FIFTH FRAMEWORK PROGRAMME
1999-2002

CATALOGUE OF SELECTED PROJECTS

Endocrine Disruption
and
Potential Endocrine Disrupters

1999 - 2000

QUALITY OF LIFE AND
MANAGEMENT OF LIVING RESOURCES
and
ENVIRONMENT AND SUSTAINABLE DEVELOPMENT
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CHAPTER I

RESEARCH PROJECTS SPECIFICALLY ADDRESSING ENDOCRINE DISRUPTION
Increasing incidence of human male reproductive health disorders in relation to environmental effects on growth- and sex-steroid induced alterations in programmed development

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Title
Increasing incidence of human male reproductive health disorders in relation to environmental effects on growth- and sex steroid-induced alteration in programmed development.

Objectives
This proposal addresses the issues of declining male reproductive health and its causes. It will use standardised methods and a central European database:

- to define the incidence of disorders of infant (urogenital malformations) and adult (low sperm count) reproductive health in different countries in Europe,
- to evaluate potential environmental/dietary causes of the disorders,
- to evaluate a novel, non-invasive method (change in finger length) for identifying the underlying causes. This stems from new evidence linking effects of sex hormones on programming genes to altered development of digits and the urogenital tract. Experiments in rats, involving manipulation of sex hormones levels in the fetus/neonate, or induction of intra-uterine growth retardation, will establish the relationship between urogenital malformations/low sperm counts and altered expression of programming genes, and whether environmental hormone disruptors or synthetic «meat hormones» can affect these pathways or the expression of other genes.

Scientific approach

Birth cohorts of boys in Denmark and Finland (countries with major differences in prevalence of male reproductive disorders) and case-control studies of boys in Spain, France and the UK will be studied to compare incidences of urogenital malformations. The underlying causes of these disorders will be investigated using detailed questionnaires on the parents (lifestyle, medical, work, diet) and biological samples from pregnant mothers and infants for analysis of hormone levels and genetic polymorphisms.

In parallel, young men from the general population (with/without proven fertility) in Denmark, Finland, France, Spain, the UK, the USA and Japan, and patients with fertility problems or testicular cancer, will be evaluated for reproductive status using standardised methods (questionnaire, hormone and semen analyses). In infants and adults, relative digit length will be determined and related to reproductive health status. Representative biological samples from patients will be analysed for oestrogenicity/anti-androgenicity using novel methods, validated in cell lines.

Additionally, animal studies will centre on experimental manipulation of the sex hormone environment of the fetus/neonate, using methods known to induce urogenital malformations and/or to reduce sperm counts. Evaluation of alterations in expression of programming genes in the reproductive tract in these animals and changes in digit length, will allow us to establish cause and effect with regard to sex hormone exposure. The same endpoints will be used to evaluate the effects of exposure to environmental chemical with hormonal activity, with particular emphasis on effects of hormones used in meat production e.g. zearalanol. Finally, as intra-uterine growth retardation (IUGR) is an important risk factor for all male reproductive disorders, the effect of experimentally induced IUGR in rats on the same endpoints will be established.
Identification of critical rat testicular genes altered after fetal androgenic disruption by flutamide/ use of dna microarray

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Title

Identification of critical rat testicular genes altered after fetal androgenic disruption by flutamide: use of DNA microarray.

Objectives:

The objective of this project is to introduce new more predictive and reliable methodologies to improve the current approach used to assess the endocrine disruption potential on the male reproductive functions of new chemicals. To achieve this general objective, a number of specific objectives are set:

1. identify genes affected by antiandrogenic disruption by using the DNA microarray methodology.
2. compare specific DNA fingerprint obtain by flutamide (which blocks the action of testosterone and dihydrotestosterone) and finasteride (which inhibits the 5α-reductase activity preventing the formation of dihydrotestosterone).
3. understand the specificity and the critical role played by each gene using different in vitro and in vivo mechanistic approaches in the different testicular cell types.
4. study the relevant genes in pathological testis (infertility and seminoma).

