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Abstracts of Theses from the Scandinavian Countries

Abstracts of Scandinavian theses on oncologic subjects are published under this heading. The full theses are as a rule published by the universities or as supplements to different journals. They can usually be obtained after contact with the author.

Environmental and genetic factors in lung cancer—Epidemiological and biomolecular studies focusing on nonsmokers

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Tobacco smoking is the major cause of lung cancer. Other risk factors include exposure to environmental tobacco smoke (ETS), dietary habits and genetic factors. When studying such risk factors, confounding from smoking presents a pervasive methodological problem. The aim of this thesis was to study some risk factors for lung cancer other than smoking, by focusing on never-smokers, and to clarify some methodological aspects of studies of passive smoking and lung cancer.

A case-control study involving 359 never-smokers (124 lung cancer cases and 235 population controls) was conducted in Stockholm in 1989–1995. The never-smoking status of participants was validated by interview with next-of-kin. Detailed information on ETS exposure was collected at personal interviews and information on dietary habits as well as residential and occupational histories were obtained. From 1992, blood was also drawn from participants, and 193 smokers (99 cases and 94 controls) were additionally recruited, for studies of genetic factors in lung cancer.

Smoking misclassification was initially studied using reports of smoking several years apart in two large survey cohorts (18419 and 9558 individuals). Approximately 5% of ever-smokers were misclassified as never-smokers on the second occasion. They were, however, mainly light smokers or long-term former smokers and in a follow-up 1961–1992 had only moderately elevated relative risk of lung cancer (1.9, 95% confidence interval 0.4–9.1). Misclassification of such smokers was shown to be unlikely to produce spurious risks large enough to explain the observed association between lung cancer and passive smoking. In the validation of never-smokers within the case-control study, only 1.2% were reported by next-of-kin to be former regular smokers and 2.6% to ever have smoked >400 cigarettes, but most had stopped long ago (median 21 years) and all had smoked less than 400 packs. These results suggest misclassification is even more limited with careful screening of smoking habits.

For passive smoking, increased relative risks were suggested for ever cohabiting with a smoking spouse (1.2, 95% confidence interval 0.7–1.9) and for ever exposure at work (1.6, 0.9–2.9). Risks tended to be more elevated in high exposure groups and with recent exposures. Both sources of ETS appeared important and considerable misclassification of total exposure occurred for each variable used separately, in particular for ever/never variables and for the less common spousal exposure used alone. When combined, the relative risk for those currently exposed to ETS from the spouse and/or at work was 2.6 (1.0–6.5) in comparison with those unexposed to either source.

Among never-smokers, a protective effect was associated with consumption of vegetables, mediated primarily by carrots. Non-citrus fruits were likewise protective, whereas citrus fruits and juice instead were associated with a suggested increase in the risk of lung cancer. A protective effect with dose-response was also seen for beta-carotene and total carotenoids. Increased risks were suggested for cultured milk products in both genders and for milk among high consumers in men only, which may be consistent with dietary fat as a risk factor for lung cancer.

The overall relative risk for lung cancer associated with the glutathione S transferase null genotype was only slightly below unity, with lower relative risk suggested among never-smokers. For N-acetyl transferase 2, there was a suggested increased risk for slow acetylators among never-smokers, whereas among smokers a steeper increase in risk with increasing pack-years of smoking exposure was suggested for rapid acetylators. Aromatic DNA adducts and mutations in the hypoxanthine-guanine phosphoribosyl transferase (*hprt*) gene in white blood cells were investigated as markers of exposure and early genotoxic effect. Adduct levels were higher in current than in former or never smokers, whereas both former and current smokers had elevated *hprt* mutant frequencies. Age affected both markers significantly, and they were also associated with smoking dose, particularly among cases.

