Trace Elements in Adolescents

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Doctoral thesis
Swedish University of Agricultural Sciences
Uppsala 2002

Acta Universitatis Agriculturae Sueciae
Veterinaria 117

ISSN 1401-6257
ISBN 91-576-6354-8

Tryck: SLU Service/Repro, Uppsala 2002

Abstract

Bárány, E. 2002. *Trace elements in adolescents.* Doctor's dissertation.
ISSN 1401-6257, ISBN 91-576-6354-8.

The major aim of the thesis was to monitor toxic and essential trace elements in a cohort of adolescents by blood and serum analyses, and describe the impact of different factors on the element concentrations. The adolescents were from the Swedish cities Uppsala and Trollhättan which represent different socioeconomic and environmental conditions, and were investigated at age 15 and 17.

It was shown that an inductively coupled plasma mass spectrometry method was suitable for simultaneous determination of 13 elements in blood or serum. The elements were cobalt, copper, zinc, selenium, rubidium, rhodium, palladium, cadmium, tungsten, platinum, mercury, thallium, and lead.

The concentrations of the toxic elements cadmium, mercury and lead were low in the adolescents. Cadmium in blood was strongly positively influenced by smoking habits and by the mother's education. In contrast to smokers, blood cadmium in non-smokers did not increase between the sampling at age 15 and that at 17. The blood lead was one of the lowest reported and decreased about 10% between the samplings, perhaps due to temporal changes in environmental exposure, or a dilution of body burden in the growing adolescents. Consumption of fish with dietary restrictions due to elevated mercury levels and other, "non-restricted", fish predicted blood mercury. Blood mercury also increased with fish consumption in those who consumed only non-restricted fish. Mercury in serum was predicted by dental amalgam, consumption of non-restricted fish, and selenium concentrations.

The levels of the essential elements cobalt, copper, zinc and selenium in blood and serum were within the reference intervals, and were not influenced by socioeconomic status. However, age, gender, and residential area had significant influences on the levels, differently for different elements. Fish consumption did not influence selenium concentrations.

A large part of the analytical data for rhodium, palladium, platinum and thallium were below the detection limits, thus restricting the interpretation of the results. There were increases in the levels of rhodium, palladium and platinum in serum, as the mean concentrations of these elements were above the detection limits at age 17 but not at age 15.

Keywords: Blood, dental amalgam fillings, essential elements, fish consumption, ICP-MS, serum, smoking, Sweden, toxic elements.

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Papers I-IV

The present thesis is based on the following papers, which will be referred to by their Roman numeral.

I. Bárány, E., Bergdahl, I. A., Schütz, A., Skerfving, S., Oskarsson, A. Inductively coupled plasma mass spectrometry for direct multi-element analysis of diluted human blood and serum. Journal of Analytical Atomic Spectrometry, 1997, 12, 1005-1009.

II. Bárány, E., Bergdahl, I. A., Bratteby, L-E., Lundh, T., Samuelson, G., Schütz, A., Skerfving, S., Oskarsson, A. Trace element levels in whole blood and serum from Swedish adolescents. The Science of the Total Environment. In press.

III. Bárány, E., Bergdahl, I. A., Bratteby, L-E., Lundh, T., Samuelson, G., Schütz, A., Skerfving, S., Oskarsson, A. Trace elements in Swedish adolescents: influence of age, gender, residential area and socioeconomic status. Submitted.

IV. Bárány, E., Bergdahl, I. A., Bratteby, L-E., Lundh, T., Samuelson, G., Skerfving, S., Oskarsson, A. Mercury and selenium in whole blood and serum in relation to fish consumption and amalgam fillings in adolescents. Submitted.

List of Abbreviations

B-	Blood
Bi	Bismuth
Cd	Cadmium
Co	Cobalt
Cr	Chromium
Cu	Copper
Ga	Gallium
Hg	Mercury
ICP-MS	Inductively coupled plasma mass spectrometry
In	Indium
LOD	Limit of detection
MeHg	Methyl mercury
Mn	Manganese
Mo	Molybdenum
Ni	Nickel
Pb	Lead
Pd	Palladium
PGE	Platinum group elements
Pt	Platinum
Rb	Rubidium
Rh	Rhodium
RSD	Relative standard deviation
S-	Serum
Sb	Antimony
Sc	Scandium
SD	Standard deviation
Se	Selenium
s-TfR	Serum transferrin receptor
Tl	Thallium
W	Tungsten
Zn	Zinc

Introduction

There is a need for knowledge of trace element levels in different population groups. One application is as reference values that can be used for detection of changed environmental exposure situations. Moreover, the levels of toxic elements may be close to concentrations where adverse effects can occur. The levels of essential elements may be used to discover deficiency, and in some cases toxicity. It is of importance to investigate various categories within the population, and to identify specific risk groups.

Adolescents constitute a little studied group that differs from adults and children. Adolescents grow rapidly, and have higher energy and nutrient requirements compared to adults. A higher energy intake may expose them to more trace elements, both toxic and essential, via diet, than adults (Mykkänen *et al*., 1986). However, deficiency of essential elements may also occur, *e.g.* of iron (Samuelson *et al*., 2000), as there are indications that many Swedish adolescents do not consume an adequate diet (Samuelson *et al*., 1996a; Höglund *et al*., 1998). Moreover, the requirements of some essential elements are higher than for adults (WHO, 1996; NRC, 2001; http://www.slv.se: Accessed 15-Dec 2001a, Table 1). For example, the requirements of Zn are higher during phases of rapid growth, and therefore adolescents are particularly sensitive to Zn deficiency (WHO, 1996). During adolescence, the biological levels of some essential elements may vary due to hormonal changes during puberty (Lockitch *et al*., 1988a; Marano *et al*., 1991).

Exposure of toxic elements and status of essential elements

Main sources and absorption of trace elements

Diet is the main source of most trace elements for the general, non-occupationally exposed population. The food items making the main contributions of trace elements to the human diet varies between elements. Toxic elements may enter the food chain through contamination of the environment, *e.g.* uptake in crop of Cd from atmospheric depositions and mineral fertilizers (Andersson and Siman, 1991), or from natural high levels in soil. Many toxic

Table 1. *Swedish recommended daily intake of some essential elements (http://www.slv.se: Accessed 15-Dec 2001a)*

Age	Iron (mg)		Zinc (mg)		Selenium (µg)	
	Male	Female	Male	Female	Male	Female
11-14	12	15	11	8	40	40
15-18	12	15	12	9	50	40
19-30	10	15	9	7	50	40
31-60	10	15	9	7	50	40
>61	10	10	9	7	50	40

elements are widespread in food products in trace amounts. Processing of foods may also increase the concentration of certain elements, and decrease that of others (Kumpulainen, 1995). Essential elements are widely distributed in most foods, as they are essential also to plants and animals used for food production.

Absorption of elements occurs via the gastrointestinal tract and the lungs. The absorbed fraction may be dependent on the route of exposure. One example is Cd, which is poorly absorbed in the gastrointestinal tract (Berglund *et al*., 1994), but 10-50% is absorbed when inhaled (Järup *et al*., 1998). Chemical species may also differ. This is exemplified by Hg, which is absorbed in the gastrointestinal tract to almost 95% in its organic forms, while only a few percent is absorbed of inorganic Hg (Clarkson, 1997). The bioavailability of the element may also differ between different food sources. Absorption of essential elements is regulated by homeostatic mechanisms in the body (WHO, 1996).

Specific exposure sources of some elements

For some elements, specific exposure sources dominate, at least in certain groups of the population. A good example of this is smoking, the main source of Cd exposure in smokers (Elinder *et al*., 1983; Moreau *et al*., 1983; Järup *et al*., 1998). In smokers, the B-Cd is 5-7 times higher (Elinder *et al*., 1983) and kidney Cd is 2-3 times higher than in non-smokers (Elinder *et al*., 1976; Nilsson *et al*., 1995).

Fish is a main exposure source for MeHg, and also of Se (Svensson *et al*., 1992). The B-Hg has been shown to correlate to fish consumption in many studies (Grandjean *et al*., 1992a, 1992b; Svensson *et al*., 1992; Bensryd *et al*., 1994; Oskarsson *et al*., 1996; Mahaffey and Mergler, 1997; Hagmar *et al*., 1998; NRC, 2000; Seifert *et al*., 2000). Hg is spread in the environment from both natural and anthropogenic sources. Methylation by microorganisms occurs in the aquatic environment (WHO, 1990). MeHg is then taken up and accumulated by aquatic organisms and fish, thus increasing levels are found in the food chain, with predatory, long-lived fish having the highest levels of MeHg (WHO, 1990; Thuvander and Oskarsson, 1998). Hg is a toxic element, and there may be a small safety margin between blood levels of the general population and levels where adverse health effects may occur in sensitive groups. Therefore, many countries including Sweden, recommend dietary restrictions on certain fish species containing high levels of Hg (http://www.slv.se: Accessed 15-Dec 2001b). The commercial sale of fish containing >0.5 mg/kg (>1mg Hg/kg for certain species) is also prohibited in the E.C. (EC, 2001). Fish in lakes in southwestern Sweden contain higher levels of Hg than fish from lakes in the southeastern parts (Bergbäck and Johansson, 1996). Another source of Hg exposure is dental amalgam fillings, which significantly influence the Hg exposure (WHO, 1991). Hg is released from the fillings and subsequently absorbed (WHO, 1991). Both total Hg in blood, serum and plasma, and inorganic Hg in blood and serum have been shown to correlate to the number of amalgam fillings (Åkesson *et al*., 1991;

Jokstad *et al*., 1992; Herrström *et al*., 1994; Oskarsson *et al*., 1996; Bergdahl *et al*., 1998: Herrström *et al*., 1997; Vahter *et al*., 2000).

The platinum group elements (PGE), *i.e.* Pt, Pd and Rh, are released in the environment through use of catalytic converters in cars (Johnson *et al*., 1975; Schäfer *et al*., 1999). Use of catalytic converters have, since their introduction in Sweden in 1987, increased steadily and become mandatory in new cars (Bil Sweden, 2000). In the year 2000, 67.5% of the cars in Sweden used a catalytic converter (Bil Sweden, 2000). Each catalyst contains 1-3 g of PGE. Emissions from the catalyst are due to the abrasion of the surface of the catalyst. Very little is known about how this influences the human exposure and concentrations in human biological samples.

Other factors influencing elements levels

Many lifestyle factors and physiological variables are of importance both for the exposure and uptake of elements. This can result in elevated biological levels of toxic elements or risk for deficiency of essential elements. Age, gender and geographical area may affect element concentrations (Christensen, 1995). Therefore, when reporting trace element levels, factors such as those mentioned above should be taken into account when appropriate, which may help in identifying risk groups. It should be mentioned, that in longitudinal studies, it might not be possible to separate the effect of "time" from the effect of "age".

Age and gender

There are large differences in many trace element levels in blood and serum between children and adults, *e.g.* due to accumulation of an element in the body. Infants and the elderly may also be risk groups to consider, due to increased susceptibility, or increased/decreased bioavailability of elements (WHO, 1996). Adolescents have, as has already been mentioned, a high recommended energy intake which may expose them to more toxic elements via diet, and a risk for deficiency of essential elements due to rapid growth. Males and females differ in factors such as intake of energy, iron status, or hormonal influence. This can affect their intakes, the exposure pattern, or the bioavailability of trace elements (Christensen, 1995; WHO, 1996).

