

## Annex I: Project Progress Summary

### *Project Progress Summary*

<u>Section 1: PROJECT IDENTIFICATION</u> Information to be provided for project identification		<b>NOT CONFIDENTIAL</b>
<b>Title of the project:</b> Comprehensive risk analysis of dioxins: development of methodology to assess genetic susceptibility to developmental disturbances and cancer		
<b>Acronym of the project:</b> Dioxin risk assessment		
<b>Type of contract:</b> shared cost		<b>Total project cost</b> (in euro) 2705693 €
<b>Contract number</b> QLK4-CT-1999-01446	<b>Duration</b> (in months) 36 Months	<b>EU contribution</b> (in euro) 1470000 €
<b>Commencement date:</b> 1 February, 2000		<b>Period covered by the progress report</b> (1 February 2000 – 31 January 2003)
<b><u>PROJECT COORDINATOR</u></b>		
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<b>Key words</b> (5 maximum - Please include specific keywords that best describe the project.). dioxins, risk analysis, cancer, developmental effects, tooth defects		
<b>World wide web address</b> (the project's www address) www.ktl.fi/ytos		

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**List of participants** Provide all partners' details including their legal status in the contract i.e., contractor, assistant contractor (to which contractor?).

Partner 1.

Coordinator. **KTL (Kansanterveyslaitos, National Public Health Institute of Finland, Department of Environmental Health)** encompasses in fact **two partners** involved in this project, first, Laboratory of Chemistry, and second, Laboratory of Toxicology and Unit of Environmental Epidemiology.

Responsible Researcher: **Professor Jouko Tuomisto**  
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Partner 2

Contractor. **Department of Cell and Molecular Biology, The Medical Nobel Institute, Karolinska Institutet**, Stockholm, Sweden

Responsible Researcher: **Professor Lorenz Poellinger**  
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Partner 3

Contractor. Institute of Dentistry, **University of Helsinki**, Finland encompasses two departments involved in this project, **Department of Pedodontics and Orthodontics and Department of Oral Pathology**.

Responsible Researcher: **Professor Satu Alaluusua**  
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Partner 4

Contractor. **Azienda "Ospedale di Vimercate", Ospedale di Desio, University Department of Clinical Pathology**, Desio – Milan, Italy

Responsible Researcher: **Professor Paolo Mocarelli**  
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Partner 5

Contractor. **University of Linköping, Department of Biomedicine and Surgery**, Sweden

Responsible Researcher: **Professor Peter Söderkvist**  
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Partner 6

Contractor. **Institute of Biotechnology**, Vilnius, Lithuania

Responsible Researcher: **Dr. Arvydas Kanopka**  
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**Section 2: Project Progress Report**

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*(2 pages maximum.. Use short sentences. Be factual. Avoid technical terms as much as possible )*

**Objectives:**

The objective is to set a scientifically defensible limit of safe exposure to dioxins, as to developmental effects and cancer. The exceptionally wide inter- and intraspecies differences in sensitivity to dioxins will be scrutinised to diminish this major uncertainty factor in risk assessment. To achieve this general objective, a number of specific objectives are set:

- 1) to study the molecular mechanisms of dioxin toxicity in a multidisciplinary manner, utilising the mutated receptor causing a remarkable resistance towards dioxin toxicity.
- 2) to resolve in population studies the sensitivity of human being to developmental effects (tooth defect and cleft palate) and cancer, and compare these outcomes to results in experimental animals
- 3) to perform an up-to-date dioxin risk assessment

## Results and Milestones:

The work in contract was divided to 13 workpackages (1: Mouse and rat transgene experiments to verify resistant or sensitive phenotypes, 2: Comparative analysis of the transactivation potential of L-E, H/W, and hamster AHRs, 3: Hamster AHR cDNA cloning, sequencing and functional characterisation with particular regard to transactivation potential, 4: Gene expression studies - Identification of biomarker genes for dioxin sensitivity, 5: Developmental toxicity of TCDD: teeth and palate as experimental organ models, 6: Teeth as an indicator of human dioxin exposure, 7: Other developmental and reproductive toxicity endpoints, 8: Cleft Palate Studies, 9: High-exposure cohort, 10: Screening for polymorphisms in the human AHR, analogous to the H/W rat mutations, 11: An *in vitro* splicing system as an aid in extrapolation of animal data to humans, 12: Risk assessments on cancer and on developmental effects, 13: Policy-driven risk analysis). All workpackages have produced new information. Many parts of information have already been published.

