SWECO Environment AB

Exposure and effect screening in urine of women

1. Metals and metabolites of phthalates, organophosphate pesticides and PAHs.

2. Endocrine disturbing effects.

Client:

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Sammanfattning

Bakgrund och metoder

Inom 2008 års screeningprogram beslutades att metaboliter samt metaller skulle mätas i urin. Orsakerna till att fokusera på metaboliter var:

- Att många ämnen metaboliseras så snabbt att det är lättare att påvisa metaboliten än modersubstansen
- Att metabolitförekomst i urin reflekterar en faktisk exponering till skillnad mot en beräknad exponering
- Att risken för extern kontaminering av prover vid provtagning och analys minimeras när metaboliter mäts
- Att metaboliter i vissa fall är mer toxiska än sina modersubstanser
- Att hälsorelaterade övervakningsprogram i länder som Tyskland, USA och Holland till stor del fokuserat på mätningar av metaboliter i urin.
- Att metaboliter relaterar till exponering på cellulär nivå eftersom ämnen måste tas upp i celler för att genomgå metaboliska processer

För att täcka in olika typer av föroreningskällor och exponeringsvägar valdes följande ämnesgrupper ut:

- Ftalater där källan ofta är plastprodukter (10 metaboliter)
- Polycykliska aromatiska kolväten (PAH) som mestadels härrör från förbränningsprocesser och där exponering ofta sker via inandning men även via kosten (15 metabolites)
- Organofosforbaserade pesticider (OP pesticider) där exponerings mestadels sker vid intag av föda (6 metaboliter)
- Metaller som reflekterar en lång rad olika källor och exponeringsvägar (Al, Cd, Co, Cr, Cu, Hg, Mn, Mo, Ni, Pb, Zn, As).

Utöver direkta mätningar av kemikalier i urin genomfördes också effektbaserade mätningar. Eftersom ett flertal av ftalaterna och OP pesticiderna samt benzo(a)pyren (en PAH) är klassade som potentiellt endokrinstörande valdes ett in *vitro test* för endokrinstörande effekter (Yeast Estrogen Assay, YES)

Urininsamlingen genomfördes inom ramen för naturvårdsverkets hälsorelaterade miljöövervakningsprogram (HÄMI), i vilket ingår ett provtagningsprogram för kadmium i kvinnors urin, som genomfördes vid institutet för miljömedicin vid Karolinska Institutet (ansvarig Marika Berglund). Ett antal kvinnor som ingick i kadmiumstudien (totalt 200 kvinnor) valdes slumpmässigt ut och tillfrågades om de ville delta i föreliggande studie. Deltagande kvinnor (20 stycken från Stockholmsområdet i åldern 20 – 29 och 50 – 59 år) fick svara på ett antal frågor som relaterade till potentiell exponering för olika typer av källor. Frågorna innefattade information om ålder och vikt, typ av boende, källa till dricksvatten, kostvanor etc.

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Syftet med förekommande undersökning var att:

- Att jämföra halter i urin med referenskoncentrationer från andra länder
- Att utvärdera samvariationen i urinhalter inom ämnesgrupper och mellan ämnesgrupper
- Få en indikation på exponeringsnivåerna hos kvinnor i Sverige för några viktiga föroreningsgrupper
- Att få en indikation på om halter av potentiellt endokrinstörande ämnen kunde kopplas till markörer för endokrinstörande (estrogena) effekter i kvinnors urin.

Resultat och rekommendationer

De huvudsakliga slutsatserna var:

- Urin tycks vara en lämplig matris inom den hälsorelaterade miljöövervakningen för att följa nivåer av en rad viktiga ämnen och ämnesgrupper hos kvinnor

- Många olika PAH- och ftalatmetaboliter som härstammar från en rad viktiga substanser inom dessa ämnesgrupper var vanligt förekommande i urin. Samtidigt var koncentrationerna av dessa metaboliter högst varierande..

- Metaboliter som härstammar från OP pesticider var inte vanligt förekommande, eftersom endast en av 6 metaboliter förekom i mer än 2 prov.

- Inga signifikanta samband mellan halter av metaller och metaboliter och information om levnadssätt och andra exponeringsparametrar som gavs av de deltagande kvinnorna kunde detekteras i det begränsade materialet.

- Halterna av vissa ftalatmetaboliter och PAH metaboliter var generellt på samma nivåer som i USA, Tyskland och Holland. Sådana jämförelser är dock behäftade med osäkerheter eftersom ett betydligt färre antal personer deltog i förekommande studie.

- Halterna av ftalatmetaboliterna MiBP and MiNP var betydligt över de nivåer som observerats i USA vilket indikerar en högre exponering för dibutylftalat och di-iso-nonylftalat.

- Halterna av ftalatmetaboliten monoetylftalat (MEP) var tydligt lägre jämfört med USA och lägre jämfört med Holland vilket indikerar en lägre exponering för dietylftalat.

- Metaboliter som härstammar från OP pesticider hade klart lägre halter jämfört med de nivåer som observerats i både USA och Tyskland.

- Halter av metaller var generellt lägre än i USA och Tyskland.

- Ftalatmetaboliter samvarierade oftast i urin vilket tyder på en gemensam exponeringskälla

- PAH metaboliter samvarierade också, men inte lika tydligt som ftalatmetaboliter.

- Det fanns inga signifikanta samband mellan metabolit- och metallkoncentrationer och endokrinstörande effekter i urin. Detta kan delvis eller helt bero på förekomsten av naturliga steroidhormoner som östradiol och östriol i kvinnors urin.

Följande rekommendationer kan ges:

1. Uppföljande studier av ftalat- och PAH metaboliter i urin bör genomföras. Dessa bör då omfatta ett större antal människor än vad som var fallet i förekommande studie, så att levnads- och exponeringsfaktorer som påverkar halterna av dessa metaboliter i urin kan identifieras.
2. I en uppföljande studie kan antalet metaboliter i varje ämnesgrupp möjligtvis reduceras baserat på resultaten från denna studie.
3. Det finns inget behov av uppföljande studier av metaboliter som härstammar från organofosfor pesticider.
4. Uppföljande studier av metaller kan vara av intresse givet att dessa inte redan ingår i existerande miljöövervakningsprogram.

Summary

Background and methods

Within the screening program of 2008 it was decided that levels of organic metabolites should be studied in urine. The reason for focusing on metabolites was:

- They reflect internal exposure at the cellular level
- The occurrence of metabolites reflects the true individual exposure
- Monitoring of metabolite exposure is not prone to external contamination
- Many metabolites are more toxic than their parent compounds
- Human biomonitoring programs in other countries has to a large degree focused on metabolites of organic substances in urine

To cover a wide group of exposure pathways, the following substance groups where included:

- Phtalates that mainly reflect exposure from substances in plastic products (10 metabolites)
- PAHs that mostly reflect exposure to airborne contaminants originating from combustion (15 metabolites)
- Organophosphate pesticides that mostly reflects exposure to food stuff in Sweden (6 metabolites)
- Metals that reflect a wide variety of exposure pathways (Al, Cd, Co, Cr, Cu, Hg, Mn, Mo, Ni, Pb, Zn, As)

To complement the exposure based screening of urine, an effects based screening was also included where the urine samples were tested for endocrine (estrogen) disturbing effects using the YES (Yeast Estrogen Screening assay) in vitro method.

The urine sampling was part of a larger sampling campaign performed by the institute of environmental medicine, at Karolinska Institutet with a focus on Cadmium. Within this sampling program a number of women were randomly chosen to receive a letter asking for their participation in the present study. From this larger subset 20 women were randomly chosen for inclusion in the present study. As part of the study, the women answered a questionnaire with a number of exposure related questions.

The objectives of the project were to:

- To broadly assess exposure levels of some important contaminant groups in the Swedish female population by examining a small subset consisting of 20 females.
- To compare exposure levels of these substance groups to exposure levels in other countries
- To statistically investigate the co-occurrence of different metabolites and metals in urine

- To explore the connection between endocrine disturbing effects and the exposure levels of these substance groups

Results

The main conclusions from this study were:

1. Urine is clearly a good matrix for human biomonitoring of organic chemicals and metals given the high prevalence of detection and the good correlations seen between many metabolites.
2. PAH and phthalate metabolites originating from many(most) of the important mother compounds as well as metals were very prevalent in the urine of the women and the levels were generally variable.
3. Metabolites of organophosphorous pesticides were not prevalent
4. No connection could be found between exposure related .information given by the women and metabolite and metal levels in urine.
5. Levels of PAH and phthalate metabolites are in general similar to reference concentrations in USA, Germany and Holland.
6. Levels of the phthalate metabolites MiBP and MiNP were clearly above levels in the USA indicating a higher exposure to the phthalates DBP and DINP
7. Levels of the metabolite Mono-ethyl phthalate (MEP) were clearly lower in the present study compared to levels in USA and below levels in Holland, indicating lower exposure to the phthalate DEP in Sweden.
8. Levels of organophosphate pesticide metabolites were clearly below levels seen in USA and Germany
9. Levels of metals were generally below levels seen in USA and Germany
10. The urinary levels of most phthalate metabolites correlated clearly
11. The levels of PAH metabolites also correlated, but not as clearly as for phthalate metabolites
12. No connection could be established between endocrine disturbing effects measured with an in vitro test, and urinary levels of metabolites and metals. A partial explanation may be the presence of natural hormones with endocrine inducing properties in women's urine.

The following recommendations were given:

5. Follow-up studies focusing on PAH metabolites and phthalate metabolites are suggested. These studies should contain larger groups of women and/or men so that exposure factors influencing metabolite concentration in urine could be established.
6. The number of metabolites in follow up studies could be reduced based on the results from this study.
7. There is no further need for organophosphate pesticide metabolite screening studies in urine.

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8. Further screening studies of metals in urine could also be of interest although the levels seen in this study were gebwerally low compared to other countries

1 Background and scope

At present there is a lack of knowledge regarding the emission, distribution and exposure for many of the chemicals emitted to the environment. The aim of the screening program financed by the Swedish Environmental Protection Agency (Swedish EPA) is to alleviate this lack of knowledge by estimating the occurrence of different chemicals in the environment and in humans

The health related screening mainly aims at investigating human exposure levels to different environmental factors/agents such as pollutants and noise. Within the health related screening program, studies on human exposure to pollutants has focused on a few selected metals (Cd, MeHg and PB) and some classical persistent organic pollutants (i.e. PCB, DDT, dioxins, PBDE) while exposure to emerging and product related contaminants has rarely been investigated[1].

Metabolites of organic substances are especially well suited as biomarkers for exposure because:

- They reflect internal exposure at the cellular level since they have undergone enzymatic transformation
- The occurrence of metabolites reflects the "true" individual exposure as opposed to exposure estimations on the basis of abiotic environmental monitoring (Angerer et al. 2007).
- Monitoring of metabolite exposure is not prone to external contamination (Angerer et al. 2007). This is especially true for substances such as phthalates and PAHs that are ubiquitous in products and the environment.
- Many metabolites are more toxic than their parent compounds. This is especially true for phtalates and PAHs (Li et al. 2008, Peck et al. 1982, Wirth et al. 2008, Meeker et al. 2009)

A few Swedish screening studies has focused on metabolites of single substance groups (Naturvårdsverket 2009), but individual exposure to several organic substance groups using metabolites as biomarkers has not been assessed previously in Sweden. Also, studies relating effects (in human matrices) to the simultaneous exposure to several different organic substances and metals has not been performed within the auspices of the Swedish screeningprogram , sometimes because a limited number of individuals are included.

In contrast, the human biomonitoring programs in USA, Germany and Holland has to large degree focused on metabolites of organic substances (Becker et al. 2003, CDC 2009, Ye et al. 2008) to estimate exposure levels.

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[1] Data from the health related screening program is available at
http://ki.se/ki/jsp/polopoly.jsp?d=19230&l=sv

Based on the above a screening study of metabolites and metals in human urine has been initiated by the Swedish EPA. The focus was on a selected subset of environmentally important organic chemicals as well as metals. To cover a wide group of exposure pathways, the following substance groups where included

- Phtalates that mainly reflect exposure from substances in plastic products, both within the household and at the workplace
- PAHs that mostly reflect exposure to airborne contaminants and food
- Organophosphate pesticides that mostly reflects exposure via food stuff in Sweden
- Metals that reflect a wide variety of exposure pathways

The substances included are presented below. Note, that originally more substances were to be included. Because of a large delay until the analytical work could proceed, it was not possible to obtain all analytical standards and consequently the number of substances decreased to give the following list:

| **Phtalate metabolites** |
| Mono-methyl phthalate (MMP) |
| Mono-ethyl phthalate (MEP) |
| Mono-isobutyl phthalate (MiBP) |
| Mono-cyclohexyl phthalate (MCHP) |
| Mono-benzyl phthalate (MBzP) |
| Mono-n-octyl phthalate (MOP) |
| Mono-2-ethylhexyl phthalate (MEHP) |
| Mono-isononyl phthalate (MiNP) |
| Mono-(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP) |
| Mono-(2-ethyl-5-oxohexyl) phthalate (MEOHP) |
| **PAH metabolites** |
| 1-Hydroxychrysene (1-chry) |
| 2-Hydoxychrysene (2-chry) |
| 3-Hydroxychrysene (3-chry) |
| 2-Hydroxyfluorene (2-flu) |
| 9-Hydroxyfluorene (9-flu) |
| 4-Hydroxychrysene (4-chry) |
| 1-Hydroxyphenanthrene (1-phe) |

2-Hydroxyphenanthrene (2-phe)

3-Hydroxyphenanthrene (3-phe)

4-Hydroxyphenanthrene (4-phe)

9-Hydroxyphenanthrene (9-phe)

1-Hydroxypyrene (1-py)

3-Hydroxybenzo(a)pyrene (3-bap)

1-Hydroxynaphthalene (1-nap)

2-Hydroxynaphthalene (2-nap)

Metabolites of organophosphorous pesticides

Dimethylphosphate (DMP)

Diethylphosphate (DEP)

Dimethylthiophosphate (DMTP)

Dimethyldithiophosphate (DMDTP)

Diethylthiophosphate (DETP)

Diethyldithiophosphate (DEDTP)

Metals

Al, Cd, Co, Cr, Cu, Hg, Mn, Mo, Ni, Pb, Zn, As

To complement the exposure based screening of urine, an effects based screening was also included in the project. Several of the substances in the substance groups are classified as category 1[2] (several phthalates and benzo[a]pyrene) or category 2[3] (organophosphorous pesticides such as parathion, malathion, methylparathion and diazinon and benz[a]anthracene) endocrine disrupters according to the EU classification (BKH 2002, DHI 2007). Consequently, the urine samples were also tested for endocrine (estrogen) disturbing effects using an *in vitro* method.

