Health of Municipal Sewage Workers

Studies of Cancer Incidence, Biomarkers of Carcinogenicity and Genotoxicity, and Self Reported Symptoms

BY

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Abstract


The occupational exposures of sewage workers are complex and variable, and include a great variety of biological and chemical agents. Previous research has focused mostly on infections and various symptoms among sewage workers, e.g. abdominal and respiratory symptoms. At several sewage plants in Sweden, concern arose about occupational cancer, specifically cancer of the stomach, the kidney, and the lung. The aim of this study was to study the cancer incidence among municipal sewage workers, some exposures that might be connected with cancer risk, and self reported abdominal and respiratory symptoms.

In a cohort of municipal sewage workers there was no increase in the overall incidence of cancer when compared with the general population. However, there was a slight increase in the incidence of prostate cancer, but not in the sites of original concern among the workers. Infection by the gastric carcinogen Helicobacter pylori (determined from the presence of IgG antibodies in serum against H pylori) was no more prevalent in sewage workers than in comparable referents. Neither were sewage workers more exposed to genotoxic agents than comparable referents, as measured by the alkaline single cell gel electrophoresis (SCG) assay performed on peripheral lymphocytes. There was no increase in the three-month prevalence of abdominal symptoms when compared with other municipal workers. Specifically, there was no difference in prevalence of the common disorders dyspepsia and irritable bowel syndrome. Sewage workers reported adult bronchial asthma significantly more than the referents.

Key words: Abdominal symptoms, alkaline single cell gel assay, asthma, cancer, comet assay, dyspepsia, genotoxic exposure, Helicobacter pylori, IBS, occupational epidemiology, respiratory symptoms, sewage workers.

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List of papers

This dissertation is based on the following papers that are referred to by their Roman numerals I – VI.


Abbreviations and units

The units used throughout the text are according to Système International d'Unités (SI).

adjOR adjusted odds ratio
b. wt. body weight
CI confidence interval
FEV$_1$ Forced expiratory volume in one second
HAV hepatitis A virus
HB hepatitis B
HIV human immunodeficiency virus
IBS irritable bowel syndrome
ICD International Classification of Diseases
IgG immunoglobulin G
ln natural logarithm
OR odds ratio
PMR proportional mortality ratio
RR relative risk
SCG assay alkaline single cell gel electrophoresis assay = comet tail assay
SD standard deviation
SIR standardized incidence ratio
SMR standardized mortality ratio
VAV Svenska vatten- och avloppsverksföreningen (The Swedish Water and Waste Water Works Association)
Introduction

Urbanization has caused many hygienic and environmental problems; to solve some of these latrines and sewage systems were invented and are still being improved. These systems are used to transport the effluents of the cities to the surrounding areas to be used for fertilization or just to be dumped. Until the 20\textsuperscript{th} century, the tasks of the sewage workers were essentially cleaning and repairing; the same task as in ancient Rome. The exposures were mainly to human excreta and domestic wastewater. With increasing demands on the wastewater systems and new environmental regulations, the transportation and treatment of wastewater has increased in technical complexity. This development has resulted in new exposures for the sewage workers, including the multitude of chemicals used in our homes and in the industries.

Sewage exposed workers in Sweden

In Sweden, the municipalities are responsible for production and distribution of municipal drinking water as well as the transportation and treatment of the municipal wastewater before it is discharged back into the environment. The drinking water plants and sewage treatment plants in about 290 municipalities in Sweden are often run by the same workers. In total, there are about 2000 workers engaged in water treatment in Sweden, of whom 50\% - 75\% are working exclusively at the sewage treatment plants. In sparsely populated areas, the sewage workers are often part time, being active also in some other profession, e.g. farming. Another 2000 municipal workers are exposed to sewage to some extent, being engaged in water duct maintenance in both the drinking water systems and the sewage systems (Personal communication with Örjan Eriksson, Swedish Water and Waste Water Works Association).

In addition to the municipal sewage plants, there are also industrial wastewater plants, e.g. at power plants, process industries, and slaughter-houses, each producing specific exposures which may be different from those of the municipal
plants. However, some of these industrial exposures may also occur at municipal sewage treatment plants since there is sometimes mixture of industrial and household wastewater.

Job titles

Different job titles are often used for the sewage-exposed workers in the literature. There is often a distinction between the workers at the sewage treatment plant and the workers in the service and maintenance of the duct system, the sewers. They are often called sewage treatment [plant] workers and sewer workers, respectively. However, the workers are often also called sewage workers with no clear distinction between the different work tasks often because individual workers may work in different parts of the sewage system.
Studies of health effects in sewage workers

In 1700, the Italian physician Bernardini Ramazzini published his book in occupational medicine called *De Morbis Artificum* (Diseases of Workers). He reviewed the ailments known to affect a number of professions, including those of the latrine and sewer-tenders. Inflammation of the eyes was considered as the hallmark of these workers, and Ramazzini alleged that they even risked blindness. He presented a theory that some “acid” evaporating from the “awful masses” affected the eyes, and he advised the workers to protect their eyes with a transparent bladder over the face, and to limit the duration of their shifts. In contrast to this theory that a chemical exposure constituted the main occupational hazard to sewage workers, the risk for infectious disease was the main issue during a good part of the 20th century. However, in the recent decades there has been a new focus on health risks connected with the chemical exposures of sewage workers.

In 1954, more than two centuries after Ramazzini, the German physician Anders reported the results from a thorough retrospective examination of the health of 449 male sewer workers in West Berlin, and concluded that the chemical and biological composition of the sewage did not present an undue risk to health. Since then, a number of studies, reflecting different aspects of the health of sewage-exposed workers, have been published.

**Microbial exposures**

Exposure to wastewater is considered to entail a risk of infectious diseases. It is reasonable to suppose that enteric viruses and bacteria would be present in sewage and, indeed, they have been found in sewage and aerosols from wastewater treatment plants. The airborne concentrations of these pathogens is variable. Pathogenic fungi have also been isolated from sewage sludge.
It is not clear, however, whether the doses of infectious agents inhaled or ingested by the sewage workers are high enough to cause clinical infection. In a study of sewage workers in three U.S. cities, there was no increased risk of infection from parasitic, bacterial, and viral infections as indicated by stool examinations, cultures, or antibody surveys. Other studies, however, have demonstrated an increased risk of various infections in sewage workers. The prevalence of infection in the general population is probably one important determinant of the degree of risk following exposure to sewage. A brief review of the literature concerning the risk of infectious diseases among sewage-exposed workers is given below.

**Bacteria**

**Leptospira**

Historically, leptospirosis (*Leptospira icterohemorragica*, Weil’s disease), which is transmitted by the urine of rats, was the bacterial infection that was of most concern in the wastewater industry, and it was regarded as an occupational disease of sewer workers. No increased risk for leptospirosis was reported in studies from Germany, USA and Denmark, however, but sewage workers in Singapore and Canada apparently did have an increased risk.

