SUMMARY

Environmental exposure is associated with a range of important diseases and it has become clear that chronic exposure to low doses - such as those relevant for the general population - can induce significant effects. Diseases and pathological conditions associated with chronic low dose exposure include cancer and endocrine disruption, which deserve more attention in scientific research and literature. This thesis aimed at discussing low dose environmental exposure of the general population in Flanders and associated health effects.

Chapter 2 gives an overview on several aspects of carcinogenesis, which are particularly important in the environment and health context. Cancer is one of the most important causes of death and lost good life years in the western world, and the incidence of several cancers increases worldwide. There is strong evidence that environmental exposure may account for up to half of the cancer risk. Cancer is a multifactorial process, which includes both DNA-reactive and 'epigenetic' (non DNA-reactive) events. Although these **epigenetic mechanisms of carcinogenesis** have received far less attention, they play an important role in the carcinogenic process. In the most strict sense of the term, these epigenetic mechanisms refer to interactions with DNA [(de)methylation] or with histones and transcription factors [(de)methylation or (de)acetylation], which leads to altered gene expression patterns. In a broader sense, epigenetic mechanisms of carcinogenesis also include other mechanisms such as the induction of proliferation (cell growth), inhibition of apoptosis (programmed cell-death), induction of oxidative stress, inhibition of gap-junction intercellular communication, and induction of telomerase activity. These key processes epigenetic in carcinogenesis were discussed, and several examples of environmental pollutants that are potent epigenetic carcinogens were given.

Chapter 3 discusses the topic of **endocrine disruption,** which has become very relevant due to an increase of incidence and prevalence of associated disorders in the last decades, and because it has become clear that many substances have endocrine disrupting properties. Endocrine disrupting substances can have (anti-)estrogenic and/or (anti-)androgenic action, disrupt thyroid and corticoid function or have other metabolic effects (for example through activation of the Aryl-hydrocarbon receptor).
Known endocrine disrupting ***substances*** (EDCs) include organochlorines, polybrominated flame retardants, perfluorinated substances, Bisphenol A, alkylphenols, phthalates, pesticides, polycyclic aromatic hydrocarbons, alkylphenols, solvents, certain metals, and some household products such as some cleaning products, air fresheners, hair dyes, cosmetics, and sunscreens. Several EDCs are active at very low concentrations. Internal exposure is not negligible and has been shown to result in detectable hormonal activity in blood.
Several important ***disorders*** have been associated with endocrine disruption, including prostate, testicular and breast cancer, diabetes, obesity, the metabolic syndrome and infertility. Epidemiological data shows increases in incidence or prevalence of these disorders, and for cancer, a higher risk of hormone-related cancers has been observed. That exposure to some agents occurring in an occupational or environmental context can induce these disorders has been shown in experiments and in epidemiologic studies.
From a ***mechanistic*** point of view, the classical idea is that endocrine disruption relies on the activation of nuclear receptors. However, several other mechanisms of endocrine disruption have been identified, including interactions with membrane- or cytosolic receptors, changes in metabolism, cross-talk between genomic and non-genomic pathways, interference with feedback mechanisms, changes in DNA methylation or histone modifications, and the induction of genomic instability. It has also been shown that effects of receptor activation can differ in function of the ligand.
These observations have led both the 'American Chemical Society' and 'the 'Endocrine Society', to issue statements recommending increased efforts to identify and study endocrine disrupting

substances, and a better information of the public in general, and of health care professionals and chemists in particular. It is also warranted to apply the precautionary principle in the absence of direct information concerning cause and effect. This is especially the case for 'newer' substances whose potential endocrine disruption properties are not or only party studied. However, it remains very difficult to determine which substances, at which point in time, and at which concentrations actually increase risk.

