Bioavailability of plasticizers in dust and food after oral administration to model organism pig

Dissertation

zum Erwerb des Doktorgrades der Humanbiologie an der Medizinischen Fakultät der Ludwig-Maximilians-Universität zu München

vorgelegt von

Veronika Plichta

Jahr
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Aus dem Institut und der Poliklinik für Arbeits-, Sozial- und Umweltmedizin, Klinikum der Universität München, LMU München
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Abstract

For decades, phthalates are widely used as plasticizers in plenty of consumer products e.g. food packaging, toys, clothing and personal care products. They are not chemically bound to the polymer matrix, so they can easily be released into the environment for example by leaching or migration. Humans are exposed to phthalates via ingestion, inhalation or dermal uptake. They are rapidly metabolized in humans and excreted via urine, mainly within two days. Phthalates act as endocrine disruptors and target mainly the reproductive system. According to US-Environmental Protection Agency (US-EPA), infants have an unintended uptake of 60 mg dust per day. Among other things, dust contains a various amount of pollutants as e.g. phthalates. Therefore, dust could pose a potential health risk to humans. Currently there are no information's about the bioavailability of phthalate in dust. Consequently in risk assessments the bioavailability is determined as 100 %. In the case of phthalates, *in vitro* digestion tests are indicating a bioaccessibility of 10 – 32 %. The aim of this study was to determine the relative oral bioavailability of certain phthalates as butyl benzyl phthalate (BBzP), bis (2-ethylhexyl) phthalate (DEHP), di-n-butyl phthalate (DnBP) and di-isononyl phthalate (DINP) in house dust after oral ingestion.

Study design: Seven five week old piglets were fed five different dust samples collected from daycare centers and one food sample. Overall, 0.43 to 0.83 g of dust samples sieved to 63 µm were administered orally. The urine was collected over a period of 38 hours. The excreted metabolites were quantified using an LC-MS/MS method.

Results: The mean uptake rate of the applied dust dose for BBzP, DnBP, DEHP and DINP is 28 ± 18 %, 52 ± 18 %, 43 ± 11 % and 47 ± 26 %, respectively. The bioavailability in food is 37 ± 23 % for BBzP, 39 ± 16% for DnBP, 53 ± 15 % for DEHP and 43 ± 13 % for DINP. No significant difference between the quantities of plasticizers excreted in urine after dust administration compared to food was observed. The metabolites showed their maximum concentration three to five hours post dose.

Conclusion: The bioavailability of the investigated phthalates is much higher compared to the bioaccessibility reported from *in vitro* digestion tests. Furthermore it

is noticeable that the bioavailability of DEHP does not vary between the dust samples. A dose dependent saturation process for DINP was observed. Besides other intake pathways, dust could pose a relevant source of phthalates for toddlers.

Zusammenfassung

Seit Jahrzenten werden Phthalate in großen Mengen als Weichmacher in (Lebensmittel-) Verpackungsmaterial, Spielzeug oder Kleidung eingesetzt und zählen daher zu einer der wichtigsten Industriechemikalien. Phthalate sind nicht an die Polymermatrix gebunden und können durch (Ab-)Nutzung relativ leicht in die Umwelt abgegeben werden. Phthalate werden als endokrin aktive Substanzen eingestuft und haben eine negative Wirkung auf das Reproduktionssystem.

Kleinkinder nehmen oral täglich 60 mg unbeabsichtigt Hausstaub auf. In Hausstaub kommen teils hohe Konzentrationen an Schadstoffen, wie z.B. Phthalate, vor. Derzeit gibt es keine Kenntnisse über die orale Bioverfügbarkeit von Weichmachern in der Matrix Staub. In einem *in vitro* Verdauungstest konnte eine Bioverfügbarkeit von Phthalaten aus dem Staub zwischen 10,2 % (DEHP) und 32 % (DMP) nachgewiesen werden. In dieser Studie soll anhand eines Tierversuchs die orale Bioverfügbarkeit von Butylbenzylphthalat (BBzP), Bis (2-ethylhexyl)phthalat (DEHP), Di-n-butylphthalat (DnBP) und Di-isononylphthalat (DINP) in Hausstaub und Lebensmitteln untersucht werden.

Studiendesign: Sieben fünf Wochen alte Schweine erhielten jeweils fünf unterschiedliche Hausstaubproben (Staub aus Kindertagesstätten auf 63 µg gesiebt; verabreicht: 0,43-0,83g) und eine Lebensmittelprobe. Der Urin wurde über 38 Stunden gesammelt. Die ausgeschiedenen Metabolite wurden mittels einer LC/MS-MS Methode bestimmt.

Ergebnisse: Die durchschnittliche Aufnahme von BBzP, DnBP, DEHP und DINP im Staub lag bei 28 ± 8 %, 52 ± 18 %, 43 ± 11 % und 47 ± 26 %. Bei der Lebensmittelprobe lag sie bei 37 ± 23 % für BBzP, 39 ± 16 % für DnBP, 53 ± 15 % für DEHP und 43 ± 13 % für DINP. Es konnte kein signifikanter Unterschied bei der Aufnahme zwischen Hausstaub und Lebensmittelprobe nachgewiesen werden. Das Konzentrationsmaximum der Phthalatmetabolite im Urin konnte drei bis fünf Stunden nach der Probengabe festgestellt werden.