**Enteric bacilli**

It seems reasonable to suppose that sewage workers would have an increased risk of infection with enteric bacilli but no cases of typhus or paratyphus were reported among the Berlin sewer workers examined in the early 1950s, and no significant increase in infection with *Salmonella* or *Shigella* was found in American sewage workers, when compared with controls.
**Legionella**

*Legionella pneumophila* is known to be spread by water aerosols, causing either pneumonia or a nonpneumonic disease (Pontiac fever). A study of American sewage workers found no increased risk for infection with *L pneumophila*\(^2\) but recently five cases of Pontiac fever have been reported in workers exposed to aerosols from a sewage plant in the food industry treating only organic industrial waste. *L pneumophila* of the same serogroup as in the infected workers was cultured from the sludge.\(^2\)

**Tuberculosis**

Tubercle bacilli are very persistent, and there have been some reports about its presence in sewage, but there is no evidence that sewage workers are at increased risk because of their occupation.\(^3\)

**Virus**

No significant seroconversion to rotavirus was seen in American wastewater-exposed workers suffering from gastroenteritis, whether analyzed for degree of wastewater exposure, wastewater aerosol exposure, or work experience, nor when compared with controls. However, there was a significant increase in seroconversion to Norwalk virus in those with higher wastewater aerosol exposure and in inexperienced sewage workers. It had been reported that there is an increased prevalence of antibodies against adenovirus and parainfluenza virus, but not influenza virus, coronavirus, or Herpes simplex virus among sewage exposed workers in Rumania when compared with unexposed workers.\(^4\)

**Hepatitis**

At the beginning of the 1950s, sewer workers in Berlin with a history of hepatitis had most often been affected before employment.\(^2\) A number of recent German studies of the prevalence of antibodies against hepatitis A virus (HAV), however, have demonstrated an increased risk of infection in both sewer and sewage plant
workers. Studies of sewage workers in Denmark, UK, France and Singapore have also reported an increased risk of HAV-infection, but there was no increase among American, Italian, or Israeli sewage workers. In one Canadian study there was no increase in HAV antibodies in sewer workers, but during a community outbreak in Canada, three cases of HAV-infection were reported among sewage workers. The risk for HAV-infection is probably dependent on the immunity of the workers, determined by both infection before employment and vaccination, and the prevalence of the infection in the population.

There are few studies of hepatitis B (HB) in sewage-exposed workers. Danish sewer workers did not have increased levels of antibodies indicating previous infection, when compared with gardeners and clerks. In contrast, however, German sewer workers appeared more likely to have had the disease than sewage treatment plant workers or medical personnel. Similarly, in a study from Greece, sewage exposed workers were more likely to have a marker of past or present HB-infection than unexposed workers.

A recent case report suggests that hepatitis C may also be an occupational hazard for sewage workers.

**HIV**

The risk for transmission of human immunodeficiency virus (HIV) from wastewater is considered unlikely, although the virus seems to survive for a limited period in wastewater. A ten-fold decline was seen in 2,9 days in the concentration of cultivable HIV-1 incubated in samples of sewage, compared with 23-30 days for poliovirus. Using the polymerase chain reaction (PCR), nucleic acids of HIV-1 virus has been detected in some municipal wastewater samples from USA; this does not necessarily equate with the presence of infectious viral particles, however.
Parasites

A significant positive correlation between the finding of protozoa in faeces from sewage workers and the duration of exposure to sewage has been reported, and the prevalence of infestation with intestinal parasites decreases with improved compliance with hygienic rules.

*Ascaris* or *Trichuris* apparently did not affect the sewer workers in Berlin in the early 1950s more often than the school children of the city, but a recent report from Egypt concluded that Egyptian sewage workers were at increased risk. A recent report from Egypt concluded that Egyptian sewage workers were at increased risk.

An increased risk for giardiasis has been reported in sewage-exposed workers in France and Germany, but not in the USA. An increased prevalence of *Entamoeba histolytica* has been reported in sewage exposed workers in France, but not in Germany.

**Chemical exposures**

Hydrogen sulphide (H₂S), from anaerobic decay of sewage, is probably the chemical substance mentioned first by most sewage workers when discussing their exposures. Exposure to H₂S at high concentrations is life threatening, but the exposures of sewage workers are normally low. The irritation of the eyes of latrine and sewer-tenders observed by Ramazzini was most likely due to exposure to H₂S.

In addition to substances of biologic origin, municipal wastewater contains man-made chemicals. Detergents and other common cleaning-agents are obvious constituents, but wastewater may also contain products that are more toxic, e.g. organic solvents. Disposal of industrial waste into municipal wastewater has been a common practice. One study of volatile organic compounds in the air at wastewater treatment plants showed considerable variability (> 10×) in the concentrations, hour-to-hour and day-to-day. The concentrations were highest at the plant with the highest portion of industrial wastewater in its influent. Since sewage workers cannot possibly be aware of all the potential chemical exposures
resulting from unexpected discharges, they may be at higher acute risk when exposed than other industrial workers with well-defined, continuous exposures. Incidents with very high exposures for industrial chemicals at sewage plants have been reported.56, 57, 58

### Health effects

#### Mortality

There are few reports concerning the overall mortality of sewage workers. In a study from the 1970s, Copenhagen sewer workers who had worked for more than 8 years in the sewers had twice the death rate of all Copenhagen males,60 while the all-cause mortality of sewage workers in Buffalo, USA was the same as that of U.S. white males. 60

#### Non-specific symptoms and some laboratory findings

Fatigue and headache are frequently reported by sewage workers, and have been noted in some studies.11, 61, 62 In one study, these complaints were positively related to increasing exposure to airborne rod-shaped bacteria, but not to spherical bacteria or endotoxin.11 Febrile attacks have been reported in workers exposed to dust from dried sewage sludge.61, 63, 64

There are a few studies of markers of inflammation or infection in sewage workers. Increased levels of C-reactive protein (CRP) in serum and fibrinogen degradation products in the urine have been observed in sewage dust exposed workers.61, 63 Increased levels of immunoglobulins in serum from sewage-exposed workers have been described, but the findings have not been consistent. 6, 13, 20, 63, 65

#### Nervous system

There has been some research on the neurotoxic effects of exposure to organic solvents in sewage. In nineteen sewage workers exposed to high levels of
benzene and toluene in the air at a sewage treatment plant receiving industrial sewage, neurobehavioral abnormalities were related to the duration of work at the plant.\(^{58}\) All the workers reported acute symptoms consistent with solvent exposure, including fatigue, light-headedness, and headache while working at the plant. In another study, where postural stability was measured as an indicator of involvement of the nervous system, increased postural sway was correlated to exposure to organic solvents.\(^{66}\) Both these reports were from plants with an admixture of industrial sewage but the low levels of organic solvents measured in the second study caused the authors to hypothesize about the presence of some as yet unidentified covariate.

**Skin and mucous membranes**

Sewage workers have reported more skin disorders than water treatment workers\(^{13, 62}\) and more inflammation of the eyes.\(^{13, 61}\) After an accidental exposure to dust containing sludge, several incinerator workers at a sewage treatment plant in Toronto, Canada were affected by irritant contact dermatitis.\(^{67}\)

**Respiratory tract**

Occupational asthma among sewage workers has been caused by sewer flies (*Psychoda alternata*).\(^{68, 69}\) Sewer workers in Berlin in early 1950s had lower than expected vital capacity.\(^{2}\) No chest symptoms and no effect on FEV\(_1\) or respiratory flow-volume curves in sewage workers was reported from one Swedish study,\(^{63}\) while sewer workers in Croatia had an increased prevalence of chronic respiratory symptoms and decreased ventilatory capacity suggesting obstructive changes.\(^{70}\) Increased risk for sore throat, but not coughing or shortness of breath, was reported among sewage workers when compared with water treatment workers.\(^{62}\) After a discharge of a large volume of hexachlorocyclopentadiene into a municipal sewage system the sewage workers complained about cough and irritation of the eyes and the respiratory tract.\(^{56}\) In a Canadian sewage plant incinerating sewage sludge, the workers at the incinerator reported acute
intermittent “influenza-like” illness with cough, sore throat and fewer more often than the other sewage workers.  

Cardiovascular system

Studies considering cardiovascular diseases in sewage workers are few in number. Sewage workers in Buffalo, USA had a similar mortality from arteriosclerotic heart disease as U.S. white males. In one other American study, however, a significantly increased proportional mortality ratio for arteriosclerotic heart disease was found for American-born, but not for foreign-born sewage workers. This may be the result of ethnic differences such as dietary habits, for example.

Gastrointestinal tract

Although gastrointestinal troubles are frequently reported by sewage workers, there are few systematic studies. German sewer workers did not have more stomach complaints or peptic ulcers during employment then before employment. Diarrhea and other gastrointestinal complaints, especially after vacations and in connection with dirty working operations, have been reported in studies of sewage workers. In one of these studies it was noted that the sewage workers had no increase in nausea or vomiting, and in one other study the gastrointestinal illness was not related with enteroviral infections.

