0.6	0.4±1.7	0.03±0.07	0.08±0.30	n.d.
	PCB-52	0.1±0.2	1.4±1.5	0.04±0.20	0.05±0.20	0.1±0.3
	PCB-101	5.6±17.5	0.5±1.0	0.10±0.30	0.40±8.30	0.5±1.5
	PCB-118	0.7±3.2	–	–	–	n.d.±0.1
	PCB-138	2.1±1.7	0.9±2.2	0.10±0.10	0.20±0.40	0.6±1.4
	PCB-153	5.6±22.6	1.3±2.7	0.20±0.20	0.20±0.50	1.2±3.4
	PCB-180	0.2±0.4	0.8±1.9	0.20±1.30	0.80±3.60	n.d.

A – chlorinated benzenes; B – organochlorine insecticides; C – polychlorinated biphenyls;
n.d. – below detection limit
Region 1 – industrial region polluted by organic chemical petrol, pesticide and rubber industries;
Region 2 – agricultural region;
Region 3 – region polluted by iron-ore mining;
Region 4 – industrial region polluted by iron-ore processing;
Region 5 – rural region with no point source of industrial pollution

polluted by chemical industry than in other investigated regions. However, concentrations of DDE and the PCB congeners 28, 52, 138, 153, and 180 were the highest in the agricultural region. Among the investigated regions, the lowest concentrations of 9 organochlorine compounds (1,2-DCBz, 1,3,5-TrCBz, TeCBz, PeCBz, p,p'-DDT, PCB-28, PCB-52, PCB-138, and PCB-153) were found in iron-ore mining or processing regions.

Exposure to organochlorine compounds can be determined in samples from various tissues, including foetal and placental tissues. Experimental findings in animals revealed that organochlorine compounds could induce oxidative stress in the foetal and placental tissue with subsequent tissue damage. The findings showed the production of superoxide anion and lipid peroxidation and DNA-single strand breaks. In our previous work we found more frequent pathological microstructural changes in the human placental samples collected from the Region 1 polluted by chemical industry than in the rural Region 5. The embryo/foetus is highly susceptible to adverse impacts of organochlorine compounds. Further research should focus on the mechanisms of these compounds in reproductive pathology. Our findings pointed to contamination of human placenta with various organochlorine compounds, depending on the predominant type of pollution in the selected regions.

Reichrtová and co-workers studied the role of organic xenobiotics in human placenta in the allergic sensitisation in newborns. (25). Although we now understand more about the genetics of atopy and the role of Th1 and Th2 cells in the control of immunoglobulin E (IgE), environmental causes of atopy are still not clear. The evidence linking patterns of foetal growth to adult diseases has focused attention on the role of the placental environment in the aetiology of atopy and atopic diseases. In our study, we gathered fresh samples of full-term placentas and umbilical cord blood n the delivery rooms of hospitals from environmentally different regions (industrial, polluted predominantly by organic xenobiotics and traffic v. rural). Data concerning parameters of placentas and newborns were collected through questionnaires. Placenta samples were analysed for 21 compounds of persistent organic pollutants (POP) using capillary gas chromatography. Concentrations of total IgE (as a biomarker of a sensitisation of newborns) in the umbilical blood samples were also determined using capillary gas chromatography. Laboratory analyses showed significantly higher concentrations of 17 out of 21 POPs investigated in the placental samples collected from the industrialized region than in from the rural region.

Total IgE level in the umbilical blood samples gathered from industrial region were also higher, perhaps as the result of organic xenobiotics. External placental parameters differed too.

Allergic diseases are on the rise in both prevalence and severity, especially in industrialised countries. Developing foetuses and young children, especially those with a genetic propensity may become even more susceptible to the effects of chemical agents, that is more prone to allergic sensitisation (24).

In general, the foetus is protected against external influences by the placental barrier, but the barrier is selective, especially for maternal immunoglobulin G (IgG) antibodies, various antigens, and chemical substances. Contrary to the transplacental IgG transport, only a low amount of immunoglobulin E (IgE) antibodies is present in the newborns, and it seems that these IgE antibodies have a foetal origin. There are environmental chemicals and drugs (such as xenobiotics) that may enhance the sensitisation to allergens of various origin in susceptible persons (due to their modulative effect on T-cells). It is therefore of great importance to understand the pathogenic mechanisms (neoantigen formation, metabolism of xenobiotics into reactive, haptenic metabolites, induction of costimulatory enzymes, and sensitisation of T-cells) involved in the xenobiotics action. Assuming that the infants undergo antigen/allergen priming *in utero*, xenobiotics may influence the response to antigen exposure and bring about allergic diseases in the early childhood.

Demonstration of benzo(a)pyrene-DNA (B(a)P) adducts in human placenta and cord blood confirmed the metabolic capacity of the placenta, the transfer of B(a)P from the mother to the foetus, and the genotoxicity of B(a)P. Organochlorine compounds accumulate in the body during the lifetime and the individual body burden increases to levels that are toxic to the organism. The offspring is exposed *in utero* through maternal transfer. Organochlorine compounds exert estrogenic effects (endocrine disruption) and a variety of associated effects such as reproductive and immune system dysfunction. Neonatal exposure to polychlorinated biphenyls (PCBs), especially to their congeners 28 and 52, were found to have persistent neurotoxic effect in adult animals. In Slovakia, these PCB congeners were detected in human breast milk as well as in cow's milk and dairy products.

Placental contamination by chemicals may act as a biological marker of exposure of the mother or foetus via placenta. Placentas collected from delivery rooms of hospitals from two Slovak regions. The samples were then analysed for 21 selected organochlorine compounds. The study was based on 2,050 full-term deliveries, randomly selected in two Slovak regions differing in the industrial potential and environmental pollution. The industrial region was represented by a city (Bratislava) polluted mainly by organic chemical industry (petrol, pesticides, and rubber industry) and by the traffic. The rural region was situated in the mountains (Stará Lubovna) away from industrial sources of environmental pollution, but with traffic that goes with tourism and a border crossing. Women were selected according to the following criteria: residence in the investigated areas at least 3 years before the conception, normal term of delivery (40±2 weeks of gestation), and non-occupational exposure to organochlorine compounds. The data from questionnaires focused on mothers (e. g. residence, smoking habit, occupation). The external parameters were analysed such as placental longest and transverse diameter and thickness and the birth weight and height of the newborns. The average age of the investigated mothers in the industrial region was 24.2 and in the rural region 22.6 years. In the industrial region 26% mothers smoked cigarettes and in the rural region 27%. The incidence rate of atopic eczema (per 10,000 children) in 1995 was 30.82 in Bratislava and 12.78 in Stará Lubovna. Samples of cord blood of newborns (N=2 050) were collected in respective maternity clinics, and sera prepared by centrifugation. Simultaneously, randomly selected samples (N=120) of full-term placentas were taken. Specimens of cord blood from 2,050 neonates were gathered for the determination of levels of total immunoglobulin E (IgE).

Table 14 shows the contents of 21 substances of organochlorine compounds in the human placental samples collected from the industrial and the rural region. Comparisons revealed that both the placental contamination with 16 (out of 21) organochlorine compounds and the cord serum IgE levels were significantly higher in the industrial region. Furthermore, the percentage of non-contaminated placental samples was significantly higher in the rural region than in the industrial. The content of all congeners of polychlorinated biphenyls (mainly PCB congeners 101 and 153) and organochlorine insecticides analysed in the placental samples was higher in the industrial region. The neonates were divided according to their cord serum IgE concentration in 3 groups: <0.7 kU/L (e.g. negative newborns), 0.7–3.5 kU/L, and >3.5 kU/L. The 2nd and the 3rd group were largely dominated by IgE-positive neonates from the industrial region (expressed in percentage) ($P<0.001$). Quite expectedly, there were less IgE-negative newborns from the industrial region (68.7%) than from the rural region (82.4%). The findings pointed to an association between organochlorine compounds and the higher levels of total IgE in newborns, suggesting a higher risk of allergic sensitisation in the industrial region. This association was supported by the higher incidence of atopic eczema recorded for population from the industrial region. The positive Spearman correlations for p,p'-DDE ($r=0.3294$, $P=0.01$) and for PCB 118 ($r=0.3482$, $P=0.006$) and cord serum total IgE level were found.

Table 14 *Median and maximum concentrations of organochlorine compounds and the percentage of samples below detection limits in human placentas collected from industrial and rural regions (24)*

	Compounds	Concentration (µg/kg)					
		Industrial region			Rural region		
		Median	Max.	% n.d	Median	Max.	% n.d
A	1,4+1,3-DCBz	1.4	218.0	19	0.8	26.9	21
	1,2-DCBz	0.8	46.9	18	7.6	64.3	18
	1,3,5-TrCBz	10.2	310.4	11	0.7	31.5	14
	1,2,4-TrCBz	0.5	41.9	14	1.9	50.5	13
	1,2,3-TrCBz	0.7	3.0	14	0.5	3.5	32
	TeCBz	0.1	12.7	28	n.d.	10.4	68
	PeCBz	0.4	102.2	23	0.2	7.0	32
	HCB	0.6	72.0	2	0.4	2.0	25
B	α-HCH	0.2	4.0	19	n.d.	5.4	70
	β-HCH	0.3	12.0	12	0.1	11.2	59
	γ-HCH	0.6	17.5	5	0.2	33.1	40
	δ-HCH	0.3	5.4	25	n.d.	3.1	65
	p,p'-DDT	0.1	3.5	26	n.d.	5.2	54
	p,p'-DDE	0.1	2.2	12	0.1	2.0	24
C	PCB-28	0.1	4.0	14	n.d.	0.2	73
	PCB-52	0.1	0.6	12	n.d.	2.0	67
	PCB-101	0.2	109.0	26	n.d.	8.9	76
	PCB-118	0.1	23.5	18	n.d.	0.4	64
	PCB-138	0.2	7.9	5	n.d.	6.4	60
	PCB-153	0.2	124.8	9	0.1	24.4	46
	PCB-180	0.1	1.9	32	n.d.	0.1	81

Industrial region: N=57; Rural region: N=63; A – chlorinated benzenes; B – organochlorine insecticides, C – polychlorinated biphenyls; Max – maximum; n.d. – below limit of detection,
% n.d. – percentage of samples below limit of detection

External parameters of human term placentas and birth parameters of neonates between the rural and the industrial region are compared in Table 15. In spite of even

Table 15 *External parameters of human full-term placentas and birth parameters compared between industrial and rural regions (24)*

Parameter	Industrial region X±SD	Rural region X±SD	Difference
Placental weight (g)	586.9±135.7	572.5±107.3	n.s.
Longest diameter (cm)	18.9±2.8	16.3±2.9	P<0.001
Transverse diameter (cm)	15.7±2.2	12.8±2.3	P<0.001
Thickness (cm)	2.3±0.6	1.8±0.7	P<0.001
Birth weight (g)	3,287.9±595.7	3,347.2±462.0	n.s.
Birth height (cm)	49.9±2.5	49.6±2.1	n.s.

n.s.– not significant

average weights of placentas in both regions, other parameters (e.g. longest diameter, transverse diameter and thickness) were significantly higher in the rural region. The comparison of birth parameters (weight and height) revealed that there were no significant differences between the regions. However, the industrial region (Bratislava) had a higher percentage of microstructural lesions diagnosed in human placentas than the rural region (Stará Lubovna). These microstructural lesions may be responsible for different external parameters of human placentas.

The comparison of biomarkers of mothers' chemical exposure (e. g. different placental metal and organochlorine content in placental samples) with the cord sera IgE levels between the two regions has led to a hypothesis of possible foetal allergic sensitisation evoked by organochlorine compounds in the placenta.

Poland

Most persistent organochlorine pesticides, excluding lindane, were banned in Poland in 1975/76. The first restrictions of use and marketing of lindane became effective in 1980 and were gradually extended until its agricultural use was ultimately banned in 1989. Unfortunately, there are no detailed data on the use and environmental release of PCBs in Poland. OCCs and PCBs in human adipose tissues were studied in Warsaw (26), Gdańsk (a chemically more detailed study), and an inland province (27).

Subcutaneous adipose tissues were taken from surgically treated patients in Warsaw hospitals (26). Samples were collected between 1989 and 1992 from male and female persons aged between 10 and 80. The total number of analysed samples was 277 (142 from male, and 135 from female persons) (Table 16).

Table 16 *Levels of organochlorine compounds in human adipose tissue from 277 patients from Warsaw hospital, Poland in 1989–1992 (mg/kg of fat) (26)*

Pollutant	Mean	Median	Maximum
PCBs	0.856	0.500	36.000
HCB	0.310	0.120	9.020
α-HCH	0.016		0.160
β-HCH	0.228	0.120	5.097
γ-HCH	0.074	0.030	2.727
p,p'-DDE	5.745	4.382	35.850
p,p'-DDT	0.537	0.478	9.600

The study has hown that age may considerably contribute to organochlorine concentrations in human adipose tissue. The same authors studied the excretion of these pollutants from human body by lactation (28). Lactation is an important factor in disposing organochlorine compounds from the female body. That is why the authors followed up the number of deliveries per woman and subsequent lactations and sought to understand their role in OCCs excretion through milk. The relationship between age, number of deliveries, and the concentrations of OCCs in mother's milk was identified by examining the findings from the analysis of 253 samples of human breast milk. The analysis included 108 milk samples from primiparae and 145 samples from multiparae (2–7 deliveries).

No decline was found in the mean concentrations of OCCs in multiparae as compared to primiparae. The reason may lie in the age of the studied women. Mean HCB and ΣHCHs concentrations were similar between the groups, and the mean p,p'-DDT and PCBs concentrations were even higher in multiparae (statistically significant only for PCBs; $P \leq 0.05$).

Women with the highest number of deliveries (over four) were reported the highest DDT levels, DDT metabolite levels, and PCB levels. Quite expectedly, the average age of those donors was also the highest – 33 years.

The concentrations of chlorinated hydrocarbons identified in human milk are the result of two processes: bioaccumulation of such compounds in the adipose tissue and the excretion of those compounds to human milk in the course of lactation. Older women may be expected to have higher concentrations of OCCs due to longer exposure. On the other hand, lactation is an important way to dispose of such compounds from the body. Daily disposal during lactation is much greater than the daily intake. Thus, the concentrations of chlorinated hydrocarbons in human milk may be expected to fall as the number of deliveries increases (28–30).

Newborns become chronically exposed to organochlorine compounds mainly through food (28, 29). Bioavailability of these compounds in breast-fed infants is higher than that in formula-fed infants. It is therefore critical that one should determine whether these compounds are hazardous to the health of infants and small children in the high risk category and identify the safety margin between the current concentrations of the OCPs in human milk and the limit beyond which health hazard is no longer acceptable.

That was the rationale of another study of infant exposure to PCBs and OCPs (HCB, HCHs, DDTs) from mother's milk (31). Samples of human milk were collected in lactarium and maternity clinic in Warsaw and from donors from different regions in Poland. The analysis comprised 462 human milk samples. To assessment of exposure of breast-fed children to the above mentioned compounds required data on ADI and FAO Guidelines to calculate the theoretical maximum daily intake (TMDI) and estimated daily intake (EDI) for the tested compounds. Table 17 shows the average concentrations of the observed compounds.

Table 17 *Average concentrations of organochlorine compounds in human milk in Poland (mg/L milk ± SD) (31)*

HCB	α-HCH	β-HCH	γ-HCH	p,p'-DDT	p,p'-DDD	p,p'-DDE	PCBs
Milk collected on the 4th day							
0.0013± 0.0008	0.0002± 0.0002	0.0014± 0.0008	0.0002± 0.0001	0.0050± 0.0024	0.0009± 0.0003	0.0211± 0.0140	0.0076± 0.0041
Mature milk							
0.0020± 0.0025	0.0005± 0.0017	0.0033± 0.0068	0.0004± 0.0014	0.0034± 0.0093	0.0004± 0.0025	0.0282± 0.0250	0.0544± 0.0814

It is worth noting that higher concentrations of PCBs and β-HCH were reported for mature milk than for milk collected on the 4th day (statistically significant, $P \leq 0.05$). In Poland ΣDDTs and PCBs had the highest share in EDI by breast-fed infants. The relation between ADI, TMDI, and EDI was calculated only for ΣDDTs and ΣHCHs, as these were the only compounds for which ADI was identified. The above mentioned calculations were then used to estimate the safety margins for ΣDDTs and ΣHCHs. These amounted to 4.2 (ADI for ΣDDTs=20 μg/kg bw per day) and 13.3 (ADI for γ-HCH=8 μg/kg bw per day), respectively. The estimated EDIs for ΣDDTs and ΣHCHs did not exceed the values assumed to be safe, that is, ADI and TMDI.

While the PCBs intake in the initial period of lactation (4th day) was relatively low and did not exceed the TMDI FAO/WAO guidelines, EDI of PCBs in the mature milk exceeded the relevant TMDI in some regions. Nevertheless, the average concentration of PCBs (1 mg/kg of fat) in human milk in Poland was lower than that found in highly developed countries. According to the literature sources, the infant average PCBs intake from human milk is 4.4 μg/kg bw per day. The EDI of PCBs in this study averaged 2.8 μg/kg bw per day.

These values of EDI for PCBs exceed the reference values of 1 μg/kg bw per day (FDA, US) or 0.6 μg/kg bw per day proposed by the National Food Agency of Denmark. However, in the light of the recent toxicological research, there is no reason why the levels of organochlorines detected in human milk should provide grounds for altering the recommendations in favour of breast-feeding. The benefits of breast-feeding for the child far outweigh possible negative impacts of compounds found in human milk (28, 29, 31).

Czaja and co-workers have also studied the differences between more and less industrialised areas of Poland (29). Higher concentrations of HCB and DDT were reported in milk of women from more industrialised areas (Table 18). Among eight chlorinated hydrocarbons examined, the concentrations of PCBs and p,p'-DDE were always the highest. Statistically significant differences were found between the mean concentrations

Table 18 *Mean concentrations of organochlorine compounds in human breast milk collected from donors living in more and less industrialised areas of Poland (mg/L) (29)*

	HCB	α-HCH	β-HCH	γ-HCH	DDT	DDD	DDE	PCBs
				More industrialised areas (N=158)				
$\overline{X}$	0.0016	0.0004	0.0019	0.0005	0.0055	0.0012	0.0254	0.022
SD	0.0012	0.001	0.0015	0.0014	0.0109	0.0033	0.0222	0.0447
Min	0.0002	0.0002	0.0004	0.0002	0.0008	0.0005	0.0009	0.001
Max	0.011	0.0108	0.0101	0.0156	0.1355	0.0381	0.1388	0.32
				Less industrialised areas (N=199)				
$\overline{X}$	0.0022	0.0007	0.0041	0.0004	0.0028	0.0006	0.0275	0.0131
SD	0.0028	0.0018	0.008	0.0011	0.005	0.0004	0.0251	0.0145
Min	0.0002	0.0002	0.0004	0.0002	0.0008	0.0005	0.0017	0.001
Max	0.0152	0.0155	0.0835	0.0083	0.0409	0.0048	0.185	0.0741

$\overline{X}$ – mean concentration, SD – standard deviation of β-HCH, HCB, p,p'-DDT, and PCBs examined in milk samples of women from more and less industrialised areas ($P<0.05$). In general, the concentrations of p,p'-DDT (banned in Poland 20 years ago) were consistently lower than those of its metabolite p,p'-DDE. The DDT/DDE ratio in the biological material dropped with time.

The β-HCH isomer showed the highest concentrations although its fraction in commercial lindane-containing pesticide products usually does not exceed 2–3%. The reason is that the excretion of the β-isomer from the human body takes five times longer than of other HCH isomers, and the ability of β-HCH to accumulate in fatty tissue is 10–30 times higher than of other isomers. The α-HCH and γ-HCH levels approximated the detection limit of the method.

Mean hexachlorobenzene concentrations were reported relatively low in human breast milk, comparable to those found in other European countries. Mean concentrations of PCBs were lower than those found in other countries (1 mg/kg of fat).

Bioavailibility of persistent organochlorine compounds found in human breast milk all over the world is very high, and their absorbtion in the infant's alimentary tract is almost complete (32). In the course of lactation, persistent organochlorine compounds dissolved in milk fat are removed from a woman's body. The infant is exposed to the compounds in question throughout the breast-feeding period.

The aim of this study was to identify possible trends in excretion of HCH isomers α-HCH, β-HCH, γ-HCH, p,p'-DDT, p,p'-DDD, p,p'-DDE, and polychlorinated biphenyls with human milk during lactation. The milk was sampled from eight lactating women from Warsaw (Table 19). The results showed individual differences in the excretion of the compounds. Subsequent milk samples collected from the same woman once a week differed markedly. The levels of compounds excreted with milk were reported to exceed significantly the daily intakes of those compounds by breast-feeding women. The findings of this study are not conclusive enough to claim that the infants' exposure to those compounds decreases or increases as breast-feeding continues. In light of the current knowledge, the advantages of natural breast-feeding outweigh the potential risk resulting from action of toxic compounds in human breast milk.