Scientific approach

The work is divided into specific parts:

1. the construction of rat testicular cDNA libraries prepared from whole testicular tissues aimed to the establishment of the µarray.
2. the gene expression analysis from pool of mRNAs isolated from treated animals aimed to identify the largest number of genes affected by flutamide or finasteride.
3. Based on their tissue up- and down-expression occurring in the different cell types, mechanistic studies aimed at increasing understanding on the mechanism of toxic actions of antiandrogens. This part is studied in the three main testis cell types (Leydig, Sertoli and germ cells) by using different approaches.
   a) cell distribution, expression and regulation of expression of genes by in situ hybridization and/or immunohistochemistry
   b) expression of genes is studied in in vitro systems under the influence of different specific hormones
   c) the coding region of each gene is introduced in an expression vector for production of transfected cell lines expressing stably the protein.
The impact of developmental exposure to weak (environmental) estrogens on the incidence of diseases in target organs later in life

Contract No: QLK4-2000-00305
Project Cost: € 1,756,761
Project Duration: 48 months
Project type: shared cost
EC contribution: € 1,550,000

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The impact of developmental exposure to weak (environmental) estrogens on the incidence of diseases in target organs later in life.

Objectives

1. Modulate estrogen levels in pregnant mice and link this to phenotypic effects in the mammary gland, testis, prostate, ovary and the brain of the offspring, to get insights in tissue specificity and sensitivity.
2. Determination of the relevant cell- and receptor involved in the effects in sensitive target tissues.
   This will be used to design tests for the most critical stages and tissues sensitive to estrogen disruption.
3. Identify molecular markers that can help to assist in diagnosis of disturbances by estrogens.
4. Further develop the identified markers for use in a clinical setting.
5. Get insight in the impact of prenatal estrogen exposure on development of disease, improve testing strategies and provide more general markers for clinical research.

Scientific approach

1. A major link between the project parts will be the use of material (tissues) of mice that are exposed to (xeno)-estrogens in a standardized manner. This will be carried out centrally, and the different tissues will be distributed to the relevant partner.
2. We will modulate estrogen levels in pregnant mice and link this to phenotypic effects in the offspring in the mammary gland, testis, prostate, ovary and brain. Each partner will have its own specific topic of investigation, and will study estrogen action in a particular target organ.
3. We will generate insight in the particular cell-and receptor type through which the effects occur in sensitive tissues. When identified, particularly sensitive tissues and end-points will be used to generate novel in vitro assays to detect endocrine disrupting chemicals.
4. Identify molecular markers that can help to assist in the diagnosis of disturbances by estrogens. Genes will be picked up that are constitutively induced after a short pulse of estrogens during development, and thus are markers of estrogen exposure.
5. Isolate human homologues of the markers identified in the animal studies (point 4) for use in a clinical setting.
6. Identification of the molecular pathways leading to prenatal disruption of hormonal imprinting, and get further insight in the possible impact of these effects on development of disease later in life. Improve current testing strategies and generate more generally applicable molecular markers of exposure.
The role of dietary phytoestrogens in the prevention of breast and prostate cancer

Contract N°: QLK1-2000-00266
Project type: Shared cost
Project Cost: €
EC contribution: €
Project Duration: 36 months
Project start date: 2001

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The role of dietary phytoestrogens in the prevention of breast and prostate cancer.

Objectives

The hormonal cancers, breast and prostate, are major causes of death in the EU. It is now clear that a diet high in plant-based foods may offer protection. This project will analyse the effects of two groups of phytoestrogens (isoflavones and lignans), on the development of breast and prostate cancer. It will study the metabolism of phytoestrogens, individual variation and its influence on cancer risk. This will also include the development of methods for the discovery of new metabolites and for the rapid measurement of bioactive metabolites. This co-ordinated approach using human patients and volunteers, animal models and in vitro techniques will result in in-depth knowledge of the role of these compounds in the development of cancer and enable informed advice to be available in Europe on the beneficial effects of phytoestrogens in the diet.

Foods such as soy, rye and flaxseed products that contain phytoestrogens (PE), may protect against breast and prostate cancer. Studies suggest that mammalian metabolites of PE may be important in cancer prevention and also that there is large inter individual variation in metabolism in humans.

The overall aim of the proposal is to investigate the preventive role of PE and metabolites on breast and prostate cancers and to assess the influence of individual variation in PE metabolism on cancer risk.

The scientific objectives are:
- To isolate and identify new metabolites and develop new methods for the rapid measurement of PE metabolites.
- To compare, using the most recent molecular and analytical techniques, the effects of PE and their metabolites on the stages of development of breast and prostate cancer.
- To analyse the influence of individual variation on biological parameters indicating cancer risk
- To use these data to make recommendations on the benefits of phytoestrogens in the diet.

Description of the work

One of the novel aspects of this project is the emphasis on comparing the biological activity of parent PE and their mammalian metabolites in order to assess the importance of the previously demonstrated, inter individual variation in metabolism.

