Master's thesis

NTNU
Norwegian University of Science and Technology
Faculty of Natural Sciences
Department of Chemistry

Selma Tofte Granerud

Analyse glycol ether and pesticide metabolites in Norwegian children and their potential determinants

Master's thesis in Environmental Toxicology and Chemistry
Supervisor: Øyvind Mikkelsen and Amrit Kaur Sakhi (NIPH)
Co-supervisor: Anders Lervik

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Kunnskap for en bedre verden

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Preface

I would like to thank my supervisor Professor Øyvind Mikkelsen for the opportunity to perform my master thesis and for his valuable guidance and moral support. Thanks to Associate Professor Anders Lervik for helping with the statistical aspect of the thesis.

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Final acknowledgements goes to my family and friends for their constant support and encouragement.

Abstract

Non persistent chemicals are widely used in consumer products and the environment. They are found in many everyday products, e.g. plastics, lubricants, solvents, plasticizers, and pesticides. Low levels of exposure to these chemicals may cause endocrine or reproductive disorders. Glycol ethers are oxygenated solvents, highly used in occupational and domestic products, and exposure to glycol ethers have been linked to increased time to pregnancy, fetal malformation, lower semen quality, menstrual cycle disorder, among other. Organophosphate (OP) and pyrethroid (PYR) pesticides are commonly used in agriculture as well as domestic and gardening use, and studies have shown the ability of multiple pesticides to affect thyroid homeostasis. Children are more exposed compared to adults as they are still developing in addition to breathe more air, drink more water, and eat more food per pound of body weight than adults. Urine samples from children in Norway (n=286), age 6-12, were analysed with ultra performance liquid chromatography-tandem mass spectrometry (UPLC-ESI-MS/MS) to look for 2-butoxyacetic acid (BAA), phenoxyacetic acid (PhAA), 3-phenoxybenzoic acid (3-PBA), 2,4-dichlorophenoxyacetic acid (2,4-D), 3,5,6-Trichloro-2-pyridinol (TCPy), cotinine, imazalil and 8-iso-Prostaglandin F2α (8-iso-PGF2α). The urine samples analysed were a pool of two spot urine samples collected at bedtime and the morning after. The urinary concentration of BAA, PhAA, 3-PBA, 2,4-D and TCPy were compared to other studies available worldwide. Further, associations between urinary concentrations and questionnaire data regarding use of cleaning products and food consumed were

studied. The detection frequency from this study were found to be with decreasing order as follows: PhAA(100%) >TCPy(58%) >BAA(44%) >imazalil(35%) >3-PBA(17%) >2,4-D(9%) >cotinine(2%) >8-iso-PGF2α(0%). PhAA was detected in 100% of the samples, all levels above LOQ (3.0 ng/mL), and had the highest median (137 ng/mL or 145.20 μg/g) compared to the other glycol ether metabolite and overall. TCPy was the pesticide metabolite with the highest detection frequency (58%) and the highest median (0.27 ng/mL or 0.31 μg/g). BAA, 3-PBA, 2,4-D, cotinine, imazalil and 8-iso-PGF2α had median below LOD, (15.0 ng/mL), (0.3 ng/mL), (0.2 ng/mL), (3.0 ng/mL), (0.1 ng/mL), (3.0 ng/mL), respectively. All compounds in this study had lower median compared to the other studies. There were no significant correlation between the glycol ether metabolites and the questionnaire data regarding the use of cleaning products, using Spearman's Rho test ($p<0.05$). Significant correlations between the pesticide metabolites with questionnaire data regarding food were observed as following: 3-PBA with cheese (-0.141, $p<0.05$) and nuts (-0.128, $p<0.05$), 2,4-D with pro-biotic yogurt (0.117, $p<0.05$), cheese (-0.140, $p<0.05$) and ham (-0.125, $p<0.05$), and TCPy with white fish (0.150, $p<0.05$), seafood (0.127, $p<0.05$), canned fish (0.186, $p<0.01$) and fruits (0.167, $p<0.01$). A trend were observed, however not significantly correlated, for all three pesticide metabolites with egg and sugar, 3-PBA and 2,4-D with red meat, and TCPy with vegetable oil. Among the glycol ether metabolites, PhAA showed a trend with the use of degreasing sprays.

Sammendrag

Ikke-persistente stoffer er mye brukt i forbruksvarer og i miljøet. De finnes i hverdagsprodukter som plastikk, smøremidler, løsningsmidler, og insektsmidler, og eksponering av disse stoffene i lave konsentrasjoner kan forstyrre det endokrine systemet og evnen til reproduksjon. Glykol etere er oksygenholdige løsningsmidler, mye brukt i flere yrker og i forbruksvarer. Eksponering av glykol etere har vært knyttet til forsinket graviditet, fostermisdannelser, lavere sædkvalitet og forstyrret menstruasjonssyklus. Organofosfat (OP) og pyretroid (PYR) insektmidler er mye brukt i jordbruk i tillegg til personlig bruk, og studier har påvist at flere typer insektmidler kan påvirke skjoldbrukskjertelens homeostase. Barn er mer eksponert sammenlignet med voksne fordi de er under utvikling. I tillegg puster de mer luft, drikker mer, og spiser mer i forhold til kroppsvekten deres. Urinprøver fra barn i Norge (n=286), alder 6-12 år, ble analysert med ultra performance liquid chromatography-tandem mass spectrometry (UPLC-ESI-MS/MS) for å se etter 2-butoxyacetic acid (BAA), phenoxyacetic acid (PhAA), 3-phenoxybenzoic acid (3-PBA), 2-4-dichlorophenoxyacetic acid (2,4-D), 3,5,6-Trichloro-2-pyridinol (TCPy), cotinine, imazalil og 8-iso-Prostaglandin F2α (8-iso-PGF2α). Urinprøvene var kombinert av to stikkprøver samlet kveld og morningen etter. Konsentrasjonen av BAA, PhAA, 3-PBA, 2,4-D og TCPy var sammenlignet med andre studier tilgjengelig over hele verden, og sammenlignet med spørreskjema data relatert til vaskeprodukter og mat, gjort med Spearman's Rho test. Deteksjonsfrekvensen for stoffene analysert i dette studiet var i synkende orden som følger: PhAA(100%) >TCPy(58%) >BAA(44%)

>imazalil(35%) >3-PBA(17%) >2,4-D(9%) >cotinine(2%) >8-iso-PGF2α(0%). PhAA var detektert i 100% av prøvene, hvorav alle konsentrasjonene var over LOQ (3.0 ng/mL). PhAA hadde og høyest median (137 ng/mL eller 145.20 μg/g) sammenlignet med den andre glykol eter metabolitten og totalt. TCPy var insektmiddel metabolitten med høyest deteksjonsfrekvens (58%) og høyest median (0.27 ng/mL eller 0.31 μg/g). BAA, 3-PBA, 2,4-D, cotinine, imazalil og 8-iso-PGF2α hadde median under LOD, (15.0 ng/mL), (0.3 ng/mL), (0.2 ng/mL), (3.0 ng/mL), (0.1 ng/mL), (3.0 ng/mL), henholdsvis. Alle stoffene analysert i denne oppgaven viste lavere median sammenlignet med de andre studiene. Det var ingen signifikant korrelasjon mellom glykol eter metabolittene og spørreskjema data som omhandlet vaskemidler($p<0.05$). Signifikante korrelasjoner mellom insektmiddel metabolittene og spørreskjema data var observert som følgende: 3-PBA med ost (-0.141, $p<0.05$) og nøtter (-0.128, $p<0.05$), 2,4-D med probiotisk yogurt (0.117, $p<0.05$), ost (-0.140, $p<0.05$) og skinke (-0.125, $p<0.05$), og TCPy med hvit fisk (0.150, $p<0.05$), sjømat (0.127, $p<0.05$), hermetisk fisk (0.186, $p<0.01$) og frukt (0.167, $p<0.01$). En trend var og observert, dog ikke signifikant korrelert, for alle tre insektmiddel metabolittene med egg og sukker, 3-PBA og 2,4-D med rødt kjøtt, og TCPy med vegetabilsk olje. For glykol eter metabolittene viste PhAA en trend med fettløselig rensespray.

Contents

List of Figures

List of Tables

1 Introduction

Some of the chemicals we are exposed to on a daily basis, both persistent and non persistent, have adverse effects. Regulations for reducing or completely stop the use of persistent chemicals with adverse effects have increased the last decades, yet, the properties that many of these persistent chemicals have are still wanted. As a result, increasing numbers of non persistent chemicals are added to the market and causes exposure on a daily basis. The half life of these chemicals are normally within a day, but if we are exposed to these chemicals constantly, we might find pseudo-persistent levels in the body that can cause damage.

In this thesis we wish to study some of these non persistent chemicals in children from Norway, age 6 to 12 years. The aim is to acquire information about the levels of glycol ether and pesticide metabolites in children from Norway and compare with other studies worldwide as well as with questionnaire data. The analysis of imazalil, cotinine and 8-iso-PGF2α were also done, but are not the main focus and will not be compared with regarding other studies or the questionnaire data. The urine samples were analysed with Ultra Performance Liquid Chromatography- Tandem Mass Spectrometry and statistical analysis of the results was performed with SPSS. To this authors knowledge, it is the first documented levels of BAA, PhAA, 3-PBA, 2,4-D and TCPy in urine from children in Norway.

2 Theory

2.1 HELIX

The human early-life exposome (HELIX) is a birth cohort study done in six countries in Europe, estimating prenatal and postnatal exposure to a broad range of chemical and physical exposures. Persistent and non persistent organic chemicals, metals, pesticides, environmental tobacco smoke, water contaminants, air pollutants, noise, ultraviolet radiation, and contact with green spaces are the focus of the study[1].

HELIX have collected urine samples from cohorts as well as surveys for epidemiological purposes. The urine samples used in this project are from a subcohort collected in 2013-2016[2].

2.2 Exposure

Non persistent compounds are widely used in the environment and are found in many everyday products, e.g. plastics, lubricants, solvents, plasticizers, and pesticides. Low levels of exposure may cause endocrine or reproductive disorders[3]. Endocrine disrupting chemicals (EDCs) modify the hormonal responses and show adverse effects at relatively low doses. Metabolic processes of xenobiotic clearance are often altered for juveniles and for advancing ages. Reduced metabolic capacity might increase toxicity[4]. For example, juvenile rodents showed a heightened susceptibility to the insecticides parathion and chlorpyrifos. This was due to delayed expression of carboxylesterase enzymes that detoxify organophosphate (OP) pesticide metabolites[5;6]. Carcinogenicity

studies are usually only performed if there is any suspect for carcinogenic effect or if the chemicals are widely used, resulting in possible chronic exposure. The studies are both costly and time consuming, since it is necessary to monitor a species for the average lifetime of the species used [4].

The body of children does not work the same way as adults. Because they are small and still developing, they are more easily exposed to environmental contaminants for several reasons. Children breathe more air, drink more water, and eat more food per pound of body weight than adults in addition to having higher hand-to-mouth activity [7]. Because they are young, children have more time to develop health conditions and diseases than adults who are exposed later in life [7]. The production, release, transport, metabolism, binding, action, or elimination of endogenous hormones that maintain normal growth and development can be altered by EDCs and increase the risk of disease. Additionally, as mentioned above, the timing of exposure is also an important factor when looking at potential effects of EDCs due to unique periods of vulnerability to environmental stressors. [8]

2.2.1 Glycol ethers

Glycol ethers are oxygenated solvents, highly used in occupational and domestic products, e.g. cleaning agents, cosmetic products, drugs, paints, inks, glues and varnishes. Some adverse effects detected from glycol ethers are increased time to pregnancy, fetal malformations, lower semen quality, menstrual cycle disorder among other [9;10;11;12].

The main route of exposure are dermal and inhalation. The parent

compounds containing a primary alcohol are metabolized to alkoxycarboxylic acids through alcohol and aldehyde dehydrogenase. A small part are metabolized into alcohol and further to CO_2 via mono-oxygenase from cytochrome P450. The ones that do not contain primary alcohol are metabolised by dealkylation[13]. Qualitatively, both dermal and oral route give the same metabolites in the urine of rats[14]. The half-life of the glycol ethers are 6-80 hours[15].

Ethylene glycol ethers are created from the reaction of ethylene oxide and monoalcohols. The metabolism of 2-methoxyethanol is well known, creating the metabolite MAA which is known to be a reproductive toxicant. MAA is the main metabolite from 2-methoxyethanol found in urine from rats, in addition to glucuronide and sulphate conjugates[14]. There is a large database of information showing that it is toxic to reproduction, both to males (testicular toxicity) and females (developmental toxicity) in rats and mice[16;17;18]. Studies in France and Germany have examined urinary biomarkers of exposure to glycol ethers and revealed that the majority of the general population, including pregnant women, is exposed[10].

2.2.2 Pesticides

Organophosphate (OP) and pyrethroid (PYR) pesticides are commonly used in agriculture as well as domestic and gardening use. They eliminate insects as a result of strong potential to disrupt the brain and nervous system of these organisms. Unfortunately, this neurotoxic effect is not selective enough as to avoid damage to other non-target species, including humans[19].

Pesticide exposures are ubiquitous and can occur through dermal, pulmonary or gastrointestinal absorption, although the latter is the main exposure route for the general population[20]. Once in the human body, OP and PYR pesticides are typically metabolized and excreted in urine within 4–48 hours after exposure, depending on the compound[21].

Organophosphates include herbicides, pesticides, insecticides and nerve gas, being utilised in agriculture, pest control, plastic making, flame retardants and for several household applications. OPs are usually considered safe for agriculture having relatively fast degradation rates. Acute or chronic exposure to OPs can produce varying levels of toxicity in humans, animals, plants, and insects. These are known to inhibit acetylcholinesterase activity, not only in insects but also in aquatic and terrestrial organisms leading to respiratory, reproductive, nervous, hepatic and renal abnormalities[22]. Experimental studies have shown the ability of multiple pesticides to affect thyroid homeostasis[23;24;25;26]. Even though some evidence have shown the disruption of the hypothalamic-pituitary-thyroid axis, effects on thyroid function at current exposure levels are largely unknown[27]. Early life exposure to OPs has been also linked to adverse neurobehavioural outcomes, obesity, and asthma[8].

2.3 Toxicokinetics

Toxicology is the study of adverse effects on chemical, physical, or biological agents in living organisms and the ecosystem, including the prevention and amelioration of such adverse effects[4]. Toxicokinetics investigates how the organism affect the toxicant, and the processes

involves absorption, distribution, biotransformation/metabolism and elimination, also known as ADME. By learning how the chemicals metabolise in the body, the right compound can be analysed since it might be the metabolite that will be present in the biological samples and not the parent compound. The most important routes of entry are the gastrointestinal tract, lungs, skin, and intravenous [4].

Absorption is when the substance enters the systemic circulation in the body. Distribution is when the substance is transferred to other parts of the body. Biotransformation is when the body reacts with the parent compound, creating metabolites, usually making it more water soluble to favor excretion. Excretion is when the metabolites and/or parent compound is removed from the body. There are also different types of toxicity that needs to be considered: immediate and delayed toxicity, reversible and irreversible toxicity, and local and systemic toxicity. For a toxic effect to happen, sufficient concentration of the active form of the chemical must accumulate at the target site for a required period of time [4]. This means that the route and site of the exposure are important factors, as well as the duration and frequency. The frequency can be acute (<24 hours), subacute (<1 month), subchronic (1-3 months), and chronic (>3 months) [4].

The toxicity of glycol ethers and OP pesticides are reversible, which means that the organs can go back to normal function after eliminating the chemical. The toxic effect can be both local and systemic. The largest concern is that the frequency of the exposure is chronic, which over time can cause a delayed toxic effect. These types of toxic effects can be difficult to detect, as a lot of confounding factors make it difficult to find the source of the problem.