Monitoring of cadmium in urine in Sweden has been intermittently performed by the institute of environmental medicine, at Karolinska Institutet since 2002 (Berglund och Åkesson 2008) and by other institutes. This constitutes a quality controlled urine

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[2] At least one study published providing evidence of endocrine disrupting effects in an intact organism. Not a formal weight of evidence approach. On the basis of the precautionary approach, substances with insufficient evidence, but chemically closely related to category 1 substances, have been categorized as category 1.

[3] Potential for endocrine disrupting effects. In vitro data indicating potential for endocrine disruption in intact organisms. Also includes effects in-vivo that may, or may not, be ED-mediated. May include structural analyses and metabolic considerations.

sampling campaign that was also used for sampling of urine within the present project.

1.1 Objectives

The objectives of the project were to:

- To broadly assess exposure levels of some important contaminant groups in the Swedish female population by examining a small subset consisting of 20 females.

- To compare exposure levels of these substance groups to exposure levels in other countries

- To statistically investigate the co-occurrence of different metabolites and metals in urine

- To explore the connection between endocrine disturbing effects and the exposure levels of these substance groups

2 Organic substance metabolites and metals

2.1.1 Phthalate metabolites

Phthalates are a family of compounds that chemically consists of dialkyl or alkyl aryl esters of 1,2-benzenedicarboxylic acid. They are ubiquitous industrial chemicals that are used as detergents fixatives, lubricating oils etc. They are also used as plasticizers in flexible polyvinyl chloride (PVC) products. The inclusion of phthalates in a wide array of products causes human exposure through multiple routes (oral, dermal, inhalation, and intravenous).

Phthalates are rapidly metabolized after intake to their respective monoester metabolites (Hauser and Calafat 2005). These metabolites can be glucuronidated (see section 3.2.1) and excreted in the urine and feces. The proportion of the diester that is transformed *in vivo* to its monoester or other oxidative metabolite varies greatly between different phthalates (Peck 1982). Measurements of phthalate metabolites in body fluids (mainly urine) are usually better biomarkers of exposure than those of the parent phthalates, partly because the latter are easily affected by laboratory contamination (Barr et al., 2003).

Figure 2.1 shows schematically how a di-alkylated phthalate is metabolized to form its phthalate monoester. This monoester is found in urine as mostly glucoronide conjugates or (to a lesser degree) as free acids. Phthalates can also be further metabolized to oxidation products (not shown here). Figure 2.1 also shows which phthalate compounds that are represented by the metabolites measured in the present project.

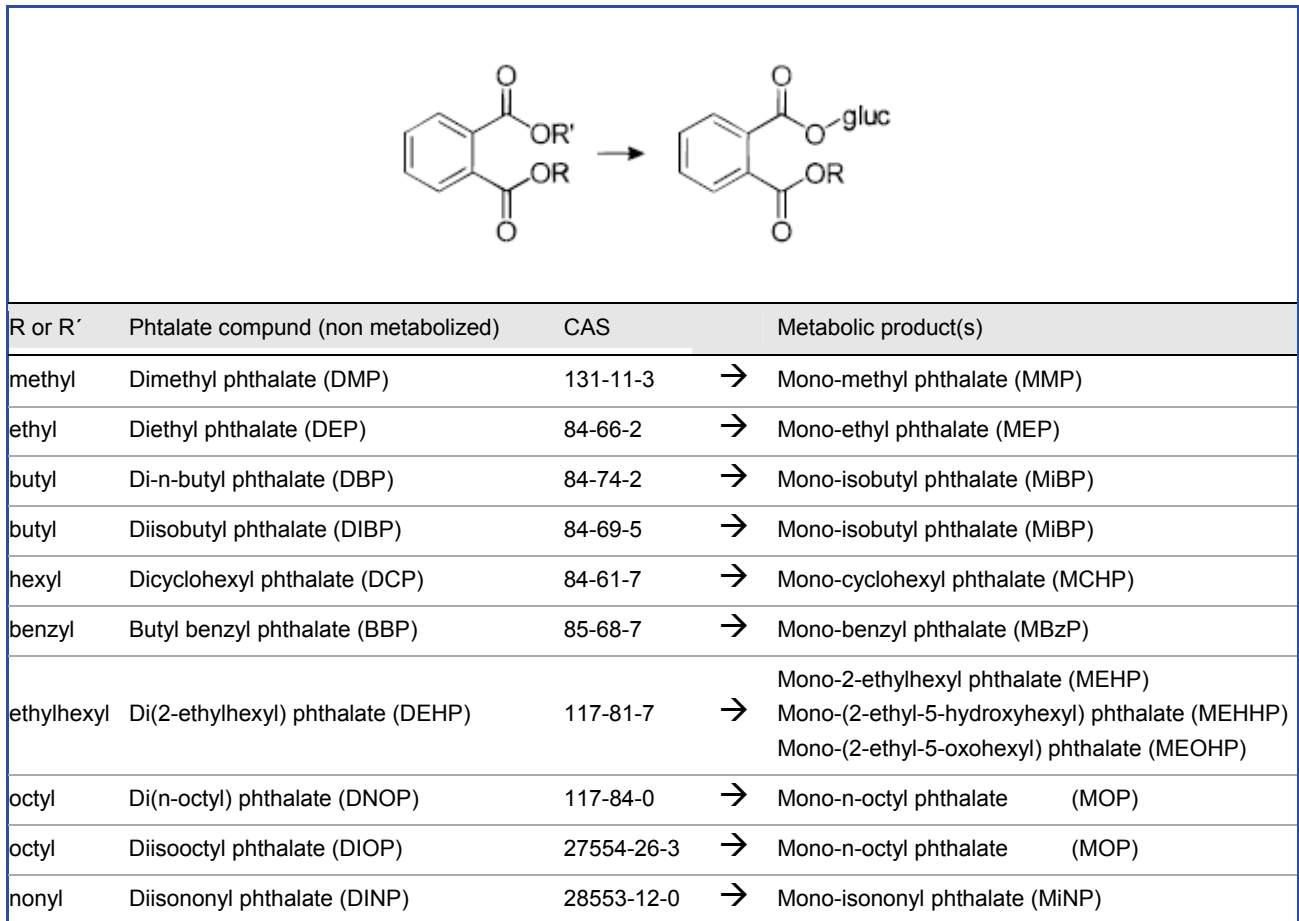| R or R´	Phtalate compund (non metabolized)	CAS		Metabolic product(s)
methyl	Dimethyl phthalate (DMP)	131-11-3	→	Mono-methyl phthalate (MMP)
ethyl	Diethyl phthalate (DEP)	84-66-2	→	Mono-ethyl phthalate (MEP)
butyl	Di-n-butyl phthalate (DBP)	84-74-2	→	Mono-isobutyl phthalate (MiBP)
butyl	Diisobutyl phthalate (DIBP)	84-69-5	→	Mono-isobutyl phthalate (MiBP)
hexyl	Dicyclohexyl phthalate (DCP)	84-61-7	→	Mono-cyclohexyl phthalate (MCHP)
benzyl	Butyl benzyl phthalate (BBP)	85-68-7	→	Mono-benzyl phthalate (MBzP)
ethylhexyl	Di(2-ethylhexyl) phthalate (DEHP)	117-81-7	→	Mono-2-ethylhexyl phthalate (MEHP) Mono-(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP) Mono-(2-ethyl-5-oxohexyl) phthalate (MEOHP)
octyl	Di(n-octyl) phthalate (DNOP)	117-84-0	→	Mono-n-octyl phthalate (MOP)
octyl	Diisooctyl phthalate (DIOP)	27554-26-3	→	Mono-n-octyl phthalate (MOP)
nonyl	Diisononyl phthalate (DINP)	28553-12-0	→	Mono-isononyl phthalate (MiNP)

Figure 2.1 Phthalate compounds and the metabolic by products. The upper part of the figure shows schematically how a di-alkylated phthalate is metabolized to form its phthalate monoester which are found in urine as glucoronide conjugates or free acids. Phthalates can also be further metabolized to oxidation products (not shown here). The lower part of the figure shows which phthalate compounds that are represented by the metabolites measured in the present project.

2.1.2 Metabolites of organophosphorous compound

Organophosphorus (OP) pesticides are commonly used as insecticides in agriculture, in residential surroundings and in public health programs to control vector-borne diseases. The most common OP pesticides are parathion, malathion, methyl parathion, chlorpyrifos, diazinon, dichlorvos, phosmet, tetrachlorvinphos, and azinphos methyl.

OP pesticides act on the enzyme acetylcholinesterase which is essential to nerve function in insects, humans, and several other animals. The way in which OP pesticides affect this enzyme varies, and consequently the potency also varies greatly between different pesticides in the group.

Most OP pesticides are composed of a phosphate, phosphorothio, or phosphorodithioate moiety that is often dialkyl substituted, where the alkyl groups

are usually dimethyl or diethyl, and an organic group. Organophosphate pesticides have a short half-life in the human body and are excreted mainly in urine (Heudorf et al. 2006).

The metabolic products can be divided into two general groups:

I. Dialkyl phosphates (DAP) which are potential metabolites of most OP pesticides (Figure 2.2). Consequently they are rarely used to follow the exposure to individual compounds; instead they are used for assessing the total exposure levels to most (all) OP pesticides.

II. Other more specific compounds, such as 4-nitrophenol (PNP) which is formed after exposure to only a few OPs (i.e. PNP is only related to parathion and methyl parathion).

This study focuses on the DAP because these reflect the total exposure, making them appropriate for a general assessment of OP exposure. The most common DAPs are dimethyl phosphate (DMP), diethyl phosphate (DEP), dimethyl thiophosphate (DMTP), diethyl thiophosphate (DETP), dimethyl dithiophosphate (DMDTP), and diethyl dithiophosphate (DEDTP). These six diesters have commonly been used as measures of OP pesticide exposure in humans, and the approach was first used more than 30 years ago (Shafik and Enos, 1969). About 75% of the U.S. EPA–registered OP pesticides form one to three of these DAP metabolites (Barr et al. 2004).
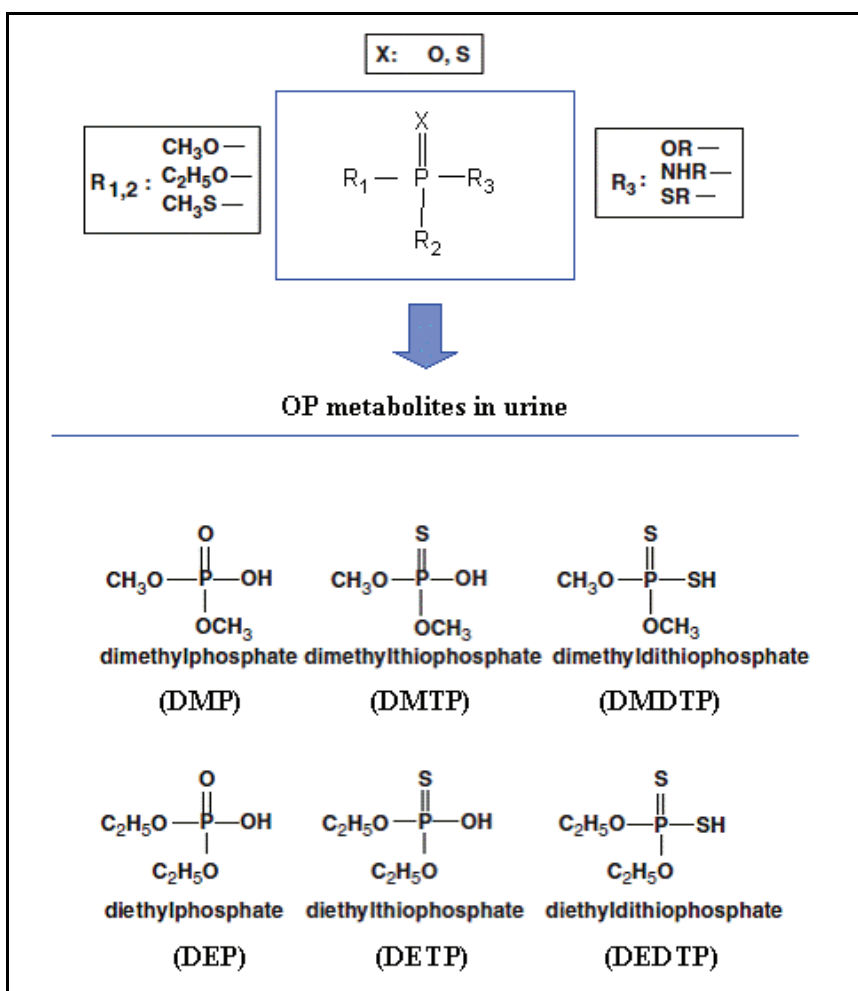
Figure 2.2 Schematic representation of OP pesticides and the metabolic by-products. The upper part of the figure shows schematically the structure of an OP pesticide. The lower part of the figure shows which metabolites that can be formed.