Socioeconomic status

Living conditions may produce differences in exposure to trace elements. In the USA, elevated B-Pb have been shown in children living in older housing, where leaded paint is more common (Pirkle *et al.*, 1998). A low socioeconomic status may positively influence the concentrations of several toxic elements in children (Zielhuis *et al.*, 1978; Osman *et al.*, 1994, 1998a), both due to a higher exposure and a low nutritional status influencing the absorption of toxic elements (Flanagan *et al.*, 1978; 1980).

Geographical area

In some areas, pollution from industries or traffic significantly contributes to the element exposure. Children living in urban areas have for example higher B-Pb than children in rural areas, because of differences in traffic intensity (Strömberg *et al*., 1995; Perrone *et al*., 1999). In the vicinity of smelters, children have higher levels of B-Pb (Strömberg *et al*., 1995; Osman *et al*., 1998a; Staessen *et al*., 2001). There are examples of successful measures to limit environmental exposure, observed as decreasing levels of biomarkers. A decrease in industrial Cd pollution has lowered the population's B-Cd in Belgium (Ducoffre *et al*., 1992). In Sweden, the B-Pb in children has decreased almost constantly since the 1970s, mainly due to reduced Pb emissions (Strömberg *et al*., 1995). However, some other elements are increasingly spread in the environment, for example W in sewage sludge (Eriksson, 2001). Moreover, certain areas receive acidic depositions in addition to atmospheric depositions from industries and combustion processes. A combination of emissions of elements and acidic deposition may be especially unfavorable, because the mobility of trace elements changes (Thuvander and Oskarsson, 1998). The west-coast of Sweden has been particularly exposed to acid depositions (Bertills and Hanneberg, 1995). The essential element Se may be more tightly bound in the ground, while toxic elements such as Cd may be more mobile if the pH in soil decreases. In Sweden, the Se content of soil is already low, which is reflected in the low Se intake by the population, as the level of Se in foods produced in Sweden is low. Individuals with a Se intake within the lower 10^{th} percentile in Sweden are close to the Nordic Nutritional Recommendations (of 1996) minimum intake value of 20 µg/day (Johnsson *et al*., 1997). Thus, it is possible that certain subgroups within the population have such a low Se intake and Se status that there is risk of future Se-related disease.

Blood and serum as media for biomonitoring

Different biological specimens are used for the assessment of human trace element exposure. Some examples are whole blood, plasma or serum, milk, hair, teeth, or kidney, depending on the element investigated (Elinder *et al*., 1988; Skerfving *et al*., 1999). They are not all relevant indicators of all elements (WHO, 1996; Drasch *et al*., 1997). Blood and serum are often used for assessment of the absorbed dose of an element in relation to health and disease, especially for toxic elements (Elinder *et al*., 1988; Skerfving *et al*., 1999). Also for essential elements, *e.g.* Se, blood and serum levels provide a reasonable estimate of the nutrient status (Alexander and Meltzer, 1995; WHO 1996). For other essential elements, *e.g.* Cu and Zn, blood or serum may not always be the relevant indicator of element intake or nutrient status, because of the homeostatic control in the body (WHO, 1996). However, deficiency or excess exposure may be reflected in blood and serum levels. Most often, essential trace elements are measured in serum and not whole blood (Oskarsson, 1995). For many toxic elements, blood has generally been used for biomonitoring purposes. This is the case for Pb, Cd and Hg (Elinder *et al*., 1988; WHO, 1996), though for Hg, blood

and serum levels reflect different Hg species. It is the unbound plasma fraction of an element that may leave the circulation and exert effects (Renwick, 1994). Therefore, serum or plasma may better reflect the distribution of toxic elements to critical organs, where health effects may occur, as has been hypothesized for Pb (Bergdahl *et al*., 1997a). For many toxic elements, the two compartments, plasma and red blood cells, are supposedly in equilibrium with each other, and therefore a relationship between them are to be expected, but has not been shown for many elements.

Analytical method for trace element determinations

Inductively coupled plasma mass spectrometry (ICP-MS)

For the determination of trace elements in biological materials, atomic absorption or emission spectrometric methods are commonly used. However, during the last decade, the use of ICP-MS has increased. The instrument consists of a sample introduction system, a plasma unit and a mass spectrometer. ICP-MS has many advantages, such as a capability for multi-element determinations and low detection limits. For determination of a single or a few elements, very low detection limits and high precision may be achieved (Bergdahl *et al*., 1997a; Farago *et al*., 1998; Moreton and Delves, 1998; Sieniawska *et al*., 1999). For multi-element determinations, such as for screening purposes in cases of unknown exposure, when the instrument is not optimized for a single element, a compromise between analytical precision and sample throughput is generally necessary. ICP-MS is nonetheless superior to many other atomic spectrometric methods when several elements are to be determined in large numbers of samples (Chan *et al*., 1998; White, 1999; Begerow *et al*., 2000).

The main drawbacks of ICP-MS can be divided into spectral and non-spectral interferences (Tan and Horlick, 1986; Vaughan *et al*., 1991; Vanhoe, 1993; Vanhoe *et al*., 1994a; Hsiung *et al*., 1997). Spectral interferences arise when atoms of different elements occur at the same atomic mass. Examples of this are polyatomic and double-charged ions, as well as isobaric overlaps. Non-spectral interferences arise from the sample matrix, and are more serious in the analysis of complex matrices, such as blood, which contain high amounts of organic and inorganic matrix components. Signal enhancement can occur, but signal suppression is more common. By use of an appropriate internal standard, and matrix matched standards, this can be controlled (Vaughan *et al*., 1991; Vanhoe, 1993; Hsiung *et al*., 1997; Chan *et al*., 1998).

Multi-element determinations

A method for multi-element determinations in large-scale investigations should meet several requirements. Firstly, the elements of interest should be possible to analyze simultaneously. Secondly, ease of sample preparation and short analysis time enables high sample trough-put, that are of crucial importance in many

research projects. A simple sample preparation can also help to minimize contamination. Possibilities of large analytical runs that does not need attendance of the analyst is preferred.

Many ICP-MS methods use digestion procedures for sample preparation. These methods are often time-consuming and therefore not well suited for large analytical series. In addition, they require extremely pure acids and usually clean room facilities in order to avoid contamination due to the extensive handling. Simple dilution of plasma and whole blood has previously been used for e.g. Pb determination (Schütz *et al.*, 1996), and proposed for multi-element determinations (Lutz *et al.*, 1991), and may constitute an attractive alternative.

A Swedish nutritional survey of adolescents

The focus of this thesis is trace elements; essential elements and toxic elements, that are of concern for human health, in two cohorts of 15-year old adolescents of both genders, participating in a large longitudinal nutritional survey (Samuelson *et al.*, 1996a). The present studies constitute a trace element study within the survey. The nutritional survey aims at investigating the change in food habits, physical activity patterns and body development during the transition from adolescence to adulthood (Fig. 1), with a first re-examination of the adolescents at age 17. These factors, which may be established early in life, might influence adult illnesses such as obesity, coronary heart disease and osteoporosis. Changes in iron status from 15-21 years of age were also studied. At the same time the social conditions of the parents were registered. The adolescents were living in two Swedish cities, Uppsala and Trollhättan. These cities represent different environmental and socioeconomic conditions, thus enabling differences related to these factors to be studied. In the present studies, data from the adolescents at age 15 and 17 are included. Some results from the other parts of the nutritional survey, which could be of importance for the interpretation of trace element levels in the adolescent study population, is presented here.

As diet is the main source of trace elements, different food habits may play a role for the biological levels of trace elements. In Sweden, it has previously been shown that children's food habits are strongly influenced by the parents and their socioeconomic status (Samuelson *et al.*, 1971; Hagman *et al.*, 1986; Sunnegårdh *et al.*, 1986), but for adolescents, contradictory results have been reported (Samuelson *et al.*, 1996a; Höglund *et al.*, 1998). In the current survey, the participating adolescents completed a 7-day dietary record and a food frequency questionnaire, and socioeconomic status was shown to positively influence only the intake of vegetables (Samuelson *et al.*, 1996a). The food habits differed in some respects between boys and girls, and boys had a higher energy intake than girls (Samuelson *et al.*, 1996a). About 40% consumed dietary supplements. Smokers consumed fewer vegetables, roots, fruits and meats than did non-smokers, and had lower intakes of *e.g.* calcium, iron, Se and vitamins (Samuelson

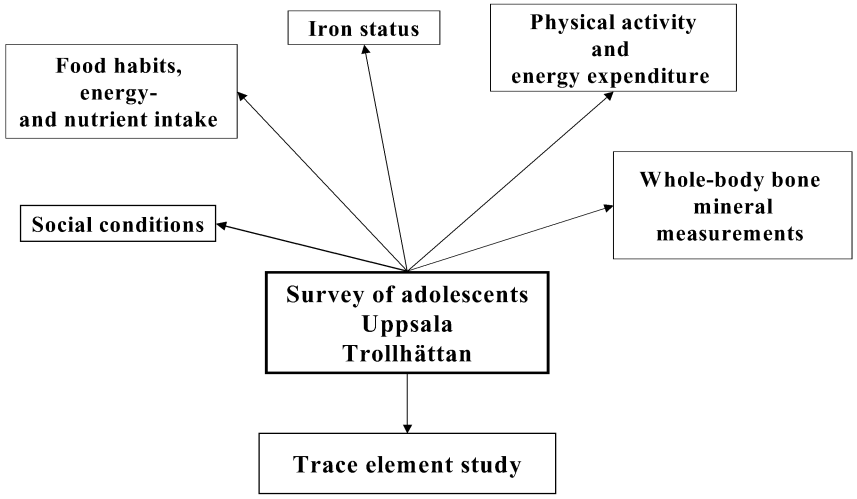

Fig. 1. The different parts of the nutritional survey. See text for details.

et al., 1996a). Between the ages 15 and 17, the consumption of fat spread, milk, and meat decreased, while the consumption of pasta, coffee and tea increased (von Post-Skagegård *et al.,* 2002).

Insufficient iron stores were prevalent among the participating adolescent girls (Samuelson *et al.*, 2000), probably increasing their uptake of other elements, e.g. Cd (Flanagan *et al.*, 1978; Berglund *et al.*, 1994). The girls lowered their iron stores, measured as increased serum transferrin receptor concentrations (s-TfR) and an increased "s-TfR/serum ferritin" ratio, between 15 and 17-years of age. The boys on the other hand, increased their serum ferritin concentrations between age 15 and 17, and the "s-TfR/serum ferritin" ratio was not significantly changed (Samuelson *et al*., 2000). Moreover, boys had significantly higher serum ferritin than girls. The iron status did not differ between the two residential areas investigated (Samuelson *et al*., 1996b; 2000). The 15-year old girls had a mean Zn intake below the Swedish nutritional recommendations, in contrast to the boys (Samuelson *et al*., 1996a). The mean intake of Se was below the recommendations in both boys and girls; however, boys had a higher intake than girls.