Table 19 *Range of average concentrations in samples of eight donors (mg/L milk ± SD) (28)*

	HCB	β-HCH	p,p'-DDT	p,p'-DDE	PCBs
Lowest concentration	0.0003±0.0002	<0.0002	0.0033±0.0019	0.0075±0.003	0.0039±0.0033
Highest concentration	0.0025±0.0008	0.005±0.0076	0.0129±0.0098	0.1387±0.1916	0.0384±0.0854

The concentrations of organochlorine compounds in breast milk depend on two processes: their lifetime bioaccumulation in the adipose tissue and the excretion with milk during lactation (33). Assuming that lactation can be an important process for eliminating organochlorine compounds from the human organism, an attempt was made to understand how successive childbirth(s) and lactation affect the organochlorine compound levels detected in human milk. The excretion of the examined compounds during lactation is much higher than the intake of these compounds with the diet, which may imply that the tissue deposits of the organochlorine compounds in women decrease with consecutive lactation.

Literature gives different perspectives on the effect of successive lactation on levels of organochlorine compound in breast milk. Some decrease in DDE, HCHs, and PCB levels in the donors' milk following consecutive deliveries was reported. The basic factors affecting the concentrations of these compounds include the donors' age, number of children, and duration of breast-feeding after each childbirth.

Another study analysed breast milk samples of two consecutive lactations taken from two donors (33). The interval between the lactations was 8 months in the first donor and 2 years in the second. Milk samples were analysed for the presence of p,p'-DDT, p,p'-DDE, p,p'-DDD, α-, β-, γ-HCH, and ΣPCBs using gas chromatography with an electron capture detector. The donor with the shorter interval between lactations showed higher mean concentrations of the examined compounds in the milk of the first lactation than in that of the second (Figure 1). These variances were statistically significant for each examined compound.

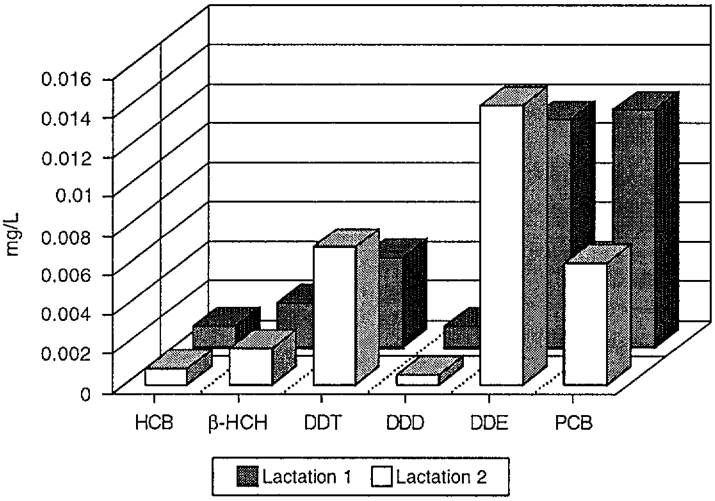

Figure 1 *Human breast milk OCP and PCB levels in a woman with the eight-month interval between two lactations (32)*

The second donor with the two-year lactation interval showed a different distribution pattern of the examined compounds. Just as the first, the second donor showed higher mean levels of HCB, β-HCH, DDD, and PCBs during the first lactation (Figure 2), but the differences were not statistically significant. At the same time, the concentrations of DDT and DDE in the second lactation were slightly higher, with the variance being statistically significant. Also noted was the absence of a decrease in DDT and DDE levels in the second donor's milk during the second lactation. This may be related to a relatively fast migration of tissue deposits of these compounds during a longer (2-year) interval between lactations.

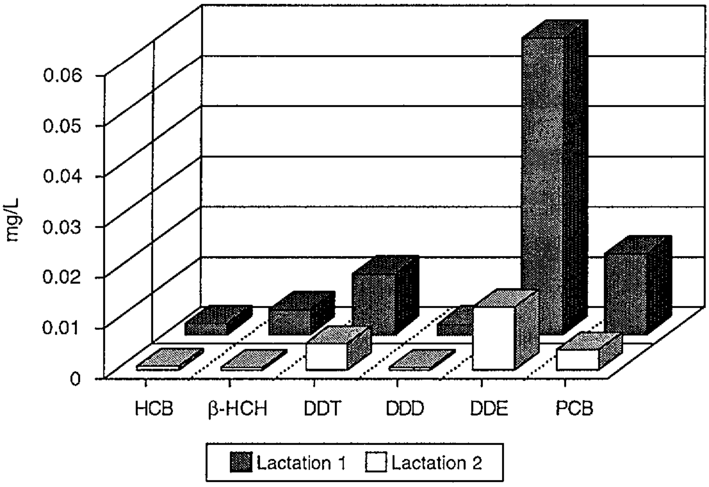

Figure 2 *Human breast milk OCP and PCB levels in a woman with the two-year interval between two lactations (32)*

The ability of a woman's organism to quickly activate tissue deposits of the organochlorine compounds has been confirmed in earlier studies (28) where mean concentrations of these compounds in primiparae and multiparae were compared, but also depended on the donors' age. No decrease in mean concentrations of the studied compounds was reported for multiparae versus primarae. Mean concentrations of HCB and ΣHCHs were similar in both donor groups, with DDT and PCBs being even higher for multiparae. Such results may be related to the age of the studied women. The number of deliveries did not affect mean concentrations of HCB and ΣHCHs. In women with the same number of children DDT, its metabolites, and PCBs increased with age. Other authors reported similar results showing that organochlorine compound concentrations in breast milk increase with the donor's age.

Age, more than the number of deliveries, seems to affect the concentrations of organochlorine compounds in breast milk. This phenomenon may imply that the deposits of these compounds recover quickly in women's tissues after lactation. This is confirmed by the results of the study in which higher DDT and DDE concentrations were detected in the second lactation milk of a woman with the longer interval between lactations.

Adipose tissue of inhabitants from Gdańsk, located at the southern coast of the Baltic Sea, and from the province of Skierniewice of the inland Poland were investigated for PCB congeners using HRGC-HRMS technique and for OCCs using HRGC-

ECD. The samples were collected during post-mortem from randomly selected donors in Gdańsk in 1990 and in the province of Skierniewice in 1979. The older data showed high concentrations of DDTs and total PCBs and much lower concentrations of HCH, HCB, and chlordane isomers (27, 34).

Table 20 *Range of levels of non-ortho coplanar PCBs and range of their 2378-TCDD TEQ in human tissues from two localities in Poland (Gdaòsk 1990 and Skierniewice 1979) (27)*

PCBs	Range of concentration (ng/kg fat wt)	TEF	Range of TEQ (ng/kg fat wt)	Mean of TEQ
77	54–500	0.01	0.54–5.0	2.3
126	41–850	0.10	4.1–85	34
169	50–390	0.05	2.5–27	13
Total	145–1,740		7.1–107.3	49.3

PCB-153 was a high contributor of the congener occupying 23% of the total PCB content, and together with PCB-138 (18%) and PCB-180 (13%) were the most prevalent members. Samples taken in Gdańsk in 1990 contained 1.5±1.3 μg/g of total PCBs on a fat basis while the 1979 samples from the inland province contained 1.2±0.4 μg/g, which seemed to indicate persistent PCB exposure in Poland.

Randomly selected post-mortem samples of liver cancer taken in Gdansk contained 4.7 μg/g of PCBs, while all other samples showed 0.75–1.9 μg/g. TCDD toxic equivalent of 13 detectable coplanar members of PCBs in adipose tissues from Gdańsk and Skierniewice was 210 pg/g and 190 pg/g on a lipid weight basis, respectively, including 45 pg/g and 59 pg/g of non-ortho, 142 pg/g and 110 pg/g of mono-ortho and 24 pg/g and 16 pg/g of di-ortho chlorobiphenyls, respectively. A fingerprint of chlorobiphenyl composition in the examined samples was virtually the same in the 1990 Gdansk human adipose tissue and the 1979 Skierniewice tissue, in spite of geographic differences and the sampling interval.

The baseline non-ortho coplanar PCB concentrations in human adipose tissue from Poland for the two localities were 10.10–20.

The recorded concentrations were equal and higher than those reported in other countries including the Czech and Slovak Republics. Both sites had comparable levels. This finding suggested a slower elimination rate of coplanar congeners and/or a continuing exposure of Polish population to PCBs.

Tanabe and Falandysz (34) also compared the previous data on organochlorine contamination in human adipose tissue of Polish cadavers reported by various researchers so far. Concentrations of PCBs were found somewhat higher than those reported earlier in other regions of Poland (Table 21). This was probably due to identification and determination of large numbers of PCB components using capillary GC in this investigation. Other organochlorine insecticide levels were rather comparable among various study periods reported for various regions in Poland. The results showed that DDT levels in the Polish environment and biota were declining at a slower rate after its withdrawal, and that the existing levels in Polish human fat were

Table 21 *Comparison of concentrations (means ± SD: µg/g lipid weight) reported in human fats in Poland (the number of samples is given in parenthesis) (34)*

Pollutant	Warsawa 1977–8 (100)	Skierniewice 1979 (12)	Poznań 1980s (53)	Gdańsk 1990 (9)
PCBs	0.19	1.2±0.44	NA	1.5±1.3
p,p'-DDE	3.330±0.199	22±16	14.095±15.798	15±13
p,p'-DDD	0.049±0.013	0.67±0.57	NA	0.013±0.007
o,p'-DDT	0.082±0.008	0.060±0.063	0.433±0.369	0.0098±0.0064
p,p'-DDT	1.011±0.078	2.0±1.2	1.772±1.767	0.67±0.38
DDTs	4.470±0.276	25±16	16.30±17.93	15±13
α-HCH	0.008±0.001	0.040±0.026	0.013±0.003	0.007±0.003
β-HCH	0.148±0.017	0.42±0.13	0.211±0.154	0.23±0.09
γ-HCH	0.022±0.003	0.068±0.025	0.020±0.018	0.004±0.002
δ-HCH	NA	0.0045±0.0057	0.045±0.025	0.002±0.014
HCHs	0.216±0.015	0.53±0.17	0.328	0.25±0.09
HCB	NA	0.36±0.22	0.221±0.143	0.26±0.23
Chlordanes	NA	0.11±0.08	NA	0.06±0.03

still higher on the international level. On the other hand, human PCB contamination corresponds to that of other industrialised nations.

Croatia

Croatia also conducted investigations of organochlorine pesticide residues and PCBs in human milk (35). The first evaluation of OCCs in breast milk took place in mid-seventies. For the last two decades OCCs and PCBs have been observed to drop in human milk samples collected in the city area of Zagreb. The first analyses of these pollutants in breast milk from two small towns in the Northern Adriatic area were carried out in 1986/87 and 1989. Both locations were approximately 50 km away from the city of Rijeka, one situated in the nearby Istrian peninsula, while the other on the island of Krk. *Frković and co-workers* (35) described OCC and PCB levels in 31 breast milk samples collected in Clinical Hospital of Rijeka.

The organochlorine pesticide levels determined in human milk from nursing women living in the Northern Adriatic region were the lowest in Croatia since the beginning of their survey in mid-seventies. Such decline could be a part of an overall tendency observed in other countries as well. Contrary to OCCs, PCB levels in breast milk were the highest ever observed in Croatia, a fact that was hard to explain at the time. Although PCB use has been restricted, there were some sources of PCBs in the environment. No statistically significant difference in OCC and PCB levels in human milk was seen between the donors' age, parity, residence, smoking habits, weight during pregnancy, and milk fat content. Good correlation was obtained between OCCs and PCBs (r=0.527, P=0.05), indicating the similar fate of those lipophilic compounds in the human body.

Generally, the levels of organochlorine pesticide residues in the breast milk from women living in that area were 10–50 times lower than was reported earlier and were comparable to the lowest levels observed in Italy and Canada. This could be the result of a constant drop observed in Croatia over the 20 years.

High levels of PCBs were found in human milk from industrialised countries, while they mostly kept below the detection limit in milk from developing countries. Average concentrations of PCBs in human milk were typically between 500 and 2,000 μg/kg milk fat (36). The mean level of PCBs (such as Aroclor 1260) reported by *Frković* (35) was 898 μg/kg milk fat and was the highest reported in Croatia. Compared to earlier results from the Nothern Adriatic region, the median value (778 μg/kg milk fat) was 56% higher than in samples from Istria location, and three times higher than in samples from Krk.

OCCs in human milk were analysed in samples collected over nine years (1987–1995) from 139 nursing mothers whose children were hospitalised for various disorders at the Department of Pediatrics, Clinical Hospital Centre in Zagreb, Croatia (37). Mothers were not occupationally or accidentally exposed to organochlorine pesticides or PCBs.

All samples contained p,p'-DDE and PCBs; the median concentrations were 318 μg/kg milk fat and 220 μg/kg milk fat, respectively. Higher levels were found in mothers (N=12) nursing neonates with impaired neurodevelopmental competencies or an inappropriate arousal reaction. No difference was observed between mothers nursing children with respiratory or gastrointestinal diseases, urinary tract infections or other infectious diseases, anaemias, prolonged neonatal hyperbilirubinaemias or when children were with dermatological findings, congenital malformations or healthy.

Levels of OCPs in human serum samples collected in Croatia have been monitored since 1975 and the levels of total PCBs since 1985. However, no data have been available on the content of individual PCB congeners (38). That is why samples of human blood serum were collected from one group of 14 donors (3 men and 11 women; between14 and 83 years old) from the general population and from another group of occupationally exposed workers (15 samples) employed between 3 months and 28 years in repairing transformers and capacitors. All donors were residents of Zagreb, Croatia. Six indicator PCB congeners (28, 52, 101, 138, 153, and 180) and some OCPs (HCB, HCHs, DDTs) were determined.

Table 22 shows median concentrations of the analysed organochlorines. All serum samples contained PCB-138, PCB-153, HCB, and p,p'-DDE. The six PCB conge-

Table 22 *Concentrations (median, μg/L serum) of PCBs and OCPs in human serum samples collected in Zagreb, Croatia (the number of positive samples is given in parenthesis) (37)*

Compound	General population (N=14)	Exposed workers (N=15)	Compound	General population (N=14)	Exposed workers (N=15)
PCB-28	0.1 (11)	0.4 (10)	HCB	0.3 (14)	0.2 (15)
PCB-52	0.7 (14)	1.6 (14)	α-HCH	0 (0)	0 (3)
PCB-101	0.4 (13)	0.6 (11)	β-HCH	1.2 (14)	0.5 (12)
PCB-138	0.5 (14)	0.9 (15)	γ-HCH	0.3 (14)	0.3 (14)
PCB-153	0.5 (14)	1.3 (15)	p,p'-DDE	3.4 (14)	4.9 (15)
PCB-180	0.3 (14)	0.9 (14)	p,p'-DDD	0.2 (10)	0 (0)
ΣPCB congener	2.4 (14)	6.6 (15)	p,p'-DDT	0.6 (14)	0 (0)
Total PCBs	NA	9 (15)	o,p'-DDT	0 (6)	0 (0)

ners were present at higher levels in the exposed workers than in the general population, but there was no difference between the two groups concerning the concentration of OCPs. All levels of OCPs and PCBs were fully within the concentration range found for the same compounds in the general population over the past ten years. The distribution pattern of PCB congeners in human serum and human milk was shown to correlate well with Aroclor 1260 (39). The sum of the six PCB congeners found in the analysed serum samples was lower than the total PCB content determined with Aroclor 1260. The same was found for human milk samples collected in Zagreb in the same year (40). This indicates that more than six analysed PCB congeners should be determined in order to assess the total PCB body burden.

Estonia

Studies concerning the concentration of OCPs in human breast milk were started in Estonia in 1971, but data about the content of PCBs in human breast milk were missing. The determination of PCBs in the breast milk of Estonian women was performed in the 80s and the results were compared with other Baltic Sea countries (41). Table 23 shows the levels of OCPs and PCBs in Estonian human milk.

Table 23 *Concentrations of OCPs and PCBs in Estonian human milk (41)*

Year	(μg/g wet weight)				$\frac{DDE}{\Sigma DDT} \times 100$ (%)
	DDT	DDE	ΣDDT	PCBs	
1971	0.026 0.004–0.050	0.099 0.021–0.230	0.125	–	79
1974	0.021 0.002–0.080	0.063 0.008–0.180	0.084	–	75
1984	–	–	0.006 0.003–0.011	0.012 0.006–0.017	95

During the years 1974–1984, decomposition of DDT into its main metabolite DDE increased in the human breast milk from 79% to 95%. As DDE is less toxic to human organism than DDT, in 1984 much less OCPs and less toxic compounds got into the organism of newborn children than between 1971 and 1974 (41).

The average OCP intake of a newborn child, weighing 5 kg and consuming 1 kg of milk a day, is 0.001 mg/kg of total DDT and 0.002 mg/kg of PCBs, that is, 5–20 times less of DDT than in the beginning of seventies. Six years after the total ban of DDT in Estonia in 1968, the content of its metabolite DDE in human milk rose up to 75–79%. The amounts of DDT and DDE consumed by newborn children in the first week of life were 5.6 μg of DDT and 15.9 μg of DDE a day. Two to four weeks after the birth, the respective amounts rose to 11.2 μg and 35.4 μg a day.

It was found that in mid-80s the average daily intake of total DDTs and PCBs by newborn children did not exceed the ADI proposed by WHO (for DDTs 0.05 mg/kg and for PCBs concentration 0.07 mg/kg per day, the exceeding of which caused the »Yusho« disease in Japan in 1968 and in Taiwan in 1979).

The potential human exposure of DDTs and PCBs through diet was also studied (41). Some authors claimed that in the Baltic Sea countries human organism received 90% of toxic compounds from fish. By the end 60s, the average DDT daily intake through diet by the residents of Estonia was 0.036 mg. According to certain data, the concentration of DDT in fish reached its peak 10–11 years after the maximum usage of DDT (in Estonia 1965).

If an average 60-kg Estonian ate 30–50 g of fish a day and if the average concentration of biphenyls and DDT in fish is not higher than it was in mid-80s (0.2 mg/kg and 0.1 mg/kg), then the concentration of DDTs and PCBs in the daily nutrition of Estonian residents was 3–5 μg/day and 6–10 μg/day, respectively (that is, if the herring accounted for the main part of the fish diet), that is 0.05–0.08 μg/kg of DDT a day and 0.10–0.17 μg/kg of PCBs a day. Similar results have been obtained in Finland and Sweden (41).

In the beginning of the 80s, the content of DDT in the daily nutrition was 10–75 μg in the former Soviet Union, 2–145 μg in the USA, 27–44 μg in Great Britain, 18 μg in Canada, 52 μg in Italy, 60 μg in the Netherlands, and 60 μg in Bulgaria. At the same time, the content of total DDTs and PCBs in the Baltic fish ranged from 0.017–0.030 mg/kg and from 0.035–0.060 mg/kg, respectively. Daily concentrations of DDTs and PCBs in fish diet decreased to 0.5–1.5 μg/day and 1.1–3.0 μg/day respectively, or 0.008–0.025 μg/kg of DDTs a day and 0.018–0.050 μg/kg of PCBs a day.

These data and an overall no-effect level for toxicity of DDT of 0.25 mg/kg body weight per day in humans suggest that the daily intake on this scale would not involve human cancer risk. Thus, if a 60-kg person consumed 30–50 g/day of fish containing 17–30 μg/kg of DDT and its metabolites, the maximal daily intake would be 0.025 μg/kg body weight. This would constitute 1/800 of the ADI and 1/12,000 of the lowest dose of non-metabolised DDT which caused liver tumours in male mice of the most sensitive strain tested (41).

Polychlorinated dibenzo-p-dioxins and dibenzofurans

Although the world was engaged in professional discussion about the sources of PCDDs/Fs, their fate in the environment, and their influence and risk to various organisms in the CEE countries, concrete information about contamination of human tissues practically does not exist (42). From 1965 to 1968, 80 workers who had worked with 2,4,5-sodium trichlorophenoxyacetate and butylester of trichlorophenoxy-acetate acid became ill (43). The cause of the illness was 2,3,7,8-tetrachlorodibenzo-p-dioxin. This intoxication occurred during the time when alkaline hydrolysis of tretrachlorobenzene at atmospheric pressure was used to increase and shorten the reaction time, and probably during the same time the mother liquor, which was originally disposed of, was put back into production. The concentration of TCDD in the work atmosphere was never measured; however, TCDD was found in final product – Arboricide E – and several years later, was also found in the building and on wall paintings. This contamination is still measurable. It was something as small »Seveso« in the former Czechoslovakia. A 10-year study was conducted for 55 exposed individuals. Most patients developed chloracne and 11 manifested porphyria cutanea tarda. Approximately one-half of the patients suffered from metabolic disturbances, i.e. pathologically elevated lipids with abnormalities in the lipoprotein spectrum, and two-fifths of the patients had pathological changes in the glucose tolerance test. One third of the patients had biochemical deviations indicative of a mild liver lesion. Histological examination revealed light steatosis, or periportal fibrosis, or activation of Kupffer cells. In 17 persons symptoms of nervous system focal damage existed, with predominance of peripheral neuron lesion of the lower extremities. Most patients suffered from various psychological disorders. By 1981 when the results were published, two patients died of bronchogenic lung carcinoma; one of liver cirrhosis; one of a rapidly developed, extremely unusual type of atherosclerosis precipu cerebri, and patients have died in traffic accidents. The conditions of most other patients improved. The measurements of air in the working hall and the content of PCDDs/Fs in walls and soil, were performed a few years ago, but results have never been published.