2.1.3 Metabolites of PAHs

Polycyclic aromatic hydrocarbons (PAHs) are formed and emitted into the environment as a result of incomplete combustion of organic materials from natural and human activities. Some PAHs are carcinogenic or co-carcinogenic compounds PAHs are also known to have endocrine disrupting activity.

PAHs are absorbed into the human body through the skin, lungs and gastrointestinal tract, and are then metabolized to their monohydroxylated PAHs. PAH metabolism is complex and occurs primarily in the liver, and to a lesser extent, in other tissues. The hydroxylated metabolites of PAHs are excreted both as free metabolites and conjugated to glucuronic acid and sulphate. PAH elimination occurs via urine and feces, and urinary metabolites are eliminated within a few days (Ramesh et al.,

2004). Consequently, measurements of urinary metabolites reflect recent exposure to PAHs.

PAHs and their corresponding hydroxylated metabolites that are measured in this study are shown in Table 2.1. As shown in the table, most of the parent PAHs can produce more than one urinary metabolite.

Table 2.1 PAH compounds and the metabolic by product measured in the present study.

Polycyclic Aromatic Hydrocarbon (CAS number)		Urinary hydroxylated metabolite
Naphthalene (91-20-3)	→	1-Hydroxynapthalene 2-Hydroxynapthalene
Phenanthrene (85-01-8)	→	1-Hydroxyphenanthrene 2-Hydroxyphenanthrene 3-Hydroxyphenanthrene 4-Hydroxyphenanthrene 9-Hydroxyphenanthrene
Chrysene (218-01-9)	→	1-Hydroxychrysene 2-Hydoxychrysene 3-Hydroxychrysene 4-Hydroxychrysene
Fluorene (86-73-7)	→	2-Hydroxyfluorene 9-Hydroxyfluorene
Pyrene (129-00-0)	→	1-Hydroxypyrene
Benzo(a)pyrene (50-32-8)	→	3-Hydroxybenzo(a)pyrene

2.1.4 Metals

Human exposure to metals is common, with wide use in industry and long-term environmental persistence. The heaviest metal exposures have usually occurred in the workplace or in environmental settings in close proximity to industrial sources. However, these sources are probably less common (in Sweden) today, and common exposure sources among the general population are instead smoking, car traffic (both from exhaust, brake discs and calipers), food and metals in products such as alloys and pigments for paints, cement, paper, rubber etc. Historical contamination of soil

and groundwater may also be locally important exposure sources. Non-industrial exposure to metals generally occurs at substantially lower levels than have been found in industrial settings (Hu 2002).

Exposure to metals can happen through a variety of routes. They may be inhaled as dust or fume. Some metals can be vaporized (e.g., mercury) and inhaled. Metals may also be ingested through food and drink. The quantity that is actually absorbed from the digestive tract can vary, depending on the chemical form of the metal and the age and nutritional status of the individual (Hayes 1997). Once a metal is absorbed, it distributes in organs, and excretion of many metals typically occurs primarily through the kidneys and digestive tract, which makes urine an appropriate sampling matrix for investigations on metal exposure in humans. Metals also tend to persist in some storage sites, like the liver, bones, kidneys and tissues such as hair and nails for years or decades (Hayes 1997).

A number of metals were included in the present study. Some of them are commonly investigated in humans matrixes (As, Pb, Cd and Hg) both because they are known to have potentially toxic effects on humans and because they are known to be elevated in humans (Hu 2002, CDC 2009). The other metals that were included are usually less investigated in humans. Copper, manganese and zinc are essential and play roles in, for example, the functioning of certain enzyme systems (Hu 2002) but they may nevertheless cause detrimental effects. Both Chromium (hexavalent chromium compounds) as well as nickel has been linked to cancer through occupational exposure.

Nevertheless, most studies of metals in humans focus on arsenic, lead, cadmium and mercury (CDC 2009).

Depending on the dose, lead exposure in children and adults can cause different health problems, ranging from convulsions and renal failure at very high doses to subtle effects on metabolism and intelligence at the low end of exposures. Children (and developing fetuses) seem to be vulnerable to the neurotoxic effects of lead where blood levels of 5-25 mg/dL have been connected to deficits in intellectual development[4]. In adults, epidemiologic studies have linked blood lead levels in the range of 7-40 mg/dL with evidence of neurobehavioral decrements and renal impairments. Also, recent measurements of bone lead levels have provided evidence that cumulative lead exposure is a risk factor for the development of hypertension, cardiac conduction delay, and cognitive impairments in adults (Hu 2002).

Mercury comes in a number of different chemical forms. Metallic mercury is not hazardous if ingested as it is not significantly absorbed in this form. Relatively

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[4] For more information see: http://www.cdc.gov/nceh/lead/about/program.htm

modest levels of occupational mercury exposure, as experienced, for example, by dentists, have been associated with measurable declines in performance on neurobehavioral tests of motor speed, visual scanning, verbal and visual memory, and visuomotor coordination. Of greatest concern however, is the sensitivity of the fetal and infant nervous system to low-level mercury toxicity. Recent research has demonstrated that low levels of mercury exposure to pregnant women through dietary intake of fish are associated with decrements in motor function, language, memory, and neural transmission in their offspring. However, these problems are associated with organic mercury, the form of mercury bioconcentrated in fish.

The toxicity of an arsenic depends on its valence state, its form (inorganic or organic), and parameters that modify absorption and elimination. Once absorbed into the body, arsenic undergoes some accumulation in the liver, spleen, kidneys, and lungs, but the major storage is tissues, such as skin, hair, and nails. Chronic exposure to arsenic results in patterns of skin hyperpigmentation, peripheral nerve damage, tingling, and weakness in the hands and feet, diabetes, and blood vessel damage. These effects have almost only been observed at workplace exposure. Chronic arsenic exposure also causes an elevated risk for developing different cancers.

The health implications of cadmium exposure are elevated by the relative inability of human beings to excrete cadmium. Occupational levels of cadmium exposure are a risk factor for chronic lung disease. Lower levels of exposure are mainly of concern with respect to toxicity to the kidney. Cadmium damages the proximal tubules of each nephron in a way that is first manifested by leakage of low molecular weight proteins and ions with possible progression over time to kidney failure. Cadmium's effect on the kidney can also have metabolic effects; The loss of calcium caused by cadmium's effect on the kidney can lead to weakening of the bones.

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3 Methods

The following sections describe the sampling procedure for urine, the analytical methods used to measure the levels of metabolites and metals in urine and the method used to quantify endocrine disturbing effects in urine.

3.1 Sampling methodology

The urine sampling was part of the national health related environmental monitoring programme run by Swedish EPA and in part performed by the Institute of environmental medicine, at Karolinska Institutet since 2002 that aims at following time trends of cadmium exposure and related effects on renal function in women 20-29 and 50-59 years of age (Berglund och Åkesson 2008). Within this monitoring program a random sample of women in the Stockholm area were asked via mail to participate in the study. Those that were willing to participate (in total 200 women) filled in a form whereafter sampling instructions and sampling vessels were sent to them. From this larger subset 20 women were randomly chosen, ten from each age group, for inclusion in the present study.

The forms consisted of a number of exposure related questions on:

- age and weight
- country of residence
- rural or city dwelling
- workplace exposure to chemicals
- smoking habits
- eating habits
- number of births
- origin of drinking water (municipal or local well)
- dwelling type (house, apartment etc)
- age of residence and age of car (based on the assumption that newer cars and houses emit more phthalates)
- agricultural workplace (because agricultural workers may be more exposed to organophosphorous compounds).

3.2 Analytical methods

3.2.1   Deconjugation

Upon exposure, environmental chemicals are metabolized. This usually includes phase II enzymatic biotransformations where the chemical becomes conjugated (joined together) with another substance. The conjugated products are larger than the original molecule (substrate) and generally polar and water soluble in nature.

Consequently, they can be readily excreted from the body. Also, conjugated chemicals have a poor ability to cross cell membranes. One of the most common molecules added to the chemical (or its phase I metabolite) is glucuronic acid. Glucuronide conjugation usually decreases toxicity, although there are some notable exceptions, for example, the production of carcinogenic substances. Another common conjugate addition is sulphate.

Conjugated chemicals will not be identified and quantified with the analytical methods used for the non conjugated chemical. This could be resolved by using two different analytical methods for the conjugated and non conjugated chemical, but an easier solution is to de-conjugate the chemical which involves removing the added molecule from the conjugated moiety.

In the present study, de-conjugation was done using enzymatic hydrolysis with glucuronidase and/or sulfatase to remove the glucuronic acid or the sulphate. The enzymes used originate from two bacteria, P. *vulgata* and E. *Coli*. Different aspects of the de-conjugation efficiency were controlled:

- enzymatic hydrolysis efficiency
- extraction efficiency
- derivatization efficiency (since many metabolites are acetylated).

This was done by adding the substrate 6-bromo-2-naphthyl-β-D-glucuronide, whereby the efficiency was evaluated by measuring the recovery of 6-bromo-2-naphthol.

3.2.2 Phthalate metabolites

Phthalate metabolites were analyzed using automated solid-phase extraction followed by quantification using isotope dilution-high performance liquid chromatography-tandem mass spectrometry (Silva et al. 2003, 2004 and 2005, and Koch et al. 2003).

Human urine (1 mL) was pretreated by adding 125 uL of 1 M phosphoric acid and vortex mixed and sonicated for 5 minutes. Following the addition of 10 nanograms of isotopically-labeled phthalates, 20 micrograms of 4-methylumbelliferone glucuronide, 250 microliters of ammonium acetate buffer (pH 6.5), and 5 microliters of β-glucuronidase (Escherichia coli – K12, Roche Biomedical). The urine was mixed and incubated at 37°C for 90 minutes to allow for the deglucuronidation of the phthalate metabolites.

After enzymatic hydrolysis, the urine was loaded on a Zymark Rapid Trace Station for automated solid phase extraction (SPE). The 60 milligram/3 mL Oasis-HLB cartridges were conditioned with HPLC-grade methanol (2 mL) and 0.1M formic

acid (1 mL). The urine was diluted with 5 mL of 0.1M formic acid and loaded onto the SPE cartridge at a rate of 0.5 mL/min. The cartridge was washed with water (1 mL) and 10% methanol in water (2 mL) at a flow rate of 1 mL/min. The phthalate metabolites were eluted with 0.5 mL of acetonitrile at a flow rate of 0.5 mL/min. The eluate was evaporated to dryness under a stream of dry nitrogen in a N-Evap (Organomation). The residue was resuspended in 85% methanol in water (200 microliteres) and transferred to glass autosampler vials.

Analysis was performed using a API 3000 liquid chromatograph/tandem mass spectrometer.

3.2.3 Metabolites of organophosphorous compounds

Organophosphorous metabolites (dialkyl phosphates) were analyzed using wet extraction and derivatization followed by gas chromatography/mass spectrometry using selected ion monitoring. (Hardt and Angerer, 2000, Hemakanthi De Alwis et al 2008).

The urine samples were collected in polypropylene bottles and stored at -20°C until sample preparation was carried out. After thawing and mixing, 5 mL of urine was pipetted into a 15-mL screw-top glass tube that already contained 4 g of sodium chloride and was spiked with 100 uL of surrogate (dibutylphosphate, 10 mg/L). Control urine was prepared in the same fashion and further spiked with 10 uL of a solution containing all dialkylphosphates (100 mg/L) for quality control purposes.

Five milliliters of ethyl acetate was added, and the sample was acidified with 1 mL hydrochloric acid (3M). The sample was vortex mixed for 3 minutes and then centrifuged for 5 minutes at 1500 rpm.

The ethyl acetate was transferred to a clean 15-screw cap tube and the extraction was repeated one more time. The extract was dried with anhydrous sodium sulfate and concentrated to 1 mL. Following transfer of the extract to a 7 mL vial the extract was concentrated to dryness using a nitrogen evaporator (N-EVAP).

Acetonitrile (1 mL), 20 mg of potassium carbonate, and 30 uL of pentafluorobenzyl bromide were added and thoroughly mixed. The vial was capped and placed in a block heater maintained at 60oC for 4 hours.

Upon cooling the solution was transferred to a 4 mL vial, 1 mL of toluene was added and the contents mixed. The derivatized extract was concentrated to approximately 0.5 mL and then adjusted to 1 mL with toluene.

Fifty microliters of internal standard (D10-phenanthrene, 40 mg/L) was added, mixed and the analysis was performed using gas chromatography/mass spectrometry using selected ion monitoring.

3.2.4 PAH metabolites

The PAH metabolites were analyzed using an automated solid-phase extraction method followed by isotope-dilution gas chromatography high-resolution mass spectrometry (Romanoff et al 2006, Smith et al (2002).