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The immunoglobulin heavy chain gene as a clonal marker in lymphoma and leukemia

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A detailed characterisation of clonal development may have important implications for our understanding of tumour progression and clinical outcome in malignancies. The immunoglobulin heavy chain (IgH) gene has been frequently used as a marker for the evaluation of clonality and clonal evolution in lymphomas and leukemias of B-cell origin. In addition, the variable heavy chain (V_H) gene usage may contribute to an understanding of the pathogenesis of these diseases.

In order to assess the V_H gene usage, V_H gene family specific polymerase chain reaction (PCR) amplification was performed in different subgroups of B-cell lymphoma (BCL). No apparent restriction in V_H gene family usage was demonstrated in follicular lymphomas, lymphoplasmacytoid lymphomas or large B-cell lymphomas, whereas a biased V_H1 utilisation was observed in chronic lymphocytic leukemia. The majority of the V_H1 rearrangements utilised the DP10 gene, which has been reported strongly associated with autoimmune disease.

The detection of clonal populations is essential for the study of clonal evolution and for the search of minimal residual disease (MRD). To evaluate the capacity of detecting PCR amplified IgH rearrangements, four different systems were compared in materials of precursor-B acute lymphoblastic leukemia (pre-B ALL) and BCL. Agarose gel electrophoresis, polyacrylamide gel electrophoresis and two techniques for single strand conformation polymorphism analysis were performed, which revealed that the latter techniques were superior in detecting clonal alterations.

Ongoing clonal evolution may hamper the detection of MRD using clone specific markers. To analyse the extent of clonal evolution, the IgH rearrangement status was characterised in

relapsing pre-B ALL and BCL. In lymphomas, alterations of the rearrangement status were mainly observed in follicular or transformed lymphomas and resulted from a low frequency of somatic hypermutations. In relapsing leukemias and one case of relapsing T-cell lymphoma, secondary rearrangement events such as V_H gene replacement, V_H to DJ_H joining and diversification of the V_H-D junction were demonstrated. The emergence of new rearrangements was also shown.

Despite these ongoing modifying events, the majority of tumour cell clones analysed were relatively stable during tumour progression, indicating that they would be eligible for analysis of MRD using the V_H gene regions as molecular markers in lymphomas and leukemias.

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Risk factors for ipsilateral breast tumor recurrence and uncontrolled local disease

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The aims of the study were to assess intrinsic and treatment related risk factors for ipsilateral breast tumor recurrence (IBTR) and uncontrolled local disease (ULD) following breast-conserving surgery of early-stage invasive breast cancer.

In a cohort of 759 women with T1–T2 tumors risk factors were evaluated. The majority of the patients (88%) had received postoperative irradiation to the breast. A continuous 1–1.5% yearly increase in IBTR was seen. Three independent risk-factors for IBTR were identified, age < 50 years, no postoperative irradiation and positive lymph nodes. Radiotherapy reduced the IBTR-rate, but the effect decreased with time. Node-negative women ≥ 50 years constituted a low risk-group for IBTR having a cumulative risk at 10 years of 9% without radiation and 5% with breast radiation.

In a case-control study 'nested' within the cohort, biological markers were assessed. Sub-groups of patients with low and high risk for IBTR were identified, namely women ≥ 50 years with proliferative index mib-1 $\leq 30\%$, who had an odds-ratio of 0.2 and patients < 50 years with tumors showing both c-erbB-2 and waf-1-immunoreactivity with an odds-ratio of 6.7.

Salvage mastectomy among patients with IBTR provided a superior local control rate compared to reexcision. A higher although not statistically significant rate of ULD was also seen in patients who had not received postoperative radiotherapy as part of their primary treatment.

In postmenopausal, node-negative breast cancer patients treated with breast-conserving surgery and radiotherapy, the addition of tamoxifen was evaluated in a population of 432 women, who constituted a separate stratum of the Stockholm Adjuvant Tamoxifen Trial. An improved event-free survival and a reduced rate of ipsi and contralateral breast tumor recurrences was accomplished in patients allocated to tamoxifen.