There are multiple names and some different estimates used to set a threshold value. Threshold of toxicological concern (TTC) is estimated such that the chance of adverse effects at exposures below TTC are considered to be low[28]. Other names for the same estimation are accetaple daily intakes (ADI), tolerable daily intakes (TDI) and reference dose (RfD)[29]. Another estimate is the minimal risk limit (MRL) which is explained to be the daily human exposure to a hazardous substance that is likely to be without appreciable risk of adverse health effects over a specified period of time, either acute (about 1 to 14 days), intermediate (from 15-364 days), or chronic (exposure for more than 364 days), cancer is not included[30]. Then there is threshold limit value (TLV) estimated to be the level to which a worker may be repeatedly exposed every day over a working lifetime without adverse health effects. Another similar to TVL is time weighted average (TWA) which estimate the same based on a 8-hour workday and a 40-hour workweek, both being more related to occupational exposure[31]. A chemical might not cause toxic effect if the chemical is excreted before for concentration reaches the range of toxic response. However, if the dosing frequency is faster than the elimination rate, then the concentration may slowly increase into the range of toxic response[4].

2.4 Compounds of interest

2.4.1 Butoxyacetic acid

Butoxyethanol is the parent compound of BAA, see Figure 2.1 for the chemical structure. It is used in cleaning products as a solvent to help dissolve grease and dirt. It is also used in nail and hair products to lower the viscosity in liquids for a smoother application. These properties are also used for industrial use in coatings and paint. The cosmetic ingredient review has concluded that butoxyethanol is safe in hair and nail products at concentrations at low levels (<10%). The U.S. occupational safety and health administration has set an exposure limit of 50 part per million (ppm) for an 8-hour workday, 40-hour workweek[32]. Thermo fisher have 20 ppm as their TWA. It is listed as being irritant to skin and eyes but not causing any developmental or reproductive effects. It is also listed as both being not carcinogenic, and for being animal carcinogenic[33]. Agency for toxic substances and disease registry have listed 0.07 mg/kg/day as a MRL when exposed up to a year[34].

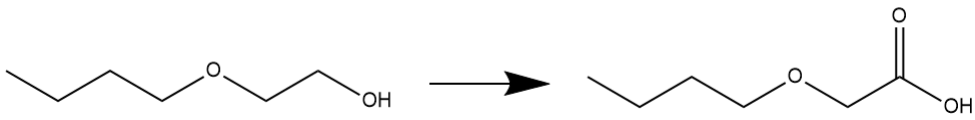

Figure 2.1 The chemical structure of butoxyethanol to the left and butoxyacetic acid (BAA) to the right.

2.4.2 Phenoxyacetic acid

Phenoxyethanol is the parent compound of PhAA, Figure 2.2 shows the chemical structure of both. It works as a stabilizer in perfumes, fragrances, soaps, and cleansers. For that reason, the name can be hidden under "fragrance" in the ingredient list[35]. In other cosmetics, it's used as an antibacterial and/or a preservative to prevent products from losing their potency or spoiling. Some glycol ethers, such as ethylene glycol ethyl ether or ethylene glycol methyl ether, have been shown to have toxic effects on reproduction and have been banned in Europe under the European regulation (EC) No. 1272/2008. However, the chemical and physical properties of phenoxyethanol differ from the glycol ethers mentioned (e.g. it is not volatile), therefore not classified as a reproductive toxicant in EC No. 1272/2008. The listed hazard statements for phenoxyethanol is eye irritation/damage (H319) and harmful if swallowed (H302)[36]. The European commission on health and food safety gives this chemical a safe rating when used in cosmetics at 1% or lower, but they also say that using several products all containing a low dose could result in overexposure[35]. Canada has given a TWA at 141 mg/m^3 for occupational exposure[37].

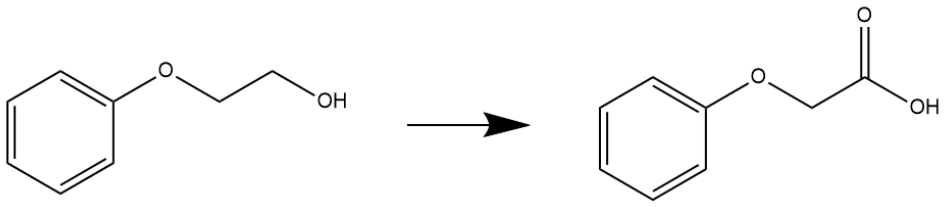

Figure 2.2 The chemical structure of phenoxyethanol to the left and phenoxyacetic acid (PhAA) to the right.

2.4.3 3-Phenoxybenzoic acid

3-PBA is a metabolite produced from several synthetic pyrethroid pesticides, cypermethrin being one of the parent compounds as shown in Figure 2.3. Synthetic pyrethroids are a class of pesticides commonly used around the world as insecticides. Pyrethroids are used in agriculture, forestry, health care settings as well as in textiles such as carpeting and clothing[38]. Household dust may be an important source of exposure, as pyrethroids have been detected in dust samples collected from homes, daycares, and other indoor environments[39]. Animal and epidemiological studies have suggested that in utero and early life exposure to various pesticides may impair neurodevelopment and cognitive-behavioral function in childhood[40;41;42]. According to EC No. 1907/2006 3-PBA causes skin irritation (H315), eye irritation (H319) and may cause respiratory irritation (H335)[43]. European food safety authority have given cypermethrin an ADI of 0.005 mg/kg body weight/day based on rat study[44].

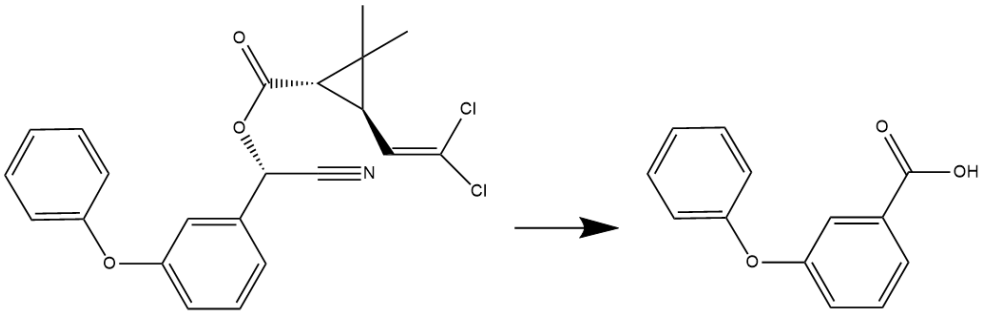

Figure 2.3 The chemical structure of cypermethrin to the left and 3-phenoxybenzoic acid to the right.

2.4.4 2,4-Dichlorophenoxyacetic acid

2,4-Dichlorophenoxyacetic acid (2,4-D) is the parent compound and the compound of interest, chemical structure shown in Figure 2.4. It has been widely used to control weeds in agriculture, forestry, and urban and residential areas. Occupational exposures to 2,4-D can occur during manufacturing and application. The general population can be exposed through food, water, dust, or residential application. The international agency for research on cancer has classified 2,4-D as probably human carcinogenic, based on evidence in humans and laboratory animals, although on limited data. There are also strong evidence that 2,4-D induces oxidative stress, and moderate evidence that 2,4-D causes immunosuppression, based on in vivo and in vitro studies[45]. Agency for toxic substances and disease registry have listed 0.2 mg/kg/day as a MRL when chronically exposed[34].

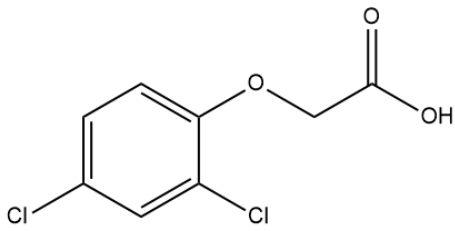

Figure 2.4 The chemical structure of 2,4-Dichlorophenoxyacetic acid.

2.4.5 3,5,6-Trichloro-2-pyridinol

Chlorpyrifos is the parent compound of TCPy, their chemical structure in Figure 2.5. It is used to control cockroaches, fleas, and termites in the house, including in some pet flea and tick collars. On the farm, it is used to control ticks on cattle and as a spray to control crop pests. Diethylphosphate (DEP) and diethylthiophosphate (DETP) are two other metabolites created from chlorpyrifos, but are unspecific which means they are also created from other pesticides[46]. The European commission withdrew all authorisations for plant protection products containing chlorpyrifos in January 2020. Experts concluded that concerns related to human health exist, especially in relation to possible genotoxicity and developmental neurotoxicity. Safe levels of exposure cannot be determined based on the available data[47]. Food and drug administration did the same in February 2022[48], with exception of food products that are not regulated by the food and drug administration.

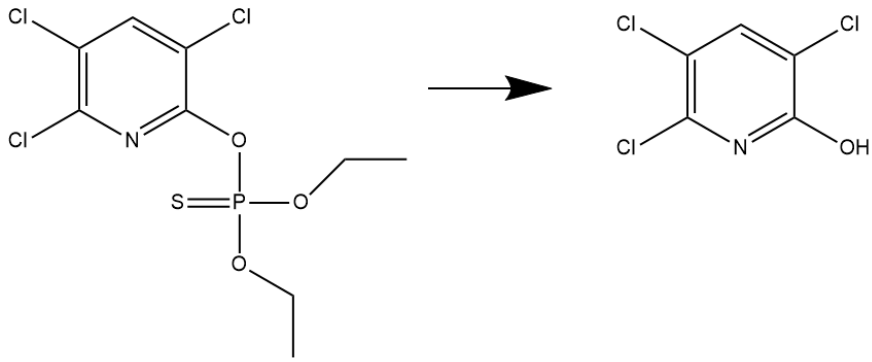

Figure 2.5 The chemical structure of chlorpyrifos to the left and 3,5,6-Trichloro-2-pyridinol to the right.

2.4.6 8-iso-Prostaglandin F2α

An increase in the oxidation of biomolecules, or "oxidative stress," is believed to be involved in the development of various pathologies, e.g., heart disease, diabetes, cancer, Alzheimer's, obesity, and many more[49]. The role of oxidative stress in disease states are widely studied, but less is known regarding oxidative stress in a healthy child[50]. Figure 2.6 show the chemical structure of 8-iso-PGF2α, a compound that has been studied as a biomarker of lipid peroxidation in over 1000 animal studies and 1000 human studies to date and has been found to correlate with a variety of diseases and exposures[51].

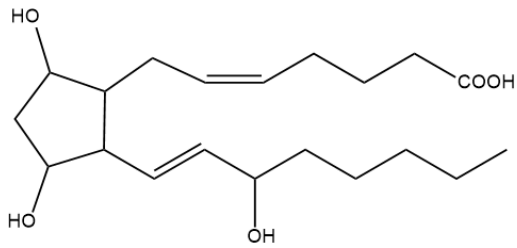

Figure 2.6 The chemical structure of 8-iso-Prostaglandin F2α.

2.4.7 Cotinine

Cotinine the metabolite formed from nicotine, their chemical structure shown in Figure 2.7. Nicotine is a chemical found in tobacco products, including cigarettes and chewing tobacco. Exposure to nicotine increases the risk for asthma, bronchitis, and pneumonia in young children[52]. The advantage of using cotinine as a biomarker of tobacco smoke and environmental tobacco smoke is it has a longer half-life (17 hours) in

comparison to nicotine (3 hours)[53]. Occupational safety and health administration has set TWA to 0.5 mg/m^3[54].

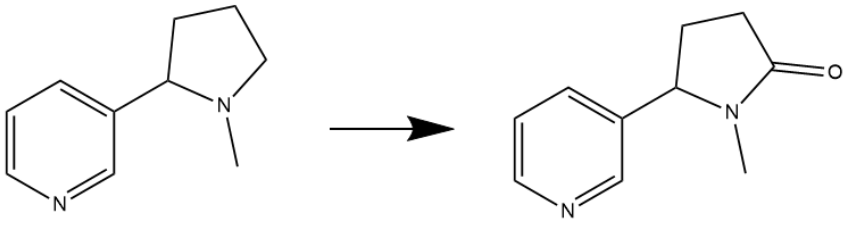

Figure 2.7 The chemical structure of nicotine to the left and cotinine to the right.

2.4.8 Imazalil

Imazalil is also known as enilconazole and is used as a fungicide for the post harvest treatment of bananas and citrus fruits, for treatment of barley and wheat seeds prior to planting, and to treat equipment and egg storage areas in chicken hatcheries. The chemical structure of imazalil is shown in Figure 2.8, and is both the parent compound and the compound of interest. Still, more than 25 metabolites have also been discovered coming from imazalil. It is categorised as harmful if inhaled (H332) or swallowed (H301) and can cause serious eye damage (H318). Additionally, likely to be carcinogenic to humans (H351)[55]. United states environmental protection agency have set 0.061 mg/kg/day based on mice studies[56].

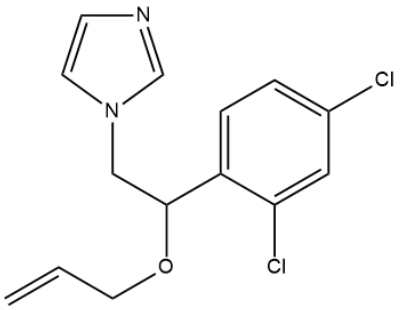

Figure 2.8 The chemical structure of imazalil.

2.5 Analysis

2.5.1 Sample preparation

Samples usually need a clean-up before analysis to remove the worst interferences while still maximising the recovery. Analysing samples with low concentrations might require a concentration step. With biological samples, it might also go through a reaction to get the analyte in the wanted form[57]. Derivatisation has been used to enhance the concentration of analytes, a method that has been used in some of the studies analysing the same metabolites as in this thesis, but with GC-MS[39;58;10;11].

Solid Phase Extraction (SPE) is commonly used in both environmental and clinical applications. It uses a solid stationary phase sorbent to retain the target, or in some cases, the interferences[57]. There are a lot of different sorbents available with different chemical properties that provide a diverse application of SPE. In online hyphenated systems like SPE-liquid chromatography (LC), low-volume cartridge or precolumn devices are commonly used[59].

A sorbent is chosen based on several factors, e.g. the sample solvent (aqueous or organic) and the analyte type (non polar, polar or ionised). If the sample is an organic solution, then polarity is the next characteristic feature to consider. If the compounds have low polarity then normal-phase sampling conditions should be considered. If the compounds have high polarity, reversed-phase sampling conditions should be considered. Ion-exchange sampling conditions is also a possibility for polar compounds if ionised, while normal-phase is also

possible for polar compounds if neutral[59]. Octadecylsiloxane- and octylsiloxane-bonded silica are sorbents abundantly used for extracting aqueous solutions and used in the application to extract non-polar and moderately polar pesticides, herbicides, food additives etc. Solvents used with this sorbent is usually methanol (MeOH) or acetonitrile (ACN). Compounds poorly extracted by octadecylsiloxane-bonded silica as a result of high water solubility can be extracted with poly(styrene-divinylbenzene) that is used on polar pesticides, herbicides, phenols and pharmaceutical compounds. Solvents used with this sorbent is usually MeOH or 2-propanol. Activated carbon is an alternative to poly(styrene-divinylbenzene), but is not as frequently used[59].

The typical protocol for analysis using SPE starts with wetting and conditioning of the sorbent. The samples are then introduced to the sorbent which retain the analytes of interest. Afterwards, a washing solvent takes the lesser retained compounds with it. Having an aqueous biological matrix with relatively non-polar analytes as an example, a washing solvent (e.g. water) that is similar to the matrix is important to not disrupt the Van der Waals interactions between the target analyte and the sorbent (e.g. C8 or C18). Lastly the analytes of interest are eluted and collected or goes directly to the analytical column. Here, the solvent must disrupt the connection to bring the analytes out. This is done by using a solvent (e.g. MeOH) that has stronger non-polar interactions with the analyte than the interactions with the sorbent. If the analytes have polar character, the sorbent (e.g. poly(styrene-divinylbenzene)) will retain the analytes with hydrophilic interactions and a non-polar solvent (e.g. ethyl acetate) will remove

interferences before eluting the analytes with a solvent (e.g. water or MeOH) that has stronger polar interaction. From this, the eluents can be analysed directly with LC-MS, or go through a concentration step[57].