Schlussfolgerung: In dieser Studie konnte gezeigt werden, dass die Bioverfügbarkeit - je nach Weichmacher im Hausstaub - bei ca. 40 % liegt und somit höher als in den *in vitro* Verdauungstests (10 – 20 %). Außerdem zeigte sich, dass die

Bioverfügbarkeit von DEHP innerhalb der getesteten Staubproben stabil und reproduzierbar ist. Bei DINP konnte ein dosisabhängiger Sättigungsprozess festgestellt werden. Zusammenfassend lässt sich sagen, dass Staub eine Expositionsquelle bei Kleinkindern darstellt.

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Abbreviation

µg/kg/d	Microgram per kilogram per day
2cx-MMHP	Mono-(2-carboxymethyl)hexyl phthalate
5OH-MEHP	Mono-(2-ethyl-5-hydroxylhexyl) phthalate
5oxo-MEHP	Mono-(2-ethyl-5oxohexyl) phthalate
5cx-MEPP	Mono-(2-ethyl-5carboxypentyl) phthalate
7oxo-MINP	Mono-(4-methyl-7oxo-octy)l phthalate
7cx-MINP	Mono (4-methyl-7-carboxy-heptyl) phthalate
7OH-MINP	Mono-(4-methyl-7-hydroxyoctyl) phthalate
95th P	95th percentile
ADD	Average daily dose
AGD	Anogenital distance
b.w.	Body weight
BBzP	Butyl benzyl phthalate
C_{max}	Concentration maximum
DEHP	Bis (2-ethylhexyl) phthalate
DINP	Diisononyl phthalate
DnBP	Di-n-butyl phthalate
EDCs	Endocrine Disrupting Chemicals
EU	European Union
FSH	Follicle stimulating hormone
GC/MS	Gas chromatography–mass spectrometry
HMW	High molecular weight
HPLC	High-performance liquid chromatography
IgE	Immunoglobulin E
IgG	Immunoglobulin G
Kow	N-octanol/water coefficient
LC-MS	Liquid chromatography–mass spectrometry
LMW	Low molecular weight
LOD	Limit of detection
LOQ	Limit of quantitation
MBzP	Mono-benzyl phthalate
MED	Median
MEHP	Mono (2-ethylhexyl) phthalate
mg/d	Milligram per day
MIN-MAX	Minimum–maximum
MINP	Mono-isononyl phthalate
MnBP	Mono-n-butyl phthalate
NOAEL	No observed adverse effect level
PBDE	Polybrominated diphenyl ethers
PVC	Polyvinyl chloride
RfD	Reference dose

$t_{½}$	Half-life time
T3	Triiodothyronine
T4	Thyroxine
TDI	Tolerable daily intake
Th2	T helper cells 2
T_{max}	Time after sample administration, when the maximum urine concentration is reached
TSH	Thyroid stimulating hormone
US-EPA	United states environmental protection agency

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1I Introduction

1I1 Phthalates

Synthetic chemical diesters of 1,2-benzenedicarboxylic acid (Figure 1), commonly known as phthalates, are widely used in the chemical industry as plasticizers to increase the flexibility and softness in plastic, e.g in polyvinyl chloride (PVC). Plasticizers can represent up to 40% of plastic products [1]. In addition to PVC products, phthalates are present in various consumer products like personal care products, children’s toys, food packaging, building materials, clothing, medical devices and in pharmaceutical products [2, 3]. There are more than 25 different phthalates in technical use. Table 1 gives an overview of the phthalates and their characteristics on which this study focuses.

Figure 1. Molecular structure of phthalate esters, R and R_1 are alkyl and aryl chains with the same or different structures [3].

Phthalates are categorized in two groups based on physiochemical properties:

- Low molecular weight (LMW) phthalates: produced from alcohols with a straight chain of one to four carbon backbones. These are primarily used in pharmaceuticals and personal care products as a solvent, e.g. in fragrances, soaps, lotions etc. LMW phthalates are slightly to moderately water soluble [3, 4].
- High molecular weight (HMW) phthalates: produced from alcohols with a straight chain or ring structure of five or more carbon backbones. These kinds of phthalates are primarily used as plasticizers in vinyl products such as flooring, wall covering, medical devices and food contact material. HMW phthalates are

water insoluble, but soluble in most organic solvents like alcohol, ether and oils [3, 4].

