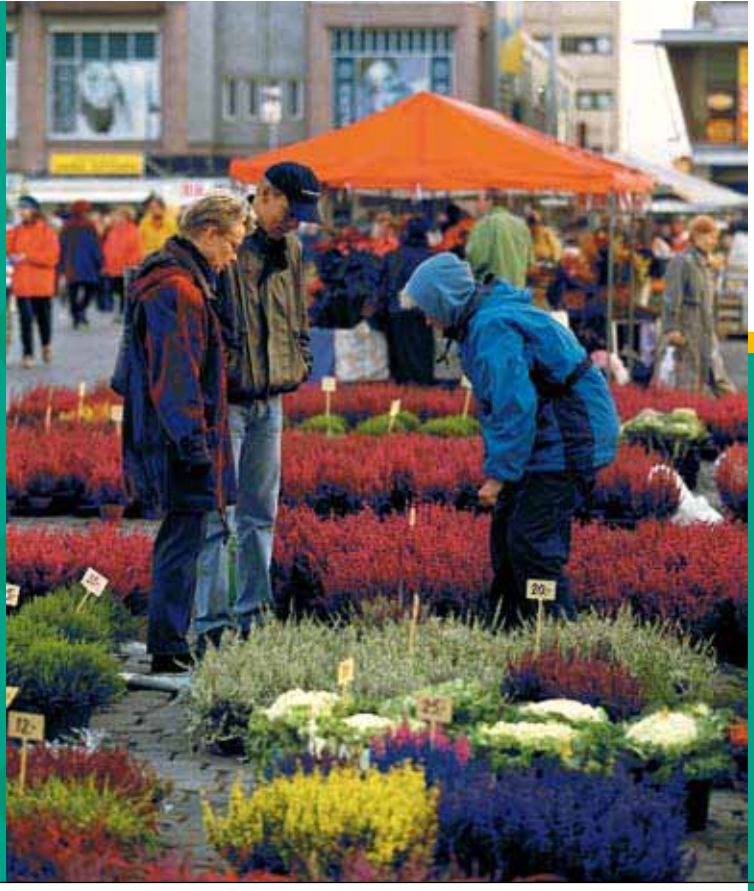

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National Public Health Institute
Department of Environmental Health
Background Material for the International Evaluation

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Ympäristöterveyden osasto
Kansainvälisen arvioinnin taustamateriaali

Kansanterveyslaitoksen julkaisuja B 12/2006

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NATIONAL PUBLIC HEALTH INSTITUTE

DEPARTMENT OF ENVIRONMENTAL HEALTH

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Abstract

This review includes the material for the evaluation of the work of the National Public Health Institute (KTL), the Department of Environmental Health (YTOS) in 1996-2005. The main purpose of the evaluation is to guide the Ministry of Social Affairs and Health for the strategic management of KTL.

A panel has been nominated to implement the evaluation. The panel members are Professor Erik Dybing, Department of Environmental Medicine, Norwegian Institute of Public Health, Dr. Robert L. Maynard, Wellington House, Department of Health, UK, and Professor Harri Vainio, Director General of the Finnish Institute of Occupational Health. The evaluation panel will examine the functions, strategic importance, scientific merits and value for money of the scientific and expert work undertaken, and make proposals for future. A special emphasis is on the relevance and effectiveness of the work and of its impact on the health of Finnish people.

The goals of YTOS are promotion of public health, reduction in environmental exposures and improved decision-making on environmental risks. These goals are pursued through scientific research, risk assessment, expert work, dialogue with the decision makers, and educating the public.

In 2005, YTOS had personnel of 121 employees, out of which 42 with governmental funding (permanent positions) and 79 with external project funding. The operating costs from the government budget has been 2.81-3.34 million Eur in 1996-2005. External funding has varied between 0.59-4.76 million Eur per year (16-60 % of the total funding), being on an average 2.34 million Eur per year (44 % of the total funding) during this same time period. YTOS has produced almost 600 international peer reviewed articles and almost 200 domestic articles in 1996-2005. Altogether 63 doctoral dissertations have been finished (the work done at YTOS) during that time.

YTOS has served the society by giving its expertise on problems related to e.g. drinking water, indoor air, risk comparisons of various energy consumption, dioxins and PCBs in fish, suburban and urban air fine particles, and contaminated soils.

YTOS has five laboratories/units: Air Hygiene Laboratory, Environmental Microbiology Laboratory, Chemistry Laboratory, Environmental Epidemiology Unit, Toxicology Laboratory. These laboratories/units at YTOS are centers of methodological expertise. Scientific research is conducted in collaborative research programmes across the laboratories/units. In this document, the material has been divided into six research programmes: 1. Air Pollution and Health 2. Respiratory Disease, Indoor Microbes and Immunotoxicity, 3. Water and Health, 4. Persistent Organic Pollutants and Health: Analysis, Concentration, Exposure and Epidemiology, 5. Persistent Organic Pollutants: Mechanisms of Health Effects, and 6. Risk Analysis.

Key words: environment, health, evaluation, air pollution, indoor air, microbes, drinking water, chemicals, risk.

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Tiivistelmä

Tämä yhteenveto sisältää kansainvälistä arviointia varten kootun materiaalin Kansanterveyslaitoksen (KTL) Ympäristöterveyden osaston (YTOS) toiminnasta vuosina 1996–2005. Sosiaali- ja terveysministeriö odottaa saavansa arvioinnista apua Kansanterveyslaitoksen strategiseen ohjaukseen.

Arvioinnin tekee kansainvälinen paneeli, jonka jäsenet ovat professori Erik Dybing (Department of Environmental Medicine, Institute of Public Health, Norja), tohtori Robert L. Maynard (Wellington House, Department of Health, Iso-Britannia) ja pääjohtaja Harri Vainio (Työterveyslaitos, Suomi). Arviointipaneelin tehtävänä on tarkastella osaston toimintaa, strategista tärkeyttä, tieteellisiä saavutuksia, tieteellisen työn ja asiantuntijatyön kustannusvastaavuutta sekä tehdä ehdotuksia tulevaisuutta varten. Erityistä huomiota kiinnitetään työn kohdentamiseen ja tehokkuuteen sekä sen vaikutukseen suomalaisten terveyteen.

YTOS:n tavoitteena on edistää kansanterveyttä, vähentää ympäristöperäistä altistumista ja parantaa ympäristöriskeihin liittyvää päätöksentekoa. Keinoina tavoitteeseen pääsemiseksi ovat tieteellinen tutkimus, riskien arviointi, asiantuntijatyö, yhteys päätöksentekijöihin ja valistus.

Vuonna 2005 YTOS:ssa työskenteli 121 henkilöä, joista 42 valtion budjettirahoituksella ja 79 ulkopuolisella projektirahoituksella. Osaston vuosittainen budjettirahoitus on ollut 2,81–3,34 miljoonaa euroa vuosina 1996–2005. Vastaavana aikana vuosittainen ulkopuolisen rahoituksen määrä on vaihdellut 0,59 miljoonasta 4,76 miljoonaan euroon (16–60 % kokonaisrahoituksesta) ollen keskimäärin 2,34 miljoonaa euroa vuodessa (44 % kokonaisrahoituksesta). YTOS on tuottanut lähes 600 kansainvälisen tieteellisen arviointikäytännön läpäissyttä artikkelia ja lähes 200 kotimaista artikkelia vuosien 1996–2005 aikana. Samana ajanjaksona YTOS:ssa tehdyn työn tuloksena on valmistunut 63 tohtorin väitöskirjaa.

YTOS on toiminut asiantuntijana lukuisissa ympäristöterveysongelmissa, jotka ovat liittyneet mm. juomaveteen, sisäilmaan, eri energiavaihtoehtojen vertailuun, silakan dioksiini- ja PCB-pitoisuuksiin, ilman pienhiukkasiin ja saastuneisiin maihin.

YTOS:ssa on viisi laboratoriota/yksikköä: ilmahygienian laboratorio, ympäristömikrobiologian laboratorio, kemian laboratorio, ympäristöepidemiologian yksikkö ja toksikologian laboratorio. Laboratoriot/yksiköt ovat YTOS:n menetelmällisiä osaamiskeskuksia. Tieteellinen tutkimus tehdään eri laboratorioiden yhteisissä tutkimusohjelmissa. Tämän yhteenvedon aineisto on jaettu kuuteen tutkimusohjelmaan: 1. Ilman epäpuhtaudet ja terveys, 2. Hengityselinsairaudet, sisäilman mikrobit ja immunotoksikologia, 3. Juomavesi ja terveys, 4. Pysyvät orgaaniset yhdisteet ja terveys: analysointi, pitoisuudet, altistuminen ja epidemiologia, 5. Pysyvät orgaaniset yhdisteet: terveysvaikutusten mekanismit, 6. Riskianalyysi.

Avainsanat: ympäristö, terveys, ilman epäpuhtaudet, sisäilma, mikrobit, juomavesi, kemikaalit, riski

Terttu Vartiainen, Matti Jantunen, Ilkka Miettinen, Aino Nevalainen, Jouni Tuomisto och Matti Viluksela
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Sammandrag

Den här genomgången innefattar material för utvärdering av arbetet på Folkhälsoinstitutet (KTL), Avdelningen för miljöhälsa (YTOS) 1996–2005. Huvudsyftet med utvärderingen är att ge social- och hälsovårdsministeriet riktlinjer för den strategiska styrningen av KTL.

Man har utsett en panel för att genomföra utvärderingen. Den består av professor Erik Dybing, Avdelningen för miljömedicin, norska folkhälsoinstitutet, Dr. Robert L. Maynard, Wellington House, Department of Health, Storbritannien, Professor Harri Vainio, chef för Arbetshälsoinstitutet. Utvärderingspanelen ska undersöka vad detta vetenskapliga arbete och expertarbete har genererat för funktioner, strategisk vikt, vetenskapliga meriter och ekonomiskt värde och ge förslag på hur det ska se ut i framtiden. Särskild tonvikt läggs vid relevansen och effektiviteten i arbetet samt dess effekt på den finska befolkningens hälsa.

YTOS mål är att främja folkhälsan, minska exponeringen för miljöfarliga ämnen samt få ett förbättrat beslutsfattande i fråga om miljörisker. Dessa mål ska uppnås genom vetenskaplig forskning, riskbedömning, expertarbete, dialog med beslutsfattarna och folkbildning.

2005 hade YTOS 121 anställda, varav 42 finansierades av regeringen (fasta tjänster) och 79 finansierades genom externa projektmedel. Driftskostnaderna från regeringsbudgeten var 2,81–3,34 miljoner euro 1996–2005. Extern finansiering har varierat mellan 0,59–4,76 miljoner euro per år (16–60 % av den totala finansieringen) och var i genomsnitt 2,34 miljoner euro per år (44 % av den totala finansieringen) under samma tidsperiod. Nästan 600 internationella granskade artiklar och cirka 200 inhemska artiklar producerades genom YTOS 1996–2005. Sammanlagt har 63 doktorsavhandlingar slutförts (varvid arbetet har skett på YTOS) under den tiden.

YTOS har tjänat allmänheten genom sin expertis om problem som rör exempelvis dricksvatten, inomhusluft, riskjämförelser av olika typer av energikonsumtion, dioxiner och PCB i fisk, luftpartiklar i stads- och lantmiljö och förorenad jord.

YTOS har fem laboratorier/enheter: Lufthygieniska laboratoriet, Miljömikrobiologiska laboratoriet, Kemiska laboratoriet, Miljöepidemiologiska enheten och Toxikologiska laboratoriet. Dessa laboratorier/enheter på YTOS är center för metodologisk expertis. Forskning bedrivs i samarbetsprogram över de olika laboratorierna/enheterna. I det här dokumentet har materialet delats upp i sex forskningsprogram: 1. Luftföroreningar och hälsa, 2. Sjukdomar i luftvägarna, mikrober inomhus och immunotoxicitet, 3. Vatten och hälsa, 4. Persistenta organiska föroreningar och hälsa: analys, koncentration, exponering och epidemiologi, 5. Persistenta organiska föroreningar: hälsoeffekternas mekanismer, och 6. Riskanalys.

Ämnesord: miljö, hälsa, utvärdering, luftföroreningar, inomhusluft, mikrober, dricksvatten, kemikalier, risk.

Content

PREAMBLE

In the early 1990s the Science and Technology Policy Council in Finland encouraged the Ministries to evaluate research institutions in their jurisdiction. In accordance to this policy, the Medical Research Council of the Academy of Finland carried out an evaluation of the research activities of KTL in 1994–1995, in response to a proposal put forward by the Finnish National Public Health Institute (KTL) and the Ministry of Social Affairs and Health (STM). The objective of this evaluation was to provide information on the public health functions, strategic importance, scientific merit and value for money of the scientific work undertaken and to make proposals for future work.

The international Evaluation Panel, chaired by Dr David Evered of the UK Medical Research Council, prepared an evaluation Report to the Academy in October 1995. For the evaluation, KTL prepared a report describing the work in the Institute. The Evaluation Panel worked via internal meetings, discussions with important stakeholders of KTL, and through site visits to the three divisions of KTL. On the basis of their evaluation, the Panel made 35 major recommendations for further development of the organization, management and work of the Institute.

Over the past 10 years, most of the recommendations made by the Panel have been implemented: the organization of KTL has been changed, several areas of research and public health work have been directed towards new priorities, and more emphasis has been put on the public health impact of the work of KTL. In addition, a renewed strategy was prepared for the Institute in 2001 during a thorough process involving the whole organization. At the end of 2003, a new Director General was appointed to KTL.

Based on these developments, and on changes in the environment, time is ripe for a new evaluation of KTL. The purpose of such an evaluation would be to evaluate the effectiveness of the work and assess the scientific and societal impact of the Institute.

KTL IN BRIEF

Strategy and functions

The mission of KTL is to protect and promote the health of the Finnish people. As a research and expert institute belonging to the Ministry of Social Affairs and Health, KTL is responsible for providing decision-makers, professionals and citizens with the best possible health-related information for their choices. A general strategy for the Institute was prepared in 2001, while detailed objectives for the work are agreed upon annually with the Ministry.

The three main areas of work in KTL have traditionally been: 1) infectious diseases and immunizations, 2) chronic diseases and health promotion, and 3) environmental health. In all these areas, both research and public health functions are carried out. Activities of the Institute include basic research, ranging from the detailed analysis of the molecular mechanisms of pathogenesis to large scale epidemiological and preventive studies and research into factors influencing health.

KTL monitors public health, diseases and their determinants through surveys and registers. Research and expert information is transferred into action by developing health-promoting

and preventive measures and by advising and collaborating with various stakeholders. National vaccine service, many centralized laboratory functions and forensic medicine investigations are some of KTL's service functions.

Several of KTL's functions are based on laws, such as surveillance of infectious diseases and protection from communicable diseases by vaccinations. In the prevention of chronic diseases KTL works in close collaboration with various Non-Governmental Organizations. In promoting healthy environment and preventing diseases KTL collaborates with environmental authorities and municipalities. A strong presence in the media is a way to reach the people. The ultimate goal is to reduce the human suffering and economic cost caused by illness and to help people enhance their quality of life.

Organization, personnel and budget

KTL's main facilities are located in Helsinki and three other facilities in Kuopio, Oulu and Turku. The Institute has 11 departments, each of which is built of various laboratories and units.

Ultimate responsibility of leading and managing the whole Institute rests on the Director General of KTL. He is assisted by the Deputy Director General, the Administrative Director and the Steering Group, consisting of the Directors of Departments. The Director General is also advised by a Scientific Council of KTL, where representatives of most important stakeholders are present.

At the end of 2005, KTL had a staff of 887 persons, of whom 367 were scientists or experts. Women make up 73% of the staff. In addition to the permanent or temporary staff, KTL is also a working place for non-paid students or scientists who pursue their studies and research together with the staff of KTL. Altogether, there were 158 PhD students working at KTL in 2005.

The total expenditure of KTL was 63 million euros in 2005, and the operating expenses were 54 million euros when the acquisition of vaccines is excluded. Most part (65%) of the operational funding comes from the national budget, 25% from external sources like the Academy of Finland, the European Union, US National Institutes of Health, or various Foundations supporting scientific research. The rest 10% of KTL's budget is covered through income from chargeable services and from miscellaneous other funding.

EVALUATION PROCESS

Scope and purpose of evaluation

The objective of the review is to provide an evaluation of the work of KTL for the Ministry of Social Affairs and Health. The evaluation should examine the functions, strategic importance, scientific merits and value for money of the scientific and expert work undertaken, and to make proposals for future work. A special emphasis will be placed on the evaluation of the relevance and effectiveness of the work and of its impact on the health of Finnish people.

The evaluation and the Evaluation Report should address the following main issues:

a.	National relevance and effectiveness of the activities
b.	Appropriateness and adequacy of the research, expert functions and services
c.	Output and quality of research activities
d.	National and international co-operation
e.	Resource allocation
f.	Research fund raising
g.	Development needs, especially regarding processes and organization

The main purpose of the evaluation is to guide the Ministry of Social Affairs and Health so that they can use them for the strategic management of KTL. The practical implementation of the results, based on the decisions made at the Ministry, is the responsibility of the Director General of KTL.

Entities to be evaluated

The first round of detailed evaluations consists of four separate, partly parallel evaluations covering the main functional areas of KTL:

a. Environmental health
b. Chronic disease prevention and health promotion
c. Infectious diseases
d. Molecular medicine – the evaluation of this area will depend on whether the results of the planned evaluation conducted by the Ministry of Education will provide sufficient information for the purposes of the Ministry of Social Affairs and Health.

After the first round has been completed, the entire Institute will be evaluated. This evaluation will focus merely on the general strategy, function and management of the Institute, with less emphasis on the evaluation of individual research and expert functions.

Information sources for the evaluation

KTL will provide the Panels following information:

Published documents concerning the whole Institute

a. Annual Report 2005
b. Kansanterveyslaitoksen toimintakertomus ja tilinpäätöslaskelmat 2005 (available only in Finnish)

c. Evaluation of the National Public Health Institute of Finland. Report of the Evaluation Panel 1995

A document prepared by the Director of each relevant Department involved in the evaluation, will be provided for the evaluation. These documents describe 1) a report on progress in research over period 1996 -2006 and research plans for the period 2007-2011, 2) the arrangement for governance and management, and 3) allocation of staff and resources, and 4) his/her plans for the future development of the Department.

Each Department involved in the evaluation will also provide the Panel a self evaluation of 1) the appropriateness of its work to the national public health needs, 2) its role in the dissemination of research results and knowledge and technology transfer, 3) a description of the interfaces between the Department and the key players in Finland and abroad.

Evaluation of the Department of Environmental Health

The evaluation process was initiated in the Department of the Environmental Health (YTOS) already in June 2006 after discussions in KTL's Steering Group and with the laboratory heads of the Department. The panel consists of three members, two from abroad and the third from Finland. All the panelists are familiar to governmental health research, and the Finnish member will provide to the panel especially the knowledge of the national needs. Prof. Erik Dybing from Norwegian Institute of Public Health, Department of Environmental Medicine and Dr. Robert L. Maynard, Wellington House, Department of Health, UK, and Prof. Harri Vainio, Director General of the Finnish Institute of Occupational Health were invited to conduct the evaluation.

The next step of the evaluation is focused on desk research, during which the Panel will examine the documentation provided to them. During the site visit in November or December 2006, Panel members will have an opportunity to interview the staff of YTOS and discuss with representatives of key stakeholders and collaborators in relevant Ministries and University of Kuopio. After the discussions, desk research and site visits, the Panel will prepare a Report to the Ministry of Social Affairs and Health and to the Director General of KTL.

1 DEPARTMENT OF ENVIRONMENTAL HEALTH (YTOS)

1.1 Objectives and organization

Motto: *"People must be able to breath, drink, eat and live in the environment trusting on its safety. This is both an individual's civil right and a prerequisite for a functioning society and economy."*

The goals of the Department of Environmental Health are (i) promotion of public health, (ii) reduction in environmental exposures and (iii) improved decision-making on environmental risks. These goals are pursued through scientific research, risk assessment, dialogue with the decision makers, and educating the public.

The main areas of the Department of Environmental Health are drinking water, chemicals, air pollution, asthma and allergies and indoor microbes. Risk analysis forms the umbrella over the most of the research done at the YTOS.

There are five laboratories/units at YTOS (Figure 1). The heads of these laboratories have regular weekly meetings. The information meetings for the who staff are also weekly.

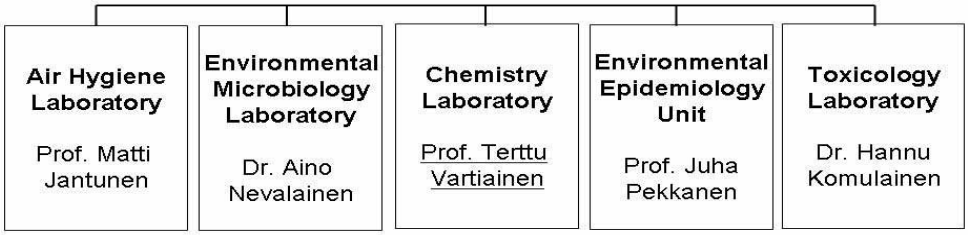

Figure 1. The laboratories/units and their heads within the Department of Environmental Health. The Head of the Department is Professor Terttu Vartiainen.

The laboratories/units at YTOS are the centers of know-how but scientific research is conducted in collaborative research programmes across the laboratories/units. In this document prepared for the evaluation of the department, we have organized the material into six research programmes (the coordinator of the documentation is in parentheses):
1. **Air Pollution and Health** (Prof. Matti Jantunen), 2. **Respiratory Disease, Indoor Microbes and Immunotoxicity** (Dr. Aino Nevalainen), 3. **Water and Health** (Dr. Ilkka Miettinen), 4. **Persistent Organic Pollutants and Health: Analysis, Concentration, Exposure and Epidemiology** (Prof. Terttu Vartiainen), 5. **Persistent Organic Pollutants: Mechanisms of Health Effects** (Dr. Matti Viluksela), and 6. **Risk Analysis** (Dr. Jouni Tuomisto).

Since all environmental problems cannot be covered with the resources available we have prioritized these six research programmes as our main focus of interest. Noise and radiation are examples of environmental health research fields that are covered by other institutions than KTL.

1.2 Staff and resources

Department of Environmental Health (YTOS) was established in 1982. The staff (3 positions) began to grow slowly after 1985 when the building '*Tutkimuskeskus Neulanen*' was completed. In 1992 the building was expanded and good animal facilities were also included in it.

In 1992, the Regional Pubic Health Laboratory, Kuopio, was closed down and 16 positions (one researcher and 15 technical assistants) were moved to YTOS. In 1996, there were 40 permanent service contracts (money coming from the government) and 91 persons with external funding. The permanent contracts have remained about the same since1992, whereas the number of persons funded with external funding has varied. The development of personnel at YTOS is shown in Table 1.

Table 1. Development of personnel of the Department of Environmental Health from 1996 to 2006.

	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006
Permanent											
Head of laboratories	6	6	6	6	6	6	6	6	5	5	5
Senior researchers	5	5	5	5	5	5	5	5	6	6	8
Researcher	1	1	1	1	1	1	1	1	1	2	1
Technical assistants	28	28	29	29	30	30	30	30	30	29	27
Together	**40**	**40**	**41**	**41**	**42**	**42**	**42**	**42**	**42**	**42**	**41**
External funded											
Senior researchers	0	0	2	2	2	3	3	3	2	2	2
Post docs	1	3	7	5	7	7	20	25	27	24	21
Students	37	40	48	42	35	29	30	26	25	21	28
Technical assistants	13	19	20	20	20	24	30	29	23	28	36
Together	**51**	**62**	**78**	**71**	**64**	**64**	**87**	**89**	**82**	**79**	**89**
Total	**91**	**102**	**119**	**112**	**106**	**106**	**129**	**131**	**124**	**121**	**130**

The operating costs (from government budget) of YTOS were 2.81 million € in 1996 and has risen to 3.14 million € in 2006 (Table 2). External funding has varied between 0.59 - 4.76 million € per year, being on an average 2.34 million € per year (Table 3).

Table 2. Governmental budget (k€) of the Department of Environmental Health from 1996 to 2006 *(not corrected for inflation).*

	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006
Salaries of permanent personnel	1220	1253	1286	1318	1401	1461	1520	1631	1700	1670	1775
Funding for running costs	673	682	639	664	650	648	638	640	634	634	634
Equipment	315	152	34	489	60	154	160	83	330	154	0
Rent	599	599	600	600	606	619	668	671	715	714	736
Total	**2807**	**2686**	**2548**	**3073**	**2720**	**2881**	**2987**	**3026**	**3338**	**3173**	**3143**

Table 3. External funding (k€) of the Department of Environmental Health from 1996 to 2006. Grants are categorized by the research programmes of the Department. The figures show the total amount of grants obtained in that year (one grant is often for 2 to 4 years).

	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005	(2006*)
Air pollution	585	404	597	53	99	195	1211	500	169	1015	116
Asthma, microbes	87	345	976	179	269	922	906	160	605	700	379
Drinking water	204	315	1259	143	97	571	126	90	374	7	248
POPs: exposure	620	107	594	52	130	1488	711	242	98	1061	52
POPs: mechanisms	243	370	654	209	3	213	938	399	90	250	632
Risk analysis	41	-	85	7	3	7	494	-	142	1723	933
Total	**1780**	**1541**	**4165**	**643**	**601**	**3396**	**4386**	**1391**	**1478**	**4756**	**2360**

**By September 2006.*

The total annual budget of the KTL/YTOS has varied between 3.66 – 7.93 million € (average 4.46 million €) during the years 1996-2006 (Figure 2).

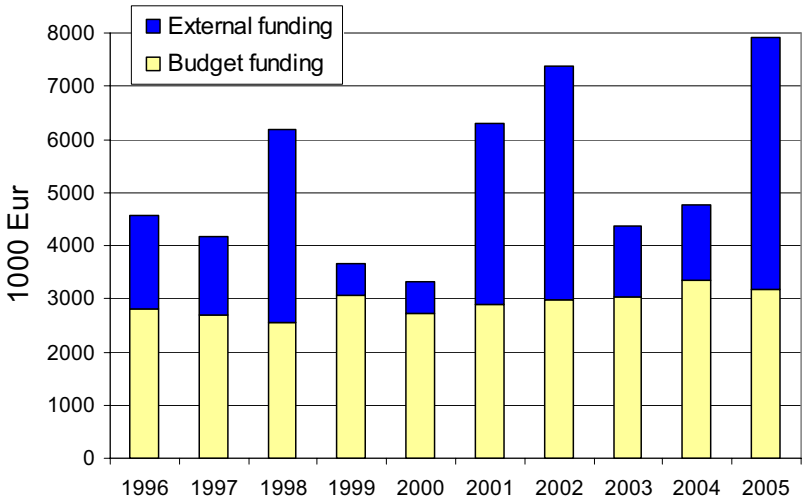

Figure 2. Total annual budget of the Department of Environmental Health in 1996-2005.

1.3 Research areas

Our main research areas are exposure to and health effects of indoor and outdoor air pollutants as well as drinking water impurities. In addition they cover chemicals in food, water and human tissues. The research includes studies on concentration distributions, determinants of exposure to chemical and biological agents, epidemiological studies and experimental studies. Research also includes syntheses in the form of risk analyses, and methodological work aiming at developing risk analysis as science. The focus is on pollutants with environmental and health-related relevance in Finland. Respiratory and cardiovascular disease and cancer are the main diseases of interest.

As to chemicals, we study persistent organic pollutants and metals, e.g. methyl mercury and organotin compounds. Studies on environmental microbes in indoor environments are mainly focused to non-infectious organisms and microbial components with immunotoxic properties. Water microbiology includes clinically significant environmental bacteria such as noroviruses, campylobacteria, legionellae and mycobacteria. Experimental studies on chemicals focus on mechanisms of toxicity (developmental effects, mutagenicity, carcinogenicity) but also *in vivo*-experiments are done in laboratory animals. The main aim of research on ambient air particles is to determine the characteristics and sources which are responsible for their health effects and to explain the mechanisms by which ambient particles damage health. The approach combines exposure assessment, experimental reseach, epidemiology, and risk analysis into a single coherent research effort. Much of our research aims to risk analysis that is our growing research programme. For this reason, we do also basic risk research. The research on risk analyses started only recently at YTOS and thus, is not represented in all the tables of this document.

The Department of Environmental Health gained a status of a Centre of Excellence for years 2002-2007 from the Academy of Finland. The general objective of the *Centre for Environmental Health Risk Analysis* is to improve environmental health risk analysis by increasing the understanding of environmental risks at all levels from molecular mechanisms to societal needs. This requires both high-quality research and better methods of risk modelling and characterization.

We have high-quality equipment for chemical, microbiological and toxicological analyses, and modern cell culture and laboratory animal facilities for *in vitro* and *in vivo* studies (see Appendix 1). We have personnel trained specifically to use the modern equipment. All our research activity is carried out according to the principles of our quality assurance system, managed by specifically trained personnel.

1.4 Quality Management

Recommendations, guidelines and legislation concerning research carried out in KTL are gathered in the '*Good Research Practice in the National Public Health Institute Handbook'* (B4/2006). The handbook is the basic manual for the quality management in KTL and it describes the practices to be followed. It gives e.g. guidance on the preparation of a study plan, on ethical issues and on funding.

In addition, YTOS has its own handbook '*Good Practices in YTOS Handbook' (2005)* (*Hyvä Toimintatapa YTOSissa*, available only in Finnish). The handbook contains general guidelines and requirements on e.g. personnel, training, facilities, equipments and archiving. The YTOS-SOPs (standard operation procedures) present more detailed guidelines for the different functions of the Department, and they also give the general formula how to write these SOPs.

Laboratory of Toxicology, especially the animal laboratory has a long history of compliance with the Good Laboratory Practice (GLP). Nowadays Laboratory of Toxicology follows the principles of the GLP, but does not have the GLP compliance status. Laboratory of Chemistry was accredited according to the standard SFS-EN ISO/IEC 17025 in 1996 (number T077 in Finland) as the first laboratory at KTL. Laboratory of Environmental Microbiology is presently preparing to achieve the similar accreditation in the near future.

1.5 Scientific impact of research

The number of scientific articles in international peer review journals and domestic articles are shown in Table 4. Each article is counted only once and included into the most relevant research programme. Thus, the sum numbers in Table 4 show the total numbers of articles published in YTOS in each year.