Czech and Slovak scientist carried out preliminary, informative measurements of contamination of human tissues by PCDDs/Fs and PCBs-dioxin in co-operation with people from CDC Atlanta, USA (42). Samples of human adipose tissue from two different sites of the former Czechoslovakia were used. Seven samples (from Prague and Michalovce) were used to determine PCDDs/Fs, PCBs and HCB, HCHs, and DDTs. The results of PCB and OCC determination confirmed the trends described by *Schoula and co-workers* (4, 11) concerning the higher level of contamination in Michalovce region. The level of planar PCBs were lower than in Poland (44).

Only 2,3,7,8-substituted congeners of PCDDs and PCDFs, especially hepta- and octachlorinated in adipose tissue, were found. This was in agreement with previous studies (45). While there were some differences in congener patterns from some countries (46), the general pattern and levels were similar to previous CDC studies (47). The I-TEQ values for the coplanar PCBs made a major contribution to the total TEQs in these samples which were similar to reports from Japan and Sweden (46). The percentage of PCDF contribution to the total TEQ in these samples was 2–3 times higher than the PCDD contribution, which was very different from data from other countries. The high I-TEQ values for the PCDFs in these samples from the Czech and Slovak Republics were due to the relatively high levels of 2,3,4,7,8-pentaCDF (range 21–44 ng/kg). The average concentration of 2,3,7,8-TCDD was 1.9 pg/g, which is lower than in other industrialised countries. Relatively high concentrations of octa-CDD (100 to 460 ng/kg) were found in human tissues, having in mind that the

Table 24 *Range of concentrations of PCDDs/Fs, PCBs, and OCCs in human adipose tissue from the Czech and Slovak Republics (N=7) (42)*

I-TEQ (ng/kg)			Total I-TEQ (ng/kg)	ΣHCHs (mg/kg)	HCB (mg/kg)	ΣDDTs (mg/kg)	ΣPCBs (mg/kg)
PCDDs	PCDFs	Planar PCBs					
6.4–10.7	12.0–25.2	6.0–32.6	31.3–59.4	0.039–0.251	0.253–3.395	0.565–9.966	1.157–3.525

ΣHCHs – sum of isomers α-HCH, β-HCH, and γ-HCHs
ΣDDT – sum of isomers and metabolites of DDT (o,p'-DDT, p,p'-DDE, o,p'-DDD, p,p'-DDD, p,p'-DDT)

isomer was found in low concentrations in pork and beef. This suggests another source on contamination (Table 24).

WHO/EURO measured the levels of PCBs and PCDDs/Fs in human milk in various European countries including the CEE region. The values of TEQ in pg/g fat for PCDDs/Fs were 18.4 pg TEQ/g fat in Uherské Hradiště and 12.1 pg TEQ/g fat in samples from Kladno. In Slovakia, the values of TEQ were 15.1 pg TEQ/g fat in district Michalovce and 12.6 pg TEQ/g fat in Nitra.

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Sažetak

PERZISTENTNI, BIOAKUMULATIVNI I TOKSIČNI SPOJEVI U ZEMLJAMA SREDNJE I ISTOČNE EUROPE – NAJSUVREMENIJI IZVJEŠTAJ – IZLOŽENOST LJUDI

Ovaj pregledni rad dio je opsežna izvještaja koji opisuje probleme vezane uz perzistentne, bioakumulativne i toksične spojeve (PBT) u srednjoj i istočnoj Europi. Opisani su problemi izloženosti ljudi u zemljama srednje i istočne Europe perzistentnim i bioakumulativnim organskim spojevima koji imaju toksična svojstva, a koji mogu uzrokovati nepovoljne učinke na zdravlje ljudi.

Ključne riječi:
bioakumulativni spojevi, izloženost ljudi, perzistentni spojevi, toksični spojevi

Requests for reprints:

Ivan Holoubek
RECETOX-TOCOEN & Associates
Veslarská 230B, 637 00 Brno, Czech Republic
E-mail: *holoubek@chemi.muni.cz*

Arh Hig Rada Toksikol 2001;52:217–227

CONFERENCE PAPER

ORGANOCHLORINE COMPOUNDS IN HUMAN MILK AND FOOD OF ANIMAL ORIGIN IN SAMPLES FROM CROATIA

BLANKA KRAUTHACKER AND ELSA REINER

Institute for Medical Research and Occupational Health, Zagreb, Croatia

Received January 2001

Human milk and food of animal origin (milk, meat, and fish) have been analysed over several decades for the DDT-complex, PCBs, HCB, and HCH-isomers. Human milk has also been analysed for PCDDs and PCDFs. Human milk samples were collected in six inland sites and one island, and food samples in marketplaces and individual households throughout Croatia. All human milk samples contained DDE and PCBs, and almost all food samples contained the DDT-complex. All analysed pools of human milk contained PCDDs and PCDFs. Levels of most analysed compounds show a decreasing trend over the past decade. The obtained data were the basis for the calculation of the daily intake of organochlorine compounds in breast-fed infants and adults.

Key words:
calculated daily intake, DDT-complex, dioxins, organochlorine pesticides, PCBs, PCDDs, PCDFs,

Organochlorine compounds listed in Table 1 are classified as persistent organic pollutants (POPs) due to their common characteristic, that is, longer persistence in the environment than their intended use (1). DDT, HCH-isomers, and HCB are pesticides; in many countries their use is restricted or even banned. PCBs are industrial chemicals. PCDDs and PCDFs, often referred to as dioxins, are unwanted by-products of various technological processes; they are not produced commercially and have no intended use.

Human exposure to these compounds is mainly attributed to the food chain, and contamination of food occurs through the pollution of the air, water, or soil. Human

Presented at the ICS-UNIDO Workshop on Contamination of Food and Agroproducts, 28 September – 1 October 2000, Varaždin, Croatia

exposure to dioxins occurs mainly in industrialised settings and in areas where waste is inappropriately burned. The toxicity of the listed compounds varies greatly, with 2,3,7,8-TCDD being the most toxic.

Table 1 *List of organochlorine compounds and abbreviations used in this paper*

Compound	Abbreviation
Hexachlorobenzene	HCB
α-hexachlorocyclohexane	α-HCH
β-hexachlorocyclohexane	β-HCH
γ-hexachlorocyclohexane	γ-HCH, lindane
1,1,1-trichloro-2,2-di(4-chlorophenyl)ethane	DDT
1,1-dichloro-2,2-di(4-chlorophenyl)ethane	DDD
1,1-dichloro-2,2-di(4-chlorophenyl)ethene	DDE
DDT + DDD + DDE	DDT-complex
Polychlorinated biphenyls	PCBs
2,4,4'-trichlorobiphenyl	PCB-28
2,2',5,5'-tetrachlorobiphenyl	PCB-52
2,2',4,5,5'-pentachlorobiphenyl	PCB-101
2,2',3,4,4',5'-hexachlorobiphenyl	PCB-138
2,2',4,4',5,5'-hexachlorobiphenyl	PCB-153
2,2',3,4,4',5,5'-heptachlorobiphenyl	PCB-180
Polychlorinated dibenzo-p-dioxins	PCDDs
2,3,7,8-tetrachlorodibenzo-p-dioxin	2,3,7,8-TCDD
1,2,3,7,8-pentachlorodibenzo-p-dioxin	1,2,3,7,8-PeCDD
1,2,3,4,7,8-hexachlorodibenzo-p-dioxin	1,2,3,4,7,8-HxCDD
1,2,3,6,7,8-hexachlorodibenzo-p-dioxin	1,2,3,6,7,8-HxCDD
1,2,3,7,8,9-hexachlorodibenzo-p-dioxin	1,2,3,7,8,9-HxCDD
1,2,3,4,6,7,8-heptachlorodibenzo-p-dioxin	1,2,3,4,6,7,8-HpCDD
Octachlorodibenzo-p-dioxin	OCDD
Polychlorinated dibenzofurans	PCDFs
2,3,7,8-tetrachlorodibenzofuran	2,3,7,8-TCDF
1,2,3,7,8-pentachlorodibenzofuran	1,2,3,7,8-PeCDF
2,3,4,7,8-pentachlorodibenzofuran	2,3,4,7,8-PeCDF
1,2,3,4,7,8-hexachlorodibenzofuran	1,2,3,4,7,8-HxCDF
1,2,3,6,7,8-hexachlorodibenzofuran	1,2,3,6,7,8-HxCDF
1,2,3,7,8,9-hexachlorodibenzofuran	1,2,3,7,8,9-HxCDF
2,3,4,6,7,8-hexachlorodibenzofuran	2,3,4,6,7,8-HxCDF
1,2,3,4,6,7,8-heptachlorodibenzofuran	1,2,3,4,6,7,8-HpCDF
1,2,3,4,7,8,9-heptachlorodibenzofuran	1,2,3,4,7,8,9-HpCDF
Octachlorodibenzofuran	OCDF

We started a monitoring programme of organochlorine compounds in human milk in 1977. Food has been systematically monitored by the Croatian National Institute of Public Health. This paper gives a selection of published data to document our general conclusions.

ANALYSIS OF COMPOUNDS

Compounds in human milk were analysed by gas chromatography and were identified and quantified by comparison with known standards (2). Concentrations of total PCBs were based on Aroclor 1260 as the standard. As the dioxins in human milk could not be analysed in our country due to the lack of equipment, the analysis of pooled samples for seven PCDD congeners and ten PCDF congeners was performed by collaborating laboratories in the USA, the Netherlands, and Sweden. The analysis of milk samples was verified through the analytical quality assurance programmes of the World Health Organization (WHO) in Geneva, the WHO European Office in Copenhagen, or the US Environmental Protection Agency.

Compounds in food were analysed by gas chromatography on capillary columns (3).

SAMPLING SITES

Human milk samples were collected in six inland sites and one island: Zagreb and Sisak (industrialised towns in the north-west of the country), Osijek (town in the Panonian plane on the river Drava close to the Danube), Jastrebarsko and Karlovac (smaller towns south from Zagreb), Labin (small town in the centre of the Istrian peninsula), and the island of Krk in the northern Adriatic. Samples were collected from mothers who were not occupationally exposed to organochlorine compounds, who were resident in the respective towns/sites for at least five years, and who were nursing one child only.

Food samples were collected in marketplaces in various towns throughout Croatia and the fish samples were also collected from individual households.

COMPOUNDS IN HUMAN MILK

All analysed samples contained DDE. Other pesticides or their metabolites (DDT, DDD, HCH-isomers, and HCB) were not found in all samples. DDD and α-HCH were the rarest. All samples also contained PCBs. Of the PCB congeners listed in Table 1, congeners PCB-153, PCB-138 and PCB-180 were found in all samples (2, 4–13).

Most data on the analysed pesticides and PCBs in human milk were obtained from samples collected in Zagreb. Table 2 illustrates the changes in concentrations over years, One can observe a decrease in the levels of DDE, PCBs, HCB, and β-HCH. A comparison of data from Zagreb with those from other sampling sites revealed no significant difference in concentrations of any analysed compound, as illus-

Table 2 *Median concentrations (μg/kg milk fat or *μg/kg milk) of organochlorine compounds in human milk. N = number of analysed samples*

	Zagreb										
Compound	1977/79* N=34	1977/79* N=37	1981/82 N=50	1985 N=18	1986/87 N=41	1987/89 N=22	1987/90 N=40	1990/91 N=30	1991/93 N=54	1994/95 N=54	1996/98 N=43
HCB	–	–	210	210	120	60	31	20	15	11	15
α-HCH	0	0	–	0	0	0	0	0	0	0	0
β-HCH	–	–	280	230	170	40	39	40	24	35	30
γ-HCH	0	0	–	0	60	0	0	0	2	5	16
DDE	30	63	1900	1060	1480	620	491	450	282	247	409
DDD	0	3	–	0	0	0	0	0	0	0	0
DDT	0	0	180	130	70	0	0	0	0	9	9
PCBs	–	–	620	440	450	290	243	230	213	212	145
Ref.	4	4	5, 6	7	7	8	9	7	9	9	Unp.

Table 3 *Median concentrations (µg/kg milk fat) of organochlorine compounds in human milk. N = number of analysed samples (4, 14)*

Compound	Karlovac 1987, N=9	Sisak 1988, N=9	Krk 1986/87, N=33	Labin 1989, N=10
HCB	75	36	100	0
α-HCH	0	0	0	0
β-HCH	45	59	100	50
γ-HCH	0	0	0	0
DDE	600	633	1080	550
DDD	0	0	–	0
DDT	31	0	160	0
PCBs	300	431	500	270

trated in Table 3 for organochlorine pesticides and PCBs in samples from Karlovac, Sisak, Krk and Labin (4, 14).

All pooled milk samples contained PCDDs and PCDFs (15–19). Of the congeners listed in Table 1, OCDD had the highest concentration (between 53 and 116 ng/kg milk fat). In seven pooled samples the concentrations of 2,3,7,8-TCDD ranged from 0.8 to 1.9 ng/kg milk fat. In five samples 2,3,7,8-TCDD was not detected, but the 2,3,7,8-TCDD detection limits in these analyses ranged between 1.0 and 9.6 ng/kg milk fat (18, 19).

Table 4 shows concentrations of dioxins expressed in 2,3,7,8-TCDD toxic equivalents (TEQ) for all analysed pools of human milk. Most data are from samples collected in Zagreb. The dioxin concentrations in samples from Zagreb are about the same as in samples collected in Labin, Jastrebarsko, Osijek, and the island of Krk. We believe that so far no general conclusion can be made concerning changes in levels over the observed period. However, a WHO comparative study of dioxin levels in different European countries drew from our data on milk samples collected in Zagreb and Krk a conclusion that the trend was slightly decreasing in Croatia (17).

A comparison of dioxin concentrations in samples from Croatia with data from other European countries indicates that our concentrations are in the lower half of the European concentration range (Figure 1).

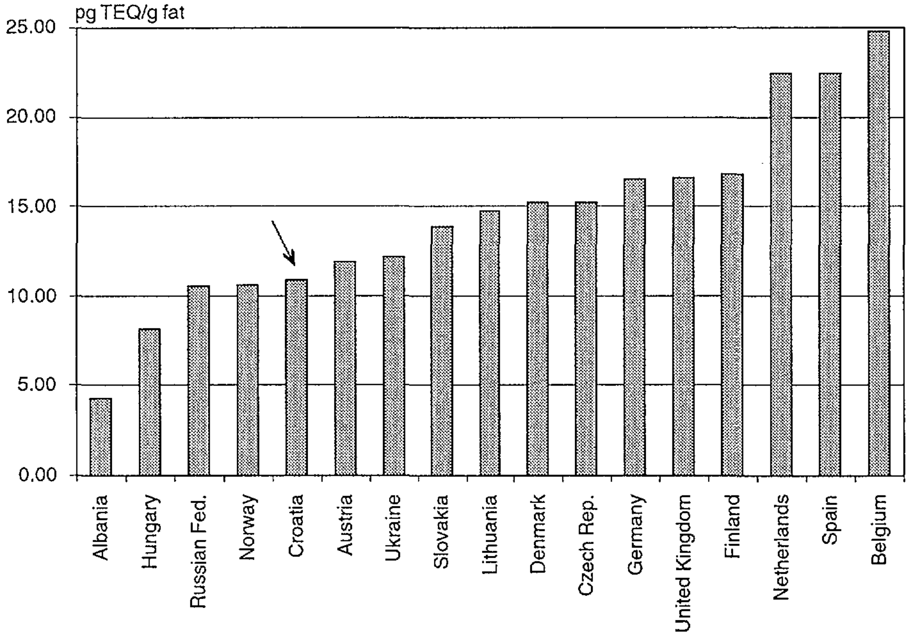

Figure 1 *Dioxin concentrations expressed in TEQ in human milk samples from different European countries collected in 1993 (17)*

COMPOUNDS IN FOOD OF ANIMAL ORIGIN

Food of animal origin has been systematically analysed for organochlorine pesticides for more than forty years. A detailed account was recently published by the Croatian National Institute of Public Health (3) on the analysis of food samples during 1986/89, and the data compared with results obtained from the analysis of food samples during 1999. Samples were analysed for DDT and its metabolites, HCB, and α- and γ-HCH. No samples contained all compounds. The frequency was the highest for DDT and its metabolites (89–98% in 1999), followed by γ-HCH (70–89%). Ten years earlier, those frequencies were about the same. The frequencies of HCB and α-HCH were between 5 and 39% in 1999, which was considerably lower than in 1986/89 (26–70%). Table 5 shows a summary of concentrations found in these samples, from which follows that levels of all compounds were invariably lower in 1999 than in 1986/89.

Table 5 *Mean concentrations (μg/kg fat and for fish μg/kg wet weight) of organochlorine compounds in food samples; number of analysed samples is given in brackets (3)*

Food and year	HCB	α-HCH	γ-HCH	DDT-complex
Fish and fish products				
1986/89 (153)	5	2	25	127
1999 (46)	0.1	0.1	0.5	4.7
Meat and meat products				
1986/89 (733)	3	2	25	75
1999 (80)	0	1	6	62
Milk and milk products				
1986/89 (438)	7	3	24	83
1999 (52)	1	1	6	35

The analysis of PCBs in food has not been very systematical (7, 20–22). Table 6 shows some data on PCBs in fish (20–22). The river Kupa south of Zagreb became polluted with PCBs due to improper storage of industrial chemicals in 1986, and this reflected in increased PCB levels in fish (21). Seawater fish contained lower PCB levels during that period (20).

Table 6 *Median concentrations or concentration ranges of PCBs in food samples*

Food and sampling year	PCBs	Reference
	μg/kg wet weight	
Fish 1987/88		
Seawater (Rijeka, Adriatic sea)	16–120	20
Freshwater (Petrinja, river Kupa)	70–1233	21
Fish 1992/96		
Seawater (Adriatic sea)	46	22
	μg/kg fat	
Pork 1992		
Fat	19	7
Fat tissue	12	7
Poultry 1992		
Fat tissue	14	7
Cow 1992		
Milk	73	7
Butter	20	7

CALCULATED DAILY INTAKE OF ORGANOCHLORINE COMPOUNDS

The calculation of the daily intake of organochlorine compounds by selected population groups relied on data obtained from the analysis of food and human milk and on questionnaires concerning food intake (Table 7).

Table 7 *Calculated daily intake (ng/kg bw) of organochlorine compounds. Individual numbers were calculated from mean or median concentrations in food, while ranges were calculated from the minimum and maximum concentrations*

Food source and population group	HCB	γ-HCH	DDT-complex	PCBs	Reference
Fish (river Kupa)					
Fishermen 1985/88	–	–	0–158	8–3550	21
Fish (from the market)					
Adults 1992	–	–	–	12	22
Adults 1992/96	0.5	5.7	10.0	–	22
Mixed diet 1987/89					
Adult women	2–700	20–600	200–1200	–	7
Nursing mothers	2–1100	30–1200	500–2400	–	7
Human milk					
Breast-fed infants					
1981/82	0–2400	0–200	700–25100	3000	7
1987/95	–	–	1620	1120	23

The intake of PCBs from fish spanned over a broad range, which was attributed to different eating habits of fishermen *vs.* other adults, and also to contamination of the river Kupa (21, 22).

The intake of organochlorine pesticides from mixed food was calculated for two groups of women of the same age: nursing and non-nursing women (7). As expected from their eating habits, nursing women had a higher intake of organochlorine pesticides. The calculated daily intake of organochlorine pesticides for breast-fed infants approached or were even higher than for the nursing women, and the intake of PCBs approached that of the fishermen eating contaminated fish from the river Kupa (7, 21). The daily intake of dioxins for beast-fed infants calculated from the data shown in Table 4 amounts to an average of 65 pg/kg body weight (bw) expressed in TEQ. Expressed in the same unit, the WHO reported in 1988 and 1989 that infants in the European countries consumed on average 70 pg/kg bw (16).

At present, guidelines concerning acceptable daily intakes (ADI) of organochlorine compounds exist only for a very limited number of compounds. Of the compounds discussed in this paper the ADI for the DDT-complex is 20 μg/kg bw (1). The ADI for HCB has recently been withdrawn (1) and there are no ADIs for PCBs (1). However, WHO experts agreed on a tolerable daily intake for dioxins to be between 1 and 4 pg/kg bw expressed in TCDD toxic equivalents (24) and for lindane (γ-HCH) 5 μg/kg bw (25). ADIs and suggested tolerable daily intakes apply to adults for a lifelong consumption, but so far there are no guidelines concerning the intake of any organochlorine compound for infants.

CONCLUSIONS

Studies conducted so far in Croatia have shown that residues of organochlorine compounds discussed in this paper are widely distributed in human milk and food of animal origin. No pronounced difference in levels was observed amongst different parts of Croatia such as rural v. urban or inland v. coastal areas. The levels of the studied compounds are comparable to levels reported in the industrialised European countries.

Having in mind that organochlorine compounds are widely distributed in the biosphere and are very stable, it seems to us that ADIs or tolerable daily intakes should be evaluated for a broad range of organochlorine compounds, including PCBs and dioxins. Some guidelines should also exist for infants whose only food is milk.