Reproductive system

Studies of reproductive outcome of wastewater-exposed workers have been carried out on few occasions and the results do not allow for any definite conclusions. In one pilot study, a negative association was found between spontaneous early fetal loss and paternal exposure to chemically contaminated municipal sewage prior to conception. No effect on fertility was seen in an analysis of males exposed to mixed industrial and domestic wastewater. The reproductive history was collected through interviews with the worker’s wives,
and live birth experience was compared with unexposed couples. In a questionnaire survey, unexposed wives of workers employed at a petrochemical wastewater treatment plant had an increased risk of spontaneous abortion when their husbands were exposed to the wastewater treatment plant around the time of conception. At the same plant, a cross sectional evaluation of sperm concentration and sperm morphology showed no differences when compared with unexposed men.

Genotoxicity and carcinogenicity

Varying mutagenic responses have been observed in wastewater and in sewage sludge. It seems that sewage sludge may be mutagenic, but there are both geographical and temporal variations. Sludge from plants processing only domestic wastewater seems to have a lower mutagenic potential than those with industrial influents. Sewage workers at 14 treatment plants in New York State had a significantly higher risk of excreting urinary mutagens determined with Ames test, both with and without in vitro metabolic activation, when compared with workers from drinking water plants.

There are also a few animal studies about carcinogenicity of sewage. A chlorination-related induction of papillomas in fish (black bullhead, Ictalurus melas) in a wastewater pond was reported, and neoplastic skin lesions were observed in tiger salamanders (Ambystoma tigrinum) living in a sewage sedimentation lagoon.

Epidemiologic studies of cancer

There are a few inconsistent reports about the increased risk of cancer in sewage-exposed workers. Sewer workers in Copenhagen had an increased death rate from pancreatic cancer, when compared with the national statistics. In a retrospective cohort mortality study of sewage plant workers in Buffalo, USA the total mortality in cancer was not increased while the mortality from cancer of the liver and the larynx was significantly increased. In one Swedish case-control
study, an increased risk of astrocytoma was noted for people “living near a municipal sewage treatment plant”.

Background of the investigation

The background to the studies presented in this thesis was an increased interest in the 1980s in the occupational health hazards of sewage workers whose numbers had increased in the preceding decades. In 1984, the National Board of Occupational Safety and Health in Sweden released regulations concerning work in sewage plants (Avloppsanläggningar AFS 1984:15). This was an impetus for continued work within other organizations. The Swedish Water and Waste Water Works Association (Svenska vatten- och avloppsverksföreningen, VAV) carried out a number of projects including the publication of a guide for the protection of sewage workers (Skyddshandbok för avloppsarbete, Publikation VAV P64, Stockholm: Svenska vatten- och avloppsverksföreningen, 1988). This document was referred to us, the Department of Occupational and Environmental Medicine, Uppsala University Hospital, for consideration. During the same period we also made an inventory of health check-ups and occupational diseases and injuries among sewage workers in Sweden at the request of the National Board of Occupational Safety and Health (Edling C, Friis L. Inventering av arbetsskador i avloppsanläggningar. Rapport från Yrkesmedicin 12/91, Uppsala: Occupational and Environmental Medicine, Uppsala University Hospital, 1991).

During the 1980s, apparent clusters of cancers had been the cause of some concern for workers at some Swedish sewage plants. The cancer sites involved the stomach, kidneys, and lungs. A request to our department for information about cancer incidence in sewage workers established the fact that there were few studies in this area and so a cohort of sewage workers was formed in co-operation with VAV with the aim of establishing cancer incidence among them. This was the beginning of the series of studies presented in this thesis.
Aims of the investigation

1. To study the incidence of and mortality from cancer among sewage workers. Specific cancer sites of interest were the stomach, kidneys and lungs. (Studies I and IV)

2. To identify potentially carcinogenic exposures among sewage workers
   2.1. To study the prevalence of infection with *Helicobacter pylori*, a gastric carcinogen, among sewage workers. (Study II)
   2.2. To study the level of DNA damage in lymphocytes from sewage workers using alkaline single cell gel electrophoresis; a biological marker for nonspecific genotoxic exposures. (Study III)

3. To study the prevalence of gastrointestinal complaints in sewage workers. (Study V)

4. To study the prevalence of respiratory complaints in sewage workers. (Study VI)
Subjects and methods

Selection of study subjects and methods in studies I and IV

The cohort

All sewage workers who had worked for at least one year during 1965 to 1985 at the municipal sewage plants in 17 small and medium sized municipalities in central and southern Sweden formed a cohort (Figure 1). In 1986 the population in these municipalities ranged from 33 000 to 157 000.

Figure 1. The 17 municipalities from which the sewage worker cohort was recruited.
Seven hundred and twelve workers (657 men and 55 women) were identified from employment records and supplementary interviews with retired workers. The data was recorded on a specific protocol filled in at each plant or the responsible municipal authority. The protocols contained the identifying data of the municipality and the worker, times of employment, information on work tasks, and smoking habits.

Exposures

Each worker’s times of employment and main work tasks were noted in the protocol. Representatives from the Swedish Water and Waste Water Works Association (VAV) and the union made a qualitative exposure classification of the work tasks in sewage treatment. Four exposure classes were determined in order of increasing exposure: laboratory work (A), work in the sewage processing plant (B), sludge pipe flushing or exhaustion (C), and work at sewage pump stations (D). The classification was based on the level of exposure to wastewater and aerosols and the estimated impurity typical of each task. If a subject had employment periods with different exposure levels, he was allocated to his highest exposure class in the analyses.

In study I, an exposure index ($EI$) was introduced for the statistical analyses. This was a weighted sum of the time of employment ($T$). The weights ($W$) were chosen as ordinal estimates of the levels of exposure: $W_1 = 0$ for laboratory work, $W_2 = 1/3$ for work at the plant, $W_3 = 2/3$ for sludge pipe flushing and exhaustion, and $W_4 = 1$ for work at the pumping stations.

$$EI = \sum_{i=1}^{4} W_i \times T_i$$
Follow up

Vital status was determined as of December 31, 1987 in study I, and December 31, 1995 in study IV. The national identification number, required for collecting data from the death and cancer registries, could not be retrieved for one individual and he was therefore excluded from the study. One individual had emigrated and contributed to the person-year calculations only up to the date of emigration.

Information on causes of death and tumors

Information on causes of death in the cohort was collected from the National Death Registry of Statistics Sweden for the period 1965 to 1987 in study I, and 1965 to 1995 in study IV. The death certificates were coded by Statistics Sweden according to the International Classification of Diseases (ICD). All codes were transformed to the 8th revision of the ICD.

Information on tumors diagnosed from 1965 to 1987 in study I, and 1965 to 1994 in study IV, and coded according to the 7th revision of ICD, was obtained from the Swedish Cancer Register.

Risk estimates and statistical methods

Mortality

Expected mortality rates were calculated from the national rates specific for calendar year, cause, gender, and five-year age groups. For study I, national death rates for the period 1965 to 1985 were available, and for study IV up to 1995. The date of death or emigration, whichever occurred first, was used as the individual end points.

In study I, only ages up to 79 were considered and the national rates for 1986 and 1987 were approximated with the 1985 data since we had national mortality data only up to 1985 but information about deaths in the cohort up to 1987.
Cancer incidence

Similarly, expected incidences of cancer were calculated using yearly national incidences specific for calendar year, site, sex, and five-year age groups from the Swedish Cancer Register. Date of death, a tumor diagnosis (the first tumor in study I, the second tumor in study IV), or emigration was used as the individual end points, whichever occurred first.

As in the mortality analyses, only ages up to 79 were considered in study I and the national rates for 1986 and 1987 were approximated with the 1985 data.