Table 4. International peer review articles and domestic articles within different research programmes at the Department of Environmental Health published in years 1996-2005. (Articles related to risk analysis are included to other research programmes).

International peer review articles	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005	**Sum***
Air pollution	8	9	10	8	17	15	13	13	13	22	**128**
Asthma, microbes	10	14	16	19	11	19	25	20	20	18	**172**
Drinking water	9	14	6	7	6	11	11	13	15	12	**104**
POPs: exposure	5	8	5	8	4	9	14	3	9	4	**69**
POPs: mechanisms	19	5	14	13	7	14	11	5	12	11	**111**
Sum*	**49**	**50**	**51**	**55**	**42**	**68**	**74**	**54**	**67**	**64**	**584**

Domestic articles	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005	**Sum***
Air pollution	-	1	1	2	2	5	4	1	12	4	**32**
Asthma, microbes	4	7	5	7	2	4	12	4	11	9	**65**
Drinking water	-	5	4	11	5	6	5	10	7	2	**55**
POPs: exposure	-	1	1	-	4	2	2	1	-	1	**12**
POPs: mechanisms	-	3	1	6	5	3	3	3	4		**28**
Sum*	**4**	**17**	**12**	**26**	**18**	**20**	**26**	**19**	**34**	**16**	**192**

*The numbers of the articles presented in Table 4 are lower than those presented by each research programme because there is overlapping between the different programmes in e.g. methods and approaches.

Our researchers are actively supervising doctoral students working at the Department. Altogether 63 doctoral dissertations have been finished (the work done at YTOS) during the last 10 years (Figure 3). On average six PhD dissertations have been completed annually.

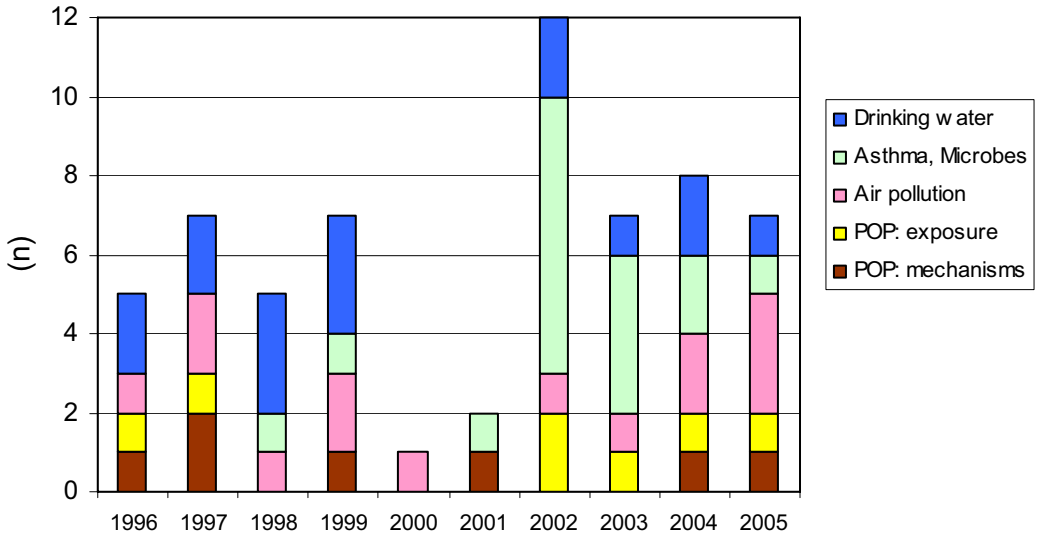

Figure 3. Finished doctoral dissertations in the five research programmes of the Department of Environmental Health during 1996 – 2005.

1.6. Social impacts of our work: examples of achievements of KTL in the Finnish Society

Finnish drinking waters were shown to be highly mutagenic, and there was concern on carcinogenic effects. KTL launched a research program including exposure monitoring, basic research, animal toxicity studies, and epidemiology to solve the importance of the problem. These results ended up to be of very important help to waterworks all around the country, many of which have changed their technology on basis of our results and often after our advice. It may be calculated that this information saves a number of cancer cases annually.

Indoor air problems were shown to be very important in Finland, and contrary to earlier wisdom, the reason was often moisture damage causing both chemical emissions and microbiological growth of mold and bacteria. This area clearly needed systematic research before any administrative measures could be effective. In this work KTL has been instrumental for both government and local authorities.

Help to authorities in rational and discussion-based handling of the difficult and highly sensitive area of storage of highly active radioactive waste. The parliament ended up to almost unanimous agreement in their decision aided by moderate and rational advice by several governmental research institutes including the Radiation and Nuclear Safety Authority (STUK) and KTL. It is remarkable that even the Green Party accepted the argumentation that it is safer to plan a permanent storage capacity instead of the present situation with temporary stores.

Help to authorities in dealing with risk comparisons of various options for energy production in Finland. These included the option of constructing a new nuclear reactor to existing nuclear power plant at Olkiluoto discussed by the Parliament in 2002. Regardless of the various opinions, discussions remained mostly quite rational and proceeded in an orderly manner differing from some other countries, and politicians accepted their duty of comparing in a science-based manner the risks of various options. This was greatly aided by the thorough expert opinions by government research institutes such as STUK and KTL.

Thorough treatise of the risks of dioxins and PCBs in fish to Finnish population has been done. Fish is important for the wellbeing of Finnish population especially because our cardiovascular mortality has been very high, and also because vitamin D intake in Finland is more dependent on fish than in most other countries. Especially after the dioxin/PCB incident in Belgium in 1999, the European population was highly worried on the risks of dioxins, but due to balanced information, public discussion in Finland remained mostly rational, and big fears were not initiated. At the same time the researchers started to work hard to define better the remaining risks and the future means to decrease them.

Suburban residential wood-burning is increasing and causes already neighbourhood air quality problems and disputes mostly in the winter. Domestic wood smoke already constitutes one-third of the total $PM_{2.5}$ emissions in Finland. Forest fires in Russia and Baltic States have also increased, and caused summertime air pollution episodes and major concern particularly in South-East Finland. KTL has raised the public awareness of the health risks of both of these biomass smoke issues, arranged frequent consultations with municipalities and patient organizations, brought the environmental health issues of wood-burning to the political agenda, prepared official statements concerning specific cases, and informed the public and susceptible population groups through the media during the episodes.

In Finland the public image of waste incineration suffers from limited experience and poor environmental track record. Yet, for many communities there appear to be few economically feasible and environmentally better alternatives to it. Also the opportunity to co-generate significant amounts of 'non- fossil' power and heat, have brought waste incineration into the agenda. To respond to public concerns, to provide guidance for the permitting and operating authorities, and based on our extensive research background on emissions of and exposures to POP and carcinogens from combustions processes, KTL has prepared several official statements of the emissions, exposure contributions, benefits and risk concerning specific plans for both national environment and public health authorities and municipalities, and participated in respective public hearings.

We assist municipal officials in the town planning questions concerning contaminated areas. We have about two hundred by dioxins and chlorophenols contaminated, otherwise excellent building areas. The question is, what areas to clean and to what extend. Wrong decisions mean either huge extra costs (millions of euros per area) or increased risk for population and contamination of e.g. ground waters.

The financial benefits of our expert work of the senior researchers on environmental health problems have been remarkable being many times more than the whole budget of YTOS.

1.7 Statements and expert assessments on environmental health questions

KTL/YTOS prepares annually about 20 written scientific statements on environmental health questions and problems to municipal and other authorities. These are given on a request, but after careful consideration. The questions to be addressed vary widely (e.g. a risk of a single contaminant in drinking water; building of houses on the land where a saw mill has previously located). Some laboratories have also tasks as a reference laboratory for e.g., water pathogens, which means frequent consultations to local laboratories. Moreover, the municipal

health authorities are advised nearly daily (daily risk assessment) in less official questions over the phone or as response to e-mails. The work has been distributed mainly between the senior experts, according to the expertise needed. Advice is also given to various professionals directly, as well as to the general public, although this direct dissemination of information takes mainly place via the web-pages of YTOS.

The expertise and authority that is needed for the expert work described above is based on scientific research at YTOS on the essential research areas in environmental health. This guarantees that the issues can be assessed through solid knowledge on the literature, methods and recent achievements of the relevant scientific research.

1.8 The responsibilities not directly related to research programmes

KTL/YTOS is a core participant in the Finnish Centre of Expertise on Chemical Threats. This is a network of expert national institutions (Finnish Institute of Occupational Health, Finnish Food Safety Authority, Finnish Defence Forces, Finnish Institute for Verification of the Chemical Weapons Convention, National Bureau of Investigation, Finnish Poison Information Centre and Ministry of Health and Social Affairs) supporting responsible authorities to improve their preparedness to chemical threats. The Centre is a backup, not the primary actor in emergency situations, and answers to non-routine difficult questions. At present it provides local health authorities, rescue services and police consultative advice on health risks during chemical incidents on request, round-a-clock. Two out of 7 experts responding to emergency calls for one week at a time are from KTL/YTOS. The emergency preparedness was started in April 2006. The members of the Centre participate also in training of the local actors in health risk -related subjects.

Other responsibilities not directly related to research are presented in the corresponding programme presentations.

1.9 Scientific courses and meetings

KTL/YTOS has participated in several scientific umbrella organisations at the Kuopio University campus area. The five collaboration organisations

- Environment, health and society programme of the University of Kuopio – YTY (1997-2000)
- Finnish Research Programme on Environmental Health – SYTTY (1998-2001)
- Kuopio University Centre for Environment, Health and Society – YTY-centre (2001- on-going)
- Graduate School in Environmental Health - SYTYKE (2002- on-going)
- Environment Risk Assessment Centre – ERAC (2005 – on-going)

are described in the Appendix 2. Four of them (YTY, SYTTY, YTY-centre, and SYTYKE) have been chaired and coordinated by the YTOS. A high number of post-graduate courses and meetings have been organised by these organisations to support doctoral training since 1997 (Table 5). In spite that doctoral training is not an essential part of a research institute's function, it is an essential part of scientific research.

Table 5. The number of meetings and post graduate-courses organized during 1997-2006. 25 % of the events had international speakers.

	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	Total
YTY (1997-2000)	1	12	8	14							**35**
SYTTY (1998-2001)		6	8	7	7	2					**30**
YTY-centre (2001-2006)					8	0	2	2	2	0	**14**
SYTYKE (2002-2006)						1	7	7	5	8	**28**
Total	1	18	16	21	15	3	9	9	7	8	**107**

1.10 Proposal for the future work and expected benefits

We continue to serve Ministry of Social Affair and Health (STM) and Finnish and the wider public in the area of environmental health in several fundamental ways: by doing high quality research that gives us the expertise and credibility to advise both the ministry and the public; by collaborating with STM in environmental health questions; by taking part in several national and international meetings as advisors, and by monitoring e.g. drinking and swimming water quality.

Future research directions

Prevention, control and risk management of environmentally-induced diseases can only be done through which is based on solid knowledge on the causal connections between exposing agents and their effects. Thus, clarification their role with well-focused research is necessary in order to implement the correct measures.

Chemicals will remain a continuous concern in our society, and both analytical and toxicological expertise is necessary both to reveal real threats and also to curb unnecessary and unfounded public concerns. This requires high sophistication, a good general knowledge, international relations, and ability to read the latest scientific achievements from scientific literature. A good generalization is that 99 % of information comes from international sources, but to be able to fully utilize those sources, one has to create 1 % of new information in our own country.

The research group on water and health has successfully influenced to drinking water policy in Finland. Although no new significant chemical hazards in drinking water are at present foreseen in Finland, the previous expertise maintains readiness to respond to new challenges as need also in the future.

One of the biggest challenges which will affect the water sector in Finland in next years is ageing – both the personnel and the infrastructure. Aging of pipeline (water/waste water) systems will increase the probability of leakages in vulnerable ground water aquifers and outbreaks of microbial contamination episodes. Thus even in future the biggest health issue in water sector will be associated with the occurrence of waterborne pathogens. The contamination of municipal water supplies and the number of opportunistic pathogens in technical water systems (bathing water, cooling water, waste water) require further research activity in Finnish context for efficient prevention and treatment of the problems and cases. One field is to improve methods in water quality analyses to identify microbiological agents causing the outbreaks. Another area which has received as yet little attention everywhere is a more comprehensive risk analysis on household water, taking into account simultaneously risks and bene-

fits of each factor and action. The work has been started in the EU-funded HI-WATE project where risks and benefits of drinking water chlorination are compared. Risk analysis is expected to provide more concrete information and new tools for decisions making in general. The group will also continue the work with a model Water Safety Planning (WSP) protocol. The group will e.g. test risk assessment and risk management protocols of drinking water production (contingency plans – protection actions).

Three major environmental health problems in developed world today are *outdoor fine particle air pollution*, adverse effects of *indoor microbial exposures* in water damaged buildings, and increased incidence of *asthma and allergies*. These areas of research have several things in common: they are all linked to airway inflammation and development of asthma and exposure occurs simultaneously indoor, and in all areas, the main piece of information needed for effective risk management is which characteristics of exposure explain the observed associations. In all areas, more close collaboration between exposure assessment, toxicology and epidemiology is urgently needed. Despite these similarities, the areas have proceeded mainly separately and developed own research traditions and methods.

Combining these traditions can potentially lead to very rapid advances in knowledge. This is a major aim within YTOS in the next years. We feel that YTOS is uniquely placed to do this. We have long experience truly multidisciplinary field works, which combine detailed exposure characterization, personal exposure assessment, and epidemiology into population-based field works. The main challenge in the future is to incorporate also toxicological approaches, i.e. in vitro and animal testing of environmental samples, into these same field works. Such a field work has already been carried out last winter looking at acute effects of outdoor air pollution and we plan similar field work looking at exposure and health effects of indoor air in problem schools. The main aim of our future research efforts are to characterize in more detail the causal microbial exposure and its mechanisms leading to both the potential protective and adverse health effects. Our main focus is on respiratory and cardiovascular disease and allergies, but hope to study also other disease, like autoimmune diseases.

Climate change is an obvious long-term threat to humankind. Even if health risks are difficult to approach by usual scientific means, this will be necessary as part of total activities in the area of air pollution. This also leads to a necessity to follow closely the most important societal activities leading to increase of greenhouse gases, i.e. traffic and energy production. This is in line with experience already achieved in air pollution field, and reduction in fossil fuel burning has a double impact on health: immediate benefit from reduced air pollution, and a long term benefit from decreased greenhouse gases. The society will need rational arguments also from health sector in the abatement of climate change.

Society needs us to predict and warn about potential new environmental health *risks* (precaution), to identify and explain and quantify - from sources via environment to exposure, mechanisms and public health consequences - the current risks, to rank and quantify the risk management potentials of alternative control measures, and to follow up policy implementation programmes for accountability. In risk analysis research, we have tried and improved this situation by specifically doing research on policy issues that have an important public health aspect. This helps getting a better view on the impact of the health issues in the larger picture. We have also developed risk analysis methods, the target being to facilitate and improve the work of future risk analyses.

Children's risk have become of more and more important concern. Some of these are real, and some are unfounded, and it will be highly important in future to be able to differentiate the true risks. Therefore increased effort should be given to developmental effects, risks of preg-

nancy, and risks of small children. This will include toxicological research, environmental analysis work, and epidemiology. This will also include research of children's microenvironment such as indoor and outdoor environments in schools, daycare centres and other places planned for children.

Department's strategy and expected benefits

"*Health in all policies*" is one of the driving forces of the policy-making in the European Union, and this topic is especially timely under the Finnish Presidency. It also very well suits the approach the Department of Environmental Health has in its work and research. There is rarely a situation when policy-makers would do environmental health policy. Instead, they are developing traffic policy, chemical registration, energy policy, tobacco policy, or waste policy. Although the focus and discussion is mainly on other issues, these are still key questions in environmental health. This situation has some important implications.

First, environmental health policy is mostly proactive influencing on other policy-making. Therefore, the time window when a particular environmental health problem is at the stage of active decision-making is usually narror and dependent on other issues than environmental health. The expert must be able to bring in her expertise whenever this situation occurs. To be able to do this, the expert must be well aware of wide range of disciplines, but at the same time she must master one or more specific fields in which she has very good view on what the suggested policies would cause, and which alternatives would be beneficial for health. The only way to fulfil this requirement is to have a wide expertise within the house and people that are encouraged to use their time in learning about other fields than their own. However, at the same time they must be top researchers in their own field and do active international collaboration. Otherwise they will not be able to gather the newest on-the-edge information that is needed to prevent the society from choosing based on outdated information.

Second, there must be enough in-house critical mass to ensure the continuity of the research and groups. In the Department of Environmental Health, most of the research is done with soft money from internationally competed sources. However, without permanent staff that can apply for funding, there would be no research funding at all, and all work would diminish to day-to-day administration and consultation. It would soon become impossible to contribute to policy-making.

Third, as there are hundreds of pieces of legislation under preparation on any given day, it is not a trivial task to know, which of them will be critical for health in the future. Wide expertise is needed to filter and select the key policy issues. Expertise in legislation processes or administration is not enough, but substantive expertise is needed even more than that. There should, therefore, be enough autonomy among the experts to choose the topics they will work at. Although it is not guaranteed that in this way there will be a top-level expert wherever one is needed, it will make it possible. When experts are truly interested in promoting public health and when they are able to choose their own topic, there is a good chance that they will do a good job in choosing an important one.

The environment, in which we work, communicate and disseminate the benefits from our work, is rapidly changing. Most of our work already deals with data assimilation, management and processing. The relative roles of laboratory experiments, field surveys and sample analyses used to be much higher. The working platform is increasingly and will be dominantly the internet. Increasingly the work will from project planning to final results and conclusions be transparent and available to anyone interested.

Appendix 1

Major equipment in KTL, Department of Environmental Health

Each laboratory has the modern basic instrumentation fitted to the work done. In addition, YTOS has the following special or large equipments:

- Two high resolution gas-chromatrograph-mass spectrometries (Autospec Ultima and VG 70-250 SE)
- Two gas chromatograph-mass spectrometries (Hewlett-Packard and Polaris Q MS-MS)
- Liquid chromatograph/MSMS (Finnigan TSQ Quantum Discovery)
- Supercritical fluid extractor (Suprex Autoprep 44)
- Accelerted solvent extractor (ASE 300)
- Ion chromatograph (Dionex DX-600)
- Total organic carbon analyzer (Shimadzu TOC-5000)
- Capillary electrophoresis (Applied Biosystems)
- Two quantitative PCR equipment (ABI Prism 7700 and Rotor-Gene 3000) and other equipment for molecular biology
- Autoclave (Steris Finn-Aqua 666E) for sterilizing culture media and clean laboratory items (e.g. pipette tips, bottles)
- Automatic media steriliser for the preparation of culture media
- Technomat Line consisting of Filling, Feeding, Embossing and Stacking Units for the preparation of culture media
- One Deko washer-thermal disinfector for laboratory glass according to the standard EN ISO 15883-1 and 15883-2, and three other Deko washers
- Pipetting robot (CAS 1200)
- Flow cytometer (CyAn ADP)
- Two Epiflourescence microscopes (Olympus BX 51 and BH2)
- Differential interference contrast microscope (Nikon Optiphot-2)
- High volume cascade impactor for particulate sampling
- Transportable particle monitoring station
- Portable particle monitors ($PM_{2.5}$, ultrafine)
- Portable monitors for gases CO, NO_2)
- Passive samplers (NO_2, VOCs)
- Lung function monitors (PEF, spirometry)
- Small animal exposure system for gaseous pollutants

Appendix 2

KTL/YTOS participation in the collaborative umbrella organizations concerning scientific research and education

Environment, health and society programme of the University of Kuopio, *YTY-centre (1997-2000)* was a joint organization of the University of Kuopio, YTOS, the Kuopio Regional Institute of Occupational Health, the Finnish Food Safety Authority and the Geological Survey of Finland. YTY contributed significantly to the training and education of the doctoral students by organizing a high number of doctoral level courses, funding the research and giving grants to doctoral students to finalize their dissertations. YTY was funded by the Ministry of Education with 320 500 €. YTY organized e.g. series of international Gordon Research Conference type workshops, Valamo conferences, on environmental health and risk assessment.

Finnish Research Programme on Environmental Health – SYTTY (1998-2001) was established by the Academy of Finland and financed by the Academy of Finland, the National Technology Agency, Finnish Work Environment Fund, Ministry of Environment, Ministry of Agriculture and Forestry, and Ministry of Transport and Communications. The aim of SYTTY was to generate scientific information that would help in analyzing connections between the environment and human health. It also aimed of developing methods and techniques for promotion of environmental health. The budget of SYTTY was about 8 million €, which was distributed to 47 projects. 15 of those were located at the YTOS. SYTTY was also coordinated by the YTOS.

Kuopio University Centre for Environment, Health and Society – YTY-centre (2001- on-going) advances scientific co-operation towards multidisciplinary research, and promotes interaction among the fields of science, education and practice. The YTY-centre also coordinates education and training, and organises courses and seminars. The Centre is a joint organization of the University of Kuopio, KTL/YTOS, the Kuopio Regional Institute of Occupational Health, the North-Savo Regional Environment Centre and the Finnish Food Safety Authority.

Graduate School in Environmental Health, SYTYKE (2002- on-going) educates experts in the field of environmental health. Graduate school combines different disciplines and substance knowledge based on the risk analysis chain of environmental health. Participating organizations in graduate school are University of Kuopio, YTOS, University of Helsinki, Radiation and Nuclear Safety Authority in Finland, Occupational health Institute, and Finnish Meteorological Institute.

Environmental Risk Assessment Center – ERAC (2005- on-going) establishes and widens the existing collaboration network between the KTL/YTOS, University of Kuopio and the Geological Survey of Finland. ERAC combines and makes the maximal use of the existing expertise on the different steps of the risk analysis chain of environmental health (emissions-transportation-exposure-effects) at these organisations. ERAC will develop, test and apply new methods in risk assessment. The budget of ERAC is 868 000 € for the years of 2005-2006.

2 AIR POLLUTION AND HEALTH

2.1. Research area and its significance

KTL air pollution and health research is an interdisciplinary programme, which focuses on those issues of particulate air pollution, which are essential for the development and implementation of science based and accountable risk mitigation policies at European, national and municipal levels. The specific tasks include

- quantification of the absolute and relative health impacts of urban air
- particulate matter (PM)
- monitoring and modeling of individual and population exposures
- determination of PM characteristics and sources that are responsible for the health effects
- explanation of the mechanisms by which PM damages health, and
- attribution of the PM exposures and health effects to the PM sources.

Our PM research is methodologically linked closely to two programme areas of the KTL Department of Environmental Health (KTL-DEH), namely those of Risk Analysis and of Respiratory Disease, Indoor Microbes and Immunotoxicology.

American and European epidemiological studies published since ca. 1990 have associated urban ambient air pollution, fine particulate matter and ozone in particular, with significant excess mortality and morbidity. Indeed, this affects a large number of individuals with cardio-respiratory disease – ca. 350 000 excess cases of death in Europe annually. This fact makes urban air pollution arguably the most important environmental health issue in Europe still today, when the levels of most pollutants are far below the levels of just a generation ago. Today, unlike 10 years ago, we start to understand the toxicological mechanisms of both long term and acute effects of fine PM on the respiratory and cardiovascular systems, adding biological plausibility to the statistical associations. New knowledge about the mechanisms helps identify the respective particle characteristics and sources, and thereby improves opportunities for targeting the risk management actions on those particle sources and exposures which are likely to pose the greatest risks.

The estimated public health impacts of air pollution are based, not on theoretical extrapolations from high concentrations or indirect evidence from experimental studies, but instead on actual urban population mortality statistics from dozens of cities around the world. Despite this fact the environmental regulators in Europe are reluctant to take decisive action for risk reduction. This is actually in contrast to their strict regulation implemented on some carcinogenic trace metal components (As, Cd, Ni) of particles, or of dioxins in food, where the evidence of human risk is much weaker and, even the highest health risk estimates, orders of magnitude lower.

The research on air pollution and health in the KTL Department of Environmental Health has targeted at building up further and more detailed epidemiological evidence, better explaining the causalities between exposures to PM of different characteristics and final health outcomes, and comparing the risk (i.e. exposure) reduction potential of different risk mitigation actions. The research has progressed on three fronts:

- Highly focused epidemiological studies in Finnish and certain Central European cities linking specific early cardiovascular morbidity indicators to exposure to urban PM fractions of different origins, composition, and different PM size fractions (coarse, fine and ultrafine).
- Experimental in vitro and in vivo studies using well-characterised, size-segregated urban PM samples collected from a number of European and Finnish cities to identify the cellular mechanisms and markers of toxicity of urban air PM in different source environments (traffic, residential heating with solid fuels, industrial).
- Exposure studies, both population-based and traffic exposure focused, aimed at attributing the exposures to sources, microenvironments and activities, and producing validated tools for predictive exposure modelling.

These three programmes are advanced in close cooperation with each other and in close collaboration with the leading European universities and research institutions.

2.2. The main achievements

The LIILA study, in early 1990's, laid foundations for incorporating personal exposure monitoring in air pollution epidemiology. It monitored CO and NO_2 exposures with several methods and assessed the associated health effects in preschool children in Helsinki. The key finding was that respiratory symptoms were most strongly associated with the personal exposure, then with NO_2 monitored at the day care centres, and only weakly with NO_2 measured at fixed-site monitoring sites. The study exemplified the need for increased accuracy in exposure assessment (Alm et al., 1998, Mukala et al, 1999, 2000).

Epidemiology of ultrafine particles and personal exposure to fine particles
After the LIILA study, our focus switched to the respiratory effects of ambient air fine PM. The PEACE project included 14 centres across Europe in mid 1990's. We were able to show that, even at the low PM levels prevailing in Kuopio, the daily variations in fine PM mass concentration ($PM_{2.5}$) and ultrafine PM number concentration were associated with changes in lung function, especially among asthmatic children (Timonen and Pekkanen 1997, Pekkanen et al. 1997, Tiittanen et al. 1999). In a study of asthmatic adults in Helsinki (Penttinen et al. 2001a, 2001b, 2006), ultrafine PM number concentration and combustion-related fine PM were more strongly associated with changes in lung function than PM_{10}, the latter being more influenced by soil materials.

Two personal exposure monitoring packages were developed with American equipment manufacturers. One consisted of $PM_{2.5}$ and VOC samplers and a real time CO monitor capable for 48-h independent operation, while the other was a simultaneous 24-h $PM_{2.5}$ sampling and real time monitoring. These, together with microenvironmental monitors formed the instrumentation core of numerous studies that followed in Finland, across Europe and in the US (Jantunen et al. 1998, Koistinen et al. 1999, Janssen et al. 2000). Personal monitors for ultrafine PM number counting were incorporated some years later.

Together with our German partner, we were the first group that used in epidemiological studies advanced aerosol spectrometers developed to monitor continuously the count/size distribution of particles in air (Pekkanen et al. 1997). Ultrafine PM number concentrations turned out to be as high in Finnish cities as in other parts of Europe, while the levels of $PM_{2.5}$ were clearly lower (Ruuskanen et al. 2001). We also quantified the large contribution of soil dust to

urban air PM in Finland (Hosiokangas et al. 1999), analysed the seasonal variation of PM (Vallius et al. 2000), and addressed the adequacy of centrally monitored ultrafine PM number concentration as proxy for exposure (Buzorius et al. 1996). The first results from epidemiological studies with personal assessment of $PM_{2.5}$ exposure among elderly cardiac patients (Janssen et al. 2000) are available and will be soon published.

In late 1990's, the focus shifted towards the cardiovascular effects of PM. We coordinated in Helsinki, Amsterdam, and Erfurt the largest ever study on the health effects of ultrafine PM (ULTRA) (Pekkanen 2000). An association was shown for the first time between ultrafine PM number concentration and $PM_{2.5}$, and the risk of myocardial ischaemia as indicated by ECG changes. $PM_{2.5}$ concentrations were also associated with increased epithelial barrier permeability and cardiorespiratory symptoms. We apportioned $PM_{2.5}$ in Helsinki to sources during two winters and have now linked also source specific $PM_{2.5}$ to respiratory and cardiovascular end points (Penttinen et al. 2006, Lanki et al. in press). The results stress the harmfulness of $PM_{2.5}$ derived from combustion, especially traffic.

We currently participate in three new EU-funded epidemiological studies, all focusing on ultrafine PM number concentrations. Two of them are panel studies utilizing our SOPs. In the first panel study, the association of PM with inflammatory markers and gene-environment interaction are scrutinised (AIRGENE). The second panel study focuses on exposure assessment to and health effects of ultrafine PM number concentrations (RUPIOH). The study demonstrated a low correlation between ultrafine PM number concentrations measured at home and at a central monitoring site. In a register-based study (HEAPSS), ultrafine PM number concentration was seen to increase the risk of recurrent myocardial infarction and other cardiovascular diseases (Lanki 2006). We have also demonstrated as association between daily stroke mortality and PM size distributions in Helsinki (Kettunen, in press).

Fine PM exposures in European urban populations

Besides epidemiology, exposure research has also other applications, such as evaluation of the relevance of environmental monitoring programmes (i.e. do they reflect actual exposures), and generation of databases and tools for risk management option evaluation and policy accountability evaluation.

For these purposes, the KTL coordinated the EXPOLIS (incl. EAS-EXPOLIS and the University of Basel coordinated EXPOLIS-IndEx) project that covered dozens of gaseous, vapour and particle phase air contaminants (Jantunen et al. 1998). It produced population representative exposure and exposure factor databases in seven cities (Athens, Basel, Milano, Praha, Grenoble, Oxford and Helsinki) (Hänninen et al. 2002 and 2004) and evaluated the applicability of personal exposure monitoring for epidemiological studies (Oglesby et al. 2000a and 2000b). It also analysed the key determinants – environmental, behavioural, socio-economic etc. - of exposure (Koistinen et al. 2001, Kousa et al. 2001, Rotko et al. 2001) and attributed the exposures to their indoor and outdoor sources (Koistinen et al. 2004). In addition, EXPOLIS project assessed the association of perceived (sensed) air pollution exposure to different actually inhaled air contaminants across Europe (Rotko et al. 2002), and produced and validated a probabilistic population technique for predictive exposure modelling (Hänninen et al, 2003 and 2005).