When the adolescents' daily energy expenditure and physical activity pattern were investigated (Bratteby *et al.*, 1997a, 1997b, 1998), a high level of physical activity was shown, but also that the 7-day dietary record underestimated their energy intake. In the results from the whole-body bone mineral measurements, higher total bone mineral content, total bone mineral density, and bone mineral area were shown in adolescents from Uppsala than in those from Trollhättan (Lötborn *et al.*, 1999). An interesting observation is, that the natural fluoride content in the drinking water in Uppsala is 10 times higher compared to the content in Trollhättan (Bratteby *et al.*, submitted).

Aims of the thesis

The main goal of the present thesis was to monitor trace elements in two populations of adolescents with a presumed different exposure to metals. Since it is well known that there are interactions between essential and toxic trace elements, both these groups of elements were included in the analysis. Some major factors that may influence trace element concentrations in blood and serum were investigated. More specifically, the aims were to:

- Evaluate the suitability of a method for large-scale multi-element analysis of human blood and serum by inductively coupled plasma mass spectrometry.

- Characterize the blood and serum concentrations of toxic and non-essential elements (rubidium, rhodium, palladium, cadmium, tungsten, platinum, mercury, thallium, lead), and of essential elements (cobalt, copper, zinc, selenium) in adolescents.

- Describe the impact of age, gender, residential area, and socioeconomic status, as well as of smoking, on the trace element concentrations.

- Assess the impact of fish consumption on mercury and selenium concentrations, and of dental amalgam fillings on mercury.

Materials and methods

The thesis is based on four studies (Fig. 2), of which the first (Paper I) concerns method evaluation, Paper II trace element levels in 15-year old adolescents as well as comments regarding the method and the analytical data. Paper III describes the impact of different factors on trace element levels in the adolescents at age 15 and 17-year, and Paper IV focuses on Hg and Se in the 17-year olds and the impact on these elements from fish consumption and dental amalgam fillings. The analytical method used is described in detail in Paper I and II, and a shorter overview is given here. All participants and their parents gave their informed consent, and the Ethics Committees at the Universities of Uppsala and Gothenburg approved the studies on adolescents.

Study group

Adolescents were selected on a random basis from two cities in Sweden: Uppsala and Trollhättan. They were 15-years of age in 1993/94 at the start of the study. Inclusion criteria were: a) living in Uppsala or Trollhättan except for distant suburban areas, b) having no chronic disease, and c) having parents born in a Nordic country. Of the 411 (69% of the invited) who chose to participate, 209 (103 boys, 106 girls) were from Uppsala, and 202 (90 boys, 112 girls) from Trollhättan. Of the adolescents who participated, 372 (171 boys, 201 girls) gave blood and/or serum for trace element analysis (Paper II and III). They completed a questionnaire about, among other things, their family and social situation, smoking habits, and alcohol consumption. The same questionnaire was sent out to the non-participants.

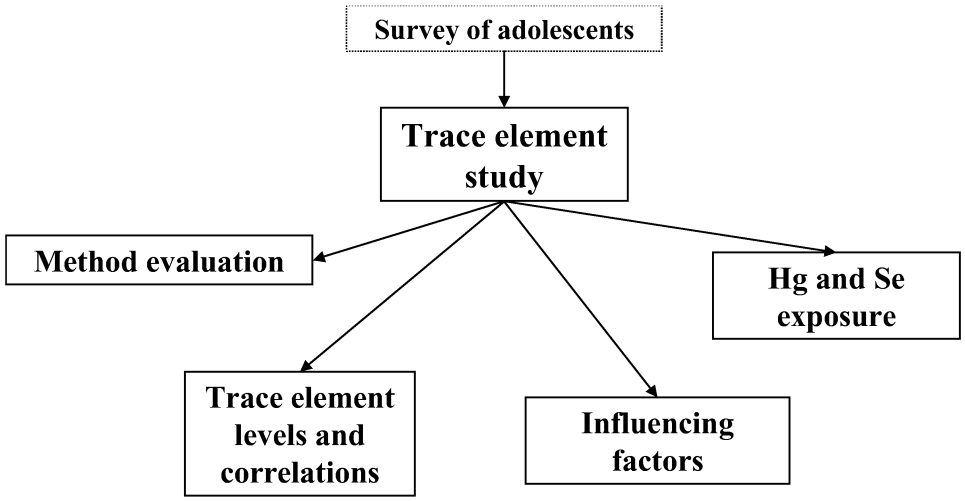

Fig. 2. The studies within the trace element part of the nutritional survey that are presented in this thesis.

There were no statistically significant differences between participants and non-participants regarding height, weight, or comparable aspects of family and social situation, and the participants can therefore be considered to be representative for their age group in the two cities. In Paper III, a comprehensive description of the participants and a drop-out analysis are made.

The adolescents had been informed of the longitudinal design of the study at the first investigation, and were invited for a second investigation at age 17 (Paper III and IV). Blood and/or serum for trace element analysis were obtained from 294 (133 boys, 161 girls) 17-year olds. These participants had lower B-Se, B-Rh, B-Pd, B-Pt, and B-Tl, and higher B-Zn, at 15-years of age, than those who did not participate at age 17. There were no differences in height, weight, or family and social situation between participants and non-participants at age 17 (Paper III).

Element exposure in the two residential areas

The two study areas used in the present work represent different environmental and socioeconomic conditions. The city of Uppsala, located in the east of Sweden, had at the time the study started, a population of about 150,000. It is an administrative and educational center. There are no significant metal emitting industries, and emissions to air in the region originate mainly from energy and heat production. The emissions from these combustion processes (to a large part burning of waste) were substantial during the 1980s. They have been drastically reduced due to better sorting of waste and refining of fumes by installation of a filter in 1992 (Table 2).

Trollhättan is a medium-sized industrial town in the west of Sweden, with a population of about 55,000 at the time the study started. The region has had several metal emitting industries, but only a few are still in use. Previous emissions to air include Cr, Mn, and Mo from the smelteries and roasting of ore. Cr is the main remaining emission, with 7000 kg emitted to air in 1994, a reduction of >90% since 1985. The area also has automobile and jet engine industries. The traffic densities in the two regions were comparable (Uppsala municipality, 1997; Trollhättan municipality, 2001), and the drinking water was of good quality in regard to metal content (Trollhättan municipality, 1994; Uppsala municipality, 1994).

Table 2. *Metal emissions to air (kg) from combustion processes for heat production in Uppsala, 1980-1995 (Stock, 1996)*

Metal	Hg	Pb	Cd	Zn
1980	130	669	36	-
1985	525	1092	43	3925
1990	73	241	10	1110
1995	14	5	0.5	35

Questionnaire regarding fish consumption and dental amalgam fillings

At the re-examination at the age of 17, 382 adolescents could be contacted, and were mailed a questionnaire regarding their fish consumption and dental amalgam. In total, 296 answers were received (Paper IV). The fish consumption was separated into two categories, fish with dietary restrictions (http://www.slv.se: Accessed 15-Dec 2001b), *i.e.* pike, perch, pikeperch, burbot, eel and halibut, and deep-sea fish, *i.e.* non-restricted fish. Fish sticks were excluded from the fish consumption because of their low fish content. Information on the number, size and location of amalgam fillings was obtained by asking the teenagers to fill in the information on a drawing of the jaws, with all surfaces of the teeth marked. They were asked to perform the inspection of the teeth themselves, by use of a mirror. The number of fillings was then estimated from the drawing (Paper IV).

Sample collection and contamination control

Blood and serum for trace element analysis were taken by venipuncture into two evacuated 7-ml tubes for whole blood (heparinized) and serum, respectively (Vacutainer, Becton Dickinson, Rutherford, NJ, USA). Stainless steel needles were used. The samples were stored in acid-washed polypropylene vials at –20 °C, and transported frozen to the laboratory. In Trollhättan, the tubes for trace element analysis were taken last, after samples were taken for other purposes within the nutritional survey. This allowed a washout of the needles, aiming at reducing contamination from them. However, the elements mainly associated with contamination from stainless steel needles are Cr, Mn and Ni (Caroli *et al.*, 1994), which were elements not determined in the samples from the adolescents. In Uppsala, the tubes were taken in a random order. Contamination from the tubes was checked for by leaching (using 0.6 M nitric acid) of a set of collection tubes and storage tubes (Paper II). After leaching, 1.4 mg/L Zn was present in nitric acid from the tubes used for blood collection, and in the serum and storage tubes, 0.21 and 0.13 µg/L Pb, respectively, were found. For all other elements, *i.e.* Co, Cu, Se, Rb, Rh, Cd, Pt, Hg and Tl, the levels were below the detection limits (Table 3) or insignificant. Contamination of W was not tested. The test represents strained conditions, and therefore the resulting concentrations most probably are considerable overestimations of the actual contamination from the tubes. All standard solutions used for calibration and internal standardization were screened by using ICP-MS, which showed only negligible contamination with other elements (Paper I).

From the 15-year olds, 343 blood samples and 355 serum samples were obtained. Both blood and serum were obtained from 327 subjects. From the 17-year olds, 286 blood samples and 255 serum samples were obtained, and both

blood and serum were obtained from 217 subjects. From the adolescents who participated at both sampling occasions (*i.e.* the same individuals at 15 and 17-years of age), 263 blood and 211 serum samples were obtained.

Sample preparation

The sample preparation consisted of simple dilution in polypropylene tubes rinsed with deionized water. Blood was diluted 10-fold, and serum 5-fold (Paper I) with an alkaline solution containing ammonia, Triton X-100, and EDTA in Millipore water. Dilution factors of 20 and 10 were also tested for blood and serum respectively (Paper I). Internal standardization was made to correct for matrix effects. The elements In and Sc were thus added to the blood samples (Paper I-IV), and to serum in the method evaluation (Paper I). Subsequently, also Bi and Ga were added to the serum samples (Paper II-IV) in order to conform to the certified method used in the laboratory at the time.

Duplicate preparations were made for blood and serum, where possible, but 212 of the 605 serum samples were analyzed in single preparation due to the small volume of sample available. For calibration, outdated blood or plasma (serum was not available) from blood donors were used. The blood or plasma was spiked with multi-element stock solutions prepared in the laboratory, in order to produce calibration curves in physiologically relevant ranges (Paper I and II).

Element analyses

Trace elements were determined by ICP-MS (Paper I and II). The instrument was fitted with an autosampler. The diluted samples were introduced into the spray-chamber in a segmented flow mode, using the dilution solution as a carrier and rinsing fluid. The autosampler was programmed to use a specific sample uptake time (20 *s*). Both peak-jumping mode and scan mode were used for acquisition (Paper I and II). Interference corrections for spectral overlap were applied, and for some elements, two or more isotopes were summarized (Paper I and II). In order to study polyatomic interferences on ^{65}Cu, ^{66}Zn and ^{82}Se, ethanol, Zn and sulphur were added to prepared blood samples (Paper I).

The analytical series included blanks, calibration, reference samples and between 60 and 80 blood or serum samples. The calibration procedure in the instrument software was used, that is a least square fit to the data points. In order to avoid errors due to memory effects and instrumental drifts in sensitivity, the duplicate blood samples were placed singularly, first in order according to case number, then in reversed order. In the serum analyses, the samples were placed adjacent to each other. The samples collected from the same adolescent at age 15 and 17 were analyzed in the same analytical series, adjacent to each other. Samples from both boys and girls were included in all analytical series, as were

samples from both Uppsala and Trollhättan. All sample results were subtracted with a blank. Detection limits were calculated as 3 times the SD of the blanks (Paper I and II, Table 3).