Table 1: Overview of the investigated phthalates, their corresponding metabolites and their characteristic [3]

Phthalate	Molecular Formula:	CAS No.	Backbone length	Log Kow	Metabolites	Uses
Butylbenzyl phthalate (BBzP)	$C_{19}H_{20}O_4$	85-68-7	C4,C5	4.48	Mono-benzyl phthalate (MBzP)	Automotive adhesives, coating, sealants and paints, plasticizer in children's toys,
Di-n-butyl phthalate (DnBP)	$C_{16}H_{22}O_4$	84-74-2	C4	4.57	Mono-n-butyl phthalate (MnBP)	Plasticizer for rubber, adhesives in textiles and leather treatments, children's toys, fragrance bases for household, personal care and cosmetic products
Diethylhexyl phthalate (DEHP)	$C_{24}H_{38}O_4$	117-81-7	C6	7.50	Mono-(2-ethylhexyl) phthalate (MEHP) Mono-(2-ethyl-5-hydroxylhexyl) phthalate (5OH-MEHP) Mono-(2-ethyl-5oxohexyl) phthalate (5oxo-MEHP) Mono-(2-ethyl-5carboxypentyl) phthalate (5cx-MEPP) Mono-(2-carboxymethyl)hexyl phthalate (2cx-MMHP)	In coating, adhesives and resins for flooring, PVC labels, fragrance bases for perfumery and cosmetic products
Di-isononyl phthalate (DINP)	$C_{26}H_{42}O_4$	285533-12-0, 68515-48-0	C8;C9	8.8	Mono-isononyl phthalate (MINP) Mono-(4-methyl-7oxo-octyl) phthalate (7 oxo-MINP) Mono-(4-methyl-7-hydroxyoctyl) phthalate (7OH-MINP) Mono (4-methyl-7-carboxy-heptyl) phthalate (7cx-MINP)	Plasticizer for PVC applications, flooring, carpet backing, lamination, toys

Di-2-ethylhexyl phthalate (DEHP) and Di-isonyl phthalate (DINP) are the principally used phthalates in industry [5]. In the European Union, the use of DEHP, DEP and DnBP is banned and often replaced by substitutes like DINP, which now accounts for 80% of the phthalate production in Europe [6]. Because of their extensive use and the resulting permanent presence in the environment, phthalates are substances of concern [7].

1I1I1 Toxicokinetics of phthalates

The majority of phthalate uptake takes place after oral ingestion. Once incorporated in the human body, phthalates are rapidly metabolized and excreted via urine. Phthalates undergo a biotransformation, which can be categorized in two phases:

Phase 1: Phthalates are metabolized into their corresponding mono-ester (primary metabolite) by hydrolysis of one of their ester bonds. The monoester of high molecular weight phthalates undergoes a further enzymatic oxidation of the alkyl chain and is finally metabolized to more hydrophilic metabolites (secondary metabolites).

Phase 2: also known as conjugation reaction; the primary or secondary metabolites conjugate with glucuronic acid and/or sulfate, both increase the water solubility and might reduce the biological activity of the phthalate metabolites. The glucuronide conjugate is finally excreted mainly with urine. The conjugation reaction is catalyzed mainly by the enzyme uridine diphosphate glucuronosyltransferase (UGT) [3].

The amount of oxidative metabolites depends on the alkyl chain length of phthalates because oxidative metabolites are more water soluble than monoesters. Water solubility decreases relative to increasing alkyl chain length. As a consequence, low molecular weight phthalates are mainly metabolized to monoesters (primary metabolite), whereas high molecular weight phthalates like DEHP or DINP mainly transform their monoester in a second step to oxidative metabolites and excrete them as secondary metabolites [8, 9]. Only 2 – 7 % of the applied dose of high molecular weight phthalates is excreted as their primary metabolite [10].

In the human body, phthalates are absorbed by the gastrointestinal tract, where they are metabolized to the corresponding monoester, which is then further transported to the liver. The monoester is transformed to the secondary metabolites mainly in the

liver. After that, the primary or secondary metabolites are transported to the kidneys where they are excreted by urine [11, 12]. During the urinary excretion, there are several maximums of metabolite elimination. The metabolism of phthalates is biphasic with a fast elimination rate within the first 24 hours after dose administration followed by a slow elimination rate. Most of the applied dose is excreted within the first 48 hours [13].

Although the urinary excretion in humans has a high individual variation of phthalates and their metabolites content, the ratio of hydrolytic and oxidative metabolites from the parent compound are highly stable in urine [14].

The individual metabolism of the investigated phthalates in this study is discussed in detail in Section 1I2I5.

1I1I2 Exposure and migration of phthalates

1I1I2I1 Routes of exposure

Due to their chemical and physical properties, phthalates are not chemically bound to the polymer, which means that they can be easily released into the surroundings by leaching, evaporation, migration and abrasion from the polymer matrix during usage [3, 4, 10]. Additionally phthalates are lipophilic, which can be an influencing factor in their leaching and environmental distribution behavior [9]. Based on their semi- to non-volatile characteristics, phthalates are mainly bound to particles like in dust instead of staying in the gaseous phase [11].

Humans are exposed to phthalates on a daily basis via several pathways like ingestion, inhalation (gaseous or particle bound), dermal absorption and intravenous injection [8]. Phthalate exposure can occur directly by using a product which contains phthalates, e.g. personal care products, or indirectly by a product where the phthalates are leached or migrate into, e.g. food through packaging material.