The experiences and results of EXPOLIS have been applied to numerous domestic (HEAT, MOBILE) and international (HEARTS, FUMAPEX, JRC-INDEX, ExpoFacts, iF, INTARESE, etc.) studies, as well as to practical risk assessment and management.

HEAT, MOBILE and smaller related studies aimed at better understanding of the air pollution exposure from traffic, and modelling of the risks of alternative urban traffic policies in the Helsinki Metropolitan Area. Findings of interest included smaller than expected benefits from CNG vs. Euro 4 diesel busses (Tainio et al. 2005), surprisingly high transition metal PM exposures in underground stations and metro trains (Aarnio et al, 2005), and the high contribution of a small fraction of high polluting vehicles on the total street traffic emissions and, thus, a great overall potential for traffic pollution reduction (Yli-Tuomi et al. 2005).

The WHO coordinated HEARTS project generated methods for the assessment of the public health impacts of air pollution, noise and accidents of alternative urban transport policies. Findings of interest included a sizable contribution of the time in street traffic to total traffic pollution exposure, a tremendous variation of ultrafine PM number concentration exposure in traffic microenvironments, and a need to reduce the exposures of public transport users and to guide pedestrians and bicycle commuters for exposure avoidance.

FUMAPEX linked weather forecasting models with ambient air quality and personal exposure modelling to evaluate various exposure mitigation alternatives for urban air pollution episodes. Of the four studied episodes in Helsinki, the highest $PM_{2.5}$ exposures were caused by long-range transported smoke from wildfires in Eastern Europe, while the lowest exposures were by a local inversion episode (Baklanov et al, 2006).

Chemical composition and effect mechanisms of urban air PM

The third critical component for understanding the risks of the urban air PM from different sources consists of the mechanisms by which the PM components cause the observed public health impacts.

In late 1990's, we published our experimental and field studies on exposures to and toxic effects of gaseous pollutants (SO2, NO2) in cold environments. This involved animal and human studies in the laboratory and human studies in indoor ice arenas. We then shifted our main scientific interest towards cell and animal toxicology of urban air PM mixtures. Our previous in-vitro and in-vivo toxicology work on indoor air bioaerosols provided the methodological basis for this enterprise. A long-term partnership was established with the Finnish Meteorological Institute (FMI) to get high-quality sampling and physicochemical characterization of urban PM for our toxicology studies.

We improved the sampling properties of the Harvard high volume cascade impactor (HVCI), developed for large capacity, size-segregated sampling of urban air PM (Sillanpää et al. 2003), and validated the PM sample preparation for toxicological studies (Jalava et al. 2005). Subsequently, we have used the HVCI in national and EU-funded projects for sampling of PM mixtures in different source environments. These studies have included episodes of long-range transported wildfire smoke to Helsinki, six European cities with different source mixtures (PAMCHAR-EU) and Helsinki during four seasons (PAMCHAR-FINE). These studies have included an in-depth chemical (e.g. ions, water soluble elements, PAHs) and source characterization of the PM mixtures (Sillanpää et al 2005a, 2005b and 2006) that were investigated in toxicological studies.

We showed that the ability of coarse (PM10-2.5), intermodal size range (PM2.5-1), accumulation (PM1-0.2) and ultrafine (PM0.2) particles to cause cytokine production (TNFα, IL-6, MIP-2) in the mouse macrophage cell line RAW264.7 reduced with smaller particle size, but that the size range had a much smaller impact on NO production and cytotoxicity. PM1-0.2 of mainly long-range origin had a lower overall toxicity compared to PM1-0.2 originating more

from local sources. We suggest that this is due to aging of the organic PM fraction (Jalava et al. 2006a). In the PAMCHAR-EU study, we observed considerable heterogeneities in the toxic activities of size-segregated PM when investigated in vitro (mouse macrophage cell line) and in vivo (intratracheal instillation in healthy mice). The PM2.5-0.2 and PM10-2.5 from springtime Barcelona and summertime Athens had the highest inflammatory activities, possibly related to high photochemical transformation of the organic PM fraction (Happo et al. 2006; Jalava et al. 2006b). Wintertime PM0.2 samples from Prague, strongly affected by domestic coal and biomass combustion, had very high cytotoxic and apoptotic activities and caused a distinct cell cycle arrest in macrophages (Jalava et al. 2006b).

2.3. Scientific impact

Table 1. Summary of the scientific output during 1996-2005

SCIENTIFIC OUTPUT	N	Comments
Peer-review papers in international journals	**116**	Number of papers between 1996 and 2005 are 5, 9, 11, 8, 11, 15, 15, 9, 13, 20
Invited lectures and chairmanships in international meetings	**93**	Chair or co-chair of 4 major international conferences of ISEE-ISEA 1999 and 2006, ISEA 2003 and ISIAQ 2000. Chair or co-chair of 5 WHO expert groups/meetings on the issues of air pollution, particulate matter, traffic pollution and exposure analysis. Chair of WHO Indoor Air Quality Guidelines GL meeting, Chair and co-chair of two joint EC-WHO Workshops on Urban Air, Indoor Environment and Human Exposure, co-chair of SGOMSEC Workshop on Exposure to Combustion Products.
Papers in domestic journals	**33**	Number of papers between 1996 and 2005 are 1, 1, 1, 2, 2, 4, 4, 2, 11, 5
Lectures in domestic meetings	**75**	Includes lectures on actual health aspects of air pollution nearly every year in the annual meetings of the Finnish Air Pollution Prevention Association and the annual seminars on Environmental Health (arranged by Uni Kuopio in collaboration with the Ministry of Social Security and Health)
Editorial tasks in international journals	**4**	Co-editor of Journal of Exposure Science & Environmental Epidemiology, guest editor of supplement issue to Scandinavian Journal of Work, Environment & Health, editorial board members of Indoor Air and BMC Public Health,
Committee memberships: domestic	**5**	Ministry of the Environment: advisory committee of the EC CAFE Programme 2002-2005, advisory committee of air pollution abatement 2005-
Committee memberships: international (EU, WHO etc.)	**22**	WHO expert groups on Update and Revision of the WHO Air Quality Guidelines for Europe (published in 2000), Systematic Review of Health Aspects of Air Pollution (2002-2004), and Global Update of Air Quality Guidelines 2005. Three EC Task Forces on indoor research and exposure modelling, and Cefic LRI Scientific Advisory Panel. COST Action 633 on Particulate matter: properties related to health effects 2002-2007.
Doctoral dissertations	**13**	1 on air chemistry, 1 on PM characteristics and sources, 5 on air pollution exposures, 3 on gaseous pollutants and toxicity, 3 on epidemiological health effects of NO_2 and PM
Supervision of dissertations: ongoing	**7**	4 on PM epidemiology, 1 on physicochemical PM characterization, 2 on PM toxicology
Opponent of dissertations	**10**	National universities 6, foreign universities 4 (e.g., University of Wageningen, NL, and Imperial College of Science, Technology and Medicine, London, UK)

2.4. Social impact

2.4.1. National

a) Municipal –level impacts

Our research has demonstrated that particulate air pollution is associated with both respiratory and cardiovascular health effects at current Finnish levels of pollution, both in the Helsinki Metropolitan area, but also in smaller cities like Kuopio. Together with international studies this has increased the awareness and also actions in the affected communities. Our research has identified and quantified the most important sources of $PM_{2.5}$ exposures in Finland, which has guided abatement efforts.

KTL has been very active in informing the public and municipal authorities through articles in domestic journals and newspapers, and through radio and TV. Our researchers have also been frequent lectures in smaller and larger meetings and courses organized by the municipalities and the Finnish Air Pollution Prevention Society. KTL has also worked closely in both research projects and practical air pollution mitigation applications especially with the city of Kuopio and the Helsinki Metropolitan Area Council (YTV). We have given advice to the local authorities on specific air pollution issues and to YTV on updating a health-based air quality index for wide use by municipalities in communication of real-time air quality information to the public.

In mid 1990's, much of our research focussed on the springtime episodes of resuspended dust. Since then, improved methods have been developed to reduce these episodes. Methods have included improved wintertime street sanding and springtime cleaning practices.

In late 1990's, central issues in our research agenda have been traffic-originated ultrafine particle number and $PM_{2.5}$ exposures in traffic microenvironments (city streets, freeways, metro stations), and during alternative means of transport (walking, car, bus, metro).

As a consequence of the EXPOLIS seven-city air pollution exposure study that centred heavily on the YTV area, and the preceding as well as subsequent smaller traffic-oriented studies and exposure modelling exercises in the same region, we have today an exceptional depth and breadth of knowledge about the air pollution exposure of the population of the Helsinki metropolitan area. It concerns the sources, microenvironments and activities to dozens of gaseous and particulate air contaminants. The studies have also produced validated population exposure models that have been used in assessments of alternative traffic policy scenarios as well as evaluation of the risk management potential of modern building and ventilation technologies for the Helsinki Region.

In 2000's, our work with smaller municipalities has focussed on problems around small-scale wood burning that is continuously increasing and causes already many problems in neighbourhoods. Domestic wood smoke also constitutes about one-third of the total $PM_{2.5}$ emissions in Finland.

In recent years, forest fires in Russia and Baltic States seem to have been increased and become less controlled. Consequently, smoke from these wildfires has raised public concerns up to the level, where it has resulted in diplomatic consultations. KTL is responding to this problem with frequent consultations with municipalities and patient organizations, and with official statements and active presence in the media during episodes that acutely threaten the

health of susceptible population groups. In this area, there is also intensive collaboration with the air quality experts of the Finnish Meteorological Institute.

Another issue that has gained rapidly increasing environmental, economical and political significance is waste incineration. In Finland, this technology suffers from small-scale application history and poor environmental track record. Yet, for many communities there appear to be few economically feasible and environmentally more benign alternatives to it. This fact, together with the opportunity to co-generate significant amounts of 'non- fossil' power and heat, have brought waste incineration into the agenda. To respond to public concerns, to provide guidance to the permitting and operating authorities, and on the foundations of extensive research conducted by us earlier on emissions of and exposures to POP and carcinogens from combustions processes, KTL has prepared several assessments of the emissions, exposure contributions, benefits and risk of such plans for both national public health authorities and municipalities, and participated in respective public hearings.

b) Other national impacts

KTL air pollution research has had a continuous impact on the formulation of country positions to the EU directives on air quality and to the national implementation of the EU legislation as well as national legislation. This has taken place via participation as health experts in the advisory committees of the Ministry of the Environment and by giving official statements on the suggested legislation in writing to the Ministry of Health and Social Welfare and the Ministry of the Environment. We are also regularly invited as experts to the hearings of the Environmental and Economics Committees of the Finnish Parliament.

Our work with YTV and non-governmental patient organizations (e.g., Pulmonary Association HeLi, Allergy and Asthma Association) in producing information leaflets, giving lectures and making directed research has been crucial in activation of the Ministry of the Environment to introduce new national legislation on combustion efficiency and emissions limits for domestic heaters.

We have written 5-10 official KTL statements per year on suggested national and EU air pollution legislation and local air pollution issues related to existing or planned industrial plants, waste incineration plants, small-scale wood burning etc. In addition, we have extensively given advice on the health aspects of these issues in unofficial person-to-person phone calls and e-mails, and by participating as health advisers in meetings on specific air pollution problems.

There has also been active information to the public on the progress in knowledge of adverse health effects and mitigation methods in relation to specific type air pollution (e.g., gaseous pollutants in ice arenas, spring dust episodes, wood smoke). This has taken place in the form of numerous interviews every year on TV and radio channels and printed media, public lectures and invited articles in the main newspapers. We have also been health advisers in a number of common language information leaflets produced by the YTV and non-governmental patient organizations (e.g., Pulmonary Association Heli, Allergy and Asthma Association) and funded by the Ministries. This kind of activity was actually crucial in activation of the Ministry of the Environment to introduce new national legislation on combustion efficiency and emissions limits for domestic heaters.

Another example is our project on the air quality and health in indoor ice arenas (JÄÄHY) that gave national recommendations on the maintenance practices of the arenas together with the Finnish Ice Hockey Association. These were made known to the arena owners and municipal authorities by a series of domestic professional articles, seminar lectures, feedback on CO and NO_2 measurements in individual arenas etc. All these actions aimed at reducing the

risk of poor air quality and adverse health effects (including acute poisonings) among the users, mostly children and adolescents, of small ice arenas. In a follow-up by 2001, a large majority of the ice arenas had changed their maintenance practices (e.g. arena ventilation) and there was a strong shift from combustion-powered to electrical ice resurfacing machines. In addition, a majority of the remaining combustion-powered resurfacers had been retrofitted catalytic converters to reduce the CO and NO_x emissions.

2.4.2. International

KTL experts on air pollution exposure and health effects have participated in and chaired subcommittees in the Update and Revision of the WHO Air Quality Guidelines for Europe (published in 2000), the more recent Systematic Review of Health Aspects of Air Pollution conducted by the WHO in 2002-2005, and the WHO Global Update of Air Quality Guidelines 2006. The outcomes from these processes have formed the scientific basis on pollution-associated health effects for subsequent European-wide impact assessments (e.g., CAFÉ) and proposals for new EU legislation on air quality.

We have chaired or co-chaired five WHO expert groups and meetings on the issues of air pollution, particulate matter, traffic pollution and exposure analysis, chaired WHO Indoor Air Quality Guidelines GL meeting, chaired and co-chaired two joint EC-WHO Workshops on Urban Air, Indoor Environment and Human Exposure, been member of three EC Task Forces on indoor research and exposure modelling, co-chaired of SGOMSEC Workshop on Exposure to Combustion Products, and been member of Cefic LRI Scientific Advisory Panel.

Especially our work on fine and ultrafine particles (WHO, 2003), exposure modelling (Hammerström et al. (WHO) 2005) and traffic PM (Krzyzanowski et al, (WHO) 2005) have made significant impacts on international assessments.

www.ktl.fi/ExpoFacts is an exposure factor database, much like the U.S.EPA Exposure Factors Handbook, and other similar data compilations. The difference is that ExpoFacts has been created for the web, where the user can browse, search and create individualised tables and download the resulting data tables with his or her own computer. It was created under the coordination and technical realisation by KTL. ExpoFacts is our first web-based database for free public access and use, and the experience has been extremely encouraging. Since 2004, it has acquired thousands of users and was thoroughly updated this autumn. (Vuori et al. 2005).

2.5. Research funding

Table 2. Project funding during 1996-present

Project name	Funding agency*	Role	KTL funding kEur	Years	Partners
Ultra/EU	EU	coordinator	106	1996-1997	
Expolis/Academy	SA	coordinator	108	1996-1997	YTV (Finland)
Expolis / EU	EU	coordinator	263	1996-1998	VTT, Univ. of Basel, EC JRC, Univ. degli Studi, Univ.Athens, Univ.Joseph Fourier, RIVM
Ultra 2/EU	EU	coordinator	287	1997-2000	
ILME sources	U.S.EPA	subcontractor	63	1997-1999	Harvard University
Ultra II	SA	coordinator	99	1998-2001	IRAS, GSF, Univ. Helsinki, Univ. Kuopio
EAS/Expolis	SA	partner	94	1998-2001	University of Basel
Expolis-Sytty	SA	coordinator	222	1998-2000	YTV
PAMTOX	SA	coordinator	137	1998-2001	FMI, YTV
Ultra Bra/SA	SA	coordinator	77	2000-2002	IRAS, GSF, Univ. Helsinki, Univ. Kuopio
Heapss	EU	partner	137	2001-2003	Roma E Local Health Authority, GSF, IMIM, Karolinska Institutet, Univ. Helsinki,
Traffic pollutants	Tekes		50	2001	YTV
Centre of excellence	Tekes, SA	coordinator	168	2002-2003	KTL epid., toxicol. and exposure teams
Rupioh	EU	partner	72	2002-2004	IRAS, Univ. Athens, Univ. Helsinki, Univ. Birgmingham, IMIM
Hearts	EU	partner	165	2002-2005	WHO/Euro, Imperial College UK, INRETS (Fr), ARPAT (Italy)
Airnet	EU	partner	70	2002-2004	IRAS, others
Fumapex	EU	partner	98	2002-2004	
PAMCHAR	EU	coordinator	521	2002-2004	FMI , RIVM , IUF, IRAS
Hearts/SA	SA	coordinator	85	2002-2004	
Airgene	EU	partner	211	2003-2005	
Exfine/SA	SA	coordinator	119	2003-2005	IRAS
Expolis-Index	Eur. Chem. Ind. Council	coordinator	65	2003-2004	Univ. Basel, Univ. Southernm Calif.
PAMCHAR/Fine	SA	coordinator	105	2003-2005	FMI, YTV, Univ. Antwerp
ENVIE	EU	partner	107	2004-2007	CSTB (Paris), Instituto de Engenharia Mecanica (Lisboa, Portugal), + 6 other
Centre of excellence	Tekes, SA	coordinator	340	2005-2007	KTL epid., toxicol. and exposure teams
Centre of excellence	SA	coordinator	156	2005-2007	Univ. Helsinki
Intake Fraction	Eur. Chem. Ind. Council	coordinator	253	2005-2007	Imperial College (UK), FMI
HIPPU	KyAmk	partner	97	2005-2006	KyAmk (Kotka, FI), FMI
PUPO-health	Tekes	coordinator	80	2005-2006	Univ. Kuopio, FMI, TTL
KCAR	Tekes	partner	66	2006-2007	Univ. Kuopio, FMI
ERA-NET Bio-energy	Tekes	partner	50	2007-2008	Univ. Kuopio, FMI, Univ. Umeå, Univ. Graz, Fraunhofer Inst
Below 50 000 €			79		
		Total	**4 552**		

* EU=European Union, SA=Academy of Finland, TEKES=Finnish Funding Agency for Technology and Innovation; FMI=Finnish Meteorological Institute, TTL=Finnish Occupational Health Institute, VTT=Technology Centre of Finland, YTV=Helsinki Metropolitan Area Council

2.6. Scientific collaboration

2.6.1 National

Our collaboration has always been very close with the University of Kuopio, especially the Department of Environmental Sciences and Department of Physics (measurement of particles), and with the Kuopio University Hospital, especially Department of Clinical Physiology (cardiovascular measurements). The same applies also to the Department of Physics of the University of Helsinki. Partners from these Departments have participated in several research projects.

Collaboration is also very close with the Air Quality Research of the Finnish Meteorological Institute in several domestic (e.g. HEAT, PAMCHAR-FINE, HIPPU, PUPO-health) and international (e.g. FUMAPEX, iF, PAMCHAR) research projects. This partner has provided modelling of air pollution (Prof Kukkonen) and physicochemical characterization of particles (Prof Hillamo).

VTT Chemical Technology unit has been our close and long-term collaborator in the analysis of indoor and in traffic exposures to VOCs, (Kristina Saarela). VTT Lab of Transport, Traffic and Logistics (Dr. Laurikko), Finnish Occupational Health Institute (Prof. Hämeri) and Helsinki Technical College (Stadia, Dr. Pirjola) have collaborated with us in Transport Exposure studies.

Collaboration has also been close with YTV, which has been an active partner in traffic pollution studies (Tech Lic. Päivi Aarnio, Tech Lic. Tarja Koskentalo, Dr. Kousa) and provided routine monitoring data for many studies.

2.6.2 International

The foundations for our international collaboration in the research of the exposure to and health effects of air pollution, and specifically PM air pollution, were laid in the European Collaborative Action (ECA) on Air Pollution Epidemiology, coordinated in KTL (1990-95), and the ECA Indoor Air Quality and its Impact on Man (1988-99), where we were represented in the Steering Committee and numerous working groups.

The KTL research teams on air pollution and health have been highly collaborative within Europe. During the period 1996-2006 the teams have co-ordinated major multicentre projects on PM epidemiology (ULTRA 1 and 2), exposure (EXPOLIS) and toxicology (PAMCHAR). In addition, the teams have been major partners (Work package leaders etc.) in numerous other projects (AIRGENE, HEAPSS, APHEA-II, RUPIOH, HEARTS, FUMAPEX, EXPOLIS-IndEx, SANCO-IndEx, EXPORED) funded by the EU 5^{th} and 6^{th} Framework Programmes. Other important collaborations have extended to the United States (Harvard School of Public Health) and to the Nordic countries (NORDAIR, NORDAIR-BIOS), the latter with funding of concerted action projects by the Nordic Council of Ministers.

We have coordinated the first multinational population based air pollution exposure study (EXPOLIS), which involved over thirty selected gaseous and particulate air pollutants. We were one of the first research teams in incorporating personal exposure assessment to PM into epidemiological field studies and in relating source-specific PM to epidemiological and toxicological health effects.

Currently, we participate as member of the Steering Committee and the Management Committee as well as chair of the health aspects working group in COST Action 633 on Particulate matter: properties related to health effects (2002-2007). This action is managed by the Euro-

pean Science Foundation and forms a multidisciplinary platform for aerosol and health researchers and modelling experts from 20 countries. It has given recommendations on research needs concerning air pollution and health to the 7th Framework Programme of EC Research. Its main interest is a better understanding of the source-, climate- and population-related heterogeneities in human exposures to and health effects of ambient air PM.

Long-term partners, multiple joint research projects

- EC/JRC/Institute for Health and Consumer Protection, Dr Helmut Knöppel, Dr Maurizio De Bortoli, Dr Stylianos Kephalopoulos, Prof Dimitrios Kotzias,(after 1996; ECA, EXPOLIS, INDEX, ExpoFacts, GN-CEM, EnVIE), 1988 -
- BGA/BUA- WaBoLu, Prof Bernd Seifert (ECA, EnVIE) 1988 -
- University of Milan, Department of Occupational Medicine, Prof Marco Maroni, Dr Paolo Carrer, Dr Domenico Cavallo, (ECA, EXPOLIS, IndEx, ExpoFacts, INDEX), 1988-
- RIVM, Dr Erik Lebret, Nicole Janssen, Flemming Cassee etc. (EXPOLIS, AIRNET, ENHIS, INTARESE, PAMCHAR) 1988 -
- University of Basel, Dept of Social and Preventive Medicine, Prof Nino Künzli. (EXPOLIS, EAS and IndEx) 1989 -
- University of Wageningen – Utrecht University, IRAS, Prof Bert Brunekreef, Dr Gerard Hoek , Dr Nicole Janssen (PEACE, ULTRA, AIRNET, RUPIOH, PAMCHAR) 1993 -
- University of Athens, Prof. Klea Katsouyanni etc. (PEACE, APHEA, EXPOLIS, RUPIOH, PAMCHAR) 1993 -
- Harvard University, Boston MA, Prof John Spengler, Prof Petros Koutrakis, Prof John Evans, Prof Joel Schwartz (PEACE, JÄÄHY, APHEA, PAMTOX) 1994 -
- GSF – Institute of Epidemiology, Dr. Annette Peters etc. (ULTRA, AIRGENE, HEAPSS, AIRNET) 1995 -
- IMIM, Barcelona, prof Jordi Sunyer (APHEA, AIRGENE, HEAPSS, PAMCHAR) 1995-
- Imperial College for Science, Technology and Medicine, Prof David Briggs (HEARTS, AIRNET, INTARESE, iF, HEIMTSA) 2000 -
- WHO/ECEH, Bonn and Rome, (HEARTS, AIRNET, ExpoFacts, ENHIS-1 and ENHIS-2) 2000 –

In addition, there have been collaborations with tens of other European and US research institutes in international projects, leading to joint peer-reviewed publications.

2.7. Proposal for the future work and expected (social) benefits (next 5-10 years)

Our future working environment and products
Society needs us to predict and warn about potential new environmental health risks (precaution), to identify and explain and quantify - from sources via environment to exposure, mechanisms and public health consequences - the current risks, to rank and quantify the risk management potentials of alternative control measures, and to follow up policy implementation programmes for accountability. All this will be done in future air pollution research.

The environment, in which we work, communicate and disseminate the benefits from our work, is rapidly changing. Our role will progress towards the developer of internet based expert services, which require continuous development - i.e. long term commitment and resources, which are only possible in the context of broad international expert networks. Our views on this development are presented in more detail in the introductory part of this evaluation material.

PM research focus
To identify, explain and quantify the complex risks of air pollution to health, we need to integrate exposure monitoring and modelling, epidemiological analyses and toxicological assessments already in the study design. In 2005-2006, we have conducted the first such field study in Kotka (HIPPU project), where we combine ambient PM characterization (FMI), personal exposure monitoring and source attribution with epidemiological follow-up and measurement of inflammatory markers among ischemic heart disease patients. Moreover, the size-segregated PM sampling with the HVCI allows subsequent in-vitro testing of the PM samples and analysing the relationship of the toxicological findings with human epidemiology.

Our work aims at strengthening the international understanding of PM compositions and sources that are hazardous to human health. This information is applied, not only for future WHO health risk assessments and updating the EU air quality legislation, but it also guides the development of future risk control technologies. In fact, integrated study approaches are needed in validating such technologies in intervention studies.

To consult the political decision makers on the risk reduction potential of alternative risk management options, and to help develop and implement accountable PM risk management policies, we develop increasingly detailed predictive PM exposure modelling techniques, which not only predict the fine PM exposures for given exposure scenarios, but actually predict the composition of the PM exposure, and link the exposures to specific sources. In the policy implementation phase, environment and exposure monitoring programmes, which are focused on the key nodes of the same mechanistic models – simplified numerical constructs of the physical/chemical/social exposure scenarios – allow the confirmation of policy's success in meeting its exposure objectives.

We expect that our research on the exposure to and health effects of urban air PM – in the context of the extensive international research effort that has focused on this topic in the course of the past 15 years - has already reached its peak. We can now monitor, sample and analyse the most relevant PM characteristics from personal to urban ambient air level, and attribute these exposures to sources and activities. An understanding about how such microgram levels of these microscopic and heterogeneous particles can affect the health and lives of such

huge numbers of individuals in the Western urban settings is emerging. Although far from complete, it is already becoming biologically plausible and consistent with the observed propagation of chronic health effects and manifestation of the acute health effects. The overall picture will need and gain a lot of new detail that reduce current uncertainties, but it is unlikely to have any dramatic change. The results of intervention studies, e.g. the Utah Valley Steel Mill Strike study and the Dublin Coal Sale Ban follow-up, also encourage this conclusion. As a consequence, we have sufficient knowledge to give specific guidance for the control of the most relevant – but not all - emissions, and EU as well as independent countries are in the verge of being able to develop science based PM risk mitigation policies and, after implementation, to verify their accountability for the expected public health benefits. The promise for public heath improvement is huge, hundreds of thousands of annual avoided excess deaths in Europe.

2.8. Personnel

From 1996 onward the personnel working in this research area at KTL has consisted of 12-25 employees at a time; four senior researchers (two part-time in this area) with their own group, 2-4 other senior or post doc researchers, 5-10 PhD students, 2-3 other students, and 2-10 other personnel working on field works, statistics, and in the laboratory. The true research group is, however, much larger due to intensive national and international collaboration. Except for the four senior researchers, practically all other personnel at KTL are on external funding.

The key senior researchers:
Matti Jantunen, MSME, MSEE, PhD, research professor: Air pollution exposure, indoor air pollution, application of exposure science in risk assessment and management

Juha Pekkanen, MD, PhD, research professor: Air pollution exposure and epidemiology, ultrafine particles

Raimo O. Salonen, MD, PhD, senior researcher: Multidisciplinary approaches in toxicological research on gaseous and particulate air pollution

Maija-Riitta Hirvonen, PhD, Docent, senior researcher: Immunotoxicology and inflammation

Otto Hänninen, MSc, PhD, postdoctoral fellow: Air pollution exposure modelling, application of exposure science in risk assessment and management

Tarja Yli-Tuomi, PhD: source-apportionment modelling, exposure measurement

Arto Pennanen, PhD: aerosol measurement

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3 RESPIRATORY DISEASE, INDOOR MICROBES AND IMMUNOTOXICITY

3.1. Research area and its significance

Epidemiology of asthma and allergies

The area started in the mid 1990's with prevalence surveys of both clinical asthma and allergy as part of epidemiological studies among children (ISAAC) and adults (ECRHS). Since then, the research has focused on risk factors of asthma and allergies.

From the very beginning, a basic starting point has been the importance of pregnancy and early life factors, as proposed in the 'Barker's hypothesis' for cardiovascular disease. Some promising findings were done, but overall the ability of birth weight etc. to explain risk of asthma and allergies appears to be less than for cardiovascular disease. In mid 1990's, the 'hygiene hypothesis' about the importance of infections and microbial exposure to development of asthma and allergies was advanced and since then this has been the main focus of research. We have used the lower risk of allergies in farms as a starting point to try to discover new risk factors of asthma and allergies. Currently, the main focus is on exposure to environmental microbes and their effect of respiratory and immune system.

Role of exposure to environmental microbes in health and disease

Exposure assessment to environmental microbes and their products has been in the focus of interest for 20 years. The role of these exposures appears to be both protective and adverse to health. The protective role of environmental microbes is currently studied as part of the epidemiological studies focusing on risk factors of allergies. The exposure is determined using house dust samples from homes and describing the microbial exposures by determining the chemical markers of microbial biomass, using QPCR for quantification of specific microbes or assay groups, and by sequencing the total microbial communities of the samples.

Environmental microbes are associated with adverse health effects in indoor environments with dampness, moisture and mold problems. Respiratory and other health effects including an increased risk for asthma have been well documented in many epidemiological studies, and the problems seem to be prevalent in most industrialized countries. Research is needed to find out the causal relationships between the exposing factors and various health effects. The series of phenomena of building dampness, exposure, health effects and effects of remediation have been extensively studied and the results widely implemented in mitigation practices. Currently, the focus is on reevaluation of microbial exposures using molecular methods and strengthening the knowledge on causal links between exposure and effects.

Specific emphasis has been put on two groups of environmental bacteria: atypical mycobacteria and actinomycetes, especially streptomycetes. We have shown that both these bacterial types grow commonly together with fungi on moisture-damaged building materials. There is growing evidence that mycobacteria may cause other kinds of health effects but traditional infections, e.g., allergic alveolitis. The role of streptomycetes is interesting due to their exceptional capacity to produce secondary metabolites, some of them being very toxic. We have shown that both mycobacteria and streptomycetes cause remarkable inflammatory responses

both *in vitro* and *in vivo*. The studies on the occurrence of these microbes in man's living environments are currently going on as well as the determinants of the exposure to them.