Most applications for online coupled systems are for aqueous samples for the analysis of biological and environmental samples. When choosing the precolumn dimensions and sorbent properties for SPE-LC, a balance between sufficient retention and efficient analyte desorption is necessary. An ideal SPE is one that gives a proper retention of the analytes of interest such that interferences can be washed and provide elution to the analytical column with 100% recovery[59].

2.5.2 Liquid Chromatography

Liquid chromatography is a separation technique based on interactions of a sample with a stationary phase and a mobile phase in a column. Most commonly used is a solid stationary phase, often with polar groups, and an organic solvent as the mobile phase. A sample is introduced to the column, being transported through the column with the mobile phase using a pump that generates high pressure. The separation is based on adsorption with the stationary phase, and the different adsorption forces are dispersion, dipolar, acid-base, complexation and more. The time between the sample injection into the system and the elution from the column is defined as the retention time. The eluate then meets the detector which will either provide signals for all the components or a selective number[60]. LC is especially useful for non-volatile organic compounds, which cannot be directly analysed by gas chromatography

as well as it is good when huge numbers of compounds are involved, such as environmental samples[61].

2.5.3 Mass Spectrometry

The mass spectrometry (MS) instrument is used to measure the mass-to-charge ratio of ions. It contains an ionization unit, a mass/charge (m/z) separation unit and an ion detector[60]. The MS will convert the analyte compound to a charged state and analyse the ions and any fragment ions that are produced during the ionisation process[62]. Afterwards, the detector converts the ion energy to electrical signals which are transferred to a computer[57]. It can present structural information, which is used to identify compounds in addition to quantification[60].

Tandem mass spectrometry (MS/MS) refers to a mass spectrometry method where two stages of mass analysis occurs. One mode is the selected reaction monitoring (SRM). Two analysers coupled in series are set to focus on selected masses of precursor ions and product ions during a fragmentation step and the specific masses will be detected[60]. This is achieved by stepping the voltages and is useful in improving the detection of targeted analytes because more detector time can be focused to detecting specific ions instead of scanning across. Ideal combinations of precursor and product ions increase the specificity of the method by finding unique m/z levels[62]. If two or more product ion masses are selected for further detection, the mode is referred to as multiple reaction monitoring (MRM)[60].

2.5.4 Liquid Chromatography - Mass Spectrometry

It is oftentimes necessary to isolate the target analyte from a sample containing thousands of other molecules. MS alone can not do this, as multiple molecules in the sample might have the same molecular weight. It is together with LC, where the compounds first separate by their physico-chemical properties and then separate with MS by their mass-to-charge ratio, that analytes can be isolated from highly complex mixtures[57]. The coupling of LC and MS has become one of the most powerful techniques for trace quantitative analysis. This was after solving the problem with introducing the flow of liquid mobile phase from the column into the vacuum in the MS by the use of atmospheric pressure ionisation (API) interface. Electrospray ionisation (ESI) and atmospheric pressure chemical ionisation (APCI) are now the most common API techniques used for quantifying small molecules with LC-MS. The main advantage of ESI for quantitative LC-MS is the low internal energy creating protonated or deprotonated molecules, increasing the sensitivity of the method. One of the major limitations is ion suppression as a result of co-eluting analytes or co-eluting matrix components. The linear range needs to be evaluated as a mass spectrometer is only linear over a certain range of concentrations before saturation occurs either in the ion source or at the detector[57]. In ESI, liquid analyte passes through a metal capillary with high voltage (capillary voltage around 4 Kv) in a chamber heated near atmospheric pressure. A fine spray of charged droplets are produced and a warm flow of nitrogen lead the droplets to a strong electric field. Because of the vacuum, the analytes are multiple charged when hitting the analyser. ESI works with online LC-MS/MS and capillary

electrophoresis for positive and negative modes[63]. Metabolites, xenobiotics and peptides are well suited for analysis with ESI, as it works well with moderately polar compounds[62].

2.6 Statistical analysis

Using statistical programs with analytical chemistry is often necessary as it can be a lot of data to handle. There are many different types of statistical programs to use, e.g. SAS (Statistical Analysis System), primarily developed for use in agriculture, or SPSS (formally short for Statistical Package for the Social Sciences), an user-friendly software package used for statistical analysis of data. SAS and SPSS does not require any programming, and makes is easier for those without any background of programming. R and Python offer more opportunities to customise and optimise the graphs compared to SAS and SPSS which only can do minor changes. That also means that it requires more training and programming to be able to use it correctly. R and Python are also free, and does not require any licenses.

Spearman's Rho test is used to look for correlations in non parametric data sets. In order to use the Spearman's Rho test, the data set needs to be either continuous or ordinal and follow a monotonic relationship. In a monotonic relationship, when one variable increases, the other variable tends to either increase, indicating a positive relationship or decrease, indicating a negative relationship. The correlation coefficients range from -1 to 1, indicating a negative or positive monotonic relationship, respectively. Values closer to -1 or 1 show a stronger relationship than values closer to 0. A curvilinear monotonic

relationships also works with Spearsman's Rho test, in addition to the straight line[64]. A p-value is a measurement used to validate a hypothesis against observed data. With a null hypothesis stating there is no relationship, the p-value is generally set to p=0.05 used as the level for statistical significance. If the p-value of the data set is lower than 0.05, the probability of stating that the data have a relationship even if the null hypothesis is true is low enough to be stated as significantly correlated. The lower the p-value, the stronger the significance[65].

Principal component analysis (PCA) is a multivariate data analysis technique used to simplify complex data sets. PCA reduces the data by projecting it to lower dimensions called principal components (PCs), revealing a simplified structure that is often visible. The hope is to remove noise from the data set and reveal meaningful information. Data presented in a 2D-plot can be interpreted as a rotation matrix that rotates data in order to get the greatest variance along principal component 1 (PC1). PC2 account for the second most variance, then PC3 and further down until all the variance in the data set are explained. Variables that are more related will reveal clustering on the plot, and variables further away from origo will explain more of the variance than variables closer to origo[66].

2.7 Quality control

An analytical measurement must produce a result that is sufficiently accurate for the user to make appropriate decisions. Several factors increasing the quality of a method is mentioned below.

2.7.1 Retention

The retention time will depends on its partition coefficient, also called the distribution constant (KD). It is between the stationary and mobile phase, also described by the following equation[67]:

$$K_D = \frac{c_s}{c_m} \tag{2.1}$$

with c_s being the analyte concentration in the stationary phase and c_m being the analyte concentration in the mobile phase. Compounds with higher KD have more affinity toward the stationary phase. As a result, they move slower through the column and their retention time will be higher than that of compounds with lower KD. Using this knowledge, identification of compounds is done by comparing the retention time of a sample to that of an external standard analyzed under the same conditions[68]. The retention factor (k) is the degree of retention in the column, defined as the time retained by the stationary phase relative to the time in the mobile phase. If eluted too fast, the retention time might not be consistent and could also co-elute with other compounds. Too much retention will result in broad peaks[69].

2.7.2 Standards

Standards are the pure form of the compounds of interest. For the sake of quantifying concentrations of an analyte, an external calibration curve is made with known concentrations first. A range of concentrations are prepared in order to generate a calibration curve[60]. The most commonly used calibration model is least squares linear regression, which calculate an equation that best fit the data. The equation is then used to predict the concentration of the analyte. Typically, the correlation coefficient r or r^2 is used to evaluate the line fitting, where the value 1 is the best fitted line. The recommended levels of concentrations spread across the concentrations range is 5, including a blank, and the set of calibrants should be injected at the beginning and end of every run. The standard is also used to confirm the compound of interest, if the peaks have the same retention time when also analysed with the same conditions[57].

The internal standard (IS) has similar chemical properties to the analyte. When using IS, it is important that the concentration added in the sample is the same as added in the standards used to make the calibration curves[60]. IS is generally added before the cleaning process, as early as possible, to control for any loss, variations, matrix effect and ionisation, while control samples are typically analysed at regular intervals to monitor precision[57]. As the concentration of the IS is known, any changes in this concentration after detection can be adjusted for[60]. An example of a good IS is the stable isotope version of the analyte since they are almost identical but can be distinguished by MS[62]."

2.7.3 Linearity

Linearity is the proportional relationship between the concentration of the analyte and the response where results can be obtained[70]. The linearity is generally obtained by the calibration curve, revealing a regression line, expressed as shown in Equation 2.2:

$$y = mx + b \tag{2.2}$$

where y is the measured response, m is the slope of the regression line, x determines the variable and b is the y-intercept[70]. The regression line is obtained by simple least squares method where the final line is obtained when the sum of the squares, created by the vertical deviation of the data point from the line, are minimised. Many software packages will provide the correlation coefficient (R) to quantify the degree of association between two variables. The squared correlation coefficient (R^2) explains the extent to which the variance of one variable explains the variance of another[71]. R is calculated as shown in Equation 2.3:

$$R = \frac{\sum_{i=1}^{n}(x_i - x)(y_i - y)}{\sqrt{\sum_{i=1}^{n}(x_i - x)^2}\sqrt{\sum_{i=1}^{n}(y_i - y)^2}} \tag{2.3}$$

where values range between -1 and 1, and values closer to 1 or -1 show stronger correlation than values closer to 0.

Simple least squares method regard all y-values as equally important. Larger deviations at larger concentrations tend to influence the regression line more than smaller deviations in smaller concentrations, leading to inaccuracy in the lower end of the calibration range. A way

to counteract this situation is to use weighted least squares linear regression (WLSLR). WLSLR is able to reduce the lower limit of quantification (LLOQ) and enables a broader linear calibration range with higher accuracy and precision.[70]. It is also possible to force the intercept of the calibration line through (0,0), but should not be used unless there is evidence that the true intercept is not significantly different from (0,0)[57].

2.7.4 Carry-over

Carry-over is when part of the previous sample appears to be present in the next sample, and can be estimated by adding a blank injections after a sample. This can happen as a result of residues from the previous sample still being in the autosampler or in the column. This can be avoided or reduced by optimising wash solvent and wash duration.

2.7.5 Limit of detection and quantification

The limit of detection (LOD) is typically defined as the lowest concentration of an analyte that can be reliably detected by the analytical process[72]. This detection limit is commonly based on the signal-to-noise ratio (S/N) which is the ratio of the intensity of the signal relative to that of the noise, noise being the fluctuation in the instrument background signal[73]. Limit of quantification (LOQ) is defined as the lowest concentration of an analyte that is quantifiable with acceptable accuracy and precision[74].

2.7.6 Precision

Standard deviation (SD) and relative standard deviation (RSD) are the most common tools for estimating the precision of a data set, potentially display random error. SD is a measure of how precise the average is, or in other words, how the dispersion of data is from its mean[75]. Equation 2.3 show how SD is obtained:

$$SD = \sqrt{\sum_{i} \frac{(x_i - x)^2}{n - 1}} \tag{2.4}$$

where x_i refers to the individual measurements and n refers to the number of measurements. The mean is represented as x, obtained as shown in Equation 2.4.

$$x = \frac{1}{n} \sum_{i=1}^{n} x_i \tag{2.5}$$

The measurement of SD has a size depending on the size of the data set which makes it hard to compare to other data sets. That is why RSD is obtained, the calculation shown in Equation 2.5:

$$RSD(\%) = \left(\frac{SD}{x} \right) \times 100\% \tag{2.6}$$

It is used in statistics to determine a standardised measure of the standard deviation-to-mean ratio that will present how precise the average of the results is[75]. Data being more spread from the mean will give a higher RSD, while less spreading from the mean will be more

precise and therefore give a lower RSD. For this reason, RSD is often used for quality control in laboratory assays[76].

2.7.7 Selectivity

Selectivity (α) is the measure of relative retention or separation of two sample components. Column packing and mobile phase composition are two important factors when looking at selectivity[77].

2.7.8 Column efficiency

Column efficiency (N) regard the separation and measures the dispersion of the analyte band trough the column. Some important parameters are flow rate, substrate particle size and column dimensions[78].

2.7.9 Resolution

Resolution (R_s) is the degree of separation between to compounds, determined by the column efficiency and selectivity. Co-elution and ion suppression are consequences of insufficient resolution[79].

2.7.10 Recovery

During the analytical process from sample preparation to analysis, loss of analyte can occur, and recovery is therefore an important part of validation of all analytical methods. Recovery is the ratio between the concentration detected and the amount from the original sample[80]. Certified reference materials are one way to determine recovery, but if that is unavailable, the recovery can be estimated by adding a

compound regarded as a surrogate for the analyte. The best type of surrogate is an isotopically-modified version of the analyte. Another very commonly applied method is spiking. A matrix blank or a matrix matching the sample can be spiked with the analyte and the recovery can then be determined after applied to the analytical procedure used. Internal standard is also a possible surrogate to determine recovery. This chemical will not have identical chemical properties to the analyte, but is often used when numerous analytes are determined in the same matrix. Even though the internal standard is not identical to the analyte, it is selected to be close, thus representing their chemical behaviour [80].

2.7.11 Matrix

Matrix-matching is preferred if possible, to ensure that all standards, quality control samples and test samples have the same matrix for a constant ion suppression.

2.7.12 Product ion

Choosing the best product ion might not always be easy, as it does not have to be the most abundant fragment. Other fragments might have a cleaner chromatogram with better signal-to-noise ratio. It is also recommended to monitor a second fragment ion to confirm the identification and check for possible interferences [62].

2.7.13 Error

Errors most often arise from random factors like operator mistakes, malfunction of instrument or other equipment, contamination of samples or calibration standards or calculations[57]. Error is the difference between the experimentally obtained value and the true value. The goal is to minimize the error as much as possible, however there are different types of error.

Gross error is the human error. This type of error can be obvious, but before removing a value, one should always statistically justify removing the value from the data set. If so, that data point will be considered as an outlier. An outlier might not always be wrong, rather an important value. In epidemiological studies, values are not necessarily normally distributed or showing a relationship, so looking for outliers are not as relevant. Systematic error is when the error from the true value is constant and is usually chemical or/and instrumental. The error can come from wrongly calibrated instrument or error in sample preparation. Random error are unpredictable high or low fluctuations in the measurement of physical properties[60]. One way to avoid error is to carry out daily instrument checks prior to using the instrument. Also inspect chromatograms visually during a run to check for acceptable separation, peak shape, etc[57].

2.7.14 Data processing

Data processing is an important step in generating quantitative data. Automated algorithms for integrating peaks are in almost all modern software packages, but it is important to still inspect every chromatogram

to ensure correctly drawn baselines. It is also important to see if the correct peak has been chosen and that the integration is consistent. After this process is completed for the standards, the ratio between the analyte and IS is used to create the calibration line and quantify the QC samples and the unknowns[57].

3 Method

3.1 Sampling

The data used in this thesis came from the HELIX project, drawn from the general population in Norway. Children were recruited and urine samples collected between the age of 6 and 12 years. The assessments of children were conducted between December 2013 and February 2016 [2]. A total of 286 urine samples are included in this study, all children from Norway. The urine samples analysed were a pool of equal amounts of two spot urine samples collected at bedtime and the morning after. A few exceptions occurred where only morning or night was collected. The urine samples were collected in CE certified polypropylene containers and have been stored in -80°C after the original project finished. When starting the thesis, the samples were moved to -20°C until analysis.