The work plan is organised to reflect the scientific objectives of the study as above.
- The use of modern, novel analytical techniques to identify new metabolites of isoflavones and ligans and to examine new methods for the rapid detection of phytoestrogen metabolites.
- A comparative evaluation of isoflavonoid and ligan PE and their metabolites for effects on the developmental stages of cancer using in vitro methods. These methods will use human breast and prostate cell lines and will examine three different stages of cancer.
- DNA damage and changes in gene expression.
- Promotion and metastasis.
- Angiogenesis
- Investigation of the effects of feeding phytoestrogens on the development of tumours in novel animal models of cancer.
- Transgenic mouse model of breast cancer.
- Human prostate cancer cell xenografts in nude mice
- Study of a group of human volunteers exhibiting large variation in the level of phytoestrogen metabolism. This will include investigations of biological endpoints which may be relevant to cancer risk e.g. hormone profiles, oxidative stress, and immune status.
- Study of tissue samples which have been previously collected from breast and prostate cancer patients. The issues will evaluate using molecular biology techniques for changes in biological character and the data compared with the blood enterolactone level of the patient.

Milestones and expected results:

- Detection of new metabolites giving a definitive metabolic spectrum for PE
- New rapid methods for the detection of metabolites
- In depth knowledge of the role of the phytoestrogens in the development of cancer in particular, those of the breast and prostate, using human, animal models and in vitro techniques.
- Details of human metabolism of phytoestrogens and how individual variation affects parameters of cancer risk
- Advice on dietary intakes of phytoestrogens for beneficial effects.
The prevention of osteoporosis by nutritional phytoestrogens

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The prevention of osteoporosis by nutritional phytoestrogens.

Objectives

Isoflavones, a subgroup of phytoestrogens mainly contained in soy and soy products, are widely held to have beneficial properties, but the evidence to date is only anecdotal. This proposal addresses the potential role of natural phytoestrogens in osteoporosis prevention among postmenopausal women living in Europe. A one-year large-scale, multicentre, randomised, controlled, intervention trial will be conducted in three European countries using specially-designed isoflavone-enriched foods, combined with rigorous assessments of the changes in bone metabolism. Acceptability of these foods among the target population (women 45 years of age and older) and expectations created by different nutritional claims will be addressed by conducting a survey in five EC state members.

The main objective of this project is to provide clear scientific evidence about the effects of soy isoflavones (IF) on bone density in Caucasian, early postmenopausal women living in Europe and perform geographical comparisons on the bone-sparing effects that IF consumption may have after menopause. For this purpose, we aim to develop IF-enriched food products that can be easily incorporated to any European diet and test IF bioavailability in different food matrices. A secondary objective is to explore the perspective of the consumers to which such foods are specifically addressed (women 45 years of age and older) in terms of acceptance, best substitution strategy, and expectations created by different nutritional claims. A third objective is to develop and test IF assays that can be used routinely by validating RIA against GC/SIM for the measurement of daidzein, genistein, cquol and ODMA in urine, and cquol and ODMA in plasma.

Description of work

Highly palatable IF-enriched foods suitable for use in human clinical trials with controlled level and composition of IF (100mg of IF in three servings) will be manufactured using two different food matrices IF composition and content as well as their organoleptic characteristics will be objectively evaluated right after production and over a period of 4 months. Successful products will be manufactured at least in three different flavours each to minimise attrition in long-term consumption. These foods will be used to evaluate the effects of food matrix on total IF plasma levels achieved after 3 days of intake in a short-term crossover study.

To test the efficacy of IF consumption (100mg/d) in preventing bone loss and other menopause-related disturbances, 300 postmenopausal women (between 12 and 60 months after last menses) from three different EC member states (3x100) will be randomly assigned to consume either IF-enriched foods or placebo foods for 12 months. The main outcome is DXA bone density at the spine, forearm and total body (at basely, 6, and 12 months). Secondary outcomes include bone turnover markers, endocrine factors involved in bone metabolism, steroid sex hormones, hot flashes (at all milestones), and vaginal epithelium maturation index (baseline and 12 months). Plasma and urinary IF (genestein, daidzein, cquol and ODMA) will be measured at all milestones (baseline, 3, 6, and 12 months). Finally, a survey in five EC member states will be conducted to investigate the acceptability that IF-enriched foods may have among women 45 years and older, and to explore the underlying motives, meanings, values, situational factors and social influences that drive the potential
consumption of IF-enriched food products. A questionnaire will be developed and validated specially for this purpose by a department specialised in consumer issues.