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Sažetak

ORGANOKLOROVI SPOJEVI U UZORCIMA HUMANOG MLIJEKA I HRANE ŽIVOTINJSKOG PODRIJETLA SKUPLJENIM U HRVATSKOJ

Humano mlijeko i hrana životinjskog podrijetla (mlijeko, meso i riba) analiziraju se već nekoliko desetljeća na prisutnost DDT-kompleksa, PCB, HCB i HCH-izomere. Humano se mlijeko također analizira na prisutnost PCDD-a i PCDF-a. Uzorci humanog mlijeka prikupljaju se na šest kontinentalnih lokacija i na jednom otoku, a uzorci hrane na tržnicama diljem Hrvatske i u domaćinstvima. Svi analizirani uzorci humanog mlijeka sadržavali su DDE i PCB, a gotovo svi uzorci hrane DDT-kompleks. Svi skupni uzorci humanog mlijeka sadržavali su PCDD i PCDF. Koncentracije gotovo svih analiziranih spojeva postupno su se smanjivale tijekom prošlog desetljeća. Na osnovi rezultata analiza izračunani su dnevni unosi organoklorovih spojeva u dojenčad i u odrasle osobe.

Ključne riječi:
DDT-kompleks, dioksini, izračunani dnevni unos, organoklorovi pesticidi, PCB, PCDD, PCDF

Arh Hig Rada Toksikol 2001;52:229-237

CONFERENCE PAPER

LEAD, CADMIUM, AND MERCURY DIETARY INTAKE IN CROATIA

MAJA BLANUŠA AND DIJANA JUREŠA

Institute for Medical Research and Occupational Health, Zagreb, Croatia

Received March 2001

This paper gives an overview of published data on levels of lead, cadmium, total and methyl-mercury in various food items, and of a daily dietary intake assessment in Croatia focusing on the last 10 years. It briefly describes the most reliable methods for quantitative analysis of lead, cadmium, and mercury in biological material. Lead and cadmium concentrations in vegetables and in organs of domestic animals refer to non-exposed rural areas. Mercury concentrations in fish and mussels refer to industrially polluted and non-polluted areas of the Adriatic. The daily dietary intake of lead and cadmium was assessed on the basis of duplicate-diet-collection and food-disappearance method. The assessment of the total and methyl-mercury dietary intake was based on dietary surveys of family seafood consumption. Lead and cadmium intake through food in the general population was 6–40% of the Provisional Tolerable Weekly Intake (PTWI), depending on the assessment method. The only Croatian population consuming more mercury through seafood is the one living in Dalmatia, approaching the PTWI defined by WHO.

Key words:
atomic absorption spectrometry, duplicate diet, fish, mussel, total and methyl-mercury, vegetable

The major route of exposure of general population to lead, cadmium, and mercury is through air and/or diet. The three metals are very toxic and their absorption and toxicity depends on dose and, among other diet constituents, on intake of essential metals through diet. Few literature data have been reported on the assessment of

Presented at the ICS-UNIDO Workshop on Contamination of Food and Agroproducts, 28 September – 1 October 2000, Varaždin, Croatia

dietary intake of lead, cadmium, or mercury in Croatia (1–4). More documented are the levels of toxic metals in various food items (1, 5–12). Most reports published by the Croatian Public Health Institutes relate to statistical evaluation of compliance of food control of toxicants with the Croatian legislation and not to the assessment of the normal population dietary intake of certain toxic metal pollutants.

The aim of this paper is to review literature data on the levels of lead, cadmium, and mercury measured in food items and on the daily dietary intake assessment in Croatia. Attention will be given to reports published in the last ten years.

METHODS

Most laboratories in Croatia use the atomic absorption spectrometry (AAS) method to analyse lead, cadmium, and mercury in food. The flame (F AAS) or the electrothermal method (ET AAS) are applied to analyse lead and cadmium and the cold vapour method (CV AAS) or the hydride generation method (HG AAS) to analyse mercury. When sampling individual food items to analyse metals (such as a vegetable), it is usually washed under the tap water, and the samples are cut to small pieces. Homogenisation is not necessary. The sampling of the duplicate or the market basket diets, however, requires homogenisation. One sample of duplicate diet contains food and drink consumed over 24 hours and collected from selected individuals. The market basket diet, also called total diet study, has the sample composed of 5–10 cooked or raw food items (13).

The destruction of the organic component of the sample depends on the method of analysis used and on the element analysed. To determine lead and cadmium by ET AAS dry ashing at 450 °C is the procedure of choice for the following reasons; at that temperature the two elements do not evaporate and ashes can be dissolved in a minimum quantity of concentrated HNO_3 and adjusted to a certain volume with deionised water to form the final 1% v/v HNO_3 solution. At this concentration of HNO_3 graphite tubes are lasting longer and the possibility to contaminate samples by large volumes of acid is smaller. Porcelain or glass crucibles should be avoided for dry ashing due to possible contamination with lead from crucibles or adsorption of lead on crucibles (14). Quartz or platinum crucibles are recommended instead.

For mercury analysis, nitric acid is successfully used in wet digestion to destroy biological materials and transform the organically bound mercury to the ionic form. This method involves heating of the sample in concentrated nitric acid up to 80 °C in a closed system. If the sample is heated in an open system, losses of mercury are inevitable (15). Another wet digestion method for mercury analysis involves addition of concentrated nitric acid and the sample treatment in a microwave oven. This method has some limitations when using the Teflon™ tubes, as these can be contaminated with mercury irreversibly. Even quartz tubes show occasional high blank values.

Distilled or suprapur HNO_3 and deionised water of 0.06 μS/cm conductivity should be used throughout the preparation phase. For the internal quality control of analysis during the analytical procedure it is also necessary to use a certified biological material (such as wheat flower, bovine liver, or fish muscle). National or international interlaboratory comparisons are recommended for the external quality control. In most references cited in this review the internal or external quality control procedures were not mentioned. The results in this report are expressed either as μg/kg wet weight of food item, μg/m^3 of air, μg/g of Hg in the hair or as μg/person of daily diet. All values are presented as mean ± standard deviation.

LEAD

The general population is exposed to lead through food, water, and air. Exposure to lead from the air is higher in urban population. Hazards from these sources have gradually diminished in Croatia due to removal of lead from gasoline. Measured concentrations of lead in the Zagreb air show a great decrease in average values over the last 10 years. Values dropped from 0.8 in 1990 to 0.09 μg/m^3 in 2000 (16, 17). The future population exposure to air lead is expected to fall further. However, concentrations of lead in different food items including vegetables are expected to remain at the present level if produced/grown in contaminated areas. Lead concentrations were measured in 81 samples of different kinds of brassicas collected in Zagreb household gardens in 1995–1996. A wide range of values was obtained from 19 to 1,871 μg Pb/kg wet weight with median value of 454 μg/kg (10). Vegetable samples (14 samples of carrot, 17 of parsley, 10 of lettuce, and 18 of potato) collected outside the Croatian capital and far from any possible pollution gave lead concentration values of 11.1±8.7 to 73.8±66.9 μg/kg (Figure 1, unpublished results obtained in our laboratory). Twen-

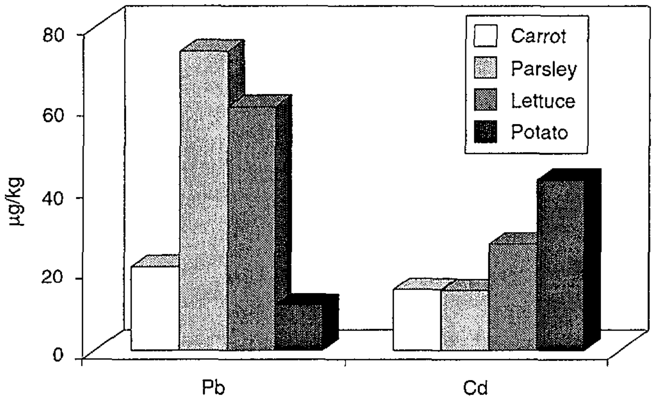

Figure 1 *Lead and cadmium in vegetable collected in rural unpolluted areas in Croatia (unpublished data; see text for details)*

ty-nine samples of the most commonly consumed Adriatic fish (Figure 2) were collected at the Zagreb market during in 2000. Lead concentrations obtained in the fish muscle ranged from 9.6 to 44.1 μg/kg wet weight (12). The concentration in the mussel tissue, however, was about 10 times higher, that is 121 to 150 μg/kg.

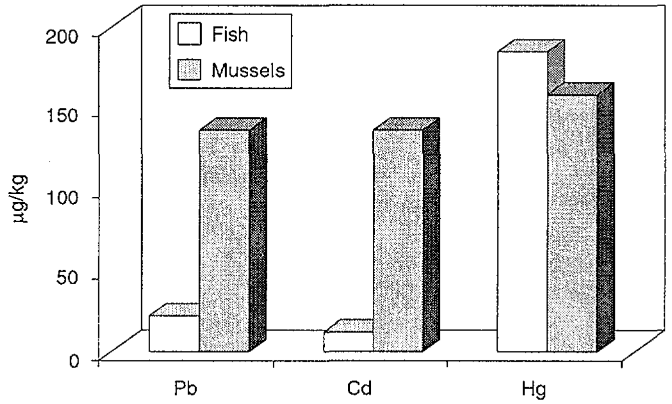

Figure 2 *Lead, cadmium and mercury in common fish and mussels from Adriatic Sea (unpublished data; see text for details)*

A study of daily dietary lead intake was performed during the 80s on a small group of women in Zagreb. The study was a part of a global-scale UNEP/WHO project (2, 18). After the international quality control of the analytical procedures, the results were compared between the participating countries. The method of duplicate diet collection and analysis of 119 samples showed that the daily dietary intake of lead in Zagreb was 15±7.2 μg/person (mean±SD). The findings in other countries varied between 26±7.9 μg/person in Stockholm and 46±18 μg/person in Beijing. Another study of daily dietary lead intake assessment in Croatia relied on lead levels in 19–22 foodstuffs. The composition of the diet and the consumption of the foodstuffs was calculated using the food-disappearance method, which relied on data published in the Statistical Yearbook of the Republic of Croatia. The daily dietary lead intake obtained by this method was 100 μg/person (4).

Absorption of lead from ingested food and water greatly depends on levels of other element present in the diet such calcium, iron, and zinc. It has been shown that dietary deficiencies in these essential elements enhance lead absorption (19). Therefore, when assessing toxic element daily intake of a certain population it is always useful to analyse the intake of other essential elements as well. During the UNEP/WHO project, zinc, copper, iron, manganese, and calcium were analysed in duplicate diets collected in Zagreb and compared to Swedish diets (Figure 3). All analysed elements in the Zagreb duplicate diets were lower than the Recommended Dietary Allowances (20) and significantly lower than in the Stockholm diets (21). The lower blood lead values found in the same study in the Stockholm population, as compared to Zagreb (18), might be partly associated with that difference in the daily intake of essential elements.

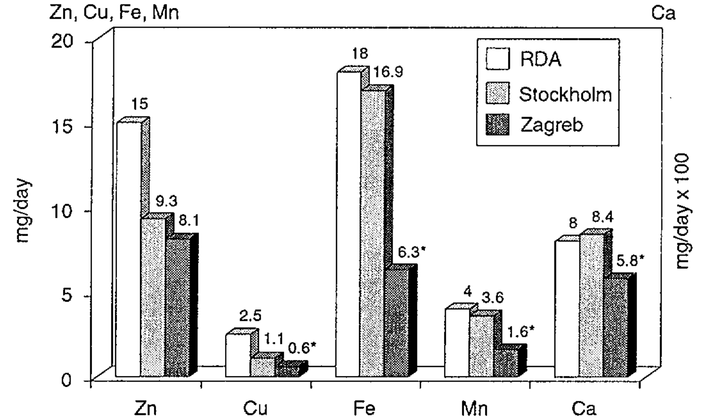

Figure 3 *Daily dietary intake of metals estimated by duplicate diet method (mg/person). *Statistical significant difference between Stockholm and Zagreb data tested by the Student's* t*-test. RDA= recommended daily allowance (adapted from ref. 21)*

Compared to the Provisional Tolerable Weekly Intake (PTWI) of 232 μg/day and adult person recommended by the Joint FAO/WHO Expert Committee on Food Additives (22), the daily dietary intake of lead found in the Croatian population makes only about 6–40% of PTWI, depending on the method applied.

CADMIUM

Cadmium is a nonessential, toxic metal to which humans are exposed through a variety of pathways including food, particularly leafy vegetables, grains, and cereals. The tobacco, itself a leafy plant, contains substantial amounts of cadmium, and its uptake is doubled in heavy smokers (19). Cadmium, which is in general lower in vegetables, muscle-meat, and fish, concentrates in shells and internal organs of different organisms. During the 80s cadmium was measured and reported in muscles, kidneys, and liver of several hundred samples taken from domestic animals (7). The range of cadmium concentrations in the muscles of cattle, pigs, lambs, fattened chicks, turkeys, and freshwater fish ranged from 17 to 102 μg/kg fresh weight. Cadmium in the liver of domestic animals was between 70 and 300 μg/kg and in the kidney from 150 to 650 μg/kg. The level of cadmium in common fish muscle from the Adriatic Sea was 5.1–49.1 μg/kg and in mussels from 130 to 142 μg/kg (Figure 2) (12). Vegetables (carrots, lettuces, and potatoes, 20 samples of each, unpublished results) collected in an non-polluted rural area in 1993–1995. contained 14.9±11.6 to 42.3±16.5 μg/kg wet weight of cadmium (Figure 1).

The UNEP/WHO study with duplicate diet collection showed a daily intake of 8.5±3.8 μg/person of cadmium (2). The other study performed using the food-disappearance method (4) reported the daily intake of 17.3 μg/person. These dietary daily cadmium intake assessments make about 20% of the PTWI (22) which is 65 μg/day and person.

MERCURY

Mercury in the form of methyl-mercury has the highest concentrations in seafood. It is concentrated from the sea with a concentration factor 10^4 to 10^5 (23). It is also considered methylated by micro-organisms in sediments and in marine organisms. Mercury accumulated in the tissues of fish usually takes the form of methyl-mercury (24). It is more toxic than the inorganic form and the population living near the coast and on the islands runs a greater risk of ingesting this highly toxic substance. However, the toxicity of methyl-mercury is moderated by the presence of selenium in fish (19). It has been found that the molar ratio between methyl-mercury and selenium in fish is 0.2–0.4 (25). Due to higher consumption of seafood, mercury monitoring of the Adriatic fish was of special interest for Croatia, as well as the mercury exposure assessment of general population in Dalmatia. Particular attention was paid to the central part of the Adriatic coast which was contaminated by mercury from a chlor-alkali plant. The earliest report on methyl-mercury concentrations in the sediment, mussel, and fish in the polluted area of the Kaštela Bay in the Central Adriatic was given by *Mikac and Picer* (26). They found 2–20 μg/kg of methyl-mercury in the sediment, 10–110 μg/kg in mussels (*Mytilus galloprovincialis*), and 102–1,448 μg/kg in various species of fish. The same area polluted with mercury from a chlor-alkali plant was further studied by scientists from the Institute of Oceanography and Fisheries in Split (8, 9, 25, 27, 28). The values of total mercury mass fractions in the contaminated area ranged from 162±5 to 1,230±70 and in the non-contaminated area from 83±6 to 380±8 μg/kg. Methyl-mercury participated with 70–97% of total mercury in fish (9). Mussels and oysters from the North Adriatic (*Mytilus galloprovincialis Lam.* and *Ostrea edulis L.*) were studied for methyl and total mercury content in different seasons by *Najdek and Sapunar* (6). Methyl-mercury varied from 36.5 to 298.9 μg/kg and total mercury from 58.3–766 μg/kg. The total and methyl-mercury content increased in correlation with the body mass. The mercury content in mussels and the most commonly consumed fish from different uncontaminated areas of the North and Middle Adriatic Sea purchased at the Zagreb market ranged from 142 to 252 μg/kg (Figure 2) (12).

The only report of mean weekly mercury intake of general population in Croatia calculated on the basis of mean annual fish consumption was evaluated by *Bošnir and co-workers* (11). Their calculation gave 19 μg of total mercury and 16 μg of methyl-mercury consumed per person and a week. It amounts to only 6–7% of PTWI (22) for the total and methyl-mercury. However, the population of the Adriatic coast and islands consuming fish 4–5 times a week is likely to have a much higher total and methyl-mercury intake. *Buzina and co-workers* compared the total and methyl-mercury content of seafood and dietary intake in polluted and control areas of the Adriatic (1). The daily intake was evaluated on the basis of dietary surveys of family seafood consumption. The results were compared with PTWI for the total mercury (300 μg Hg per person) and for methyl-mercury (200 μg Hg per person). The respective percentages of subjects from the industrially polluted area whose dietary intake exceeded PTWI was about 20% for the total mercury and 16% for methyl-mercury. In the control area, about 5% of adult male population consumed more than the total mercury PTWI and about 13% above the methyl-mercury PTWI. These values were compared to the values of total and methyl-mercury in hair (3). The concentration of the total mercury found in hair was 1.3–12.9 μg/g and of methyl-mercury 1.1–10.8 μg/g. It was concluded that it did not reach the critical level at which the toxic effect of mercury could be expected.

CONCLUSIONS

The daily lead and cadmium diet intake of general population in Croatia is much lower than the PTWI values proposed by WHO. Population living on the Adriatic coast and islands consumes more total and methyl-mercury through seafood than the inland population and its daily intake of mercury is closer to PTWI.

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Sažetak

UNOS OLOVA, KADMIJA I ŽIVE HRANOM U HRVATSKOJ

Prikazani su podaci o unosu olova, kadmija te ukupne i metil-žive hranom u općoj populaciji Republike Hrvatske. Posebna pozornost posvećena je podacima objavljenim u posljednjih deset godina. Opisane su najčešće primjenjivane metode za kvantitativno određivanje ovih metala u biološkom materijalu. Navedeni su objavljeni i vlastiti neobjavljeni podaci o koncentracijama olova i kadmija u povrću, ribama, školjkama i organima domaćih životinja sakupljenim u neonečišćenim seoskim područjima. Također su navedene koncentracije žive u ribama i školjkama iz onečišćenih i neonečišćenih područja uz Jadransko more. Dnevni unos olova i kadmija hranom procjenjivan je različitim metodama: metodom analize elemenata u sakupljenim uzorcima dvostrukog dnevnog obroka i proračunom iz podataka o potrošnji hrane prema statističkom godišnjaku Republike Hrvatske. Dnevni unos ukupne i metil-žive određen je prehrambenom anketom o navikama uživanja morskih plodova. Zaključeno je da unos olova i kadmija hranom u općoj populaciji iznosi 6-40% od privremeno dopuštene količine tjednog unosa hranom prema Svjetskoj zdravstvenoj organizaciji (*provisional tolerable weekly intake*, PTWI), ovisno o metodi određivanja. PTWI vrijednosti jesu 1.600 µg olova i 450 µg kadmija po osobi. Populacija koja unosi najviše ukupne i metil-žive je ona koja živi u Jadranskom priobalju i na otocima. Njihov unos hranom je blizu PTWI vrijednosti koje iznose 300 µg ukupne žive i 200 µg metil-žive po osobi.

Ključne riječi:
atomska apsorpcijska spektrometrija, dvostruki dnevni obrok, metil-živa, povrće, ribe, školjke

Requests for reprints:

M. Blanuša, Ph. D.
P. O. Box 291, HR–10001 Zagreb, Croatia
E-mail: *mblanusa@imi.hr*

Arh Hig Rada Toksikol 2001;52:239–251

CONFERENCE PAPER

PERSISTENT, BIOACCUMULATIVE, AND TOXIC COMPOUNDS IN CENTRAL AND EASTERN EUROPE – HOT SPOTS

IVAN HOLOUBEK, ANTON KOČAN, IRENA HOLOUBKOVÁ, KLARA HILSCHEROVÁ, JIŘI KOHOUTEK, JERZY FALANDYSZ, AND OTT ROOTS

Recetox-Tocoen & Associates, Brno, Czech Republic

Received November 2000

The sources and environmental levels of the PBTs in the countries of Central and Eastern Europe are broadly described. Most of the countries in the region produce and/or formulate pesticides. The pesticide registration is a primary requirement for import, production and distribution. The special attention must be given to unwanted pesticides. The problem of unwanted and expired pesticides pose the greatest danger to the natural environment and people which is brought about by the use of chemicals in agriculture in CEE countries. Countries still have not solve the problem of safety storage for PBTs and other chemicals classified as poisons and they have no special sites or facilities for destruction of these chemicals. This region has very specific problems of environmental pollution, which are the results of the recent wars. Destruction of industrial facilities and spilling of chemicals have the worst effect for the environment (Bosnia and Herzegovina, Croatia, Serbia and Montenegro).

Key words:
environmental pollution, organochlorine pesticides, PCBs, POPs, transboundary pollution

Organic substances that are persistent, bioaccumulative, and posses toxic characteristics likely to cause adverse human health or environmental effects are called PBTs (persistent, bioaccumulative, and toxic substances). In this context, »substance« means a single chemical species, or a number of chemical species, which form a specific group by virtue of (a) having similar properties and being emitted together into the

Presented at the ICS-UNIDO Workshop on Contamination of Food and Agroproducts, 28 September – 1 October 2000, Varaždin, Croatia

environment or (b) forming a mixture normally marketed as a single product. Depending on their mobility in the environment, PBTs could be of local, regional or global concern (1).