The precision of the method was between 1% relative standard deviation (RSD) and 11% RSD in blood (Paper I and Table 4), and 1 and 6% RSD in serum. For each element, a limit was set for the deviation accepted between the results of the duplicate sample preparations (Paper II). The limits were both in relative terms (percentage) and in absolute terms, depending on the concentration range of the element. For major elements, the criteria was: less than 20% difference between duplicates, and for the other elements, a difference of less than 0.5 μg/L was accepted. These criteria for analysis acceptance resulted primarily in reanalysis of a sample, but if the reanalysis also failed the criteria, the specific element result was rejected (Paper II and III).

Reference material

Several reference materials were used in order to check the accuracy of the method and of the analyses. Both certified and uncertified reference material (but with recommended levels) as well as interlaboratory comparison samples were used (Paper I and II). The results are presented in the papers, respectively, and in Table 4. For the elements Rh, Pd, W, and Tl, no reference samples in blood or serum were available.

Statistics

Statistical analysis was preformed using the statistical software Minitab (release 13, Minitab Inc., State College, PA, USA). Non-parametric methods (Spearman's rank correlation, Mann-Whitney U-test) were generally used, so that occasional outliers would not unduly influence the results. This was done even though a normal distribution is approached in samples as large as the present one. In Paper III and IV, some continuous variables were logarithmically transformed in order to conform with the requirements of the statistical tests preformed, *e.g.* residual distribution. In Paper IV, the parametric 2-sample t-test was subsequently used on the log-transformed variables. Analysis of variance (ANOVA) was used to evaluate variation of predictors between subgroups. Linear regression was used to evaluate the effect and significance of one or more (multiple stepwise regression) independent variables on a continuous outcome variable. Statistical significance was defined as $p<0.05$, except in the ANOVA, where $p<0.01$ was used to allow for multiple inference. When multiple comparisons otherwise were made (Paper III), correction for multiple inference was made (Holm, 1979). All sample results were used for calculations, also results below the detection limits.

Results and discussion

Multi-element determination by ICP-MS

Challenges encountered in multi-element determinations

When the analytical work of this investigation was started, few ICP-MS multi-element methods were published that could be suitable. The sample preparation was one critical step, as complicated and time-consuming digestion or deproteinization methods were not feasible for us with such large numbers of samples. Also, each preparation step results in an increased risk for contamination. Therefore, simple dilution based on published work (Shuttler and Delves, 1986; Delves and Campbell, 1988; Lutz *et al*., 1991; Schütz *et al*., 1996) was the method we chose, and it was evaluated for use in large-scale investigations (Paper I). The method seemed appropriate for rapid screening of large sample series, but on the other hand, the detection limits of the current method are higher than what could be expected from different methods with more elaborate sample preparations and with focus on only one or two elements. Since Paper I was published, some other multi-element methods aimed at large-scale investigations have been presented (Case *et al*., 2001; Forrer *et al*., 2001).

The quality check of the analytical results had to follow a strict protocol, in order to handle the almost 8200 element results from the blood samples (including standards and reference samples), and the approximately 7600 element results from the serum samples. However, we could not employ the same quality criteria as when determining only one element, as 13 elements were analyzed in each sample, and too strict criteria would result in numerous reanalyses. The resulting analysis acceptance criteria were thus a compromise between analytical precision and sample throughput.

Choice of analytes

When we started working on the multi-element method currently used, the elements of most interest were primarily chosen as analytes (Paper I). They included 5 essential elements; Co, Cu, Zn, Se, Mo, toxic elements of environmental or occupational concern; Cd, Hg, Pb, Ni, and elements increasingly emitted in the environment; Pt, Pd, Rh, Ga, Sb, W, Tl, Sn, or with no defined biological role; Rb. However, due to analytical considerations, 13 elements were chosen for the study of trace elements in blood and serum from adolescents (Paper II-III). These analytical considerations included unacceptable number of reanalyses, lack of agreement with reference material, and the fact that reference material did not exist for many elements.

Other analytes of great interest were Mn, an essential element, and Cr, both elements emitted around Trollhättan for a long time due to the smelter industries.

Due to spectral overlap from iron, Mn could not be determined by the current method, and Cr could not be determined due to the high carbon content of the sample matrix (Paper I).

Limits of detection

In order to lower the detection limits in blood, the instrument's peak-jumping mode was used in blood for elements of low signal and of special interest (Paper I and II). In general, the scan mode is more efficient for multi-element determinations, and was also used for analyses of the blood samples (Paper II). After the number of analytes had been limited to the investigated 13, only the peak-jumping mode was subsequently used for serum (Paper II).

The limits of detection (LOD) in the analyses of the adolescents' blood and serum samples are shown in Table 3. They were calculated as 3 SD of all the blank samples of all the analytical runs. These LODs differ, for some elements with a factor of 10, from the LOD shown in Paper I. The reason for this was that the LOD in Paper I was calculated differently, namely 3 SD of one blank with 10 consecutive repeats, representing more the instrumental LOD, while the LOD in Paper II represent analytical "routine" conditions. Both ways to calculate LOD are used (Vanhoe *et al*., 1994b; Vanhoe *et al*., 1995; Schütz *et al*., 1996; Osman *et al*., 1998a), as well as other definitions (Schütz *et al*., 1996; Bergdahl *et al*., 1997a; Bergdahl *et al*., 1996; Ellingsen *et al*., 1993). It is however obvious that the definition of LOD can be crucial in trace element analysis, as many elements concentrations are close to or lower than the current method's LOD. The intended use of the LOD is what should determine the definition.

In Paper I, mean results below the LOD are indicated as not detected, while in the other papers, it is indicated as less than (<) LOD. This can also be regarded as a matter of convention. Because of the blank variability, a single result below the LOD cannot individually be separated from zero. However, for large numbers of samples, the mean value can be separated from zero, even if below the defined LOD. Consequently, it can also be argued that the mean values should then be presented. However, for the present use, *i.e.* to indicate levels in a general population, the detection limits, range, and percentage above the detection limits can provide enough information.

Reference material

For most elements, the analysis results of the reference materials were in agreement with the recommended or certified concentrations (Paper I and II; Table 4). However, for some elements, the levels found were outside the given ranges. In blood, the intra-day variation was less than 11% for all elements, while the inter-day variation was less than 9%. In both cases, Se analyses showed the largest variation (Table 4). In serum, the intra-day variation was less than 6% for all elements, and the inter-day variation was not investigated.

Table 3. *Limits of detection in blood and serum for 13 elements (µg/L; mg/L if indicated)*

Element	Blood detection limit	Serum detection limit
Co	0.2	0.2
Cu (mg/L)	0.02	0.004
Zn (mg/L)	0.09	0.006
Se	10	5
Rb (mg/L)	0.005	0.0005
Rh	0.1	0.1
Pd	0.2	0.2
Cd	0.2	0.4
W	0.2	0.04
Pt	0.1	0.1
Hg	0.7	0.3
Tl	0.06	0.2
Pb	1.0	0.1

The concentration of Co in serum reference material seemed to be overestimated by the current method at low Co levels, but in good agreement at higher levels (Paper I and II). This positive bias may arise from polyatomic interferences (White, 1999). The overestimation seemed rather uniform (see further section Nutritional status of essential elements), and should therefore not have any significant impact on the conclusions regarding how different factors influenced the concentration, as derived from the studies. The reported concentration of S-Co may however be somewhat too high.

Cu and Zn concentrations found in blood were in good agreement with the certified levels (Paper I), and in serum the same was found for recommended levels and intercalibration samples (Paper II), but the certified material "Second generation" human serum was slightly overestimated (Paper I). For Se, the concentrations reported in Paper I were generally too high, both in blood and in serum. Se was still determined in the study of adolescents, and here Se concentrations were much more in agreement with the recommended value (Table 4) and intercalibration samples (Paper II; see further section Specific comments on the analyses of some elements).

The Rb concentrations agreed well with certified and recommended levels (Paper I and II). In the Seronorm blood reference sample batch 205052, there was quite a large difference between the Rb result reported in Paper I and Paper II. The reason for the lack of agreement of the Rb results is not clear, and the Seronorm reference material has no recommended level for Rb. In general, there was a low variation in Rb results, and we are not aware of any significant spectral interferences on the Rb isotope determined. Perhaps inhomogenity of the reference material contributed to the different results (Friese *et al*., 2001), or some contamination from repeatedly using the same batch of material (Rodushkin *et al*., 2000). Krachler *et al* (1998) found a lower level in the same Seronorm batch, using a sample digestion method and ICP-MS.

The Cd concentrations were in good agreement with recommended or certified levels (Paper I and II). For Pt, only the serum intercalibration samples had recommended levels, and the concentrations were in several cases lower than the present LOD (Paper II). The results from the Hg reference material determinations differed between Paper I and II for Seronorm batch 203056, but not for Seronorm batch 205052 (Table 4). In Paper I, memory effects may have contributed to the higher concentration found. However, the analysis result agreed with the recommended concentration of Hg in the analyses of the adolescents' samples (Paper II). For Tl, the added amount to Seronorm batch 203056 was the only reference value, but the analysis result agreed reasonably well with this level (Paper I and II; Table 4).

The current method has been used extensively for determination of Pb in blood and plasma (Schütz *et al*., 1996; Bergdahl *et al*., 1997a; 1999). In blood, the analysis results were generally in good agreement with recommended levels (Paper I and II), although overestimated in Seronorm batch 205052 in Paper II (Table 4). In serum, no reference material is available.

Table 4. *Concentration of two reference materials of whole blood (µg/L, mg/L if indicated) and blood analysis precision*

Element	**Seronorm 205052**			**Seronorm 203056**			**Intra-day variability %RSD (Paper I)**	**Inter-day variability %RSD**[a]
	Recom-mended conc.	**Result Paper I**	**Result Paper II**	**Recom-mended conc.**	**Result Paper I**	**Result Paper II**		
Co	<1	0.2	0.33	6	6.2	5.4	1	3
Cu (mg/L)		0.82	1.0		0.88	0.79	2	5
Zn (mg/L)		6.6	8.0		6.3	5.6	2	6
Se	83 (79-87)	120	110	93 (89-97)	140	96	11	9
Rb (mg/L)		1.4	1.8		2.1	1.9	9	4
Rh		<0.08[b]	<0.1		<0.08	<0.1	5	3
Pd		0.2	<0.2		0.4	0.25	8	4
Cd	0.9 (0.8-1.0)	0.7	1.2	6.4 (5.9-6.8)	6.0	5.7	2	2
W		0.4	0.51		0.5	0.28	9	4
Pt		0.3	<0.1		<0.2	<0.1	9	5
Hg	3 (2-4)	3.2	3.8	9 (8-9)	12	8.6	3	5
Tl		<0.05	0.071	+5	5.4	4.9	2	3
Pb	35 (31-41)	34	46	383 (361-396)	380	380	6	8

[a]Calculated as the relative standard deviation of a high standard analyzed as a sample.
[b]< denotes less than detection limit.