Urine samples (3 mL) were aliquoted into test tubes and spiked with 30 uL of an isotopically C13 labelled internal standard mixture (10 pg/uL) and 20 uL of 6-bromo-naphthol-β-D-glucuronic acid (133 mg/L in acetate buffer). Following the addition of 10,000 units of glucuronidase/sulftase (P. vulgata, 200 uL, 10,000 units in 0.4 M acetate buffer, pH 5.0) the samples were incubated (37°C) overnight.

Samples were mixed, allowed to equilibrate, then extracted on the Rapid Trace SPE work station. Some samples were filtered prior to cleanup. Cartridges (Focus 60 mg) were preconditioned with methanol (1 mL, 16 mL/min), followed by purified water (1 mL, 16 mL/min). Samples were added to the cartridge at 1 mL/min, rinsed using purified water (1 mL, 10 mL/min), and followed by methanol/sodium acetate buffer (3 mL, 4:6 by volume, pH 5.0, 10 mL/min). The sorbent was dried by vacuum drying for 30 min. Finally, the cartridge was eluted with dichloromethane (3 mL, 0.5 mL/min). Ten microliters of nonane was added prior to concentration to ~0.5 mL using a nitrogen evaporator (N-Evap). Following transfer of the extract to a taped HRMS vial (100 uL), the extract was concentrated just to dryness. Following reconstitution with 5 uL of toluene and 10 uL MSFTA, the samples were incubated at 600C for 1 hour. Fifteen microliters of internal standard (D10-phenanthrene, 10 ng/mL) was added prior to analysis using gas chromatography/high resolution mass spectrometry.

3.2.5 Metals

12 elements were analysed in urine by sector field ICP-MS as described previously (Rodushkin et al. 2001). The sector field ICP-MS instrument used was the ELEMENT (Thermo Fischer, Bremen, Germany) equipped with an ASX 500 sample changer (CETAC Technologies Inc., Omaha, USA). In this study the device was operated in low resolution mode (LRM, m/Δm about 300) and medium resolution mode (MRM, m/Δm about 4400).

Chemicals and reagents

All calibration and internal standard solutions used were prepared by diluting 1 g/l single-element standard solutions (SPEX Plasma Standards, Edison, NJ, USA), taking into account inter-element compatibility. Concentrations in the calibration

standards were checked by means of quality control samples that were prepared by diluting 10 mg/l multi-element standard solutions (PE Pure Plus, Atomic Spectroscopy Standard, Norwalk, USA). Analytical grade nitric acid (Merck, Darmstadt, Germany) was used after additional purification by sub-boiling distillation in a quartz still.

For dilution of urine samples, blanks and standards Milli-Q water (Millipore Milli-Q, Bedford, USA) additionally purified by sub-boiling distillation in a Teflon still (Savillex Corp., Minnetonka, Minnesota, USA) was used.

Sample preparation

Urine samples were collected in 100 ml Nalgene polyethylene bottles (Nalge Nunc International, Rochester, NY, USA) and were stored in a refrigerator prior to analysis. The urine reference material was reconstituted with ultra pure water according to the manufacturer's instructions (Sero A/S, Billingstad, Norway).

Al, As, Cd, Co, Cr, Cu, Mn, Mo, Ni, Pb, Zn

A 0.5 ml aliquot of urine was transferred into a disposable 10 ml Nalgene polypropylene autosampler tube and made up to 10 ml with 0.14 mol/l HNO3 in ultra pure water. A set of preparation blanks was prepared by pouring 100 ml aliquots of ultra pure water into Nalgene bottles inside the sampling area followed by dilution as described for a urine sample. The resulting solutions were spiked to 20 μg/l each of Sc, In and Lu as internal standards. Prior to use, plastic labware was thoroughly cleaned in a sequence using detergent, water, a mixture of nitric (1.4 mol/l) and hydrochloric (1.1 mol/l) acids (1:1 v/v, Merck, analytical grade) followed by soaking in distilled nitric acid (0.7 mol/l) and a final rinse with de-ionized water.

Hg

Digestion of urine (1 ml of urine and 1 ml of 14 mol/l nitric acid) was accomplished using a microwave oven (MDS-2000, CEM Corporation, Matthews, USA) equipped with 12 low-volume PFA lined vessels (ACV 50) with safety rupture membranes, for 1 h at 600 W power. The digest was diluted to 10 ml. Two or more blank digests were also prepared with each batch of urine samples using 1 ml of water instead of urine. The resulting solutions were spiked to 20 μg/l with internal standard solution containing In and Lu.

3.2.6 Test for endocrine disturbing effects

The following section describe the general methodology and the specific analytical procedure for assessing endocrine disturbing effect in the urine.
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3.2.6.1   THE YES ASSAY

Endocrine disrupting effects were tested for by using the Yeast Estrogen Screening assay (YES) assay. The method is based on the work of Routledge et al. (1996), and it is a standard method for screening liquid samples for substances that exert an estrogenic effect.

The method uses the yeast *Saccharomyces cerevisiae*. The DNA sequence of the human estrogen receptor (hER) is integrated into the yeast genome. This sequence is linked to the lac-Z gene for ß-galactosidase. The hER is expressed in a form that binds with the estrogen response elements (ERE) within a hybrid promoter on the expression plasmid. Estrogen binds to the estrogen receptor (expressed by hER) and this ligand complex binds to the ERE elements on the hybrid promoter linked to the gene for ß-galactosidase. The enzyme is synthesized and secreted into the medium and metabolizes the substrate chlorophenol red-ß-D-galactopyranoside (CPRG) causing a change in colour from yellow to red. The intensity of the red colour is measured spectrophotometrically.

In summary, a compound with estrogenic effects (ability to interact with the human estrogen receptor) will cause the yeast cells to metabolize a substrate in the growing medium which will produce a red color that is measured/quantified using spectrometric methods

Data are usually reported as estrogen equivalent (EE) in μg/L for liquid samples. When environmental samples are tested following a 100-fold solvent concentration step, the detection limit of the original sample is lowered to about 1 ng/L estrogen equivalent (EE)/L. The EC50 for 17βestradiol by this assay is reported as 2.2 ± 0.22 x 10-10 M or 27 to 120 ng/L (Beresford et al., 2000).

3.2.6.2   ANALYTICAL PROCEDURE

Following enzymatic deconjugation as described earlier, the urine samples were screened for estrogenic activity in a 96 well plate using the yeast estrogen screen (YES).

189.8 milligrams of L-11 (glucuronidase/sulfates) were dissolved from limpets in 10.0 mL of 0.4 M acetate buffer. To one set of urine samples 200 uL of glucuronidase/sulfatase was added to 3 mL of urine. The samples were incubated (on a shaking incubator) at 37 ^{o}C overnight. To another set of urine samples (3 mL) 20 uL of 6-bromo-2-naphthol-glucuronide and 200 uL of glucuronidase/sulfatase was added. These samples were also incubated (on a shaking incubator) at 37 ^{o}C overnight.

For quality control (QC) the following was prepared:

- 4 method blanks, using (3 mL) water instead of urine
- 4 control serum (3 mL)
- 4 control serum with 10 nanograms of estradiol (3 mL)

Glucuronidase/sulfatase was added to all of the QC controls.

4 Results

For human biomonitoring studies it is relevant to compare the levels found with results from other studies in other countries, and this has been the general practice in many large human biomonitoring studies. This approach aids the identification of elevated levels and can also be connected to a discussion on chemical usage and the correlation between discontinued usage and levels in humans.

The urine sampling in this study is done as part of a larger sampling program with a focus on cadmium (see section 3.1), but it was not deemed necessary to compare the levels in the present study with the levels seen in that study, given the large difference in number of sampled individuals (20 vs 200).

In the following sections levels of metabolites and metals are compared to levels found in a number of large studies from other countries. The aims of these studies are usually to obtain representative levels of chemicals and metabolites in the urine of national populations and they are consequently appropriate for comparison purposes. The studies that are used for comparison have in general used the same, or very similar analytical methods, as the ones used in the present study. Consequently the comparisons are valid from a analytical point of view.

Concentrations are compared to data from both the general population and women since the present study only encompasses women. Since the actual data from other countries are not available[5] from the studies in it has not been possible to do statistical comparisons to assess whether the levels in Sweden differ to the levels in other countries. A qualitative assessment has been used, and when the levels in the present study are deemed to be above the levels in other countries, this is marked by bold numbers in the table. The large difference in the number of subjects/samples has to be taken into consideration when comparing results. In most cases, studies from other countries encompass a much larger number of subject/samples.

The results are presented and compared both as unadjusted values and as creatinine adjusted values[6].

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[5] Only statistical representations of the data such as percentiles are presented, and the actual raw data is not presented anywhere.
[6] Creatinine is produced at a fairly unvarying rate by the body and is filtered out of the blood by the kidneys. Creatinine can thus be used as an indicator of the diluteness of the urine and chemical concentrations in the urine is usually presented both as is, and creatinine adjusted.

4.1 Phthalate metabolites

Levels of phtalate metabolites are compared to levels found in American studies of more than 4000 people (HANES project, CDC 2009) and levels in urine of (pregnant) women from Rotterdam (Generation R study, Ye et al. 2008). The comparisons are presented in Table 4.1 and Table 4.2.

It is apparent that the levels of MiBP and MiNP in the present study of Swedish women are much higher than the levels in the American HANES study. On the other hand MiBP levels are similar to what was found among pregnant women in Rotterdam (Ye et al. 2008).

Levels of MEP seem to be lower in Sweden, especially in comparison with the 95th percentile. It should be noted that extreme values (which tend to influence the 95th percentile) are more likely to be absent when a small population is sampled which may explain the relatively lower value of the 95th percentile in the present study. No other clear differences can be observed; although the levels of MEOHP seem to be higher in Swedish women.

Table 4.1 Non adjusted levels (50th, 75th and 95th percentiles) of phthalate metabolites in the present study of Swedish women compared to levels in the general population and women from USA and pregnant women from Rotterdam..

Country	Population	Percentile	Concentration of metabolites[1] (µg/L)									
			MMP	MEP	MiBP	MCHP	MBzP	MEHP	MOP	MEOHP	MiNP	MEHHP
Sweden (present study)	Female	50th	2.8	80	99	0.20	16	3.0	<LOD	25	23	32
		75th	3.9	270	170	0.29	29	8.6	<LOD	75	79	94
		95th	11	640	250	0.36	38	37	<LOD	220	220	300
	Observations above LOD		20	20	20	8	20	20	0	20	20	20
USA [2]	Female	50th	1.20	182	4.10	<LOD	13.3	1.80	<LOD	13.7	<LOD	19.4
		75th	3.60	478	8.00	<LOD	33.3	4.90	<LOD	29.5	<LOD	46.4
		95th	15.3	2590	20.5	.300	101	27.8	<LOD	143	<LOD	214
	All	50th	1.30	174	4.20	<LOD	14.3	1.90	<LOD	14.4	<LOD	21.2
		75th	3.90	502	8.40	<LOD	32.3	5.30	<LOD	31.4	<LOD	49.1
		95th	16.3	2700	21.3	.300	101	31.0	<LOD	157	1.00	266
Netherlands [3]	Female	50th	<LOD	117.0	42.1		7.5	6.9	<LOD	14.5		14.0
		75th	3.5	425.0	72.8		16.8	17.3	<LOD	27.4		30.0
		95th	20.1	1150.0	249.0		95.8	82.8	<LOD	104.0		86.2
	All	50th										
		75th										
		95th										

1 MMP = Mono-methyl phthalate   MCHP = Mono-cyclohexyl phthalate   MEHP = Mono-2-ethylhexyl phthalate   MEOHP = Mono-(2-ethyl-5-oxohexyl) phthalate
MEP = Mono-ethyl phthalate   MBzP = Mono-benzyl phthalate   MiNP = Mono-isononyl phthalate
MiBP = Mono-isobutyl phthalate   MOP = Mono-n-octyl phthalate   MEHHP = Mono-(2-ethyl-5-hydroxyhexyl) phthalate

2 CDC (2009)

3 Ye et al. 2008.

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Table 4.2 Creatinine adjusted levels (50th, 75th and 95th percentiles) of phthalate metabolites in the present study of Swedish women compared to levels in the general population and women from USA and pregnant women from Rotterdam..

Country	Population	Percentile	Concentration of metabolites[1] (µg/g creatinine)									
			MMP	MEP	MiBP	MCHP	MBzP	MEHP	MOP	MEOHP	MiNP	MEHHP
Sweden (present study)	Female	50th	3	81	**86**	0.26	15	3.3		23	**22**	30
		75th	5.2	180	**150**	0.39	27	7.5		53	**56**	66
		95th	7.6	600	**220**	0.54	47	32		250	**270**	230
	Observations above LOD		20	20	20	20	8	20	20	0	20	20
USA[2]	Female	50th	1.53	181	4.00	<LOD	13.9	2.16	<LOD	12.7	<LOD	18.7
		75th	3.87	508	6.73	<LOD	27.9	4.40	<LOD	26.6	<LOD	39.3
		95th	16.5	2250	15.7	.500	73.4	27.0	<LOD	118	<LOD	171
	All	50th	1.53	153	3.57	<LOD	12.6	1.89	<LOD	12.1	<LOD	17.7
		75th	3.45	452	6.21	<LOD	24.6	4.31	<LOD	24.3	<LOD	35.8
		95th	13.5	2040	15.4	.450	70.0	25.4	<LOD	118	2.92	182
Netherlands[3]	Female	50th	<LOD	222.0	57.1		11.7	9.9	<LOD	20.9	<LOD	20.3
		75th	3.9	487.0	99.4		22.6	20.9	<LOD	30.7		32.3
		95th	30.3	1810.0	327.0		84.7	88.7	<LOD	126.0		99.5
	All	50th										
		75th										
		95th										

[1]
MMP = Mono-methyl phthalate
MEP = Mono-ethyl phthalate
MiBP = Mono-isobutyl phthalate

MCHP = Mono-cyclohexyl phthalate
MBzP = Mono-benzyl phthalate
MOP = Mono-n-octyl phthalate

MEHP = Mono-2-ethylhexyl phthalate
MiNP = Mono-isononyl phthalate
MEHHP = Mono-(2-ethyl-5-hydroxyhexyl) phthalate

MEOHP = Mono-(2-ethyl-5-oxohexyl) phthalate

[2] CDC (2009)

[3] Ye et al. 2008.