- Ingestion: is the main route of exposure to phthalates. It occurs mainly via dietary intake and to lesser extent via pharmaceuticals, nutritional supplements, and mouthing or sucking children toys. The systemic bioavailability is assumed to be 50 % for adults and 100 % for children / infants [15].
- Inhalation: occurs through house dust and also through medical devices, e.g. breathing tubes (bioavailability from 75 % (adults) to 100 % (children and infants)

- Skin absorption: occurs through direct contact with products like clothes, personal care products, etc. which contain phthalates. In general the absorption of phthalates through skin is limited (bioavailability lays around 5 %).
- Intravenous: occurs through medical devices when phthalates migrate from e.g. infusion bags into the fluid which is intravenously administered to the patient [9].

Ingestion of food containing phthalates and absorption through the use of personal care products are the major contributors to phthalate exposure, whereas inhalation is negligible [16].

In addition to the different exposure pathways mentioned before, the phthalate body burden is influenced by hand to mouth activity, mobility, diet, personal care and hygiene practices. Therefore infants and toddlers have a higher risk of an increased phthalate intake than adults, based on their high food and water requirements per unit of body mass, their increased hand to mouth activity and their higher ventilation rate [17].

1I1I2I2 Mouthing – another exposure pathway for infants

Young children use mouthing as a part of exploring their environment. Mouthing is defined as activities where fingers or objects are put into the mouth or touched with the mouth, which includes licking, sucking, chewing, biting but excludes eating and drinking. An important and limiting factor of phthalates exposure is the duration of mouthing, which is equal to the exposure time. As demonstrated in studies [18, 19], mouthing objects like toys, fingers and pacifiers and mouthing time differ by age. Between 0 and 18 months the average mouthing time of toys is 20 mins, whereas between 18 and 36 months, it is only five minutes [20]. Sucking and licking behaviors are the most common way of mouthing. It was also shown that mouthing objects change by age. In the first three months, the main mouthing objects are fabrics (80 %), while around 6 to 9 months, plastic toys account for 50 % of the objects mouthed and remain the main mouthing object until the age of five. A 6 to 9 months old child mouthed around 26 different items per day, whereas only 3 items were mouthed by a 1 to 3 month old child. The highest mouthing time (5 hours) was found at age 6 to 9 months and two year old children [18]. In general the hand to mouth frequency decreased by increasing age [21, 22]. Besides the age of the child and the duration,

another important factor of the frequency of mouthing is the location, indoor and outdoor. It was shown that indoor hand to mouth frequencies was 2.4 times higher than outdoors with two-year old children.

In two meta-studies, Xue et al. [21, 23] calculated the frequency of hand to mouth and object to mouth. The average indoor hand to mouth contact for toddlers ranged between 12.7 and 19.6 contacts per hour, whereas the mean indoor object to mouth contact is higher and varies between 15 and 26.6 times per hour. At 1 to 2 years, the outdoor object to mouth rate was three times lower than the indoor rate.

In addition to mouthing, crawling and sitting on the floor increases the accessibility to house dust. Compared to older children and adults, infants and toddlers have a greater exposure risk of indoor pollutants in dust.

Including mouthing, the average daily dust ingestion of an infant or toddler is around 50 mg/d whereas an adult only ingests around 1 mg/d [24, 25]. Furthermore, infants and toddlers could be exposed through using teething rings, pacifiers or the nipple of baby bottles, which could contain certain phthalates. Since 1999 the use of phthalates in certain baby articles were restricted to less than 0.1 % of the object weight [20].

In conclusion, mouthing of objects, like toys etc. which contain phthalates or are covered by dust can be a potential source of the phthalates exposure for infants and toddlers.

1I1I2I3 Phthalate migration

The common method for measuring phthalate migration from toys to saliva is the head over heels agitation method. Five sample pieces 2 mm thick and 23 mm in diameter are removed from the surface of the investigated toy. Each piece is put in a flask which is filled with 25 ml of saliva simulant solution. In a head over heels rotator, this flask is rotated for 30 min at 60 rpm. After this, the saliva simulant solution was prepared for analyzing by HPLC or GC/MS [26] (detailed description in TNO Report [27]). Pfaff et al. [28] concluded in their migration experiment with plastic toys that the average DEHP and DINP migration was around 10 $\mu g/cm^2/h$. In an *in vivo* experiment by Fiala et al. [29], the migration behavior of DINP was investigated by sucking and biting on a toothing ring. The result of that study showed that the average release of DINP through sucking was 1.38 $\mu g/min/10cm^2$, whereas when

chewing was included, it amounted to 2.22 µg/min/10cm². During this procedure 36 % of the DINP content of the toothing ring was released. Furthermore, it showed that the migration behavior of DEHP is similar to DINP. The higher the DEHP and DINP content of a product, the higher the migration rate. In addition to the phthalate content, it has been concluded that the migration rate is also influenced by factors like surface roughness, coating type, thickness of the object, and the surrounding temperature [26, 30]. Further tests also showed that in a saliva simulant solution the migration rate was higher than in water and additionally that sucking and chewing increase the migration process compared to static conditions [29].

1I1I2I4 Methods to calculate phthalate exposure and intake

There are two tools to calculate the human phthalate exposure, biomarker studies and indirect studies. In biomarker studies, the total daily intake was back calculated using the metabolite concentration in urine. Whilst in the indirect studies, the concentrations in the environmental media and food combined with e.g. ingestion rates are used to calculate the phthalate intake. Biomarker studies are susceptible to physiology and do not provide any information about exposure sources. In addition, the toxicokinetic properties of each phthalate has to be known for an adequate calculation. In the indirect studies, the main focus is on dietary intake, which is calculated by a database of phthalate concentration in food. A few indirect studies include other pathways like inhalation or dermal contact [31].