A subclass of PBTs called POPs (persistent organic pollutants) is a group of compounds which are prone to long-range atmospheric transport and deposition (1, 2). The global extent of POP pollution became apparent with their detection in areas such as the Arctic, where they have never been used or produced, at levels posing risks to both wildlife (3) and humans (4).

The UN-ECE initiative, which started within the UN-ECE region (comprising Eastern and Western Europe, Canada, and the USA) in 1992, prepared the Protocol on POPs (2). The Protocol includes 16 POPs and its main objective is to control, reduce, or eliminate discharges, emissions, and losses of POPs. Beside the UN-ECE initiative, a similar programme of the United Nations Environment Programme (UNEP) started in the co-operation with the International Forum for Chemical Safety (IFCS) (5) which included 12 POPs.

The expert groups of both international bodies call for new data on exposure and fate assessment. Especially for data which are available for a particular region, as they should be used in the assessment process. International experts strongly recommend to study the fate and distribution of selected chemicals in different regions using compartment mass balance models which must be verified by the real measured data. The most serious data gap for the prediction of environmental behaviour are degradation rates and their regional variability based on specific transport conditions. More data need to be collected in this area. The main topic of further research are the deposition/emission processes, transformation processes, and bioavailability of POPs and PBTs in terrestrial ecosystems (1).

PBT COMPOUNDS

The sources and environmental levels of PBTs in the countries of Central and Eastern Europe have broadly been described by *Holoubek and co-workers* (6). The first complex description of the state of environmental pollution with PBT types of compounds in Central and Eastern European countries (CEECs) was prepared by *Heinisch and co-workers* (7). Heinisch and his co-workers continued that investigation and published many other reports such as the three-volume comparison study of the situation in Germany, mainly in Bavaria, and the new countries including the Czech Republic (8–10).

This region has very specific problems of environmental pollution, which are the results of the recent wars. The destruction of industrial facilities and spilling of chemicals have had the worst effect for the environment in Bosnia and Herzegovina, Croatia, Serbia, and Montenegro. Furthermore, the bizarre usage of transformer oil as a diesel fuel and anti-lice shampoo containing lindane against pests in gardens seems to have been inevitable (11). Beside the above mentioned sources of contamination in Bosnia and Herzegovina, a specific problem was the so-called pharmaceutical waste. During the war, huge amounts of drugs were received by those countries as humanitarian aid. Part of those drugs had already expired when they arrived, while others were not used for various reasons. The total waste drugs according to the WHO estimation are 800 t. The major part is still not adequately treated in Bosnia and Herzegovina. Disposal such as through incineration and legal and illegal dumps can be the source of environmental contamination, including the one by PBT compounds.

Most countries in the region produce and/or formulate pesticides. Pesticide registration is a primary requirement for import, production, and distribution. During the period of centralised economy in this region, the import was monopolised by relevant state bodies. By the end of the 80s, many private companies and minor distributors were involved in import and distribution of pesticides.

Special attention must be given to unwanted pesticides. Unwanted and expired pesticides pose the greatest danger to the environment and people. Many countries still have not solved the problem of safe storage for pesticides and other chemicals classified as poisons and they have no information concerning the quantity of pesticide and chemical (e.g. PCBs) waste. Many CEE countries have no special sites for dangerous materials or incinerators in which these types of chemicals could be safely burnt.

The potential risk is linked with storage of unused PBT pesticides. In this respect, Poland has evidenced the storage of over 60,000 t of unused pesticides (mixture of different pesticides including different PBT compounds). The situation is probably very similar in many other CEE countries. These were identified as possible hot spots in the region.

Polycyclic aromatic hydrocarbons (PAHs)

The main sources of polycyclic aromatic hydrocarbons (PAHs) in the region are thermal plants and district heating, waste incineration, road traffic and some industrial technologies (e. g. high-temperature coal carbonation, catalytic cracking of crude oil, and aluminium production).

A limited amount of non-carcinogenic PAHs (naphthalene, anthracene, phenanthrene, pyrene, and carbazole) is produced industrially in pure form (DEZA Valašské Meziříčí, Czech Republic). They usually serve as the material for synthesis of dyes, pesticides, and pharmaceuticals (12).

The major sources of PAHs in the Czech Republic (CR) are PAH production and their use as intermediates, production of carbon black, metallurgy, production and use of coke, asphalt, and coal tars, catalytic cracking, heat coal conversion processes, waste waters, landfills, combustion of wastes and fossil fuels, cement production in dry or wet process kilns, petroleum refineries, crematoria, forest fires, and smoking. The situation in other CEE countries is very similar. The annual emissions of PAHs in the former Czechoslovakia were estimated to about 215 t (13) or 378 t (14).

One of the major PAH sources in the Czech Republic are coal-fired stations burning mainly the lower quality brown coal. The coal used in the Czech Republic has in some cases low heat capacity, high content of water and ash, and the sulphur content that can be over 2% (brown coal from northwest Bohemia). The estimated average emission factor of ΣPAHs in Czech power plants (45 μg/kg with the range 25–60 μg/kg) is practically identical with the value of 42 μg/kg found in the UK (15). The annual emission of 1,381 kg (765–1,842 kg/year) represents approximately one third of the amount emitted in the UK.

Among different kinds of coal, sorted brown coal with the average heating capacity of 18 MJ/kg, is mostly used in heating of Czech residential buildings. Sorted black coal, coke, and brown or black coal briquettes are combusted less often. The estimated emission factor of ΣPAHs of 2–50 mg/kg for residential heating with brown coal is comparable with the value of 56.4 mg/kg (15) and the range of 1.8–30 mg/kg reported in the UK (IEA, 1993) and with the range of 3–70 mg/kg found in the Netherlands (16).

The estimated emission of PAHs from residential heating is about 560 t/year. Assuming that the emission caused by residential heating accounts for about 80% of the annual PAH emissions, the estimation for the Czech Republic would be approximately 700 t/year. This value corresponds to the sum of individual major increments (annual emissions from coal-fired stations, residential heating, PAHs and carbon production, and coke production) which is approximately 735 t/year.

Hungary showed a decreasing trend in total annual PAH emissions from 137.1 kt in 1980 to 61.7 kt in 1996. The crucial point was the shutting down of coke production in 1993 (17).

Organochlorine pesticides (OCPs)

Most countries in the region produce and/or formulate pesticides (18, 19). Many country lack the required experience, and a large part of the farming population has insufficient education and training in protection of plants (20).

Pesticide concentrations in the Danube and its tributaries show significant differences between the Danube basin countries in the number and types of detected pesticides (18). The cumulative number of analysed pesticides was 76, but the residues were found of only 36 pesticides. The most frequently detected are organochlorine compounds and triazines. DDT and metabolites, HCH and isomers, and atrazine and metabolites were found in more than 50% of all samples.

Special attention must be given to unwanted pesticides. Unwanted and expired pesticides pose the greatest danger to the natural environment and people. The amount of unwanted pesticides in Poland is estimated to 60,000 t – about 10,000 t in tombs, another 25,000 t in storehouses, and about 25,000 t at private farms (21). The inventory of banned organochlorine pesticides in stocks in Bulgaria in 1996 showed about 35 t, which is a relatively high quantity for the small territory such as Bulgaria (20).

The potential risk is linked with the storage of unused PBT pesticides. In this respect, Poland has evidenced the storage of more than 60,000 t of unused pesticides (a mixture of different pesticides including other PBT compounds). The situation is probably very similar in many other CEE countries (22). These were identified as possible hot spots in the region.

In some countries of the region (such as Bulgaria) it was recognised that concentrations of PBT pesticides decreased in environment to such levels that no further systematic monitoring is needed (20). That is not true for Albania (23) or Romania (18, 19, 24) where relatively high concentrations are still measured in water and sediments (DDT and other chlorinated pesticides). In the Slovak Republic, the measured data have shown high exposure to hexachlorobenzene (HCB) from an unknown source, which resulted critically high concentrations of that compound in human tissue (22, 25–27).

Many pesticides from the UN-ECE list of POPs have never been used in many countries from the region (aldrin, chlordane, mirex, heptachlor, and toxaphen). These were banned or restricted in several countries of the region, as well as in some other countries in the world. Hungary was among the first in the world to ban or severely restrict chlorinated pesticides, that is, as early as 1966 (22).

DDT is no longer used in many countries, including the CEE countries. It was banned in the Czech part of the former Czechoslovakia in 1974 and in the Slovak part in 1976. However, it is still used in some African, Asian, and South American states as a cheap and efficient insecticide. Some countries in the region have inventories of use and import of pesticides. For example, in 1997 Croatia imported more than 503 t of pesticides containing PBTs as active substances (22). Estonia was one of the first countries in the Baltics to ban chlorinated pesticides (in 1968) (28). In 1957, 226 tons of pesticides, mainly DDT and lindane were used in Estonia. As pests do not reproduce in cold climates as much as in warm climates, the usage of pesticides in Estonia did not exceed 0.7–1.0 kg per ha in mid 60s (among them, 0.03—0.06 kg per ha were chlorinated pesticides).

The distribution of DDT, DDE, DDD, DDMU, HCH isomers as well as the PCB congeners in aquatic and terrestrial ecosystems suggest input (i.e. the perpetrator) as well as date of the process. *Heinisch and Kettrup* (29) demonstrated it on the distribution pattern of DDT metabolites after their massive application in forests of the German Democratic Republic (GDR) in 1983/4 and compared it with earlier applications and with the distribution pattern of HCH isomers in the surroundings of factories or as consequences of lindane applications.

The situation in the former GDR is not discussed in this report, but that application influenced the contamination levels in the former Czechoslovakia and the described approaches serve as a very useful tool to interpret the history and the state of contamination. The comparison of the levels of contamination was published in the Report of IOC GSF, Neuherberg, Germany (8–10).

More than 20 years after the severe restrictions of DDT worldwide, increased contamination levels can still be found in different matrices. A simple substance distribution pattern of the DDT parent substance and metabolites is able to suggest the source. We will describe two examples.

In 1983/4, 260,000 ha of forests of the former GDR were treated with high amounts of DDT/lindane preparations using aircrafts to combat the black-arched moth (*Lymanthria monacha*). Before that, in 1975, a gradual ban of DDT was passed, which mainly worked well. The levels of ΣDDTs and the percentage of the parent substance DDT usually decreased after their banning. The decrease in the former GDR from 1976 to 1982 had one simple reason – until 1992 no new DDT was imported into the region.

In 1984 and 1985 not only the ΣDDTs concentrations increased in many observed matrices and commodities, but also the percentage of the parent substance DDT. This suggested a new DDT input in the region from 1983. The impact was detectable not only in the region where it was used, but also throughout the former GDR and former Czechoslovakia, and now 15 years later in the Czech Republic.

For example the DDT levels in the herring matrix from waters around the Isle of Rügen were elevated and in 1986, the observed value of DDT in herrings reached the maximum as a result of transport up to the Baltic Sea with a time-lag effect. After a sharp decrease in ΣDDT contamination values and in the percentage of the parent substance DDT between 1971 and 1985, there was a sharp increase in 1986, especially in the percentage of the parent substance DDT.

In the south-east GDR – the district of Chemnitz (the former Karl-Marx-Stadt) received about 10% of DDT/lindane preparations used for combating *Lymanthria monacha* in 1982–1985 – there was apparently a drift leading to »co-treatments« of the North-Bavarian border areas. In 1983 and 1984, the used amounts of DDTs were 120 and 480 t, respectively. Using the analysis of kidney fat of deer and red deer in 1985, *Hecht* found clear gradual loads of ΣDDTs from the north to the south in 1996 (7).

Other border-crossing co-treatments have been registered in the former Czechoslovakia. Table 1 describes higher levels ΣDDT residues with high percentages of DDT in soil samples from the boundary mountain Krušné hory (Erzgebirge Mountains), Krkonoše Mountains, Lužické hory and in the north-east Bohemian cities of Ústí nad Labem, Teplice, and Karlovy Vary. These levels decreased from the former GDR towards the inner parts of the Czech Republic (Prague, Kladno, Príbram, South Bohemia, and the south border Šumava Mountains). The trend is evident in the levels of ΣDDTs, and mainly the percentages of the parent substance markedly decrease, which means the SDDT residues were of older origin. Especially low were the ΣDDT

Table 1 *ΣDDTs and percentages of the parent substance DDT in soil samples from the Czech Republic (ng/g). Sampling took place in 1995 (6, 44)*

Sampling site	DDT	DDE	DDD	ΣDDTs	% DDT in ΣDDTs*	References
Mumlava, Krkonoše, 1,190 m	33	8	n/a	41	80	(44)
Pudlava, Krkonoše, 1,140 m	179	70	n/a	249	72	
Pašerácký chodníček, 1,310 m	99	36	n/a	135	73	
Jedlová, Lužické hory, 710 m	302	84	n/a	386	78	
Lesná, Krušné hory, 800 m	2,390	795	n/a	3,185	75	
Načetín, Krušné hory, 710 m	4,013	1,164	n/a	5,177	78	
Ústí nad Labem (M)	1,133	1	1	1,135	100	(6)
Teplice (M)	1,207	146	256	1,609	75 (89)	
Karlovy Vary	398	122	36	556	72 (77)	
Sokolov (M)	522	209	40	771	68 (71)	
Cheb (M)	264	167	25	456	58 (61)	
Kladno (M)	63	53	7	123	51 (54)	
Praha (M)	1,044	1,054	1	2,099	49 (50)	
Mníšek pod Brdy (M)	651	245	4	900	72 (73)	
Příbram (M)	28	31	1	60	47 (47)	
Boubín, Šumava	11	7	n/a	18	61	(44)
Český Krumlov, M	1	1	1	3	33 (50)	(6)
Prachatice, M	1	1	1	3	33 (50)	
Košetice	0.2	9	0.2	1.3	15	

(M) – maximal value from 5–60 samples
* number without parantheses – original number of *Heinisch and Kettrup* (8);
in parantheses – ΣDDTs=DDT+DDE, same values for all samples from various authors (6, 8, 44)

residues and the percentage of the parent substance in the south of the Czech Republic, in Šumava Mountains, in the towns of Český Krumlov, Prachatice, and in the area of TOCOEN/CHMI regional background observatory Košetice.

The total amount of the technical DDTs used in Poland from 1974 to 1980 was 48,152 t and the annual rate was up to 3,881 t. HCB was imported and used in Poland as fungicide and the application rates were relatively small (the total import was 187.6 t between 1962 and 1972) (29).

The soil contamination by hexachlorocyclohexane and consequently the HCH cumulation in agricultural products were of great interest due to their long persistence in our ecosystem (30). In the Slovak Republic, hexachlorocyclohexane was synthesised in the former CHZJD factory in Bratislava. During the years 1956–1966 the factory produced more than 13,000 t of γ-HCH. Some additional raw HCH was imported from the former Yugoslavia. After isolation of the gamma isomer, it was formulated into different pesticides in same factory. The produced pesticides were partly intended for domestic use. A very rough estimate of the annual consumption of lindane-containing pesticides in the Slovak Republic was about 1 t/year. That amount gradually decreased. Lindane-based formulations also dropped. For instance, the List of Permitted Pesticides for 1972 quoted 11 pesticides on the basis of lindane. Those were insecticides for the most important crops (potatoes, beet roots, rapeseeds, and hops), staining agents for treating seeds of cereals, maize, legumes, sugar beet, cucumber, cotton, hemp, rapeseed, water melon, soil desinsectants for growing beet roots, sugar beet, cereals, maize, tobacco, hops, young fruit, trees and vines, and fumigant.

The application of all these pesticides was possible only with some restrictive precautions. The wide and systematic utilisation of lindane containing insecticides was banned in 1974. The year 1992 was the last to see permitted use of lindane seed treating agents, such as formulations Lindane WP 80 for rapeseed and Lindane 50/35 WP for flax and hemp seeds. The List of Permitted Pesticides for 1993 included a new lindane-containing pesticide Emdenit, intended for controlling pine insects, but only for two years.

HCB had been used worldwide as a fungicide for agricultural purposes since 1915. That compound was also registered in Poland and sold as a fungicide under the trade name »Snieciotox«. Around 1980 HCB became unpopular and finally was withdrawn from agriculture in Poland (31).

Pentachlorobenzene (PeCBz) is a substrate used for the synthesis of the fungicide pentachloronitrobenzene (PeCNBz) and, apart from this application, it has not found any other special application. Pentachlorobenzene, like HCB, is a technical impurity in some chemical formulations (up to 0.06% in technical formulation of HCB, and was also found in technical formulation of PCNBz). Another source of environmental pollution with HCB and PCBz are high temperature processes such as municipal solid waste incineration. All theoretically possible congeners of chlorobenzene were identified in flue gas and fly ash from the municipal solid waste incinerators, and the contribution of PCBz and HCB was up to ~50% and ~13%, respectively. Hexachlorobenzene is highly persistent under environmental conditions. Nevertheless, depending on the environmental matrix, HCB slowly undergoes abiotic (photochemical) and biotic degradation (it is mainly metabolised by bacteria and lower fungi in soil and sediments as well as by man and animals). Pentachlorobenzene is an intermediate product of metabolism of HCB and lindane (γ-HCH).

Polychlorinated biphenyls (PCBs)

In the former Czechoslovakia, PCBs were manufactured in a chemical plant in eastern Slovakia under the commercial name Delor from 1959 to 1984. From the total 21,482 t produced (and about 1,600 t of PCB wastes), 46% was exported and the rest was intended for the home market. In both the Czech Republic and Slovakia, PCB formulations may currently be used only in closed systems and they are being gradually replaced. Currently, waste landfills are considered to be the most relevant source of environmental pollution by PCBs in these countries. The estimated contribution of applied paint to total PCB pollution in Slovakia is about 5% and that of industrial and municipal waste incinerators 9%.

It was recently rather unknown that Poland had its two own technical PCB formulations – Chlorofen, which is similar in appearance and composition to Aroclor 1262, and Tarnol, which is similar to Aroclor 1254 (32).

Tarnol, also called Chlorowany bifenyl, was a low chlorinated technical PCB formulation manufactured between 1971 and 1976 by the company Zakłady Azotowe in Mościce near the city of Tarnów in south-east Poland (31, 33). The mixture is similar in its physical appearance and properties to the well-known foreign technical PCB mixtures such as Aroclor 1248, Clophen A 40, Phenoclor DP-4, Fenchlor 42, or Kanechlor 400. Tarnol was the product of the »anti-import philosophy«, which was of the government agendas in the 1970s. The total quantity of manufactured Tarnol is 679 t. Tarnol was a colourless clear liquid of density 1.45–1.47 g/ml at 20 °C. The chlorobiphenyl isomer and congener composition of Tarnol are unknown in detail. According to the manufacturer, this mixture was composed mainly of trichlorobiphenyls with di-, tetra- and pentachlorobiphenyls as minor constituents. Nevertheless, the composition of Tarnol was not confirmed using the capillary gas chromatography and low/high resolution mass spectrometry (HRGC-LR/HRMS) for analysis. No official data on the use of the Tarnol were released – it seems that it was used exclusively as dielectric fluid mainly for transformers, but its use as a dielectric in capacitors is also likely.

Chlorofen was a highly chlorinated (63.6% Cl) PCBs formulation manufactured in the town of Ząbkowice Śląskie in the south of Poland. The mixture was a light to dark-brown sticky and viscous resin mainly composed of PCB congeners with 5–9 chlorine atoms that comprised 99.55% of total PCBs. The average number of Cl per biphenyl molecule in Chlorofen is 7.3 and the average molecular weight is 405.4. Chlorofen contains at least 59 PCB congeners with the major components such as PCBs nos. 153 of hexa-, 176, 180 and 187 of hepta-, 194, 195, 198, 201/196 of octa-, and 206 of nonachlorobiphenyls.

Industrialised countries such as the USA and Western European countries have some legislative measures to control the flux of PCBs in the environment. One important aspect is control of PCB sources such as transformers, capacitors, and electric motors. The materials containing more then 50 $\mu g/g$ are subject to regulations. These regulations have been adopted by the CEE countries. In Poland, the waste oils containing PCBs have been included on the list of hazardous substances since 1993, but the flux of these pollutants has not been the subject of any regulation to the present day (34). Recent data (35) indicate that the national power plants use about 1,400 t of transformer and capacitor oils. However, the amount of the waste industrial oils (transformers, capacitors, motors etc.) cannot even be estimated. An assessed percentage of PCB-contaminated equipment is: transformers (0.38%), capacitors (35–50%), and other electromagnetic equipment (25–50%). An assessed amount of PCB contaminated oil/capacitors/other electromagnetic equipment is up to 10,000 t (31). The determination of PCB levels in random samples of waste motor and transformer oil collected from different regions in Poland showed that these concentrations did not exceed the limit value of 50 μg/g in most samples (34).

Although other countries of the region did not produce PCBs, they can still be found in many closed systems, dumps, and environmental matrixes. For example, Croatia imported more than 2,000 t of PCB oils from various countries in 1997 (36).