Mammograms from 69 patients with invasive breast cancer, operated on with breast-conserving surgery between 1987–1990 were blindly reevaluated for prediction of IBTR. The study cohort was divided into two groups of 34 and 35 patients, respectively. Each group was matched according to age, the time at risk and to presence or absence of IBTR. The mammographic reinterpretation correctly predicted an IBTR in 81%. Mammographic characteristics, such as diffuse microcalcifications, multifocality, solitary densities of stellate type near the nipple and/or solitary densities with spicula in the vicinity of the retroareolar region, correlated with the ability of the tumor to recur locally.

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Positive and negative angiogenic factors in patients with malignant disease

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In this thesis, the levels of positive and negative angiogenic factors were studied in tissue, serum and urine from patients with malignant disease.

The angiogenic factors VEGF (vascular endothelial growth factor) and bFGF (basic fibroblast growth factor) were studied in breast carcinomas. In one of the materials studied, we did not find a correlation between any of these factors and the number of microvessels in the tumors. A correlation was found, however, between strong staining of bFGF in the stromal compartment of breast carcinomas and the levels of a metalloprotease expressed by stromal fibroblasts, stromelysin-3.

In a material of small (≤ 1 cm), mammographically detected breast carcinomas, low intratumoral microvessel densities were detected. Small breast tumors were found to show low expression of VEGF by immunohistochemical staining. These findings are of interest, since they imply that the excellent prognosis of patients with small breast carcinomas may be related to the limited angiogenic potential of their tumors.

Other groups have reported that tissue VEGF is a prognostic marker for some malignancies. Determination of serum VEGF has also been suggested to be of prognostic value. In the present study, we examined the relationship between tissue and serum VEGF using samples from patients with head and neck carcinoma. We found no correlation between the levels of tissue and serum VEGF in the same patients.

Serum levels of VEGF were generally increased in patients with malignant tumors. Serum VEGF levels correlated with tumor stage in patients with head and neck carcinoma. Serum VEGF may turn out to be a useful tumor marker for some malignancies. In our material, we found that VEGF and bFGF were not coexpressed in the same sera.

Western blotting experiments demonstrated, for the first time, the presence of angiostatin, an inhibitor of angiogenesis, in the urine of some patients with solid tumors. N-terminal sequencing suggested that the structure of angiostatin from cancer patients is similar to that described in Lewis carcinoma bearing mice. The levels of urine angiostatin were found to be dependent on kidney function.

In conclusion, increased levels of VEGF, bFGF and angiostatin could be demonstrated in samples from cancer patients. Although these factors are likely to have important physiological functions, the prognostic value of their determination in cancer patients remains to be established.

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Psychological responses to gastrointestinal cancer

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The overall aim of the present thesis is to gain knowledge about psychological distress and adjustment in gastrointestinal cancer patients (colon, rectum, gastric, pancreatic or biliary) at various phases of their disease.

Reactions to the diagnosis, anxiety, depression and coping were investigated in newly diagnosed patients (n = 139). Repeated assessments were performed throughout the first year after the diagnosis. Only a limited group reported high levels of anxiety (17%) and depression (21%) close to the diagnosis. Patients with colon or rectal cancer, most of whom were potentially cured, had a more confronting attitude to their diagnosis and reported more 'Fighting Spirit' than patients with gastric and pancreatic/biliary cancer. These responses were associated with better emotional well-being. The former group also reported less 'Hopeless/helplessness' and 'Anxious preoccupation', which were related to higher levels of psychological distress. There were no changes over time in mean levels of anxiety and depression and virtually no changes in mean values of the coping subscales. In a separate group (n = 141), overall levels of anxiety, depression and worry were low in conjunction to a medical follow-up control visit approximately two years after diagnosis.

Levels of anxiety and depression at diagnosis predicted a similar status six months later. A model based on standardised cut-off scores of moderate or high levels of anxiety or depression and intrusive thoughts close to the diagnosis was used to identify patients with prolonged psychological distress.