In a longitudinal study, with the aim to compare element concentrations over time, the inter-day variation could pose a problem. It can however be overcome, for example, as was the case in the analysis of the adolescents' samples, by analyzing a single persons samples from both sampling occasions in the same analytical run. Then, only the intra-day variation (controlled through the analysis acceptance criteria) has to be taken into account. It was also important to mix samples from boys and girls, as well as from the two cities, so that any systematical errors would not produce false differences between groups.

Specific comments on the analyses of some elements

Can we trust the Se results?

Selenium analyses suffer from polyatomic interferences and a low degree of ionization in the ICP (Goossens *et al*., 1993; Niu and Houk, 1996; Sieniawska *et al*., 1999). It may also be speculated that the interferences differ between different ICP-MS instruments, thus making relevant corrections more difficult. The polyatomic interferences ZnO and SO_3 for ^{82}Se were investigated (Paper I), but contributions from them could not be identified. Attempts were also made to increase the degree of ionization by addition of ethanol (Niu and Houk, 1996), but this was also without positive results (Paper I). In the first study, Se analyses did not agree with reference material levels (Paper I), but in most cases did so in the following studies (Paper II). The reason for this is not known. It can be speculated that the interferences of Se changes over time. However, the present results were in the highest range of the recommended interval, and a slight overestimation of Se cannot be excluded.

Why did we not continue with Ni analyses?

An attempt was made to analyze Ni in the method evaluation (Paper I). Because of lack of agreement with reference material and the fact that the samples from the adolescents were taken with stainless steel needles, a major cause of Ni contamination (Caroli *et al*., 1994), no attempt was made to analyze Ni in these samples. Ni also suffers from polyatomic interferences, and low Ni concentrations tend to be overestimated (White, 1999). It is however interesting to note, that the low Ni concentration found in the reference material IAEA A-13 (Paper I), has been confirmed by other investigators (Rodushkin *et al*., 2000). Their report also highlights how easily reference materials are contaminated when the same batch of sample is in frequent use.

Why are Rh and Pd determined?

Most Rh and Pd results were below the detection limits, and also no reference material existed for these elements. It has been shown previously, that Rh and Pd suffer from spectral interferences when analyzed in human blood by ICP-MS (Begerow and Dunemann, 1996; Rodushkin *et al*., 1999), but by using UV photolysis for sample digestion in combination with magnetic sector field ICP-

MS, better results have been achieved (Begerow *et al.*, 1997). In this way, LOD far below the ones in the present work are made possible. However, even if the LOD of the current method are above the levels that can be expected in the general population, they can still be used in conjunction with the range as an indication of the level in the population (see further section Limits of detection).

Biological monitoring of trace elements and factors influencing their levels in adolescents from Uppsala and Trollhättan

For the elements Cu, Zn, Se, and Rb in blood and serum, and Pb in blood, all results were above the LOD, and for Co and Hg, in blood and serum, and W and Pb in serum, most results were (Paper II and III). This was also the case for B-Cd in smokers (Paper III). For the blood levels of the elements Rh, Pd, W, Pt, Tl, as well as Cd in non-smokers, the interpretation of the results were more restricted, as more than 50% of the results were below the LOD (Paper II and III). The same was true for serum levels of many of these elements (Paper II and III). In the next part of the Results and discussion section, each element is discussed separately. Some unpublished data are also included. For more specific results and further detail, the reader is referred to the individual papers.

Toxic elements

Cadmium

The concentration of Cd in blood was strongly influenced by smoking (Paper III; Fig. 3), and results for smokers and non-smokers are here discussed separately.

Table 5. *Median concentrations of 13 elements in blood and serum of 15-year old boys and girls (µg/L, mg/L if indicated)*

Element	**Blood**		**Serum**	
	Boys (n=104-153)[a]	**Girls (n=139-190)**	**Boys (n=157-164)**	**Girls (n=181-191)**
Co	0.31	0.31	0.48	0.49
Cu (mg/L)	0.90	0.93	1.0	1.1
Zn (mg/L)	6.1	6.1	1.0	0.97
Se	110	110	110	100
Rb (mg/L)	2.9	2.8	0.24	0.23
Rh	<0.1 [b]	<0.1	<0.1	<0.1
Pd	<0.2	<0.2	<0.2	<0.2
Cd	<0.2	<0.2	<0.4	<0.4
W	<0.2	<0.2	0.082	0.091
Pt	<0.1	<0.1	<0.1	<0.1
Hg	1.2	1.1	0.43	0.44
Tl	<0.06	<0.06	<0.2	<0.2
Pb	20	15	0.29	0.31

[a] The difference in n depends on the analysis acceptance criteria.
[b] < denoted less than detection limit.

The median concentration of B-Cd in the non-smokers was below the rather high detection limit of 0.2 µg/L (Paper III). This corresponds well to the levels previously reported in Swedish children and adolescents; median <0.2 µg/L in 14- 15-year olds (Granvik *et al*., 1988), geometric mean 0.14 µg/L in 4-11 year olds (Willers *et al*., 1988), geometric mean 0.08 µg/L in 7-10 year olds (Willers *et al*., 1992), and median 0.13 µg/L in 9-10 year olds (Osman *et al*., 1998a). Some recently reported European data give the geometric mean concentrations reported for German children aged 6-14; 0.14 µg/L (Hoffmann *et al*., 2000), and for Belgian 17-year olds; 0.27 µg/L (Staessen *et al*., 2001). In Swedish adult non-smokers, the reported median concentrations of 0.2-0.3 µg/L (Elinder *et al*., 1983; Vahter *et al*., 1996; Olsson *et al*., submitted) was only somewhat higher than in the presently investigated adolescents.

The smokers differed significantly in B-Cd from the non-smokers (Paper III; Fig. 3). Smoking was the strongest predictor of B-Cd, dominating the Cd exposure and giving rise to B-Cd four times higher in these young smokers compared to their non-smoking counterparts. Due to the increased exposure of the smokers, a wide range in the concentration of B-Cd was observed. There was also a significant difference between the two residential areas (Paper III). The median B-Cd concentration in the smokers in Uppsala was 0.21 µg/L (range <0.2-2.6), and in Trollhättan 0.44 µg/L (range <0.1-2.6). This is explained by the more frequent smoking of the smokers in Trollhättan (Bárány *et al*., unpublished results).

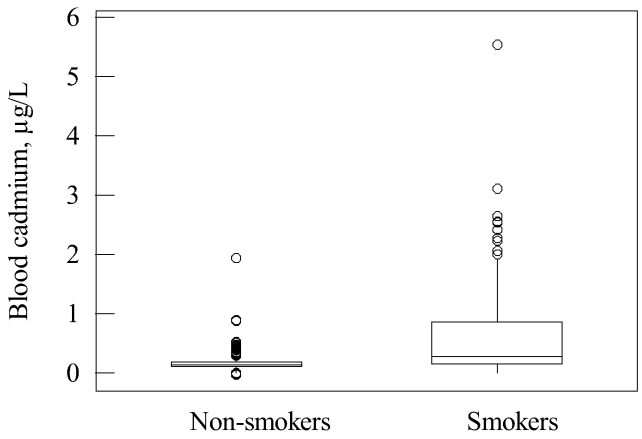

Fig. 3. Blood Cd concentration (µg/L) in 15- and 17-year old adolescents. Note that most persons were represented twice, at both 15 and 17-years of age (n= 444 non-smokers and 155 smokers). The box is defined by the upper and lower quartiles and with the median marked by a subdivision of the box. The whiskers are the lines that extend from the top and bottom of the boxes to the lowest and highest observations, excluding outliers (circles) with values that are more than 1.5 box-lengths from the median.

The level of Cd in Swedish arable soil has increased 30% during the last century, due to atmospheric depositions and Cd in mineral fertilizers used agriculturally (Andersson, 1992). On the other hand, recent data indicate a reduction in Cd concentrations in kidney cortex, which is where Cd is accumulated, in non-smoking Swedes during the last decades (Friis *et al*., 1998). The health risk for the Swedish population due to Cd exposure has been debated because of the limited number of subjects in the study by Friis *et al* (1998; Friis and Edling, 1998; Vahter *et al*., 1998). In the current study population, Cd in blood did not increase in non-smokers (intra-individual difference) during the study period, that was however relatively short, only 2 years (Paper III). As B-Cd in the general, non-smoking population, is thought to reflect body burden, *i.e.* accumulated exposure, as well as recent exposure (Berglund *et al*., 1994), an increased exposure was not supported in the present study. During the 1990s, a decline in atmospheric deposition of Cd was also indicated as measured in forest topsoil (Hedlund *et al*., 1997). However, a dilution of body burden during the growth-intense adolescent years could possibly mask an increased exposure.

The adolescents with university-educated mothers had higher B-Cd than those with mothers with primary school education (Paper III), in contrast to what has been shown previously regarding socioeconomic status and B-Cd (Osman *et al*., 1998a). This was not dependent on reported smoking habits (Paper III; Bárány *et al*., unpublished results). It is possible that the mother's education was a proxy for some other variable, probably associated with dietary Cd intake, as diet is the main source of Cd in non-smokers (WHO, 1992). Speculatively, frequent consumption of vegetables contributed to this relationship, as consumption of vegetables was more frequent in the highest socioeconomic group and no other differences in dietary habits between the groups were found (Samuelson *et al*., 1996a). However, the reported smoking habits were not validated (*e.g.* by analyzing urinary cotinine), and it is possible that smoking was underreported, especially in the group with university-educated mothers. When the data from the sampling at 15- and 17-years were considered together, a negative correlation was found between serum ferritin and B-Cd (Bárány *et al*., unpublished results), as has been shown previously (Flanagan *et al*., 1978; Berglund *et al*., 1994). Although iron deficiency affects Cd uptake, the difference between the socioeconomic groups could not be explained by differences in serum ferritin. The serum ferritin concentration did not differ between the socioeconomic groups (Samuelson *et al*., 1996b; 2000), and thus did not contribute to the differences caused by socioeconomic status.

Serum Cd is seldom analyzed, as the main part of the Cd is in the erythrocytes (Järup *et al*., 1998). Our analysis results of S-Cd were mostly below the detection limit of 0.4 μg/L, and were not influenced by smoking (Paper II and III). The latter fact may indicate a lower reliability in the serum analyses, as B-Cd was so strongly influenced by smoking. Also, no correlation was found between blood and serum levels of Cd, probably because of the uncertainty in the S-Cd analyses.

In Egyptian adolescents, aged 14-18 years, the concentration of S-Cd ranged from 0.5-3.1 μg/L, with a geometric mean of 1.2 μg/L (Hossny *et al*., 2001), which seems comparatively high.

Lead

The median blood Pb was 16 μg/L in the 15-year old adolescents, and 15 μg/L in the 17-year olds ($p<0.009$). Boys had higher concentrations than girls (Paper III; Fig. 4; Table 5), median 20 vs. 15 μg/L in the 15-year olds ($p<0.0005$), 16 vs. 14 μg/L in the 17-year olds ($p=0.022$). The difference can probably partly be explained by the difference in hematocrit and by the higher food intake in boys (Iyengar and Woittiez, 1988; Skerfving *et al*., 1999).