A part of PCB amounts from various countries of the region was exported to France for destruction. Part of the PCB amount was destroyed legally and a part probably illegally during the period of main economic changes in the early 1990s. Unknown amounts of PCBs are still in various environmental compartments. The recent inventory of PCBs in Slovakia (37) gave the following actual PCB equation for this country:

PCBs (wastes from production – 1,606 t)+PCBs (products – 4,071 t)=PCBs (still used – 960 t)+PCBs (eliminated – 368 t) + PCBs (disposed – 1,605 t) + PCBs (rest – 2,744 t).

In Croatia, there are 405 users of 22,532 PCB capacitors and 293 users of PCB transformers (36).

The use of PCBs in Slovenia increased after 1960, when ISKRA condenser factory was built in Semič, Bela Krajina (about 80 km south-east from the capital Ljubljana) (38). PCBs were introduced into the production process in 1962 (until 1970 Clophen A-50 and A-30 supplied by Bayer, the former Federal Republic of Germany, and between 1970 and 1985 Pyralen 1500 supplied by Prodelec, France). The consumption of PCBs by ISKRA in 1962–1985 totalled about 3,700 t with a PCB waste rate of 8–9% in the form of waste impregnates, condensers, and so on. By 1974, 130 t of waste containing about 70 t of pure PCBs were dumped on various waste sites within five km from the factory. After 1975 waste impregnates were collected and sent to France for treatment (170 t), whereas smaller waste condensers were still disposed of at local waste sites. Measurements in 1982 showed very high concentrations of PCBs in environment (air, water, sediments) as well as in food and in animal and human tissues (39).

Polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDDs/Fs)

The main sources in the region of PCDDs/Fs, mainly by-products of chemical industry, are typical for industrialised countries: production of chlorophenols and their derivatives (former Czechoslovakia, USSR, GDR, Poland, probably Romania), processes using chlorinated catalysts and solvents used such as pulp and paper production, bleaching based on chlorine treatment, metallurgy, where metal chlorides are used, magnesium production, metal scrap recycling, municipal, hospital, hazardous, and industrial waste incineration; solid fossil fuel combustion (coal, wood, peat), internal-combustion engine operation, leaded petrol use with the addition of chlorinated compounds; dry distillation (dry cleaning), and fires (forest, agriculture).

Despite the two decades of worldwide interest and intensive studies of PCDDs/Fs, there are practically no data available on these compounds in environmental matrices in the CEE countries. Limited information exist in the Czech Republic, Slovakia, Poland, Slovenia, whereas other countries lack information altogether (40).

The knowledge of sources of environmental pollution and emission rates of PCDDs/Fs in Poland is also described as extremely limited (41). In the past, some technical products potentially contaminated with dioxins were used. For example, a popular wood preservative of the Xylamit series contained technical pentachlorophenol (similar products were also seen in the former Czechoslovakia), and Masc grzybobójcza (fungicidal ointment) contained waste products of the distillation of technical chlorophenols and was used for technical purposes. Technical pentachlorophenol (e.g. Antox), and herbicides such as 2,4-D; 2,4-DP; MCPA, MCPP, and Dicamba were also used in the past in Poland. Some efforts have been undertaken to elucidate the status of PCDDs/Fs in some of those formulations. However, detailed composition and concentrations of contaminating dioxin residues remain unknown.

Polychlorinated naphthalenes (PCNs)

Virtually nothing is known about the type and quantity of PCNs potentially manufactured in the CEE countries. From PCB formulations produced in the former Czechoslovakia and USSR, we can roughly assess a 0.0067% content of PCNs in the PCB mixtures. In the case of the former Czechoslovakia, we can guess the potential release into the environment of about 1.6 t of PCNs during between 1959 and 1984, and secondary inputs a year after the ban (from disposals, combustion, incinerators, contaminated soils, and sediments).

1-chloronaphthalene was utilised in Xylamits, a popular wood (and other purpose) preservative widely applied in the past in Poland and also containing technical pentachlorophenol together with waste products of chlorophenol distillation and other substances (42).

2-chloronaphthalene was produced and used as a solvent in Poland (43). It is possible that in the chemical plant in Tarnów-Mościce in southern Poland (the manufacturer of the technical PCB formulation Tarnol) some higher chlorinated naphthalenes were produced on a small scale in the past (1930–1950?).

Acknowledgement This paper was produced as part of the following projects: TOCOEN (Toxic Organic COmpunds in the ENvironment), BETWEEN (The relationships BETWEEN environmental levels of pollutants and their biological effects), and research projects which are funded by the Ministry of Education, Czech Republic ENVIRONMENT – CARCINOGENESIS – ONCOLOGY (CEZJ071400003).

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Sažetak

PERZISTENTNI, BIOAKUMULATIVNI I TOKSIČNI SPOJEVI U ZEMLJAMA SREDNJE I ISTOČNE EUROPE - KRITIČNA MJESTA

U radu su iscrpno opisani izvori i razine u okolišu perzistentnih bioakumulativnih i toksičnih spojeva (PBTs) u zemljama srednje i istočne Europe. U većini zemalja te regije proizvode se i/ili formuliraju pesticidi. Registracija pesticida osnovni je uvjet za uvoz, proizvodnju i distribuciju. Posebna pozornost mora se posvetiti nepoželjnim pesticidima. Nepoželjni pesticidi i oni kojima je istekao rok upotrebe najveća su opasnost za okoliš i ljude, uzrokovana primjenom kemijskih sredstava u poljoprivredi u zemljama srednje i istočne Europe. U većini zemalja još nije riješen problem sigurnosti skladištenja PBTs-a i ostalih kemikalija klasificiranih kao otrovi, a također nema posebnih mjesta ni uređaja za uništavanje takvih kemikalija. Ova regija ima i specifičan problem onečišćenja okoliša kao posljedicu nedavnog rata. Razaranje industrijskih postrojenja i rasipanje kemikalija ima najgori učinak na okoliš (Bosna i Hercegovina, Hrvatska, Srbija i Makedonija).

Ključne riječi:
onečišćenje okoliša, organoklorovi pesticidi, PCB, perzistentni spojevi, prekogranična onečišćenja

Requests for reprints:

Ivan Holoubek, Ph. D.
Recetox, Department of Environmental Chemistry and Ecotoxicology
Veslarská 230B, 63700 Brno, Czech Republic

Arh Hig Rada Toksikol 2001;52:253-280

CONFERENCE PAPER

OVERVIEW OF REMEDIATION TECHNOLOGIES FOR PERSISTENT TOXIC SUBSTANCES

ANDREA LODOLO, EDUARDO GONZALEZ-VALENCIA, AND STANISLAV MIERTUS

International Centre for Science and High Technology, United Nations Industrial Development Organization, Pure and Applied Chemistry, Trieste, Italy.

Received October 2000

This paper gives a review of established and emerging technologies for the treatment of wastes and soils contaminated by Persistent Toxic Substances which include the Persistent Organic Pollutants. The technologies are classified as biological, physico-chemical, and thermal treatments, describing main unit operations and comparing technical, social and environmental limitations, including some potential risks and environmental impacts. Estimated overall costs, cleanup times, reliability, and maintenance levels are also presented in order to assess advantages and limitations of each technology.

Key words:
PCBs, pesticides, POPs, PTS, remediation, soil, treatment, waste

Remediation of polluted sites has become a crucial issue because of the increasing global awareness of pollution, overexploitation of natural resources, and adverse effects of unclean processes on environment and human health. This has increasingly led to the issuing of tighter norms and regulations related to storage, transportation, treatment, and disposal of liquid and solid waste, as well as remediation of contaminated sites. These measures are also significantly influencing the development and the use of hazardous compounds to comply with regulatory limits.

Presented at the ICS-UNIDO Workshop on Contamination of Food and Agroproducts, 28 September – 1 October 2000, Varaždin, Croatia

Stringent cleanup standards are being directed to protect soil, vegetative and ecological systems and to prevent contamination of groundwater.

The purpose of this paper is to review the existing technologies for the treatment of waste and PTS contaminated soils, presenting their limitations and some technical, environmental, social and economic criteria for the evaluation and the selection of a suitable technique. This paper, of which the first part overviews general properties of PTS, is mainly focused on remediation technologies addressing problems generated by already produced PTSs (e.g. pesticides and PCBs). Technologies to prevent PTS formation and effects (such as dioxins and furans) are not dealt with in this review.

Some key criteria for selecting an appropriate remediation approach are presented in the section about remediation technologies, including overall costs, cleanup time, reliability, and maintenance. The technologies discussed have been classified as »established« and »emerging/innovative« in order to differentiate between those which have demonstrated their full-scale application and those which have proved their efficiency on a laboratory or pilot level only.

Each section is divided in physico-chemical, thermal, and biological techniques, although some technologies combine thermal and chemical or thermal and biological methods. Each technology is basically described in operational conditions and through unit operations. The main limitations of each technology are also presented and classified as technical/economical, social, and environmental (including environmental impacts and safety risks). The list of technologies discussed is not exhaustive since many are currently being developed and others are variations of the existing ones.

Finally, some recommendations and conclusions are presented in order to stress the importance of considering both ratable and non-ratable criteria during the selection of technologies and while assessing and performing PTS remediation projects.

SUMMARY OF PROPERTIES OF PTS

PTS is a group of substances with specific characteristics of persistence, bioaccumulation, and toxicity. The group of PTS is integrated by the POPs (Persistent Organic Pollutants) and some inorganic compounds (mercury, cadmium, lead, and compounds). Table 1 gives a list of PTSs. POPs are highly stable organic compounds used as pesticides, herbicides, fungicides, or in chemical industry. They are also generated as byproducts of combustion and industrial processes. They persist in the environment, accumulate in the fatty tissues of living organisms, and are toxic to humans and wildlife. POPs are typically semi-volatile; they travel long distances and condense over colder regions of the planet. They are classified through lipophilicity, persistence (resistance to photolytic, chemical, and biological degradation), and toxicity.

The Convention on Long-Range Transboundary Air Pollution (LRTAP) has defined criteria and procedures for adding substances to the Protocol on Persistent Organic Pollutants. A party proposing to add a substance to the list of POPs must provide the LRTAP Executive Body with a risk profile on that substance and information related to these four characteristics (1):

Table 1 *List of Persistent Toxic Substances*

Aldrin	Dieldrin	Hexachlorobenzene, HCB
Chlordane	Endrin	Mirex
Chlordecone	Heptachlor	Polychlorinated biphenyls (PCBs)
DDT	Hexabromobiphenyl	Toxaphene
Dioxins and furans	Polychlorinated napthalenes	Polycyclic aromatic hydrocarbons
Polychlorinated benzenes	Polychlorinated paraffins	Kepone (chlordecone)
Polychlorinated phenols	Polybrominated compounds	Lindane
Isodrin	Parathion	Malathion
Alkyl-lead	Mercury and compounds	Octachlorostyrene
Dinitropyrene	Cadmium and compounds	Benzopyrene

- ☐ the potential for long-range transboundary atmospheric transport; vapour pressure below 1,000 Pa and an atmospheric half-life greater than two days or monitoring data that evidence the substance is found in remote regions.
- ☐ persistence; a half-life in water greater than two months, soil and sediment half-lives greater than six months or, alternatively, evidence that the substance is otherwise sufficiently persistent to be of concern.
- ☐ bioaccumulation; evidence that the fish bioaccumulation factor is greater than 5,000 or the log Kow is greater than 5 or, if those values are not achieved, other factors that could make the substance of concern.
- ☐ toxicity; potential to affect human health and/or the environment.

After a technical review, the parties to the protocol meeting within the LRTAP Executive Body decide by consensus whether a substance is within the scope of the protocol and whether to adopt the proposal to to add that substance in the protocol.

REMEDIATION TECHNOLOGIES

Waste and soil remediation technologies can be classified according to their development status. »Established technologies« are those having demonstrated full-scale applications and removal efficiencies whereas »emerging/innovative technologies« refer to methods having few or no reported full-scale applications, but have a proven pilot- and laboratory-scale removal efficiency.

Tables 2 and 3 present some criteria that can be considered to select a remediation technology. The overall cost includes design, construction, operation, and maintenance of the remediation program, but it does not include transportation costs (if it is done off-site), previous assessments or post treatment costs, and manpower (since this cost considerably varies from country to country). The reliability and maintenance criteria refer to the level of process complexity and the ease to maintain it.

Other criteria should also be considered when selecting a remediation technology, such as the technique's ability to clean up to a desired level (minimum pollutant concentration achievable by the technology), community acceptability, applicability, post-treatment costs, soil quality required after the intervention (in case of soil pollution), environmental impacts, and risks of remediation activities/processes.

Table 2 *Some criteria to assess established remediation technologies*

Remediation technology	Overall cost * (USD/ton)	Clean-up time	Reliability and maintenance (level)
Physico-chemical			
Landfill cap system (in or ex)	N.A.	–	Varies
Vapour extraction (in)	S	M to L	Average
Vapour extraction (ex)	S	M to L	High r. and low m.
Solidification/Stabilization (ex or in)	S to M	S to M	High r. and low m.
Thermal technologies			
Combustion systems (ex)	M to L	S to M	Average
Thermal desorption (in or ex)	S to M	S to M	Average
Pyrolysis (ex)	M to L	S	Average
Biological technologies			
Bioventing (in)	M	M to L	Low r. and high m.
Composting (ex)	M	M to L	Average
Biopiles (ex)	S	S	Average
Land farming (ex)	S	M to L	High r. and low m.

(in)=*in situ* (ex)=*ex situ*; S=short term <6 months, M=medium, 6 to 12 months, L=long, >12 months
*Cost (USD): S<$150, M=$150–$300, L>$300
r.=reliability; m.= maintenance; T.D.=thermal desorption; N.A.=not available

Table 3 *Some criteria to assess innovative and emerging remediation technologies*

Remediation technology	Overall cost * (USD/ton)	Clean-up time	Reliability and maintenance (level)
Physico-chemical			
Base catalysed dechlorination (ex)	M to L	S	Average
Electrochemical oxidation (in)	M to L	S to M	Low r. and high m.
Solvent extraction (ex)	M to L	S to M	Average
Solvated electron (ex)	L	S	Average
Supercritical water oxidation (ex)	S to M	S	Average
Solar detoxification (ex)	N.A.	S to M	N.A.
Gas phase chemical reduction (ex)	L	S to M	High m.
Catalytic hydrogenation (ex)	N.A.	S	High r. and low m.
Thermal technologies			
T. D. – Catalysed dehalog. (ex)	M to L	S	Low r. and high m.
T. D. – Pyrolysis (ex)	L	S	Average
T. D. – Retort system (ex)	M to L	S to M	Average
Plasma ARC Systems (ex)	L	S	Average
Vitrification (in or ex)	M to L	S	High r. and low m.
Biological technologies			
Bioslurry (ex)	M	S to M	Average
Enhanced bioremediation (in)	S	L	Low r. and high m.
Phytoremediation (in)	S to M	L	Average

(in)=*in situ* (ex)=*ex situ*; S=short term <6 months, M=medium, 6 to 12 months, L=long, >12 months
*Cost (USD): S<$150, M=$150–$300, L>$300
r.=reliability; m.= maintenance; T.D.=thermal desorption; N.A.=not available

ESTABLISHED TECHNOLOGIES – PHYSICO-CHEMICAL TECHNIQUES

Table 4 shows the main limitations of physico-chemical techniques established so far.

Landfill cap system

Landfill capping is one of the most common forms of remediation technologies. It is used to cover buried waste materials in order to prevent contact with the environment and to effectively manage the human and ecological risks associated with a remediation site. The design of landfill caps is specific and depends on the intended functions of the system. The most critical components of a landfill cap are the barrier layer and the drainage layer. Landfill caps can range from a one-layer system of fertile soil to a complex multi-layer system of soils and geosynthetics. In general, less complex systems are required in dry climates and systems that are more complex are needed in wet climate; the system complexity also depends on the type of waste (phase,

Table 4 *Main limitations of established physico-chemical technologies*

Landfill /Cap Systems	Solidification/Stabilization	Vapour extraction (SVE)
Technical/Economic		
The toxicity is not reduced and pollutants are not destroyed with these methods	The solidified material may hinder future site use if carried out *in-situ*. The process is not effective in immobilizing organic waste.	Low permeabilities, high humidity content and soil heterogeneity limit the performance. The method is only suitable for medium to high volatile compounds.
Social		
In some cases this methods may attract public opposition.	In some cases this methods may attract public opposition.	Usually does not attract public opposition.
Environmental/Risk		
Precautions must be taken to ensure the cap is not damaged by land use activities. Several semivolatile pollutants may evaporate more rapidly with increased moisture in soils and sediments (2). Potential leaking of hazardous compounds.	Precautions must be taken to minimise components leaching from stabilised media. Environmental conditions may affect the long-term immobilization of contaminants. There is no reduction of pollutants toxicity.	Potential releases of hazardous compounds during excavation and materials handling. Exhaust air from SVE requires secondary treatment.

hazardous or not hazardous, etc.). The materials used in the construction of landfill caps include low- and high-permeability soils and low-permeability geosynthetic products. The low-permeability materials prevent water to pass into the waste. The high permeability materials drain and collect water that percolates into the cap.

Landfill caps may be temporary or permanent. Temporary caps can be installed before permanent closure to minimise generation of leachate until a better remedy is performed. These caps are usually used to minimise infiltration when the underlying waste mass is undergoing settling. A more stable base will thus be provided for the final cover, reducing the cost of the post-closure maintenance. Landfill caps can also be applied to waste masses too large for other treatments. Disposal in a landfill is not a proper method for liquid pesticides or highly mobile waste. Inorganic pesticides or liquid pesticide waste containing about 5 percent organic material should be solidified or stabilised prior to disposal in a landfill (3).

Solidification/Stabilisation

This method for treating hazardous waste or highly contaminated soils is based on solidification or reduction in mobility of contaminants that are mostly heavy metals. The aim is to prevent contaminated materials from affecting the surrounding environment. The contaminated soil is mixed (either *in-situ* or *ex-situ*) with binding materials such as cement, pozzolanas, thermoplastics, fly-ash, lime-kiln dusts, and low-cost silicate-containing by-products to produce a stabilised mass (solidification) or less solid material that binds liquids and reduces mobility of contaminants (stabilization). The solidified material may hinder future site use if carried out *in-situ*. Environmental conditions may affect the long-term immobilisation of contaminants. The process is not effective in immobilising organic contaminants (4).

Vapour extraction

Soil vapor extraction (SVE) is a well-established, economic and efficient technique for the removal of volatile organic compounds (VOCs) and some halogenated organics. The technology can be used for treating contaminants *in-situ* or *ex-situ*. *In-situ* SVE is performed by means of a vacuum system that uses extraction wells to create a concentration gradient that enhances gas phase volatiles removal from soil through the extraction wells. During *ex-situ* SVE the excavated soil is placed over a network of aboveground piping where vacuum is applied to encourage volatilization of organics. The soil piles may be sealed with geomembranes to avoid volatile emissions and soil saturation due to percolation. This technique has an advantage over *in-situ* method as a result of increased passageways able to collect leachate and a making possible a more uniform treatment (5).

THERMAL TECHNIQUES

COMBUSTION SYSTEMS

High temperature incineration

This has been one of the most applied remediation technologies for the treatment of a variety of contaminant sources including several POPs. It is a high temperature (870 °C to 1200 °C) destructive *ex-situ* treatment of polluted soil; the waste and/or contaminated soil are fed into the incinerator, under controlled conditions; the high temperatures in the presence of oxygen volatilise and combust the contaminants into innocuous substances. Though variety of designs are available most incinerator designs are fitted with rotary kilns, combustion chambers equipped with an afterburner, a quench tower and an air pollution control system. Removal efficiencies of more than 99.99% are feasible. For PCBs and dioxins the high temperature incinerators can achieve destruction and removal efficiencies up to 99.9999% (6).

Modern incinerators are commonly described as destroying pesticides, PCBs, and similar chemicals very efficiently. However, recent tests suggest that incinerators achieved destruction efficiencies that are lower than those achieved by certain non-combustion technologies. In addition, some incinerators burning POPs (e.g. pesticides and PCBs) and other waste are associated with the spread of undestroyed and newly formed POPs (e.g. dioxins and furans) into the surrounding environment, contaminating air, soil, vegetation, wildlife and human populations (7).

The USEPA has approved high efficiency incinerators to destroy PCBs with concentrations above 50 mg/kg. Incinerators destroying liquid PCBs must meet technical requirements, for instance, 2-sec residence time at 1200 °C and 3% of excess oxygen, alternatively, 1.5-sec residence time at 1600 °C and 2% of excess oxygen in the stack gases. The destruction and removal efficiency (DRE) for non-liquid PCBs must be equivalent to 99.9999% (<1 mg/kg) (Table 5).

Table 5 *Main limitations of established thermal technologies*

Combustion Systems	**Thermal Desorption**	**Pyrolysis**
Technical/Economic		
Require cleaning systems for heavy metals. Need strict control to prevent dioxins formation. Older types of cement kilns are not suitable.	Require dewatering to achieve proper soil moisture levels. It must be linked to a post treatment.	Does not attack inorganic compounds. Performance depends on the soil moisture content, which has correlation with overall cost.
Social		
In many cases may attract public opposition.	If it is linked to combustion systems may present public opposition.	Usually does not attract public opposition.
Environmental/Risk		
Emission of combustion products. Potential release of toxic compounds (dioxins, furans, chlorinated compounds).	Potential of fugitive emissions. Emission of combustion gases and potential formation of dioxins (when linked to combustion systems).	Require controls and systems to prevent dioxins formation. Needs control of combustion gases.