A psychometric analysis was performed of the Mental Adjustment to Cancer (MAC) scale (n = 868 patients with various cancers). The reliability of the original subscales was satisfying. A confirmatory factor analysis revealed a factor structure including 28 of the original 40 items in four factors. Both versions of the MAC confound coping efforts and emotional outcomes, preventing analyses of coping-outcome relations.

The main conclusion is that a majority of gastrointestinal cancer patients cope well with their disease in the short as well as in the long run.

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Molecular mechanisms of tumor development in hyperparathyroidism

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This thesis is based on molecular characterization of parathyroid tumors and families with hyperparathyroidism (HPT).

Loss of heterozygosity (LOH) analysis and comparative genomic hybridization (CGH) were used in an attempt to identify recurrent chromosomal alterations in different types of parathyroid tumors. These included familial and sporadic, benign and malignant, as well as primary, irradiation associated and secondary tumors. Hitherto unidentified tumor suppressor genes were implicated on chromosomes 1p, 6q, 9p, 11p, 11q, 13q, 15q, 18q and X, as well as a putative oncogene locus represented by a gain of DNA copy number on chromosome 19. Since many genes responsible for familial cancer syndromes also are mutated in their sporadic counterparts, another approach was to search for deletions of chromosomal regions to which familial syndromes are mapped. The 11q13 region, which harbors the multiple endocrine neoplasia type I (MEN 1) tumor suppressor gene, is deleted in the majority of parathyroid tumors from MEN 1 patients. Similarly, a third of tumors from patients with sporadic primary HPT showed LOH at 11q13 and in half of these cases a somatic *MEN1* mutation could be demonstrated. The sporadic adenomas with *MEN1* involvement also displayed a significantly higher number of CGH aberrations as compared with the non-*MEN1* associated group ($p < 0.05$). The genetic profile obtained for the radiation associated adenomas closely resembled that of the ordinary adenomas with involvement of the *MEN1* gene, i.e. multiple CGH alterations and frequent losses of 11q. Indeed, inactivating mutations of the *MEN1* gene were identified in four of the eight irradiation associated tumors analyzed.

Changes in the calcium receptor (CaR) have been proposed to be responsible for the increase in set-point of parathyroid hormone secretion seen in primary HPT. The level of *CaR* mRNA in the adenomas was in the range of 41–98% (median of 64%) of the expression level in the normal parathyroid tissue. The mRNA level was not found to correlate with serum calcium, parathyroid hormone or adenoma weight. It seems likely that the reduced levels of receptor mRNAs and protein is a secondary phenomenon, rather than a primary event.

Two families with HPT-JT syndrome were investigated and in both families renal hamartomas or cystic kidney disease were prominent associated features, possibly representing a new phenotypic variant of the HPT-JT syndrome. A sex dependent penetrance of HPT was seen, resulting in mainly male affected cases. Three large previously unreported FIHP families in whom the disease was linked to the chromosome 1q21–q32 region were analyzed and two of these families were considered to be true FIHP families, i.e. there was no evidence of jaw or renal lesions despite careful radiological investigations. In the third family, a parathyroid cancer and two cases of polycystic kidney disease were found and that family was therefore considered as being affected by the classical HPT-JT syndrome. The same sex-dependent penetrance of HPT was seen in the FIHP families as in the HPT-JT families. LOH analysis showed loss in the 1q region of the wild type allele in the renal hamartomas and in some of the parathyroid tumors, including the parathyroid cancer, suggesting that this gene is a tumor suppressor gene.

Parathyroid cancer is a rare cause of primary HPT. Nevertheless this group creates diagnostic and therapeutic problems, since in the absence of invasion of adjacent organs or structures and/or metastases, the diagnosis of parathyroid cancer cannot be definitely established based on histopathology. The expression of Ki-67, RB and Gelatinase A were investigated as possible tumor markers in parathyroid cancers. However, none of these markers was suitable for reliable differentiation between benign and malignant parathyroid tumors.

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Etiology of oral cancer

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Oral cancer is a disease with increasing incidence in most West European countries. In Sweden in 1995, intraoral cancer accounted for 1.4% and 0.8% of malignant tumours among men and women respectively. The disease has a bad prognosis and approximately half of the patients will die of their disease.