Clark et al. [31] performed a comparative study of biomonitoring and the indirect method and concluded that in many cases both methods agree with each other and discrepancies are explainable through regional differences, focusing on one metabolite, not including all pathways or lack of information. Both methods have their advantages as well as their disadvantages, but there is a tendency that for low molecular weight phthalates the biomarker method is a better choice, because for those phthalates is it difficult to identify all the exposure source, whereas for high molecular weight phthalates, either method is adequate. Furthermore, the indirect method is useful to detect new exposure sources while the biomarker method quantifies the amount of exposure.

For calculating phthalate intake, the following equations are used:

The daily intake of phthalates is estimated by the following equation [32]:

$$D = \left(\frac{\text{Curine} \;\; x \; \text{UV}}{\text{Fue}}\right) x \left(\frac{\text{MWp}}{\text{MWm}}\right)$$

D = daily intake, C urine = metabolite concentration in urine in µg/l, UV = excreted urinary volume per day, Fue = molar fraction of excreted metabolites in relation to the corresponding parent compound, MW_p = molecular weights of phthalates and MW_m =molecular weight of the corresponding metabolite.

To estimate the phthalate intake through dust ingestion the following formula is used [24]:

$$E_{dust} = \frac{C_{dust} \text{ x } q_{dust}}{bw} \text{ x } r_{uptake}$$

E_{dust} = phthalate intake caused by dust in mg/kg b.w. per day, C_{dust}: phthalate concentration in dust in mg/kg, q_{dust}: amount of the daily dust ingestion in kg/d, r_{uptake}: the fraction of the amount of phthalates transferred into the body.

This equation can be modified for other ways of ingestion, e.g. food or exposure through toys.

1I1I3 Health effects of phthalates

Due to the extensive use of chemicals, the exposure of humans to various industrial chemicals has increased in the last decades. Several of such man-made substances have been shown to be toxic in animal studies and also have an impact on human health, respectively [33]. As shown *in vivo* and *in vitro*, several phthalates have an antiandrogenic activity and possible estrogenic actions and therefore phthalates are classified as endocrine disrupting/modulating agents, which target mainly the male reproductive system [16].

Endocrine-Disrupting Chemicals (EDCs) are natural or man-made substances which alter the normal function of the hormone system, by inhibition, mimicry or modification of the hormone. In addition, EDCs can modify the production of hormones in endocrinal glands and the metabolism of hormones [34].

1I1I3I1 Reproduction and development

Some phthalates cause reproductive and developmental toxicity. They are able to cross the placental barrier. Gestational exposure can reduce the Leydig cell testosterone production, which could cause malformation in genital development or a

shortened anogenital distance (AGD) [16]. In addition, it was discussed if abnormalities like hypospadias, cryptorchidism and malformation of the epididymis, vas deference, seminal vesicles and prostate are effects of the so- called “phthalate syndrome” or “testicular dysgenesis syndrome”. Especially DEHP, DnBP and BBzP are associated with these abnormalities [35].

At the present state of knowledge, it is not clear if prenatal phthalate exposure is associated with gestational length or anthropometry of newborns. In recent years, several studies have been published, but the results are inconclusive [36, 37].

Semen parameters

The primary target of e.g. DEHP are Sertoli cells in the testes. Furthermore, it is well known that the metabolite MEHP is responsible for the effects in the testes. The Sertoli cells are an important part of spermatogenesis by determination of the amount of germ cells. The amount of Sertoli cells in an adult man is influenced by the proliferation of Sertoli cells in puberty. The Leydig cells, responsible for the production and secretion of testosterone and stimulation of sperm production, were also negatively affected by phthalates. It has been shown that DEHP and DnBP reduce sperm motility [3, 38]. Duty et al. [39] observed a dose response relation between MBzP (metabolite of BBzP) and sperm concentration and motility. Furthermore, a correlation between phthalate exposure and DNA damage in human sperm was reported. A repeated DEHP dose results in seminiferous tubular atrophy induced by the loss of meiotic and post meiotic cell populations in the seminiferous epithelium [3, 40, 41].

Ovary

Some phthalates are suspected of disrupting the development of ovaries and oocyte, acceleration of the primordial follicle recruitment, targeting growing follicles, inhibiting the growth of antral follicles, disrupting oocyte maturation and ovulation and altering the post-ovulatory process. Further, several studies indicated, that phthalates disrupt the production, action and secretion of several sexual hormones by altering mRNA, protein and steroidogenic enzymes, resulting in a decreased estradiol level. But the mechanism of action is still not clear and further research is needed [42].

1I1I3I2 Thyroid hormone system

The thyroid hormone system is involved in many physiological processes like metabolism, brain and organ development and fetal and child growth. Phthalates are suspected of affecting the thyroid signaling through various mechanisms, by interfering with the binding between T_3 and transport proteins and the plasma membrane uptake of active T_3, respectively. An inverse relationship between urinary DEHP-metabolites and total and free T_4 levels, total T_3, thyroglobulin and increased TSH level was observed. Those results indicated that phthalates negatively affect the thyroid system and metabolism, which can cause a negative impact for the developing fetus and growing child [16, 43, 44].