**Milestones and expected results:**

We expect to produce IF-enriched foods at least in 2 food matrices with 3 flavours each that meet the characteristics specified by the investigators (M1). IF bioavailability in these foods will be tested in 42 postmenopausal women (14x3) recruited in 2 months on cross over study (M4). We expect to reach plasma levels of total IF in the high nM range with both foods tested (M5). If mean differences are greater than 0.15µM/L total plasma IF, only one food will be selected for the main intervention trial, in which 3x100 subjects will be recruited in 6 months and randomised to consume placebo foods or IF-enriched foods for 12 months (M8). It is expected to find differences between groups in total IF plasma levels (= 300-400nM/L), in bone resorption by month 3, and in BMD by month 12 (M9, M10). Simultaneously, a survey will be conducted in five EC state members to assess acceptability of IF-enriched foods (M8).
CHAPTER II

RESEARCH PROJECTS
RELEVANT TO ENDOCRINE DISRUPTION
Comprehensive risk analysis of dioxins: development of methodology to assess genetic susceptibility to developmental disturbances and cancer

**Contract N°:** QLK4-1999-01446  
**Project type:** shared cost

**Project Cost:** € 2,705,693  
**EC contribution:** € 1,470,000

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**Project start date:** February 2000

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Title

Comprehensive risk analysis of dioxins: development of methodology to assess genetic susceptibility to developmental disturbances and cancer.

Objectives

The objective is to set a scientifically defendable 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:

To study the molecular mechanisms of dioxin toxicity in a multidisciplinary manner, utilising the mutated receptor causing a remarkable resistance towards dioxin toxicity.

To resolve in population studies the sensitivity of human being to developmental effects (tooth defect and cleft palate) cancer, and compare these outcomes to results in experimental animals.

To perform an up-to-date dioxin risk assessment.

Scientific approach

The work can be divided to five major parts, all of which contain input by several partners. Mechanistic studies aimed at increasing understanding on the mechanism of toxic actions of dioxins. This is necessary, because there are several serious gaps in the information, and a science-based risk assessment is not possible without understanding the basics. This part utilises mutated dioxin receptor genes which the partners have demonstrated in experimental animals, and by means of molecular biology techniques attempt, to pinpoint the critical steps in toxic mechanisms.

Human levels in population are established by measuring dioxin-like compounds in fat (breast milk, surgical samples, placentas).

The sensitivity of human beings to the developmental effects (especially tooth defects in children) is scrutinised in population studies, and compared with the sensitivity of normal and mutated animals to find out the capability of data to predict human effects.

The risk of cancer is studied in human populations by correlating the cancer risk with dioxin concentrations in the body. Especially important is soft tissue sarcoma which is thought to be associated with dioxin exposure.

A comprehensive risk assessment exercise is performed on the basis of the whole data set obtained from the proposed studies directed at critical data gaps, and also fully utilising previous information. In an attempt to help decision makers, this continues with a policy-driven risk analysis exercise to illuminate all factors needed in the decision-making process.
Developmental neurotoxicity of polybrominated diphenylether: mechanisms and effects

Contract No.: QLK4-1999-01562  
Project Cost: € 1.554.092  
EC contribution: € 800.000  
Project Duration: 36 months  
Project start date: February 2000

EC scientific officer: Callum SEARLE

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Title

Developmental Neurotoxicity of Polybrominated Diphenylether: Mechanisms and Effects.

Objectives

The goal of the proposal is to elucidate the effects of developmental exposure of animals to flame retardants Polybrominated Diphenyl Ethers (PBDE) on CNS. Epidemiological studies indicate a marked increase of PBDE levels in breast milk. In contrast, effects of PBDEs on the developing CNS are largely unknown. Therefore, the aims of the proposal are:

i.) to assess several neurobehavioral endpoints after perinatal treatment of animals for characterisation of possible impairments;
ii.) to relate neurobehavioral effects to electrophysiological effects in the different brain areas;
iii.) to compare perinatal with adult exposure in order to examine if the developing CNS is particularly susceptible to PBDEs;
iv.) to compare different animal species for generalisation of the findings;
v.) to characterise mechanisms of PBDE neural toxicity at cellular and molecular levels including neurotransmitters, receptors and intracellular signal transduction pathways;
vii.) to examine if exposure alters sexual differentiation of the brain.

Different dose levels of PBDEs will be studied. PBDE exposure will be compared with PCB-induced effects to relate findings to a better examined substance group.