3.2 Sample preparation

3.2.1 Calibration standards

Standards from Cambridge Isotope Laboratories were already dissolved in ACN with exact concentrations. Other standards were weighed, and exact concentrations were prepared by dissolving in ACN. All the standards were combined together and working standards were prepared in the following concentration range: (a) 0.8-3200 ng/mL for pesticide metabolites (TCPy, 3-PBA and 2,4-D) and cotinine, (b) 2.4-9600 ng/mL for glycol ether metabolites (BAA and PhAA). These

working standards were further used in the sample preparation to get calibration curves in the following range: (a) 0.04-2400 ng/mL for pesticide metabolites (TCPy, 3-PBA and 2,4-D) and cotinine, (b) 0.12-7200 ng/mL for glycol ether metabolites (BAA and PhAA). The internal standard solution was prepared by combining different internal standard stock solutions. Calibration standards were prepared in water with the following procedure: To 300 µL water, standard, internal standards, buffer and formic acid were added. The calibrated standards were not incubated. Table 3.1 show the standards and other materials used in this method, including the supplier.

Table 3.1 Purchased chemicals and materials for determination of glycol ether pesticide metabolites, cotinine and 8-iso-PGF2α in urine.

Materials	Supplier
3,5,6-Trichloro-2-pyridinol (TCPY) unlabelled and labelled	CIL (Cambridge Isotope Laboratories) Inc, Andover, MA, USA.
3-Phenoxybenzoic acid (3-PBA) unlabelled and labelled	CIL (Cambridge Isotope Laboratories) Inc, Andover, MA, USA.
2,4-Dichlorophenoxyacid (2,4-D) unlabelled and labelled	CIL (Cambridge Isotope Laboratories) Inc, Andover, MA, USA.
Cotinine unlabelled and labelled	CIL (Cambridge Isotope Laboratories) Inc, Andover, MA, USA.
Methoxyethoxyacetic acid (MEAA) 2-2 unlabelled	TCI (Tokyo Chemical Industry), Tokyo, Japan.
Methoxyacetic acid (MAA) unlabelled and labelled	TRC (Toronto Research Chemicals), Toronto, Canada.
Ethoxyacetic acid (EAA) unlabelled	TCI (Tokyo Chemical Industry), Tokyo, Japan.
2-methoxyproprionic acid (2-MPA) unlabelled	TRC (Toronto Research Chemicals), Toronto, Canada.
2-butoxyacetic acid (BAA) unlabelled	TRC (Toronto Research Chemicals), Toronto, Canada, while C13 was from CDN Isotopes, Quebec, Canada.
Creatinine unlabelled and labelled	TRC (Toronto Research Chemicals), Toronto, Canada.
Ethoxyethoxyacetic acid (EEAA) unlabelled	QMX Laboratories, Essex, UK.
Phenoxyacetic acid (PhAA) unlabelled	QMX Laboratories, Essex, UK, while labelled was from TRC (Toronto Research Chemicals), Toronto, Canada.
n-propoxyacetic acid (PrAA) unlabelled	QMX Laboratories, Essex, UK.
8-iso Prostaglandin F2α unlabelled and labelled	Cayman Chemical Company, Ann Arbor, MI, USA.
SPE: Hypercarb guard column	Thermo Scientific ,San Jose, CA, USA
Analytical column Acquity Premier HSS T3 1.8 µm, 2.1 x 100 mm	Waters Corporation Milford, MA, USA.
MeOH/ACN/Water lcms grade	J.T. Baker, Gliwice, Poland
Formic acid 98%	VWR Chemicals, EC
β-Glucuronidase	Helix pomatia from Sigma Aldrich St. Luis, Mo, USA
Ammoniumacetate powder of >99% purity	Sigma–Aldrich (St. Louis, MO, USA)

3.2.2 Urine samples

The biological samples are regarded as hazardous, so when working with the samples, it was done in the contamination lab with protective clothing including disposable lab coat and disposable gloves. Preparation of the biological samples was performed in contamination fume hoods, and when taken out, the bottoms were wiped with surface disinfectant before leaving them on any other surface. All equipment that had been in contact with the biological material were discarded in specific containers.

For the sample preparation of the urine samples the following procedure was done: 300 μL of urine was added 75 μL of ACN, 75 μL of 20% MeOH in water, 75 μL of enzyme and 45 μL of IS. The enzyme mix was made by 110 mg enzyme (βL-Glucuronidase) with 5.5 mL 1M ammonium acetate pH 5, and rested for 1 hour. The samples were mixed and then incubated at 37°C for 24 hours. Two in-house control samples (not spiked) and two spiked blanks were prepared together with the urine samples, included in each run. After incubation, the samples were centrifuged for 10 minutes at 14 000 rpm and the supernatant was transferred to sample glass vials. 30 μL of 100% formic acid was added to the sample, and then stored in -20°C until analysis.

3.3 LC-MS analysis

The analysis was performed with on-line column switching UPLC-ESI-MS/MS. The online SPE was a Hypercarb guard column with 100% porous graphitic carbon (Thermo Scientific ,San Jose, CA, USA). The analytical column used was C18 Acquity Premier HSS T3 1.8 µm, 2.1 x 100 mm (Waters Corporation Milford, MA, USA.). The samples were injected on to the SPE column using a quarternary pump (loading pump). The initial setting for the quarternary pump was 100% water and the flow rate was 0.5 mL/min. The sample was washed for 2 min and eluted with a binary (separation pump). The initial setting for the binary was mobile phase A (95%, 0.1% formic acid in water) and mobile phase B (5%, 100% ACN). The flow rate was 0.3 mL/min. A gradient was run between mobile phase A and mobile phase B to elute the analytes from the SPE into the analytical column and further to the mass spectrometer (method article under preparation). The Xevo TQ-XS MS Detector was on ES- on every compound except imazalil that was on ES+. The source temperature was 150°C, desolvation temperature was 500°C, cone gas was 170 L/hour and desolvation gas was 700 L/hour.

The instrument was checked before each run, writing down the pressure of the SPE catridge and the of the analytical column. After a few samples were analysed, the chromatograms were inspected visually to check for acceptable separation and other errors. The calibration standards were analysed in the beginning and end of every run.

3.4 Data treatment

Each chromatogram was visually inspected to make sure the software program integrated the right peak and that the integration was consistent. The limits of detection was estimated by a signal to noise ratio of 3. The limits of quantification was estimated by a signal to noise ratio of 10. Weighted least squares linear regression was used, and all calibration curves had R >0,98 except one for cotinine, all shown in Appendix 6.2. The method is fully validated, recovery and precision (relative standard deviation) were 80-120% and <20% , respectively for all the compounds (method manuscript under preparation). Recovery was calculated from spiking analytes in urine samples.

4 Results and discussion

4.1 Determination of concentrations

Urine samples from children in Norway (n=286) were analysed and concentrations of the following compounds were detected: BAA, PhAA, 3-PBA, 2,4-D, TCPy, imazalil, cotinine and 8-iso-PGF2α. The detection frequency (N>LOD(%)), mean, median, minimum (min), maximum (max), standard deviation, LOD, and LOQ are given for the compounds presented in Table 4.1. All concentrations below LOD are replaced with LOD/2, marked with "^{a}". The median concentrations were lower than the mean, probably as a result of a few high values increasing the concentration of the mean. Values from biomonitoring studies are not necessarily normally distributed, which is why the median might give a better picture of the concentrations relative to the mean. The detection frequency from this study were found to be with decreasing order as follows: PhAA(100%) >TCPy(58%) >BAA(44%) >imazalil(35%) >3-PBA(17%) >2,4-D(9%) >cotinine(2%) >8-iso-PGF2α(0%). The highest median concentration was observed for PhAA (137.00 ng/mL or 145.20 μg/g) and was also observed in all samples (n=286) above LOQ (3.0 ng/mL). Creatinine adjusted values are shown in Table 4.2.

Table 4.1 Concentrations (ng/mL) of metabolites in urine from children in Norway (n=286). DF=detection frequency, LOD=limit of detection, LOQ=limit of quantification. [a]Number is estimated as a result of being below LOD.

Compound	DF>LOD (%)	Mean (ng/mL)	Median (ng/mL)	Min (ng/mL)	Max (ng/mL)	LOD (ng/mL)	LOQ (ng/mL)
BAA	44	22.75	7.50[a]	7.50[a]	249.87	15.0	45.0
PhAA	100	424.50	137.00	14.27	6682.00	0.6	3.0
3-PBA	17	0.30	0.15[a]	0.15[a]	5.12	0.3	1.0
2,4-D	9	0.14	0.10[a]	0.10[a]	1.89	0.2	0.6
TCPy	58	1.04	0.27	0.10[a]	26.44	0.2	0.6
8-iso-PGF2α	0	1.50[a]	1.50[a]	1.50[a]	1.50[a]	3.0	10.0
Cotinine	2	1.78	1.50[a]	1.50[a]	31.77	3.0	10.0
Imazalil	35	0.19	0.05[a]	0.05[a]	5.34	0.1	0.3

Table 4.2 Creatinine adjusted concentrations (μg/g) of metabolites in urine from children in Norway (n=286). DF=detection frequency, LOD=limit of detection, LOQ=limit of quantification.

Compound	DF>LOD (%)	Mean (μg/g)	Median (μg/g)	Min (μg/g)	Max (μg/g)	LOD (ng/mL)	LOQ (ng/mL)
BAA	44	24.89	12.62	3.20	311.97	15.0	45.0
PhAA	100	487.49	145.20	11.98	13073.78	0.6	3.0
3-PBA	17	0.32	0.18	0.06	4.54	0.3	1.0
2,4-D	9	0.16	0.11	0.04	2.58	0.2	0.6
TCPy	58	1.05	0.31	0.04	18.93	0.2	0.6
8-iso-PGF2α	0	1.74	1.59	0.64	5.98	3.0	10.0
Cotinine	2	2.06	1.61	0.64	53.09	3.0	10.0
Imazalil	35	0.18	0.07	0.02	4.10	0.1	0.3

BAA was detected in 44% of the samples. LOD and LOQ ended up being higher compared to the other compounds, as a result of lower signal-to-noise ratio. TCPy was the pesticide metabolite with the highest detection frequency (58%) and the highest median (0.27 ng/mL or 0.31 μg/g). As the EU regulation did not stop the authorisation of chlorpyrifos until 2020, it is not surprising to see levels of TCPy as the samples are from 2013-2016. Nevertheless, experts have concluded concerns related to human health, and no safe levels of exposure can be determined based on the available data[47]. It would be interesting to look at urine samples collected after 2020 to see if the regulations have affected the concentrations detected. We would not expect to find many samples with levels of 8-iso-PGF2α causing oxidative stress. 8-iso-PGF2α was detected in a few samples, but as shown in Table 1, non of them were above LOD. The same assumption were for cotinine, yet a few samples were above LOD. One possible reason for the few detected levels of cotinine is passive smoking.

Concentrations of the in-house control samples are shown in Table 4.3., where PhAA had the lowest RSD. 3-PBA, 8-iso-PGF2α and imazalil were not detected in the in-house control samples. BAA, 2,4-D, TCPy and cotinine had their mean below their LOQ which might explain why their RSD was higher than that of PhAA. The spiked blanks were below the detection limits for all compounds except BAA, as shown in Table 4.4.

Table 4.3 Concentrations of non-spiked in-house control samples (ng/mL). 3-PBA, 8-iso-PGF2α and imazalil were not detected. SD=standard deviation, RSD=relative standard deviation.

Run	BAA (LOQ=45.0)	PhAA (LOQ=3.0)	2,4-D (LOQ=0.6)	TCPy (LOQ=0.6)	Cotinine (LOQ=10.0)
Run 1 K1	16.39	2341.30	0.25	0.35	1143.07
Run 1 K2	10.71	2212.06	0.26	-	1121.56
Run 2 K1	25.45	2561.87	0.24	0.30	1239.83
Run 2 K2	30.66	2584.95	0.20	0.22	1016.27
Run 3 K1	26.04	2582.02	-	0.67	1959.69
Run 3 K2	49.73	2618.40	0.20	-	1288.84
Run 4 K1	16.29	2328.16	0.22	0.35	857.21
Run 4 K2	-	2318.59	-	0.64	608.57
Run 5 K1	18.24	2265.58	-	0.15	1166.28
Run 5 K2	19.61	2226.96	-	0.17	512.29
Run 6 K1	16.43	2210.48	0.13	0.36	783.59
Run 6 K2	24.08	2051.75	0.15	-	695.42
Mean	23.05	2358.51	0.20	0.36	1032.30
SD (%)	10.52	184.97	0.05	0.19	388.57
RSD (%)	45.62	7.84	22.79	51.91	37.64

Table 4.4 Concentrations of spiked blanks (ng/mL). SD=standard deviation, RSD=relative standard deviation.

Run	BAA	PhAA	3-PBA	2,4-D	TCPy	8-iso-PG	Cotinine	Imazalil
Run 1 IS 1	4.68							
Run 1 IS 2	9.06							
Run 2 IS 1								
Run 2 IS 2	5.70							
Run 3 IS 1	4.46							
Run 3 IS 2								
Run 4 IS 1								
Run 4 IS 2	19.68							
Run 5 IS 1		0.55						
Run 5 IS 2								
Run 6 IS 1		0.15						
Run 6 IS 2	16.40							
Mean	10.00							
SD (%)	6.53							
RSD (%)	65.29							

4.2 Comparison to other studies

There are a few studies who have looked at the same metabolites in children, shown in Table 4.5.

Table 4.5 Comparing concentrations (ng/mL) of metabolites in urine with other studies. ^{c}SG corrected (ng/mL). ^{d}Creatinine adjusted(μg/g). DF = Detection frequency. N = total participants.

Study	Sampling year(s)	Country	N	Age (years)	Compound	DF(%)	Median	creatinine adjusted	LOD (ng/mL)
Garlantézec et.al.[81]	2009-2012	France	110	6	BAA	96	14	-	3.0
			110	6	PhAA	100	141	-	3.0
Werthmann et.al.[82]	2014-2015	USA	68	7-12	3-PBA	95	0.49	-	0.1
Calafat et.al.[83]	2013	USA	122	3-5	3-PBA	71	0.46	0.9^{d}	0.1
			122	3-5	2,4-D	62	0.21	0.5^{d}	0.15
Song et.al.[84]	2018-2019	China	139	0-7	2,4-D	100	0.12	0.13^{c}	0.01
Calafat et.al.[83]	2013	USA	122	3-5	TCPy	89	0.9	2.0^{d}	0.1
Morgan et.al.[39]	2000-2001	USA	129	1-5	TCPy	100	5.3	7.3^{d}	2.0

The french study by Garlantézec et al.[81] looked at PhAA and BAA among others in urine from children. Table 4.5 shows that the detection frequency is 100%, same as our study. BAA had however higher detection frequency (96%) compared to our study (44%). This could be the result of their LOD (3.0 ng/mL), possibly including lower levels of BAA that our study were not able to detect as a result of higher LOD (15 ng/mL). The median of PhAA and BAA from the french study was 141 ng/mL and 14 ng/mL, respectively. In comparison, our study found 137.00 ng/mL of PhAA and 7.50 ng/mL of BAA. However, the median of BAA was estimated as a result of being below LOD. Calafat et al.[83] and Werthmann et al.[82] had detection frequency of 3-PBA at 71% and 95%, respectively. Both frequencies higher compared to our study (17%). As

with BAA, the median of 3-PBA was estimated as a result of being below LOD (0.15 ng/mL). In comparison, Calafat et al.[83] and Werthmann et al.[82] had higher median of 3-PBA, 0.46 ng/mL and 0.49 ng/mL, respectively.