1I1I3I3 Asthma and Allergies

Phthalates have been associated with modulation of the immune system. Reviewed by Bornehag et al. [33], experimental studies showed that phthalates have an adjuvant effect on Th2 cells (differentiation or production) and enhanced the level of Th2 cells promoted immunoglobulins IgG and IgE. IgE has an essential role in allergies and asthma. Several epidemiological studies indicate a possible correlation between asthma, allergies, rhinitis, eczema or wheezing symptoms and phthalate – especially DEHP exposure [17]. Another study by Bornehag et al. [45] showed that high concentration of DEHP in house dust is associated with asthma and high levels of BBzP are related to allergic and eczematous symptoms.

1I1I4 Biomonitoring data of phthalate levels in urine

The Human Biomonitoring Commission advises two Human BioMonitoring values (HBM1 and HBM2) to assess the pollutant exposure in the general population. The HBM1 value is a control value, where no negative health effects are expected, whereas the HBM2 value is an intervention level. The (HBM I) for the sum of DEHP metabolites in urine is set for children (6-13 y) at 500 µg/l, for women in childbearing age 300 µg/l, and for the rest 750 µg/l [47]. The German Environmental Surveys (GerES) IV, a representative study, showed that 1.5 % of the investigated children exceed the HBM I value [46]. Additionally, GerES indicated that children have up to

four fold higher urinary phthalate metabolite levels than adults. The study also shows that the concentration levels decreased by increasing age [46].

Table 2 gives an overview of the urinary phthalate metabolite concentration of several biomonitoring studies conducted on children. In nearly all urine samples the phthalate metabolites were detectable [42]. Moreover, it shows that highest concentrations were observed for DEHP metabolites, followed by DnBP and DINP metabolites [46].

Table 2. Phthalate metabolite concentration in urine [µg/l]

Source	MEHP			5oxo-MEHP			5OH MEHP			5cx-MEPP			MnBP		
	MED	Min-Max	95thP.	MED	Min-Max	95thP	MED	Min-Max	95thP	MED	Min-Max	95thP	MED	Min-Max	95thP
Fromme et al, 2013 n=663 [47]				17.9	0.8-168	58.2	16.5	0.8-225	60.2				32.4	2.3-221	124
Langer et al, 2014 n=441 [48]	4.7	-	14.6	17.6	-	71.3	33.2	-	115.9	34.5	-	135.8	80.1	-	242.7
Becker et al, 2004 n=254 [49]	7.18	0.74-223	29.7	97.7	<0.5-1420	139	52.1	1.86-2590	188	-	-	-	-	-	-
Koch et al. 2007 n=239 [50]	-	-	-	-	-	-	-	-	-	-	-	-	166	15.5-3193	624
Koch et al. 2003 n=85 [51]	10.3	<LOQ-177	37.9	36.5	0.5-544	156	46.8	0.5-818	224	-	-	-	-	-	-
Kasper-Sonnenberg et al. 2012 n=104 [52]	4.0	<LOQ-27.7	17.1	26.4	1.9-135	88.8	31	1.6-163	88.2	42.1	7.3-259	127	54.2	2.0-274	148
Becker et al. 2009 n=599 [46]	6.7	<LOD-319	25.1	36.3	<LOD-2490	123	46	<LOD-3640	164	61.4	<LOD-4490	209	93.4	<LOD-1090	310
Larsson et al. 2017 n=113 [53]	1.5	<LOD-11	5.6	12	1.5-82	37	17	1.8-133	56	16	2.4-100	52	54	3.9-327	141

	MINP			7oxo MINP			7OH-MINP			7cx-MINP			MBzP		
	MED	Min-Max	95thP	MED	Min-Max	95thP	MED	Min-Max	95thP	MED	Min-Max	95thP	MED	Min-Max	95thP
Fromme et al, 2013 n=663 [47]	-	-	-	4.6	0.2-486	29.9							11.6	0.8-311	80.7
Langer et al. 2014 n=441 [48]	-	-	-										13	-	74.1
Becker et al, 2004 n=254 [49]	-	-	-										-	-	-
Koch et al. 2007 n=239 [50]	-	-	-										18.8	0.93-744	123
Koch et al. 2003 n=85 [51]	-	-	-										21	1.2-268	146
Kasper-Sonnenberg et al. 2012 n=105 [52]	-	-	-										11.7	0.5-368	62.9
Becker et al. 2009 n=599 [46]	-	-	-	5.4	<LOD-86.7	28.9	11	<LOD-198	50.6	12.7	<LOD-195	58.9	18.1	<LOD-468	76.2
Larsson et al. 2017 n=113 [54]	-	-	-	5.6	0.7-453	35	12	1.3-1100	93	17	1.7-2300	128	8.6	1-95	45

All studies collected morning urine, except of Fromme et al.[47] and Larsson et al.[54] (spot urine samples); all urine samples were collected from children, MED: Median, Min-Max: minimum to maximum, 95th P.: 95th Percentile.