Scientific approach

For the examination of developmental neurotoxicity of PBDEs the congener 2,2’,4,4’,5-pentaBDE (PBDE 99) will be studied since this congener is the one of the most abundant PBDEs. Research will be conducted in rats and in mice as well as in vitro models for the characterisation of underlying mechanisms. Animals will be exposed perinatally at different dose levels of PBDE 99. In addition, a PCB-exposed group will be used to compare effects of PBDE exposure to the known effects of PCB exposure. Several neurobehavioral tests will be conducted in different laboratories. These are active and passive avoidance, catalepsy, social and sexual interaction, spatial learning and memory, sleep/wake cycle, and circadian rhythms. Alterations in these behavioural tests should result in a profile of effects which can be related to effects on different parts of the brain, e.g.: passive avoidance is dependent on the amygdala and spatial learning on the hippocampus. Behavioural results will be related to neurophysiological functions in different brain areas, in particular, to long-term potentiation which is an electrophysiological correlate of neuronal plasticity. Underlying effects of PBDEs on glutamatergic and dopaminergic neurotransmitters and receptors will be studied ex vivo and in vitro. In addition, cholinergic receptor density and binding will be examined ex vivo. Several signal transduction pathways in neurons and glia cells will be investigated in an ex vivo approach. Sexual differentiation of the brain will be studied ex vivo by determination of enzymatic targets of steroids and anti steroid-dependent gene regulation. The outcome will be related to sex-behaviour. All groups will supply blood and tissues samples which will be collected by the coordinating institute for chemical analysis to determine internal exposure to PBDEs in dams and offspring.
Genetic polymorphisms and biomonitoring of styrene

Contract N°: QLK4-1999-01368
Project type: shared cost
Project Cost: € 2.194.850
EC contribution: € 1.360.000
Project Duration: 36 months
Project start date: February 2000

EC Scientific Officer: Callum SEARLE

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Title

Genetic polymorphisms and biomonitoring of styrene.

Objectives

The following questions will be studied:
Effect of CYP2E1, EPHX, GSTM1, GSTP1 and GSTT1 genotypes on macromolecular adduct levels and on cytogenetic parameters in human whole-blood lymphocyte cultures in vitro
Effect of the genetic polymorphisms on internal dose by comparing styrene (and SO) concentrations in personal breathing-zone air samples with different genotype constitutions;
Effect of the genetic polymorphisms on the biologically effective dose of styrene by determining styrene oxide adducts in haemoglobin and DNA of mononuclear blood cells in volunteers and workers with a known genotype constitution;
Effect of the genetic polymorphisms on the biologically effective dose of styrene by determining chromosome aberrations, micronuclei and sister chromatid exchanges in peripheral lymphocytes of styrene exposed workers and unexposed controls with known genotype composition;
Assessment of CYP2E1 phenotype.

Scientific approach

The present project aims at the clarification of the potential role of the genetic polymorphisms in CYP2E1, EPHX, GSTM1, GSTP1 and GSTT1 genes in determining individual responses to styrene exposure. The blood samples of exposed subjects will be studied for adducts in DNA and blood proteins, and cytogenetic changes in peripheral lymphocytes. Cytogenetic alterations will be examined both by microscopic methods and new molecular genetic techniques. The exposure to styrene will also be assessed by personal air sampling and urine metabolite analysis.

The project is built upon co-operation between ten European laboratories, with the idea that techniques developed and experience gained in one laboratory could be utilised in another. To this end, the tasks of different partners have been organised into 5 work packages, which complement each other.

The objectives of the workpackage 1 are:

to develop alternative methods to assess CYP2E1 phenotype, which would be more convenient for implementation in large epidemiological studies and in occupational medicine;
to clarify the still unclear correlation between epoxide hydrolase genotypes and phenotypes.

The main objective of workpackage 2 is to examine the influence of polymorphisms for CYP2E1, GSST1 and EPHX on the interpretation of biomarkers of styrene exposure.

In workpackage 3 the macromolecular adducts in the leucocytes of styrene exposed subjects will be examined.
In the workpackage 4 the cytogenetic parameters will be analysed in the leucocytes of styrene exposed subjects.

In workpackage 5 the genotyping for CYP2E1, EPHX, GSTM1, GSTP1 and GSTT1 genes will be performed using the total white blood cell DNA from all the study subjects. The genotype data will then be correlated with the data from biomonitoring studies using parametric and non-parametric approaches. The extent of the association between exposure and outcome will be evaluated, with particular concern for the presence of effect modification attributable to susceptibility biomarkers. Potential confounders investigated through the questionnaire as well as the interaction terms will be evaluated by means of multivariable regression analysis, i.e., Poisson regression, Logistic regression and Log-linear models. The effect of the exposure in the genotype subgroups will be estimated for each biomarker in terms of frequency ratio with its 95% confidence interval.
Evaluating human health risk from low-dose and long-term PCB exposure

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Evaluating human health risk from low-dose and long-term PCB exposure.