Statistical methods

Cause-specific standardized mortality ratios (SMR) and standardized incidence ratios (SIR) were calculated. The corresponding 95% confidence intervals (CI) were calculated according to the Poisson distribution, or to the chi-square distribution when the expected values were greater than 10 in study I, and the normal distribution when the expected values were greater than 15 in study IV.

In study I, logistic regression analyses were performed to investigate possible exposure-response relations. Separate models for the diagnoses of interest were applied with the alternative exposure variables total time of employment and exposure index EI, respectively. Odds ratios (OR) with 95% CI were calculated from the logistic regression models.

In study IV, exposure-response relations were evaluated by calculation of the SIR:s and 95% CI:s for exposure-class stratified data.

The term significant indicates that the 95% CI of the risk estimate does not include 1.
Selection of study subjects for the studies II, III, V and VI

Studies II, V, and VI

All 156 workers employed at the municipal wastewater plants in ten municipalities in mid-Sweden were invited to participate in studies II, V, and VI. One hundred and forty-seven referents selected from among municipal laborers in the same municipalities were also invited to the study. In addition to group matching for geography and socio-economic status, the referents were also matched for age (± 5 years). One hundred and fifty-one (97%) sewage workers and 138 (94%) referents participated. The mean age of the participating sewage workers was 46.5 years (standard deviation (SD) = 9.95), and of the participating referents 45.4 years (SD =10.4). Nine of the participating sewage workers, and

Figure 2. The municipalities from which the subjects for study II, V, and VI were recruited.
one of the referents, were women. Because of the natural differences in abdominal symptoms between the genders, the female workers were all excluded from the study of abdominal symptoms (Study V) to avoid possible confounding. Information about present and previous occupational exposures to sewage was collected from a questionnaire given to each participant.

Study III

A subgroup of the participating subjects in study II consisting of workers in Uppsala, 38 sewage workers and 38 of the referents, was invited to participate in study III; thirty-five (92%) sewage workers and 30 (79%) referents agreed to do so. The referents were selected from among municipal construction workers supposedly not exposed to sewage, and they were matched for age (± 5 years) and present smoking habits (tobacco smoker / non-smoker). Matching for gender was not possible in all due to limited numbers; thus, five female employees at the sewage plant but only one female referent participated in the study. The mean age of the 65 subjects was 45.7 years (SD 8.7 years), and 18 were smokers.

Methods in study II

An increased incidence of stomach cancer in sewage workers was reported in study I. The bacterium *Helicobacter pylori* is a recently recognized gastric carcinogen, and it has been suggested that it may be transmitted by the faeco-oral route. When *H pylori* colonizes the gastric mucosa, seroconversion result and detection of serum immunoglobulin G (IgG) antibodies against *H pylori* is an established method for diagnosing the infection.

Analysis of IgG antibodies against *Helicobacter pylori*

A blood sample for serum analysis was collected from each worker by a routine venepuncture. After centrifugation, the serum samples were kept at -18°C, and analyzed in batches. IgG antibodies against *H pylori* were determined using the
HM CAP™ immunoassay (Enteric Products, Inc., NY, USA) according to the instructions of the manufacturer. In a few cases with borderline results the sera were reanalyzed using a commercial immunoblot technique (Genelabs Diagnostics, Singapore).

Statistical analyses

Statistical analyses with 2-by-2 table statistics, and logistic regression were performed. Relative risks (RR) with 95% confidence intervals (CI) were calculated in the 2-by-2 table analyses. Odds ratios (OR) and adjusted odds ratios (adjOR) with 95% CI were calculated from the logistic regression models. The term significant indicates that the 95% CI of the risk estimate does not include 1.

Methods in study III

Assessment of exposure and confounding

Data about work place exposures, medical history, and life style factors were collected through a questionnaire and supplemented by a personal interview. This revealed that some of the chosen referents had occasional exposure to waste water in their present job.

Occupational exposure

At least 8 hours of occupational exposure to waste water or sludge each week during the two weeks preceding the blood sampling was required for being considered as exposed. To evaluate possible bias due to misclassification, three different models for allocation into exposure groups were used in the statistical analyses. In the first exposure classification (A) all sewage workers and all construction workers who had worked in sewage contaminated environments, and had been exposed according to the criterion above, were considered as exposed. The remaining workers were all classified as referents. For the second classification (B), only sewage workers meeting the above criterion were
classified as exposed, while all others were used as referents. For the third exposure classification (C) the referents were restricted to those who had absolutely no occupational sewage exposure, while the exposed were the same as in B.

Confounding

No indication on confounding was found in this study. Information was collected about possibly confounding exposures during the week preceding the blood sampling. The confounders considered were tobacco smoking, alcohol consumption, occupational exposure to organic solvents, ongoing infections, heavy physical exertion, intake of vitamins, and medical procedures causing genotoxic exposure, such as ionizing radiation or cytostatic medications. A two sample t-test performed on ln of standardized mean tail moment, the dependent variable in this study, grouped on each dichotomous confounder variable resulted in p-values >0.15 for all variables but intake of vitamins (p=0.11). However, those who consumed vitamins had on average more DNA damage, which was contrary to our a priori assumption that certain antioxidant vitamins might protect against DNA damage. Linear regression with the dependent variable and the exposure variable (classes A, B, and C respectively in separate analyses) testing one of the potential confounders at a time did not indicate confounding in this study.

Blood sampling and isolation of lymphocytes

Each sewage worker and his or her matched referent were invited for blood sampling on the same day. Venous blood (5 ml) was collected by routine venepuncture between 8 and 10 a.m. The samples were coded and kept on ice until the lymphocytes were isolated (usually within 2 hours) on a density gradient. The isolated single cell suspension mainly consisted of viable lymphocytes (>99% viability).
SCG assay and evaluation of DNA damage

The alkaline single cell gel electrophoresis (SCG) assay, also called the comet assay due to the appearance of the treated cells, was performed following a procedure described in detail elsewhere. Briefly, a mixture of a freshly prepared suspension of lymphocytes and low melting point agarose was layered onto a microscope slide precoated with agarose. After lysis, the slides were transferred to a separate tank containing an alkaline electrophoresis buffer to unwind the DNA before they were transferred to the electrophoresis unit. After electrophoresis at high pH, the slides were neutralized, dried at room temperature, and kept in a sealed container before analysis. The slides were stained with ethidium bromide in water and examined at 500× magnification with a fluorescence microscope attached to a black and white video camera connected to a computer-based image analysis system. For each subject, 50 ‘comets’ per slide, 3-4 slides per subject, were randomly captured at a constant depth in the gel, avoiding the edges of the gel, occasional dead cells (< 1% of all cells), and superimposed comets. An image analysis program with a special application for the comet assay was used for automatic analysis of the digitally stored images. The image analysis program computed the numerical measures tail moment, tail inertia and tail length, which were used as indicators of DNA-damage.

Validation of the SCG assay

All electrophoresis sessions in the study were successfully performed, and there were no indications that the results were invalid. To ensure the validity of the laboratory work and the electrophoresis, one mouse exposed to a reference mutagen (cyclophosphamide) and one unexposed mouse were included in each experiment (18 - 22 g female mice C57BL/6, B&K, Uppsala, Sweden). Sixteen hours before each occasion of blood sampling, one mouse was given an intraperitoneal injection of physiological saline (10 µl/g b. wt.) and at the same
time another was injected with a freshly prepared solution of cyclophosphamide in physiological saline (150 mg/kg b. wt.). The mice were sacrificed and 0.5 ml blood was collected and treated in parallel with the human samples.  

**Statistical methods.**

After decoding the samples, the potential DNA damaging effect of sewage work was analyzed. The mean and median values of each subject’s tail moment, tail inertia, and tail length were standardized by division by the corresponding value for the untreated mouse on the same day. The rationale for this standardization was to compensate for possible drift in the laboratory conditions between different days.