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4.2 Metabolites of organophosphorous pesticides

Levels of OP metabolites are compared to levels found in American studies of more than 4000 people (HANES project, CDC 2009) and levels in urine of (pregnant) women from Rotterdam (Generation R study, Ye et al. 2008). The comparisons are presented in Table 4.1 and Table 4.2.

The results show that the levels of metabolites originating from organophosphorous pesticides in the present study of Swedish women are lower than, or equal to, levels in other countries. It is also apparent that DMTP is the most prevalent metabolite both in Sweden and other countries.

Table 4.3 Non adjusted levels (50th, 75th and 95th percentiles) of organophosphorous pesticides metabolites in the present study of Swedish women compared to levels in the general population and women from USA and pregnant women from Rotterdam.

Country	Population	Percentile	Concentration of OP metabolites[1] (µg/l)					
			DMP	DEP	DMTP	DETP	DMDTP	DEDTP
Sweden (present study)	Female	50th	1	1	3	1	<LOD	<LOD
		75th	1	2	6	1	<LOD	<LOD
		95th	1.1	2.1	21	1.8	<LOD	<LOD
	Observations above LOD		1	7	16	2	0	0
USA[2]	Female	50th	<LOD	<LOD	1.86	<LOD	<LOD	<LOD
		75th	4.18	4.39	5.21	.780	.690	<LOD
		95th	14.8	13.5	33.8	2.57	5.07	.240
	All	50th	<LOD	<LOD	1.90	<LOD	<LOD	<LOD
		75th	3.99	4.54	5.65	.830	.640	<LOD
		95th	14.8	15.7	31.1	2.80	5.05	.320
Netherlands[3]	Female	50th	10.8	2.0	10.6	0.8	0.4	0.0
		75th	21.8	3.4	19.5	1.8	0.8	0.1
		95th	35.8	12.9	45.3	14.1	2.3	0.3
	All	50th						
		75th						
		95th						

1 Dimethylphosphate = DMP   Dimethyldithiophosphate = DMDTP
   Diethylphosphate = DEP)   Diethylthiophosphate = DETP
   Dimethylthiophosphate = DMTP   Diethyldithiophosphate = DEDTP

2 CDC (2009)
3 Ye et al. 2008.

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Table 4.4 Creatinine adjusted levels (50th, 75th and 95th percentiles) of organophosphorous pesticides metabolites in the present study of Swedish women compared to levels in the general population and women from USA and pregnant women from Rotterdam.

Country	Population	Percentile	Concentration of OP metabolites[1] (µg/g creatinine)					
			DMP	DEP	DMTP	DETP	DMDTP	DEDTP
Sweden (present study)	Female	50th	0.9	0.9	2.7	<LOD	0.9	<LOD
		75th	0.9	0.9	5.3	<LOD	0.9	<LOD
		95th	1.0	1.0	18	<LOD	1.6	<LOD
	Observations above LOD		1	1	7	16	0	2
USA [2]	Female	50th	<LOD	<LOD	1.88	<LOD	<LOD	<LOD
		75th	5.00	4.87	6.00	0.750	5.60	<LOD
		95th	16.0	13.8	32.6	2.60	5.88	0.500
	All	50th	<LOD	<LOD	1.75	<LOD	<LOD	<LOD
		75th	3.86	4.42	5.21	0.700	0.500	<LOD
		95th	14.6	13.2	30.4	2.62	5.27	0.410
Netherlands [3]	Female	50th	17.6	3.0	15.5	1.1	0.6	0.1
		75th	24.2	5.5	26.1	2.6	1.1	0.1
		95th	45.2	18.6	52.1	11.6	2.6	0.4
	All	50th						
		75th						
		95th						

1 *Dimethylphosphate = DMP   Dimethyldithiophosphate = DMDTP*
Diethylphosphate = DEP)   Diethylthiophosphate = DETP
Dimethylthiophosphate = DMTP   Diethyldithiophosphate = DEDTP

2 *CDC (2009)*
3 *Ye et al. 2008.*

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4.3 PAH metabolites

Levels of PAH metabolites are compared to levels found in American studies of more than 4000 people (HANES project, CDC 2009) and levels in urine of approximately 500 people from Germany (The GerES III study, Becker et al. 2003, Wilhelm et al. 2008). The comparisons are presented in Table 4.5 and Table 4.6.

The comparisons show that the levels of PAH metabolites in the present study of Swedish women are equal to the levels in the American HANES study and the German GerES III study. One exception may be 9-flu that seem to be higher (although this is not statistically proven).

It is also apparent that the chrysene and benzo(a)pyrene metabolites are less prevalent both in Sweden, USA and Germany (i.e. they have a low detection frequency in the present study and a 50th percentile < LOD in USA and Germany).

Table 4.5 Non adjusted levels (50th, 75th and 95th percentiles) of PAH metabolites in the present study of Swedish women compared to levels in the general population and women from USA and Germany.

Country	Population	Percentile	1-chry	2-chry	3-chry	4-chry	2-flu	9-flu	1-phe	2-phe	3-phe	4-phe	9-phe	1-pyr	3-bap	1-nap	2-nap
							Concentration of PAH metabolites[1] (µg/L)										
Sweden (present study)	Female	50th	<LOD				0.19	**0.64**	0.15	0.07	0.13	0.009	0.008	0.004	<LOD	1.78	3.76
		75th					0.27	**1.34**	0.27	0.08	0.18	0.01	0.00	0.05	<LOD	3.69	6.74
		95th		0.02			0.50	**1.77**	0.58	0.16	0.23	0.08	0.04	0.09	<LOD	15.18	9.45
	Observations above LOD		0	1	0	0	15	15	18	17	18	5	1	8	0	18	18
USA[2]	Female	50th					0.23	0.23	0.14	0.05	0.10	0.02		0.08			
		75th					0.49	0.44	0.26	0.10	0.19	0.05		0.16			
		95th					2.31	1.10	0.59	0.25	0.53	0.14		0.50			
	All	50th	<LOD	<LOD	<LOD	<LOD	0.28	0.27	0.17	0.06	0.12	0.03	0.04	0.09	<LOD	1.89	2.44
		75th	0.02	<LOD	<LOD	<LOD	0.68	0.54	0.29	0.12	0.22	0.05	0.10	0.19	0.04	5.28	6.38
		95th	0.12	0.03	0.04	<LOD	2.67	1.39	0.63	0.29	0.65	0.15	0.39	0.57	0.21	23.30	28.10
Germany[3,4]	Female	50th														3.60	2.10
		75th															
		95th														29.9	16.90
	All	50th							0.39	0.25	0.31			.14		5.0	3.6
		75th															
		95th							1.42	0.85	1.14			.73		19.7	17.1

1
1-Hydroxychrysene = 1-chry
2-Hydoxychrysene = 2-chry
3-Hydroxychrysene = 3-chry

2-Hydroxyfluorene = 2-flu
9-Hydroxyfluorene = 9-flu
4-Hydroxychrysene = 4-chry

3-Hydroxyphenanthrene = 3-phe
1-Hydroxyphenanthrene = 1-phe
2-Hydroxyphenanthrene = 2-phe

4-Hydroxyphenanthrene = 4-phe
9-Hydroxyphenanthrene = 9-phe
1-Hydroxypyrene = 1-py

3-Hydroxybenzo(a)pyrene = 3-bap
1-Hydroxynaphthalene = 1-nap
2-Hydroxynaphthalene = 2-nap

2 CDC (2009)

3 Becker et al. (2003)

4 Wilhelm et al. (2008)

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Table 4.6 Creatinine adjusted levels (50th, 75th and 95th percentiles) of PAH metabolites in the present study of Swedish women compared to levels in the general population and women from USA and Germany.

Country	Population	Percentile	Concentration of PAH metabolites1 (µg/g creatinine)														
			1-chry	2-chry	3-chry	4-chry	2-flu	9-flu	1-phe	2-phe	3-phe	4-phe	9-phe	1-pyr	3-bap	1-nap	2-nap
Sweden (present study)	Female	50th		<LOD			0.15	0.44	0.13	0.06	0.10	0.007	0.006	0.008	<LOD	1.50	3.10
		75th					0.23	1.10	0.24	0.07	0.16	0.01	0.00	0.04	<LOD	3.00	5.40
		95th		0.01			0.44	1.60	0.50	0.14	0.20	0.07	0.02	0.07	<LOD	10.0	7.80
	Observations above LOD			0	1	0	0	15	15	18	17	18	5	1	8	0	18
USA[2]	Female	50th					0.21	0.23	0.15	0.05	0.10	0.02		0.08		2.04	2.68
		75th					0.42	0.40	0.24	0.09	0.18	0.04		0.14		6.68	6.43
		95th					2.22.	1.02	0.51	0.20	0.50	0.12		0.44		24.7	22.5
	All	50th					0.22	0.23	0.14	0.05	0.10	0.02		0.08		2.1	2.56
		75th					0.50	0.41	0.22	0.09	0.17	0.04		0.15		6.56	6.34
		95th					2.07	1.10	0.49	0.21	0.50	0.11		0.42		21.8	19.9
Germany[3,4]	Female	50th															
		75th															
		95th															
	All	50th															
		75th															
		95th															

1
1-Hydroxychrysene = 1-chry
2-Hydoxychrysene = 2-chry
3-Hydroxychrysene = 3-chry

2-Hydroxyfluorene = 2-flu
9-Hydroxyfluorene = 9-flu
4-Hydroxychrysene = 4-chry

3-Hydroxyphenanthrene = 3-phe
1-Hydroxyphenanthrene = 1-phe
2-Hydroxyphenanthrene = 2-phe

4-Hydroxyphenanthrene = 4-phe
9-Hydroxyphenanthrene = 9-phe
1-Hydroxypyrene = 1-py

3-Hydroxybenzo(a)pyrene = 3-bap
1-Hydroxynaphthalene = 1-nap
2-Hydroxynaphthalene = 2-nap

2 *CDC (2009)*

3 *Becker et al. (2003)*

4 *Wilhelm et al. (2008)*

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4.4 Metals

Levels of Metals are compared to levels found in American studies of more than 4000 people (HANES project, CDC 2009) and levels in urine of approximately 500 people from Germany (The GerES III study, Becker et al. 2003, Wilhelm et al. 2008). The comparisons are presented in Table 4.7 and Table 4.8

The levels of Cd, Co, Hg, Mo and Pb seem to be lower in the present study of Swedish women compared to the levels in the American HANES study and the German GerES III study. As on the other hand exhibit roughly the same levels in Sweden and the Hanes and GerES III studies.

For the other metals there were no national percentile levels available for comparison. Instead comparisons can be made to scientific studies.

Campbillo et al. (1999) tested the usage of electrothermal atomic absorption spectrometry with a fast-programme methodology for the analysis of aluminium in urine and found levels of 2.3 – 14 µg/l in four control subjects. Sjögren et al. (1985) investigated the levels of aluminium in industrially exposed workers. In the large control group (49 men and 29 women) he found a median level of 4 µg/l in urine. These levels are lower than the median levels of aluminium found in this study (19.4 µg/l). The reason remain unknown, but may be due to the fact that different analytical methods were used (ICP-MS in this study versus AAS in other studies).

The median urine level of chromium in the general population in USA was 0.4 µg/l in 1988 with a range of 0.24–1.8 µg/l (ATSDR 2009a) while the median level of copper in urine of the US population has been estimated to be 18 µg/l (Georgopoulos et al. 2001). These levels are well above the levels seen in the present study and it may indicate generally decreasing concentrations since 1988 (chromium), 2001 (copper) and/or lower levels in USA compared to Sweden.

Manganese was detected at quantifiable levels in urine samples from 73% of 496 participants in a monitoring study of the US population (ATSDR 2009b). The mean urinary manganese concentration in these 496 individuals (aged 6–88 years of age) was 1.19 µg/l, which is comparable to the levels seen in the present study.

For Nickel there exists different estimates of levels that can be expected in the general population; A review of studies of nickel concentrations in humans before 1994 indicated that the most reliable reference values were 1–3 µg/L for nickel in urine (ATSDR 2005a). Later studies indicated that levels in individuals not occupationally exposed to nickel are generally <2 µg/L and can range as high as 9–10 µg/L (95% upper confidence limit) in healthy adults (ATSDR 2005a). These

levels are higher than the median levels seen in the present study indicating decreasing concentrations and/or lower levels in USA compared to Sweden.

For Zinc there is little data on zinc levels in human biological matrices, and even less data on levels in urine (ATSDR 2005b). Twenty workers in a zinc foundry in Baiyin, China were investigated for exposure to zinc oxide fumes. Concentrations of zinc in urine for these workers averaged 240 µg/l during the period of an 8-hour shift (ATSDR 2005b). Levels of Zinc were investigated in urine of 200 randomly selected male volunteers comprising 100 smokers and 100 non-smokers smokers in Abeokuta city, Nigeria (Opeolu et al. 2007). The levels of Zinc were 235 – 664 µg/l in the non smoking group. Although these levels are above the median levels in the present study, it is doubtful whether the groups of people investigated in these studies are comparable to the women studied here. Consequently it is not possible to conclude whether the levels seen in this study comprise normal, elevated or lower concentrations than what could be expected in the general population of Sweden.