The mean B-Pb level decreased 10% during the two-year study period (Paper III). Use of Pb in petrol was banned in Sweden right before the second sampling period. The Pb concentration in blood of Swedish children has been monitored for several years, and an almost constant decrease has been reported during the years 1978-1994 (Strömberg *et al*., 1995). For children living in a rural area of Sweden, the geometric mean concentration of 22 μg/L was the most recently reported, and in an urban area 24 μg/L (Strömberg *et al*., 1995). The presently reported level in the 17-year olds (both residential areas can be regarded as urban) was the same as that reported by Pirkle *et al* (1998) in 12-19 year old adolescents in the USA, and by Staessen *et al* (2001) in Belgian 17-year olds living in a rural area. This is less than half of the concentration found in other recent studies from Europe (Osman *et al*., 1998a; 1998b; Beneš *et al*., 2000; Seifert *et al*., 2000), however these studies included younger children. Therefore, in addition to a probable lower environmental exposure and a different exposure pattern (younger children may be more exposed to soil and dust while playing), an age-dependent dilution of body burden in the adolescents is possible. In Sweden, Pb has not been used in plumbing or paint, which are important sources of exposure in many parts of the world. There was however a range in exposure levels within the presently studied group, as indicated by the ratio of 10 between the highest and the median B-Pb in the 15-year olds (Paper II, Fig. 4).

There was a significant difference in B-Pb of 15% between the two residential areas, not dependent on socioeconomic differences (Paper III). Although the traffic density was similar as mentioned in the Materials and methods section, and the drinking water had similar (low) metal levels (Trollhättan municipality, 1994; Uppsala municipality, 1994), the adolescents in Uppsala had lower B-Pb concentrations. The reason for this can only be speculated on. Possible reasons are industrial emissions in Trollhättan, differences in lifestyle not investigated in the nutritional survey, differences in the Pb content of the food consumed in the two areas, or a possible interaction with Se (Gustafsson *et al*., 1987; Osman *et al*., 1998b). Pb, as well as several other toxic metals, interacts with Se (Magos and Webb, 1980; Ellingsen *et al*., 1995; Ellingsen *et al*., 1997; Peraza *et al*., 1998), and S-Se levels indeed differed between the two residential areas (Paper

Fig. 4. Blood Pb concentrations (µg/L) in 15- and 17-year old Swedish boys and girls. The box is defined by the upper and lower quartiles and with the median marked by a subdivision of the box. The whiskers are the lines that extend from the top and bottom of the boxes to the lowest and highest observations, excluding outliers (circles) with values that are more than 1.5 box-lengths from the median.

III). A negative correlation was observed between Pb in blood and serum and S-Se in the adolescents studied (Bárány *et al*., submitted), which could indicate such an interaction. The difference between the adolescents' Se concentrations in the two cities may thus play a role in the difference in Pb concentrations. Wine consumption, which is a well-known source of Pb exposure in adults (Elinder *et al*., 1983), can however not explain the difference. Wine consumption was not statistically significantly related to B-Pb, and moreover, wine consumption was more frequent among adolescents from Uppsala (10% reported wine consumption, usually 2-3 times/month, Samuelson *et al*., 1996a) than among those from Trollhättan (5% reported consumption, similar frequency as above).

There was also a significant correlation between the Pb concentrations in blood and serum (Paper II and Table 6), despite the large difference in concentrations, with a ratio of 56. The relationship between blood and plasma Pb has been thoroughly investigated by Bergdahl (1997b), but in a higher concentration range.

Table 6. *Statistically significant Spearman's rank correlation coefficients (rs) between blood and serum concentrations for each element*

Element	**Co**	**Cu**	**Zn**	**Se**	**Rb**	**W**	**Hg**	**Pb**
rs	0.21	0.62	0.17	0.31	0.56	0.31	0.52	0.15
p	<0.01	<0.01	<0.05	<0.01	<0.01	<0.01	<0.01	<0.05

Mercury

Speciation of Hg into MeHg and inorganic Hg can indicate the source of exposure, as MeHg originates from fish consumption (WHO, 1990) and inorganic Hg mainly originates from amalgam (WHO, 1991). In the present study, speciation was not possible due to the analytical technique used. MeHg is however mainly contained in the erythrocytes (Kershaw *et al*., 1980), while inorganic Hg is approximately equally distributed between serum and erythrocytes (Barregård *et al*., 1992). By comparing Hg concentrations in serum and whole blood (or ideally, erythrocytes) information can thus be obtained about the major Hg contributor. However, MeHg is demethylated in the body, and subsequently distributed as inorganic Hg, which may make the interpretation more difficult. Among the current adolescents, a mixed exposure to the two sources was indicated by the relationship between the B-Hg and S-Hg concentrations (Paper II).

The B-Hg concentration is strongly dependent on fish intake (Svensson *et al*., 1992), which was also shown presently, with B-Hg levels ranging from <0.7-5.8 μg/L in the 17-year olds depending on fish consumption (Paper IV). In Swedish adults consuming no fish, a B-Hg of 1.8 μg/L has been reported (Svensson *et al*., 1992) compared to the concentration 0.74 μg/L in the corresponding group of 17-year old adolescents (Paper IV). Certain fish species contain higher levels of Hg than others, and the distribution of the chemical form of Hg also differs between species (Cappon and Smith, 1981; Cappon and Smith 1982). Predatory fresh-water fish from Hg-contaminated lakes usually have the highest levels of Hg, and the largest proportion of MeHg (Cappon and Smith, 1981; Cappon and Smith 1982; Ohlin, 1993). The Swedish National Food Administration recommends that certain fish species are not to be consumed more than once a week, and not at all by pregnant or lactating women, or by women planning a pregnancy (http://www.slv.se: Accessed 15-Dec 2001b). In the present study (Paper IV), it was shown that both the species with dietary restrictions and other fish species (*i.e.* non-restricted fish) influenced the levels of B-Hg in the 17-year old adolescents, even though their fish consumption was low.

The median level of S-Hg was 0.43 μg/L in the 17-year olds, of whom 61% had no amalgam fillings (Paper IV). Of those who had, the median number of fillings was 2.0±1.5 (range 0.5-9). In Swedish adolescents taking part in studies of immune-related diseases, the median plasma Hg was 0.26-0.42 μg/L (Herrström *et al*., 1994; Herrström *et al*., 1997). The S-Hg is generally much higher in Swedish adults (Åkesson *et al*., 1991; Svensson *et al*., 1992; Bergdahl *et al*., 1998). Dental amalgam, Se concentrations, consumption of non-restricted fish, and residential area influenced S-Hg (Paper IV). The fact that Se concentrations (both blood and serum Se) explained S-Hg indicates an interaction between these elements (Paper IV). Using B-Se instead of S-Se as a predictor of S-Hg gave the greatest explained variance in the 17-year olds (Paper IV). However, the explained variance was very low, only 1.7% for S-Hg vs. B-Se when 15-year and

17-year olds were analyzed together (Fig. 5). That Hg influences Se through some interaction in the body has been shown previously (Ellingsen *et al.*, 1993; Ellingsen *et al.*, 1995), and Se modifies the toxic effects of Hg in animals (Parizek and Ostadalova, 1967; Ganther *et al.*, 1972). One suggested explanation for the interaction is through complexing of Hg and Se with Selenoprotein P in serum (Yoneda and Suzuki, 1997). Another reason to a correlation is the exposure from the common source, fish consumption, of both Hg and Se (Svensson *et al.*, 1992). However, in the current adolescents no relationships were found between fish consumption and Se concentrations (Paper IV).

In blood, the influence on Hg from fish consumption was greater from fish with dietary restrictions, as shown in the multiple regression analysis. In serum however, non-restricted fish contributed, although not in those who only consumed such fish. Perhaps the larger proportion of inorganic Hg in such fish species (Cappon and Smith, 1981; Cappon and Smith 1982) contributed to this relationship. It may also be that the dietary questionnaire gave uncertain answers (Hallgren *et al.*, 2001), at least for restricted fish, which is consumed more seldom. Moreover, the Hg levels of fish show considerably inter-species and intra-species variation (Ohlin, 1993; NRC, 2000).

The reason for the difference in S-Hg (Paper III and IV) between the residential areas is not clear. There were no differences in fish consumption or in number of amalgam fillings between the adolescent groups from the two cities. However, in Uppsala, large quantities of inorganic Hg have earlier been emitted from combustion processes for heat production (Table 2), which could contribute to the S-Hg levels. In addition, the lower S-Se concentrations in Trollhättan (Paper III and IV) may also be of importance.

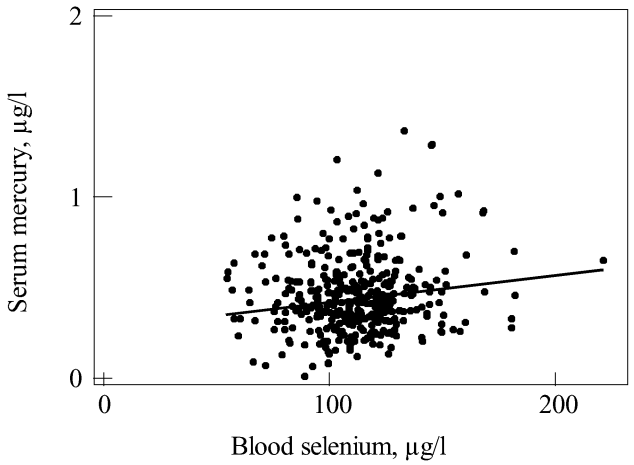

Fig. 5. The relationship between log serum Hg and blood Se in adolescents (log S-Hg = -0.53 + 0.014*B-Se, r^2=1.7, p=0.007). The relationship is plotted on the log-scale. Note that many persons are represented twice, at 15- and 17-years of age (total n=450).

The absolute majority of the investigated adolescents had B-Hg levels below 4.4 μg/L, which corresponds to the Reference dose (RfD) recommended by the US National Research Council (Mahaffey and Mergler, 1997; NRC, 2000, Paper II-IV). The RfD is what presumable can be consumed throughout life without an increased risk for adverse health effects, even in sensitive groups. One of the presently studied 15-year olds exceeded this level. His B-Hg had however decreased from 6.1 μg/L to 1.6 μg/L at the sampling at 17-years of age. Moreover, one 17-year old girl from Uppsala had a B-Hg of 5.8 μg/L. She had no excessive fish consumption (her reported consumption was 6 non-restricted and 0.2 restricted fish meals/month), and no dental amalgam fillings. The reason for her high B-Hg is therefore not clear.

Essential elements

Nutritional status of essential elements

The distributions of the essential elements' concentrations were generally narrow, with a ratio between the highest and the median concentration ranging from 1.5-2.8 (Paper II). This is what can be expected, as the essential elements are regulated by homeostatic mechanisms. For the performance of essential function, an optimum systemic supply is maintained by regulation of absorption, excretion and tissue retention, thereby adapting to varying intakes of essential elements. Interpretation of the analytical data from monitoring of blood or serum therefore requires considerations of the physiological relevance and limitations of the concentrations (WHO, 1996).

For the blood and serum concentrations of essential elements, socioeconomic factors did not have any impact (Paper III). Dietary supplements could have influenced the levels, and may perhaps account for some of the differences between the residential areas. The group of adolescents who took such supplements regularly or sometimes (n=165) was compared with those who never took supplements (n=235), but there were no differences in element levels. Even if it could not be demonstrated, an influence of dietary supplements cannot be ruled out, as many different non-specified supplements were used, of which some may influence level of specific elements.