1I1I5 Short description of certain phthalates

According to the Annex XVII to Regulation (EC) No 1907/2006 of the European Parliament and of the Council, toys which contains DEHP, BBzP or DnBP in a concentration greater than 0.1% by weight of plastics is not allowed to be placed on the EU market. DINP is forbidden to have higher concentration of 0.01% per weight in toys and childcare articles [14]. BBzP, DEHP and DnBP are also classified as substances of very high concern and are listed in REACH [54].

1I1I5I1 Butyl benzyl phthalate (BBzP)

Butyl benzyl phthalate is produced by esterification of phthalic anhydride. The resulting monobutyl ester of the phthalic acid reacts with benzyl chloride to form BBzP. The physicochemical properties of BBzP are described in Table 3. From 1994-1997 36,000 tons p.a. of BBzP were produced and used within the European Union. The use and production of BBzP has decreased since 2004 (19,500 tons p.a.), because it was classified as toxic, possibly causing harm to unborn children (R61), risking impaired fertility (R62) and effecting the environment negatively, especially aquatic organisms (R50-53). According to Directive 76/769/EEC, the marketing and use of BBzP and preparations containing BBzP intended for consumer use is prohibited, which means it is banned in cosmetic products, toys and childcare articles [55]. BBzP is mainly used as a plasticizer in PVC products, especially in flooring. Besides PVC, sealants, adhesives, paints, ink and lacquers contain BBzP [56].

Table 3. Physicochemical characteristics of BBzP [56]

IUPAC Name	Benzyl butyl phthalate	
CAS Number	85-68-7	
Molecular formula	$C_{19}H_{20}0_4$	
Molecular weight	312.35 g/mol	
Physico-chemical properties	Physical state:	Clear oily liquid
	melting point	< -35°C
	boiling point:	370°C at 10.10 hPA
	relative density	1.116g/cm³ at 25°C
	vapor pressure	0.00112 Pa
	water solubility	2.8 mg/l at 20-25°C
	Partition coefficient n-octanol/water	4.84
Structural formula		

Toxicokinetics of BBzP

For the general population, the most probable means of BBzP exposure are ingestion and inhalation of indoor air. The absorbed BBzP is metabolized to mono butyl phthalate (MnBP) and mono benzyl phthalate (MBzP) in the gut wall and/or in the liver (Fig. 2). In rats, where most toxicokinetic studies have been made, the ratio of MnBP and MBzP is 3:1. On the contrary, in humans, the main metabolite and therefore an adequate biomarker seems to be MBzP. Based on limited data, the half-life time seems to be less than 24 hours [56].

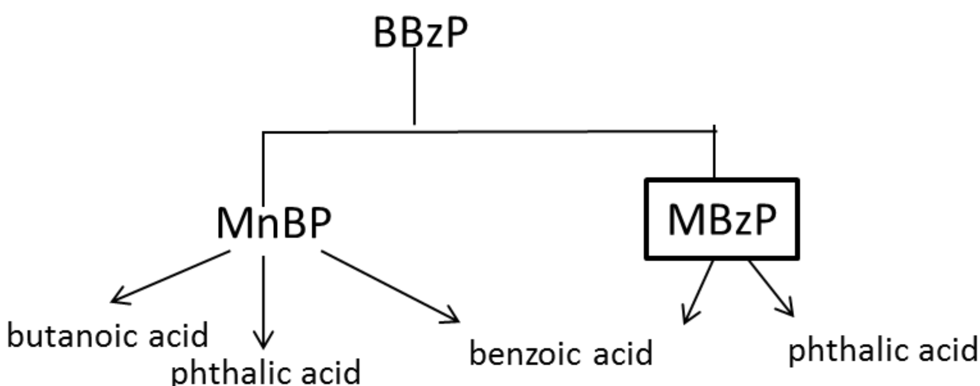


Figure 2. Schematic view of the Metabolism of BBzP [56]. BBzP: Benzylbutyl phthatale, MnBP: mono-n-butyl phthalate, MBzP: mono-benzyl phthalate. The framed metabolite MBzP is qualified as a biomarker.

Health effects

This phthalate has a low acute toxcixity (oral LD_{50} 20,400 mg/kg). Repeated dose toxicity studies (mainly in rats) resulted in decreased body weight gain, negative effects to organs as liver, kidney, spleen and pancreas and male genital tract as testes, epididymis and prostate. Besides altering the semen parameters (already mentioned above), BBzP is inversely related to the anogenital index (AGI). Boys with a high prenatal maternal urinary MBzP concentration had a higher probability of a lower AGI [56].

BBzP is associated with rhinitis, eczema (atopic dermatitis), allergy and a higher risk of asthma especially for children (reviewed by Bekö [57]). In an epidemiological study conducted by Bornehag et al. [45], a relationship between the BBzP concentration in children's bedroom dust and diagnosed rhinitis or eczema was determined. Several *in vivo* and *in vitro* studies indicate a weak estrogenic as well as an anti-androgen-like activity after a high dose of BBzP [56].