Table 4.7 Non adjusted levels (50th, 75th and 95th percentiles) of metals in urine in the present study of Swedish women compared to levels in the general population and women from USA and Germany.

Country	Population	Percentile	Concentration of metals (µg/L)											
			Al	As	Cd	Co	Cr	Cu	Hg	Mn	Mo	Ni	Pb	Zn
Sweden (present study)	Female	50th	19	8.1	0.045	0.16	0.002	4.2	0.15	1.3	19	0.83	0.28	140
		75th	56	16	0.071	0.28	0.14	4.9	0.35	1.9	31	1.6	0.36	180
		95th	120	65	0.25	1.4	0.33	7.3	0.53	3.9	45	2.6	0.54	300
	Observations above LOD		20	20	13	20	7	20	19	18	20	20	19	20
USA [1]	Female	50th		6.90	.210	.340			.430		37.9		.540	
		75th		15.0	.450	.580			1.07		67.3		.920	
		95th		60.5	1.20	1.47			3.54		127		1.82	
	All	50th		7.70	.210	.330			.420		44.5		.640	
		75th		16.0	.450	.520			1.00		78.5		1.04	
		95th		65.4	1.15	1.16			3.19		138		2.29	
Germany [2,3]	Female	50th												
		75th												
		95th												
	All	50th		3.3	0.22				0.4			3.0		
		75th												
		95th		18.9	0.96				3.3					

1 *CDC (2009)*

2 *Becker et al. (2003)*

3 *Eilhelm et al. (2004)*

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Table 4.8 Creatinine adjusted levels (50th, 75th and 95th percentiles) of metals in urine in the present study of Swedish women compared to levels in the general population and women from USA and Germany.

Country	Population	Percentile	Concentration of metals (µg/g creatinine)											
			Al	As	Cd	Co	Cr	Cu	Hg	Mn	Mo	Ni	Pb	Zn
Sweden (present study)	Female	50th	23	10	0.0	0.2	0.0	3.8	0.2	1.4	21	0.9	0.2	130
		75th	50	16	0.1	0.3	0.1	4.4	0.4	2.1	27	1.4	0.4	200
		95th	85	61	0.3	0.9	0.5	5.9	0.7	4.8	31	3.6	0.6	240
	Observations above LOD			20	20	13	20	7	20	19	18	20	20	19
USA [1]	Female	50th		7.33	.253	.361			.545		41.1		.649	
		75th		14.4	.487	.554			1.06		61.4		1.03	
		95th		58.4	1.06	1.29			2.77		122		1.96	
	All	50th		7.04	.208	.290			.447		39.2		.622	
		75th		14.1	.412	.455			.909		58.3		.979	
		95th		50.4	.940	1.02			2.35		120		1.97	
Germany [2,3]	Female	50th												
		75th												
		95th												
	All	50th												
		75th												
		95th												

1 *CDC (2009)*

2 *Becker et al. (2003)*

3 *Eilhelm et al. (2004)*

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4.5 Endocrine disturbing effects

For the YES assay there are no national reference values to use for comparison. Instead, the levels (expressed as estradiol equivalents per L (EEQ/L), see section 3.2.6) were compared to EEQs in urine caused by the presence of endogenous hormones such as estradiol, estrone and estriol. These are naturally occurring estrogenic compounds (NEs) that are responsible for a large part of the endocrine inducing effect seen in the YES assay, and it is consequently valid to compare results from an YES assay of urine with the levels of these. NE levels were calculated from Liu et al. (2009) which provides data on the urinary excretion of natural estrogens. By dividing the daily excretion (EEQ/day) with an average urinary discharge of 1 – 1.5 L/day the concentrations of natural estrogens expressed as estradiol equivalents per L (EEQ/L) could be calculated.

Table 4.9 Levels of Estrogenic effects expressed as estradiol equivalents per L (EEQ/L) in the present study compared to natural levels in women..

	Estrogenic effects (EEQ/L)
Premenopausal women[1]	6.6 – 10
Postmenopausal women[1]	2.9 – 4.4
Pregnant women[1]	2900 - 4300
Subject 1	2
Subject 2	1.7
Subject 3	8.5
Subject 4	1.7
Subject 5	1.9
Subject 6	4.4
Subject 7	0.5
Subject 8	8.6
Subject 9	9.1
Subject 10	7.3
Subject 11	<0.0027
Subject 12	6.1
Subject 13	3.8
Subject 14	4.9
Subject 15	5.1

	Estrogenic effects (EEQ/L)
Subject 16	3.7
Subject 17	<0.0027
Subject 18	<0.0027
Subject 19	<0.0027
Subject 20	<0.0027

[1]Calculated from data in Liu et al. (2009)

4.6 Statistical evaluations

A very efficient way of exploring the co-relationship between chemical constituents in the urine is to use Principal Component Analysis (PCA) which is a method that can be used to identify co-varying variables in data sets with many variables. In this way, the original variables (=substances in urine and YES results) are projected into linear combinations of the variables. These linear combinations are denoted principal components.

Before PCA analysis, concentration data were log transformed and standardized to the mean 0 and variance 1. Only substances with more than 15 detects were used in the PCA analysis.

The resulting PCA model, explained 53 % of the variation with the first two principal components (Figure 4.1). The resulting PCA plot is shown in Figure 4.2. In the PCA plot, variables close to another tend to co-vary more than variables at larger distances. Also, variables that are close to zero on the (principal component) scale tend to contribute less to the overall variability than those that are further from zero.

The co-variability is also graphically presented in Figure 4.3 - Figure 4.5. Note that specific metals has been included in the PAH and phthalate metabolite co-variability graphs (Figure 4.3 and Figure 4.4) because these metals tend to co-vary with some of the organic metabolites.

The most prominent feature of the multivariate evaluation is the close co-variability between certain PAH metabolites (Figure 4.2 - Figure 4.3) and some phthalate metabolites (Figure 4.2 and Figure 4.4) as well as the fact that metals do not co-occur (Figure 4.2 and Figure 4.4).

The co-variability between concentrations in urine and exposure related information (see section 3.1) was evaluated using two different methods; For parametric exposure information (i.e. weight, length and age), multiple regression was used with chemical concentrations as the dependent variable. For non-parametric exposure

information (rural or city dwelling, workplace exposure to chemicals, smoking habits, eating habits, origin of drinking water, dwelling type, age of residence and age of car) non-parametric methods were used:

- logistic regression with chemical urine concentrations as the dependent variable
- kruskal wallis ANOVA & median test