Research on environmental health effects of emissions of biological particles and odors has been recently initiated. Together with epidemiological population studies this research provides knowledge needed in waste treatment future strategies and area planning.

Mechanisms of health effects

During the last ten years research activities have focused on cytotoxicity and immunological defense mechanisms of adverse health effects caused by diverse population of the microbes in indoor air. Specific emphasis has been to develop a new systematic research strategy for identification of causative constituents. The methods for these studies includes human and mouse cells cultures, intratracheal instillation to the mouse lungs as well as nasal lavage, induced sputum and blood samples collected from exposed subjects. In addition, several biochemical methods for analysing inflammatory responses and cytotoxicity induced by environmental samples in various experimental settings are used.

Since exposure to a single microbial agent in indoor air is very unlikely the toxicological studies have concerned both the effects caused by single microbes and interactions of them. These interactions can change the characteristics of the microbes, which may explain the various outcomes of apparently similar microbial exposures in different exposure situations. However, the activated mechanisms in exposed respiratory cells and tissues as well as causative constituents and sources are not well understood.

Current focus is on in-depth mechanistical studies on the links between immunotoxicity, inflammation, genotoxicity and oxidative stress activated by microbial cells or components. These toxicological data on activated mechanisms of both the cytotoxic and inflammogenic effects on mammalian cells is combined with comprehensive analysis of microbial characteristics of the same samples.

3.2. The main achievements

Epidemiology of asthma and allergies

We started our research effort in the mid 1990's with studies on the prevalence of asthma. Given the problems in the comparability of definitions of asthma, this is best done within international studies, i.e. ISAAC among children (Pekkanen et al. 1995) and ECRHS in adults (Vartiainen et al. 1999). We established that prevalence of asthma in Finland is similar to countries in continental Western Europe, lower than in Brittain, but higher than in Central and Eastern Europe. Hay fever, but especially atopic dermatitis, is higher than in other parts of Europe. We have also actively compared (Remes et al. 2002), reviewed (Pekkanen and Pearce 1999), and also proposed new approaches to measurement of asthma (Pekkanen et al. 2005).

Given the importance of perinatal risk factors on the development of several chronic diseases, we then focused to study these factors in asthma and allergies. In collaboration with University of Oulu, we utilized the very large birth cohorts born in 1966 and 1985/86 in Northern Finland and their follow-ups. We identified the importance of gestational age, infections during pregnancy, mode and complications of delivery, and anthropometry to future development

of asthma and allergies. We found no effect of maternal hormone status on development of asthma. Overall, the findings were less strong and consistent than for some other chronic disease, like cardiovascular disease.

Therefore, we turned our attention to other determinants, especially microbial exposures in early childhood. This line of research started with our studies confirming that children born on farms have less allergies and common atopy also in Finland and also in Kuopio area. Current studies are focused on finding reasons for this lower risk. First we observed that also in Finland, the low risk is mainly explained by contact with live-stock, but did not observe an association with farm milk, as suggested by other groups. No other farm characteristic explained the lower risk. We also confirmed that living on farm in childhood is protective independent of current farm exposure.

Currently, we are running birth cohort studies to the effect of microbes in more detail. Birth cohorts are intensive international and national collaborations between epidemiologists, immunologists, and exposure assessors. In a small pilot study, we recently observed that microbial exposure in the first 3 months increases the production of IFN-gamma by stimulated peripheral leukocytes. We have also observed a suggestion in adult farmers that current farm exposure may also be protective of common atopy and allergic symptoms.

Exposure to environmental microbes

Microbial exposure as a possible protective factor for allergy in farming environments.
The current studies on microbial exposure are aiming to determine differences in microbial levels and composition between urban, rural and farming living environments and revealing the determinents of microbial exposure. Sampling and analysis methods are also systematically validated. Farming has been found to be associated with endotoxin levels of dust together with cleanliness of the home (Hyvärinen et al. 2006). Farming was also shown to be one of the main determinants of 3-OHFAs and ergosterol, which are chemical markers of the biomass of gram-negative bacteria and fungi (Hyvärinen et al., submitted). The study on reproducibility of endotoxin determination in dust samples showed that bed dust samples have the highest reproducibility followed by floor dust and settled dust samples with moderate reproducibility. Bed samples did not however reflect the farming status of the home and hence floor samples were shown to the best choice for epidemiological purposes (Hyvärinen et al. 2006).

This research is currently in an intensive phase of laboratory analyses and subsequent data analysis and reporting. Collecting environmental samples from a number of large epidemiological field studies in Finland and as international collaboration have been completed, resulting in a total of several thousand samples, and another set of approximately 1000 samples will be collected starting 2007. The main sample matrices are house dust and stable dust. Due to the serious limitations of culturing methods in quantitative analyses, chemical and DNA-based methods to characterize microbial exposure have been validated: ergosterol for fungi, muramic acid for bacteria and 3-OH fatty acids for gram-negative bacteria. Quantitative PCR is used for quantifying individual species and assay groups, and large scale sequencing for identifying the species profiles of the samples.

Indoor dampness, microbial exposure and adverse health effects.
The research line on adverse health effects of microbial exposures started with extensive characterization of indoor microbes and their association with indoor dampness and moisture and their health effects. Adverse effects of dampness, moisture and mold on health of adults and children were shown, and recently, moisture damage in the living quarters was shown to

be risk factor for asthma among small children. Higher concentrations of microbes in indoor air and unusual mycobiota and bacterial flora was also associated with dampness or moisture damage of the building although the causal factors of the health effects are not yet fully known. We have also shown that among the microbial ecosystem of moldy building materials, also amoebae can be found which may indicate the presence of Chlamydia and other bacteria that are dependent of their protective effect.

The microbiological results have been used to formulate guidance documents for local health authorities to manage indoor air problems (see Section Social Impact). The high prevalence of moisture faults in the Finnish housing stock was shown, indicating that the associated health effects are a real public health issue. Several intervention studies on effects of renovation in day-care centers and schools have shown that the elimination of the moisture and mold has beneficial effects on the users' health. The effect can also be seen in microbial exposures that decrease to normal after remediations.

Personal exposures to airborne microbes was studied using 80 teachers as experimental individuals. Personal exposure turned out to be higher than the concentrations measured with stationary measurements at home or work. Levels of proinflammatory cytokines (IL-6, TNF alpha) and nitric oxide (NO) in nasal lavage were connected with measured microbial concentrations in personal samples.

Current applications of molecular methods in microbial exposure assessment will deepen the understanding of the microbial exposures in health and disease by providing new insight to the microbiological world of the human intimate environment, both qualitatively and quantitatively. Among the non-culturable methods in current use are determination of chemical markers, quantitative PCR, large-scale sequencing of environmental microbes and DNA-fingerprinting.

Research on Healthy Housing and School Environment
Projects focusing on development of healthy housing information system and the importance of school indoor air quality on the students' academic performance are currently starting. The information collection will be based on an internet-based questionnaire dealing with issues of healthy housing, such as condition of the residence, air quality, traffic, noise and safety issues. At the moment, the questionnaire is being developed.

With recently obtained funding, a study focusing on school indoor environment and its effects on the students' academic performance will be in full activity in 2007. The performance data will be obtained from the national testing programmes, and measurements of indoor air quality are carried out simultaneously with the testing.

Effects of biowaste treatment
Environmental health effects of biowaste treatment are being studied with environmental measurements and with epidemiological methods. Two series of sampling campaigns have been carried out: A monitoring program of 13 months in three sampling sites (landfill, urban city and natural background) to find out the variation of concentrations of microbes and particles, and of the immunotoxic potential of the airborne particle material. The second campaign was done in three different sites of biowaste treatment, sampling air from three different distances from the source. The results of these studies are in the analysis and reporting phase. The data collection phase of a telephone interview-based study (N=1000) on the perceived effects of odor, respiratory symptoms and general well-being of individuals residing nearby biowaste treatment sites has been completed. The population sample was drawn using GIS-based information about the proximity to the source. The study material is currently being analysed and reported (See also the evaluation material on Risk analysis).

Cytotoxic and inflammogenic effects of microbes

Proposed toxic mechanisms behind the health effects associated with bioaerosol exposure are cytotoxicity, inflammation associated injury, oxidative stress and genotoxicity. Our studies on cellular mechanisms activated by indoor air microbial samples have shown that the spores of gram-positive bacteria, especially actinobacteria, show inflammatory and cytotoxic activities resembling those of endotoxin both in the mouse and human cells *in vitro* and in the mouse lungs *in vivo* (Hirvonen et al. 1997a,b Huttunen et al. 2003, Jussila et al. 2003). Furthermore, *Streptomyces californicus*, gram negative bacteria *Pseudomonas fluorescens* and toxic fungus *Stachybotrys chartarum* have clearly the most prominent immunotoxic effects and interactions with each other. We have also shown that the characteristics of the building material have a crucial role in immunotoxic potency of the microbes (Murtoniemi et al. 2003). However, our studies on bioactivity of spores of microbes grown on plasterbord revealed that the use of biocides has to be carefully evaluated since incomplete prevention of microbial growth by biocides may even increase the harmfulness of the microbial spores.

In search for causative agents behind the reported symptoms and diseases in mouldy buildings, we have shown the role of microbial interactions on their immunotoxic potential. After extensive testing of microbial strains isolated from moldy buildings, we have identified the highly potent interaction between the bacterium Streptomyces californicus and the fungus Stachybotrys chartarum, which significantly affects the potency of the spores of these microbes to cause apoptosis and cell cycle arrest in immunological cells (Penttinen et al 2004). In addition, the microbial interactions significantly upregulate or downregulate the production of inflammatory mediators in macrophages. These interactions are not only seen during the co-exposure to separately grown microbes, but especially when microbes were co-cultivated. In co-cultivation. Furthermore, we have shown that interactions during the co-cultivation of these two microbes stimulate the production of currently unidentified compounds including substances with cytostatic properties. Although the mechanism of cell death caused by these co-cultivated microbial spores resembles the apoptotic pathway of the well known cytostatic compounds, doxorubicin and actinomycin D, the causative agent for the cytostatic effect remains unidentified at present.

Inflammatory responses associated with microbial exposure

Upper airways: We have shown an association between increased production of inflammatory markers in the nasal lavage (NAL) fluid, especially IL-6, high prevalence of respiratory symptoms in occupants, and exposure to moisture-damaged indoor environments (Hirvonen et al, 1999). The concentrations of nitrite, IL-6 and TNFα in NAL samples of the school personnel were significantly increased during the working period compared to vacation. A similar difference was observed in the concentrations of IL-4 in the NAL samples of office employees. Reported respiratory symptoms were more frequent during the working periods than during the vacation. On the contrary, no statistically significant differences between work and vacation in the NAL outcomes or reporting of symptoms were observed among sawmill workers exposed to high concentrations of microbes. When NAL and *in vitro* methods in the assessment of inflammatory and cytotoxic potential of the airborne particle material collected with personal samples were compared, both methods discriminated between subjects with high or low microbial exposure, differences detected with the *in vitro* method being statistically significant.

Lower airways : Our results on the inflammatory responses in lower aiways of the exposed subjects revealed that IL-6 concentrations in induced sputum from the personel of the moisture damaged shool building were higher when compared to the values from the personel of

the reference school. Interestingly, similar decrease in IL-6 levels to control level during the vacation as we detected NAL samples in the previous study, was not seen in IS samples. This suggessts that cytokines levels in upper airways do not predict the levels in lover airvay

3.3. Scientific impact

The scientific output of the research on respiratory disease, indoor microbes and mechanisms of their health effects is presented as numbers in Table 1.

Table 1. Summary of the scientific output during 1996-present

SCIENTIFIC OUTPUT	N	Comments
Peer-review papers in international journals	**172**	Does not include papers published in 2006
Invited lectures and chairmanships in international meetings	**31**	
Papers in domestic journals	**24**	
Lectures in domestic meetings	**138**	
Editorial tasks in international journals	**3**	
Committee memberships: domestic	**2**	
Committee memberships: international (EU, WHO etc.)	**6**	EU; SCHER Expert group on risk assessment of indoor pollutants and on air fresheneres, WHO; Working group on the document on Biological Agents in Indoor Environments, Working group on Disease Burden due to Inadequate Housing, Working group on Guideline Values of Indoor Air Pollutants, US National Academies of Science, Institute of Medicine; Committee on Damp Indoor Spaces and Health; Swedish Real Estate Administration; expert group on indoor problems of the Moderna Museet and Arkitekturmuseet
Doctoral dissertations	14	
Supervision of dissertations: ongoing	**12**	
Opponent of dissertations	**3**	
Reviewer of PhD thesis, evaluation of docentship	**7**	
Reviewer of scientific articles (number of Journals)	**20**	

3.4. Social impact

3.4.1 National

b) Municipal –level impacts

Research on the problem of dampness, microbial growth and associated health effects has produced a lot of knowledge that has been directly implemented into practical work of diagnosing and management of the cases. Our research and our active dissemination of information actually initiated the processes that led to public recognition of the indoor mould problem and its health risks. The research has also provided the foundation of identifying and assessment of this complex problem.

Recognition of the problem. Identifying and correction of the faults in the building codes and other regulatory material that previously often led to false practical solutions and moisture problems have been one of the very concrete results of the recognition of the problem. Identification of indoor dampness and mould as an emerging public health issue has been a focus of numerous newspaper and magazine articles as well as radio and TV programmes, which has facilitated public awareness of the problem and thus its prevention and control.

Estimation of the prevalence of the problem. Studies on the prevalence of dampness and moisture problems have facilitated the estimations of the extent of the problem. Prevalence studies have been made with random samples of single-family houses and apartment buildings. Many organizations, e.g., municipalities, school districts, housing organizations and companies have then systematically evaluated the condition of their building stock, and many types of long-term renovation planning have been initiated.

Guidelines for authorities. Based on our research, a guideline document of microbial indoor air quality for local health authorities have been prepared in collaboration with the Ministry of Social Affairs and Health (3rd updating 2003). A handbook-style booklet has also been prepared to give guidance in technical details of the measurements and the interpretation of results. These documents are in extensive use not only among the municipal authorities but also among practitioneers in occupational health and among indoor air consultants. An additional document on special aspects concerning school buildings is at its final stage and will be published early 2007. These documents, based on the research carried out at KTL/YTOS and prepared by us, have made it possible to use standard procedures to handle individual cases and interpret the results of the measurements. This has had an extensive impact on the risk assessment and management of individual buildings and the health issues of their occupants.

We have collaborated with the Institute of Occupational Health in identification of the occupational health risks connected with dismantling and renovation work of mouldy building structures. This research, showing the importance of working practices such as protection of other than working facilities from fungal contamination, and personal protection of the workers, has led to guidance documents given by the Ministry of Labor, and to training of professional groups in question.

Training and education. A remarkable amount of work has been put on training efforts for various professional groups and also general public. This training has been organized together with the University of Kuopio, and targeted e.g. for health inspectors, physicians and other health personnel, professionals in building, construction and maintenance; lawyers and teachers. A year ago, an international Nordic (NIVA) training course (NIVA) on mouldy buildings

and their health effects, identifying and management of the problems was organized in collaboration with the Finnish Institute of Occupational Health, target groups being mainly scientists and personnel within occupational hygiene and health. The scientists of the research group have also been invited as speakers in numerous training courses organized by municipalities, county administration, patient organizations, summer universities and scientific societies.

A senior researcher of the group has been an active consultant of the University of Kuopio and Ministry of Social Affairs and Health in the organization of the annual courses of Environmental Health (2 days in spring) and Environmental Medicine (2 days in late fall). She has been the leader or key planner of the programme of these events for over 25 years. Scientist of the Department have been frequent speakers of these training events, which are generally regarded as the best national training courses in the field.

Implementations in prevention of allergy. The long tradition of research within exposure assessment to environmental microbes and its connections to health is nowadays applied to allergy research, a major challenge of which is to reveal the protective effect against allergy of farming environment. To implement the results of current allergy research into practice, a working group on prevention of allergies, founded by KTL, has started its work this year.

c) Other national impacts

Recognition of dampness, moisture and mould problems as a health risk has led to an industry developed into diagnosing and renovation practices. There is an increasing need for special professionals to design and carry out the renovations of existing buildings in need of "mould remediation", which is often a result of faulty "repair" with insufficient understanding of the moisture physics of the building structures. Hence, the knowledge of health effects of such faulty practices is increasing the pressure for more sophisticated solutions in building practices. This is not only a national issue, but a largely recognized need internationally as well.

3.4.2. International

Participation in international expert groups and committees has been an important part of the implementation of research knowledge into practice (see Table 1). Among the organizations where our experts have been working are WHO and working groups within EU and US National Academies of Science.

3.5. Research funding

The research funds that have been raised during the period 1996-2006 are shown in Table 2. Shown as separate projects are only those with a budget over 50000 €; the rest are grouped together at the end of the Table.

Table 2. Project funding during 1996-present

Project name	Funding agency*	Role*	KTL funding €	Years	Partners
Method development	STM	Leader	129504	1997	
Biological exposure assessment	TSR	Leader	100912	1997-2000	
Senior researcher	SA	Leader	60699	1997-1998	IMIM, Barcelona
Personal exp to microbes	SA	Leader	193029	1998-2001	
School interventions	SA	Leader	111306	1998-2001	municipalities
Mycobacteria in moluldy materials	TSR	Leader	65593	1998-1999	
Fetal environment	SA	Leader	107842	1998-2001	Univ of Oulu
Health effects of mould	SA	Leader	358273	1998-2001	
Effects on joints			75684	2000-2002	Society for Healthy Housing
Building interventions	Tekes	Leader	84093	2000-2001	Consulting eng.companies
Senior researcher	SA	Leader	93882	2001-2002	
Surface probiotics	Tekes	Partner	550000	2001-2004	Univ of Oulu
Lactobacillus surface structures	Tekes	Partner	92503	2001-2004	Univ of Oulu
Coordination	SA	Leader	73919	2001-2002	
Toxicity and inflammatory responses	SA	Leader	126140	2002-2005	Univ of Kuopio
Amoeba-project	SA	Leader	126141	2002-2004	Inst. Occup Health
Microbes and allergy	Mela	Leader	50000	2002-2007	PASTURE team
Biological and fine particles	Tekes	Leader	124000	2002-2003	Univ of Helsinki
Pasture	EU	Partner	292961	2002-2005	Munich, Basel, Utrecht, Marburg
Pasture/domestic	KYS	Partner	50456	2002-2003	Munich, Basel, Utrecht, Marburg
Senior researcher	SA	Leader	97980	2002-2003	IMIM, Barcelona
Guidelines for schools	STM		60000	2003	
Effects of mold on joints	TSR		100000	2003-2006	
Senior researcher/AH	SA		153000	2004-2007	
Indoor microbes MOBI	STM		73000	2004	
Biowaste	STM		70000	2004	
Biofine2/Tekes	Tekes	Leader	203100	2004-2005	HY
Senior researcher (UH)	SA		156090	2005-2008	
Indoor microbes MOBI	STM		100000	2005-2007	
Biowaste 2005-6	STM		200000	2005-2007	
Indoor microbes chip development	Tekes	Leader	116700	2005-2006	HY
Gabriel	EU		95833	2006-2010	
Microbial exposure in health and disease	SA		339040	2006-2009	
Total			**4631680**		

* EU=European Union, SA=Academy of Finland, STM= Ministry of Social Affair and Health, TSR=The Finnish Work Environment Fund, Mela=Farmers' Social Insurance Institution, Tekes=Finnish Funding Agency for Technology and Innovation

3.6. Scientific collaboration

3.6.1 National

- Dept of Pediatrics and Dept of Pulmonology, Kuopio University Hospital. Several collaborative projects
- Dept of Clinical Microbiology, University of Kuopio and Kuopio University Hospital. Several projects on Immunotoxicity *in vitro* and *in vivo*
- Dept. of Environmental Sciences, University of Kuopio. Several projects in microbial exposure assessment.
- Dept. of Pathology and Forensic Medicine, University of Kuopio and Kuopio University Hospital, Several projects on Immunotoxicity *in vitro* and *in vivo*
- Kuopio Regional Institute of Occupational Health, Kuopio, Finland . Several projects on occupational microbial exposure and inflammatory responses
- Institute of Applied Technology, University of Kuopio. Research on proinflammatory activity of probiotics
- University of Helsinki, Departments of Physical Sciences and Institute of Biotechnology. Collaboration in research on microbial fine particles
- Asthma and Allergy Association. Collaboration in a study on children's asthma and dampness

3.6.2 International

- University of Lund, Department of Medical Microbiology, Dr. Lennart Larsson.
  Close collaboration in the determination of chemical markers of the microbial communities. This work is part of the exposure assessment in the asthma and allergy research.
- University of Tulsa, Tulsa, OK, USA, Prof. Richard Shaughnessy and IIT (Illinois Institute of Technology), Prof. D. Moschandreas. Collaboration in school indoor environment and academic performance.
- U.S.EPA, National Exposure Research Laboratory. Dr. Stephen Vesper and Dr. Richard Haugland. Collaboration in the development of PCR techniques for indoor microbes.
- PASTURE birth cohort on allergies: University Children's Hospital Munich, Munich, Germany, Institute for Risk Assessment Sciences, Utrecht, NL, University of Basel, Basel, Switzerland, Children's Hospital Schwarzach, Austria, Department of Epidemiology, University of Ulm, Germany
- IMIM, Barcelona, Spain. Epidemiology of asthma (ECRHS and Spanish birth cohort, INMA)

3.7. Proposal for the future work and expected (social) benefits (next 5-10 years)

This research area on asthma and allergy, assessment of microbial exposures in connection to health and disease, and mechanistic work aiming to revealing of the mechanisms that lead to respiratory inflammation and subsequent disease, is at its very active phase. Parts of this research, such as the microbial exposure assessment, represent pioneer work which is now at its rapidly rising phase. It is to be expected that this research area will be active and grow in the next ten years. A major development will be a more close collaboration between research focusing on indoor and outdoor particles of either microbial or non-microbial origin. The impor-

tance of this knowledge to the society is remarkable given that the focus is on the most important environmental health issues in the developed world.

Specifically, the research questions to be answered during the next 3-5 years are the following.

The main focus of future research efforts are to characterize in more detail the causal microbial exposure and its mechanisms leading to both the potential protective and adverse health effects. Of special interest will be the etiology of asthma and allergies, where microbes can provoke lung inflammation and symptoms, but exposure in early childhood can on the other hand direct the development of the immune system away from allergies. This also raises the need to study the possible role of microbial exposures in autoimmune diseases.

The effect of microbial exposure on the development of asthma and allergies will be examined with several epidemiological studies. As part of the international PASTURE birth cohort study, detailed microbial exposures and immune system responses have are currently being measured at birth and one years of age in 400 Finnish children. In addition to questionnaires, blood sampling will be organized at age 4 (starting in 2007) and a detailed clinical examination at age 7. As it appears that endotoxin is not able to explain the protective effect of microbial exposure, the major aim is to identify the causal microbial exposure. The adverse effect of indoor microbial exposure in moisture damaged buildings is studied e.g. in a follow-up of our case-control study on asthma.

The currently used non-cultural methods to characterize microbial exposures will be thoroughly validated during this period. As a result, a deeper understanding of the role of microbial groups, various microbial components and of their metabolites in various health effects has been achieved. This characterization also includes large-scale sequencing of environmental microbes in man's intimate environment. This work will provide potential tools in identification of connections between the etiology of various diseases and exposure to environmental microbes, many of which have remarkable inflammatory and toxic potential.

An intervention study on health effects of moisture and mould exposure is being initiated late this year. The study will be done in collaboration of the Finnish Society for Pulmonary Disabled, which will select serious cases of mould-related houses and their owner families for the study. The families are helped to make thorough renovation in their home, and our role is to monitor the microbial exposures and the occupants' health throughout this process. A part of this study will be assessment of the exposure to microbial toxins which is considered a potential emerging issue of indoor air research. This research will strengthen the understanding on causal relationships between exposing agents in mouldy buildings and health effects, and clarify the importance of microbial toxins in relation to the health effects.

Our interest and the social need of knowledge on the effects of indoor environmental factors on health and well-being will lead to more extensive studies on these connections; the studies on healthy housing will be linked to e.g., sociological and architectural research on housing and the results be applied in area planning of the society. Studies on effects of ventilation efficiency and indoor air quality on students' academic performance will be crucial in decision-making of school building design and maintenance.

To build a more detailed understanding how microbial exposure contributes to mechanisms of the adverse health outcomes and to develop a new systematic research strategy for identification of causative constituents, the research on the toxicological mechanisms will be progressed on following three fronts:

The ongoing research on cellular mechanisms of indoor air microbes will be further expanded by in-depth mechanistical studies on the links between immunotoxicity, inflammation, genotoxicity and oxidative stress. This information is crucial for the development of biomonitoring methods for confirming causality in the adverse health effects and for risk assessment and prevention of the health consequences among the exposed individuals. The relation of microbial characteristitics to induced immunotoxicological responses in mammalian cells offers a new view for evaluation of the adverse health effects induced by inhaled particles.

The role of microbial interactions in the indoor exposures are of interest since these effects may explain the various outcomes in different exposure situations. Our ongoing studies on the role of microbial interactions on induced inflammogenic and cytotoxic responses will be focused on identification of produced immunotoxically active components and agents including microbial toxins. The microbial interactions may have significant, and currently unknown role in induced responses since the detected immunotoxic effects caused by cytostatic compounds can impair the ability of macrophages to protect the host against biological agents.

The immunotoxicological methods will be also applied on multidisciplinary studies combining analysis of toxicological characteristics of microbial exposures in different indoor and out door environments, analysis of microbial characteristics of the same samples and epidemiological data collected from the exposed persons. This provides a unique opportunity to understand how microbial emissions contribute to the adverse health outcomes and to develop a new systematic research strategy for identification of causative constituents. These multidisciplinary data will bring new perspective to risk management combining the strengths of indoor and outdoor air research.

3.8. Personnel

From 1996 onward this research area has consisted of approximately 35 employees at a time; three senior reasearchers with their own group, 11 senior reasearchers or post doc researchers with backgrounds e.g., in microbiology, toxicology and clinical sciences. 15 researchers are at different phases of PhD training, either currently working on it or completed during the time period presented. In addition, a statistician, a civil engineer, a secretary and 9 persons in laboratory tasks or data management are working in this research area. Of the whole group, eight individuals are funded by the government and the rest of this personnel are funded by external funding.

The key senior researchers:

Prof. Juha Pekkanen	asthma and allergy, environmental epidemiology
Doc. Aino Nevalainen	microbial exposure assessment, indoor air
Doc. Maija-Riitta Hirvonen	mechanisms of health effects
Doc. Anne Hyvärinen	microbial exposures, chemical markers of microbes
Dr. Ulla Haverinen-Shaughnessy	healthy housing, school studies; data analysis
Dr. Marjut Roponen	cytokines and allergy
Dr. Kati Huttunen	toxicity and bioactivity of environmental samples
Dr. Tuula Putus	symptom questionnaires, school environment and health

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4 WATER AND HEALTH

4.1. Research area and its significance

The main goal of the water and health research group is to improve public health by developing and applying methods to detect chemical and microbiological agents posing a health threat and to apply them to risk assessment. This knowledge is applied to affect all bodies operating in drinking water sector to carry out decisions which promote public health.

Water chemistry

As to chemical impurities in drinking water, disinfection by-products are a concern in water distributed by waterworks, fluoride, arsenic, natural uranium and radon in private (drilled) wells in certain areas in Finland. At present, 2.1 million people are drinking chlorinated water and thus potentially exposed to any disinfection by-product. The number of persons exposed to fluoride, uranium and arsenic over the health based guideline values are approximately 200 000, 40 0000, 20 000, respectively. Some get exposed to indeed high concentrations. YTOS has addressed by research all these substances except radon (Radiation and Nuclear Safety Authority, STUK, is responsible for radiation) with the view, how serious and wide health problems they may cause. The project on disinfection by-products was launched about 20 years ago , studies on arsenic and fluoride were done in the 1990's and most of the work on uranium has also been done. We have not foreseen any new chemical impurity in Finnish drinking water, which would require research activity from KTL at present (and is justified). However, we are still partners in an EU projects (HI-WATE and INTARESE) studying disinfection by-products in Europe. In this project YTOS is responsible for risk benefit assessment of disinfected drinking water and for the chemical analysis of strong drinking water mutagen 3-chloro-4-(dichloromethyl)-5-hydroxy-2(*5H*)-furanone (MX).

The "MX-project" has been one major effort in YTOS and 4 senior scientists (one full time), 7 Ph.D-students, 3 graduate students and 3 technicians have worked in it since 1996, full or part time depending on the position. Two senior scientists, one Ph.D student and one technician have participated in studies concerning well water. KTL funded itself a notable part of the MX-research. Other significant sources were the Academy of Finland and TEKES. The research on well waters was funded mainly by the Ministry of Social Affairs and Health.

Water microbiology

Many factors have changed enhancing the survival and growth conditions of microbes in Finnish drinking water distribution systems during the last three decades. The trend has been to improve drinking water quality by using (cheap and simple) ground water (technique) instead of surface water as a drinking water supply. Also, a common negative attitude against chlorination, has prevented the use of chlorine disinfection. Moreover, at the same time drinking water consumption has decreased, causing increasing retention times in distribution systems. The contacts from health authorities and water companies have proven that problems related with microbial growth in distribution systems occur in Finland. The research concerning the impacts of the properties of water (water treatment) on microbial growth, formation of biofilms and the fate of different microbes in water and biofilm environment was started in 1988 in YTOS. This research has involved experiments in both laboratory and municipal wa-

ter system scale. The research concerning drinking water biofilms will focus in future more to the fate of pathogenic microbes in biofilms, and to the effects of pipeline materials.

In Finland, a significant public health risk is associated with the waterborne outbreaks. Since 1997, when KTL got the responsibility to take care the waterborne outbreaks, this monitoring system has detected yearly from 5 to 11 waterborne outbreaks. These outbreaks have resulted on average 2,100 officially reported illness cases/year. The most significant microbial agents which have caused outbreaks are noroviruses and campylobacters. The microbiological drinking water problems are mainly associated with the drinking water produced in the small ground water works. The general good quality in these several hundreds ground water supplies has created an illusion that ground water is safe in all times without any protective actions As an official body taking care of waterborne outbreaks, KTL has a unique opportunity to focus its research into the activities benefiting best the public health. The work done in KTL creates tools for solving of drinking water contamination cases. The improvements of microbiological methods can prevent humans from waterborne exposure to intestinal pathogens.