For all the essential elements, significant relationships were found between an element's concentration in blood and that in serum (Table 6). From investigating the ratio between the blood and serum concentrations, Zn was to the largest part contained in the erythrocytes, Cu and Se were almost equally distributed, while Co was higher in serum (Paper II). However, as mentioned above (in section Reference materials), S-Co might be somewhat overestimated. Despite this, the relationship between blood and serum was significant. This may indicate that a possible overestimation was relatively uniform. This may also be supported by the analysis data, as there was a narrow range in S-Co concentrations, and <1% of the element results were excluded due to failure of analysis acceptance criteria

(Paper II). Thus, conclusions regarding the influence of predictors on S-Co can still be drawn.

Cobalt

Exposure to Co is usually assessed by analysis of blood or urine samples (Christensen, 1995; WHO, 1996). Urine seems to better follow peaks in the Co exposure (Alexandersson, 1988). However, there is a high degree of conformity between both blood and urine Co concentrations and Co exposure (Alexandersson, 1988). Mineral supplements containing inorganic Co may influence the blood and urine Co significantly (Christensen, 1995).

The concentration of Co in blood was previously reported to be near 20 μg/L (Iyengar and Woittiez, 1988), but more recent data reports means of 0.18-0.50 μg/L in adults (Alexandersson, 1988; Minoia *et al*., 1990; Christensen *et al*., 1993; Poulsen *et al*., 1994). This is in good agreement with the level found in the adolescents, 0.32 μg/L (Paper II, Table 5). In serum, the present mean level of 0.49 μg/L (Paper II) is slightly higher than that (0.1-0.45 μg/L) reported by other investigators (Iyengar and Woittiez, 1988; Minoia *et al*., 1990; Caroli *et al*., 1994).

The levels differed between boys and girls (Paper III, Table 5); both B-Co and S-Co were higher in the girls. A suggested reason to gender differences (Christensen *et al*., 1993; 1995; Kristiansen *et al*., 1997) is the increased uptake of Co in iron deficiency (Schade *et al*., 1970; Thomsen *et al*., 1971; Flanagan *et al*., 1980), which is a plausible explanation, as low iron stores was more prevalent in the girls (Samuelson *et al*., 1996a; 2000). Iron and Co shares the same uptake mechanism in the gastro-intestinal tract (Gunshin *et al*., 1997), and in the current adolescents, a negative correlation was found between serum ferritin and Co in both blood and serum (Bárány *et al*., unpublished results).

Copper

The most commonly used indicators of Cu status is S-Cu, serum ceruloplasmin (which binds 90-95% of Cu in plasma) and erythrocyte superoxide dismutase (Pettersson and Sandström, 1995). The investigated adolescents' median S-Cu concentration, 1.0 mg/L (Table 5), and range of S-Cu (Paper II) were similar to those reported for Finnish (Laitinen *et al*., 1989), Italian (Perrone *et al*., 1998) and German adolescents (Rükgauer *et al*., 1997). Using a definition of hypocupraemia as S-Cu <0.8 mg/L (WHO, 1996), 3% of the 15-year olds and 5% of the 17-year olds had a low Cu status. Regarding the upper range, the 97.5th percentile was the same as that reported for Danish adults (Grandjean *et al*., 1992a), and adverse chronic effects are rare due to the homeostatic control (Pettersson and Sandström, 1995). The Cu nutritional requirements are not well defined (WHO, 1996; NRC, 2001).

The concentration of Cu increased in the girls with age (Paper III). This can probably be attributed to hormonal changes, which increases ceruloplasmin levels (Denko and Gabriel, 1981; Lockitch *et al.*, 1988b). The highest levels were found in 17-year old smoking girls (Paper III). Smoking may also increase both ceruloplasmin and S-Cu concentrations (Davidoff *et al*., 1978).

Zinc

Plasma or serum Zn is believed to reflect Zn status (Laitinen *et al*., 1989), but the relevance of serum or plasma levels can be questioned as they may easily be affected by conditions unrelated to Zn status, *e.g.* fever or other stresses (WHO, 1996). At present, the reversibility of symptoms related to Zn deficiency (*e.g.* growth retardation or impaired resistance to infections) by Zn supplementation is considered a useful indicator of marginal Zn status (WHO, 1996). Excessive Zn intakes lead to disturbances of the Cu metabolism (Fischer *et al*., 1984, Yadrick *et al*., 1989).

The concentration of Zn in blood of the adolescents was somewhat higher than those reported for children (Osman *et al*., 1998b; Beneš *et al*., 2000), and more like those reported for adults (Minoia *et al*., 1990; Hamilton *et al*., 1994). The results for Zn in blood were perhaps affected by contamination (see section Sample collection and contamination control). A slight age-dependent increase in the concentration of Zn in serum has been reported (Rükgauer *et al*., 1997), but rather the opposite was indicated in adolescents from Uppsala in the present study (Paper III), although the time span was limited. The adolescents' mean concentration of S-Zn, 0.99 mg/L (Paper II), corresponded well to the mean levels of 0.90-0.98 mg/L reported for Finnish (Laitinen *et al*., 1989), and Italian (Perrone *et al*., 1998) adolescents, but was somewhat higher than the 0.88 mg/L reported in German adolescents (Rükgauer *et al*., 1997).

A gender-dependent difference, as found in the investigated adolescents (boys had 106% of the girls mean S-Zn concentration; Paper III), was also reported by Laitinen *et al* (1989), but not by other investigators (Lockitch *et al*., 1988a; Rükgauer *et al*., 1997; Perrone *et al*., 1998). In the study group, girls had a lower Zn intake than boys, below the Swedish nutritional recommendations, as reported by Samuelson *et al* (1996a). The S-Zn also seemed to decrease in the girls, although not statistically significantly (Paper III), to a level in the low end of the reference range for adults (Iyengar and Woittiez, 1988; WHO, 1996). However, the Zn requirements of girls are lower than boys' requirements, and because of the homeostatic mechanisms, there is an inverse relationship between the efficiency of Zn absorption and current Zn intake as well as existing Zn status (WHO, 1996). Accordingly, the absorption of Zn in the girls should have adapted and become greater if needed to better fulfill the nutritional needs. On the other hand, the suggested decrease in S-Zn from 15-years to 17-years of age may indicate that the homeostatic mechanisms have not been sufficient in order to maintain the Zn status. More specific investigations including Zn

supplementation would be needed in order to conclude if the girls' mean S-Zn concentration reflected a marginal Zn status.

Selenium

Different indicators of Se status are used, and are useful at different levels of exposure. Glutathione peroxidase (GSH-Px) is a Se dependent enzyme present in various tissues in the human body. In blood, GSH-Px in blood, plasma, and platelets seem to be saturated at different levels of Se intake, and can therefore be used as functional indicators of Se status (Alexander and Meltzer, 1995). At lower exposures, blood and serum levels usually reflect Se intake. Therefore, blood and serum Se concentrations vary between different populations of different countries and areas, reflecting the Se available in soil for plant growth and the corresponding variations of foods (Iyengar and Woittiez, 1988; Alexander and Meltzer, 1995; SCF, 2000). Plasma or serum levels are usually somewhat lower than blood levels, as was the case in the present studies (Paper II-IV, Fig. 6).

The mean B-Se in the presently investigated 15-year old adolescents was 110 µg/L, and S-Se 100 µg/L (Paper II). B-Se in adults from Sweden was 102 µg/L in one study (Hansson *et al.*, 1989), but 176 µg/L in another (Rodushkin *et al.*, 1999). In Swedish 9-10 year old children, a mean B-Se concentration of 130 µg/L has been reported (Osman *et al.*, 1998b). The latter was however analyzed by the same method as reported here, and an overestimation of Se cannot be excluded (Paper I; Osman *et al.*, 1998b; 1998c), as mentioned above (see further section Can we trust the Se analyses?). In the presently investigated adolescents, both blood and serum Se increased a few percent with age (intra-individual difference) (Paper III). Few reference values are available for adolescents, but from Germany, S-Se concentrations of 60-92 µg/L have been reported for adolescents,

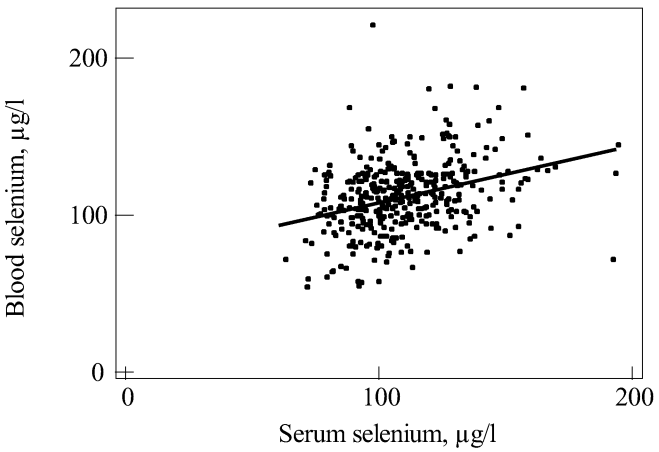

Fig. 6. The relationship between blood and serum Se in 15 and 17-year old adolescents (B-Se=73+0.35*S-Se, r^2=10%, p<0.0001). Note that most persons are represented twice (n=374).

with age-dependent increases (Lombeck *et al*., 1977; Rükgauer *et al*., 1997). Similar results were reported from Belgium, the Netherlands and France (van Caillie-Bertrand *et al*., 1986; Malvy *et al*., 1993). The age-dependent increase was also found in Canadian children and adolescents, and the reference interval for 15-19 year olds was 102-185 µg Se/l in serum (Lockitch *et al*., 1988a). In Swedish adults, reported mean plasma or serum Se range from 77-86 µg/L (Gustafsson *et al*., 1986; Hansson *et al*., 1989; Svensson *et al*., 1992), in Danish 84 µg/L (Grandjean *et al*., 1992a), in Norwegian 100 µg/L (Rahil-Khazen *et al*., 2000), and a general review states a median of 96 µg/L in adults (Iyengar and Woittiez, 1988).

Boys had higher Se levels than girls (Paper III), and a higher Se intake (Samuelson *et al*., 1996a). This is in accordance with the assumption that Se levels reflect Se intake. However, Se concentrations were not influenced by fish consumption (Paper IV), which is a major dietary source of Se (Svensson *et al*., 1992; Johnsson *et al*., 1997). However, the bioavailability of different Se compounds differs (SCF, 2000). Fish-Se has been reported to influence plasma Se at levels <100 µg/L, but not at higher levels, >120 µg/L (Alexander and Meltzer, 1995), but in this case, the reason may be the relatively low fish consumption.

There was a significant difference between the residential areas (Paper III and IV), indicating lower S-Se levels in Trollhättan, although the intake did not differ (Samuelson *et al*., 1996a). It can be speculated, that an interaction with Pb as mentioned above (see section Lead), decreased the uptake of Se with subsequent lower Se concentrations in the more Pb exposed adolescents from Trollhättan. However, it is questionable at these low exposure levels of Pb.