Objectives

The overall objective of this project is to evaluate the human health risks of low-dose and long-term exposure to a group of persistent organochlorine pollutants, including polychlorinated biphenyls (PCBs) and their metabolites, organochlorine pesticides, polychlorinated dibenzo-\(p\)-dioxins (PCDDs) and dibenzofurans (PCDFs) within a population that has been exposed to these chemicals as a result of environmental pollution.

Scientific approach

The following parameters will be investigated in human subjects living in both a polluted and a background area:
the levels of PCBs, their metabolites (PCB-OH, PCB-MeSO\(_2\)) coplanar PCBs, PCDDs, PCDFs, and organochlorine pesticides (HCB, HCH, DDT, and DDE isomers)
estrogenic activity of sera by \textit{in vitro} bioassays,
dioxin-like activity of blood by \textit{in vitro} bioassays,
antibodies against insulin-producing cells, thyroperoxidase and TSH-receptor,
broad spectrum tumour marker levels,
thyroid hormone, TSH and testosterone (in males) levels,
prevalence of several gonadal and reproductive disorders,
behavioral and cognitive disorders in children,
sensorineural hearing function in children,
exposure of children to other neurotoxic effects that may act as confounders (Hg, Pb, Mn, Cd, Se),
incidence and prevalence of certain types of tumours, allergy, diabetes mellitus, birth defects, and endocrine disorders.

Through the use of a multidisciplinary approach, we expect this project to have two major achievements. The first is to obtain information contributing to the further elucidation of the PCB toxicity (acting alone or in combination with other organochlorines) that will be useful for the assessment and management of environmental hazards. The second achievement will be to define, validate, and apply biomarkers that will be relevant to the assessment of health risks for both adult and juvenile populations.
REMPHARMAWATER - Ecotoxicological assessment and removal technologies for pharmaceuticals in wastewaters

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**Title**
Ecotoxicological assessments and removal technologies for pharmaceuticals in wastewaters (REMPHARMAWATER)

**Objectives**
The presence of pharmaceuticals in natural and drinking waters has been reported in recent years in the literature. Sewage treatment plants were pointed out as the major source of the discharge for pharmaceuticals to the environment. Although these compounds have been found in the environment at very low concentrations, damages to man and living organisms can not be ruled out as demonstrated for other xenobiotics. Few indications are available for pharmaceuticals on their persistence in the environment, toxicity and removal mechanisms. No systematic investigations have been carried out in Europe. The present project will contribute to an evaluation of the threat by pharmaceuticals present in the environment to human and animal health and, more generally, to the environment. In addition, advanced technologies to remove these chemicals at their sources of discharge, thus preventing their entering into the environment, will be developed.

**Scientific approach**
The prevention of pollution of water resources and more generally the protection of the environment are the ultimate goals of the present project. This aims specifically at: i) assessing the presence of pharmaceuticals in waste waters and sludges of municipal sewage treatment plants (STP) in participating countries (Italy, Greece, France, Sweden) and identifying those at environmentally relevant concentrations; ii) assessing the fate of pharmaceuticals in conventional biological processes; iii) evaluating the persistence of these pharmaceuticals in the environment, that is their refractoriness to undergo biotic and abiotic degradation process; iv) evaluating the ecotoxicity of pharmaceuticals found in STP effluents with respect to living organisms such as algae and invertebrates and fishes; v) evaluating the possibility of removing the pharmaceutical substances in STP effluents by means of integrated biological processes or AOP techniques.

These aims will be achieved through the adoption of a combination of chemical and ecotoxicological methods.

**Expected impacts**
Generally speaking it can be expected that the results of this project will draw the attention of Authorities which have the responsibility of public health and environmental protection on the problems related to the presence of pharmaceuticals in water. Specifically it can be expected that these results will stimulate European and National Authorities to prepare new directives and/or regulatory issues on the quality of drinking water and/or treated waters and ecotoxicological risk assessment procedures before marketing new drugs.