A relevant health risk is formed by the microbes, which can multiply in water system and cause infections. Such microbes are Legionella and mycobacteria. Legionnaires' disease (LD) caused by *Legionella pneumophila*. In Finland about 20 LD are yearly recognised, but the true number of cases is closer to 100 - 200. Legionella research began in YTOS in 1987, and several groups of different water systems have been studied. Research on the occurrence of *Legionella* bacteria in process waters and waste water systems of Finnish forest industry has been started.

Environmental mycobacteria can cause lung infection similar to tuberculosis and infections also in lymph nodes, skin and other soft tissues. The risk for infections caused by these bacteria is increasing since the immunocompromized population is increasing, due to disease or treatments. The number of isolations of environmental mycobacteria from clinical samples in Finland is 400-500 per year. Water systems are nowadays regarded as one of the most important sources for mycobacterial infections and knowing the factors affecting the growth and/or persistence of mycobacteria in water systems is essential. The mycobacterial research began in YTOS in 1989 with studies on the occurrence and species variation of mycobacteria in natural Finnish waters and soils.

4.2. The main achievements

Water chemistry

Disinfection is necessary to guarantee the hygienic quality of drinking waters processed especially from surface waters. Chlorine is the most common disinfectant used although it is known to produce chlorinated disinfection by-products (DBPs) in the presence of natural organic matter (NOM). Concerning the disinfection by-products, during the first 10 years the focus of the research was on 3-chloro-4-(dichloromethyl)-5-hydroxy-2(*5H*)-furanone (MX), its concentrations, stability, basic toxicology etc. Then we showed that MX is a multisite carcinogen in rat and the cancer risk in man is associated in a dose-dependent manner with mutagenicity of water. Concentrations of the other main by-products (trihalomethanes, halogenated acetic acids, brominated furanones, bromate) were shown to be mostly below the guideline values in water and their formation depends e.g. on the humus content of the water and on the chlorine dose. E.g. splitting of the humus by ozonation decreased the by-product

formation. MX and some of its congeners revealed to be both genotoxins and tumor promoters in mammalian cells in vitro. Altogether, the studies yielded a comprehensive understanding for the problem and practical ways to mitigate it in Finland as far as possible. Toxicological aspects produced 2 Ph.D.-theses and epidemiological aspects one.

Because many chlorinated DBPs have mutagenic and/or carcinogenic potency the use of other disinfectants has increased. Simultaneously enhanced removal of NOM has become more important. Ozonation and activated carbon filtration have proved to be useful methods for these purposes. We studied the removal of NOM and the formation of DBPs with pilot-plant experiments using ozonation, ozone with hydrogen peroxide oxidation (O_3/H_2O_2), granular activated carbon filtration (GAC), and preozonation combined with carbon filtration called biologically activated carbon (BAC). Furthermore the characteristics of NOM and the concentrations of DBPs were studied in 35 Finnish drinking waters. Studied DBPs were trihalomethanes, haloacetic acids, adsorbable organic halogens (AOX), and mutagenicity.

O_3/H_2O_2 oxidation was more powerful method to reduce the concentration of total organic carbon (TOC), the formation of AOX, and mutagenicity than ozonation alone. Preozonation improved the removal of TOC in BAC filter by increasing the biological activity in the filter. BAC filtration decreased the formation of chloroform, AOX, and mutagenicity during subsequent chlorination. However, the formation of DBPs after BAC filtration was higher than after ozonation, probably because some humic compounds or microbiologically degraded compounds were released from the filter. The GAC filter without preozonation removed TOC less than BAC filter.

In drinking waters produced from surface waters the most efficient removal of TOC was measured in waterworks using BAC filtration. The concentrations of DBPs were much lower after chloramine disinfection than obtained with free chlorine. Ozonation and/or activated carbon filtration followed by chloramine disinfection reduced the concentration of DBPs to level obtained in chlorinated artificially recharged groundwaters. Conventionally purified and chlorinated surface waters had the highest concentrations of TOC and DBPs.

Research on disinfection by-products required development of analytical methodology that focused on precursors of DBPs, actual DBPs, and different organic compounds acting as surrogates for microbial growth. New methods developed in this context include e.g. the use of capillary electrophoresis and ion chromatography for the determination of different inorganic and organic anions, and a broad method development study for the determination of brominated analogues of MX. Even though the research on DBPs has ceased in YTOS, the method for the determination MX is still used in the EU-project HI-WATE.

These chemistry-related studies have produced 3 Ph.D.-theses.

People drinking water from drilled wells containing abnormally high arsenic had an increased frequency of chromosome aberrations in peripheral lymphocytes and bladder cancer risk was increased already at very low arsenic levels (around 1 µg/l). Arsenic was a subject of one Ph.D.-thesis.

In epidemiological studies on uranium in water from drilled wells, uranium was associated with increased excretion of calcium and phosphate in urine and with elevated blood pressure. No kidney damage occurred but there were signs on effects on bone turnover. Hair was a significant excretion route for uranium.

Water microbiology

Microbial growth and biofilm formation
The major discovery which was published in 1996 in *Nature* was that in most of the Finnish drinking water the microbial growth is limited by phosphorus. This changed our research scope which was earlier based on the common hypothesis that only organic carbon can regulate microbial growth in drinking water. Three years later we reported a novel, very sensitive bioassay for detection of microbially available phosphorus (MAP) in water. The modification of assimilable organic carbon measurement and MAP method have enabled us to study in details the interactions between different water treatment techniques and microbial growth/biofilm formation potential.

We have also studied the effects of pipeline materials and nutrients on the growth of biofilms. One of the discoveries was that the copper material which is commonly believed to prevent microbial growth, can only slow down the microbial growth. In a few months time microbes can grow on copper material as well as on plastic pipeline material. At the same time with pilot and laboratory scale, intensive work was done in close cooperation with the largest Finnish waterworks. These studies found out how quickly the biofilms consisting of diverse group of microbes can recover after mechanical cleaning and how they and the drinking water distribution system itself affect the water quality. The research concerning the microbial community structures using phospholipid fatty acid (PLFAs) techniques indicated that differences in water and biofilm microbial communities depend water quality (hot – cold water, age of water) and pipeline material. In EU-funded SAFER project we studied the growth of biofilms and how pathogenic microbes survive in drinking water biofilms. The project proved clearly that biofilms extent the persistency of pathogens in water systems. During the SAFER project, new rapid techniques e.g. FISH microscopic detection methods were developed for Legionella bacteria and mycobacteria in water and biofilms.

The research done in microbial growth and biofilm formation has increased the understanding of the factors affecting drinking water quality. These studies have combined both basic research and practical applications. Biofilm research has brought four Ph.D. theses , two from microbiological quality of drinking water and hot water, one from microbial nutrients in drinking water and one from identification of microbial communities.

Indicator bacteria and waterborne pathogens
The research among the indicator bacteria (coliforms, E. coli, enterococci), which was started in present form in year 2002, has showed that YTOS has achieved a good national position. The group has tested and compared different techniques to detect indicator bacteria and thus enhanced the knowledge about which methods are best suitable for certain applications of water microbiological analyses. The most important achievements so far consist of the national instructions about water microbiological methods to the local laboratories and health care officers during water quality monitoring. The created ability and readiness to do analyses and to give knowledge in waterborne outbreaks is also important for Finland.

The main aims of the research group have been to develop detection methods that are novel and best suitable for the detection of the waterborne pathogens. The scope of the analytical methods is based on our own experience: noroviruses and *Campylobacter* sp. have been the causative pathogens in most of the waterborne outbreaks. This explains why our novel detection techniques include real-time PCR detection (qPCR) applications for campylobacters and noroviruses. A qPCR method for adenoviruses was developed to detect and use adenoviruses as a human faecal contamination indicator. Methods for genomic fingerprints of bacteria for

source tracking of microbiological contamination of drinking water have also been taken in use in the research group.

Two Ph.D students are focusing on the occurrence and survival of indicator bacteria, Campylobacters and noroviruses.

We have started to study the extent of molds and fungi in drinking water because no systematic or comprehensive study about the occurrence of them was ever done in Finland. Our survey study showed that about 10% of Finnish water works may have problems related to fungi/actinomycetes. Field sampling results from selected municipalities showed that the occurrence of fungi is mainly related to raw water. The concentration of fungi mainly decreased in the network, while the number of actinomycetes increased in the network. Identification of the isolated strains is under way. A series of experiments to elucidate the survival of fungi and molds in water as well as the effect of disinfection on survival was carried out in summer 2006. Three M.Sc. theses are being written from the results of these experiments.

Pathogens able to multiply in water systems
Legionella: The studies concerning occurrence/survival of legionella in different water systems have increased the amount of both scientific and practical knowledge.

Now we know where legionella bacteria can grow and we have also been able to show how growth of legionellae could possibly be prevented. The research group has also been able to show the sources of some most interesting legionellosis cases.We have shown that legionellae bacteria persist commonly in Finnish hot water, cooling water and waste water systems, where prevailing warm water temperatures favour the growth legionellae .

We have also shown with the newest gene technology methods the source of the first legionellosis outbreak to be the hospital hot water system, and that a new-born child got her legionellosis from her home hot water system with low water temperatures. New FISH-method for the detection of Legionella in biofilms and waters without culture has recently been developed and are applied in current studies.

Two Ph.D. theses have been done about legionellae.

Mycobacteria: Our earliest studies have shown that the Finnish natural environment is rich with mycobacteria and that low pH, high content of organic matter and high area of peatlands in drainage area favor the occurrence. Our later studies have shown that mycobacteria occur in many types of water systems: drinking water, hospital water, swimming pool water, cooling water and waste water systems with active sludge. Drinking water studies have also shown that mycobacteria adhere easily to drinking water biofilms and persist there. Hospital water studies have shown that tap and shower heads are easily contaminated with mycobacteria even though the circulating hot water does not contain culturable mycobacteria and that it is extremely difficult to eradicate mycobacteria from the systems. Waste water systems have shown that mycobacteria in active sludge pools get aerosolized to the near environment. All studies have included development of culture techniques to improve the detection of mycobacteria which are slowly growing. In addition, a new FISH-method has recently been developed for the detection of *M. avium* in biofilms and waters and is applied in current studies. Quantitative PCR-method for the analyzing of mycobacteria is also under development.

In all, our studies have given a comprehensive view of the occurrence, concentrations and species of mycobacteria in Finnish natural and man-made water environments and the factors affecting them. Two Ph.D. theses have been done, one on the occurrence of mycobacteria in natural waters and soils and the other on identification and characterization of mycobacteria.

Expert work on waters

Since 1997 YTOS has been responsible for collecting data related to waterborne outbreaks and assisting different bodies in these cases. At National Public Health Institute (KTL) the advisory work is co-ordinated between the departments of Environmental Health and Infectious Disease Epidemiology. The aim is to help the municipal health protection authorities and water works to investigate and to finish these outbreaks.

Six researchers from YTOS participate in detection and consulting work concerning waterborne outbreaks. In addition, one researcher is providing help when the sources of legionelloses are tried to be solved and also to be cleaned.

After joining in the European Union, Finland and Finnish water works had to adopt new monitoring and reporting systems for drinking water quality. Like other member states in the union also Finland has to regularly submit to the European Commission a report on drinking water quality in Finland. YTOS collects the information on monitoring results and produces the report to the Commission at three-year intervals. YTOS also produces to the Commission annual reports on bathing water quality. One specialist from YTOS participates in these EU reporting tasks.

4.3. Scientific impact

The KTL-group on disinfection by-products has been the leading group internationally in elucidation the role of MX in the cancer risk. The studies on effects of natural uranium in drinking water are largest and cover the widest exposure range. They are pioneer work on the subject. Studies on the cancer risk at low exposure levels of arsenic fill the required data gap and have been well taken. Lately our biofilms studies have been focused on the survival of pathogens in biofilms and the effects of household plumbing materials (copper and polyethylene) on water quality. We have found that pathogens survive in extended times in biofilms and that pipeline materials affect the growth of biofilms. One surprising result was that copper pipes decreased microbial numbers only for a few months, after which the number of bacteria in biofilms of copper pipes was in same level as in plastic pipes. However, the population structures were different in biofilms on these materials. Pipeline material affected also disinfection efficiency of chlorine against biofilms and bacteria in water. Material studies have close links to practical applications and practices and have been interested widely among the industry and scientific community.

Table 1. Summary of the scientific output during 1996-present

SCIENTIFIC OUTPUT	N	Comments
Peer-review papers in international journals	**96**	
Invited lectures and chairmanships in international meetings	**28**	
Papers in domestic journals	**55**	
Lectures in domestic meetings	**200**	e.g. Finnish Water and Wastewater Association, Terveystarkastajapäivät, Hygieenikkoeläinlääkäripäivät, lääninhallitusten koulutuspäivät
Editorial tasks in international journals	**0**	
Committee memberships: domestic	**5**	e.g. Vesiyhdistys, Vesimikrobiologian standardoimisryhmä
Workshop and committee memberships: international (EU, WHO etc.)	**5**	European Microbiology Advisory group (EMAG), International Water Association (IWA), EU´s Bathing Water Committee, Society for Applied Microbiology (SfAM)
Doctoral dissertations	**16**	
Supervision of dissertations: on-going	**2**	Tarja Pitkänen and Leena Taskinen, Kuopio University
Opponent of dissertations	**3**	*Technical University of Denmark (2004), Royal Institute of Technology, Sweden (2004), Riku Vahala, Helsinki Univ. of Technology*
Referee tasks in international scientific journals	**20**	e.g. Water Research, Chemosphere, FEMS Microbiology Ecology

4.4. Social impact

4.4.1. National

d) Municipal –level impacts

Water chemistry
The project on drinking water by-products has changed drastically the drinking water policy in Finland. Where possible the raw water source has been changed to ground water, chlorination practices were changed and alternative methods to chlorination have been increasingly used. The associated cancer risk has dropped approximately to one third. Characterization of both the arsenic and uranium problem in drinking water has helped the municipal authorities to instruct better the use of private wells to reduce the health risk.

Water microbiology
Waterborne outbreaks. Since 1997 YTOS has been responsible for taking care the waterborne outbreaks in Finland. The consultancy from YTOS is frequently asked like the numerous waterborne outbreaks show. The main aims are to provide knowledge for the local health protection authorities and water works which is needed to investigate and to stop these outbreaks. The water research group is producing in co-operation with the Finnish Environmental Institute (SYKE) a guideline for design, maintenance and operation of small water treatment plants.

Waterborne pathogens. YTOS provides and maintains analytical methods which are necessary to identify microbiological agents causing the outbreaks as described in the previous chapter.

The financial support from Ministry of Social Affairs and Health has enabled development and maintenance of the analytical capabilities at YTOS. The methods have been applied not just in waterborne outbreaks, but also in microbial water contamination cases. These tools have been useful for source tracking of pathogens in water systems.

Indicator organisms. The work done on indicator organisms has had significant national influence: health care officers and laboratory microbiologists ask advices, which are given by lectures, national publications, official instructions, web site and most often by personal communication. Our laboratory also helps local laboratories with identification and storage of bacterial strains and we participate to the national standardization of water microbiological methods.

Microbial growth/biofilm formation. The research concerning survival/growth of microbes in water environment has helped waterworks and engineers in planning and maintaining drinking water distribution systems. National authorities have obtained locally relevant information of the functions of water systems, which help them to evaluate water quality results. Our decisions and advices concerning water microbiological methods have economic influence on those who need to use these methods for water quality monitoring.

Legionella and mycobacteria: Our publications, reports and lectures have increased the knowledge of different stakeholders, such as waterworks personnel, health officers, medical, constructing and industrial professionals, on legionella and environmental mycobacteria and associated health risks. The research done in hot water systems and cooling water systems has greatly helped industry and authorities to find balance between the economical aspects and microbiological health risks.

Entire research group. The main results of the studies have been published not just in the scientific journals but also in domestic professional journals. The researchers of the group have given several lectures in national level for the authorities, industry and personnel operating in the water services sector. The true impact of Water and health - research group is manifested by the high number of the invited domestic lectures.

b) Other national impacts

YTOS has been participated many research projects funded by TEKES. In these projects close co-operation has been done with private companies operating in water sector. The research co-operation has enhanced technique applied in drinking water distribution systems (e.g. Suomen Pipeline Cleaning Ltd, Outokumpu Poritube Ltd, Uponor Ltd).

4.4.2. International

One researcher from YTOS has been a member of work groups preparing the IARC monograph on arsenic and MX and updating WHO guideline values on drinking water contaminants. A researcher from YTOS is a member in European Microbiology Advisory Group and participates also the work done in ISO and CEN standardization committees. One specialist has been a member in EU bathing water committee since 1997.

The group has also participated in WHO Environmental Health Indicator, ENHIS and CEHAPE programs by providing the information collected in drinking water quality, bathing water quality and waterborne outbreak issues, and in the work related to the Protocol on Water

and Health (WHO, UNECE) where the main objective is to prevent and reduce water-related diseases in Europe. One specialist from YTOS has been participated in the revision of the Bathing Water Directive 76/160/EEC. A new directive, Directive 2006/7/EC of the European Parliament and of the Council concerning the management of bathing water quality and repealing Directive 76/160/EEC, came into force in March 2006.

The group organized a scientific international drinking water conference at Kuopio in 2003. The conference was a part of EU-funded WEKNOW. 132 participants from 22 nations attended the conference.

The main goal of the research group has been to publish the most important results always in the scientific international journals. The researchers of the group have participated in many international congresses as invited speakers. Nearly all of the group´s researchers have acted as reviewers of international scientific journals.

4.5. Research funding

Table 2. Project funding during 1996-present

Project name	Funding agency*	Role	KTL funding: k€	Years	Partners
Brominated by-products	Tekes	leader	55,5	1998-99	
Water knowledge network (WEKNOW)	EU	partner	83,2	2002-2005	7 EU partners
Green house gases of lakes	SA	leader	171,6	1998-2000	Univ.Joensuu/KuY
Phosphorus and microbes	SA	leader	140	1998-2001	
Nitrification bacteria	SA	leader	117	1997-1999	KuY
Emerging waterborne pathogens	STM	leader	159	2001-2006---	HY
Norovirus diagnostics	STM	leader	212	2004	HY
Deposits of distribution networks	TEKES	partner	75,9	1996-1998	Suunnittelukeskus Ltd
Control of networks (Vesikko)	Tekes	partner	111,6	2006-2008	TKK
Monitoring of water quality (Vainu)	Tekes	partner	119	2004-2006	KUY, Savonia-AMK
Pilot water works and distribution network (TARVE)	Tekes	partner	104,6	2001-2003	KUY, Savonia-AMK
Distribution networks	Tekes	leader	55,3	2001-2002	
Chlorinated by-products in drinking water	Tekes	leader	65,6	1998-1999	
Brominated by-products II	Tekes	leader	67,3	1999-2000	
Biofilms in drinking water networks	Tekes	leader	67,1	1996-1997	
Deposits of networks: effect of removal	Tekes	partner	55,3	2001-2002	Suunnittelukeskus Ltd
Legionella in cooling waters	Unibase Ltd	leader	69,2	2006	Sonera Ltd
Cemfec	EU	partner	86,2	2001-2003	
Monitoring of drinking/bathing water quality	STM	leader	480	1998-2006---	STM
Below 50 k€			707,4		
TOTAL			3002,8		

* EU=European Union, SA=Academy of Finland, STM= Ministry of Social Affair and Health, Tekes=Finnish Funding Agency for Technology and Innovation, KuY=University of Kuopio, HY=University of Helsinki

4.6. Scientific collaboration

4.6.1. National

The research group has co-operated intensively with all major Finnish research institutes, universities, ministries and the biggest water works. The lists includes the most active and significant partners.

Water chemistry

In the disinfection by-product project the Department of Pathology, University of Kuopio was responsible for all histopathology in animal experiments, the Department of Organic Chemistry, Åbo Akademi for synthesis of some chlorohydroxyfuranones and their analyses in water. The Cancer Registry in Finland provided data on cancer frequencies for epidemiological studies and participated in data analysis. Concerning the work on arsenic and uranium, Radiation and Nuclear Safety Authority, Finland has been a key collaborator. It has provided data on uranium and arsenic concentrations and coordinated the uranium studies. The University hospitals of Kuopio and Tampere have performed analyses on biological specimens and the researchers have represented clinical expertise in human studies.

Water microbiology

With Kuopio university/Department of Environmental Sciences the co-operation has begun in 1980´s. The partnership with Kuopio university has included intensive exchange of information and knowledge in many drinking water/biofilm projects.

The best example of co-operation with Savonia University of Applied Sciences are the test facilities including pilot scale waterworks and distribution system (total 600 m long) at Kuopio city. It was built in 2002 in co-operation with University of Kuopio and Savonia University of Applied Sciences. In these facilities we have been able to study the effects of pipeline material and water flow regime on drinking water microbiological quality and formation biofilms.

The water research group started the co-operation with Finnish Environmental Institute (SYKE) in 1980´s. The main subject in the research projects led by SYKE was the artificial recharge technique (ARG). SYKE provided the planning, sampling and physico-chemical planning of ARG projects.

The effects of ozonation, GAC-filtration and nanofiltration on microbiological stability of drinking water have been studied intensively with Technical University of Helsinki (HUT). HUT usually provided the planning, technical design, sampling and physico-chemical analyses in these projects.

The co-operation with Institute of Biotechnology at Helsinki University consist of sequencing services dealing with campylobacter, mycobacteria and norovirus strains and with species identification of coliform bacteria found from water samples.

YTOS co-operates in many research projects dealing with the detection and occurrence of pathogenic microbes (especially campylobacters and noroviruses) in drinking water with researchers at Helsinki university Department of Food and Environmental Hygiene.

Oulu University, Bioengineering group: Peter Neubauer and his co-workers have developed new detection methods for legionellae which has been applied for samples of ongoing studies done in YTOS.

Geological Survey of Finland (GTK) has been an important co-operator in mycobacteria research. Their role in the research has been the sampling and geochemical analyses that were used to study the factors affecting the occurrence of mycobacteria in waters, soils and sediments.

4.6.2. International

Water chemistry

We have had good cooperation with Harward School of Public Health; Massachusetts, USA and Univ. of Cologne, Germany, concerning disinfection by-products The research on disinfection by-products, arsenic and uranium has been finished at KTL except two partnerships in the EU-projects (INTARESE and HI-WATE) related to disinfection by-products. No new chemical contaminant/threat is foreseen which would deserve research at KTL at present. Implementation of the work already done to practical instructions e.g. for owners of the private wells is more important at this stage.

Water microbiology

YTOS was very successful in the EU´s 5th framework program. The EU funded research projects SAFER and WEKNOW enabled good co-operation with the leading European drinking water research institutes.

In SAFER project, led by NanCIE research institute (France) we were able to develop new rapid techniques for detection of pathogenic bacteria in water and biofilms. Intensive co-operation resulting scientific publications has been included in studies concerning the fate of legionella and mycobacteria in drinking water biofilms and development of FISH-based methods for *M. avium and Legionella pneumophila* bacteria.

Co-operation with the_University of Duisburg-Essen in SAFER project has been intensive, including e.g. exchange of samples and biofilm research knowledge. Our group has now a three-year experience about student exchange with the University of Duisburg-Essen.

The water research group has actively participated in the work done in the European Working Group for Legionella Infections (EWGLI). The co-operation forms have been the exchange on knowledge concerning legionella case studies and reports about the cases. In Scandinavia, there is a co-operation network of legionella researchers in Swedish, Danish, and Norwegian Public Health Institutes. Forms of the co-operation are the Nordic Legionella Meetings.

Veterans Affairs Medical Center (Boston, USA) and Dartmouth-Hitchcock Medical Center, (Lebanon, USA). Co-operation included shared samples of swimming pool waters which were analyzed by different mycobacteriological methods in USA and Finland.

Mycobacteria Reference Center, Tuscany (Italy), Institut Pasteur, Mycobacterium National Reference Center, Paris (France),_Statens Serum Institut, International Reference Laboratory of Mycobacteriology, Copenhagen (Denmark). YTOS has made collaboration with the insti-

tutes in the description of novel mycobacterial species isolated from environmental, clinical and veterinary samples.

The research group participated actively the EU-funded WEKNOW-project. The aims of the project led by KIWA Water Research institute was to include all stakeholders involved in the process of supplying drinking water to European citizens in order to maximise the impact of European research and effort in all areas related to drinking water.

4.7. Proposal for the future work and expected (social) benefits (next 5-10 years)

Water chemistry

The research on disinfection by-products, arsenic and uranium have been finished at KTL except two partnerships in the EU-projects (INTARESE and HI-WATE) related to disinfection by-products. No new chemical contaminant/threat is foreseen which would deserve research at KTL at present. Implementation of the work already done to practical instructions e.g. for owners of the private wells is more important at this stage.

Water microbiology

In order to maintain the status of institute capable to help the municipalities in various microbial threats originating from water systems (drinking water, hot water, cooling water, process water or waste water) our group has to continue the maintenance and development of modern analytical techniques for the detection of waterborne pathogens. This will include quantitative PCR detection methods for pathogenic agents: campylobacteria, noroviruses, adenoviruses, legionella and mycobacteria. In future our study will continue in biofilm research in pilot scale water distribution system and in applying the new detection methods of pathogenic microbes in real water and biofilm samples.

Water research group will also start the work concerning the risk analysis. This work will include comparison the chemical risks and microbiological benefits of drinking water disinfection. The work has been started in EU-funded HI-WATE project. The group will also continue the work with Water Safety Planning (WSP). Risk assessment and risk management protocols of drinking water production will be tested in future.

In future the research interests of Legionella-bacteria will be directed on waste and process water systems prevailing in forest industry. The microbial risks associated with legionellae among the workers and outsiders will be elucidated. Experimental biofilm studies with mycobacteria will continue. They will help to understand the factors affecting the occurrence and persistence of mycobacteria in water distribution systems. Studies on active sludge systems of forest industry will also continue and they will help to evaluate the possible health risk associated with mycobacteria in these systems.

4.8. Personnel

From 1996 onward the research group has been consisted of 15-18 employees: head of the department, a chemist, a senior researcher, 5 Post Doc researchers, 2 Ph.D. students, 2-3 M.Sc students and 2 assistant persons of which 5 persons get funding from government and the rest 10 get funding from other external sources like EU, ministries, TEKES, funding organizations etc.

The key senior researchers:

Prof. Terttu Vartiainen	(disinfection by-products)
Doc. Hannu Komulainen	(chemical impurities)
Doc. Ilkka Miettinen	(microbial growth, biofilms)
Ph.D. Markku Lehtola	(biofilm studies, FISH-methods)
Ph.D. Eila Torvinen	(mycobacterial studies)
Ph.D. Jaana Kusnetsov	(legionella studies)

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5 PERSISTENT ORGANIC POLLUTANTS AND HEALTH: ANALYSIS, CONCENTRATIONS, EXPOSURE AND EPIDEMIOLOGY

5.1. Research area and its significance

Polychlorinated dibenzo-*p*-dioxins, dibenzofurans (PCDD/Fs, dioxins), polychlorinated biphenyls (PCBs), polychlorinated diphenyl ethers (PCDE), polychlorinated naphthalenes (PCN), and their brominated analogues like polybrominated diphenyl ethers (PBDEs) are widespread environmental contaminants. Due to their lipophilicity and persistency they accumulate in the food chain and they and their methoxy and hydroxyl metabolites can be found in animal samples.

Some of those compounds such as PCDD/Fs have never been intentionally manufactured but exists in burning gases or as by-products or contaminants in pesticides or other chemical products. Others have been used in variety of applications e.g. dielectric fluids in transformers, hydraulic systems, and paints (PCBs) or as flame retardants (PBDEs).

The most potent of these compounds exert several toxic effects in experimental animals such as immunosuppression, body weight loss, enzyme induction, developmental defects and tumor promotion. In humans, accidental or occupational exposures to high doses of PCDD/Fs and/or PCBs have caused lesions of skin, chloracne, developmental defects, and increased the risk of cancers. PCBs are suspected to cause neurobehavioural effects as well as to function as endocrine disrupters.

Finland has not been thought to be severely polluted by persistent organic pollutants (POPs), excluding the Baltic Sea that is contaminated with PCBs and pesticide DDT and its metabolites, but to our surprise, also our nature and humans contained those compounds sometimes at quite high concentrations. The production and use of chlorinated chemicals was found to be a significant source of PCDD/F releases to soils and sediments. The manufacture of a Finnish chlorophenol formulation Ky-5 and vinyl chloride monomer between the 1940s and mid-1980s has been identified as potential source of PCDD/F contamination in the Gulf of Finland. Moreover, Ky-5 has been widely used in sawmills as a fungicide, which has resulted in leakages into soil and sediments. Proper management of these contamination problems and prevention of human exposure to PCDD/Fs requires in-depth knowledge of the concentrations and sources of pollution and development of appropriate techniques for environmental cleanup.

Organotin compounds (OTCs) are used in a variety of industrial, domestic and consumer products. The main use of tributyltin (TBT) and triphenyltin (TPhT) has been in antifouling paints of marine ships owing to their strong biocidal activity toward aquatic organisms. This has caused global OT contamination of marine areas with high shipping activity. OTs are extremely hazardous to some aquatic species even at very low concentrations. Their observed effects include malformations, growth retardation and reproductive effects, e.g. imposex. TBT and TPhT are endocrine disruptors in several marine species and recent *in vitro* studies suggest that these compounds might affect the endocrine system of humans as well. Due to the observed adverse effects their use has been restricted by many nations. However, TBT deposited in sediments will remain an important source for marine biota for many years to come.

Our aim has been to analyse POP concentrations, mainly PCDD/Fs and PCBs, but also e.g. organotin and in the future also organomercury, in Finnish nature, food and humans, to calculate and measure their concentrations in humans and to do their epidemiology. Knowledge about the levels of intake and body burden of POPs in a population helps to focus efforts to diminish population exposure to these hazardous compounds. The effects of already applied measures to limit the population exposure to these contaminants can be judged by examining the temporal changes in intakes and body burdens. History and current occurrence of these compounds provide a way to assess future development of concentrations. A highly exposed group of people would be the best target group to study hazardous effects of PCDD/Fs and PCBs and therefore finding such a group is essential for any epidemiological study (our fishermen's study).