Based on the pooled group data from the 12 different sampling occasions, the standardized mean tail moment, tail inertia and tail length were compared using a t-test for independent samples.

Pairs of exposed and unexposed subjects were matched for age, smoking habit, and day of blood sampling, for comparison of the standardized mean tail moments using a t-test for paired data. After testing the normality of the data (Kolmogorov-Smirnov and Shapiro-Wilk tests), normality was rejected in each exposure class for the standardized mean tail moment as recorded, but not in natural logarithmic ($ln$) units. Thus, the paired t-tests were performed for $ln$ of standardized mean tail moment.

The term significant indicates that the p-value (2-tailed) of a statistical test was < 5%.

**Methods in studies V and VI**

The studies V and VI were cross-sectional analyses of abdominal symptoms and respiratory symptoms, respectively, using self-administered questionnaires for data collection.
The questionnaire and statistical methods used in study V

The abdominal symptom questionnaire used in this study had been validated earlier, and also shown to be a useful tool in diagnosing dyspepsia and irritable bowel syndrome (IBS). Questions asked about the 3-month prevalence of 24 different abdominal symptoms, life style factors, and medical and occupational histories.

AdjOR with 95% CI were calculated from logistic regression models for symptoms or diagnoses, with employment as sewage worker as a proxy for exposure, and the confounder variables age, tobacco smoking, use of wet snuff and regular (at least weekly) alcohol consumption. All variables, except age, were dichotomous. The term significant indicates that the 95% CI of the risk estimate does not include 1.

The questionnaire and statistical methods used in Study VI

The prevalence of respiratory symptoms, sick history, medication, smoking habits, and the two-year prevalence of symptoms referable to the airways, skin, and the eyes both at work and not at work were collected with a previously published questionnaire. Subjects smoking at least one cigarette per day were defined as current smokers. A history of atopy was defined as having suffered from at least one allergic disease (asthma, hay fever, or eczema) or the presence of allergic disease in either parent.

Differences in proportions were assessed using the chi-square test or Fisher’s exact test, and RR with 95% CI were calculated. Logistic regression modeling was used for multivariable analysis, and adjOR and 95% CI were calculated from the logistic models. Backwards elimination of the least significant variable was performed, but tobacco smoking was forced into all models. The term significant indicates that the 95% CI of the risk estimate does not include 1 or that the p-value in a statistical test is <5% (two-tailed).
Results

In the first analysis of the sewage workers cohort (study I) the total cancer incidence was not increased. However, there were inconclusive indications of increased incidence of cancer of the nervous system, stomach, and kidney. This was the stimulus for the succeeding studies. In study II, the prevalence of infection with *Helicobacter pylori*, a recognized gastric carcinogen, was analyzed. There was no difference between the sewage workers and the referent workers. In study III, strand breaks in DNA, analyzed with the single cell gel electrophoresis assay, was used as a nonspecific marker of genotoxic exposure. No difference in level of DNA-damage was found between the sewage workers and a reference group of municipal construction workers. In study IV, a follow up of the sewage workers cohort was performed with an increased number of person-years. Again, there were inconclusive findings of increased incidence of some cancers; of the stomach, nose, and prostate. The previously observed increased incidence of cancers of the nervous system and the kidneys had decreased.

Study V showed that the sewage workers were less affected by nausea than comparable controls. The sewage workers had a numerical increase in the three-month prevalence of diarrhea and no significant deviation in the prevalence of dyspepsia or irritable bowel syndrome.

In study VI, the sewage workers reported significantly more bronchial asthma than the controls, and they reported increased frequencies of some respiratory symptoms consistent with an obstructive lung disorder.
Study I

Mortality

The total mortality of the sewage worker cohort was lower than expected (SMR=0.75, 95% CI 0.58 – 0.97), mainly due to a significant decrease in cardiovascular deaths (SMR=0.61, 95% CI 0.39 – 0.91). The cancer mortality was the same as in the reference population (SMR=1.08, 95% CI 0.68 – 1.67). The number of person-years of observation was 9 534.

Cancer incidence

There was an increased incidence of cancers of the nervous system, stomach, and kidney (Table 1). However, the incidence of all cancers combined was not increased.


<table>
<thead>
<tr>
<th>Cancer site (ICD – 7)</th>
<th>O</th>
<th>E</th>
<th>SIR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>All sites (140 – 209)</td>
<td>37</td>
<td>36.1</td>
<td>1.02</td>
<td>0.72 - 1.38</td>
</tr>
<tr>
<td>Stomach (151)</td>
<td>6</td>
<td>2.20</td>
<td>2.73</td>
<td>1.00 - 5.94</td>
</tr>
<tr>
<td>Lungs (162.0 – 162.2)</td>
<td>3</td>
<td>4.27</td>
<td>0.70</td>
<td>0.15 - 2.05</td>
</tr>
<tr>
<td>Kidneys (180)</td>
<td>3</td>
<td>1.79</td>
<td>1.68</td>
<td>0.35 - 4.90</td>
</tr>
<tr>
<td>Nervous system (193.0)</td>
<td>3</td>
<td>1.37</td>
<td>2.19</td>
<td>0.45 - 6.39</td>
</tr>
</tbody>
</table>

(O=observed number of cancers, E= expected number of cancers, SIR=standardized incidence ratio, CI=confidence interval)
When introducing a 10 years induction-latency period there were only slight changes in the standardized incidence ratios.

Logistic regression analyses were performed to investigate possible exposure-response relations. In separate models for all cancers combined, cancer of the nervous system, stomach, kidney, and lung, respectively with the alternative exposure variables, total time of employment and exposure index, the only significant relation was between kidney cancer and the exposure index.

Thus, study I resulted in inconclusive indications of increased incidence of cancers of the nervous system, stomach, and kidney.

**Study II**

In an attempt to substantiate the observed numerical increase of cancer of the stomach in study I, the prevalence of infection with the gastric carcinogen *Helicobacter pylori* was analyzed. There was no difference in immunization against *H pylori* between the sewage workers and the referents (Table 2, relative risk (RR) 0.98, 95% CI 0.68 – 1.5). Nor was there any difference between the sewage workers and the referents in an age stratified analysis (<31 years, 31 – 50 years, and >50 years), although the prevalence increased with age in both groups in an expected manner. Thus, there was no indication on increased risk for *H pylori* infection in the oldest sewage workers.
Table 2. Occurrence of IgG antibodies to Helicobacter pylori in 151 sewage workers and 138 referents.

<table>
<thead>
<tr>
<th>Group</th>
<th>Seropositive subjects (N)</th>
<th>Seronegative subjects (N)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sewage workers</td>
<td>43</td>
<td>108</td>
</tr>
<tr>
<td>Referents</td>
<td>40</td>
<td>98</td>
</tr>
</tbody>
</table>

(Seropositive = antibodies against H. pylori in serum, seronegative = no antibodies against H pylori in serum)

A multivariable logistic regression analysis was performed with immunization against *H pylori* as the dichotomous dependent variable and employment as sewage worker as a dichotomous exposure variable and the confounder variables age, gender and geographic region. This resulted in an adjOR for being sewage worker of 0.90 (95% CI 0.53 – 1.5), again indicating no increase in the risk of *H pylori* infection among the sewage workers.

**Study III**

To identify potential exposure for unspecific genotoxic carcinogens, single strand breaks in peripheral lymphocytes were analyzed with the SCG assay in sewage workers and referents. In a pooled analysis of all lymphocytes from the exposed sewage workers and the referents, respectively, there was no difference in degree of DNA-damage, no matter what exposure classification was used to group the workers. Nor was there any difference in standardized mean tail moment in a paired comparison, with the subjects matched for age (± 5 years), tobacco smoking, and day of blood sampling (Table 3).
Table 3. Comparison in pairs of DNA-damage (ln of standardized tail moment) in peripheral lymphocytes from sewage exposed workers and referents matched for age, tobacco smoking, and day of blood sampling. Three alternative exposure classifications, A, B, and C were used.