Novel technologies developed within the present project are practically applicable and therefore expected to be adopted for wastewater treatment. These technologies can be used for up-grading the already-existing wastewater treatment plants and for the ones to be constructed in connection with the urban wastewater treatment Directive (UWWT Directive).
POSEIDON - Assessment of technologies for the removal of pharmaceuticals and personal care products in sewage and drinking water facilities to improve the indirect potable water reuse

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Assessment of technologies for the removal of pharmaceuticals and personal care products in sewage and drinking water facilities to improve the indirect potable water reuse - POSEIDON

Objectives

Municipal wastewater contains a multitude of persistent organic compounds derived from domestic application such as active ingredients in pharmaceuticals and personal care products, which are used in large quantities throughout the world. Here both groups will be collectively referred to as "Pharmaceuticals & Personal Care Product ingredients" (PPCPs). PPCPs passing wastewater treatment systems are continuously infused to the environment via WWTP discharges and are present in the feeding water (groundwater, bank filtrates, surface water) of waterworks. In some cases even drinking water is contaminated with PPCPs. Pharmaceuticals are designed to induce specific biological effects at specific target organisms for a limited period of time. The continuous, wide spread, long-term exposure of PPCPs to the environment and humans, although at low concentration levels, may result first in gradual almost hardly detectable changes, however, in the long run significant impacts on the environmental and human health can not be excluded. In particular, the release of antibiotics into the environment may induce the development of resistant bacterial strains. To reduce the risks of unforeseeable long-term side effects of PPCPs to the environment and human health, and to circumvent a life-long consumption of low doses of potentially toxic PPCPs through drinking water, POSEIDON develops methods which will reduce the uncontrolled releases of PPCPs to the environment via wastewater. Further, POSEIDON intends to enhance efficient and unpolluted water supply and to specify the potential risks of PPCPs to the environment.

Scientific approach

The scientific objectives and approach can be summarised as follows:

Comprehensive studies to assess and improve the PPCP removal efficiency of conventional and advanced wastewater as well as drinking water technologies.
Investigations of conventional and advanced drinking water treatment with respect to their efficiency in eliminating of selected PPCPs.
Investigation of source control by urine separation, thus reducing the contamination levels of pharmaceuticals in wastewater, and hence facilitating the treatment.
Studying of irrigation in agricultural areas to assess the pollution of groundwater by PPCPs.
Detailed analysis of selected PPCPs in wastewater and drinking water treatment processes to elucidate fate and - if possible - degradation pathways.
Performance of Environmental Risk Assessments (ERAs) in relation to conventional and advanced wastewater technologies. The following steps of an ERA will be outlined:
Hazard identification to specify the environmental compartment which might be at risk when PPCPs are released into the environment.
Exposure assessment to determine the predicted environmental concentration in the compartment of concern.
Effect assessment to determine the predicted no-effect concentration in the compartment of concern.
Risk characterisation to quantify the risk which might be posed to specific environmental compartments by PPCPs.
Completion of a strategy for indirect potable water reuse of treated urban wastewater considering the distinct PPCP contamination in the wastewater, and thus combining wastewater and drinking water technologies.

Expected impacts:

Since elevated efficiency in wastewater treatment guarantees lowered pollution of the environment and reduced efforts for drinking water treatment, the main topic of *POSEIDON* is based on the PPCP removal in wastewater treatment including the aspect of sustainable source control. In accordance with European policy, the sustainability of water supply is the essential element of *POSEIDON* and is highlighted by the following aspects:

Improvement of wastewater treatment which guarantees that the contamination of the environment with PPCPs is reduced and that the costly end-of-pipe approach (drinking water treatment) can be at least partly reduced.
Integration of urine separation as a novel approach to reduce the amounts of PPCPs into the wastewater treatment and hence reducing treatment requirements and costs.
Augmentation of water supply by indirect potable water reuse of treated wastewater in areas where the growth of urbanised population has exceeded the quantity of available natural water sources.
Development of guidance for competent European authorities on the performance of ERAs specific for PPCPs.

The precautionary principle for providing drinking water is applied by testing the removal efficiency of existing facilities and by delivering options for optimised treatment sequences. Otherwise, a non-calculable risk of drinking water consumer and the aquatic biota might be emerging. After finalizing *POSEIDON* the end-users (e.g. waterworks, wastewater treatment facilities) have the opportunity to use the results for minimizing their loads of PPCPs by optimizing the treatment processes. In areas with water scarcity *POSEIDON* will provide data on the efficiency of PPCP removal at affordable costs for the planned indirect reuse of water, i.e. for augmentation of water supplies with reclaimed water derived from treated municipal wastewater. Furthermore, *POSEIDON* gives the end-users an instrument to assess the risks caused by PPCP contamination of soil and groundwater.
FAMIZ - Food web uptake of persistent organic pollutants (POP) in the Arctic marginal ice zone of the Barents Sea