Our research group started to study POPs in 1987 when the Laboratory of Chemistry got the first high resolution mass spectrometry. However, the personnel was quite limited until the mid of 1990s and we did not have any official quality standard. Our first step was to raise the quality of chemical POP analyses to a level which is as high as in the best laboratories in the world. We have succeeded quite well, the laboratory of chemistry has been accredited since 1996 and we have been successful in all international intercalibrations (see Appendix 2). Nowadays, we analyse e.g. EU food monitoring samples not only from Finland but also many sample sets from Sweden and Estonia as well.

An epidemiologist started to study POPs in the late 1990s. She has started to create an epidemiological/nutritional research group around POPs. She gets funding from the Academy of Finland, and so do some students in her group. She is responsible for the small area epidemiology in KTL.

5.2. The main achievements

Chemical analyses of POP

Analytical processes of POPs are normally labor-intense and time consuming methods based on high volume solvent extractions. These methods, like the modification of Method USEPA1613 previously used in the laboratory of chemistry, produce primary and secondary waste such as toxic solvents and chemicals. We have developed a method using Supercritical Fluid Extraction (SFE) for the simultaneous extraction and cleanup of POPs from soil and sediment samples, which significantly reduces analysis time and is almost solvent-free. An Accelerated Solvent Extraction (ASE) -method has been developed for the extraction and cleanup of different POP from biological matrices. During this development work also cleanup and fractionation phases following ASE were significantly rationalized. Combined this has resulted in significant time savings and reduced the use of solvents and chemicals in the analysis of POPs from biological matrices.

Concentrations in the environment and remediation

The concentrations and sources of PCDD/Fs in the Gulf of Finland and Gulf of Bothnia were surveyed by analysing sediment samples. Lake sediments from an area with no known industrial sources were analysed to identify natural formation processes and pre-industrial sources of PCDD/Fs. In addition, experiments were carried out to investigate and develop a method

for the removal and decomposition of PCDD/Fs in contaminated soils. The method was based on the use of vegetable oils and sunlight.

There has been a significant reduction e.g. in the PCDD/F and PCB environmental levels due to control measures. Concentrations have declined by as much as 90% in the environment from those of the late 1960s. Today the major sources of PCDD/Fs and PCBs are combustion processes (waste incineration and backyard burning), metal industries, contaminated soil and sediments, and landfill sites with contaminated material (Isosaari et al. 2002).

Sediment studies showed that the most heavily polluted sediments were located within 12 km from the Kymijoki River inlet. However, the impact area of the Ky-5 releases extended over a distance of 75 km. Low concentrations of mainly highly chlorinated dibenzo-*p*-dioxins were detected in lake sediments that had been formed up to 8 000 years ago. The contamination of sediments of the River Kymijoki is reflected as elevated levels of POPs in water in this river as indicated by mussel incubation.

Analyses of herring from different areas in the Baltic Sea indicated that most POPs are evenly distributed in the aquatic environment in the Gulfs of Bothnia and Finland. Although the levels of POPs have decreased from those in the 1960s, they are still higher in the Baltic Sea than in reference areas, the levels in Baltic ringed seals being threat to their well being. Differences between seal species are partly explained by food sources of seals (Kiviranta et al. 2003).

Concentrations in fish and food: intake of POPs

To obtain knowledge about concentrations of POPs in fish, about 300 fish samples, containing 1500 individual fish, were analysed for PCDD/Fs, PCBs and PBDEs. This was done to report for EU those concentrations and to improve our own knowledge. Different fish species accumulated different PCDD/Fs congeners, and different concentrations. The highest concentrations were found in Baltic herring and in Baltic salmon and the concentrations were age dependent. All other fish samples contained PCDD/Fs below the Council Regulaton (EC) No 2375/2001 of 4 pg TEQ/g fresh weight and also Commission regulation No 199/2006 maximum level for dioxins and dioxin like PCBs of 8 pg /g fresh weight.

Consumer safety of farmed salmon has been studied in two large projects. Safety can be improved by determining the transfer efficiency of hazardous pollutants from fish feed to salmon.

We have evaluated the characteristics of average intake of PCDD/Fs and PCBs in Finland. Intake studies revealed that the average adult Finnish intake of PCDD/Fs and PCBs was 1.5 pg WHO-TEq/kg bw/day which is below the suggested tolerable daily intake (TDI) of 2 pg WHO-TEq/kg bw according to EU SCF. When comparing to European countries, the intake of PCDD/Fs was similar and the intake of PCBs was slightly lower in Finland. An annual decrease of 6% in the PCDD/F intake during the 1990s has occurred. Fish and fish products contributed most (60%-95%) to the intakes in Finland (Kiviranta et al. 2004).

Concentrations in breast milk and epidemiology

A survey of Finnish breast milk samples from two locations has been conducted since 1987 (Vartiainen et al. 1997). A decline from 1987 to 2000, similar to other countries was detected in the breast milk concentrations of PCDD/F and PCBs, being annually 5% and 6%, respectively. In the year 2000, the average concentration of $WHO_{PCDD/F}$-TEq was 9.4 pg/g fat, and WHO_{PCB}-TEq 5.9 pg/g fat, both concentrations being close to European levels (Kiviranta et al. 1999).

Of those children born in 1987 and whose mothers' milk were analysed, teeth were studied for the presence of hypomineralised enamel defects. Hypomineralization defects were observed in newly erupted first permanent molars that undergo mineralization during the first two years of life, the period for maximal PCDD/Fs exposure of the child via mother's milk. The frequency and severity of these defects correlated with the total individual exposure calculated for each participating child from the PCDD/F concentration in mother's milk and the duration of breast feeding. Although the study population is relatively small (102 children), the effect is clear and statistically significant. In fact, this is the first evidence about an association between PCDD/F exposure and a developmental defect observed in humans at normal background exposure levels (Alaluusua et al. 1999). Of the children born 1990 or later, no association to the defects in teeth occurred (unpublished data).

Concentrations in humans and epidemiology

Adipose tissue concentrations of PCDD/Fs and PCBs were measured in the general population, and concentrations in three geographical areas were compared. Adipose tissue concentrations of PCDD/Fs (median 24 pg $WHO_{PCDD/F}$-TEq/g fat) and PCBs (median 17 pg WHO_{PCB}-TEq/g fat) in the general Finnish population were comparable to European concentrations (Kiviranta et al. 2005). The population based concentration frequency graph suggested that exposure of Finnish population to these contaminants have been declining during the last decades. Professional fishermen were shown to represent a highly exposed population (Kiviranta et al. 2000) being at a same level as in Seveso contaminated area B. However, the difference between Finnish fishermen and Seveso population is that fishermen's exposure is lifetime but Seveso exposure was a single accident.

Adipose tissue concentrations of Finnish population above were obtained during a study in which we studied the association between soft tissue sarcoma risk and individually measured dioxin exposure in a general population with no known exposure to dioxin other than exposure via food. This was a multicenter case-control study with 110 patients and 227 matched controls. No increased risk associated with increased dioxin concentration was observed. Actually the highest risk of sarcoma was found at low levels of dioxin. Later also non-*ortho*-PCBs were included in the exposure pattern, but the results of the study remained unchanged (Tuomisto et al. 2004).

In the EU project EXPORED (Exposure – outcome relationships in male urogenital malformations with special reference to endocrine disrupters), the objective is to establish the relationship of male urogenital malformations with exposure to potential endocrine disrupters during pregnancy. Cohort studies in Denmark and Finland, two countries with different incidences of male urogenital disorders, such as cryptorchidism and hypospadias are included. In the EU project EDEN (Endocrine Disrupters: Exploring Novel Endpoints, Exposure, Low-Dose- and Mixture Effects in Humans, Aquatic Wildlife and Laboratory Animals), we continue with the same cohorts by correlating concentrations of POPs in adipose tissue samples from boys diagnosed with undescended testes (chryptorchidism) and their controls (boys undergoing hernia operations) and adipose tissue specimens from women with breast cancer, and from women without neoplasms of the breast.

These studies have produced 9 Ph.D. theses.

Nutrition, environment and health

Evidence is accumulating that fish consumption is positively associated with many aspects of human health and wellbeing. However, there are also important safety considerations relating to toxic contaminants of fish. The picture gets more complicated as relatively little is known about health effects of fish and new scientific data keeps emerging. Furthermore, an objective

optimal solution cannot be based on scientific risk assessment only. The *Nutrition, environment and health – research project* contributes to a balanced risk analysis of dietary fish intake, shedding light to various potential health effects of the composition of diet as well as its individual nutritious or toxic components.

The research objectives are: (i) to measure levels of environmental contaminants, nutritional compounds and their relations with characteristics of fish consumption; (ii) to investigate selected biological mechanisms that may explain the observed association between methylmercury and cardiovascular health; (iii) to explore potentially hazardous effects of environmental contaminants originating from fish in diet (including diabetes); (iv) to study potentially beneficial effects of fish-derived fatty acids and other compounds (decreased depressiveness, improved self-reported health and well-being); and (v) to conduct an overall risk analysis of the health effects of fish consumption in Finland.

For this purpose, we have compiled new epidemiological data sets at the KTL Department of Environmental Health. The fisherman family study is a retrospective study that includes register data for 36,097 people (20% fishermen), 418,000 person years, 1,060 incident cancers and 1,510 deaths. We have also conducted a health questionnaire study with 1,429 respondents, and an in-depth study of 310 volunteers with extensive questionnaire information, dietary information, electrocardiogram and carotid ultrasound recordings, and biological samples allowing measurements of hazardous and beneficial compounds and genetic structure. We also have permission to use KTL Health 2000 survey data (Cardiovascular Disease and Diabetes substudy) which has been collected with a similar protocol and includes data for 1,295 subjects. The multidisciplinary team consists of about 15 researchers with backgrounds in medicine, public health, environmental chemistry as well as nutritional and environmental sciences.

Data collection, storage and cleaning have been finalized, most of the chemical analyses have been finalized and reporting of the study has been started. The first statistical analyses have confirmed that the average fish consumption, the intake of fish-derived fatty acids, and the serum dioxin concentration were significantly higher among the fishermen and their wives than among the general male and female populations. Further, the fishermen had significantly lower mortality from all main causes of death. The wives had a similar mortality pattern, but the relative risks were closer to unity. Among the fishermen, significantly lower relative risks were observed for cancers of the stomach, colon and lung.

Organotin studies

The ongoing project funded by Finnish Academy of Sciences "*Organotin compounds in the environment, humans, and possible effects on human endocrine system*" has four subprojects where we study: 1) environmental levels and average population intake. In this subproject, sediment OT concentrations in one of the most contaminated sea area and its surroundings in Finland are measured. Finnish market basket was analysed for OTs to calculate the dietary intake. The intake for average population was very low, only 1% of the tolerable daily intake proposed by the European Food Safety Authority. Fish contributed 81% of this intake (Rantakokko et al. 2006). 2) blood concentrations of OTs from fishermen from Baltic Sea and freshwater regions in the context of the above mentioned Fishermen's Study. We try to find associations between OTs blood levels and various health risk patterns. 3) the relationship of male urogenital malformations with exposure to potential endocrine disrupters (OTs in this case) during pregnancy in the context of the above mentioned EXPORED-project. 4) the relationship between OT exposure and semen quality in fertile men, in Finland and Denmark. We also study the relationship between OT exposure and semen quality in young men represent-

ing general population, in Finland and Denmark. General population may contain individuals with so severely impaired semen quality, that fertility is seriously decreased.

According to literature and our market basket study, fish is by far the most important food group that contributes to the intake of OTs. In the project funded by EVIRA (Finnish Food Safety Authority) "*Levels of organic tin compounds in domestic lake- and seafish"* we study levels of OTs in fish from sea- and freshwater areas. Within the projects almost 900 fish samples will be collected, which will be pooled to 270 samples and analysed for OTs.

5.3. Scientific impacts

Summary of the scientific impact is presented in Table 1.

Table 1. Summary of the scientific output during 1996-present

SCIENTIFIC OUTPUT	N	Comments
Peer-review papers in international journals	**69**	Number of papers between 1996 and 2005 are: 5,8,5,8,4,9,14,3,9, and 4
Invited lectures and chairmanships in international meetings	**5**	
Papers in domestic journals	**12**	Number of papers between 1996 and 2005 are: 0, 1, 1, 0, 4, 2, 2, 1, 0, and 1
Lectures in domestic meetings	**Over 100**	e.g. many yearly Science days (Lääketiedepäivät, Itä-Suomen lääkäripäivät,) lectures in Universities,
Editorial tasks in international journals		
Referee tasks in international journals	**24**	Referee in Environmental, natural and epidemiological journals
Committee memberships: domestic	**20**	e.g. Chairman of the Research Council for Environment and Natural Resources t the Academy of Finland 1998-2000, Chairman of the Research Council for Biosciences and Environmental Research at the Academy of Finland 2001-2003, Sciences and Technology Policy Council of Finland (Tiede ja teknologianeuvosto) 2005-2008, Mikkeli Highschool, Environmental Technology Council chair 2006-2008, Member of Scientific Advisory Board for Defence (MATINE) 2006-2009, Member of National Ethical Committee (TENK) 2003-2008, Member of Expert Group of Metrology 1994-1998, East Finland Chemical Society, chairman 1998-2000, Member of Expert Group of Metrology 1994-1998, Chairman of the Management Board of the Finnish Environment Institute 2000-2004, Finnish Environment Institute Advisory board member 2004-20007
Committee memberships: international (EU, WHO etc.)	**5**	Member of the European Science Foundation, Standing Committee for Life and Environmental Sciences (LESC) 1998-2003, Member of Expert Group in Nordic Council of Ministers 1994-2000, Member of the European Science Foundation, Subcommittee (EERO) 1998-2000, Who human milk study, corresponding from Finland since 1987, Member of several EU subcommittees
Doctoral dissertations	**7**	Number of dissertations between 1996 and 2005 are: 1, 1, 0, 0, 0, 0, 2, 1, 1, and 1
Supervision of dissertations: ongoing	**5**	Four in the fishermen study and one in organotin study
Opponent of dissertations	**5**	Once in Sweden, others in Finland

5.4. Social impact

5.4.1. National

a) Municipal level

POPs and especially dioxins and chlorophenols are problem for many municipalities. In Finland, about 23 400 000 kg chlorophenols (Ky-5) containing about 1500 kg PCDD/Fs or 20 kg dioxin as TEq has been produced. About 250 saw mills have used chlorophenols in large scale. Soils have sometimes been cleaned from chlorophenols but dioxins are present or have been carried out to dumping areas. Contaminated former saw mills areas have been taken for permanent settlements or new settlements are planned to those areas.

We have given tens of statements concerning dioxin/chlorophenol problems. In most cases, we visit several times the place and sometimes also measure contaminant concentrations. Sometimes we measure the exposure of inhabitants e.g. take blood samples for chemical analyses. We also follow the impact of whole cleaning process and also the decrease in exposure of the target population. We discuss with the local inhabitants in organized meetings, give advice and follow the whole procedure. Such large and labour intense cases have been in Oulu, Lappeenranta, Joensuu and Jyväskylä cities.

b) Other national impacts

The burning of wastes almost stopped in Finland in 1986 when Kyläsaari waste incineration plant was pulled down. We have studied dioxin, PCB and other harmful missions of incineration plants in many projects, also in full scale plants. The Ministry of Social Affairs and Health has based their statements to Finnish Parliament on our knowledge.

Co-operation with other research institutes in matters regarding POP problems
The other part of dioxin contamination is the river Kymijoki. Ky-5 was synthesized in the former Kymi- Kymmene, nowadays UPM-Kymmene, and wastes of the process can be found by the river Kymijoki. Together with Finnish Environment Institute (SYKE), we have worked out dioxin problem by the river. This includes possible cleaning of the river, exposure of population and epidemiological studies, and many other aspects by the river. We have given several reports or commentaries from this case for Finnish parliament.

Co-operation with national authorities in matters regarding EU legislation
National authorities, the former National Food Agency, currently Finnish Food Safety Authority, Ministry of Trade and Industry, and Ministry of Agriculture and Forestry have used the expertise of the Laboratory of Chemistry as advisor in matters concerning EU legislation of POP contamination, regarding especially PCDD/Fs, PCBs, and PBDEs. One example of such an action is our participation as invited expert into one of the Scientific Co-operation projects: SCOOP Task 3.2.5, “Assessment of dietary intake of dioxins and related PCBs by the population of EU member states”.

Leader of this group has been involved in several scientific positions in Finland, e.g. chair of two councils of the Academy of Finland during 6 years and member of Sciences and Technology Policy Council of Finland chaired by Prime Ministry. She also is chair and/or member of several advisory boards in Research Institutes and High Schools.

5.4.2. International impact

As shown in Table 2, our group researchers have been members of several EU groups, mainly dealing with harmonization of analytical procedures or sampling. We gave data for EU SCALE: Integrated Monitoring of dioxins & PCBs in the Baltic Region.

WHO mother milk studies
Much effort has taken the WHO's international studies of human milk on the levels and trends of dioxins and PCBs in human milk in 1987, 1992, and 2000. We have performed all those three rounds collecting mother milk samples from two locations, in Helsinki and Kuopio. In 2005 WHO announced the initiation of the 4th round of surveys of human milk for Persistent Organic Pollutants (POPs). Again we participate in this study and the collection of mother milks in currently on going in three towns in Finland, in Helsinki, Kuopio, and Rovaniemi.

We also have been involved in WHO Environmental Health Indicator program.

5.5. Research funding

Table 2. Project funding during 1996-present

Project name	Funding agency*	Role	KTL funding k €	Years	Partners
Budapest	STM		70	2005-2007	
Eden	EU	Partner	527	2002-2006	From 15 countries
Expored	EU	Partner	631	2001-2005	From 3 countries
Dapaff	EU	Partner	110	2001-2002	
Inlakes fishes: POPs	EVI		227	2001-2003	
Organotin in environment and humans	SA		393	2005-2008	
Fisherman	SA		361	2001-2003	
Fisherman2	SA		70	2004	
Contaminated soils	SA		179	1996-1998	
Sarcoma; exposure	SA		107	1997-1999	
Dioxins and PCBs, risk for humans	SA		249	1998-2000	
Bottom sediments;River Kymijoki	STM		126	1998-2001	
NFA/Dioxins	NFA		54	2005	
Remediation of river Kymijoki	SYKE		52	2006-2007	
Research in SYKE	SYKE		174	2002-2008	
PCDD/F-research	SYKE		394	1990-2001	
Dioxins in sediment	YM		76	1998-1999	
Dioxmode	SA		106	2003-2006	
Mechanisms of cancer of dioxins	SA		87	1998-2001	
Organotin/EVIRA	EVI		54	2005-2008	
Fishermen study	SA		145	2005-2007	
SA post	SA		311	2005-2010	
Below 50 k€			537		
Total			**5156**		

EU=European Union, EVI= National Food Agency, NFA=National Food Agency, Sweden, SA=Academy of Finland, STM= Ministry of Social Affair and Health, SYKE= Finnish Environment Institute, YM=Ministry of Environment

5.6. Scientific collaboration

5.6.1. National collaboration

The national co-operation has been wide involving several research institutes and Universities in Finland. Here is a list of the most frequent partners:

Finnish Food Safety Authority (EVIRA), former National Food Agency (EVI) With EVI/EVIRA our co-operation has long traditions starting in 1980s by analysing food samples and assessing the Finnish intake of PCDD/Fs. Since then the intake studies have been expanded to cover PCBs. The development of the legislation in EU, covering PCDD/Fs and PCBs in feed and food, has resulted that nowadays we analyze the national monitory samples of feed and foodstuffs for PCDD/Fs and PCBs, and also for PBDEs.

Finnish Environment Institute (SYKE) With SYKE we have a long lasting co-operation involving POP analytics in contaminated soils, sediments as well as in biota. The sediments of river Kymijoki have been the major concern in Finland

Finnish Game and Fisheries Research Institute (RKTL) In those fish contaminant studies together with KTL and EVIRA the RKTL has had a crucial role in sampling of fish material.

Universities of Joensuu, Turku, and Jyväskylä and the Finnish Institute of Marine Research we have studied the contamination of bottom sediments in all major sea areas around Finland.

University of Helsinki, With the Institute of Dentistry in the University of Helsinki we have had a long lasting very fruitful co-operation. This co-operation has demonstrated that dioxins are associated with dental defects of the first permanent molars.

5.6.2. International collaboration

The international activity and co-operation can be divided in three major areas (not in priority order):

1. Co-operation with Nordic countries in projects funded by NMR
2. Co-operation with European countries in projects funded by EU
3. Individual research projects with foreign partners

Co-operation with Nordic countries in NMR-projects
Laboratory of chemistry is actively participating in Nordic Council of Ministers projects with other Nordic countries in projects:

- assessing risks of PCDD/Fs and PCBs
- collecting data of contaminants in different matrices
- harmonising sampling and analysis methods
- evaluating future consequences of EU legislation in Nordic perspective
- trying to find a common Nordic view of sampling and analysis of different contaminants aiming ultimately to impact EU legislation

Co-operation with European countries in EU-projects
Since accession of Finland into EU the laboratory of chemistry has participated in several EU-funded projects.

In "Dioxin risk assessment"-project (QLK4-CT-1999-01446) our task was to provide exposure data of PCDD/Fs and PCBs to risk assessors.

In EXPORED (QLK4-CT-2001-00269) project we surveyed exposure-outcome relationships in male urogenital malformations (hypospadias and cryptorchidism) together with Finnish, Danish, and German partners. Our task was to analyse PCDD/F, PCB, and PBDE occurrence in placenta and mother milk samples collected by hospitals in Turku and Copenhagen.

In another EU-project, EDEN (QLRT-2001-00603), we continue analysing those EXPORED subjects but now the matrice is human tissue from those boys operated for chyptorchidism. Other samples in EDEN were fat samples from southern Spain women and fish tissue samples from the Netherlands. Our aim was to demonstrate the occurrence of potential endocrine disrupters in a variety of environmental samples.

Quite different EU-project from the above mentioned projects was the DAPAFF-project (Q5CR-2000-70418) in which the purpose was to measure the efficiency of transfer of dioxins and DL-PCBs from fish feed to edible flesh of salmon. In addition to those contaminants we measured the bioaccumulation potency of PBDE congeners into salmon. In this project we had partners form Norway and UK.

The very recent and just initiated EU –project in which the laboratory of chemistry is participating is the BENERIS project. It deals with risk benefit analysis of eating fish among other things. In BENERIS our main co-laboratories are from Denmark, Ireland and the Netherlands.

Individual research projects with foreign partners
In the fishermen study we have had fruitful cooperation with the University of Lund, prof Lars Hagmar's group. Lars died of cancer on June, 2006, but we will continue with his group; concentrations of POPs in fishermen in Sweden and Finland, cancer epidemiology, mortality epidemiology etc. We also have several single research projects with different partners in Spain, Sweden, and Estonia. These projects have included analyses of mother milk and foodstuffs.

5.7. Proposal for the future work and expected (social) benefits (next 5-10 years)

Exposure assessment has been the main focus in this project. To do that well, we must have good analytical facilities and high quality, e.g. the analytical laboratory and relevant analyses must be accredited. This has demanded much resource and we will continue this way. Until now, dioxins, planar PCBs and PBDEs have been the most important substances to analyze from human. Now we will expand to other and newer pollutants.

Research of PCDD/Fs and DL-PCBs continues within the Beneris-project, where POP exposure of fetus is estimated with the measurement of placenta samples. Existing contaminant data in food will also be used to estimate the personal exposure inside the Finnish population. WHO has mandated fourth monitoring round of mother milks, whereby 400 milk samples will be analyzed for PCDD/Fs and DL-PCBs. Chemistry laboratory was recently nominated as the

national reference laboratory for PCDD/Fs and DL-PCBs in food and feed. Thus the measurement of PCDD/Fs and DL-PCBs from food samples within EU monitoring and analytical services for food and feed samples also continues.

Method development with LC-MSMS will be conducted to expand the list of persistent organic contaminants. A very demanding development work is underway for methylmercury. It has typically been measured with gas chromatography in combination with highly specific detectors like inductively coupled plasma mass spectrometry or cold vapor atomic fluorescence spectrometry. No practical method has been published for methylmercury with a universal analytical tool like LC-MSMS. Other technically more straight forward contaminants to be analyzed with LC-MSMS are different perfluorinated compounds (perfluorooctanesulfonate and perfluorooctanoate) and brominated flame retardants (tetrabromobisphonol-A, and different isomers of hexabromocyclododecane). One of the key applications of these methods will be to study the exposure and possible health effects of the subpopulation of Finnish fishermen to these contaminants. Also the exposure of general population to these compounds will be studied.

Another future use of LC-MSMS techniques will be in the identification and characterization of biologically active molecules. One field of application will be molecules that have different health effects for people living in the moisture damaged buildings. Another application is to find possible indicator molecules associated with different pathogens in drinking water.

In the fishermen study, during the next five years we will focus on reporting the results of the Hazardous and beneficial components in fish – study as scientific articles. Two doctoral dissertations on the topics of Beneficial and hazardous components in fish and their effects on cardiovascular health and Dietary characteristics of fish intake in fishermen and general population have been started, and a third on the relations of dioxins, PBDE:s and organotin compounds and serum biomarkers of health status is under consideration. Postdoctoral researchers will be reporting on the levels of pollutants in fishermen and their wives, and additional papers are planned on effects on cancer and subjective health and wellbeing.

We also aim to connect exposure analyses to several, for us new diseases, as diabetes and other endocrine diseases. We do that in our fishermen's study but we have started to do that also with other groups from KTL, University of Helsinki (IDEFIX study) and University of Kuopio.

5.8. Personnel

From 1996 onward our research group has been 20-25 employees: head of the laboratory, two senior researchers, two to three chemists, 2-4 students and about 10 technical assistants and the secretary. Six persons, head of laboratory, secretary, and 4 technical assistants, get funding from the government and the rest, about 15 persons get funding from external sources, the Academy of Finland, EU, ministries, TEKES etc.

The key senior researchers:
Prof. Terttu Vartiainen (exposure)
Doc. Pia Verkasalo (epidemiology)
Doc. Jaana Koistinen (POP analyses)
Ph.D. Hannu Kiviranta (chemistry, epidemiology)
Ph.D. Panu Rantakokko (water chemistry, organotin, POPs)
Ph.D. Päivi Ruokojärvi (quality control)

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Appendix 2

Laboratory of Chemistry has almost 20 years experience in analyses of polychlorinated biphenyls (PCBs) and dioxins (polychlorinated dibenzofurans, PCDFs, and dibenzo-*p*-dioxins, PCDDs), and also several years of experience in analyses of polybrominated diphenyl ethers (PBDE).

Laboratory has successfully participated in international quality control studies of PCBs and dioxins since 1992 and later also of polybrominated diphenyl ethers (see Table below). Laboratory was accredited in 1996 with No T077 in Finland (SFS-EN ISO/IEC 17025).Besides dioxins, PCBs and PBDEs, the scope of accreditation covers nowadays also organotin compounds, halogenated acetic acids and chemical testing of some water parameters (total organic carbon, anions and bromate). The scope of accreditation for persistent organic compounds (POPs) is flexible i.e. different matrix (in addition to present human and animal samples, soil, sediment etc) can be included in the scope of accreditation after validation. Laboratory fulfills the strict requirements of European community in analysis of dioxins and dioxin-like PCBs in food and feed (directives 2005/7/EC, 2002/70/EC, 2002/69/EC), and is the national reference laboratory in Finland for dioxins in food.

Table: Participation of Laboratory of Chemistry in POP interlaboratory studies in the past five years

Intercalibration	Year	Number of participants	Matrix of sample (analytes)
FOOD 2001 (Norwegian Institute for Public Health)	2001	55	Beef, breast milk, cod liver (PCDD/Fs, PCBs)
FOOD 2002	2002	46	Egg, tuna fish, pork meat (PCDD/Fs, PCBs)
Umeå, 7th round of the international intercalibration study	2002	86	Fish, ash, soil/sediment (PCDD/Fs, PCB)
LGC: International study on ISO/DIS 18073 water quality -determination of PCDD/FS	2002	15	Water (PCDD/Fs)
FOOD 2003	2003	77	Turkey, salmon, cheese (PCDD/Fs, PCBs)
FIRE 2003	2003	11	Egg, fish, turkey, human milk (PBDEs)
CIL 2003	2003	53	Fish (PBDEs, DDT)
Difference 2004	2004	15	Herring, pork, milk, fish oil, feed (PCDD/Fs, PCBs)
Örebro 9th round of the intercalibration study	2004	103	Soil, ash (PCDD/Fs, PCBs)
Proficiency test 2	2004	11	Fish oil (PCDD/Fs, PCBs)
FOOD 2004	2004	73	Chicken, salmon, palm oil (PCDD/Fs, PCBs, PBDEs)
Örebro 10th round of the intercalibration study	2005	101	Sediment, river clay (PCDD/Fs, PCBs)
FOOD 2005	2005	87	Herring, cod liver oil, reindeer (PCDD/Fs, PCBs, PBDEs)
FOOD 2006, WHO 2006, Örebro 10th round of the intercalibration study	2006	Results yet to come	Breastmilk, egg, halibut filet, oil, sediment, sludge, river clay (PCDD/Fs, PCBs, PBDEs, DDTs)

6 PERSISTENT ORGANIC POLLUTANTS: MECHANISMS OF HEALTH EFFECTS

6.1. Research area and its significance

Studies on toxic effects and mechanisms of persistent organic pollutants (POPs) were initiated in 1984, and for the first ten years the main objective was to clarify the mechanisms of acute toxicity and the wasting syndrome caused by the dioxin model compound TCDD. During the whole research program the main interest has been focused on polychlorinated dibenzo-*p*-dioxins and furans (PCDD/Fs), the most potent and the most relevant group of POPs from the Finnish point of view. These studies were undertaken, because (1) significant sources of exposure, such as Baltic fish and old contaminated sawmill areas, had been identified in Finland, (2) these compounds are highly potent, persistent and accumulative, (3) there are wide inter- and intraspecies differences in sensitivity to toxic effects of these compounds, and (4) there are huge differences in limit values set by the authorities in different countries, mainly because of lack of consensus within the scientific community about the most significant and most relevant toxic effects and their underlying mechanisms. Therefore the health risks to the Finnish population were potentially important but highly uncertain. These facts emphasize the potential toxicological significance of PCDD/Fs from the public health point of view on one hand, and the shortage of knowledge that would enable accurate risk assessment on the other hand.