The minimum observed S-Se concentrations were both in boys from Trollhättan; in the 15-year olds it was 63 µg/L, and in the 17-year olds 75 µg/L (Paper II and III). It is not clear at what levels deficiency occur, but there may be negative health effects below a plasma Se level of 50 µg/L (Alexander and Meltzer, 1995). Thus, even though the Se intake in boys and girls of the present study was below the Swedish nutritional recommendations as reported by Samuelson *et al* (1996a), no clear deficiency could be established, even considering a slight overestimation of Se results. However, for the adolescents with the lowest Se concentrations, there is not a wide margin. On the other hand, toxicity of Se occurs at much higher intakes and serum concentrations than reported here (Alexander and Meltzer, 1995; SCF, 2000).

The elements Rb, Rh, Pd, Pt, Tl, and W

Rubidium

The biological role of Rb, if any, is not clear. Essentiality has not been proven, and no toxic effects are described in the literature. It is however interesting to

note that so little is known about an element that is present in such high levels in human blood (Table 5). The concentrations of Rb in blood and serum (Paper II and III) corresponded well to reported levels in adults (Minoia *et al*., 1990; Hamilton *et al*., 1994; White and Sabbioni, 1998; Rodushkin *et al*., 1999). It is interesting to note, that the impact of the determining factors investigated in Paper III were very similar in blood and serum, indicating an equilibrium between blood and serum, which is also supported in the strong relationship between blood and serum levels (Table 6). However, the reasons for the differences between subgroups of adolescents described are not obvious, but remain to be elucidated.

Platinum-group elements: Rhodium, palladium and platinum

The PGE are discussed together, as they are spread in the environment in much the same way, and from the same source (catalytic converters in cars). In the present studies (Paper II and III), the mean blood levels of the PGE were all below the LOD (Table 3 and 5). Previous reports range in mean B-Pt levels from 0.0009 μg/L (Begerow *et al*., 1997) to 0.56 μg/L (Vaughan and Florence, 1992) in persons not occupationally exposed. B-Pd was 0.05 μg/L in two studies (Begerow *et al*., 1997; Rodushkin *et al*., 1999), and B-Rh less than 0.0009 μg/L (Rodushkin *et al*., 1999). Few reports concern serum levels. Messerchmidt *et al* (1992) reported plasma Pt levels in non-exposed subjects ranging from <0.8-6.9 ng/l. The S-Pt concentration in the 17-year olds seems high, and may be due to a hitherto unidentified source of contamination, but could possibly reflect a new source of exposure. Mean S-Pd was 0.20 μg/L (LOD 0.2 μg/L) and 0.23 μg/L in 15 and 17-year olds in Uppsala, respectively (Paper III). Mean S-Rh was 0.11 μg/L (LOD 0.1 μg/L) in 17-year old adolescents in Uppsala and girls in Trollhättan. In serum, all PGE increased (intra-individual difference) with age/time in the adolescents.

Many studies have reported increasing PGE concentrations in the environment after the introduction of catalytic converters, *e.g.* increases in road dust and river sediments (Wei and Morrison, 1994; Schäfer *et al*., 1999). Traffic-density related air levels of Pt (Schierl and Fruhmann, 1996), Pt and Rh (Gómez *et al*., 2001), or soil and dust levels (Farago *et al*., 1998) have also been shown. However, Farago *et al* (1998) found no difference between B-Pt levels in motorway maintenance workers and college staff in a pilot study. The bioavailability of the PGE in their emitted form is also not clear (Vaughan and Florence, 1992; Zereini *et al*., 1997; Artelt *et al*., 1999), but the absorption of Pt seems to be greater when inhaled than when ingested (Artelt *et al*., 1999). This may be due to that the fraction of emitted Pt that consists of particles small enough to reach the alveoli after inhalation has been reported to be substantial (Gómez *et al*., 2001). The increasing serum concentrations shown in Paper III may be related to an increasing environmental contamination of the PGE, but much more work must be carried out before firm conclusions can be drawn.

Thallium and tungsten

The median concentration of B-Tl in the adolescents was less than 0.06 µg/L (Paper II and III), in accordance with a previous study, where B-Tl was reported to be 0.057 µg/L in adults (Sabbioni *et al*., 1994). The 97.5th percentile was 0.08 µg/L in the present study, indicating a low variation in analysis results. In serum, very few results were above the LOD and the uncertainty in the results is considerable (Paper II and III).

The B-W was below the LOD of 0.2 µg/L, a level that was exceeded by very few individual analysis results (Paper III). Minoia *et al* (1990) reported a mean B-W concentration of 0.39 µg/L, and Rodushkin *et al* (1999) 0.036 µg/L. It is however notable, that in Paper I and the study by Rodushkin *et al* (1999), the same level of W (0.2 µg/L) was found in the reference material IAEA A-13 (no recommended or certified level of W). In serum, the mean concentration in the adolescents at 15-years of age was 0.14 µg/L, while Minoia *et al* (1990) reported a mean serum level of 0.045 µg/L. Unfortunately, the contamination of W was not investigated in the present study (Paper II), and the variations with age and residential area were extensive (Paper III). There was however a significant correlation between blood and serum concentrations of W (Table 6), which indicates a reasonable reliability in the W results, or a very uniform contamination. The latter seems improbable, as blood and serum samples were collected and stored in different tubes, and analyzed separately. Increases in W contamination from sewage sludge used on arable land have been shown in the Swedish environment (Eriksson, 2001), but in the present studies, both blood and serum levels of W decreased (Paper III). Thus, the present results should be interpreted carefully.

Concluding remarks

The current method for multi-element determination by ICP-MS, using dilution as preanalytical step, was useful for large-scale analysis of blood and serum samples (Paper I). The detection limits were sufficient to determine the elements Co, Cu, Zn, Se, Rb, Hg, and Pb in blood and serum (Paper II and III). Moreover, B-Cd in smokers could be determined. In blood, the largest part of the analysis results for the elements Rh, Pd, Cd in non-smokers, W, Pt, and Tl, were below the detection limits. This was also the case for most of these elements in serum. Lack of reference material also restricted the interpretation of these element results.

Very low concentrations of the toxic elements Cd, Hg and Pb were observed in the present studies, especially in the case of Pb (Paper II and III). There was however a wide range in exposure levels, indicating a large variation. Cd in blood was strongly positively influenced by smoking habits and by the mother's

education (Paper III). In contrast to smokers, B-Cd in non-smokers did not increase between the samplings. The mean B-Pb was one of the lowest reported, and also decreased about 10% between the sampling of the adolescents at 15-years and that at 17-years (Paper III). This might be due to temporal changes in environmental exposure, since the use of leaded petrol was decreasing, and was banned in Sweden right before the second sampling period. However, age changes may reflect not only temporal changes in environmental exposure but also a dilution of body burden in these growing adolescents. Moreover, there was a difference between the genders, as B-Pb was higher in boys than in girls.

B-Hg was predicted by consumption of fish, both fish with dietary restrictions due to elevated Hg levels, and other "non-restricted" fish (Paper IV). There was a linear increase in B-Hg with increasing fish consumption in the group of adolescents who consumed only non-restricted fish. However, consumption of ≥6 meals/month of non-restricted fish did not result in statistically significantly higher mean B-Hg than no fish consumption at all. The individual B-Hg concentrations at age 17 were all but one below the level corresponding to the US National Research Council's RfD. Hg in serum was predicted by dental amalgam, consumption of non-restricted fish, and Se concentrations. S-Hg was also lower in Trollhättan. There were no differences in consumption of fish or in number of amalgam fillings that could explain this difference. The lower S-Se in Trollhättan (Paper III and IV) and the higher Hg emissions in Uppsala during the 1980s from combustion processes for heat production may contribute to the differences in S-Hg.

The levels of the essential elements Co, Cu, Zn and Se in blood and serum were within the reference intervals (Paper II). The essential elements showed a lower variation in concentrations than the toxic, probably explained by the homeostatic control in the body. Socioeconomic status did not influence the essential elements' concentrations (Paper III). However, age and gender had significant influences on the levels. The S-Se concentration was lower in Trollhättan than in Uppsala, and several interactions between Se and toxic elements were indicated (Paper III and IV). Fish consumption did not influence Se concentrations (Paper IV).

The interpretation of the results for the elements Rh, Pd, W, Pt, and Tl were limited by the large percentage of analytical data below the detection limits (Paper II and III). Increases in the levels of Rh, Pd and Pt in serum were however indicated, in intra-individual increases and as the mean concentrations of these elements were above the detection limits at the second sampling (Paper III). The relations between blood and serum levels of these elements, as well as the impact on biological levels of them from increasing use of and thereby emission from catalytic converters in cars needs further investigation.

Residential area, *i.e.*, living in Uppsala as compared to Trollhättan, had an impact on all 13 elements investigated (Paper III). The differences between the cities went however in different directions, and there was no clear evidence that the higher metal exposure in Trollhättan caused these differences.

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Acknowledgements

This work was carried out at the Department of Pharmacology and Toxicology at the Swedish University of Agricultural Sciences, Uppsala, the Department of Occupational and Environmental Medicine, University Hospital, Lund, and the Department of Food Hygiene, Swedish University of Agricultural Sciences, Uppsala. I wish to express my sincere gratitude to all who have supported me in this work and in various ways contributed to this thesis, especially:

Professor *Agneta Oskarsson*, my supervisor, for being an encouraging and inspiring person, with an excellent knowledge of diverse research areas. Without Agnetas positive and generous support, it is fair to say that this thesis would never have been written.

Dr *Ingvar Bergdahl*, my co-supervisor, whom equipped with a thousand Why?, How? and How come? has ensured that my mind got it's weekly dose of exercise, for being a great friend, coach and mentor.

Professor *Gösta Samuelson*, my co-supervisor, for optimistic interest in my work, and help in elucidating problems encountered under way.

The late Associate Professor *Andrejs Schütz*, also one of my co-supervisors, for welcoming me to the laboratory and for kind support and interest in my work.

My other co-authors, Professor *Staffan Skerfving*, for support and constructive criticism, Dr *Thomas Lundh*, for your work in the lab and for interesting discussions, and Associate Professor *Lars-Eric Bratteby*, who started the nutritional survey together with Gösta.

Anna Akantis, at the Department of Occupational and Environmental Medicine, for expert technical assistance and many laughs. All personnel involved in the adolescent study, especially *Heléne Enghardt-Barbieri*.

For statistical advise, *Patrik Öhagen*, at the Department of Epidemiology, and my colleague, PhD-student *Anna Lindén*.

Soon–to-be-Dr *Ing-Marie Olsson*, and Dr *Christian Lindh*, for social support, encouragement and occasionally a scientific discussion or advice.

My other colleagues at the Department of Pharmacology and Toxicology, and at the Department of Occupational and Environmental Medicine.

Anna-Lena Bergdahl for your hospitality and friendliness during my visits to Lund. All my other friends, for good times.

My parents, *Anders* and *Marie-Louise*, for constant support, encouragement, and fruitful discussions, for always believing in my abilities, for help with baby-sitting, and Sunday dinners. My brother and sister-in-law + my sister and her boyfriend, for enjoyable family gatherings.

Most of all, I would like to thank my family, *Stefan* and *Love*, for love and support in all ways, and for creating the best home anyone can wish for when absorbed in writing a thesis.