In the context of the human biomonitoring programme of the Flemish Environment and Health Study, we intended to investigate whether residence in areas polluted by heavy industry, waste incineration, a high density of traffic and housing or intensive use of pesticides, could contribute to the high incidence of cancer observed in Flanders (**chapter 4**). For more than 1500 residents aged 50–65 from 9 areas with different types of pollution, internal exposure was measured, including cadmium, lead, p,p'-DDE, hexachlorobenzene, PCBs and dioxin-like activity (Calux test) in blood, and cadmium, t,t'-muconic acid and 1-hydroxypyrene in urine. Several biomarkers of effect were also measured, including tumor associated proteins (prostate specific antigen, carcinoembryonic antigen and p53 protein serum levels), and biomarkers for (oxidative) DNA-damage (number of micronuclei per 1000 binucleated peripheral blood cells and the comet assay in peripheral blood cells, and 8-hydroxy-deoxyguanosine in urine).
Overall significant differences between areas were found for carcinoembryonic antigen, micronuclei, 8-hydroxy-deoxyguanosine and DNA damage. Compared to a rural area with mainly fruit production, effect biomarkers were often significantly elevated around waste incinerators, in the cities of Antwerp and Ghent, in industrial areas and also in other rural areas. However, differences were also observed between the local districts within the main areas, and even within these local districts. For example, within one industrial area, DNA strand break levels were almost three times higher close to industrial installations than 5 kilometres upwind of the main industrial installations ($p < 0.0001$).
Positive exposure-effect relationships were found for carcinoembryonic antigen (urinary cadmium, t,t'-muconic acid, 1-hydroxypyrene and blood lead), micronuclei (PCB118), DNA damage (PCB118) and 8-hydroxy-deoxyguanosine (t,t'-muconic acid, 1-hydroxypyrene). Also, we found significant associations between values of PSA above the p90 and higher values of urinary cadmium, between values of p53 above the p90 and higher serum levels of p,p'-DDE, hexachlorobenzene and marker PCBs (PCB 138, 153 and 180) and between serum levels of p,p'-DDE above the p90 and higher serum values of carcinoembryonic antigen. Significant associations were also found between effect biomarkers and occupational or lifestyle parameters.
These observations indicate that low levels of internal exposure and residence near waste incinerators, in cities, or close to important industries, can be positively correlated with biomarkers associated with carcinogenesis and thus probably contribute to risk of cancer.

In the following literature review **(chapter 5)**, we discussed opportunities of gene expression as a biomarker. Many (environmental) exposures and effects are associated with changes in gene expression. As a result, gene expression analysis has proven useful in the identification of the modes of action of toxicants, in dealing with interspecies variability, low dose extrapolation (due to the often higher sensitivity), in the development of specific gene expression fingerprints (which allow to distinguish between tissues exposed to different types of chemicals, or to distinguish between several clinical effects), and in research on combined exposures. The use of gene ontology and pathway analysis has provided new possibilities for biological interpretation of gene expression patterns, and the systems biology-, or integrated approach (involving transcriptomics, proteomics and metabolomics) further increases opportunities for biomarker development. However, at the moment this is expensive and still suffers from several difficulties, as was discussed.
There are still challenges concerning the actual use of gene expression, since there are important factors potentially influencing gene expression which are still poorly investigated. These include personal (inter- and intra-individual variation, sex, age), lifestyle (nutrition, smoking) and sampling

characteristics (time of day, season, …). These factors have to be taken into account and require additional research.
Thus, the use of gene expression as a biomarker in environment and health context is promising, since it can inform us on a wide range of biological processes at once, but some challenges remain, and continued research is needed in order to overcome these challenges. At the moment, the number of biomonitoring studies using gene expression is still limited, even though gene expression analysis may be very useful for evaluation of environmental (low levels of) exposure, in a context relevant for the general population.

In the last part of this thesis **(chapter 6)**, we investigated genome wide gene expression changes associated with a range of environmental pollutants, including cadmium, lead, PCBs, dioxin, hexachlorobenzene, p,p'-DDE, benzene and PAHs, using a subset of the adults from the Flemish biomonitoring population. Gene expression levels were measured in peripheral blood cells of 20 adults with relatively high and 20 adults with relatively low combined internal exposure levels, all non-smokers aged 50-65. Pearson correlation was used to analyze associations between pollutants and gene expression levels, separately for both genders. Pollutant- and gender-specific correlation analysis results were obtained. Interestingly, for organochlorine pollutants, analysis within genders revealed that genes were predominantly regulated in opposite direction in males and females. Significantly modulated pathways were found to be associated with each of the exposure biomarkers measured. Pathways and/or genes related to estrogen and *STAT5* signaling were correlated to organochlorine exposures in both genders. Our work demonstrates that gene expression in peripheral blood is influenced by environmental pollutants. In particular, gender specific changes are associated with organochlorine pollutants, including gender specific modulation of endocrine related pathways and genes. These pathways and genes have previously been linked to endocrine disruption related disorders, which in turn have been associated with organochlorine exposure. Based on our results, we recommend that males and females be considered separately when analyzing gene expression changes associated with exposures that may have endocrine disrupting properties. These results point to the existence of gene expression changes associated with low internal exposure to several environmental pollutants (which has also been demonstrated in other studies) and in particular to gender-specific changes, including gender-specific modulation of endocrine related pathways and genes. A further reduction of pollution levels in Flanders is thus warranted.

In **conclusion**, we have shown that low dose environmental exposure to several pollutants was associated with significant effects in the general Flemish population, with implications on carcinogenesis and endocrine disruption, which were in agreement with other studies. Low dose effects and gender specific effects should receive more attention in research. Biomonitoring studies using gene-expression analysis could further improve insight in low dose effects of environmental pollution. In view of these results, and according to the precautionary principle, policy should at least aim at further lowering the exposure of the general population to all kind of pollutants, and especially pay attention to new developed chemical compounds that are introduced on the market or used in production processes.