The most important results are:

*Dioxin receptor and related biochemistry:* New information on the transcriptional machinery involved in the actions of AH (dioxin) receptor emerged. The AH receptors of hamster, a resistant species, and of guinea pig, a sensitive species, were cloned, and interesting similarities and differences with human receptor noted. Dioxin receptor synthesis in two rat strains was found to be upregulated after both acute and repeated TCDD administration, which perhaps contributes to continued toxicity.

*Tooth development and other developmental effects:* TCDD was shown to totally arrest the development of rat third molar tooth, if the dose was large and/or timing was during a critical period, and disturb the development at lower doses. AH receptor and ARNT protein expressions were shown to take place at critical sites and during critical time periods during tooth morphogenesis. High accidental exposure to TCDD was shown to associate with tooth defects in children younger than 9.5 years at the time of accident. Also mouse salivary gland and prostate development were shown to be very sensitive to dioxin exposure.

*High exposure populations:* Dioxin exposures and concentrations in high-exposure populations were characterised and analysed, notably among Finnish and Swedish fishermen who exhibit very high concentrations. Small area statistics on health (SMASH)-system is being developed and used for investigating cancer in populations living close to the polluted Kymijoki river. Contamination by many simultaneous compounds (dioxins, chlorophenols, methyl mercury) may cause problems for interpretation. Work on possible health effects continues.

*Genetic variation in population:* AH receptor polymorphisms were searched for in Swedish population, and very few polymorphisms were found. No genetic predisposition for lung cancer for any of the AH receptor SNP's could be disclosed.

*Cancer:* Case-control study revealed no increased risk of soft-tissue sarcoma when concentrations were measured in contrast to work history data in previous studies. This is in line with our experimental data that tumourigenicity is a high-dose effect (relative to other dioxin effects).

*Dioxin risk assessment:* Dose-response studies and molecular studies made it possible to divide the effects to dioxin I effects and dioxin II effects. The former include CYP1A1 induction and several developmental effects, and they are low-dose effects and exhibit no or modest sensitivity differences between species and strains. Dioxin II effects include liver damage, bilirubin increase, tumour promotion, and acute lethality, they are often high-dose effects, and sensitivity differences between species and strain may be huge. The sensitivity differences seem to be efficacy differences rather than potency differences. This classification, among other things, suggests that carcinogenicity may be a high-dose phenomenon, perhaps even secondary to organ toxicity. An obvious implication is that developmental effects are likely to be more important and carcinogenicity less important in dioxin risk assessment.

**Benefits and Beneficiaries:**

A clear view is starting to emerge as to the relative importance of developmental effects and cancer in dioxin risk assessment. As yet it seems that several developmental effects (tooth development, especially rat third molar, development of ventral prostate, possibly other organs developing from epithelial "budding", bone development) are highly sensitive to dioxins at certain stages of development. This sensitivity is in line with tooth defects found in children both among Seveso accident victims and previously in normal population. When dioxin concentrations in breast milk have decreased, no dioxin-associated increases in tooth defects could be demonstrated later. On the other hand, sensitivity to cancer effects seems lower than previously believed. Therefore we believe that dioxin risk assessment should be based on developmental effects rather than cancer.

The overall conclusion is that developmental effects as the critical endpoint has gained support in this project. There are well-documented adverse developmental effects at exposures not much higher levels than those currently seen. In contrast, dioxin cancer risk seems lower than previously thought, and even a zero-effect is quite possible until very high exposures. This supports the principles of the latest WHO dioxin risk assessment based on developmental effects rather than cancer.

These findings will be directly available and useful for dioxin risk assessment and risk management in European Union and other international bodies.

**Future Actions (if applicable):**

Academy of Finland has nominated the coordinator institute the Centre of excellence in environmental health risk analysis. Within this framework risk analysis work will continue. Partners of this contract will also continue in other related projects of dioxin risks or toxicity BONETOX (Contract No. QLK4-CT2002-02528), EXPORED (QLK4-CT2001-00269), and EDEN (QLK4-CT2002-00603). More information from [http://europa.eu.int/comm/research/endocrine/index\\_en.html](http://europa.eu.int/comm/research/endocrine/index_en.html)