None of these tests showed any significant effect of the exposure parameters on the concentration of metabolites and metals in the urine. A PCA plot separating the women on the two first principal components show that there was no grouping of women as a results of the chemical composition in the urine (Figure 4.6).
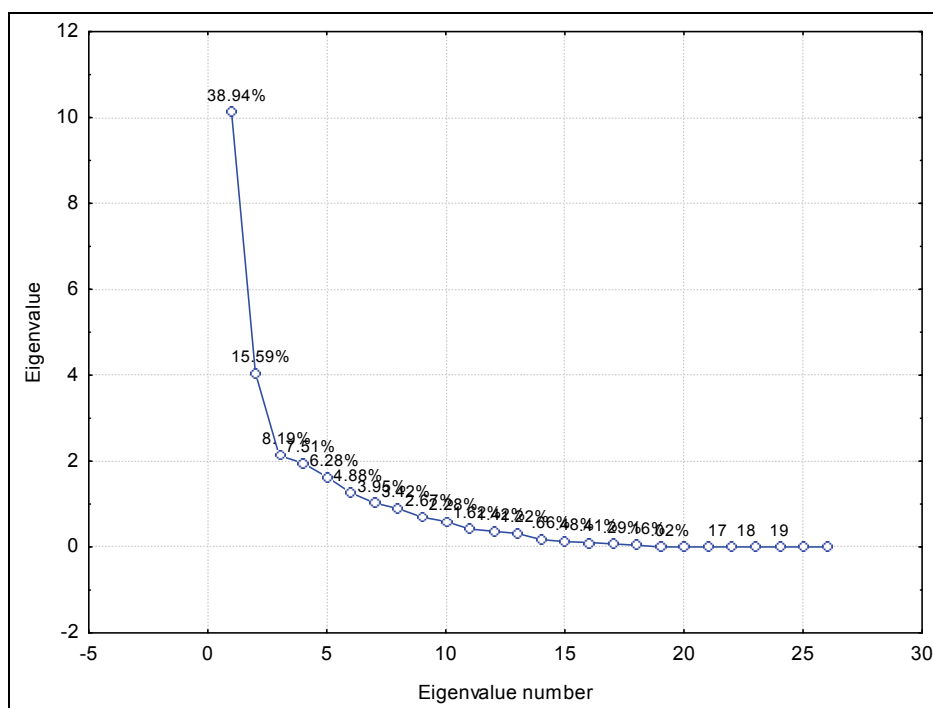
Figure 4.1 Graph showing how much of the total variation in the data set that is explained by each principal component. Eigenvalue number is equal to the principal component number.
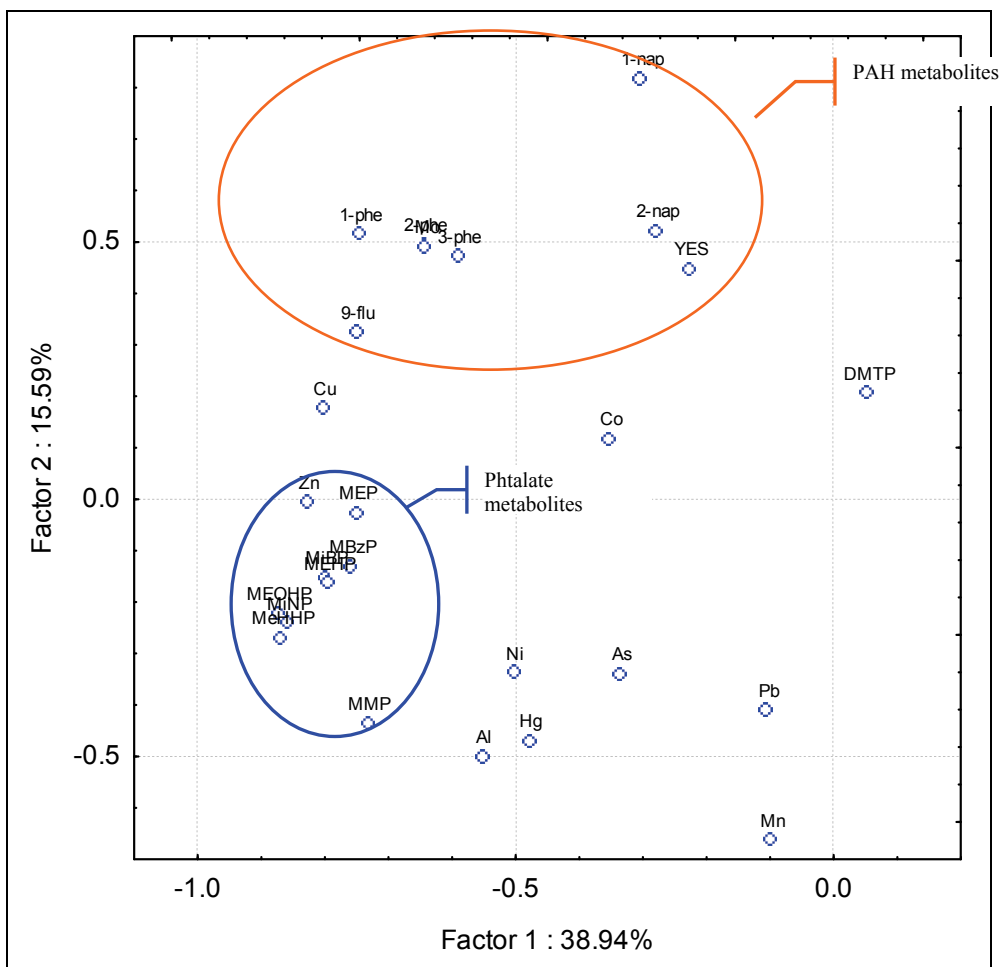
Figure 4.2 PCA plot of the urinary concentrations of metabolites and results from YES assay showing loadings of separate substances along the first two principal components. The first principal component (PC1, y axis) explained 39% of the variation of the levels of substances while the second component (PC2, x axis) explained 16%.. PAH metabolites and phthalate metabolites are clustered and separately marked.
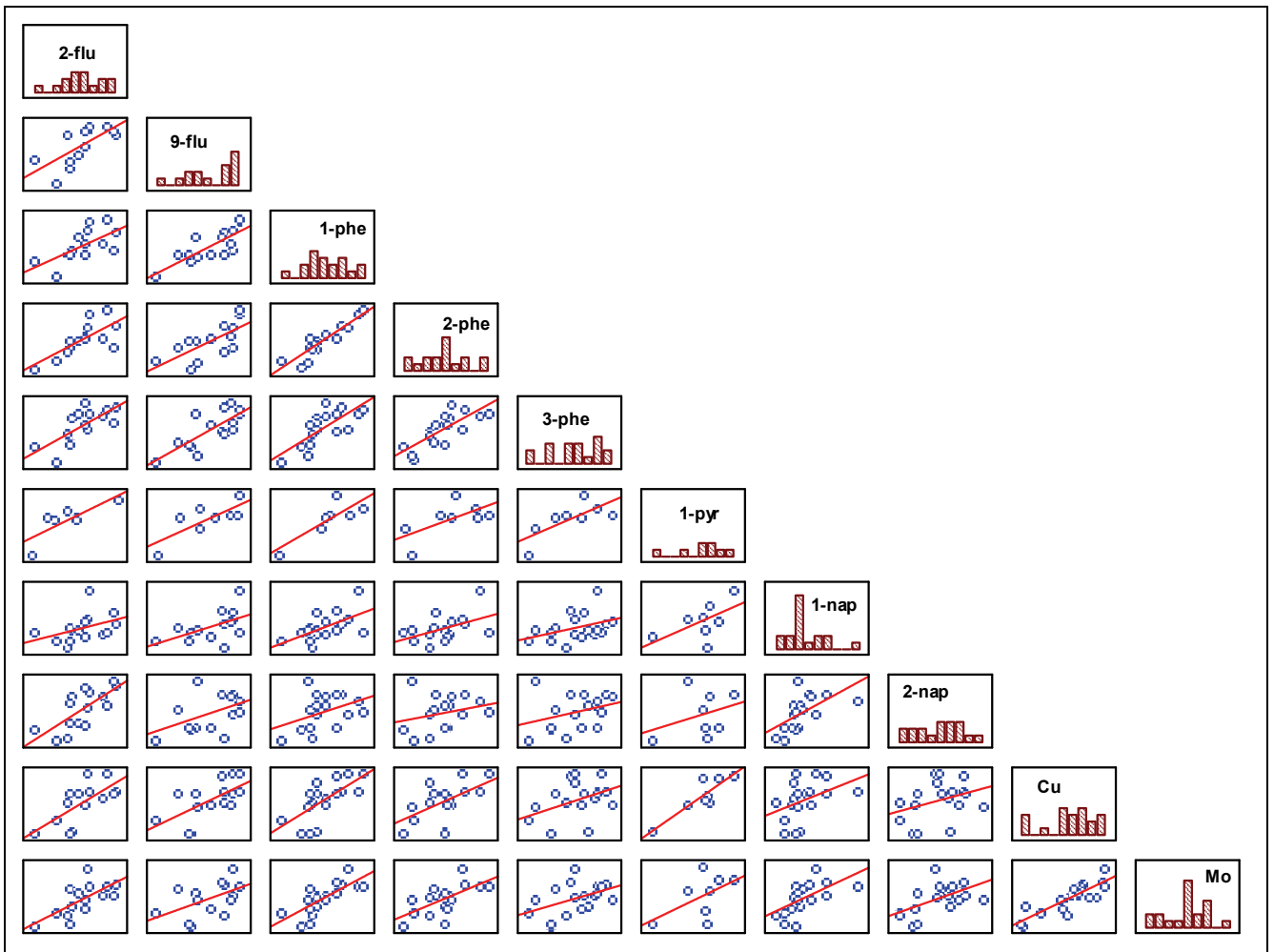
Figure 4.3 Co-variability between PAH metabolites and two metals. Graphs are based on log transformed data
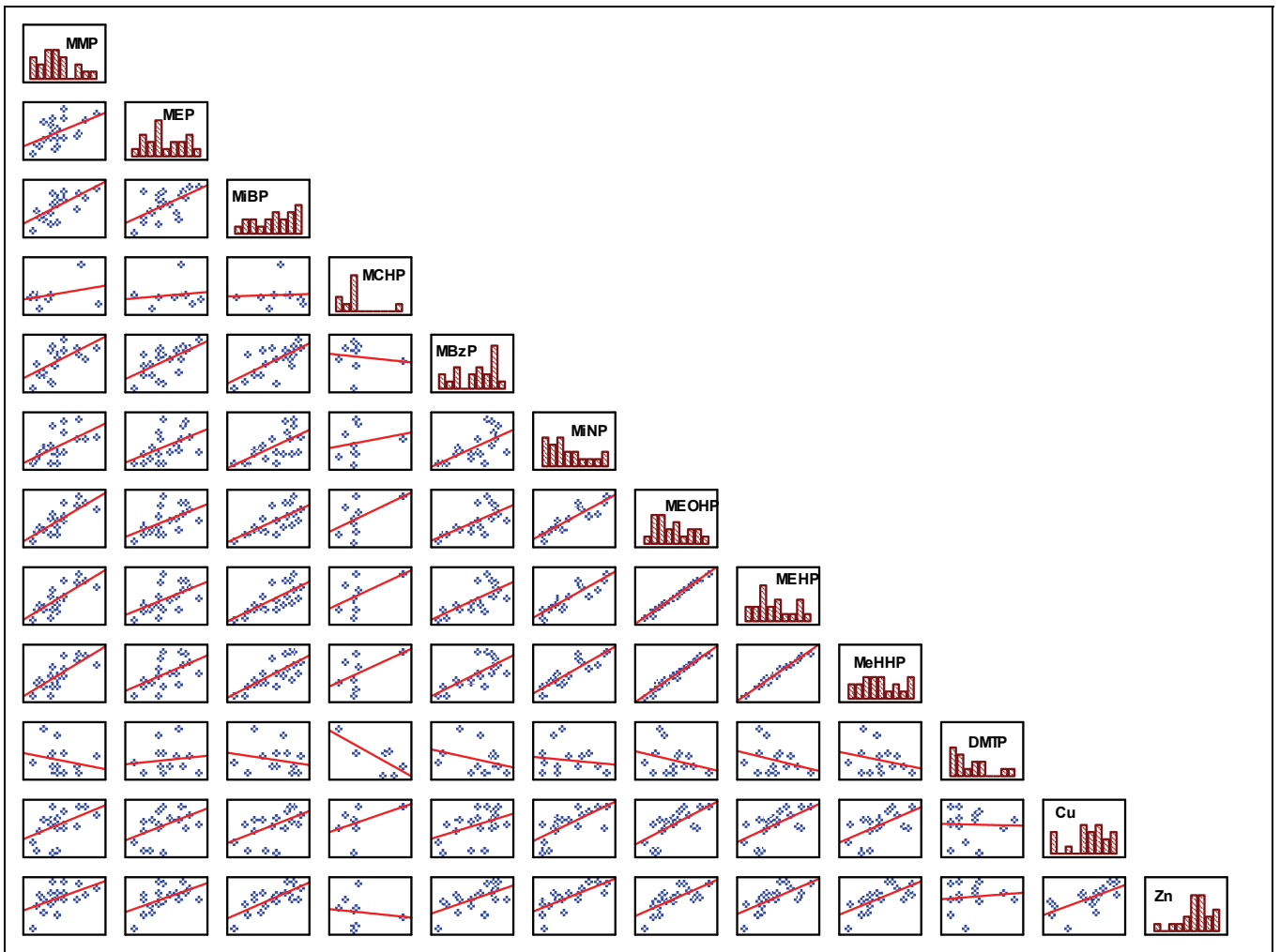
Figure 4.4 Co-variability between Phatakte metabolites and two metals. Graphs are based on log transformed data
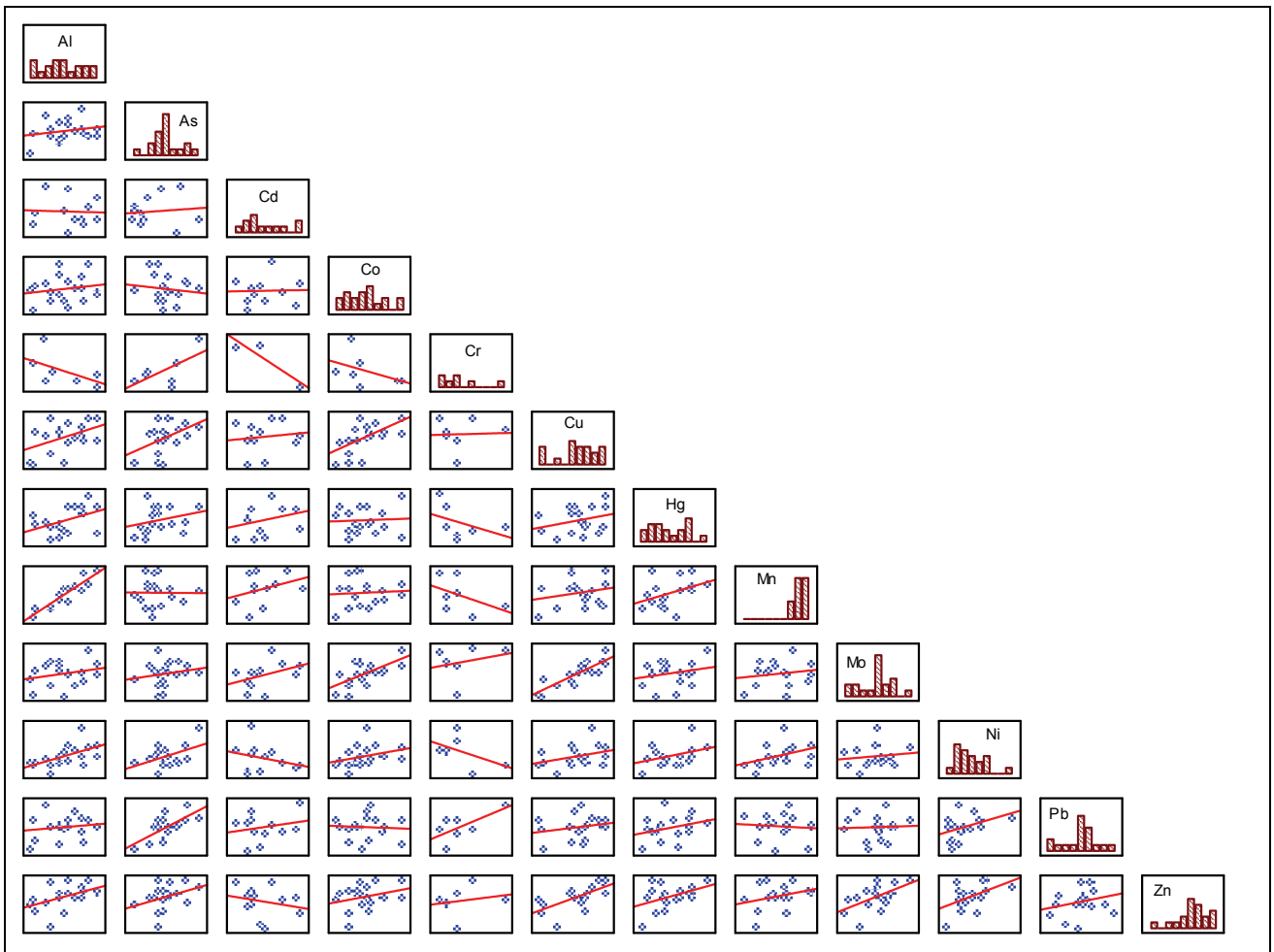
Figure 4.5 Co-variability between metals. Graphs are based on log transformed data
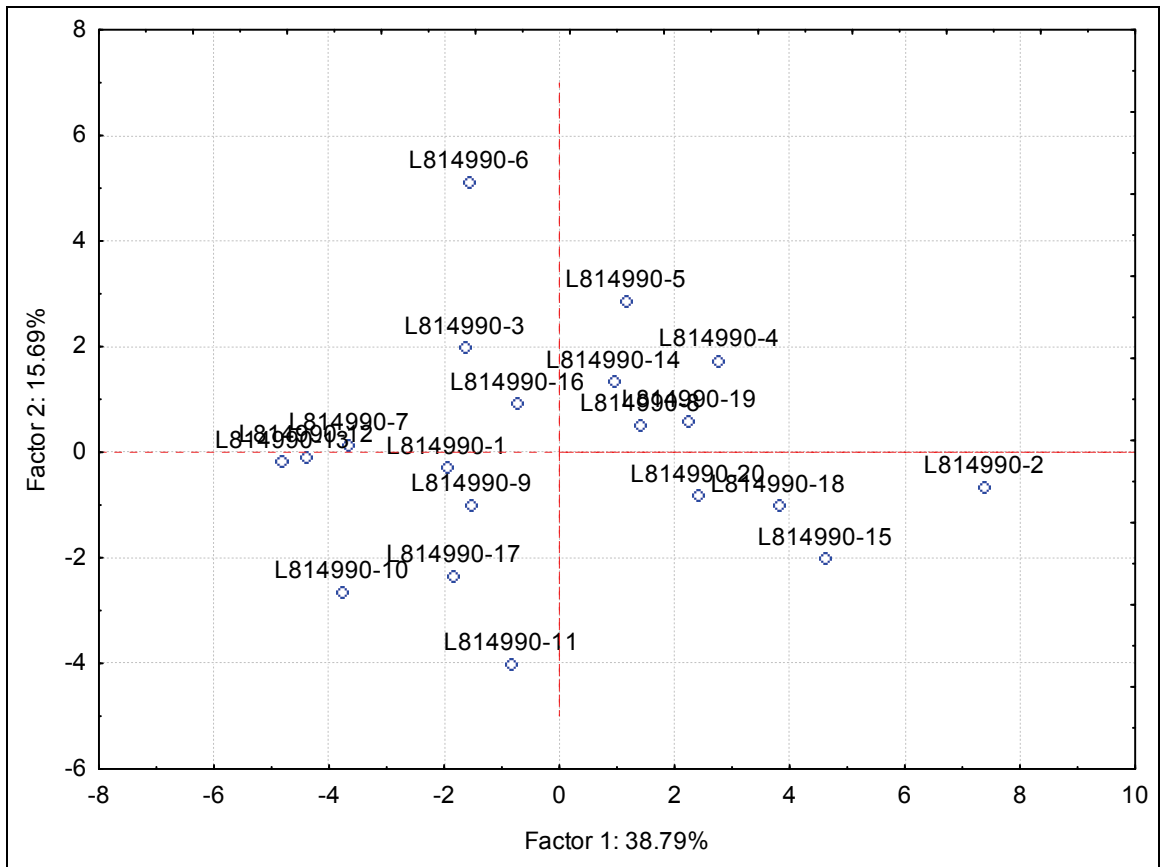
Figure 4.6 Projection of the women on the fist two principal components.