Cement kilns

The main processes employed in making cement clinker can be classified as either »wet« or »dry« depending on the method used to prepare the kiln feed. In the wet process the feed material is slurried and fed directly into the kiln. In the dry process, the kiln exhaust gases are used to dry raw material while it is being milled.

At very high temperatures of the cement kiln, and with the available long residence times, very high destruction efficiency is possible for hazardous waste. The highly alkaline conditions in a cement kiln are ideal for decomposing chlorinated organic waste. Chlorinated liquids, chlorine and sulphur are transformed in chlorides and sulphates. The quantities of the inorganic and mineral elements added in treating chlorinated waste are limited (usually is a small fraction of the large feed requirements of a commercial kiln). No liquid or solid residues requiring post treatment and/or disposal are generated since all residues are bound within the product.

The most appropriate waste to be processed in cement kilns are those which provide additional energy value as a substitute fuel, or material value as a substitute for portions of the raw material feed (e.g. calcium, silica, sulphur, alumina, or iron). Liquid waste or low ash waste can be relatively easy burnt in cement kilns. The material is fed in dry or in slurry form (especially for the »wet« process), or as a fuel supplement into the burning zone of the kiln. In this zone, high destruction efficiencies are achievable at a temperature of 1450 °C, as the gas passes though the kiln.

For the typical counter current process configuration, polluted-soils and solid waste cannot be fed into the firing end of the kiln, since they would discharge in the clinker without adequate treatment; in addition, they cannot be fed into the cool end of the kiln, as the waste would volatilise and would not be adequately destroyed. There are two suitable options for feeding the waste. The first one consists of feeding solid material to the middle of the kiln through a specially designed hopper; the kiln temperature at feeding point is approximately 1100 °C and increases as the materials pass further down the kiln. This involves a major modification of the rotary kiln. Monitoring and verification that complete destruction of stable chlorinated compounds such as PCBs occurs with the desired efficiency is required (8).

The second option includes a pre-treatment of the solid waste (e.g. thermal desorption, as the approach taken in Catalysed Dehalogenation systems). After such treatment, the material can be utilised as a raw material substitute, and the condensate can be incorporated in the liquid feed stream.

When properly operated, destruction of chlorinated compounds in cement kilns can be >99.00% complete with no adverse effect on the quality of the exhaust gas (9). The contribution of waste materials to the exhaust gases are relatively minor given that the waste are only used as a minor supplement to the main energy or raw material stream.

Thermal desorption

Thermal desorption is an *ex-situ* process to remove volatile and semi-volatile contaminants that are sorbed on the waste, by heating to temperatures (between 170 to 550 °C) high enough to volatilise the contaminants. Thermal desorption is not a standalone technology, and must be followed by a subsequent system to treat the off-gas (which is normally captured by a carrier gas or vacuum system) in order to remove particulates and contaminants. Wet scrubbers or fabric filters are one of the best units to remove particulates while contaminants are removed through condensation followed by carbon adsorption, or through a secondary combustion chamber or a catalytic oxidiser such as an afterburner. Thermal desorption may use either direct/indirect heat exchange or air/inert gas to transfer vaporised contaminants from the contaminated medium.

Thermal desorption has been widely applied to treat tar-contaminated soils, refinery waste, wood-treating waste, creosote-contaminated soils, hydrocarbon-contaminated soils, nonhalogenated VOCs, SVOCs, PAHs, PCBs, pesticides, mixed (radioactive and hazardous) waste, synthetic rubber processing waste, and paint waste. The bed temperatures (from 170 to 550 °C) and residence times used by thermal desorption systems will volatilise selected contaminants and drive off water, but typically will not oxidise or degrade organic compounds. Thermal desorption followed by direct combustion (e.g. using an afterburner) can be compared to an incineration system, and it can potentially have acceptability problems with local communities if used to treat hazardous waste.

There are different thermal desorption units available, including, Direct Fired (e.g. natural gas) rotary units, Indirect Fired, Hot oil Rotary Screw units, Molten Metal (e.g. tin) bath units and Infra Red heated batch units. Although thermal desorption units are commonly available, some systems may not be appropriate for treating chlorinated waste streams (10).

Pyrolysis

Pyrolysis is an established *ex-situ* remediation technology. It is a technique of chemical decomposition where the hazardous organic compounds are transformed, under pressure and heat, into gaseous components such as methane, carbon monoxide, hydrogen and a residue of ash and carbon contents. The technology is useful in the treatment of pesticides contained in oily sludge, sediments and soils. This technology is usually linked to a pre treatment technology such as thermal desorption or soil vapour extraction. Further discussion regarding Pyrolysis is provided on Thermal Desorption Integrated Technologies section.

BIOLOGICAL TECHNIQUES (Table 6)

Table 6 *Main limitations of established biological technologies*

Bioventing	**Composting /Biopiles**	**Land farming**
Technical/Economic		
Soil heterogeneity and low permeability may reduce efficiency. Low moisture content can limit biodegradation.	Large space area is needed. Existence of metals may affect the clean up performance. The final volume increases due to amendment addition (for composting). Medium to long term time to reach clean up levels.	Chlorinated and nitrated compounds may affect pollutants degradability. Climatic conditions may increase time required to clean up. Not suitable for PCBs.
Social		
No public opposition.	No public opposition (with proper odours and emissions control).	No public opposition.
Environmental/Risk		
Potential fugitive emissions of by products or hazardous compounds. Requires off gases trapping systems.	The risk of fugitive emissions may limit the treatment of pesticides. Requires odour control and off gasses trapping systems.	Potential release of VOCs or hazardous compounds during tilling.

Biological techniques use microorganisms or enzymes to degrade chemical contaminants. The key factor for bioremediation is identify and/or develop the appropriate bacteria/fungi and the deep understanding of how they survive, reproduce and grow on optimal conditions. Parameters like temperature, humidity, pH, available oxygen, rH, substrate, soil/ waste properties and degradation metabolites, must be controlled and understood in order to obtain effective results.

EPA has chosen bioremediation as a primary reasonable remedy to treat organic contaminants (including some POPs) in soils, sludge, and sediments at wood-treating sites (11). Bioremediation technologies were selected as treatment techniques for 17 of 47 sites in the US Office of Technology Assessment Wood Site Remediation survey. Biological techniques have lately been encouraged because allow accomplishing soil sustainability needs, since the decontaminated soil can be reused or restored to its original use.

Bioventing

This method uses air supplied through injection wells and, in some cases, circulated through vacuum extraction. The airflow increases the volatilisation of organic contaminants while simultaneously creates a proper environment for the aerobic biodegradation of the less volatile organics. Although there are many variants of this technology, the basic principle is to deliver optimised airflow rates to provide enough oxygen to the zone of contamination and, if requested, to add nutrients to sustain and promote biological degradation of organic compounds by the naturally occurring soil microorganisms. The optimal flow rates maximise the biodegradation while vapours move slowly through biologically active soil and minimise volatilisation of contaminants (12). There are different methods used to supply oxygen to the subsurface, including vertical and horizontal bioventing wells; in some cases bioventing is combined with SVE (Soil Vapour Extraction) to increase the control of the flow of the injected air.

The method is applicable for the removal of organic compounds with moderate to low volatility such as petroleum hydrocarbons, oils and lubricants. *Marley and Hoag* (13) demonstrated 99% removal of gasoline using this technique. Bioventing is not appropriate for the treatment of metal and inorganic contaminants.

Land Farming

Land farming is a well-known remediation technology for the treatment of petroleum hydrocarbons contaminated soils. It is a technique designed to enhance the microbial degradation of contaminants through periodic tilling to induce aeration, controlled moisture content and addition of nutrients such as nitrogen and phosphorus. *Pope and Matthews* (14) proposed a relatively standard operational methodology for this technology. The contaminated soil is usually excavated onto a designed lined bed (to avoid leaching) and mixed with a controlled amount of nutrients and soil additives such as bulking agents. Bioaugmentation of microbial culture also can also be performed to enhance the degradation rate.

The treatment is appropriate for small quantities of pesticides that can be diluted and applied to land at controlled rates. Some pesticides are biodegradable, they decompose through the action of naturally occurring microorganisms in soil. Land treatment is appropriate for pesticides that are susceptible to biodegradation in a short period of time (less than 26 weeks), under either aerobic or anaerobic conditions. The soil microorganisms' activity can be enhanced through the addition of biologically active materials such as compost, sewage sludge, or night soil.

Composting

Composting is an *ex-situ* solid-phase remediation technology. Unlike land farming, this technique requires thermophilic (55 to 65 °C) conditions due to the increased biological activity in the degraded organic material. The contaminated soil is excavated and mixed with bulking agents and organic additives (such as wood chips and vegetative waste) to improve soil structure for aeration and drainage. Proper additive selection ensures adequate porosity and provides a balance of carbon, nitrogen and phosphorous to promote thermophilic microbial activity. The system is optimised by controlling (via irrigation) moisture content, pH, temperature and nutrients (15), as well as the optimal carbon-to-nitrogen ratio. At the end of the process, organic-rich compost remains; this material can then be placed back onto the contaminated site, providing a fertile soil for reforestation.

The composting process is applicable to soils contaminated with biodegradable organic compounds, heavy oils, PAHs, and munitions (explosives) waste such as 2,4,6-trinitrotoluene (TNT), hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX), and octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX). It was reported that on a field experiment using compost, TNT reductions were as high as 99.7% in 40 days while removal efficiencies for RDX and HMX were 99.8% and 96.8%, respectively (16).

Biopiles

Biopiles or »engineered biopiles« are a modification of land farming method for petroleum hydrocarbons decontamination which gives the advantage of a relatively small land-space needed, as well as of capturing and treating volatile organic compounds. It is a full-scale *ex-situ* bioremediation technology in which the polluted excavated soils are stockpiled into a heap within the treatment bed in order to prevent further contamination and includes a delivery aeration system. In addition, features an irrigation/nutrient supply system applied to the treatment heap and a leachate collection system used to recycle the collected fluid. Moisture, heat, nutrients, oxygen, and pH are controlled parameters to enhance biodegradation of the contaminants. This process normally reduces the contaminants to carbon dioxide and water within three to six months of operation.

For PCBs the process requires the anaerobic dechlorination in a first stage, where the chlorinated congeners are reduced to less than three chlorines per biphenyl molecule. After an aerobic stage is used to degrade PCB congeners that contain three or fewer chlorines per each biphenyl molecule (17). HCH removal has also been reported using combined anaerobic-aerobic processes.

EMERGING AND INNOVATIVE TECHNOLOGIES – PHYSICO-CHEMICAL TECHNIQUES (Table 7a)

Table 7a *Main limitations of emerging/innovative physico-chemical technologies*

Base catalysed dechlorination (BCD)	**Electrochemical oxidation**	**Solvent extraction** **Chemical dehalog.** **Radiolytic degradation**
Technical/Economic		
Not economical to treat large volumes of aqueous waste. The waste may require pre-dilution to achieve required destruction efficiencies. Overall efficiency is limited by thermal desorption efficiency. Energy costs to treat pesticides waste may be higher, due to the solvents distilled from the mixture.	Highly dependent on soil moisture content. Requires neutralization of treated soil.	Less effective when treating weight organic and hydrophilic compounds. Requires secondary treatment (including extracted metals). Soil types and moisture may impact efficiency.
Social		
Generally not regarded adversely by community.	No public opposition.	No public opposition.
Environmental/Risk		
Potential to form dioxins and furans is low, since the system operates under an inert atmosphere and the process should dechlorinate dioxins. Exclusion of air is required to prevent auto ignition of hot oil. Alkaline pre-treatment and solvent extraction imply fire and explosion risks.	Acids' handling implies spill risk.	Solvent extraction implies fire and explosion risks. Must be assured the proper handling, recycling and disposal of used solvents.

Base Catalysed Dechlorination (BCD)

The Base Catalysed Dechlorination (BCD) system was developed to treat halogenated organic compounds. It is claimed that BCD is applicable for treatment of waste that contains up to 100,000 mg/kg of halogenated aliphatic or aromatic organic compounds such as PCBs. The formation of salt within the treated mixture may limit the concentration of halogenated material able to be treated. *Rogers* (18) reports a reduction of chlorinated organics to less than 2 mg/kg. The BCD process can involve direct dehalogenation or can be linked to a pre-treatment method such as thermal desorption that yields a relatively small quantity of a condensed volatile phase for separate treatment by the BCD process.

The BCD technology involves the addition of an alkali or alkaline earth metal to the polluted material that contains one or more halogenated or non-halogenated organic contaminant compounds. The BCD patent states that the alkaline chemical can be added to the contaminated medium in an aqueous solution, or in a high boiling point solvent. When the solid chemical is added as a suspension in water, the water helps to distribute the metal compound homogeneously throughout the contaminated medium.

A compound able to provide hydrogen ions to react with the contaminants is added to the mixture when hydrogen ions are not already present in the contaminated material. The hydrogen donor compound may include the high boiling point solvent in which the alkali or alkaline earth metal compound is added, or it may include aliphatic alcohols or hydrocarbons, amines or other alike compounds. A source of carbon (such as sucrose) must be added to activate these compounds to produce hydrogen ions.

The mixture is heated and maintained enough time to totally dehydrate the medium. After the water is removed from the medium during the dehydration step, the alkali is concentrated to a reactive state. The medium is further heated at temperatures from 200 to 400 °C with enough time (from 0.5 to 2 hours) to produce a reductive decomposition of the pollutants.

The mixture is neutralised by the addition of an acid. Depending on the nature of the feed material, the added substances and the site use, it may be possible for the treated material to be returned to the site, although may exist land use limitations if the material is oily and/or has a high salt content. The BCD process can reduce PCB from 10,000 mg/kg to below detectable limits in approximately 2 hours (18).

The BCD process mainly involves chlorine stripping; when treating chlorinated aromatic hydrocarbons the removal of chlorine atoms causes an increased concentration of lower chlorinated species. This does not generally represent a problem for PCBs treatment, but with components such as dioxins, the lower congeners (e.g. TCDD) can be more toxic than the highly chlorinated congeners (e.g. OCDD); the process must be therefore well monitored to ensure that the reaction continues to completion.

The BCD system is not appropriate for treating large volumes of aqueous media (including wet sludge) because of the cost of evaporating water. The technology is applicable for low volatility organic liquids and high volatility organic liquids.

Electrochemical Oxidation

Electrochemical Oxidation was initially developed for the high-efficiency conversion of several radioactive organic wastes into environmentally acceptable waste streams. In tests with chemical warfare agents, this process, also called Mediated Electrochemical Oxidation (MEO), was successfully applied in destroying an organophosphorous nerve agent to non-detectable levels after one hour and an organochlorine agent (mustard) after two hours (10).

The system includes an electrochemical cell used to generate oxidizing compounds at the anode in an acid solution (typically nitric acid). These oxidisers and the acid attack any organic compounds, converting most of them to carbon dioxide, water and inorganic ions, at low temperature (<80 °C) and atmospheric pressure. The organic content of the feed, which may be soluble or insoluble organic liquids or solids, can vary from 5 to 100% without affecting the process. In the same manner, the water content of the waste can vary over a wide range. Some compounds destroyed by this process include aliphatic and aromatic hydrocarbons, phenols, organophosphorous and organosulphuric compounds, chlorinated aliphatic and aromatic compounds.

Electrokinetics

This technology is used to remove heavy metals from contaminated soil. However, it can also be used for the treatment of radionuclides and organic contaminants. The technique is based on ion and water migration in an electric field. The movement of water is called electro-osmosis, and ion movement electromigration. This technique is mainly applied to metallic contaminants in the soil matrix. It involves the application of DC potential across the contaminated zone using electrodes in the ground.

To solve the limitations of electrokinetics technology, Surfactant-enhanced electrokinetics works by injecting surfactant into the soil at one of the electrodes. This reduces the interfacial tension between the contaminant and soil matrix: the surfactant can then extract the organics from the soil surface and carry them towards the cathode. Control of the direction and rate allows maximum contact between the surfactant and soil particles, maximising movement of the organics. The performance of the technique is highly dependent on the soil moisture content and is also limited by soil heterogeneity.

Solvent Extraction – Chemical Dehalogenation – Radiolytic Degradation

This *ex-situ* physico-chemical process reduces the volume of the pollutant that needs to be destroyed. The technology uses an extracting chemical to dissolve target contaminants from soils in a final solution suitable for treatment with recovery of the solvent. This process produces relatively clean soil or sediment that can be returned to the original site or disposed on landfill. In some practices, prior to the solvent extraction, a physical separation technique may be used to screen the soils into coarse and fine fractions, in order to enhance the kinetics of the extraction process. This pretreatment technology is very useful in mitigating organic waste and heavy metals.

Solvent extraction technology can be applied to soils contaminated with volatile and semi-volatile organic compounds and other higher boiling point complex organics, such as polynuclear aromatic hydrocarbons (PAHs), petroleum hydrocarbons, pesticide/insecticide, polychlorinated biphenyls (PCBs), dioxins, and pentachlorophenol (PCP). Recent US EPA regulatory guidelines allow solvent extraction applications with nonharmful solvents for the removal of PCBs (19).

Solvent Extraction techniques are cost-effective methods to treat materials contaminated by PCBs and by other chlorinated compounds, but the main limitation is that the contaminants transferred into the solvent must be destroyed through a secondary method. Different approaches have been developed to combine solvent extraction with other techniques like chemical dehalogenation with immobilised reagents (CDP) and gamma-ray irradiation. Recent studies show that PCB concentration in transformer oil was reduced from 700 mg/kg to non-detectable levels in less than 5 minutes using chemical dehalogenation. While the results for radiolytic degradation showed that the PCB concentrations decreased with an increased γ-ray dose, nearly 60 mega rads were needed to degrade PCBs from 300 mg/kg down to 1 mg/kg in solvent saturated soil (20).

Solvated Electron

This technology uses sodium metal dissolved in liquid anhydrous ammonia to produce a dark blue solution of solvated electrons; the solvated electrons act as dehalogenating agents. Solvated electron solutions are rapidly formed when alkali or alkaline earth metals are dissolved in ammonia or in some amines, forming solutions containing the metal cation and free electrons.

Halogens can be separate from organic halides to yield a fully substituted parent hydrocarbon and a metal halide. The treatment of waste with »solvent electron« is performed with low to medium temperatures and the conversion of the waste occurs in seconds. It has been stated that there is no need for pre-treatment, although some dewatering of sludge and/or sediments might be required.

The method is claimed to be applicable to treat halogenated hydrocarbons, pesticides, dioxins, PCBs, herbicides, CFCs, and chemical warfare agents. It is also stated that waste have been successfully treated in bulk pure material, soils, sludge, sediments, porous and non-porous surfaces, oils, contaminated vessels, hardware, and contaminated clothing. Some tests had confirmed that Solvated Electron process destroys the three chemical agents that comprise 85 percent of all US chemical weapons stockpiles (21).

The process design employs a cement mixer like reactor in which contaminated material and liquid ammonia are mixed. The ammonia completely disperses the soil, including the clays and washes the contaminant from the soil. After brief mixing, a reactive metal charge is added (commonly calcium). The electrons released from the calcium rapidly dehalogenate the contaminants. Ammonia is recovered for further use, and the soil is deodorised. The decontaminated soil is suitable for return to the site, suitable for agricultural use since is enriched in nitrogen from trace amounts of residual ammonia. The method is able to treat soils with up to 25% water content (Table 7b).

Supercritical water oxidation

Supercritical water oxidation (SCWO) is an *ex-situ*, high temperature and pressure technology that uses the properties of supercritical water to destroy organic compounds and toxic waste. Under supercritical conditions, carbon is converted to carbon dioxide and hydrogen to water; chlorine atoms derived from chlorinated organic compounds to chloride ions, nitro-compounds to nitrates, sulfur to sulfates, and phosphorus to phosphate.

The properties of super critical water are used to operate this process. Gases like oxygen and organic substances are completely soluble in super critical water, whereas inorganic salts present reduced solubility under supercritical conditions. Organic sub-

Table 7b *Main limitations of emerging/innovative physico-chemical technologies*

Solvated electron	Supercritical water oxidation	Solar detoxification – Photochemical degradation
Technical/Economic		
May require a pre-treatment for dewatering of sludge and/or sediments.	The end products (ash and brine) require proper disposal. Limited to treat liquid waste with solids sizing less than 200?m. Applicable to waste with organic content less than 20%.	The photolysis rates for pesticides are highly dependent on latitude, season and other meteorological conditions.
Social		
No public opposition known at this stage.	Not known public opposition at this stage.	No known public opposition.
Environmental/Risk		
Ammonia is a volatile liquid; toxic and fire risks. Calcium metal combined with hydrogen may form explosive mixtures.	Due to the high temperatures and pressures used in this technology, requires specialised control equipment, reactor materials and safety practices.	Low environmental impact due to limited use of chemicals and low off-gas generation rates.

stances dissolve in the super critical water, and oxygen and the organic substances are brought into intimate single phase contact at temperatures and molecular densities that allow the conventional oxidation reactions to carry out rapidly to completion.