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Abstract

The Arctic has a symbolic value as being a pristine area untouched by human industrial activities. Field data indicate that Arctic food webs are remarkably efficient in accumulating persistent organic pollutants (POP). This has major implications for the health of indigenous people and commercial fishing values. The objectives of the project are to understand why the Arctic systems are so vulnerable to POP contamination; which are the major input process for different POP and which physical-chemical properties of the different POP are the most important for bioaccumulative potential. FAMIZ will provide process based predictive models of POP accumulation in Arctic food webs.
BEEP - Biological effects of environmental pollution in marine ecosystems

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Abstract

Biological markers allow the direct determination of pollutant impact of living organisms in aquatic systems. While new emerging biomarkers are actually under evaluation, some common markers are in a validation-phase and may be used as assessment tools for the quality of the marine environment. The goal of this research programme is to evaluate the potential of using biological marker determined in marine organisms as a means of assessment of chemical contamination and to investigate the socio-economic implications for certain selected zones. The results of the BEEP project will be of importance for various Community policies including Water Quality Objectives and fishing policies. It will provide scientific and technical (and to an extent societal approaches) tools for regional development policies and management of coastal and marine areas.
BIOCET – Bioaccumulation of persistent organic pollutants in small cetaceans in European waters: transport pathways and impact on reproduction

Contract No: EVK3-CT2000-00027  Project type: shared cost
Project Cost: €1.616.510  EC contribution: 1.183.996 €
Project Duration: 36 months  Project start date: February 2001

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Abstract:

This proposal aims to quantify and model the transport pathways and impact on reproduction of bioaccumulation of persistent organic pollutants (POPs) by four species small cetaceans in European Atlantic waters. Following a 36 month workplan, a partnership 5 European countries will sample and analyse small cetaceans collected by strandings networks. Females in good condition will provide samples for detailed individual studies while wider sampling and access to historical material will help quantify population parameters. Data will be collected on histopathology, age, reproduction, diet; POP and toxic element burdens. Reproductive success will be estimated from individual past-pregnancy indices and population pregnancy rates. Results will be synthesised to identify vulnerable populations and to produce simple empirical models of bioaccumulation in individuals from each population in relation to dietary inputs.
INTERPOL – Impact of natural trawling events in resuspension, dispersion and fate of pollutants

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Abstract:

The Mediterranean coastal zones experience intense trawling activity and severe storm surges inducing significant resuspension-release of nutrients, pollutants and toxic elements. Due to their oligotrophic character the resuspension processes are expected to play a key role in the carbon, nutrients and pollutants recycling in the Mediterranean coastal ecosystem. INTERPOL project, bringing together complementary expertise and know-how, in an unprecedented way, will provide new and unique data towards the understanding of the environmental impact of resuspension processes in the Mediterranean coastal ecosystem. It will also help to constrain and to identify the complex routes of pollutants transfer within the marine system. These are important steps towards a better assessment of the marine cycling of natural and anthropogenic substances, including toxic ones, and thus towards a better protection of the Mediterranean from pollution.
ADIOS – Atmospheric deposition and impact of pollutants, key elements and nutrients on the open mediterranean sea

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Abstract:

ADIOS is a focussed pan-European study of the atmospheric deposition and impact of pollutants, key elements and nutrients on the Mediterranean open sea. The extent to which atmosphere represents an important pathway for various pollutants and natural substances from Europe and North Africa towards this ecologically sensitive marine environment, and subsequently affects its biogeochemistry remains largely unknown. The magnitude and quality of atmospheric deposition will be assessed by an atmospheric sampling network encompassing the whole basin, as well as by the extensive use of Sea WIFS data and dust transport-deposition modelling. Biogeochemical processes, levels and ecotoxicological effects induced by natural and anthropogenic aerosols will be examined, from surface down to the deep sediments, in two central sites of the Eastern and Western basins, using oceanographic surveys, moored instrumentation and 3D ecohydrodynamical modelling.

Web-site: http://adios.univ-perp.fr/
Airwin – structure and role of biological communities involved in the transport and transformation of pops at the marine-water interface

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Abstract:

The aim of this proposal is to investigate the structure of biological communities living and growing in the sea surface microlayer (SML) and their role in the transport and cycling of natural organic matter and xenobiotics. This research proposal will provide original data on the identification of organisms living in the SML. It will provide both scientific and biotechnological communities with a collection of organisms with information on their role in the transformation of specific pollutants.