The aim of this investigation was to focus on the relationship between oral cancer and a suite of potential risk factors. The prime focus was to investigate the risk of snuff use but smoking tobacco, alcohol consumption, infections, dental factors, dental x-ray, iron deficiency, occupations and occupational exposures were also studied using the case-control design. The study was conducted in Northern Sweden and encompassed 410 cases and as many controls. Furthermore, two molecular epidemiological studies were done regarding the relationship between exposure factors and certain biological parameters of tumours.

The use of oral snuff is an increasingly common habit in Sweden. Contrary to previous American studies of cancer risk from oral snuff use, this study showed no elevated risk for oral cancer. Smoking was an important risk factor for oral cancer, but this was restricted to current smokers. Alcohol consumption showed a clear dose–response relationship to oral cancer, and the combination with smoking increased the risk further.

Moreover, the case-control study strongly indicated recurrent HSV-1 infections as an independent causative factor in oral cancer, particularly when the infection was on the lip. Dental factors like different kinds of fillings, fixed prosthesis or removable dentures were not associated with increased risks, nor were dental x-rays.

Pulp industry workers and wood or wood product workers were found to have increased risk for oral cancer. Smoking and alcohol in addition increased the risk. Exposure to phenoxyacetic acids indicated an increased risk.

Oral infections, and HSV-I infections in particular, were associated with an increased risk for oral cancer in patients with p53 positive tumours as demonstrated by immunohistochemistry (IHC). However, this relation was not found in patients with p53 mutated tumours confirmed by PCR techniques. This suggests that HSV-1 infection, directly or indirectly, can inactivate p53 function by binding of wild type protein. No association between smoking and p53 positive tumours was found.

A new non-random deletion in exon 8 in oral squamous cell carcinoma was found. The cause and clinical significance of this new 14 base pair deletion is unknown. Eighty percent of the patients with the deletion were women. No correlation was found for the deletion group with the known risk factors for oral cancer such as smoking.

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Cancer risks in Swedish pesticide applicators in agriculture, forestry and gardening—A toxicological and epidemiological approach

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The aims of this thesis were to study the cancer risks in Swedish pesticide applicators, which is one of the occupational groups that is most exposed to pesticides in Sweden. This could be done by utilizing a complete register kept by the Swedish Board of Agriculture which include 20245 subjects with licenses issued between 1965 and 1976, for epidemiological studies. This was possible by linkage between different registers and identifying cancer cases in the Swedish Cancer Register; to compare cancer risk in farmers—an occupational group with a similar life style, but with less use of pesticides; to study the effect of use of protective equipment as regards cancer risk when handling pesticides; to update and perform a critical analysis of the overall risk of cancer after occupational use of pesticides; to study the use and composition of phenoxy acid herbicides in Sweden. This is of significant value because phenoxy acid herbicides have been used to a great extent for several decades, and are thought to be risk factors of malignant lymphoma (Hodgkin's disease, non-Hodgkin's lymphoma), and soft tissue sarcoma; and to test the hypothesis that exposure to phenoxy acid herbicides and chlorophenols is a risk factor of malignant lymphoma and soft tissue sarcoma.