<table>
<thead>
<tr>
<th>Exposure class</th>
<th>Pairs (N)</th>
<th>Paired difference (control – exposed)</th>
<th>95% CI</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>17</td>
<td>-0.009</td>
<td>-0.57 – 0.55</td>
<td>0.97</td>
</tr>
<tr>
<td>B</td>
<td>11</td>
<td>0.07</td>
<td>-0.64 – 0.78</td>
<td>0.83</td>
</tr>
<tr>
<td>C</td>
<td>6</td>
<td>0.54</td>
<td>-0.47 – 1.6</td>
<td>0.23</td>
</tr>
</tbody>
</table>

Study IV

Study IV was a follow up of the previous cohort analysis with an additional 9 years of follow-up in the analyses of cancer incidence. However not reported in the previously published paper, mortality was also analyzed with a further 10 years of follow-up. The total mortality was still lower than expected, while the total cancer incidence was not different from expected.

Mortality

The mortality of the cohort was significantly lower than expected (SMR 0.80, 95% CI 0.67 – 0.95) during the period 1965 - 1995. However, there was no significant deviation from the expected mortality for any specific cause of death. The cohort had accumulated 15 169 person-years of observation for this analysis.

Cancer incidence

The total cancer incidence was not significantly different from expected (Table 4). However, cancer of the prostate and of the nose were significantly more
frequent than expected in the cohort. The incidence of stomach cancer almost reached the conventional level of statistical significance.


*Person-years of observation: 13 452.*

<table>
<thead>
<tr>
<th>Cancer site (ICD – 7)</th>
<th>O</th>
<th>E</th>
<th>SIR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>All sites (140 – 209)</td>
<td>77</td>
<td>66</td>
<td>1.2</td>
<td>0.92 - 1.5</td>
</tr>
<tr>
<td>Stomach (151)</td>
<td>8</td>
<td>3.5</td>
<td>2.3</td>
<td>0.99 - 4.5</td>
</tr>
<tr>
<td>Nose and sinuses (160)</td>
<td>2</td>
<td>0.17</td>
<td>12</td>
<td>1.5 - 44</td>
</tr>
<tr>
<td>Lungs (162.0 – 162.1)</td>
<td>7</td>
<td>7.1</td>
<td>0.99</td>
<td>0.40 - 2.0</td>
</tr>
<tr>
<td>Prostate (177)</td>
<td>21</td>
<td>13</td>
<td>1.6</td>
<td>1.0 - 2.5</td>
</tr>
<tr>
<td>Kidneys (180)</td>
<td>3</td>
<td>2.4</td>
<td>1.2</td>
<td>0.26 - 3.7</td>
</tr>
<tr>
<td>Nervous system (193.0)</td>
<td>4</td>
<td>2.3</td>
<td>1.8</td>
<td>0.48 - 4.5</td>
</tr>
</tbody>
</table>

*(O=observed number of cancers, E= expected number of cancers, SIR=standardized incidence ratio, CI=confidence interval)*

When accounting for 10 and 20-year induction-latency periods, respectively, there was no significant increase in all cancers combined. There was a small increase in the SIR of prostate cancer when a 20-year induction-latency was assumed (SIR= 1.9, 95% CI 1.0 – 3.1). No other specific cancer incidence was significantly increased in these analyses. When analyzing the effect of the exposure levels there was no consistent pattern indicating any exposure-response relations.
Study V

The sewage workers had a significantly decreased risk for nausea (Table 5). The adjOR for the three-month prevalence of diarrhea was slightly increased for the sewage workers, but not significant. There was also a tendency towards increased risk for IBS and peptic ulcers during the present employment among the sewage workers.

Table 5. Frequencies, adjusted odds ratios (adjOR, adjusted to age, tobacco smoking, use of wet snuff, and weekly alcohol consumption), and 95% confidence intervals (CI) for the three-month prevalence in sewage workers, compared to unexposed referents, of the diagnoses dyspepsia and irritable bowel syndrome (IBS), three abdominal symptoms, and the cumulative incidence of peptic ulcers during the present employment period.

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Sewage workers (n=142)</th>
<th>Referents (n=137)</th>
<th>adjOR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dyspepsia</td>
<td>33</td>
<td>36</td>
<td>0,85</td>
<td>0,49 – 1,5</td>
</tr>
<tr>
<td>IBS</td>
<td>11</td>
<td>8</td>
<td>1,4</td>
<td>0,55 – 3,7</td>
</tr>
<tr>
<td>Nausea</td>
<td>2</td>
<td>10</td>
<td>0,20</td>
<td>0,043 – 0,96</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>21</td>
<td>13</td>
<td>1,7</td>
<td>0,82 – 3,7</td>
</tr>
<tr>
<td>Pain in the stomach</td>
<td>29</td>
<td>25</td>
<td>1,2</td>
<td>0,67 – 2,2</td>
</tr>
<tr>
<td>Peptic ulcer</td>
<td>5</td>
<td>3</td>
<td>1,4</td>
<td>0,31 – 6,1</td>
</tr>
</tbody>
</table>

Study VI

The sewage workers reported adult asthma significantly more often than the referents, 6.7% and 1.4% respectively. Although not significant, the sewage workers also reported chronic bronchitis (defined as cough and phlegm for a minimum of 3 months a year for at least 2 successive years) and symptoms with
There was a possible relation to obstructive pulmonary disease (wheezing when infected, wheezing in the night, and shortness of breath in the night) more frequently. There were no differences in the symptoms referable to the respiratory tract, skin or the eyes when at work.

Table 6. The prevalence of symptoms and disorders in the respiratory tract, the eyes, and the skin of sewage workers and referents without exposure.

<table>
<thead>
<tr>
<th></th>
<th>Sewage workers (n=149)</th>
<th>Referents (n=138)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atopic disease during childhood or in parents</td>
<td>33</td>
<td>38</td>
<td>0,3</td>
</tr>
<tr>
<td>Adult asthma</td>
<td>10</td>
<td>2</td>
<td>0,03</td>
</tr>
<tr>
<td>Wheezing</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>when breathing cold air</td>
<td>5</td>
<td>4</td>
<td>0,8</td>
</tr>
<tr>
<td>when infected</td>
<td>32</td>
<td>20</td>
<td>0,1</td>
</tr>
<tr>
<td>Chronic bronchitis</td>
<td>9</td>
<td>4</td>
<td>0,2</td>
</tr>
<tr>
<td>Dyspnea when walking fast</td>
<td>14</td>
<td>16</td>
<td>0,5</td>
</tr>
<tr>
<td>Easily irritated by smoke, exahusts, or solvents</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>in the respiratory tract</td>
<td>29</td>
<td>32</td>
<td>0,4</td>
</tr>
<tr>
<td>in the eyes</td>
<td>21</td>
<td>31</td>
<td>0,06</td>
</tr>
<tr>
<td>During the last 2 years experienced:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wheezing in the night</td>
<td>11</td>
<td>6</td>
<td>0,3</td>
</tr>
<tr>
<td>Dyspnea in the night</td>
<td>10</td>
<td>4</td>
<td>0,1</td>
</tr>
<tr>
<td>Rash or itching in the face or the hands</td>
<td>28</td>
<td>30</td>
<td>0,5</td>
</tr>
</tbody>
</table>
The multivariable analysis of risk factors for adult asthma revealed significant relations with an atopic disposition (adjOR 6.8, 95% CI 1.9 – 25) and employment as a sewage worker (adjOR 5.3, 95% CI 1.1 – 26) but not for current smoking (adjOR 0.35, 95% CI 0.042 – 2.9).
Discussion

Studies I – IV indicate that Swedish sewage workers do not have a general increased risk for cancer. Nor were the initial concerns about an increased risk for cancer of the stomach, kidneys, and lungs conformed. If the clusters perceived by the sewage workers were real, they were probably due to chance, or at least not caused by generally occurring exposures among sewage workers. Study V showed no clinically significant abdominal complaints among sewage workers. Study VI indicated that sewage exposed workers may be at increased risk from bronchial asthma.