Calafat et al.[83] and Song et al.[84] showed detection frequency of 2,4-D to be 62% and 100%, respectively. In comparison, our study had a detection frequency of 9% for 2,4-D, the lowest detection frequency of the pesticides. The median of 2,4-D in our study was 0.1 ng/mL, an estimated value as a result of being under LOD, whereas Calafat et al.[83] got 0.21 ng/mL and Song et al.[84] got 0.12 ng/mL. Morgan et al.[39] and Calafat et al.[83] had detection frequency of TCPy of 100% and 89%, respectively, higher than obtained in our study (58%). The median of TCPy in our study (0.27 ng/mL) was lower compared to both Morgan et al.[39](5.3 ng/mL) and Calafat et al.[83](0.9 ng/mL).

4.3 Comparison to questionnaire data

Questionnaire data related to expected sources for the compounds in the thesis were compared with the concentrations found in the samples. Using SPSS, the comparisons were done with Spearman's Rho test ($p<0.05$ and $p<0.01$). Answers of the questions used in the comparisons are under Appendix 6.2, Table 6.1 showing answers related to the use of cleaning products and Table 6.2 showing answers related to food. PCA was also done, but since PC1 and PC2 did not explain much of the variance for either of the comparisons, the plots were added in Appendix 6.4. Correlation and clustering between the questionnaire data themselves were not discussed, as the focus was to

look for any correlation and clustering between the questionnaire data and the compounds. Comparisons between the glycol ether metabolites and questionnaire data regarding use of cleaning products are shown in Table 4.6. There were no significant correlation, either at $p<0.01$ or $p<0.05$. Figure 6.9 in Appendix 6.4 shows the PCA plot of BAA, PhAA, and the questions regarding the use of cleaning products. PC1 explain 24% of the variance and PC2 explain 11% of the variance. Neither BAA nor PhAA were clustered with any of the cleaning products, which could strengthen the findings from Table 4.6 which revealed no significant correlation. Both BAA and PhAA are also close to origo, meaning they did not contribute a lot to the explanation of the variance in PC1 or PC2 compared to the other factors.

Table 4.6 Correlation coefficients from comparisons between the glycol ether metabolites (BAA and PhAA) and questionnaire data regarding use of cleaning products with Spearman's Rho test.*-Correlation is significant at the 0.05 level (2-tailed).**-Correlation is significant at the 0.01 level (2-tailed).

Product	BAA	PhAA
BAA	1.000	0.060
PhAA	0.060	1.000
Bleach	-0.061	-0.057
Ammonia	-0.068	-0.054
Solvents/ spot removers	-0.043	0.068
Polishes/ waxes	-0.062	-0.036
Floor cleaning liquids	0.010	0.031
Electronic air fresheners	-0.012	-0.048
Perfumed cleaning products	0.029	-0.060
Furniture sprays	0.051	-0.034
Multipurpose sprays	0.036	0.018
Degreasing sprays	-0.097	0.075
Glass cleaning sprays	-0.014	0.037
Air refreshing sprays	0.072	0.035
Other sprays	0.003	0.001
How often is your child present while cleaning	-0.020	0.012

Comparisons between the pesticide metabolites and questionnaire data regarding food are shown in Table 4.7. The Spearmans' Rho test revealed significant correlations between the following: 3-PBA with cheese (-0.141, $p<0.05$) and nuts (-0.128, $p<0.05$), 2,4-D with pro-biotic yogurt (0.117, $p<0.05$), cheese (-0.140, $p<0.05$) and ham (-0.125, $p<0.05$), and TCPy with white fish (0.150, $p<0.05$), seafood (0.127, $p<0.05$), canned fish (0.186, $p<0.01$) and fruits (0.167, $p<0.01$). In addition, 3-PBA was significantly correlated to both 2,4-D (0.493, $p<0.01$) and TCPy (0.188, $p<0.01$), as well as 2,4-D was significantly correlated to TCPy (0.165, $p<0.01$). It was not the main focus to look for potential correlations between the pesticide metabolites themselves, but the findings were noteworthy to mention. Figure 6.10 and Figure 6.11 in Appendix 6.4. show the PCA plot of the pesticide metabolites with the questionnaire data. In this plot, PC1 explain 8% of the variation and PC2 explain 7% of the variation. The total explanation of PC1 and PC2 are lower compared to the PCA plot of the glycol ether metabolites. As with the glycol ether metabolites, the pesticide metabolites are close to origo, meaning they do not contribute a lot to the explanation of the variance in PC1 and PC2 compared to some of the questionnaire data. Considering the significant correlations from Table 4.7, the negative correlation between 3-PBA and nuts along PC2 is the most visible in the plot. There is also 85% of the variance in the data set that is not explained, which is why this plot might not strengthen the findings in Table 4.7. Another important aspect that should be taken into consideration is the detection frequency of the pesticide metabolites. While TCPy are detected in 58% of the samples, 3-PBA and 2,4-D were detected in 17% and 9%, respectively. The

median of 3-PBA and 2,4-D were also estimated as a result of having levels below LOD. That creates uncertainty and should be taken into account when looking at the results. Further studies should to be done in order to potentially strengthen the findings.

Table 4.7 Correlation coefficients from comparisons between the pesticide metabolites (3-PBA, 2,4-D and TCPy) and questionnaire data regarding food with Spearman's Rho test.*-Correlation is significant at the 0.05 level (2-tailed).**-Correlation is significant at the 0.01 level (2-tailed).

Product	3-PBA	2,4-D	TCPy
3-BPA	1.000	0.493**	0.188**
2,4-D	0.493**	1.000	0.165**
TCPy	0.188**	0.165**	1.000
Milk	0.089	0.065	0.006
Yogurt	0.056	0.027	-0.014
Pro-biotic yogurt	0.041	0.117*	0.090
Cheese	-0.141*	-0.140*	-0.051
Egg	-0.007	-0.095	0.064
Poultry	0.014	-0.005	-0.059
Red meat	0.016	-0.050	-0.022
Cold meat	-0.023	0.011	-0.034
Ham	0.040	-0.125*	0.027
White fish	0.093	0.014	0.150*
Fatty/ oily fish	-0.064	-0.046	0.025
Canned fish	0.010	-0.020	0.186**

Continued on next page

Table 4.7 *(Continued)* Caption for the multi-page table

Product	3-PBA	2,4-D	TCPy
Seafood	-0.053	-0.104	0.127*
Dairy dessert	-0.046	-0.070	-0.046
Raw vegetables	0.025	0.082	0.049
Cooked vegetables	0.016	0.059	0.103
Potatoes	0.103	0.071	0.015
Pulses	-0.026	0.017	0.022
French fries	0.073	-0.058	0.003
Fruits	0.099	0.075	0.167**
Fresh juice	-0.037	0.032	-0.006
Nuts	-0.128*	-0.089	0.074
Canned fruit	0.075	0.026	0.028
Dried fruit	-0.030	0.014	0.019
White bread	0.007 -	0.017	-0.057
Dark bread	0.027	-0.016	-0.005
Sugar-sweetened cereal	-0.081	-0.065	-0.036
Other breakfast cereal	0.064	0.069	0.103
Rice and pasta	0.003	-0.029	0.016
Crispy bread/ corn cakes	-0.081	0.021	-0.037
Plain biscuits and cookies	0.021	-0.057	-0.024
Pastries	-0.011	-0.062	-0.026
Chocolate	-0.057	-0.027	-0.087
Sugar/honey/jam	0.040	-0.042	0.086
Sweets	-0.072	-0.104	0.021

Continued on next page

Table 4.7 *(Continued)* Caption for the multi-page table

Product	3-PBA	2,4-D	TCPy
Soda	0.020	-0.070	-0.031
Diet soda	-0.008	-0.085	-0.102
Olive oil	-0.115	-0.013	0.030
Vegetable oil	-0.088	-0.054	0.016
Butter	-0.080	-0.090	-0.044
Margarine	0.020	0.012	0.004
Dressing/ketchup/ mayonnaise	0.025	-0.026	0.063
Salt snacks	-0.041	-0.060	0.050

As chlorpyrifos creates the metabolites DEP and DETP in addition to TCPy, concentrations of DEP and DETP from an earlier study done on the samples were compared to the concentrations of TCPy. The Spearman's Rho test showed significant correlation between TCPy and both DEP (0.260, $p<0.01$) and DETP (0.306, $p<0.01$). As TCPy is the specific metabolite of chlorpyrifos, the prediction was that DEP and DETP should also be detected. This would not work the other way around as DEP and DETP are metabolites from other parent compounds as well as chlorpyrifos.

In addition to the significant correlations revealed, some trends were visible even though not significantly correlated. The median of the three pesticide metabolites against the question related to the amount of egg eaten are shown in Figure 4.1.

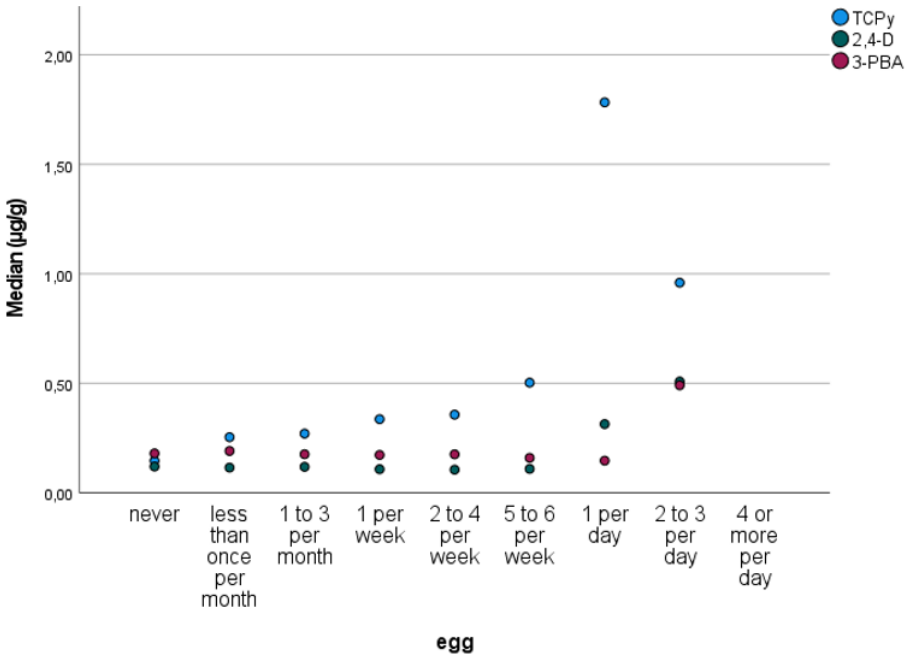

Figure 4.1 Median (creatinine adjusted) of TCPy (blue), 2,4-D (green) and 3-PBA (red) correlated with the amounts of egg eaten. The x-axis show the answer alternatives from the questionnaire, an overview of how many answered the alternatives are shown in Table 6.2.

Looking at Figure 4.2, the levels of TCPy were more stable compared to 2,4-D and 3-PBA which showed a trend when increasing the amount of red meat eaten.

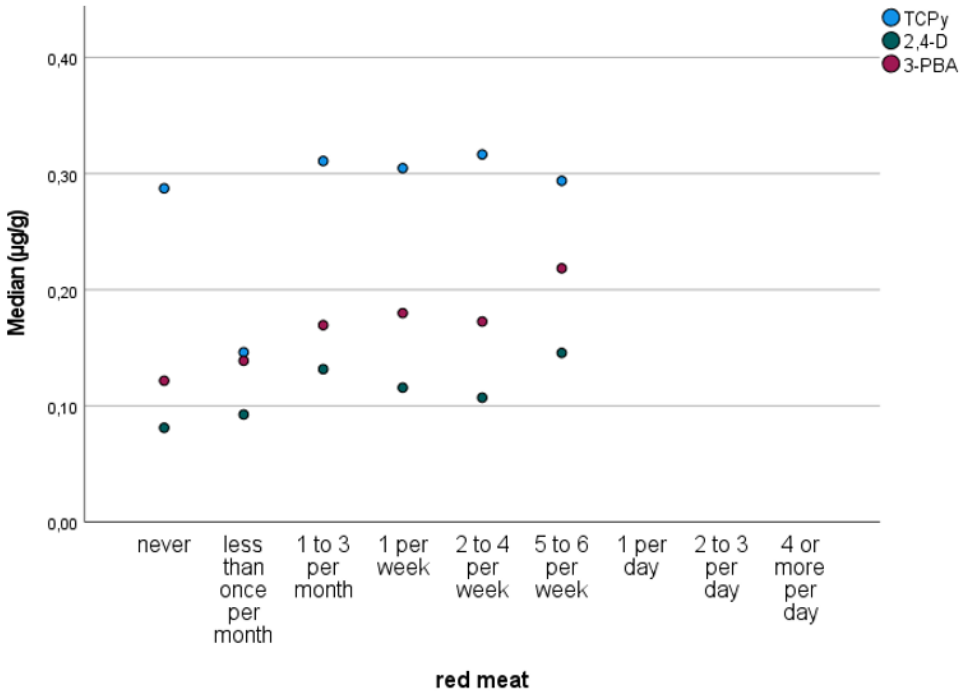

Figure 4.2 Median (creatinine adjusted) of TCPy (blue), 2,4-D (green) and 3-PBA (red) correlated with the amounts of red meat eaten. The x-axis show the answer alternatives from the questionnaire, an overview of how many answered the alternatives are shown in Table 6.2.

Figure 4.3 shows the comparison between the pesticide metabolites and sugar eaten (meaning sugar, honey and jam). Here, TCPy show more of a trend compared to 3-PBA and 2,4-D. The metabolites might be related to all three sources which is referred to in this question.

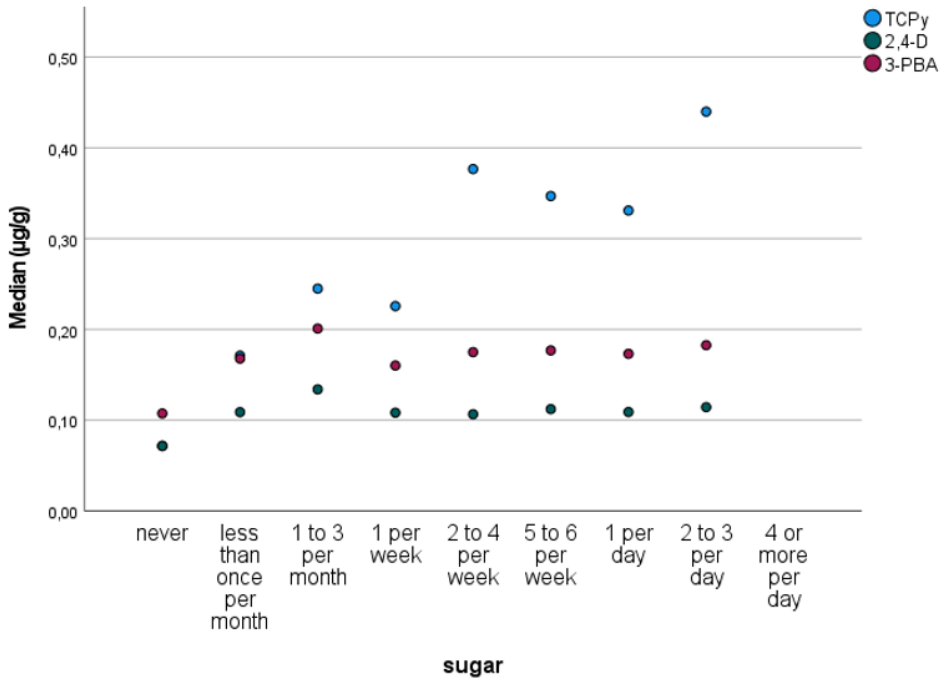

Figure 4.3 Median (creatinine adjusted) of TCPy (blue), 2,4-D (green) and 3-PBA (red) correlated with the amounts of sugar eaten. Sugar represents the amount of sugar, honey and jam eaten. The x-axis show the answer alternatives from the questionnaire, an overview of how many answered the alternatives are shown in Table 6.2.