Due to the exceptionally high mortality from cardiovascular diseases in Finland the societal need for improved risk assessment culminates in the question of using fish as a part of healthy diet. The National Public Health Institute has been successful in reducing cardiovascular diseases mainly due to dietary recommendations that include increased consumption of fish and vegetables. Therefore, it is important that dietary recommendations are in agreement with scientifically valid and accurate tolerable daily intake values for PCDD/Fs. Another need for improved risk assessment stems from the increasing demand to use contaminated sawmill areas for housing.

The goal of this research is to improve the accuracy of risk assessment of PCDD/Fs by (1) identifying the key toxic effects of PCDD/Fs, (2) clarifying their mechanisms, and (3) defining the molecular basis and significance of sensitivity differences among different species and individuals.

6.2. The main achievements

Our research on mechanism of dioxin action has largely utilized the animal model discovered by Raimo Pohjanvirta and Jouko Tuomisto during the first years of this area of research. The model is based on >1000-fold sensitivity difference between exceptionally TCDD resistant Han/Wistar (H/W) rats and TCDD sensitive Long-Evans (L-E) rats in acute lethality and many other, but not all toxic effects of TCDD. This model has proved to be highly useful for studying the genetic basis of sensitivity differences and mechanisms of various dioxin-induced toxic effects.

Aryl hydrocarbon receptor (AHR) structure and dioxin sensitivity

Most dioxin effects have long been known to be transmitted through a specific "dioxin receptor", AHR. The exceptionally wide interstrain difference between L-E and H/W rats in sensitivity to the acute lethality of TCDD was initially perplexing because our early studies had demonstrated that both strains responded in a practically identical manner to the best-established AHR-mediated impact of TCDD, induction of certain enzymes metabolising foreign compounds. Furthermore, a number of other biochemical and even toxic responses (e.g. atrophy of thymus gland) to TCDD were manifested normally in H/W rats. However, two facts pointed to a critical involvement of the AHR in the strain difference: 1) The divergence was specific to dioxins, and 2) among dioxins, it correlated with affinity to the AHR. In mid-1990's, a specific antibody to rat AHR became available, enabling a specific detection of the receptor (Western blot). The analyses revealed that the AHR of the TCDD-resistant rat strain, H/W, is smaller than that of other strains. Yet it behaved normally in forming a protein complex binding to respective DNA, a step necessary to initiate expression of AHR-regulated genes. These features prompted us to clone and sequence the AHRs from H/W and L-E rats. We discovered that there was an important single nucleotide mutation in H/W AHR gene. It resulted in generation of 3 different mRNAs, subsequently resulting in two AHR protein species, both truncated at the C-terminus within the transactivation domain with either 43 (deletion variant) or 38 (insertion variant) amino acids missing (and 7 novel amino acids introduced at the C-terminus in the latter case). This part of the protein molecule is especially important for interactions with other proteins, and together with them in initiating the reading of genes regulated by AHR. Hence we had found a tool to explain, why different genes might respond differently in H/W rats from other strains, and also a tool for explaining why TCDD does what it does.

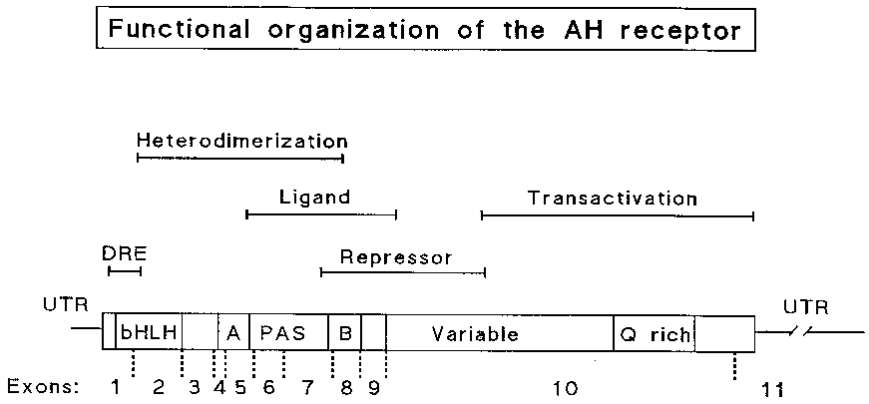

Figure 4. Simplified diagram of the functionally salient structural elements of the AHR

Subsequent genetic experiments confirmed that the mutated AHR was by far the most important reason for the exceptional TCDD resistance of H/W rats, but there was a contributing modifier gene (gene "*B*") whose identity and function are unknown as yet. Extensive long-term breeding studies succeeded in segregating these two resistance genes into new rat lines (see below), A (H/W-type *AHR*, wild-type *B*), B (wild-type *AHR*, H/W-type *B*) and C (wild-type *AHR* and *B*; sensitivity similar to that of L-E rats). It was further demonstrated that in crosses between L-E and H/W rats, TCDD resistance was inherited as a dominant trait.

To extend these findings beyond the rat model, we next cloned the AHR from the most TCDD-susceptible species, guinea pig, and from the most TCDD-resistant species, hamster. Hamster resembles the H/W rat in its selective responsiveness to the wide range of adaptive and toxic changes elicited by TCDD. Therefore, it was rewarding to find out that this similarity held also in the case of the mediator molecule. As in H/W rats, the transactivation domain of the hamster AHR was substantially restructured. However, in hamster AHR the underlying reason was not a splicing error but rather an incorporation of short glutamine-coding DNA repeats in exon 10 at the glutamine (Q)-rich region (see Figure 4). This subdomain is functionally essential to the receptor and thus it is conceivable that its remarkable expansion accounts for the TCDD resistance of this species.

In TCDD-sensitive guinea pigs, the C-terminal Q-rich region of the AHR was only about half of that in hamsters. Interestingly, the closest homolog of the guinea pig receptor turned out to be the human AHR suggesting that the human AHR is fully capable of mediating major toxic impacts of dioxins.

In an attempt to elucidate the unknown gene B of H/W rats (see above), we cloned and sequenced (and measured mRNA expression levels of) several other proteins related to AHR. Although these studies disclosed important novel features of these factors, gene B remained elusive.

The pivotal role of the AHR in TCDD toxicity as well as its wide-scale involvement in physiological processes became obvious by our microarray and qRT-PCR analyses of liver gene expression changes upon TCDD exposure in AHR wild-type (C57Bl/6J) and AHR-deficient (AHRKO) mice. While TCDD influenced expression of over 400 genes in the wild-type animals, only a handful of genes responded to it in AHRKO mice. Importantly, almost as high a number of genes (392) were affected by AHR alone in the absence of ligand suggesting multiple functions of the AHR in normal development.

Recently, L-E and H/W rats were utilized in a study comparing the effects of TCDD on liver gene expression by DNA microarray and proteomics approaches. We were able to show that most of the changes seen at the protein level were also found in microarrays, which suggests that TCDD modulates the functions of these genes. A subset of hepatic proteins were differentially regulated between the two strains by TCDD (e.g. oxidative stress-related proteins) suggesting that they may reside along the pathways crucial to TCDD toxicity.

Even though both the rat strain (L-E vs. H/W) and line (A, B and C) models have proved effective tools in mechanistic studies on TCDD toxicity, a major drawback of them is a formidable background variation in constitutive gene expression levels. This problem prompted us to develop a knock-in mouse model. To this end, we transferred each of the 3 rat AHR variants (wild-type, deletion and insertion) to C57Bl/6J mouse pronuclei by microinjection, selected construct-positive animals by genotyping, and eliminated the innate mouse AHR by breeding the mice with AHRKO animals. At present we have a breeding colony of mice with rat receptors of all 3 lines. The first pilot experiment recently conducted with them showed that the AHRs are fully functional and suggested that it is the insertion variant that is responsible for the exceptional resistance to TCDD of H/W rats.

Development of rat lines A, B and C

Our previous studies indicated that the exceptional resistance of H/W rats is based on two alleles, the mutated $Ahr^{hw/hw}$ allele, and to a lesser extent, the allele $B^{hw/hw}$ that has not yet been identified. Using classical cross-breeding and selection based on AHR structure and resistance

to TCDD lethality these H/W rat resistance alleles were further segregated into different rat lines each harbouring the resistant allele from only one of these genes. Of the new rat lines A, B and C, line A has the mutated *Ahr* allele and the wild-type *B* allele (genotype $Ahr^{hw/hw}B^{wt/wt}$). Line B rats lack the resistant *Ahr* allele, but they are homozygous for the resistant *B* allele (genotype $Ahr^{wt/wt}B^{hw/hw}$), and line C rats lack the both resistance alleles (genotype $Ahr^{wt/wt}B^{wt/wt}$). These rat lines exhibit highly different LD50 values for TCDD: >10 000, 830 and 40 μg/kg in males, respectively, and >2 000, 410 and 19 μg/kg in females, respectively. Experiments on neonatal A and B rats indicated that the resistance of line A rats to a huge dose of TCDD (1000 μg/kg) develops immediately after birth, between postnatal days (PND) 2-5. In contrast, the resistance of B rats develops much later, between PND 14-28. Therefore, the $Ahr^{wt/wt}$ allele accounts for the high resistance to lethality already during early neonatal life, but the $B^{wt/wt}$ allele results in moderate resistance that develops by weaning.

In subsequent studies these rat lines were used to study the effects of TCDD on dose-responses of different short-term endpoints of dioxin toxicity. In addition, the rat lines were used to study the effects of TCDD on the development of teeth, bone and the male reproductive system, and to evaluate the roles of the resistance alleles in sensitivity to these low dose developmental endpoints. Because the safety margin of harmful effects of dioxins is still very narrow, it is highly important to understand the sensitivity to each toxic effect separately to be able to protect the population.

Characterization of Dioxin type I and II effects for short-term endpoints

Endpoint dependence of TCDD resistance was further studied by determining dose-responses for several characteristic short-term effects in H/W and L-E rats as well as in line A, B and C rats. These studies indicated that the endpoints can be divided into two categories. Type I endpoints show similar sensitivity in these rat strains and lines, i.e. they are independent on the genotype variation. These endpoints include CYP1A1 induction, thymus atrophy and incisor tooth defects. On the other hand, for type II endpoints the efficacy (magnitude of effects) is suppressed in rat lines with the resistance alleles, while the potency for these effects is less affected. The $Ahr^{hw/hw}$ allele is the most important factor, but the $B^{hw/hw}$ allele has only a minor influence on the efficacies of most endpoints. Type II endpoints include weight loss, increased serum transaminase (ASAT) activity, increased serum bilirubin and free fatty acid concentrations, and mortality. Decreased efficacy of effects mediated via the mutated AHR suggests that genes responsible for effects are not fully functional. It seems evident that the mechanistic differences leading to type I and type II effects are localized to the AHR transactivation domain and to the machinery responsible for transcription activation.

Tumour promotion and carcinogenicity: experimental studies, mechanisms and epidemiology

Experimental studies and mechanisms

IARC has classified TCDD as a human carcinogen (group 1), and induction of liver tumours by TCDD in rats was used as the basis for PCDD/F risk assessment for a long time, until the end of the 1990's. Because the significance of species and strain differences in sensitivity to tumourigenic endpoints was not known we carried out a liver tumour promotion study using an initiation / promotion protocol for comparing the sensitivity of H/W and L-E rats. A remarkable sensitivity difference of about 100-fold in induction of AHF by TCDD was found between L-E and H/W rats. Interestingly, dose-responses for hepatotoxicity were identical with those of AHF induction in each strain exhibiting the same 100-fold sensitivity difference. This suggests that the induction of liver tumours is related to hepatotoxicity, but not to

CYP1A1 induction and thymic atrophy that were equally sensitive in both strains. The results imply that genetic differences may account for significant interindividual / intraspecies sensitivity differences in dioxin-induced carcinogenesis. The exceptional resistance of H/W rats is associated with an altered AHR transactivation domain. If hepatotoxicity is the cause of tumour formation, there is all reason to expect a practical threshold for carcinogenic dose. This would mean change in the risk assessment as to carcinogenicity.

Using a combination of *in vivo* and *in vitro* studies we studied further the effects of TCDD on the tumour suppressor gene p53 and its downstream targets. Pretreatment with a low dose / concentration of TCDD attenuated the p53 response to genotoxic and cytotoxic agents and decreased the amount of apoptotic cells in the liver. No clear sensitivity differences between H/W and L-E rats were observed, but studies in AHR-knockout mice and their wild-type littermates indicated that this response is AHR-mediated. Further studies revealed important aspects on the mechanism of action. Altogether, these studies indicate that TCDD attenuates the net response in downstream targets of p53 and thereby potentially impairs cellular adaptation to DNA damage. These changes are likely to play a role in TCDD-induced toxicity and carcinogenesis.

Epidemiology

In 1996, we started a joint effort with the unit of epidemiology to characterize the carcinogenic potential of dioxins at the general population exposure levels. This was seen important, because the hypothesis of dioxin carcinogenicity in humans was based on two sources of information: a conclusive body of evidence showing that dioxins (notably TCDD) are animal carcinogens, and a few epidemiological studies that were based on high exposures after occupational or accidental incidents (e.g. Seveso accident). It was therefore not clear whether dioxins would be associated with carcinogenicity at low background levels in humans.

To study this question, a case-control study was launched. Soft tissue sarcoma was selected as the endpoint of interest, because that had been previously associated with dioxin exposure. We were able to collect the majority of all soft tissue sarcoma patients in southern Finland during 1996-1998. Patients coming to appendicitis operation were used as controls, as a surgical procedure was needed to obtain the adipose tissue samples. The outcome was surprising, as there was a negative trend of sarcoma related to increased dioxin exposure. Although the effect was not statistically significant, this study gave evidence that the soft tissue sarcoma risk is not increased due to general background levels of dioxin, and therefore a non-linear dose-response should be considered.

Developmental toxicity of dioxins

Studies carried out in early 1990's at Prof. Richard E. Peterson's laboratory had identified the male reproductive system as a highly sensitive target of dioxin toxicity. These novel findings questioned the use of liver tumour promotion as the basis for dioxin risk assessment. Sensitivity and irreversibility of developmental defect together with potentially high perinatal PCDD/F exposure emphasize the significance of developmental defects in general from the risk assessment and risk management points of view. We initiated our developmental toxicity studies in order to investigate the role of dioxin resistance alleles in sensitivity to the male reproductive system defects. At the same time we examined two completely new developmental endpoints, teeth and bone (for details, see below). Overall, these studies indicated that tooth development and bone development – together with male reproductive system development – belong to the highly sensitive developmental endpoints of PCDD/Fs. Based on our findings, tooth development is the most sensitive and the most relevant of these endpoints as it has been observed also in humans. Moreover, unlike liver tumour promotion and several high dose

endpoints, sensitivity to most defects of tooth and the male reproductive system development is not significantly modified by genetic factors (dioxin resistance alleles). However, the resistance alleles seem to decrease sensitivity to altered bone development.

Tooth development: epidemiology, experimental studies and mechanisms

Case reports about dental changes observed in human infants after accidental exposures to high doses of dioxin-like compounds led us to investigate the effects of PCDD/Fs on tooth development in children after prevailing background exposure via mother's milk (Alaluusua et al. 2000; more details in the research programme Persistent Organic Pollutants and Health: Analysis, Concentrations, Exposure and Epidemiology).

Based on these findings we initiated experimental studies to establish the biological plausibility of the developmental tooth defects and to determine dose-responses, the critical window of sensitivity and the role of the resistance alleles in sensitivity to these defects. Pregnant line A, B and C rats were given a single oral dose of TCDD (0.03-1.0 µg/kg) at different time points during pregnancy or lactation and tooth development studied in the offspring. TCDD at 1 µg/kg completely prevented the development of the lower third molars in 50-100% of line C and Sprague-Dawley rats, and in 5-6% of line A and B females. TCDD also dose-dependently delayed the eruption and diminished the size of the third molars in all rat lines. Therefore, impaired tooth development is one of the most sensitive endpoints of TCDD-induced toxicity. Mineral composition studies on enamel and dentin of third molars showed a slight dose-dependent decrease in enamel calcium weight percentage that was significant at the highest dose level. Toxicokinetic data indicate that the maternal lowest observable adverse effect level (LOAEL) 0.03 µg/kg is likely to result in a maternal lipid based tissue concentration that is very similar with the mother's milk PCDD/F concentrations associated with molar mineralization defects. The resistance alleles $Ahr^{hw/hw}$ and $B^{hw/hw}$ slightly decrease the sensitivity to arrested third molar development, but they have no influence on the decrease in size.

Time-course studies indicated that the critical window of sensitivity for the third molar development is during early morphogenesis, from tooth initiation (gestation day 20) to the early bud stage (postnatal day 0), and that the primary target is the dental epithelium. *In situ* hybridization and immunohistochemical studies confirmed that the basic elements of dioxin signalling are expressed in dental epithelium (but not in mesenchyme) during the bud stage, but not thereafter. Further *in vitro* studies clarified the roles of growth factors, apoptosis and cell proliferation in the process.

We also studied the influence of perinatal TCDD exposure on dental caries susceptibility and mineral composition of tooth enamel in rats. At weaning the offspring were challenged with cariogenic treatment including sugar-rich diet and inoculations with cariogenic *Streptococcus mutans* bacteria. TCDD treatment dose-dependently increased cariogenic lesions in the enamel starting at the lowest maternal dose used, 0.03 µg/kg, and at the highest maternal dose (1 µg/kg) the lesions extended through the enamel to dentin more frequently. Thus, perinatal TCDD exposure renders rat molars more susceptible to caries.

Overall, the experimental studies showed that the molar development is a previously uncharacterized and highly sensitive target of dioxin toxicity. Toxicological significance of this finding is emphasized by the developmental similarity of human teeth with rodent molars, and the results strengthen the biological plausibility of the PCDD/F-associated molar defects observed in children. Moreover, developmental tooth defects are excellent biomarkers of dioxin exposure, because teeth, unlike bone, do not undergo remodelling, and therefore the defects are

permanent. It is unlikely that genetic differences would play a significant role in sensitivity to PCDD/F-induced dental defects.

Further evidence about the role of PCDD/Fs in dental aberrations was obtained recently in studies carried out on the Seveso population exposed to TCDD during childhood in a chemical accident 25 years earlier. Prevalence of developmental enamel defects as well as missing permanent teeth was higher in the exposed population than in controls, and the prevalence was significantly correlated with serum TCDD levels. The study indicated that developmental dental aberrations, which are permanent in nature, were related to childhood exposure to TCDD. This supports our previous findings that dioxins can interfere with human organogenesis, and therefore tooth development is a superior endpoint for human risk assessment.

Bone development

Our observations on dental effects of PCDD/Fs led us to initiate experimental studies on effects of TCDD on bones and bone development. We analysed long bones of rats exposed to TCDD as adults or *in utero* / lactationally using peripheral quantitative computed tomography (pQCT), three-point bending test and histomorphometry. These studies showed for the first time that TCDD treatment impairs bone quality and bone modelling at relatively low dose levels. The changes included dose-dependent decreases in mechanical strength as well as altered geometry and bone mineral density. LOAEL for bone effects was 1.7 µg/kg (total dose) in adult animals, and L-E rats were more sensitive than H/W rats (type II effect). After *in utero* / lactational exposure the changes in offspring were observed at the maternal dose level of 1 µg/kg, which indicates that bone development is also a relatively sensitive developmental endpoint. Most of the changes observed in the offspring were recovered at the age of 1 year. The resistance alleles $Ahr^{hw/hw}$ and $B^{hw/hw}$ decrease clearly the sensitivity to the bone effects, and therefore genetic differences in general may have some influence on sensitivity to these effects.

In subsequent studies we analysed the possible role of steroid hormones and retinoid status in dioxin-induced bone defects, alterations in gene expression as well as the effects of TCDD on cultured bone cells and differentiation of bone marrow stem cells to osteoblasts and osteoclasts *in vitro*. These studies are ongoing, and the results obtained so far suggest that the bone changes are not associated with steroid hormone levels. On the other hand, retinoid metabolism is dose-dependently altered already at the lowest dose-levels and during early time points, and therefore altered retinoid metabolism could be causally related to the bone changes. Quantitative RT-PCR analysis on humerus collected on PND 7, 35 and 70 revealed no significant changes in the expression levels of osteocalcin, runt-related transcription factor 2 (RUNX2) or estrogen receptor beta (ERβ). Using immunohistochemistry and RT-PCR we showed that AHR and ARNT are present in bone forming osteoblasts and bone resorbing osteoclasts. In subsequent studies we showed that exposure of mature osteoclasts to TCDD *in vitro* does not influence their bone resorbing activity. However, differentiation of bone marrow stem cells to osteoclasts is highly sensitive to TCDD as low picomolar concentrations impair the differentiation as indicated by the decreased amount of active osteoclasts and decreased area of resorption pits. Similarly, low concentrations of TCDD impair the differentiation of bone marrow stromal stem cells to osteoblasts by inhibiting the expression of differentiation markers characteristic for the main stages of osteoblast differentiation (RUNX2, alkaline phosphatase and osteocalcin) without affecting the proliferation of the cells. We are currently carrying out a proteomic analysis on the osteoblasts at different stages of development as well as on the bones and livers of the rat offspring exposed to TCDD *in utero* / lactationally. Preliminary data on TCDD-treated osteoblasts showed a strong induction of calcium/phospholipid binding protein, up-regulation of proteins involved in mitochondrial energy pathways and decreased expression of cytoskeletal proteins.

Development of the male reproductive system

Effects of *in utero* / lactational exposure of A, B and C line rats to low doses of TCDD on male reproduction parameters were assessed in the offspring at different ages. Serum testosterone concentration, male sex organ weights, as well as testicular and cauda epididymal sperm numbers were analyzed. Decreased sperm numbers was the most sensitive endpoint, and the most consistent and significant effects were observed in the weight of prostate lobes. The growth of the male reproductive organs was not affected by the resistance alleles Ahr^{hw} and B^{hw}, but the effects on sperm parameters were slightly modified by the resistance alleles. Thus the intraspecies genetic differences in C-terminal transactivation domain of AHR appear to modify the sensitivity to only certain dioxin-induced male reproductive effects.

Accumulation of biliverdin and hepatic peliosis – a new type of dioxin-induced liver toxicity

During cross-breeding of H/W and L-E rats a new type of liver toxicity characterized by dark green or black pigment and swollen livers was found. This "black liver syndrome" was seen most frequently in intermediately resistant rats with at least one resistance allele ($Ahr^{hw/hw}$ or $B^{hw/hw}$) after a large dose (≥300 μg/kg) of TCDD and a follow-up period more than three weeks. It is thus a type II dioxin effect. Bilirubin metabolism was studied as a potential source of the pigment. The main source of bilirubin is the heme from degrading erythrocytes. TCDD had no influence on the life span of erythrocytes in C rats, which also show large increases in serum bilirubin. The pigment fractions were separated by thin layer chromatography and then analyzed by HPLC and electrospray mass spectrometry. The pigment was found out to consist of biliverdin and several biliverdin-related compounds. In liver histopathology, progressive sinusoidal distension and hepatic peliosis with membrane-bound cysts were seen. The primary biochemical alteration leading to this syndrome remains to be elucidated.

Regulation of feed intake

One of the most striking behavioural changes emerging in TCDD-exposed animals is a severely diminished feed consumption leading to a dramatic (up to 60%) loss of body weight and emaciation; this has been dubbed the wasting syndrome. Surprisingly little is known about its pathogenesis. Therefore, we undertook a venture to analyze the effects of TCDD on hypothalamic mRNA levels of a large number (ca. 20) of hypothalamic factors critically involved in the regulation of food intake and body weight using TCDD-sensitive L-E and TCDD-resistant H/W rats. The analysis was carried out by quantitative real-time (reverse transcription-)PCR, and the physiological satiety substance, leptin, was employed as a positive control. We found out that TCDD mainly modified expression of orexigenic (feeding stimulatory) factors causing an initial suppression followed by reversal to enhanced expression by 96 h. The latter was also seen in feed-restricted controls advocating its secondary nature as a compensatory reaction. The brief duration of the changes recorded does not lend strong support for a primacy of hypothalamic factors in the wasting syndrome, but on the other hand it is possible that strictly localized alterations were missed by examining the entire hypothalamus instead of individual nuclei.

Recently, we have commenced an analysis of the microstructure of feeding behaviour in TCDD-treated animals. To this end, we monitor control and TCDD-treated rats continuously for periods of 1 to 2 weeks using an automatic analyzer and dust-free precision-weight pellets that enable recording of feed intake at the accuracy of 45 mg. Our preliminary data indicate that TCDD exposure leads to reduced meal sizes and to a decelerated eating rate in conjunction with an increased number of feeding bouts. This suggests that TCDD does not impair hunger sensation but rather enhances the satiating potency of feed.

6.3. Scientific impact

Table 1. Summary of the scientific output during 1996-present

SCIENTIFIC OUTPUT	N	Comments
Peer-review papers in international journals	111	2006 not included
Invited lectures and chairmanships in international meetings	18	
Papers in domestic journals	28	2006 not included
Lectures in domestic meetings	226	
Editorial tasks in international journals	6	Pharmacological Reviews, Pharmacology & Toxicology, Toxicological Letters, Toxicology, Environmental Toxicology and Pharmacology
Referee tasks in international journals (number of journals)	25	
Committee memberships: domestic	20	Board member of the National Agency for Medicines; Permanent Expert Advisor of the National Authority for Medicolegal Affairs; Director of the Finnish Research Programme on Environmental Health (SYTTY); Working Group on the Environmental Impact of Veterinary Drugs (Ministry of Agriculture); Review Committee of Ethical Questions in Research, University of Kuopio 2000-2006; Institutional Ethics Review Board of KTL (chairman); Review Group on Chemical Substances (Ministry of Social Affairs and Health); Working Group for Collaboration of Governmental and Local Authorities in Environmental Health (Ministry of Social Affairs and Health); Permanent Advisory Group on Dioxins (Ministry of Social Affairs and Health); Permanent Expert Advisor of the National Agency of Medicines; Working Group for Development of Training in Toxicology, University of Kuopio; Working Group for the Internal Development of the KTL (chairman); Expert Advisory Committee on Toxicology (TOXAS), the National Product Control Agency for Welfare and Health, Ministry of Social Affairs and Health (member and scientific secretary); The Finnish Scientific Committee on Health Effects of Chemicals, Ministry of Social Affairs and Health; Permanent expert advisor of the Advisory Committee on Chemicals, Ministry of Social Affairs and Health; Novel Food Board, Ministry of Trade and Industry; Working group on the Coordination of Alternative Methods to Animal Experiments, Ministry of Agriculture and Forestry
Committee memberships: international (EU, WHO etc.)	13	Steering Committee of the European Science Foundation: Programme of Fellowships in Toxicology; Alternate member of the Scientific Advisory Forum of the European Food Safety Authority; Technical Working Group on Integrated monitoring of dioxins & PCBs in the Baltic Region (chairman), DG Environment SCALE-Program; EC representative in the EC/US workshop on dioxin risk assessment; Nordic Working Group on Food Toxicology and Risk Evaluation (NNT) ; Nordic Expert Group "WHO assessment of the health risks of dioxins:Re-evaluation of the tolerably daily intake (TDI)"; The external peer review panel on the U.S. Environmental Protection Agency dioxin reassessment documents "Integrated Summary and Risk Characterization for 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and related compounds" and "Toxicity Equivalency Factors (TEF) for Dioxin and Related Compounds"; Scientific Advisory Committee of the European Centre for the Validation of Alternative Methods (ESAC), Commission of the European Communities; Scientific Program Committee for the 10th International Congress of Toxicology; Scientific Committee on Health and Environmental Risks (SCHER) of the European Commission DG SANCO; Grant Reviewing Committee on Environmental Toxicology of the Swedish Research Council for Environment, Agricultural Sciences and Spatial Planning (FORMAS)
Doctoral dissertations	8	
Supervision of dissertations: ongoing	6	
Opponent of dissertations	1	

6.4. Social impact

6.4.1. National

a) Municipal –level impacts
We are regularly involved as invited speakers or advisers in various municipal and local seminars and courses dealing with environmental health, toxicology, risk assessment, POPs, management of polluted sites, spatial planning etc.

b) Other national impacts
We have been or are currently members of several national working groups and committees related to toxicology, environmental health and risk assessment. Most of these are ministerial advisory groups or boards, or related to research programs or other activities of the Academy of Finland. In addition, we are regular lecturers and members of education development at different Finnish universities and we serve regularly as reviewers, opponents or mentors of theses.