Comments on validity

Comments on validity in studies I and IV

Selection

Loss to follow up, which might cause systematic errors in cohort studies, was minimal.

Selection bias may also be introduced in occupational cohort studies if incomplete registers of previously employed workers have to be completed by, for instance, interviews with retired workers. People with severe diseases are more likely to be remembered by their colleagues, and this could lead to an overestimation of possible risks. The great effort given to finding all eligible individuals to the cohort leads us to believe that there were no serious errors due to recall bias in the cohort analyses.

The sewage worker cohort showed a significantly decreased total mortality. This might be due to the use of data from the general population for the calculation of expected numbers. The general population includes people with higher morbidity and mortality than usually found in workers with long-term employment, leading
to an underestimation of the risk for the workers. This is often called the “healthy worker effect”.

Geographical differences in mortality and morbidity rates may cause systematic errors in cohort studies if the study cohort and the reference population are from different areas. Our use of national rates for calculating expected values, while the cohort was selected exclusively from small to medium sized cities, may be one source for such errors. The three largest cities in Sweden, not included in this study, differ in some mortality and morbidity rates from the rest of the country. Specifically, lung cancer is more incident in the largest cities, and thus our study might underestimate the risk of lung cancer. The differences in the incidence of the other cancers of interest in this study are less pronounced.

**Confounding**

Tobacco smoking is an important confounder in cancer studies. There was information about smoking for 76 % of the workers in the cohort. Fifty per cent were smokers, or had stopped smoking less than ten years previously. Although deficient, these data do not indicate that the cohort members smoked less than the general population during the period in question. Underestimation of cancer risks due to less smoking is thus not probable.

**Misclassification**

Misclassification of exposure may introduce errors in assessment of exposure-response relations. The exposure classification used for the sewage worker cohort was crude, and no hygienic measurements were performed. Random misclassification of individual workers would “dilute” possible relations, thus explaining why no relations were found.

Chemical carcinogenesis is considered to require several decades from the primary exposure until the onset of clinical disease. Therefore, studies of cancer incidence reflect effects of possible carcinogenic hazards encountered several decades ago. Since no specific details could be obtained about historical
exposures in the cohort, the assessment of exposure was based on job titles or job tasks. Specific carcinogenic exposures that might have occurred only at a few plants were thus impossible to identify. Such exposures have been described, e.g. cyclophosphamide in wastewater from hospitals. Furthermore, the limited numbers of cases and numbers employed at each individual plant were too small to allow meaningful analyses of the individual plants.

Comments on validity in studies II and III

Selection
In cross-sectional studies, systematic differences between participants and non-participants could cause either underestimation or overestimation of risk estimates. For instance, exposed subjects with perceived health effects may be more motivated to participate than unexposed. However, this selection mechanism was likely in neither study II nor III. The participation rates were high and almost equal for exposed and unexposed workers in study II (97% and 94%, respectively). In study III, the participation rate was slightly lower and there was a difference in participation rates (92% exposed vs. 79% unexposed). However, an increased level of single strand breaks in the DNA of lymphocytes, the dependent variable under study, is not noted by the affected person.

Confounding
There were several potential confounders identified *a priori* for studies II and III. Confounding was controlled for either by matching or statistical evaluation. Socioeconomic status, age, and geography were considered important in study II. In study III, tobacco smoking, alcohol consumption, occupational exposure to organic solvents, ongoing infection, heavy physical exertion, intake of vitamins, and genotoxic medical procedures were considered. Perfect matching regarding gender was not possible, but there were few women in both studies. Furthermore, there were no previous reports indicating that gender was of
importance when studying the prevalence of infection with *H pylori* or strand breaks in DNA.

*Misclassification*

In studies II and III, it was noted that occasional sewage exposure also occurred among some of the controls. This introduced the risk of underestimating the risk estimates, and was considered in the statistical analyses. Different models for exposure classification were tested and multivariable analyses were performed. There were no indications that the conclusions of studies II and III were affected by misclassification of exposure.

*Validity of laboratory methods in study III*

Potential sources of error in the SCG assay were analyzed in a previous study, and the results were applied in study III. Day to day variability in the laboratory procedures and possible long-term variations, e.g. possible seasonal variations, were controlled by analysis of matched pairs of exposed and unexposed workers on the same day. Positive and negative mouse controls were used to validate the laboratory work.

Although sensitive to several genotoxic exposures, the use of the lymphocyte as the target cell in the SCG assay may not reflect DNA damage in other cells of interest. For instance, it has been shown that oral exposure of mice to benzo(a)pyrene does not lead to increased DNA damage in peripheral lymphocytes as determined with the SCG assay while the mouse hepatocytes do show such damage. Regarding the cancers of concern among the sewage workers, analysis of cells from the stomach, kidneys and lungs would perhaps have been more appropriate. However, collection of viable cells from internal organs from healthy workers would be impracticable.
Comments on validity in studies V and VI

Selection
The response rates for the sewage workers and the controls were high in both studies V and VI, 91 % vs. 93 % and 96 % vs. 94%, respectively and serious selection bias caused by non-responding was not considered likely.

Confounding
One potential source for confounding in study V, the asymmetrical distribution of women between the exposed and the controls, was avoided by excluding all female subjects. Since most women have periodic abdominal distress, there may have been a risk for false associations.
In study VI, confounding by age, residence, and smoking was controlled for by matching. Furthermore, multivariable analysis, controlling for these confounders and atopic disposition, also showed an increased risk of asthma among the sewage workers.

Misclassification
Misclassification of disease may cause systematic errors in cross-sectional studies of exposed and unexposed workers. Similarly, when self-reported symptoms are studied, over-reporting of symptoms due to awareness of exposure may cause systematic errors. The only significantly increased risk found in studies V and VI was an increased prevalence of asthma among the sewage workers. Misclassification may have contributed to this finding.
Comments on the results

Comments on the results in studies I and IV

Gastric cancer

Although an increase in the risk of gastric cancer was not confirmed for the cohort, it cannot be ruled out entirely. There are a number of other, albeit inconclusive, reports about gastric cancer in sewage-exposed workers. In an analysis of the Cancer-Environment Registry of Sweden, an increased risk for gastric cancer was found for waterworks industries (SIR=1.7; p<0.01). Since the workers at waterworks are often engaged at both water plants and sewage plants, this could be interpreted as an indication of an increased risk connected with sewage exposure. Based on three cases, there was a numerical increase of the standardized mortality ratio (SMR= 2.0; 95% CI 0.39 – 5.7) for gastric cancer in U.S. sewage workers. In one other American study, the proportional mortality in gastric cancer of foreign-born sewage workers in Chicago was significantly higher than in the US white male population (Proportional mortality ratio [PMR]=4.3; 95% CI 2.1 – 8.9), while American-born sewage workers were not at increased risk. This difference may be due to ethnic differences, e.g. the prevalence of infection with *H pylori*. A significant increase in mortality from gastric cancer in workers in garbage-handling, waste incineration and sewage treatment was reported from Italy (SMR=3.0; 95% CI 1.2 – 6.4). The mixed exposure of the studied workers makes interpretation of this study difficult. In one case-control study in U.S., an increased risk of gastric cancer was found in water and sewage treatment plant operators (OR=5.2; 95% CI 1.1 – 25.0). This result is based on only three cases, which explains the wide confidence interval.

Kidney cancer

The original hypothesis of an increased risk of cancer of the kidney among sewage workers was not supported. Nor have other studies of cancer in sewage
workers given support for an increased risk of kidney cancer. In one recent British case-control study of urothelial tumors and occupation there was an increased risk among sewage workers, although these tumors of the urinary tract are of different origin than tumors of the kidney parenchyma.