The comparison between the pesticide metabolites and use of vegetable oil are shown in Figure 4.4. As with the sugar, TCPy is showing more of a trend.

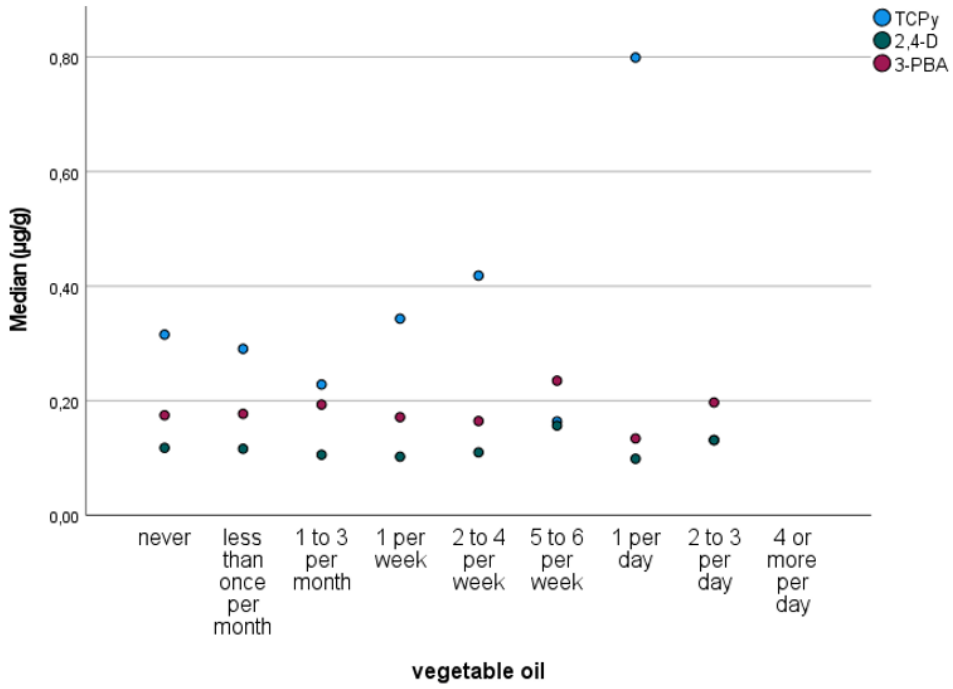

Figure 4.4 Median (creatinine adjusted) of TCPy (blue), 2,4-D (green) and 3-PBA (red) correlated with the amounts of vegetable oil eaten. The x-axis show the answer alternatives from the questionnaire, an overview of how many answered the alternatives are shown in Table 6.2.

There was only one graph (Figure 4.5) showing a trend between concentrations of glycol ether metabolites and the use of cleaning products, and that was the use of degreasing sprays. The trend was stronger for PhAA compared to BAA. It would be interesting to see if there would be any trend or correlation between the use of liquid soap, face cream and hand cream with the glycol ether metabolites, as children may be more exposed to the glycol ethers that way compared to cleaning products.

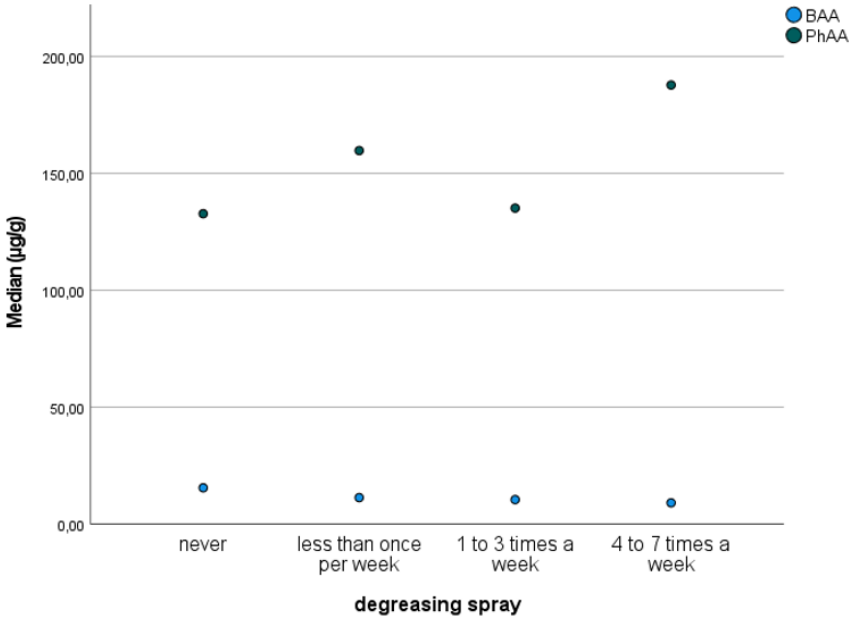

Figure 4.5 Median (creatinine adjusted) of PhAA (green) and BAA (blue) correlated with the amounts of degreasing sprays used. The x-axis show the answer alternatives from the questionnaire, an overview of how many answered the alternatives are shown in Table 6.1.

4.4 Method performance

The method used for this project did not work for all compounds that were initially added. In the end, BAA and PhAA were the glycol ether metabolites included, while methoxyacetic acid (MAA), methoxyethoxyacetic acid (MEAA), n-propoxyacetic acid (PrAA), ethoxyacetic acid (EAA), ethoxyethoxyacetic acid (EEAA) and 2-methoxyproprionic acid (2-MPA) were eliminated. For the pesticide metabolites, 3-PBA, 2,4-D and TCPY were included while glyphosate (GLY) and aminomethylphosphonic acid (AMPA) were eliminated. For the fungicides, imazalil was included while boscalid was eliminated. For the oxidative stress biomarkers, 8-iso-PGF2α was included while 8-hydroxy-2'-deoxyguanosine (8-OH-DG) was eliminated. The reasons for eliminating the mentioned compounds were poor retention, poor ionisation in MS and difficulty in finding an adequate product ion. Very polar molecules are not preferred with either GC or LC. Glycol ether metabolites have been analysed by GC-MS as well [10;81;11;12;9;39], though the samples are then often derivatised as well before analysis.

The ideal situation is to use urine when creating the calibration curves to achieve matrix-match. Yet, urine is complex, and there are compounds in the urine that could affect the results as not every compound need incubation. Even the compounds that needs incubation could also have some concentration in free form which affects the calibration curve. This was noticed early in PhAA, and the calibration curve for this compound was made with water as matrix from the beginning. While analysing the samples, the question of whether water could be a better matrix for the other compounds was raised as well, and so two runs were analysed with

both water and urine as matrix. After careful consideration, water was chosen for the matrix in the calibration curve for all compounds. The mean was calculated from the slope and intercept gathered from the two runs using water as matrix for all compounds, the calibration curves are shown in Appendix 6.3.

As the compounds that are analysed in this study are non persistent, their short half-lives can affect the concentrations detected and exposure can vary every day. Samples taken over a period of time is therefore ideal to get a better picture of what the exposure levels are. The samples used in this study only represent one day. In addition, not all the urine from the day is collected, meaning that adjusting for creatinine is important. If all the urine from one day were collected, adjusting for creatinine is not necessary. Yet, as this was the first study of BAA, PhAA, 3-PBA, 2,4-D and TCPy in children from Norway, the levels observed can help determine if a more comprehensive study should be performed.

The linear area for imazalil was revealed to be 0.1-100 ng/mL as a curve was observed when including concentrations up to 2400 ng/mL. Even though the calibration curve had R >0,98, the preparation to determine imazalil might include other preparation steps that can improve the results. Our samples were only incubated for 24 hours, even though it was recommended 48 hours[85]. Imazalil was detected, but most concentrations were found below LOQ and maybe 48 hours is necessary to get increased concentration.

5 Conclusion and further work

Urine samples from Norwegian children were analysed for BAA, PhAA, 3-PBA, 2,4-D, TCPy, 8-iso-PGF2α, cotinine and imazalil. The levels of BAA, PhAA, 3-PBA, 2,4-D and TCPy were also compared to similar studies and to questionnaire data. The analysis revealed that all compounds except 8-iso-PGF2α were detected in the samples. PhAA was detected in all samples above LOQ, and showed the highest median compared to the other compounds analysed. From the pesticides, TCPy had the highest detection frequency and median. These findings highlight that further analysis of TCPy should be done, as the pesticide is no longer authorised in the EU and US due to the inability to find a safe level. Future studies might be able to examine whether new regulations affect the levels observed. BAA, 3-PBA, 2,4-D, cotinine and imazalil all had median below LOD, leading to estimated values. In the comparison to other studies, it was revealed that all the other studies had higher median when compared to the same compounds in our study. In addition, all compounds, except PhAA, showed lower detection frequency in our study compared to the other studies. A Spearman's Rho test showed no significant correlation between the glycol ether metabolites and the questionnaire data regarding the use of cleaning products. Between the pesticide metabolites and the questionnaire data regarding food, the following showed significant correlation: 3-PBA with cheese and nuts($p<0.05$), 2,4-D with pro-biotic yogurt, cheese and ham($p<0.05$), and TCPy with white fish, seafood($p<0.05$), canned fish, and fruits($p<0.01$). In addition, 3-PBA was significantly correlated with both 2,4-D and

TCPy ($p<0.01$), as well as 2,4-D with TCPy ($p<0.01$). Comparing the pesticide metabolites between themselves were not the main focus, but the results were noteworthy to mention. A few trends were visible, however not significantly correlated, regarding PhAA and the use of degreasing sprays. For future studies, correlating concentrations of glycol ethers to questions related to face cream, hand cream and liquid soap could be of interest, as these products might be a bigger source of exposure for children relative to cleaning products. Trends between pesticide metabolites and questions related to food were visible for egg, red meat, sugar and vegetable oil. Future studies should further investigate the findings.

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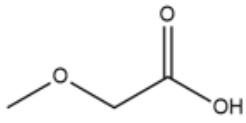
6 Appendix

6.1 Chemical structures



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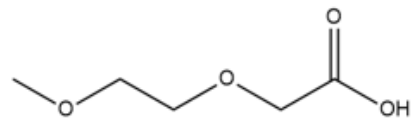

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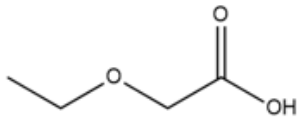

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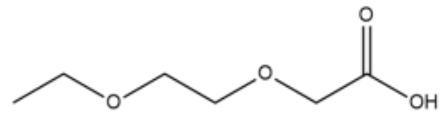

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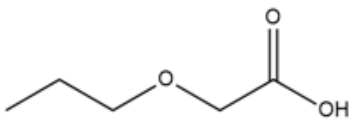

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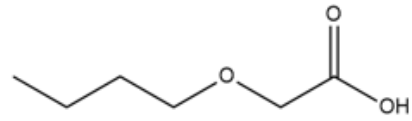

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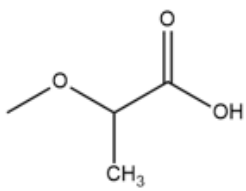

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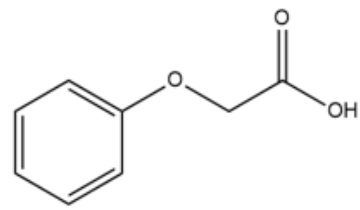

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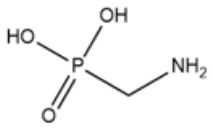

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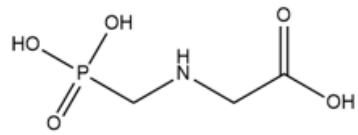

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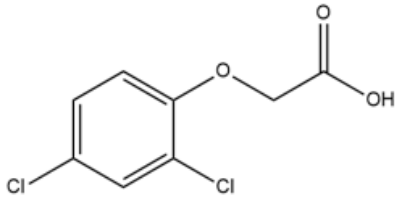

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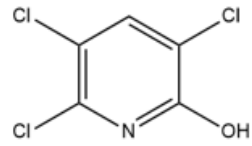

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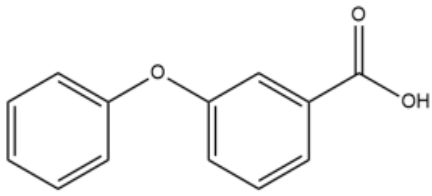

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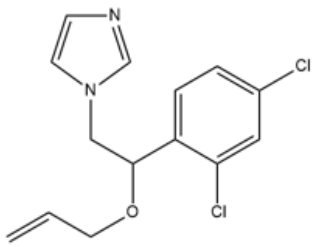

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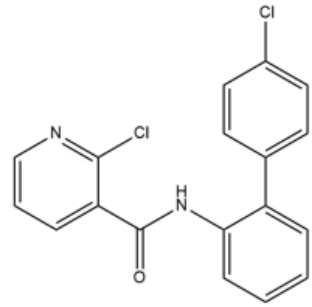

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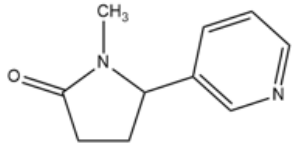

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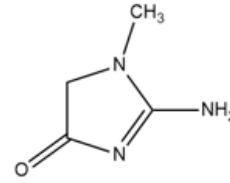

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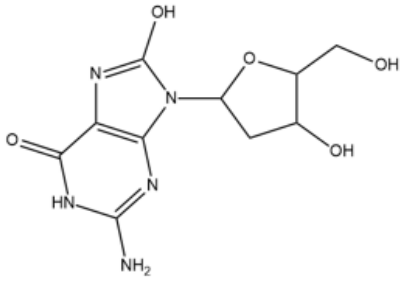
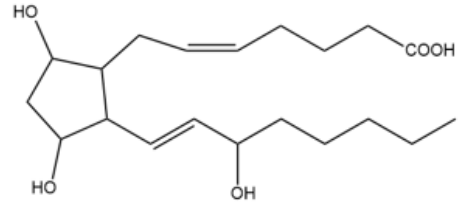


8-hydroxy-2′-deoxyguanosine (8-OH-DG)



8-iso-Prostaglandin F2α (8-iso-PG F2α)

6.2 Questionnaire data

The different cleaning products and food groups included in this thesis are presented in Table 6.1 and Table 6.2, respectively. The tables show the frequency of each cleaning product used inside the participants house and all food groups eaten by each child.

Table 6.1 Answers from questionnaire data related to cleaning products (n=286). The participants answered how often the following cleaning products are used inside the house.

Product	never	less than once a week	1 to 3 times a week	4 to 7 times a week
Bleach	59	175	51	1
Ammonia	106	169	8	1
Solvents/ spot removers	124	134	12	4
Polishes/ waxes	186	93	0	0
Floor cleaning liquids	5	143	132	6
Electronic air fresheners	218	60	0	1
Perfumed cleaning products	78	101	77	15
Furniture sprays	222	61	0	0
Multipurpose sprays	52	90	111	32
Degreasing sprays	116	111	35	16
Glass cleaning sprays	22	148	105	11
Air refreshing sprays	228	47	7	2
Other sprays	177	57	12	3
How often is your child present while cleaning	33	184	65	3

Table 6.2 Answers from questionnaire data related to food (n=286). The participants got asked to think about what the child had eaten in the last year and mark the frequency of each food group that fits best to the child.