Studies on the risk of cancer after exposure to pesticides were reviewed concerning epidemiological and experimental animal studies. It was found that further epidemiological studies with detailed exposure assessment for individual pesticides were needed, taking into consideration work practices, use of protective equipment, and other measures to reduce risk. Calculations of the total composition of commercial phenoxy acid herbicides purchased in Sweden from the late 1940s and onwards have been performed. It was found that the estimated sales increased until the early 1980s and Swedish studies had previously found that old formulations mainly of 2,4,5-trichlorophenoxy acetic acid contained toxic dioxins such as 2,3,7,8-tetra-chlorodibenzo-p-dioxin, as well as chlorophenols, chlorocresols and dimethylaitrosamines in some products. Register studies using the pesticide applicator cohort with a random sample of the cohort and then regarding inquires on the use of pesticides and protective equipment, tobacco habits, and occupations during the 1950s, 1960s, and 1970s were analyzed for risk of malignant lymphoma and soft tissue sarcoma. Although there was rather poor use of protective equipment during pesticide application during the 1950s and 1960s these studies with a mean observation time of 13.9 and 12.2 years, respectively, and corresponding person-years 282000 and 248000 of follow-up could not find any increased risk of soft tissue sarcoma or non-Hodgkin's lymphoma and found only a non-significant increased risk of Hodgkin's disease. The overall risk of cancer was analyzed on the same cohort and a statistically significant decreased risk was found. Significantly decreased risks were also found for liver, pancreas, lung, and kidney cancers. No significant increase in risk was found. Higher risks for pesticide applicators than for agricultural workers were found for testicular cancer, tumors of the nervous system and endocrine glands, and for Hodgkin's disease. The unclear etiology of the increasing incidence of prostate cancer worldwide has provoked much attention and several incidence and mortality studies have found an association between farming and prostate cancer. This study, a follow-up study of Swedish pesticide applicators until 1991, found a statistically significant increase in risk of prostate cancer compared with the general population. The aim of the case-control study was to test the hypothesis that occupational exposure to phenoxy acid herbicides is a risk factor for malignant lymphoma and soft tissue sarcoma and that the use of protective equipment would reduce the risk of cancer. The case-control study was performed nested within the cohort of Swedish pesticide applicators. No increased risk due to the use of phenoxy acid herbicides was found, but a striking finding was that cases wore protective equipment considerably less often than controls and that poor protection increased the risk of malignant lymphoma and soft tissue sarcoma compared to non-users of phenoxy acid herbicides.

This thesis concludes that no evidence of association between the use of any pesticide and increased risk for malignant lymphoma or soft tissue sarcoma, or other cancers, was found but supports the recommendation that protective equipment should be worn in order to minimize the uptake of potentially dangerous pesticides.

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Epidemiological studies including new methods for cluster analysis of acute childhood leukaemia and brain tumours in Sweden

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The aetiology in childhood cancer is essentially unknown. Epidemiological investigations as to whether the incidence rates have changed for paediatric cancers or whether clustering of cases occur may give clues to possible causal factors. One of the main purposes of the present work was to develop improved methods for spatial epidemiological investigations, especially cluster analyses and apply the methods evolved to the two most common forms of childhood malignancies: leukaemia and brain tumors.

Population-based materials of all cases of acute childhood leukaemia during the period 1973 to 1994 and registered brain tumours during the period 1973 to 1992 among children under 16 years of age were analysed. A geographical information system (GIS) was utilised in the management of spatially referenced data on patients and population. Analyses of geographical clustering in space, space–time and of space–time interaction were conducted by essentially new statistical methods, evolved in the work, namely a spatial scan statistic and a modified Knox test. For the brain tumours, analyses of temporal trends were performed by a logistic regression procedure.

No statistically significant geographically localised clusters of childhood leukaemia in Sweden were detected in space or in space–time. Statistically significant space–time interaction was found for acute lymphoblastic leukaemia (ALL) in the analyses using the modified Knox test statistic ($p = 0.01$). Incidence rates in population centres, constituting 1.3% of Sweden's land area and approximately 80% of the population, compared to the rest of Sweden showed a statistically significant excess of (ALL), (OR 1.68, 95% CI = 1.44–1.95) but not of acute non-lymphoblastic leukaemia (ANLL), (OR 1.13, 95% CI = 0.98–1.32).

There was no statistically significant increase in the incidence of acute childhood leukaemia in areas contaminated due to the Chernobyl reactor accident. Statistically significant increasing time trends were observed for the group of childhood malignant brain tumours as a whole ($p = 0.0001$) largely caused by an increase for the astrocytoma subgroup ($p = 0.0001$). The increase of astrocytoma rates was significantly larger for girls than for boys ($p = 0.021$).