5 Discussion

5.1 Exposure

There was a large variability in the levels of metals and metabolites in urine within the group of 20 women. This may be due to individual differences in metabolism (Moore and Gould 1984). For instance, PAH metabolite profiles in urine have been shown to vary between individuals experiencing similar exposures within the same workplace (Jacob and Seidel 2002). The variability is also most likely related to exposure conditions (Van Rooij et al. 1994, Grimmer et al. 1997, Jacob and Seidel 2002).

The study group was fairly uniform (same sex and all living in the Stockholm area), but each individual will have a number of exposure related living conditions and activities that influence exposure levels. Despite this, statistical tests could not discern any connection between information on exposure related issues given by the women, and the chemical levels found in urine.

A dependence of PAH metabolite urinary levels on smoking habits could have been expected since several studies have found such connections (Campo et al., 2006; Nan et al., 2001, Li et al. 2008, Jacob and Seibel 2002). A likely reason for an absence of this connection was that the study group in the present study was to small to discern statistical differences between groups with different smoking habits. PAH exposure is also expected to be higher in urban areas. It is interesting that one of the two women living outside of urban areas had concentrations < LOD for all PAH metabolites. This could not be evaluated statistically given a small N.

Phthalates are commonly used in polyvinyl chloride based plastic products, such as inflatable toys, plastic bags, garden hoses and toys (ATSDR, 2001). Since they are not chemically bound to the plastics, phthalates are emitted into the environment during use or disposal, and different phthalate esters have been measured in foods, indoor and ambient air, indoor dust (Clark et al 2003). People are exposed through ingestion, inhalation, and, to a lesser extent, dermal contact with products that contain phthalates. Dietary sources have been considered as the major exposure route, followed by inhaling indoor air (Clark et al. 2003). Since phthalates are metabolized and excreted quickly their urinary levels probably reflect recent exposure.

In the present study one question on eating habits was posed, but 19 of the 20 women answered that they had a mixed food intake. A more detailed questionnaire could perhaps have found a connection between eating habits and urinary levels of phthalate metabolites. It is also believed that phthalates are emitted to a larger degree from newer products, and questions were posed on whether the women lived in

newer or older houses. Five of the women were living in newer houses, but it was not possible to discern higher phthalate metabolite levels in these women. Again, the study group may have been too small to discern differences that are actually present.

Exposure to OP insecticides mainly occurs by ingesting contaminated food. In Sweden, most OP pesticides are banned from use, and these substances have only been detected on very few occasions in the Swedish environment despite several thousand analyses of OP pesticides in surface water, ground water and drinking water within the regional monitoring programme during the 1990s[7]. Consequently, food ingestion may be the only exposure pathway in Sweden. The most commonly occurring OP metabolite, DMTP, is known to be the degradation product (in humans) of more than ten different OPs, and it is consequently not possible to draw conclusions on which OP pesticides in food that causes the occurrence of DMTP in urine (CDC 2009). Since all the specific OP metabolites are not well known, this is generally considered to be a good reason why DMTP and the other DAP metabolites should be used for estimating OP exposure in humans (Heudorf et al. 2006, Barr et al. 2004).

Most metals were common in urine, and these originate from a very wide variety of sources. Two of the more toxic metals, mercury and arsenic, were found in all women

This study focuses on the DAP because these reflect the total exposure, making them appropriate for a general assessment of OP exposure

Exposure to arsenic can occur through multiple pathways and sources, some of which are (CDC 2009):
- Consumption of drinking water, especially from locally supplied water, since arsenic levels may be naturally elevated locally and/or regionally
- Foodstuff such as meats, grain, and produce.
- Children may ingest contaminated soils
- Dust exposure to contaminated soil as well as dermal contact with CCA-preserved wood structures.
- Workplace exposure, especially at smelters
- Tobacco smoking

Only one women had non-municipal drinking water, and arsenic levels were not elevated in her urine. Also, there was no connection between smoking and As levels.

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[7] Based on searches in the pesticide database kept by the Water Quality Management unit at the Swedish University of Agricultural Sciences (http://pesticid.slu.se/)

Elemental mercury was used in Sweden to produce chlorine gas and caustic soda for industrial applications. Other major uses have been electrical equipment and thermometers, as well as dental amalgam. Inhalation of elemental mercury originating from dental amalgam has been a very important source of mercury exposure, although this is declining since amalgam is replaced with plastic fillings. Elemental mercury may also be emitted to the air in Sweden from the combustion of coal and solid-waste incineration. Transnational and global atmospheric transport occurs and elemental mercury is deposited on land and water. The ingestion of methyl mercury, predominantly from fish and other seafood, constitutes the main source of dietary mercury exposure. However Hg in urine mainly reflects inorganig Hg exposure and only to a minor degree MeHg exposure. The questionnaire was missing questions on dental fillings and fish consumption, which may have clarified the pattern of Hg concentrations seen in urine.

5.2 Co-occurrence

PAH metabolites and phthalate metabolites were common in urine, but they did not correlate. This may be a result of differing physicochemical properties which influence both the uptake and distribution in the body, as well as enzymatic transformation (Liu et al. 1998). However, the main reason is probably different exposure pathways and different sources (see section 5.1), the end result being that women exposed to high levels of one contaminant group is not necessarily exposed to high kevels of other contaminant groups.

Within the contaminants groups, there were interesting patterns. The phthalate metabolites MEHP, MEOHP and MEHHP are metabolic products of DEHP (Figure 2.1), and their urinary concentrations correlate strongly (p<0.001, r2>0.99; see also Figure 4.4). Several other phthalate metabolites (MMP, MEP, MiBP and MBzP) originating from "modern" phthalates correlated clearly, most likely because they originate from the same products/sources. It is also interesting that the metabolites of DEHP, that is being phased out, correlates clearly with other "modern" phthalates (Figure 4.4). One reason for a decreased correlation between different phthalate metabolites are markedly different times from exposure to metabolite excretion between different phthalates (Gender et al. 2004).

Within the PAH metabolite group there was a co-variability between metabolites originating from the same PAH parent compound, but also between metabolites originating from different parent compounds. The PAHs clustered less clearly in the PCA analysis compared to phthalate metabolites (Figure 4.2). This indicates that different PAHs originate from a wider variety of sources compared to phthalates and/or that uptake and metabolism of differs more for PAHs compared to phthalates.

Other studies have found a clear influence of age on the levels of OP metabolites where the levels decreased significantly with increasing age except for DEDTP (Becker et al. 2007). No such age dependent pattern could be discerned in the present study, probably because of the low OP metabolite levels and the low number of women studied.

5.3 Levels

On the one hand, it is interesting that such a wide variety of metabolites and metals are seen in a large portion of the urine samples. On the other hand, the levels in Sweden are in most cases equal to or below levels seen in other countries.

The only clear exception was the phthalate metabolites MiBP and MiNP that were clearly above level in the USA (Table 4.1 - Table 4.2) while MiBP were somewhat above levels in Holland. Both of these originate from fairly new phthalate compounds (DINP $\rightarrow$ MiNP, DBP $\rightarrow$ MiBP) that have been introduced on the market within the latest 5 – 10 years where DINP is a replacement for DEHP. Other studies have also found higher levels of MiBP and MiNP in European countries compared to USA (CDC 2009).

Mono-ethyl phthalate (MEP) levels were clearly lower in the present study compared to levels in USA. The parent compound, diethyl phthalate (DEP) is a solvent used in many consumer products, particularly those containing fragrances both in Sweden[8] and USA (CDC 2009). The large differences in levels may reflect different usage patterns of fragrance products between USA and Sweden and/or different usage of DEP in the products (i.e. different concentrations).

Levels of OP metabolites were consistently below levels in Germany and the USA (Table 4.3 - Table 4.4) which probably reflects non-existent usage of OP pesticides in Swedish agriculture, and possibly a different pattern of fruit- and vegetable import.

PAH metabolite levels were in general equal to or lower compared to the levels in USA and Germany. This is despite that women in the present study mostly lived in a large urban area while the American and German studies encompassed a much wider variety of living conditions. The reason for this discrepancy remains unknown. The levels of metals were also consistently lower compared to USA and Germany. For instance, the levels of mercury were lower in Sweden despite generally elevated mercury levels in limnic fish in Sweden, However this may be explained by the fact that levels in fish relate to methyl mercury while levels in urine mainly reflects inorganic Hg exposure. .

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[8] Information from the Swedish product registry: http://apps.kemi.se/kemistat/start.aspx

5.4 Endocrine disturbing effects

Several of the phthalates and benzo[a]pyrene as well as several of the OP pesticides are classified as endocrine disrupters (BKH 2002, DHI 2007). For instance, in vitro studies show that certain phthalates can bind to estrogen receptors (CDC 2009) and cause ovarian abnormalities in female animals at high doses (CDC 2009). However, no clear connection between phthalate exposure and actual endocrine disturbing effects in humans seem to have been established (Jonsson et al. 2005, Duty et al. 2004).

In general, the levels of endocrine disturbing effects were very high which is probably caused by high levels of naturally occurring estrogenic compounds in female urine (Liu et al. 2009). The levels were in fact so high that the urine samples had to be diluted before they could be analysed using the YES assay. The endocrine disturbing effects were also much higher compared with levels measured in sewage water and effluent using the YES assay (Huggett et al. 2003, Sapozhnikova et al. 2005).

At the same time, there was no correlation between urinary metabolite or metal levels and endocrine disturbing effects expressed as estradiol equivalents. One reason may be that the naturally occurring estrogenic compounds concealed any effects caused by potentially endocrine disturbing synthetic chemicals. From an environmental perspective however, natural estrogenic compounds may be as important as the synthetic; It has been recognised that an important part of the endocrine disturbing effects observed both in sewage water and effluent derives from naturally occurring estrogenic compounds originating from humans, since their estrogenic potencies are several orders of magnitude higher than that of known estrogen disrupting compounds (Benjamin et al. 2007, Travis et al. 2006, Lintelmann et al. 2003). Accordingly, an important part of the suspected endocrine disturbing effects seen in downstream biota probably also originates from natural estrogenic compounds (Travis et al. 2006, Lintelmann et al. 2003). The results from this study are in accordance to this, given the high levels of endocrine disturbing effects seen in the urine, and the fact that urine is a major contributor to the waste stream reaching waste water treatment plants.

6 Conclusions and recommendations

Firstly, it should be noted that finding a number of organic substance metabolites and metals in urine does not mean that the levels of the parent compounds or metals are associated with any undesirable health effects. Instead, studies of contaminants in urine serve as a basis for establishing reference values which can be used to monitor long term exposure levels, and in separate cases, determine if people have been exposed to higher levels than are found in general.

The main conclusions from this study are:

1. Urine is clearly a good matrix for human biomonitoring of organic chemicals and metals given the high prevalence of detection and the good correlations seen between many metabolites.
2. PAH and phthalate metabolites originating from many(most) of the important mother compounds as well as metals were very prevalent in the urine of the women and the levels were generally variable.
3. Metabolites of organophosphorous pesticides were not prevalent
4. No connection could be found between exposure related .information given by the women and metabolite and metal levels in urine.
5. Levels of PAH and phthalate metabolites are in general similar to reference concentrations in USA, Germany and Holland.
6. Levels of MiBP and MiNP were clearly above levels in the USA indicating a higher exposure to DBP and DINP
7. Mono-ethyl phthalate (MEP) levels were clearly lower in the present study compared to levels in USA and below levels in Holland, indicating lower exposure to DEP in Sweden.
8. Levels of OP metabolites were clearly below levels seen in USA and Germany
9. Levels of metals were generally below levels seen in USA and Germany
10. The urinary levels of most phthalate metabolites correlated clearly
11. The levels of PAH metabolites also correlated, but not as clearly as for phthalate metabolites
12. No connection could be established between endocrine disturbing effects measured with an *in vitro* test, and urinary levels of metabolites and metals. A partial explanation may be the presence of natural hormones in womens urine with endocrine inducing properties

The following recommendations are given:

1. Follow-up studies focusing on PAH metabolites and phthalate metabolites are suggested. These studies should contain larger groups of women and/or men so that exposure factors influencing metabolite concentration in urine could be established. The number of metabolites could be reduced based on the results from this study.

2. Given that OP pesticide exposure mostly occur via ingestion (indicating that women from urban areas are as exposed as women from rural areas) and the low levels seen in this study, there is no further need for OP metabolite screening studies in urine. This is also supported by the fact that the higher levels seen in USA and Germany are still below risk levels (CDC 2009).

3. Studies of metals in urine could also be of interest, especially given the relatively low costs of metal analysis in urine. Given the multitude of possible sources and exposure pathways for metals, such studies need to be much more extensive than the present if connections between exposure factors and urinary metal levels are to be established.

7 References

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