Product	never	less than once a month	1 to 3 a month	once a week	2 to 4 a week	5 to 6 a week	1 per day	2 to 3 per day	4 or more per day
Milk	22	9	5	13	23	39	62	99	14
Yogurt	13	17	48	42	103	31	32	0	0
Pro-biotic yogurt	70	69	57	27	33	10	17	3	0
Cheese	9	7	20	21	81	50	40	56	2
Egg	11	24	69	96	78	5	2	1	0
Poultry	3	11	66	142	64	0	0	0	0
Red meat	2	5	25	113	139	2	0	0	0
Cold meat	1	1	35	74	108	35	23	8	1
Ham	48	39	42	52	66	21	14	4	0
White fish	5	13	76	132	58	2	0	0	0
Fatty/ oily fish	19	16	60	146	44	1	0	0	0
Canned fish	115	34	35	35	42	12	10	3	0
Seafood	143	97	39	6	1	0	0	0	0
Dairy dessert	11	53	130	67	23	2	0	0	0
Raw vegetables	3	3	10	16	54	66	66	68	0
Cooked vegetables	8	13	22	38	111	41	47	5	0
Potatoes	9	10	30	67	155	11	3	0	0
Pulses	60	69	83	48	23	2	0	0	0
French fries	39	166	76	6	1	0	0	0	0
Fruits	2	3	2	4	20	45	90	115	4
Fresh juice	2	16	28	50	106	27	42	14	0
Nuts	60	64	72	38	38	10	3	0	0
Canned fruit	205	72	5	2	0	0	1	0	0
Dried fruit	62	92	60	36	27	6	1	1	0
White bread	26	86	96	48	27	0	1	2	0
Dark bread	1	2	1	4	21	30	29	178	20
Sugar-sweetened cereal	105	89	35	26	19	6	6	0	0
Other breakfast cereal	23	30	46	37	66	27	36	15	6
Rice and pasta	0	1	3	30	212	35	4	0	1
Crispy bread/ corn cakes	8	23	58	60	95	25	13	4	0
Plain biscuits and cookies	7	48	118	83	29	1	0	0	0
Pastries	0	25	135	105	20	1	0	0	0
Chocolate	5	13	50	135	72	5	6	0	0
Sugar/honey/jam	1	28	46	52	86	34	25	14	0
Sweets	2	11	37	206	30	0	0	0	0
Soda	49	63	77	78	19	0	0	0	0
Diet soda	128	93	35	23	7	0	0	0	0
Olive oil	4	12	13	16	92	88	57	4	0
Vegetable oil	46	58	55	52	54	8	12	1	0
Butter	19	15	22	28	66	42	44	47	3
Margarine	63	50	43	34	37	15	23	19	2
Dressing/ketchup/ mayonnaise	8	18	45	86	101	18	10	0	0
Salt snacks	5	28	95	140	16	0	2	0	0

6.3 Calibration curves

The following calibration curves are the ones used to determine the concentration of the compounds in this thesis. Water is used as matrix for all calibration curves.

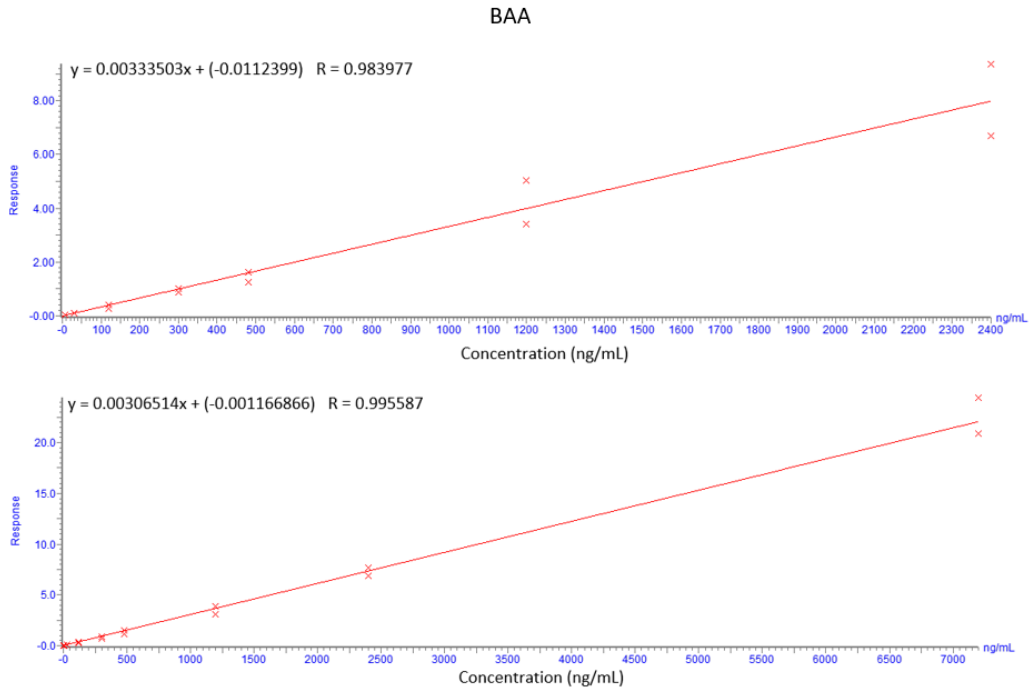


Figure 6.1 Calibration curves of butoxyacetic acid (BAA), the relationship between concentration (ng/mL) and response.

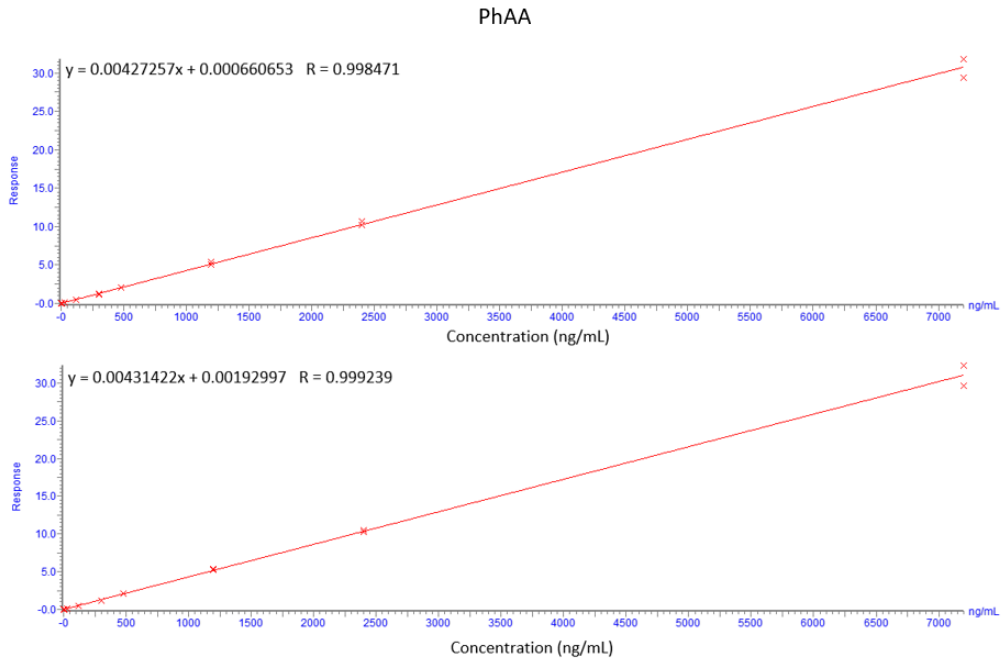


Figure 6.2 Calibration curves of phenoxyacetic acid (PhAA), the relationship between concentration (ng/mL) and response.

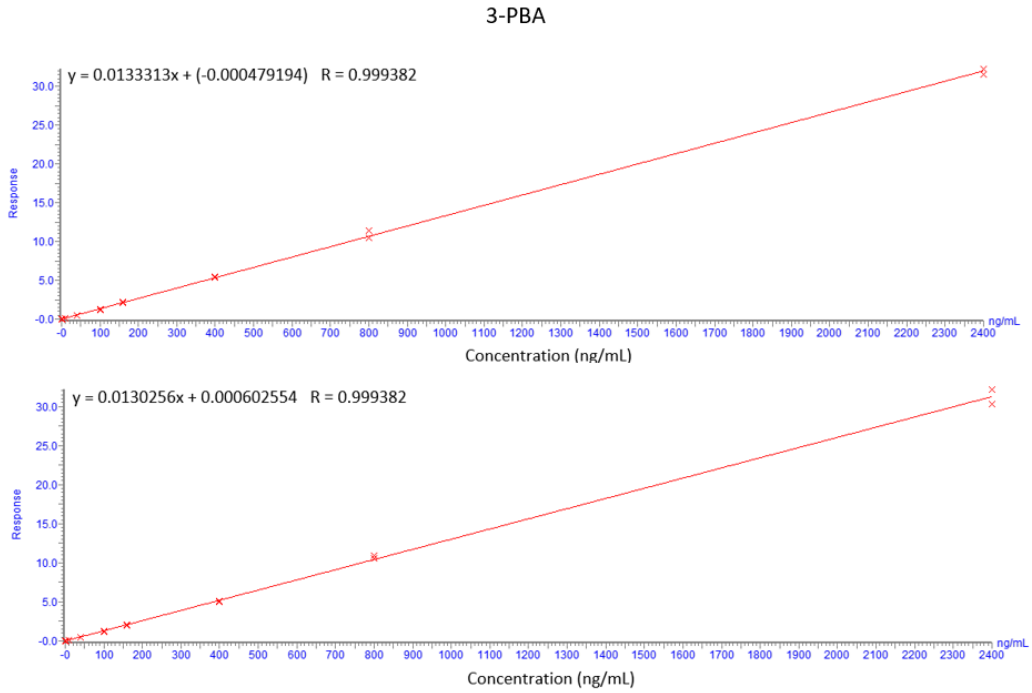


Figure 6.3 Calibration curves of 3-phenoxybenzoic acid (3-PBA), the relationship between concentration (ng/mL) and response.

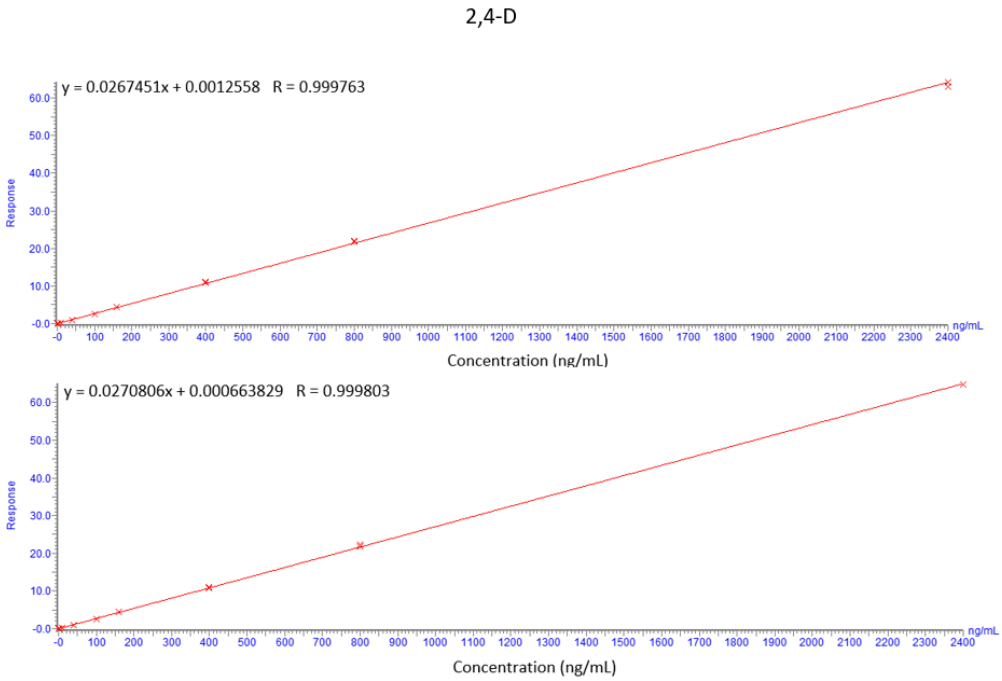


Figure 6.4 Calibration curves of 2-4-dichlorophenoxyacetic acid (2,4-D), the relationship between concentration (ng/mL) and response.

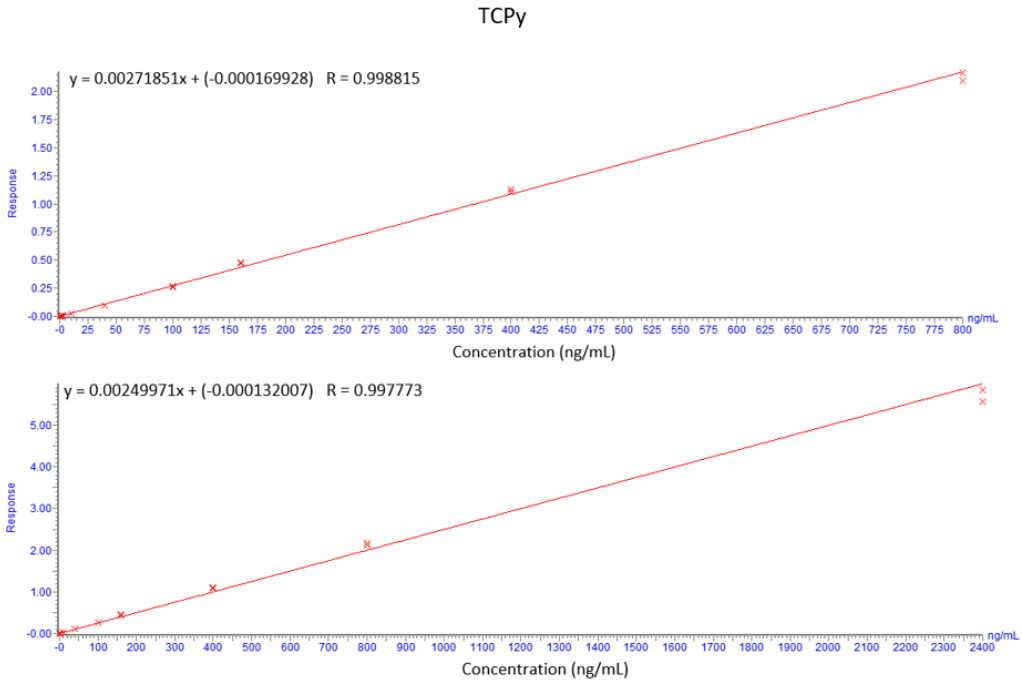


Figure 6.5 Calibration curves of 3,5,6-Trichloro-2-pyridinol (TCPy), the relationship between concentration (ng/mL) and response.

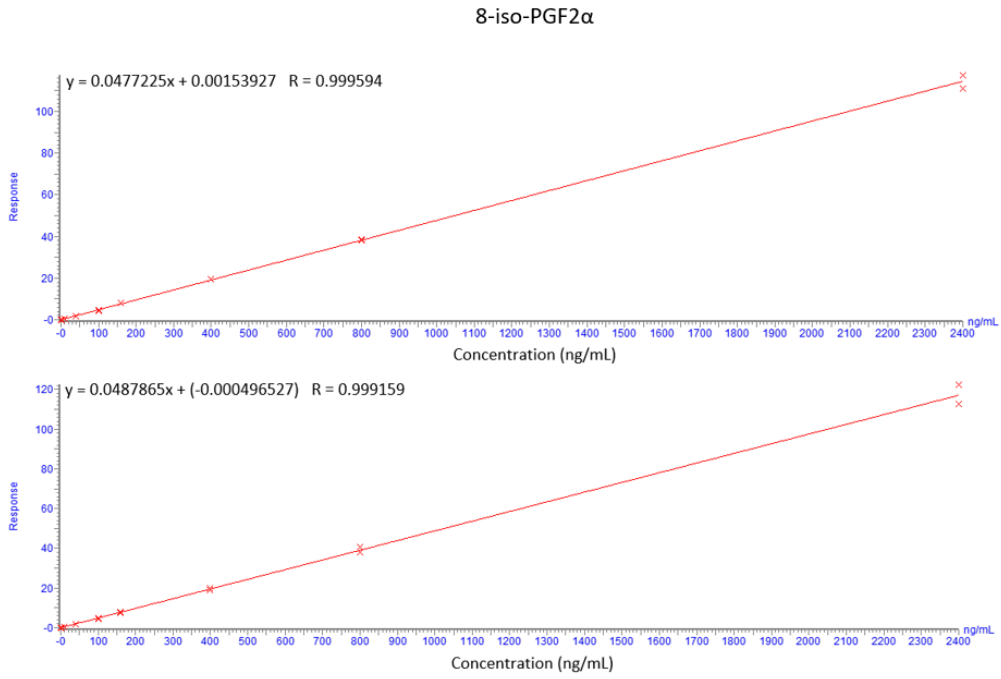


Figure 6.6 Calibration curves of 8-iso-Prostaglandin F2α (8-iso-PGF2α), the relationship between concentration (ng/mL) and response.