No geographically localised clusters were found for acute childhood leukaemia and childhood brain tumours. The space-time interaction found for ALL indicates that environmental factors may be of importance to the aetiology of childhood ALL. No increased risk for ALL or ANLL was found after the heavy fallout in parts of Sweden after the Chernobyl reactor accident. The statistically significant increase of brain tumours, notably astrocytomas in girls, indicates the possible importance of some environmental factors to the aetiology. Within the project important improvements to the methodology of spatial analyses have been developed, which may possibly set a new standard for investigations of disease clusters and clustering.

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Liver tumour promoting effects by polychlorinated biphenyls

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PCBs cause a number of toxic effects and are present in a variety of biological samples including fish, meat and dairy products. Cancer is a serious toxic effect by PCBs that has been observed in animal experiments but the knowledge about PCB induced carcinogenicity is still scarce.

This thesis is focused on tumour promotive effects by PCBs in rat liver. The ultimate goal was to generate data, which would improve risk assessments of these substances. The specific aims were to analyse the tumour promotive effects of some individual PCBs in livers of rats, to study interactive effects of such substances on tumour promotion, and to study foci growth with a biologically based cancer growth model, using experimental data on cell proliferation and foci occurrence for the selection of growth parameters in the model.

The tumour promoting potency of two PCB congeners, 2,3,4,5,3′,4′-hexachlorobiphenyl (PCB156) and 2,4,5,3′,4′-pentachlorobiphenyl (PCB118), was studied individually in a two-stage initiation/promotion rat liver bioassay. The effect of PCB156 was studied after 20 weeks of administration and PCB118 after 20 and 52 weeks of administration. Both substances enhanced the occurrence of preneoplastic lesions in rat liver and relative potencies to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) based on the tumour promotive effect were calculated to be 0.0001–0.001 for PCB156 and < 0.00002 for PCB118.

Cell proliferation in focal and non-focal tissue was analysed after treatment with PCB118 for 20 or 52 weeks. A strong correlation between proliferation and foci occurrence was found in focal and non-focal tissue. Experimentally derived foci- and proliferation data were used to simulated foci growth over time with a biologically based cancer growth model. The simulation indicated that there are at least two types of initiated cells differentially affected by PCB118 and that increased cell proliferation occurs in conjunction with the initiating treatment and in the beginning of PCB treatment.

Interactive effects between compounds were investigated for two different types of mixtures. A complex mixture of PCBs, dioxins and dibenzofurans, similar to that in fish, was studied. The tumour promotive effect was somewhat lower than expected, suggesting that the TEF concept, which is presently used for risk assessment of these substances, overestimates the risk. These results could be explained by the conservative TEF values and/or by weak antagonism between the substances in the mixture. Mixtures of three PCB congeners 3,4,5,3′,4′-pentachlorobiphenyl (PCB126), 2,3,4,3′,4′-pentachlorobiphenyl (PCB105) and 2,4,5,2',4′,5′-hexachlorobiphenyl (PCB153), representing different types of chlorine substitution in the *ortho* position, were studied and the results were analysed with a multivariate method of analysis. Weak antagonism was demonstrated between PCB126 and PCB105 and between PCB126 and PCB153. Collectively, these data suggest antagonistic effects on tumour promotion by mixtures of polychlorinated aromatic hydrocarbons.

In summary, the present study shows that PCB156 and PCB118 can stimulate the growth of preneoplastic lesions in rat liver. The tumour promotive potency of PCB156 is 1000–10000 times less than TCDD and the potency of PCB118 is > 50000 times less than TCDD. The PCB118 promoted foci growth was successfully modeled by a biologically based cancer growth model. At the dose levels tested, additivity or weak antagonism could be demonstrated after exposure to mixtures of polychlorinated aromatic hydrocarbons. Based on these results, there is no immediate reason to change the application of the TEF concept, given the uncertainty of the estimated TEFs and the importance of protecting the general human population through conservative risk assessments.

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