Process residues are contained if the waste contains inorganic salts or organics with halogens, sulphur or phosphorous. The effluent gases contain no oxides of nitrogen or acid gases such as hydrogen chloride or sulphur oxide. The process does not generate particulates and less than 10 mg/kg carbon monoxide has been measured.

It has been stressed that this system must be constructed of materials capable of resisting corrosion caused by halogen ions. The precipitation of salts may cause plugging problems in the system (22). Destruction and Removal Efficiencies of greater than 99% have been reported for the treatment of numerous hazardous organic compounds. SCWO can be applied to aqueous waste streams, sludge and contaminated soils. It is also applicable to treat acrylonitrile wastewater, cyanide wastewater, pesticide wastewater, PCBs, halogenated aliphatics and aromatics, and organic nitrogen compounds.

Solar detoxification –Photochemical degradation

Sunlight energy can be used to degrade organic compounds of synthetic and natural origin. Short wavelengths (295–400 nm) of solar spectrum are greatly attenuated by the atmosphere, such radiation is able to generate direct and indirect photolytic processes that can degrade soil and surface waters polluted with POPs (mainly pesticides). Since the mentioned wavelengths are attenuated more strongly than longer visible wavelengths, the rate of photolysis of pesticides is highly dependent on latitude, season and other meteorological conditions; thus, in tropical regions photochemical processes are a key factor to assess pesticides fate and degradation (23).

Solar energy is used to degrade hazardous organic chemicals by direct thermal decomposition or by photochemical reaction. Some advantages include savings in fuel use, improved thermal destruction of contaminants, and a reduction in exhaust gas volumes, including PICs (products of incomplete combustion). These processes can use either thermal energy or a range of photochemical reactions.

In order to use efficiently solar energy is required to concentrate the solar radiation to achieve high temperatures to decompose or destroy the contaminants. Solar radiation is reflected by mirrors (heliostats) and absorbed by a receiver reaching temperatures of up to 2,300 K. No auxiliary fuel is required and it has been demonstrated to show an improvement in the destruction and removal efficiency (DRE) of organics, including pesticides, by a factor of 100 or more against conventional thermal technologies. High destruction efficiencies can be achieved at a temperature of 750 °C that is lower than the temperature required for thermal incineration.

The main photochemical processes that aid thermal treatment in solar detoxification include photocatalytic oxidation using titanium dioxide (TiO_2) as a catalyst. Ultraviolet radiation is used to promote an oxidation reaction in photocatalytic reactions using a catalyst such as TiO_2 in the presence of oxygen. The reactivity of singlet oxygen, irradiated with visible light in the presence of dissolved oxygen, is used in the dye-sensitiser processes. The reactive species produced can then react with contaminant molecules in the waste.

Oxidative degradation of pesticides, including lindane in contaminated water has been tested with direct sunlight in a solar furnace. Singlet oxygen was effective against some of the pesticides but reacted slowly or not at all with others. All pesticides were degraded by OH radical generating agents (such as methylene blue). Each system has different capabilities, that need to be taken into consideration when making comparisons.

Gas Phase Chemical Reduction

Gas Phase Chemical Reduction (also known as Eco Logic Process) has been developed as an alternative to incineration technologies. The technology is based on gas-phase thermo-chemical reaction of hydrogen with organic compounds. Hydrogen combines with organic compounds at 850 °C or higher, in a reductive reaction to form lighter hydrocarbons (mainly methane). For chlorinated organic compounds, such as PCBs, the products are methane and hydrogen chloride. The reaction is carried out with water that functions as a reducing agent and generates hydrogen. The technology is a hydrogenation process and adds hydrogen atoms to any incompletely hydrogenated organic molecule, dechlorinating molecules and breaking down aromatic rings, therefore is non selective in its treatment of organic substances.

The process can quantitatively convert PCBs, PAHs, chlorophenols, dioxins, chlorobenzenes, pesticides and herbicides, to methane. The yield will be determined by the concentration of organics in the waste. Approximately 40% of the methane produced can be further converted to hydrogen through the water shift reaction and non-reacted methane is converted to hydrogen in the catalytic steam reformer. Therefore, the process can operate with the hydrogen produced itself (10).

The gas phase reduction process is likely to be preceded by a thermal desorption unit when treating solid waste. There is potential for the removal of organic contaminants from the solid material to be improved in the chemical reduction process, as the thermal desorber will operate under a reducing hydrogen atmosphere, offering simultaneous destruction. The technology needs water in its operation and therefore can process waste with relatively high water content. This aspect provides an advantage over other thermally based processes that require treatment for sludge with high water content (Table 7c).

Table 7c *Main limitations of emerging/innovative physico-chemical technologies*

Gas phase chemical reduction	Catalytic hydrogenation
Technical/Economic	
Pollutants such as sulphur and arsenic may inhibit treatment. Sulphur in combination with iron may produce slimes that require additional centrifuge separation. The existence of irregular solids may also limit waste treatment due to materials handling. May need to be linked to special waste handling facilities in order to improve waste material handling.	Potential poisoning of catalysts may decrease or nullify process efficiency.
Social	
Generally not regarded adversely by community.	No public opposition.
Environmental/Risk	
Potential fugitive emissions of PCBs, pesticides or dioxins. The handle, use and storage of hydrogen within the process represent fire and explosion risks. The facilities must be subjected to an internal hazardous operations reviews and specialised process control to prevent release of waste materials during a process upset.	Gaseous products may generate safety and toxicity hazards. Combustion products may require scrubbing that would generate aqueous waste.

Catalytic Hydrogenation

The destruction of halogenated waste by hydrogenation in the presence of noble metal catalysts has been studied for many years. Noble metal catalysts are particularly susceptible to poisoning by a several substances found on waste, thus limiting the applicability of the technology. It has been developed a process for the regeneration of PCB contaminated transformer fluids using hydrogenation catalysts based on metal sulphides, which are extremely robust and tolerant to most catalyst poisons (24). The process is also claimed to destroy a wide range of chlorinated hydrocarbons, forming hydrogen chloride and light hydrocarbons as by-products.

In different trials relatively high concentrations of pure POPs compounds were treated in a hydrocarbon solvent and all were destructed to levels below the detection limit of analysis, presenting destruction efficiencies from 99.9996% (for hexachlorobenzene) to 99.99999% (for 1,2,3,4-TCDD). It is claimed that the variations in destruction efficiencies reflect the differences in the instrument detection limits rather than real differences in the extent of destruction (25). Most off-gases are recycled through the reactor, although purge gases are discharged through a catalytic combustion chamber.

Different surveys have shown that successful dechlorination of polychlorinated aromatic compounds by using Ni catalysts requires severe reaction conditions, high temperature and high hydrogen pressure. Pd, Ru catalysts that permit successful dechlorination of polychlorinated aromatic compounds under mild conditions are not developed for large-scale applications because of their high cost (26).

Recent surveys have shown the preparation of a selective catalyst to convert environmentally problematic compounds into useful products, allowing to perform liquid phase hydrodechlorination under mild conditions, using bimetallic catalysts consisting of nickel or copper associated with palladium, supported on a high-surface area carbon. The results show that such bimetallic systems permit to carry out liquid phase hydrodechlorination of hexachlorobenzene under mild conditions (P_{H2} 1 atm, t=50 °C), and that the method of catalysts preparation has a strong effect on their selectivity (27).

THERMAL TECHNIQUES

Thermal Desorption Integrated Technologies

This section includes the technologies involving thermal desorption as a pre-treatment-separation technique integrated with a post treatment-destruction technology (Table 8).

Thermal Desorption – Catalysed Dehalogenation

This system is composed by a thermal desorption system linked to the Base Catalysed Dechlorination (BCD). The system uses an indirectly heated thermal desorber to split organic compounds from contaminated media (28). The system is designed to achieve feed material temperatures of up to 510 °C allowing an effective treatment of soils and sludge polluted with a wide range of low and high boiling point compounds. The system is applicable for hydrocarbons, pesticides, herbicides, PCBs, coal by-products, wood treating compounds, dioxins, and furans. The gases produced during

Table 8 *Main limitations of emerging/innovative thermal technologies*

Thermal desorption integrated technologies	**Plasma Arc Systems**	**Vitrification**
Technical/Economic		
Overall efficiencies of methods are limited by thermal desorption efficiency, that depends on soil type and conditions.	The removal of volatile metals and particulates formed from inorganic components may require treatment; these additional steps may increase the cost. This process usually has a relatively high capital and operating cost. Some systems are limited to treat liquids and gases. Solids can only be treated after extraction or by forming slurry mixtures.	Vitrification is a destructive process and the soil can no longer be used for agricultural purposes. The vitrified matrix may hinder future use of the site if done *in-situ.*
Social		
In some cases may attract public opposition.	Generally not regarded adversely by community.	No known public opposition.
Environmental/Risk		
Combustion of off-gases requires control and emissions treatment. Process conditions must be selected and controlled in order to minimise the risk of dioxin and furan formation, and require pollution control equipment to treat these in the event that small quantities are formed.	The absence of combustion gases results on a gas emission smaller than for incineration systems. A surge tank is provided to contain any uncontrolled release of gases from the treatment chamber. The use of mechanical seals and operation of the unit at slight negative pressures should prevent any fugitive emissions.	Cautions must be taken to prevent fugitive emissions of vaporised organics. The vitrified nature of the formed matrix greatly reduces any potential leaching of metals or other residual pollutants.

the process are treated by a vapour recovery system that includes an oil venturi, an oil scrubber, water scrubber, condensing unit and vapour phase carbon adsorption unit.

Contaminants and moisture volatilised from the contaminated material are entrained in the off-gas and are condensed and recovered by the scrubbers/condensers. The condensed mixture is separated and the organic contaminant is collected for recycling via solvent recovery, fuel substitution or treatment using the BCD process. Separated water can be treated by liquid phase carbon adsorption and sand filtration. Most of the treated water can be recycled back to the process for use in the scrubbers and cooling conveyor.

Thermal Desorption – Pyrolysis

The PCS (*Product Control Someus*) Technology is based on thermal desorption combined with flash pyrolysis technique, and followed by combustion. The main operational units of the system include indirectly heated rotary reactor, indirectly cooled solid material cooler, and multi venturi scrubber, pyrolysis gas combustion chamber, water treatment, auxiliary equipment and automatic operation with continuous monitoring.

The rotary reactor is the main component of the system. Waste is partially vaporised in a reductive environment under low vacuum conditions (0 to 50 Pa). The reactor is cylindrical in shape, arranged horizontally and rotates around its axis. The operating temperature in the reactor ranges from 450 to 800 °C. The waste may be introduced directly, or after drying in a desorber. If needed, the waste is ground in a mill in order to homogenise to a size less than 5 mm. The waste is decomposed into solid and vapour phases which include heavy metals in water insoluble form, high boiling point organics in the solid phase, and volatile organic compounds, volatile heavy metals and halogens in the vapour phase.

After the pyrolysis, the vapour phase is combusted and rapidly cooled; the gas stream is cleaned in a gas scrubber before emission. Although dioxin and furan gases are not generally formed in a reductive environment, it is possible that they could be formed following the combustion step. Therefore, after combustion the resulting gases must be treated by scrubbing. The scrubber process water is cleaned, neutralised, and water recirculated.

The process applications include the conversion to energy of waste such as solid hazardous waste, PCB contaminated soil, mercury contaminated soil, hospital waste, municipal solid waste, sewage sludge and coal. In addition, the technology can treat a full range of chlorinated hydrocarbons, organochlorine pesticides, organic and/or inorganic materials combined with contamination of organics, halogens and heavy metals. Although, this technology is not applicable for treatment of liquids (water, flammable liquids and solvents), explosives and/or materials with highly oxidizing nature under heat treatment and materials that cannot be decomposed by thermal treatment at 600 °C.

Thermal desorption – Retort System

This technology is adapted to treat contaminated soils containing volatile organic compounds (VOCs) or some semi-VOCs. The process has been configured for the treatment of pesticide contaminated soils, especially for dip sites.

The system involves an indirectly fired retort that is used to remove the volatile materials through an off gas-vent, leaving the treated soil for return to its original site. The retort operates on a continuous basis under negative pressure, and under neutral conditions (i.e. neither oxidizing, nor reducing) resulting in some leakage of air into the system. The treated soil leaves the retort via an overflow washer from where it is transferred to a stockpile.

The retort contents are indirectly heated. A combustion chamber surrounds the retort and the components are initially brought up to operating temperature by heating a batch charge of inert material. When this mass is at opening temperature, feed is started. Bed temperatures are monitored to ensure that conditions are maintained by varying either the feed rate or the firing rate; temperatures are set in the range of 400–700 °C depending on the residence time required, type of contaminant and soil properties. To treat organochloride pesticide contaminated soils, the retort usually operates with a bed temperature of 450 to 500 °C (10).

Within the retort the pollutants are volatilised and/or decomposed and separate as part of the off-gas. The off-gases are then drawn by a fan through a hot gas filtration system that removes particulate matter, allowing the cleaned gases to go to an afterburner for the residual organics destruction. The afterburner is designed to operate at 1,100 °C with a two-second-residence time. From the afterburner, the gases are quenched to minimise dioxin and/or furan formation.

The retort process is only able to treat solids and sludge, although liquids (e.g. pesticides formulations) could be treated by first producing a slurry. Treatment of low volatility compounds such as PCBs is not proposed on the current development status.

Plasma ARC Systems

This technology uses high temperatures (around 10,000 °C) for pyrolysis, which result from the discharge of a large electric current in an inert gas, to convert hazardous chemicals such as PCBs, pesticides, CFCs, halon gases into innocuous and safe-emitted end products. The destructive process is made possible by the conversion of the hazardous compound by the superheated cloud of gas or plasma into atomic elements and subsequent treatment converts the atomic forms into innocuous substances.

A thermal plasma field is created by directing an electric current through a low-pressure gas stream. Plasma arc ranges can reach 5,000 to 15,000 °C. There are different variations of plasma arc processes like PACT (Plasma Arc Centrifugal Treatment) (29), PLASCON (In-Flight Plasma Arc System) and STARTECH (Plasma-electric waste converter) (10).

Vitrification

The soil is treated with high temperature to cause melting and forming a glass when cooled. This technology can either be carried out *in-situ* or *ex-situ*; consists on inserting graphite electrodes into the contaminated encased area and energizing with a high electrical resistance heating (more than 1,700 °C) to melt soil into a molten block. It is applicable for the treatment of organics (including pesticides and PCBs), inorganics and radionuclides. The organic contaminants will normally be destroyed while the inorganics will be trapped into the vitrified matrix. The Plasma Arc Centrifugal Treatment (PACT) mentioned above is a combination of Plasma Arc and Vitrification techniques (10).

BIOLOGICAL TECHNOLOGIES

Biological techniques are commonly carried out with indigenous microorganisms since these present superior performance due to the better survival rates compared to strains taken from geographically different locations (non-indigenous inoculants). However, some studies (30) have illustrated that the use of indigenous microorganisms for bioremediation and as hosts for developing genetically engineered organisms does not provide any advantage in dynamic and highly competitive environments. Thus, the survey recommends that the site must be engineered to provide temporal advantages for the non-indigenous microorganisms, or the known inoculant must be able to degrade a specific compound better than the native strain (Table 9).

Table 9 *Main limitations of emerging/innovative biological technologies*

Phytoremediation	Bioslurry	Enhanced bioremediation
Technical/Economic		
The process may take years to achieve regulatory limits. Few data exist yet as input to standardization and regulatory acceptance.	Dewatering soil fines after treatment can be expensive. It is required an acceptable method for disposing of non recycled wastewater.	Water-based solution circulation may move pollutants to underlying ground water. Clogging may occur. It is not suitable for low permeability soil. High metal and chlorinated organic concentrations may be toxic to the organisms. This technology is not effective at low temperatures.
Social		
Not regarded adversely by community.	Not regarded adversely by community.	Not regarded adversely by community.
Environmental/Risk		
Appropriate waste management and disposal is required to prevent contamination of the food chain.	Cautions and operational conditions must be set to prevent potential fugitive emissions of pesticides.	Some POPs may be formed under anaerobic conditions. The mobilization of contaminants may affect surrounding environment (air and groundwater).

Bioslurry

This is a technique for sites that require greater process control, more complete and faster degradation rates. The contaminated soils are mixed with water to form a slurry in order to allow contact between microorganisms and contaminants. The slurry is then fed into a bioreactor where a controlled amount of air is supplied for mixing and aerating; inoculation may be performed to enhance treatment. If conditions (temperature, nutrient concentration and proper aeration) are optimised, slurry processes are faster than other biological processes. The treated slurry is suitable for direct land application, similar to composted soils (17). The clean-up time is less than twelve months. Slurry-phase bioreactors are used to remediate soils and sludge contaminated with explosives, petroleum hydrocarbons, petrochemicals, solvents, pesticides and other organic chemicals. Bioslurry is favoured over *in-situ* biological techniques for heterogeneous soils, low permeability soils and areas where underlying groundwater is difficult to capture.

Enhanced bioremediation

Enhanced bioremediation, also called biostimulation or bioaugmentation, is a process to increase the biodegradation rate of contaminated soil by the addition of nutrients and oxygen. The activity of microflora and fauna may be stimulated by circulating water-based solutions through the contaminated soils and/or addition of indigenous/ inoculated microorganisms, engineered microbial species or seeding with pollutant degrading bacteria so as to enhance biological degradation of contaminants or immobilization of inorganic contaminants (17). Although it could be done in anaerobic conditions, it is more advantageous when oxygen is not limiting in order to prevent the formation of persistent by-products such as vinyl chloride resulting from the anaerobic degradation of trichloroethylene.

This technique can be used *in situ* to treat soils contaminated with different pollutants such as petroleum hydrocarbons, solvents, pesticides, wood preservatives and/or nitrotoluenes.

Phytoremediation

This is an emerging cost-effective technology for in-situ treatment of hazardous contaminants in soils and water. The technique makes use of specific plants and planting techniques to accelerate the rate of degradation, accumulation, removal, transformation, stabilisation and destruction of targeted soil, water and even airborne contaminants (31). Although it is most suitable for sites with shallow contamination of organics and metals, some practice now make use of deep rooted plants such as poplars and alfalfa to attack, mitigate and contain pollutants located many feet below the surface.

The degradation process stimulates micro-organisms through the release of carbon-containing nutrients from their roots. The zone closely associated with the plant root, the rhizosphere, has many more metabolically active micro-organisms, as a result of the naturally released nutrients that they use for energy and other biological activity. It is this symbiotic relationship between plants and microbes that is responsible for the degradation process. Other known mechanisms whereby plants are able to effect remediation process are hydraulic barrier/containment, phytovolatilisation, phytoaccumulation, and phytodegradation (32).

RECOMMENDATIONS AND CONCLUSIONS

The difference between technologies that only separate and/or concentrate a pollutant (e.g. solvent extraction and thermal desorption) and those which destroy the contaminant (e.g. pyrolysis, oxidation, reduction, and biodegradation) must be considered when setting site-remediation goals. Those technologies that only immobilise contaminants (e.g. landfill cap systems, stabilization, and vitrification) should also be clearly differentiated. Current soil sustainability trends have enhanced the use of non-destructive technologies making it possible to reuse the treated soil.

Several ratable and non-ratable criteria should be considered for the selection of the most suitable technology. Non-ratable, or relative criteria, include public acceptability and risk and environmental impacts (which depend on the specific geographic site location). Ratable criteria may include the applicability of the method (in accordance with its development status), overall costs, minimum achievable concentration, cleanup time, reliability, maintenance, post treatment costs, and ability to use soil after treatment. Social, environmental, technical, and economical criteria should be considered during the technology selection process; the more criteria are involved, the better performance is obtained. In case of choosing more than one technology to treat a specific waste or soil, limitations, impacts and risks related to the combined methods should be considered. Environmental impact and risk assessments must be carefully considered in order to avoid or control the emissions of PTS during the remediation process.

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Sažetak

PREGLED TEHNOLOGIJA REMEDIJACIJE PERZISTENTNIH OTROVNIH TVARI

Ovaj članak donosi pregled postojećih i novonastajućih tehnologija obrade otpada i tla onečišćenog perzistentnim otrovnim tvarima uključujući i perzistentna organska onečišćivala (engl. *persistent organic pollutants*). Autor dijeli tehnologije obrade na biološke, fizikalno-kemijske i termalne te opisuje rad osnovnih uređaja i uspoređuje tehnička, društvena i ekološka ograničenja, uključujući moguće rizike i učinke na okoliš. Članak također razmatra ukupne procijenjene troškove, vrijeme potrebno za čišćenje te razine odgovornosti i održavanja dajući na uvid prednosti i ograničenja svake tehnologije zasebno.

Ključne riječi:
otpad, PCBs, pesticidi, poliklorirani bifenili, POPs, tlo

Requests for reprints:

Andrea Lodolo, Ph. D.
International Centre for Science and High Technology
United Nations Industrial Development Organization (ICS-UNIDO)
Pure and Applied Chemistry
Padriciano 99, 34012 Trieste, Italy
E-mail: *lodolo@ics.trieste.it*