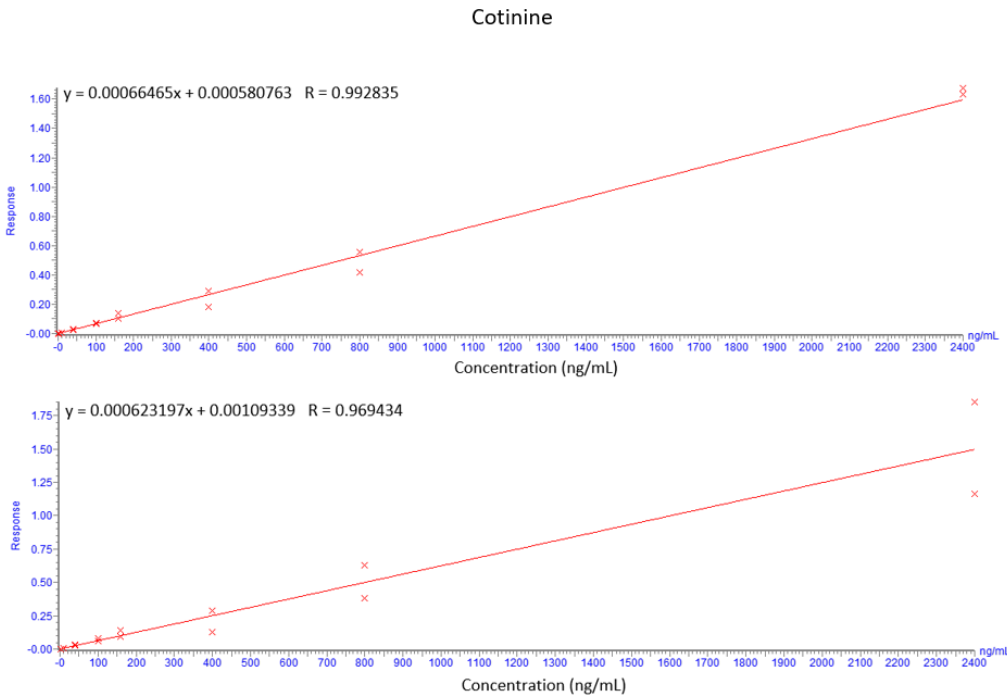


Figure 6.7 Calibration curves of cotinine, the relationship between concentration (ng/mL) and response.

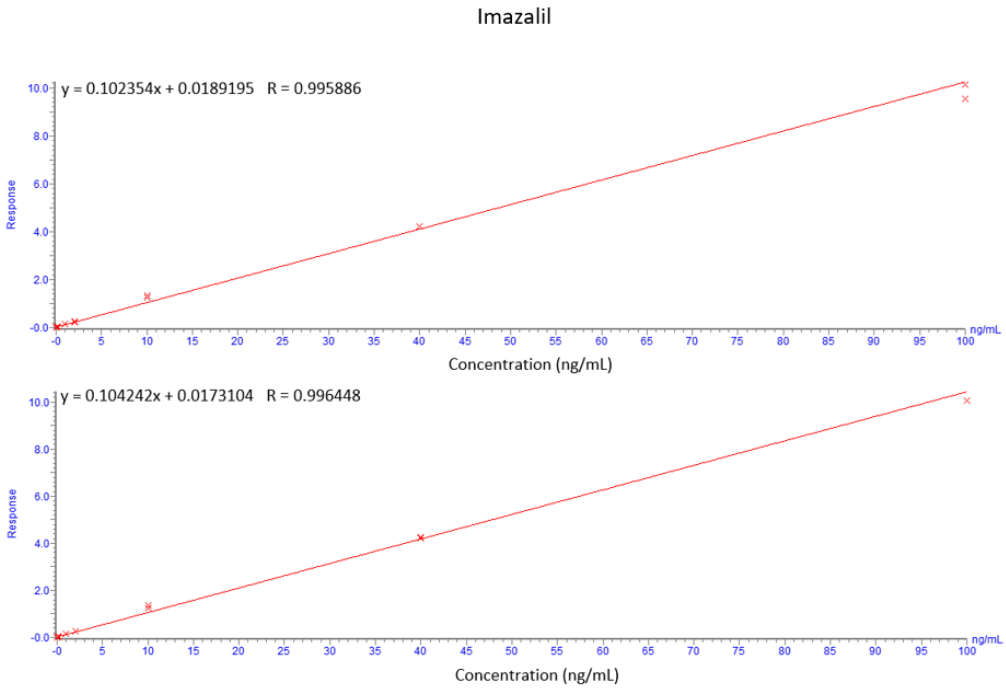


Figure 6.8 Calibration curves of imazalil, the relationship between concentration (ng/mL) and response.

6.4 PCA plots

Figure 6.9 shows PCA plot for BAA, PhAA and questionnaire data regarding use of cleaning products. Table 6.1 shows answers related to cleaning products. Figure 6.10 PCA plot for 3-PBA, 2,4-D, TCPy and questionnaire data regarding food. Table 6.2 show answers related to food. Figure 6.11 shows the same plot as Figure 6.10, but zoomed, in order to separate the factors in the plot.

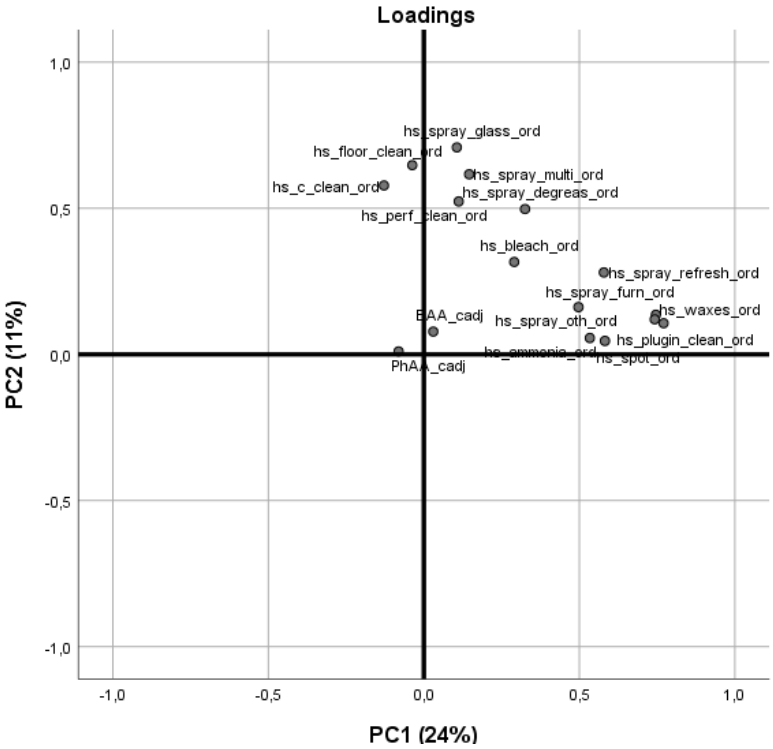


Figure 6.9 Principal component analysis (PCA) containing PC1 and PC2 for butoxyacetic acid (BAA), phenoxyacetic acid (PhAA) and questionnaire data regarding use of cleaning products. Table 6.1 show answers related to cleaning products. Variables with shortened names: hs_spray_glass_ord=glass cleaning sprays, hs_floor_clean_ord=floor cleaning liquids, hs_c_clean_ord=how often is your child present while cleaning, hs_spray_multi_ord=multipurpose sprays, hs_spray_degreas_ord=degreasing sprays, hs_perf_clean_ord=perfumed cleaning products, hs_spray_refresh_ord=air refreshing sprays, hs_spray_furn_ord=furniture sprays, hs_spray_oth_ord=other sprays, hs_waxes_ord=polishes/waxes, hs_plugin_clean_ord=electronic air fresheners, hs_spot_ord=solvents/spot removers, BAA_cadj=creatinine adjusted BAA (μg/g), PhAA_cadj=creatinine adjusted PhAA (μg/g).

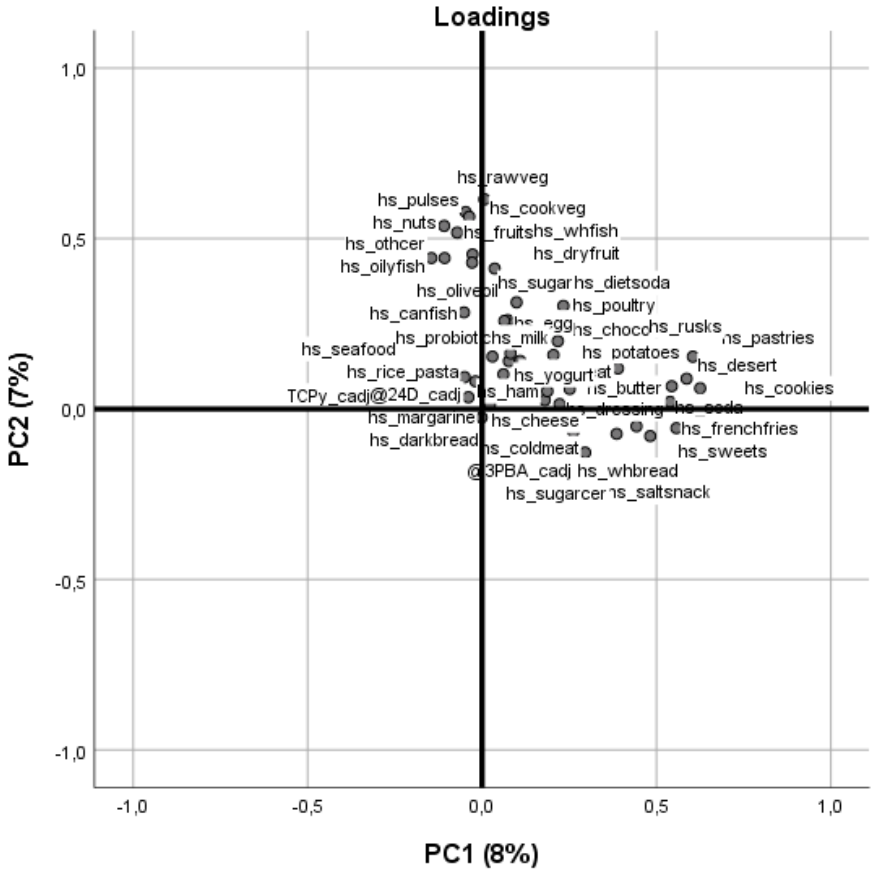


Figure 6.10 Principal component analysis (PCA) containing PC1 and PC2 for 3-phenoxybenzoic acid (3-PBA), 2-4-dichlorophenoxyacetic acid (2,4-D), 3,5,6-trichloro-2-pyridinol (TCPy) and questionnaire data regarding food (Table 6.2 show answers related to food). Variables with shortened names: hs_rawveg=raw vegetables, hs_cookveg=cooked vegetables, hs_oilyfish=oily/fatty fish, hs_whfish=white fish, hs_othcer=other breakfast cereals, hs_sugar=sugar/honey/jam, hs_canfish=canned fish, hs_veg oil=vegetable oil, hs_canfruit=canned fruit, hs_rusks=crispy bread/corn cakes,hs_probiotic=pro-biotic yogurt, @24D_cadj=creatinine adjusted 2,4-D (μg/g), TCPy_cadj=creatinine adjusted TCPy (μg/g), @3PBA_cadj=creatinine adjusted 3-PBA (μg/g), hs_dressing=dressing/ketchup/mayonnaise, hs_sugarcer=sugar-sweetened cereals, hs_whbread=white bread, hs_choco=chocolate, hs_cookies=plain biscuits/cookies, hs_desert= dairy dessert. For a better view of the variables, see Figure 6.11

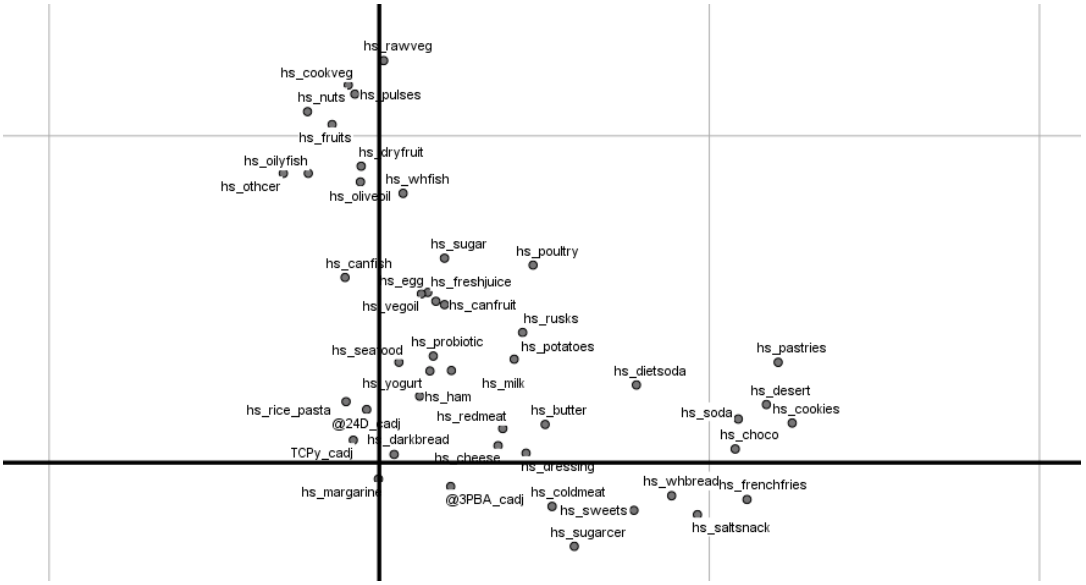


Figure 6.11 Zoomed in Principal component analysis (PCA) containing PC1 (8%,vertical) and PC2 (7%,horisontal) for 3-phenoxybenzoic acid (3-PBA), 2-4-dichlorophenoxyacetic acid (2,4-D), 3,5,6-trichloro-2-pyridinol (TCPy) and questionnaire data regarding food (Table 6.2 show answers related to food). The light grey lines closest to origo represent 0.5, the next line represent 1.0. Variables with shortened names: hs_rawveg=raw vegetables, hs_cookveg=cooked vegetables, hs_oilyfish=oily/fatty fish, hs_whfish=white fish, hs_othcer=other breakfast cereals, hs_sugar=sugar/honey/jam, hs_canfish=canned fish, hs_veg oil=vegetable oil, hs_canfruit=canned fruit, hs_rusks=crispy bread/corn cakes,hs_probiotic=pro-biotic yogurt, @24D_cadj=creatinine adjusted 2,4-D (μg/g), TCPy_cadj=creatinine adjusted TCPy (μg/g), @3PBA_cadj=creatinine adjusted 3-PBA (μg/g), hs_dressing=dressing/ketchup/mayonnaise, hs_sugarcer=sugar-sweetened cereals, hs_whbread=white bread, hs_choco=chocolate, hs_cookies=plain biscuits/cookies, hs_desert= dairy dessert.

Selma Tofte Granerud

Analyse glycol ether and pesticide metabolites in Norwegian children and their potential determinants

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