**Lung cancer**

The hypothesis of an increased risk of lung cancer was not corroborated in the cohort analysis confirming the finding of other studies.\(^60, 71, 100\) Recently, one cluster of five cancers in sewage workers at one plant in Italy was reported, of which three were lung cancer.\(^103\) The studied plant received industrial wastewater and there was incineration of sludge, probably causing exposures not occurring at most municipal sewage plants in Sweden.

**Prostate cancer**

There was a marginally significant increase of cancer of the prostate in the cohort. Those affected were older men, apparently with an age distribution in accordance with expected, with only one case before the age of 60 years and a maximum incidence above 70 years of age. Recently, one study of occupation and prostate cancer was performed by analysis of the Swedish Cancer-Environment Registry.\(^104\) Unfortunately, sewage workers are not coded specifically in this register, so this study gives no reliable information about sewage workers. Since no other studies have reported an increased risk of prostate cancer among sewage workers, and no known risk exposures for prostate cancer have been identified for sewage workers, the increased prostate cancer incidence was probably a chance finding.

**Cancer of the nose and sinuses**

The finding in study IV of an increase in cancer of the nose and the sinuses is not conclusive since it is based on only two cases. Considering the exposures via aerosols and gases in the sewage treatment environment, this observation may
still be of interest. However, cancers at this site have not been reported in other studies of sewage workers.

**Exposure-response relations**

There were no consistent exposure-response relations in the analyses of the exposure-stratified sub cohorts. The exposure index used in study I was an exposure-level weighted time of exposure. It was used in an attempt to identify possible exposure-response relations for specific tumors as a means to focus the future search for specific carcinogenic exposures in the complex environment under study. Without confirmed cancer risks from the cohort analysis this exercise may be questioned. The finding of a significant correlation of the exposure index with cancer of the kidney in study I was probably a spurious finding, since, eventually, there was no support for such a cancer risk in study IV.

**Comments on the results in study II**

There was a numerical, but non-significant, increase in cancer of the stomach in the cohort. The prevalence of infection with the bacterium *Helicobacter pylori*, a recently recognized gastric carcinogen, was studied in an attempt to substantiate the numerical increase of stomach cancers. However, the prevalence was similar in both sewage workers and controls. *Helicobacter pylori* causes a chronic infection of the gastric mucosa lasting for decades, and there is a cohort phenomenon with a decreasing prevalence with increasing year of birth. This allowed us to assess the risk of infection at possible past exposures to this bacterium, but the oldest sewage workers did not differ from their controls. Although seemingly not causing infection in these workers, this study does not exclude the presence of the bacterium in wastewater. DNA of *Helicobacter* species has recently been found in Swedish drinking water and wastewater. Since *H pylori* mainly seems to infect children, wastewater
contaminated drinking water may still constitute a public health hazard, although not an occupational health hazard.

Comments on the results in study III

The SCG assay was used to measure exposure for genotoxic agents. Since we studied DNA damage in sewage workers at only one plant during a limited period, the result should be interpreted with some caution. Specific genotoxic exposures may have been present at some plants, or during limited periods, that were not brought to light by this study. As illustrated by the speed of DNA-repair in lymphocytes from cyclophosphamide exposed mice and human lung cancer cells exposed for ionizing radiation in vitro, the SCG assay seems to reflect genotoxic exposure during the last hours or days, depending on the nature of the specific exposure.

Finally, the SCG-assay is not able to detect exposure for epigenetic carcinogens. Exposure for carcinogens not damaging the genetic material can thus not be excluded by study III.

Comments on the results in study V

The significant finding of study V, less nausea among the sewage workers, was interpreted to be a result of selection of people with higher tolerance against nauseating stimuli in this type of job. However, this finding was not confirmed in one recent study from USA in which the one-year prevalence of nausea, abdominal pain, bloating, diarrhea, gastroenteritis, and headache was higher in sewage exposed workers when compared with unexposed. But in a recent Swedish study, where questions about gastrointestinal symptoms were posed, diarrhea, but not nausea was reported significantly more by sewage workers than controls. Based on a small number of cases, there was a slight increase in the frequency of a history of peptic ulcer among the sewage workers during the present
employment. Disregarding the weakness in this association, this finding may be of interest when connecting it with the similarly weak indication of an increased incidence of stomach cancer in the sewage worker cohort. There is a common risk factor for both these diseases, namely infection with \textit{Helicobacter pylori} and this was an additional stimulus to carry out study II. However, as shown in study II, there was no increased risk of infection with this bacterium among the sewage workers, and the excess of peptic ulcers in sewage workers is probably the result of chance.

The questionnaire used in study V asked about the three month prevalence of abdominal symptoms and so was not designed to record transient gastrointestinal troubles, e.g. diarrhea, after returning to work after absence or after certain operations. In spite of that, there was a numerical increase in the three month prevalence of diarrhea, which probably reflects this previously reported association.

**Comments on the results of study VI**

The frequency of self-reported asthma in the sewage workers was at the same level as in the general Swedish adult population, while the controls were less affected.\cite{110,111} When studying populations consisting of employed workers, one usually expects a “healthy worker effect” resulting in reduced morbidity or mortality rates when comparing with the general population. In this specific case, one might expect that workers with respiratory sensitization – asthmatics, for example – would tend to leave occupations that involved exposure to irritants prematurely. Thus, the observed prevalence of self-reported asthma in the sewage workers, which is equal to that in the general population, might be interpreted as being actually higher than expected. The comparison with the unexposed controls gives support for the conclusion that sewage workers have an increased risk of asthma.
Although some other studies report an obstructive pattern of reduced pulmonary function in sewage exposed workers, there is no consistent support for the conclusion of study VI. Nevertheless, sewage workers are exposed to substances with the potential to cause inflammation and respiratory symptoms, and, in theory, these substances may either cause or aggravate asthma.

Since this study was performed via a questionnaire, one may question the reliability of the reported diagnoses of asthma. One validation of self-reported asthma through questionnaires in relation to a clinical diagnosis was performed in a review, and the sensitivity of self-reported asthma was found to be high, although the specificity may be lower. The questionnaire used in study VI was validated in house painters and it was found valid for screening obstructive airways disease in that group. The question about adult asthma was significantly related to bronchial hyperresponsiveness in the house painters. In study VI, 50% of all who reported having asthma also reported the use of medication given for asthma. Credibility is also added by the increased frequency, even though not significant, of some asthma-related symptoms in the sewage workers (wheezing and dyspnea).
Conclusions

The sewage workers:

- had the same cancer incidence, and cancer mortality, as the general population.
- had no significant alteration in the incidence of the cancers of initial concern for these studies; cancer of the stomach, kidney, and lung. However, a slightly increased incidence of cancer of the prostate was observed.
- had the same prevalence of infection with *Helicobacter pylori* as comparable workers without sewage exposure.
- had the same level of DNA damage in the peripheral lymphocytes as comparable workers without sewage exposure, when measured with the SCG assay.
- were significantly less affected by nausea than comparable controls. There were no significant differences in the three month prevalence of other abdominal complaints or diagnoses.
- had significantly more self-reported asthma than comparable workers. In a multivariable analysis of self-reported asthma, the risk estimate for sewage work was of the same magnitude as that for having an atopic disposition.
- had no significant deviations in the prevalence of work related symptoms referable to the respiratory tract, the eyes or the skin.

Although the results concerning cancer in sewage workers seem reassuring, the exposures at certain plants or situations may entail increased risk. Identifying possible cancer risk at one or a few sites is very difficult, since the exposures are complex and varied and only a small number of workers are exposed. Identification of specific exposures is an alternative way for risk assessment, feasible even at individual plants. To protect the workers, it seems prudent to
avoid disposal of known toxic substances, e.g. industrial waste, into municipal sewage.

The conclusion about increased risk for asthma reported in this thesis is not conclusively supported by other reports. More information is required before specific recommendations can be given, e.g. by performing prospective studies of asthma incidence in sewage workers.
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