JoukoTuomisto has participated in the work of the following national committees or working groups:

- Board member of the National Agency for Medicines 1996-2000
- Permanent Expert Advisor of the National Authority for Medico legal Affairs 1996-2004
- Director of the Finnish Research Programme on Environmental Health (SYTTY), 1997-2002
- Working Group on the Environmental Impact of Veterinary Drugs (Ministry of Agriculture) 1998-1999
- Review Committee of Ethical Questions in Research, University of Kuopio 2000-2006
- Institutional Ethics Review Board of KTL, chairman 2001-2005
- Review Group on Chemical Substances (Ministry of Social Affairs and Health), deputy member 2001-2004
- Working Group for Collaboration of Governmental and Local Authorities in Environmental Health (Ministry of Social Affairs and Health), 2002-2004
- Permanent Advisory Group on Dioxins (Ministry of Social Affairs and Health) 2002-
- Permanent Expert Advisor of the National Agency of Medicines 1999-2004
- Working Group for Development of Training in Toxicology, University of Kuopio, 2004-2004
- Working Group for the Internal Development of the KTL, chairman 2005-2006

Raimo Pohjanvirta has participated in the work of the following national committees or working groups:

- Expert Advisory Committee on Toxicology (TOXAS), the National Product Control Agency for Welfare and Health, Ministry of Social Affairs and Health 1997-present
- EC representative in the EC/US workshop on dioxin risk assessment 1997
- Present Vice Member of the Board of Veterinary Pharmaceuticals for the National Agency for Medicines 1999-
- Member of the Nordic Working Group on Food Toxicology and Risk Evaluation 2000-2002
- Vice Member of the Executive Board of Kuopio University Centre for Environment, Health and Society (YTY-centre) 2001-2002
- Member of the Expert Group on Food Contaminants for the Food Consultative Board of the Ministry of Trade and Social Affairs 2001-present

Matti Viluksela has participated in the work of the following national committees or working groups:

- Expert Advisory Committee on Toxicology (TOXAS), the National Product Control Agency for Welfare and Health, Ministry of Social Affairs and Health (scientific secretary), 1995-
- The Finnish Scientific Committee on Health Effects of Chemicals, Ministry of Social Affairs and Health, 1998-2004
- Advisory Committee on Chemicals, Ministry of Social Affairs and Health (permanent expert advisor), 1999-
- Novel Food Board, Ministry of Trade and Industry, 2000-

- Working group on the Coordination of Alternative Methods to Animal Experiments, Ministry of Agriculture and Forestry (2001-2002)

6.4.2. International

JoukoTuomisto has participated in the work of the following international committees or working groups:

- Member of the Steering Committee of the European Science Foundation: Programme of Fellowships in Toxicology (1991 – 1997)
- Alternate member of the Scientific Advisory Forum of the European Food Safety Authority (2002-)
- Technical Working Group on Integrated monitoring of dioxins & PCBs in the Baltic Region, (chairman), DG Environment SCALE-Program 2003-2005

Raimo Pohjanvirta has participated in the work of the following international committees or working groups:

- EC representative in the EC/US workshop on dioxin risk assessment (1997)
- Member of the Nordic Expert Group "WHO assessment of the health risks of dioxins; Re-evaluation of the tolerably daily intake (TDI)" (1999)
- Member of the Nordic Working Group on Food Toxicology and Risk Evaluation (NNT) (2000-2002)

Matti Viluksela has participated in the work of the following international committees or working groups:

- Member of the Nordic Expert Group "WHO assessment of the health risks of dioxins; Re-evaluation of the tolerably daily intake (TDI)" (1999)
- Member of the external peer review panel on the U.S. Environmental Protection Agency dioxin reassessment documents "Integrated Summary and Risk Characterization for 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and related compounds" and "Toxicity Equivalency Factors (TEF) for Dioxin and Related Compounds" (2000)
- Member of the Scientific Advisory Committee of the European Centre for the Validation of Alternative Methods (ESAC), Commission of the European Communities (1996-2004)
- Member of the Scientific Program Committee for the 10th International Congress of Toxicology (2004)
- Member of the Scientific Committee on Health and Environmental Risks (SCHER) of the European Commission DG SANCO (2004-)
- Member of the Grant Reviewing Committee on Environmental Toxicology of the Swedish Research Council for Environment, Agricultural Sciences and Spatial Planning (FORMAS) (2006-)

6.5. Research funding

Table 2. Project funding during 1996-present

Project name	Funding agency*	Role	KTL funding k €	Years	Partners
Dioxins and anorexia	SA	Project leader	202	1996	
Population exposure	STM	Project leader	50	1997	
Dioxin risk assessment	EU	Coordinator	491	1997-1999	4: S, D, FI
SYTTY- Finnish research programme in Environmental Health	SA	Coordinator	471	1998-2001	
Determinants of sensitivity to dioxin-induced health effects	SA	Project leader	159	1998-2001	4: DK, USA, FI
Ah receptor and dioxin sensitivity	SA	Project leader	111	1998-2001	2: CA, FI
Dioxins	SA	Project leader	202	1999-2001	
Dioxin risk assessment	EU	Coordinator		2000-2003	5: S, I, LIT, FI
In vitro testing methods, developmental toxicity	TEKES	Project leader	97	2001	
Molecular mechanisms of dioxins	SA	Project leader	110	2001-2002	1: CA
In vitro testing methods, developmental toxicity	TEKES	Project leader	225	2002-2004	
CoE for Environmental Health Risk Analysis / Dioxins	SA	Coordinator	706	2002-2004	
BONETOX (Bone development and homeostasis - critical targets in toxicology)	EU	Partner	399	2003-2006	8: S, D, F, I, FI, USA
Accumulation of persistent environmental contaminants to osprey	Nessling Foundation	Project leader	90	2004-2006	2: FI
CoE for Environmental Health Risk Analysis / Dioxins	SA	Coordinator	250	2005-2007	
ATHON (Assessing the toxicity and hazard of non-dioxin-like PCBs present in food)	EU	Partner	632	2006-2009	12: S, D, NL, B, I, UK, N, CA

* EU=European Union, SA=Academy of Finland, STM= Ministry of Social Affairs and Health, TEKES=Finnish Funding Agency for Technology and Innovation

6.6. Scientific collaboration

6.6.1 National

University of Helsinki, Department of Food and Environmental Hygiene and Finnish Food Safety Authority, Helsinki; Prof. Raimo Pohjanvirta

- Experimental studies on AHR structure, animal models and regulation of feed intake

University of Helsinki, Institute of Dentistry, Department of Pedodontics and Orthodontics
Prof. Satu Alaluusua, Dr. Pirjo-Liisa Lukinmaa

- Experimental and epidemiological studies on the effects of POPs on tooth and tooth development

University of Turku, Department of Physiology, Department of Anatomy, Department of Biology; Prof. Jorma Toppari, Dr. Jorma Paranko

- Experimental studies on the effects on male and female reproductive system and steroidogenesis

University of Oulu, Department of Anatomy and Cell Biology; Prof. Juha Tuukkanen

- Experimental studies on bone and tooth development, bone cell differentiation, material properties of bone and enamel, biological imaging

6.6.2 International

University of Toronto, Department of Pharmacology; Prof. Allan B. Okey

- Microarray studies, determination of AHR relative expression levels, transactivation studies

Karolinska Intitutet, Institute of Environmental Medicine; Prof. Helen Håkansson

- POP toxicology, bone effects, retinoids, risk assessment

Prof. Johan Högberg

- Mechanisms of tumor promotion, role of p53 in carcinogenesis and developmental defects

Department of Environmental Health Sciences, Insittute di Richerche Farmacologische ”Mario Negri”: Dr. Roberta Pastorelli, Prof. Roberto Fanelli

- Proteomics analyses of samples from *in vivo* and *in vitro* experiments

School of Veterinary Medicine, Department of Food Toxicology: Prof. Heinz Nau

- Studies on xenobiotic effects on retinoid metabolism, role of retinoid metabolism in developmental defects

University of Wisconsin (Madison), School of Pharmacy, Molecular and Environmental Toxicology Center, Madison, WI, USA: Prof. Richard E. Peterson

- Experimental studies on male reproductive toxicology

Institut de Génétique et de Biologie, Moléculaire et Cellulaire, Illkirch, France; Dr. Norbert Ghyselinck

- Role of retinoid pathways in developmental defects

6.7. Proposal for the future work and expected (social) benefits (next 5-10 years)

Our principal aim is to update and develop further our expertise in mechanistic toxicology and chemical risk assessment by carrying out high quality scientific research of high relevance for toxicology and risk assessment within the field of environmental health. This activity is essential for our national role as experts and advisers in environmental health and toxicology. We are currently working on preliminary analyses and study plans to extend our research to investigate the role and significance of epigenetic effects of persistent organic pollutants. Several recent studies have highlighted this new area of research, and due to our previous experience and the continuing exposure to POPs we will start this endeavour by using POPs as model compounds. Epigenetic alterations are potentially a highly significant mechanism of toxicity due to the stability and heritable nature of these alterations. For example, exposure of only the

gestating rat dam to endocrine disruptors has been recently shown to trigger fertility problems in multiple generations probably via altering DNA methylation, which is the main epigenetic regulatory mechanism. However, the effect of POPs on epigenetic mechanisms is a totally uncharacterized field. Therefore, our objective is to find out if epigenetic mechanisms, especially changes in DNA methylation, are a target for POPs. We aim to elucidate the toxicological significance and consequences of epigenetic alterations and also the role of AHR in them. The model substance used first in these studies is TCDD and two different approaches will be used: the DNA methylation patterns will be compared with and without TCDD-treatment in differentiating mesenchymal stem cells *in vitro* and in germ cells *in vivo*. These epigenetic studies will introduce our institute into a new field of research, the environmental epigenetics.

The new knock-in mouse lines expressing all 3 variants of the rat AHR offer intriguing opportunities to explore the role of the transactivation domain structure in various manifestations of dioxin toxicity mediated by the AHR. Of special importance will be a scrutiny of the type I and type II effects to see which of them, and to what extent, can be reproduced in these transgenic mice. The genetically neutral background enables unbiased analysis of differentially expressed genes by microarrays in the liver and other tissues relevant for TCDD toxicity. Furthermore, as there is also a recently introduced "humanized" mouse model available (expressing human AHR), it is now for the first time possible to directly compare the impacts of TCDD mediated by rat, mouse or human AHRs *in vivo*. This will potentially be of high significance for dioxin risk assessment.

The pathogenesis of the wasting syndrome poses a particularly interesting challenge to modern toxicology. Apart from basic toxicology, elucidation of its mechanism would also benefit physiological research on body weight regulation because TCDD is the most potent anorexigen and leptogen (weight-reducing agent) known. With overweight and obesity gaining ever-increasing prominence as health risks to the general public, a thorough mechanistic understanding of food intake control systems is urgently needed. We aim at studying effects of TCDD on gene expression of neurotransmitters and their receptors in hypothalamic nuclei intimately involved in feeding and body weight regulation by microarrays and qRT-PCR. Techniques to repress activities of key metabolism genes selectively in liver or brain (such as siRNA in a suitable promoter context) can be used to assess the relative roles played by these organs in the wasting syndrome.

6.8. Personnel

The size of the research group is about 10 persons, including the (former) director of the department, 2-4 senior researchers, 1-2 post docs, 2-4 doctoral students and 2 technicians. Most of the funding is from external sources.

The key senior researchers:

Prof. Jouko Tuomisto

- director of the Department of Environmental Health, retired 2004, still active
- toxicology, risk assessment, pharmacology, neuropharmacology, environmental medicine, epidemiology

Dr. Merja Korkalainen

- molecular biology, cell culture models, toxicology

Prof. Raimo Pohjanvirta

- employed by KTL until year 2000; close collaboration continues
- molecular biology, toxicology, pathology

Dr. Jouni Tuomisto

- full time in this group until year 2000, thereafter mainly in risk analysis, but partly involved in POP mechanisms
- risk analysis, risk assessment, toxicology, epidemiology,

Dr. Matti Viluksela

- toxicology, developmental toxicology, risk assessment

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7 RISK ANALYSIS

7.1. Research area and its significance

The amount of scientific information is increasing rapidly. However, it is also fragmented in smaller and smaller disciplines with specialized experts. This is also true with the society as a whole. Although pieces of information can be found easily in the Internet and using other modern tools, it is often still very hard to get an overview and get a synthesis of a complex environmental health issue. Risk assessment and risk analysis are becoming more and more important fields when societies try and understand health and environmental effects of various activities. Formal and scientifically based methods are needed, as causal chains of the remaining problems are increasingly complex and span across several scientific disciplines and governmental sectors. For example in the European level, the REACH chemical initiative has changed the focus of chemical risks from single pre-marketing assessments to complex environmental and health assessments of existing substances. Therefore, there is a great need to develop risk analysis methods that provide guidance and scientific understanding to policy-makers. The main challenges lie within the interface of science and policy, and therefore a wide multidisciplinary approach must be used. There are two large research needs: methods for estimating the value of scientific information for decision-making, and methods for explicitly describing the actual decision situations in a way that scientific understanding can be utilised.

Understanding of a particular risk develops simultaneously at various levels and using differently structured methods. At one end there is a "discussion layer": political and public unstructured discussions about risks of a hazard with a wide interest on e.g. economical consequences of available decision options, public health, and social justification equity. At the other end there is a "modelling layer": models dealing with specific questions such as air concentrations of a pollutant from a specific source or risk-benefit analyses of particular actions. There is a need for methods facilitating the flow of information and understanding between the existing layers.

We have started to develop a method of structured deliberation as an intermediate layer in the field of risk assessment. It describes a risk situation in a formal manner by using a diagram method developed by us. It is an enhanced causal diagram that contains items along the causal pathway (or network) from emissions to dispersion to exposure to effects. It is designed to describe also other than causal connections such as values and arguments (Tuomisto and Tainio, BMC Public Health 2005: 5: 123)

Centre of Excellence on Environmental Health Risk Analysis
We have obtained the status of a Centre of Excellence nominated by the Academy of Finland for years 2002-2007. The general objective of the *Centre for Environmental Health Risk Analysis* is to improve environmental health risk analysis by increasing the understanding of environmental risks at all levels from molecular mechanisms to societal needs. This requires both high-quality research and better methods of risk modelling and characterization.

Environmental health risk analysis must be based on high quality multidisciplinary science. The Centre uses dioxins and urban air fine particles as two different examples of environmental pollution and challenges for risk assessment and management. The data on dioxins comes mostly from experimental studies on animals, whereas most air pollution studies are epidemiological. It is useful to study if these methodological differences have led to differ-

ences in regulation. We study the human exposure, toxicology, epidemiology, and risk modelling of these pollutants and use this work to evaluate and develop general methods and principles for environmental health risk analysis. Research on fine particulate air pollution focuses currently on exposure and health effects of particles from specific sources, especially traffic. Research on dioxins focuses on effects on development, liver toxicity, and wasting, and their molecular basis. Risk analysis methods are examined both in theory and real-life case studies in order to develop better modelling tools to deal with complex exposures and outcomes, and to incorporate risk tradeoffs and uncertainties.

Geoinformatics and remote sensing (GIS)
Today geoinformatics and remote sensing (GIS) are applied worldwide in collecting and analysing environmental data. In Finland, unlike in most countries, there are also valuable nationwide population and health registers, which can be combined to environmental datasets using location coordinates. Recent years have revealed an increasing need to use geoinformatics in a range of environmental epidemiological studies lead by KTL Department of Environmental Health. Geographic information systems can potentially provide very powerful tools for research and practice of environmental health as well as environmental health risk analysis.

7.2. The main achievements

Risk assessment and risk communication has been an important part of the work by the researchers since the foundation of the Department of Environmental Health. This has been a way to use the expertise in practical problems and decision-making situations in Finland. As an example, we published a booklet about dioxins *(Synopsis on dioxins and PCBs)* after the Belgian dioxin crisis in 1999. The aim was to publish easy-to-use information about dioxins in general, their emissions, exposures, and health effects, and major issues related to the Belgian incident itself. The booklet was also published in the Internet.

The scientific effort to do serious methodological work in the field of risk analysis started in 2000, when two things happened. First, the Department decided to apply for a Centre of Excellence for Environmental Health Risk Analysis from the Academy of Finland (and received the funding next year); and second, a research collaboration started with the Harvard Center for Risk Analysis at the Harvard University, Boston. The basic idea was to make risk analysis research in areas where there was a strong research expertise within the Department, namely dioxins and fine particles. Thus, an intensive in-house collaboration between toxicologists, epidemiologists, and exposure assessors was the basis of the risk analysis research.

Health impact assessment of fine particles has been a major topic during the whole risk analysis research period. We have for example estimated the exposure to fine particles emitted from buses in the Helsinki metropolitan area in 2001 (see Municipality impact), and performed impact assessments for the whole country. The project found out that the emission sources with major concern were domestic wood combustion and vehicular traffic. This was due to both large emissions and emissions occurring close to the population breathing zone. This branch of research continues, with the focus on these two emission sources and exposure situations near (less than 10 km) the source.

We have built capacity to model complicated environmental health risks. Most commonly, the models are probabilistic Monte Carlo simulation models developed in Analytica, which is a program with a versatile graphical interface. This interface facilitates the combination of other graphical methods such as directed acyclic graphs and extended causal diagrams. We have

also developed general modules in Analytica, such as for calculating correlated variables, life expectancy, or value of information. These modules are subsequently used in several risk assessments.

A major effort regarding fine particle risks was a formal elicitation of expert judgement. We interviewed six top fine particle experts in Europe and asked their views on a) the magnitude of health effects related to population exposure, b) differences in toxicity of different particles, and c) timing of health effects occurring after the exposure (or cessation of exposure). In this way, it was possible to obtain new information about questions that do not yet have measured data from studies.

Another project related to fine particles is a *composite traffic project* where we estimated health effects of urban car traffic. We developed a new theoretical traffic system that aggregates trips into common vehicles, and estimated it costs compared to current alternatives. (For further details, see Municipality impact.) Our model has been offered as one approach for case studies in INTARESE, and the collaboration is starting in other cities than Helsinki.

In 2004, Hites and co-workers published a study about persistent pollutant concentrations in wild and farmed salmon in Science. This paper caused outrage as it claimed that farmed salmon should be avoided due to pollutant contamination. We published a commentary in Science. Using a formal risk assessment method called *value-of-information* analysis, we were able to show that net benefits of salmon outweigh the risks, and that the critical questions related to the salmon recommendations are actually political in nature, in which Hites' paper had only a minor, if any, contribution.

Methodological research has been a major underlying theme during the whole Centre of Excellence period. The work has mostly been in-house method development in small steps. However, the start of INTARESE in November 2005 pushed methodological work into full speed. There have been several new innovations about how to make risk assessment in a more participatory and still more consistent way. The major challenge during the last months and next year have been and will be to publish these innovations and test them in practice in own work and within collaboration projects, notably INTARESE and BENERIS (www.beneris.eu). (For further details, see Proposal for future work.)

Our experiences of the development and use of a small area statistics of health system (SMASH) at KTL Department of Environmental Health have enabled geographical specificity to be incorporated into studies on environmental determinants and cancer risk. This is possible by combining the location coordinates of buildings with environmental data. The same principle of linking environmental data with geocoded population and health data can theoretically be used in any study applying geoinformatics in environmental epidemiological studies. More recently, we have been developing further the KTL ADP infrastructure to allow more efficient GIS- related research and to allow more environmental and health data to be incorporated to SMASH system and research.

We have used GIS in epidemiological studies also in the fields of power lines and cancer, several small area of health case studies, and production of cancer maps for Finland, Belarus and the Ohio State, US (http://www.cancerregistry.fi/eng/statistics/graphics.html). We have also used GIS in epidemiological sampling of a study on biowaste treatment and health effects, contributed to mapping of infectious diseases, and conducted a pilot study on associations between socioeconomic status and environmental exposures in Finland.

7.3. Scientific impact

Table 1. Summary of the scientific output during 1996-present

SCIENTIFIC OUTPUT	N	Comments
Peer-review papers in international journals		
Invited lectures and chairmanships in international meetings	**5**	
Papers in domestic journals	**4**	
Lectures in domestic meetings	**20**	
Editorial tasks in international journals		
Committee memberships: domestic		
Committee memberships: international (EU, WHO etc.)	**2**	
Doctoral dissertations		
Supervision of dissertations: ongoing	**7**	topics: fine particles, dioxins, risk-benefit analysis for fish, contaminated soils, risk assessment methodology, mechanisms of biliverdin accumulation due to dioxin, geographical information systems in health assessment
Opponent of dissertations		

7.4. Social impact

7.4.1. National

a) Municipal –level impacts

Gasbus project was about comparing different engine techniques in urban buses and their health impacts in the Helsinki area. A Finnish report was written and published in the publication series of the Ministry of Health and Social Affairs.

Composite traffic research about different urban transportation system showed that trip aggregation has real potential in reducing several pressures of car traffic in cities, including fine particles, carbon dioxide, traffic jams, and overall costs of transportation. These results were disseminated (in addition to a scientific article) in newspapers and TV news, and we also organised a special stakeholder event. The discussions and comments from the stakeholders (including administration from ministries and local transportation planning offices and companies), had a major impact on model updates and further research questions asked. The updated model version is finished, and the second round of stakeholder events will be organised in the near future.

b) Other national impacts

We have estimated the total health effects of primary fine particles in Finland. Mortality was used as the indicator, as previous studies have shown that it is the dominant impact when various health endpoints are summarized. A recent EU-level CAFÉ report estimated that the number of premature deaths is 1300 per year in Finland, and more than 300 000 per year in the EU. Our estimates give new insight into these numbers, because we can estimate the effects in a more disaggregated way due to more detailed data in Finland. For example, we have a detailed and comprehensive estimates about emissions per activity sector. In addition our resolution is more precise (5-10 km vs 50 km in CAFÉ). These and other recent results from Finland have emphasized the role of domestic wood combustion, and an active discussion has started on the necessity and possibilities to reduce these emissions. The Ministry of Environment is planning emission standards for small-scale wood burning devices.

A recently started project examines the ecological and health risks of metals due to metal industry, and contaminated soils especially. The approach utilizes several kinds of methods including particle characterization from air samples, exposure modeling, biogeochemistry, risk communication research, and value of information. This work is carried out in close collaboration with metal industry and authorities, which enhances the use of existing information and measurements. Collaboration also facilitates the use of project results in practical decision-making within the industry and by the authorities.

The role of geoinformatics is rapidly evolving in epidemiological research as well as in practical health - related applications. We seek to combine the use of traditional statistical methods, geoinformatics, and intelligent data mining. We also hope to turn them into a routinely used tool in epidemiological studies and risk analysis. The research creates an important environmental health database and multidisciplinary networks that will enable effective conduction of new environmental health studies and also rapid inquiries into health concerns in environmentally pollution areas.

7.4.2. International

Risk analysis has had a major impact on fishery policies in Europe, and especially in the Baltic Sea area. Since the so-called Belgian dioxin crisis in 1999, dioxin regulations have been actively kept on political agenda in the European Union. An example is dioxin limit values in foods, and notably fish that is a critical food item in this respect. Finland and Sweden had a five-year derogation (2001-2006) to use Baltic herring although it regularly exceeded the dioxin limit value. Our commentary in Science (Tuomisto et al, 2004) and other commentaries and expert opinions related to dioxins in fish, had an impact on the dioxin directive by the European Union. In 2005, the derogation was extended to new countries and fish species, especially because of the improved scientific evidence and benefit-risk analyses produced in KTL and other institutes.

In the future, we may come to see powerful GIS applications in spatio-temporal data mining of epidemiological databases using intelligent methods, in large-scale environmental health monitoring, and in practical location-based services that provide environmental information for citizens.

7.5. Research funding

Table 2. Project funding during 1996-present

Project name	Funding agency*	Role	KTL funding k€	Years	Partners
Natl' Environ. Health Program	STM	Coordinator	60	1998-2000	
CoE for Environmental Health Risk Analysis (CEHRA)	SA	Coordinator	1513	2002-2004	
Micorbes and Man	SA	Partner	335	2002-2008	
KOPRA	Tekes	Partner	131	2004-2005	Finnish Environmental Institute, Finnish Meteorological Institute
Scientific uncertainties in decision-making	SA	Project leader	155	2005-2007	
CEHRA	SA	Coordinator	174	2005-2007	
Environmental Risk Assessment Centre (ERAC)	Tekes	Partner	223	2005-2006	GTK, KuY
ERAC (doctoral education)	GTK	Coordinator	267	2005-2007	GTK, KuY
CEHRA	SA	Coordinator	406	2005-2007	
Doctoral education of environmental health	KuY	Partner	186	2005-2006	KuY
Academy researcher fellowship	SA	Project leader	310	2005-2010	
INTARESE	EU	Partner	437	2006-2009	Imperial College, RIVM, Utrecht University
BENERIS	EU	Coordinator	4964	2006-2009	TUDelft, FSAI, DFVF
Small research grants	various	varous	**433**	1997-2007	various
Total			**3768**		

* EU=European Union, SA=Academy of Finland, STM= Ministry of Social Affair and Health, GTK= Geological Survey of Finland, KuY=University of Kuopio,

7.6. Scientific collaboration

7.6.1 National

The Finnish Meteorological Institute and the Finnish Environment Institute have closely collaborated with KTL in KOPRA project related to fine particle emissions, dispersion, and health effects. This work continues actively in a new project, PILTTI, which focuses on traffic and domestic combustion emissions.

Environmental Risk Assessment Centre is a cooperation project between KTL, Geological Survey of Finland, and the University of Kuopio. It develops infrastructure for risk assessment work, gives university level education on risk assessment, develops methods in risk assessment, and performs research and case studies according to the new methods. The cooperation has started in 2005 and it is rapidly growing.

The University of Kuopio has a graduate school SYTYKE for environmental health. A large proportion of the students involved come from KTL/YTOS and/or the Centre of Excellence.

Finnish Cancer Registry: development of the small area statistics of health system to assess local cancer maps; producing cancer maps and animations

University of Kuopio, Environmental Informatics Unit: sharing methodological and scientific knowhow e.g. on studies of environmental equity

Geological Survey of Finland: enhancing the ADP infrastructure and researcher networking via the ERAC-projects

7.6.2. International

We have ongoing collaboration in a project "*Expert judgement of uncertainty in particulate matter mortality effects"* with Dr John S. Evans (Harvard University) and Prof Roger Cooke (Delft University of Technology, the Netherlands). In the project we interviewed the most prominent PM epidemiologists and toxicologists in Europe and in Mexico City.

INTARESE risk assessment project is the largest collaboration related to KTL risk research at the moment. It has more than 30 partners involved. KTL has a major role especially in method development, and KTL is actively collaborating in this area with Imperial College (the coordinator), University of Stuttgart (responsible for the final output, toolbox for risk assessment), RIVM (developing risk assessment framework), and Utrecht University (developing health impact assessment and exposure-response functions).

BENERIS is another project with major collaboration, as it is coordinated by KTL. Other partner are Delft University of Technology (NL; Bayesian probability methods, expert elicitation), Oy Foodfiles Ltd (FI; clinical nutrition research , consultation), Food Safety Authority of Ireland (IE; nutrition research, dissemination of scientific results), Food Research Institute of Denmark (DK; nutrition research), Food Safety Authority of Denmark (DK; food safety, administration), Lendac Ltd (IE; information technology), Fundación Privada para la Investigación Nutricional (ES; nutrition research).

Imperial College, Small Area Statistics of Health Unit: collaborations in the development of a new small area statistics of health system (RIF)

7.7. Proposal for the future work and expected (social) benefits (next 5-10 years)

Risk analysis is a new area of research in KTL. It has been actively pushed forward during the last five years. During the last year, the number of researchers focussing on risk as the main topic has increased from three to nine. Now there are clearly signs of critical mass, where the work of individual researchers have started to promote the work of other researchers and the whole group. The funding opportunities in this area are good, and therefore we expect that this research in KTL is expanding.

Risk research is done by combining methodological work together with real-life risk assessments and risk modelling. Topical cases important for public health are chosen, and these are

examined with a top-down approach: first, an overview is built, and then the research continues with more detailed analysis on the parts that were found critical in the first phase. Fine particle risk assessment is an example of previous work. Monte Carlo methods will be a crucial part of the work in the future, but also other statistical methods are being applied. Fine particles and dioxins will be research topics for several years, but other issues are likely to come up during the next years, contaminated soils being one of the new areas.

The major objectives for the next five years relate to the methodological work in risk assessment. There is a special need for integrative methods that would help in synthesising existing but fragmented information. To this aim, we are developing a methodological framework that is flexible enough to be able to handle different kinds of pieces of information. The linking and smooth interfaces between various methods are therefore crucial.

The methodological framework is being built on causal diagrams, or directed acyclic graphs. These methods have clearly improved during the last ten years, especially due to the linkages to Bayesian probability theory. Causal chains are the skeleton in the Intarese approach, and in BENERIS, Bayesian graphs and tools have a central role. In addition, we have extended the use of these graphs in a way that also non-causal connections and relations are being represented in a similar way. This enables the use of conceptual diagrams together with mathematically explicitly defined probability relations.

Another area of methodological development relates to the acceptability of risk assessments. Stakeholder involvement has been seen as a crucial part of risk analysis, but methods for doing that systematically have not been well developed. We will develop this area by introducing argumentation theory in risk assessment. This kind of approach is completely novel, and the potential benefits are high. The basic motivation comes from the idea that the acceptability issues, which the risk assessment will inevitably face after it is released, can in part be incorporated in the work of performing risk assessment. In this way, the critical issues can be tackled in the analysis and produce a more comprehensive and relevant picture about the risks. This is not a simple task, as the integrity of the assessment must be maintained. The involvement of non-scientists must be organised, processed, and documented using as strict quality standards as the other parts of the assessment. Argumentation theory offers tools to organise and document non-scientific issues, and resolve disputes that may arise during the process in a coherent and transparent way.

To open the risk assessment process to participants outside the small group of risk assessors of scientist will create several practical problems. There must be a functional system that is able to collect the contributions, store them properly and have a version control of each persons' contributions. In addition, some parts of the assessment may require different levels of protection. These practical problems are being dealt with using new tools developed especially for collective, multi-person tasks about a given topic. The most developed computer tools include Mediawiki, which is the program that is used to build Wikipedia, the Internet encyclopedia. This program is extensively used in several projects, and we are also developing new features to it, especially for the needs of risk assessment and representation of scientific information in a coherent manner. The first public websites have recently been opened, and we are collecting feedback to further develop the methods of collective work.

We will also be working to strengthen and rationalize the GIS-infrastructure at KTL Department of Environmental Health, to develop the use of the small area statistics of health –system particularly by adding environmental data to the analyses, and to strengthen the budding collaborations on the use of satellite data as well as hierarchical models in epidemiological studies. We also

wish to promote small area statistics of health – methodology as a routine approach in environmental risk analysis. The topics in this area will cover computational methods and geoinformatics, environmental equity, and biowaste and health effects in nearby residents.

7.8. Personnel

The core group in risk analysis research currently consists of an academy researcher, a post doc, five doctoral students, a statistician, an information technologist, and a risk communication researcher. Their main task is to develop risk assessment methods, and to apply the methods in practical risk assessments (fine particles, dioxins, contaminated soils as main topics, and some smaller topics such as municipal waste incineration). In addition, senior researchers within the Centre of Excellence participate in the methodological development and discussions. This happens especially within a large risk research project INTARESE project (www.intarese.org; see International collaboration).

Our Spatial analysis for environmental epidemiology and risk analysis (SPEAR) –team currently includes 5 researchers (principal investigator, three starting PhD-students and one research assistant) who, however, are relatively experienced with the use of geographic information systems and artificial intelligence.

The key senior researchers:
- Docent Jouni Tuomisto (risk analysis, dioxin and fine particle health impact assessment, contaminated soils)
- Docent Pia Verkasalo (GIS, epidemiology of dioxins and fish)
- PhD Anna Karjalainen (risk analysis)

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