1I1I5I2 Di-n-butyl phthalate (DnBP)

DnBP production occurs through the reaction of phthalic anhydride with n-butanol in the presence of concentrated sulphuric acid (catalysator). DnBP is used as a gelling aid (for cellulose ether, polyvinyl acetate dispersion), a lubricant (textile manufacture), an antifoam agent or a solvent (oil-soluble dyes, insecticides, peroxides etc.). The enteric coating material in medications or food supplements also contains DnBP. Formerly, DnBP was added to cosmetic products to enhance durability and smoothness but since April 2005, it is no longer available on the European market. However, this plasticizer can be found in a wide range of end products like textiles, coatings and the primary packaging of medicines [58, 59].

Based on the European restriction, the production and use of DnBP has decreased from 26,000 tons in 1998 to 10,000 tons in 2007. The physicochemical characterization of DnBP is given in Table 4.

Table 4. Physicochemical characteristics of DnBP

IUPAC Name	1,2-Benzendicarboxylic acid dibutyl ester	
CAS Number	84-74-2	
Molecular formula	$C_{16}H_{22}O_4$	
Molecular weight	278.34 g/mol	
Physico-chemical properties	Physical state:	Oily liquid
	melting point	-69°C
	boiling point:	340°C at 1,013hPa
	relative density	1.045g/cm³ at 20°C
	vapor pressure	9.7±3.3x10^{-5} hPa at 25°C
	water solubility	10 mg/l at 20°C
	Partition coefficient n-octanol/water	Log K_{ow} 4.75
Structural formula		

Toxicokinetics of DnBP

The exposure to DnBP occurs mainly through oral uptake, where it is rapidly absorbed and mainly excreted in urine within 48 hours. Fecal excretion is very low and negligible. Compared to oral uptake, dermal absorption is with 2.4 µg/cm²/hour relatively slow. After dermal exposure to rats, 60 % of the dose was excreted within 7 days [60].

The metabolism of DnBP was investigated by Koch et al. [59]. Di-n-butyl phthalate is metabolized to mono-n-butyl phthalate (MnBP) and then further oxidized to its metabolites 3OH–mono-n-butyl-phthalate (3OH-MnBP) and 4OH-mono-n-butyl-

phthalate (4OH-MnBP). The latter is then metabolized to 3-carboxy-mono-propylphthalate (MCPP) (as shown in Figure 3). In this study, it was postulated that 84% of the applied DnBP doses was excreted as the primary metabolite MnBP, with a half-life time of 2.6 hours. Therefore MnBP is an appropriate biomarker for biomonitoring studies.

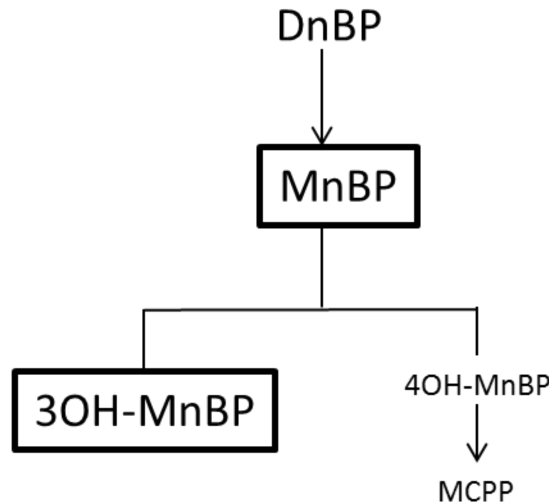


Figure 3. Schematic view of the DnBP metabolism (according to Koch et al. [59]). DnBP: di-n-butylphthalate; MnBP: mono-n-butylphthalate; 3OH-MnBP: 3OH-mono-n-butylphthalate; 4OH-MnBP: 4OH-mono-n-butylphthalate; MCPP: 3carboxy-mono-propylphthalate; the framed metabolite are qualified biomarkers.

Health Effects

DnBP is classified as reprotoxic. DnBP seemed to have more of an anti-androgenic effect than estrogenic. Reproductive toxicity studies in male rats showed increased incidence of undescended testes, hypospadias, malformation of reproductive organs and nipple retention. The underlying mechanism might be a decrease in the fetal testicular testosterone production in Leydig cells. DnBP is associated with sperm motility and weak evidence of an altered morphology was found [39]. In a Chinese case control study, a relationship between a higher meconium DnBP metabolite level and lower birth weight in infants was found [17]. The No observed adverse effect level (NOAEL) ranges between 20 to 50 mg/kg body weight per day [61].

1I1I5I3 Bis (2-ethylhexyl) phthalate (DEHP)

The production of DEHP occurs through the esterification of phthalic anhydride with 2-ethyl-hexanol [15]. DEHP is mainly used as plasticizers in polymer products, especially in flexible PVC (up to 30 % DEHP content), which is used in many different products as toys, building material (flooring), cables and medical products (tubes, blood bags, etc.). Besides PVC, it is also used in polymer and non-polymer formulations and products like sealants, paints lacquers or ceramics. In 1997 the volume of production of DEHP was estimated to be 595,000 tons p.a. in Western Europe. Since 2004 the use of DEHP has decreased to 221,000 tons p.a., while the production and use of phthalates to substitute DEHP, DINP and DIDP has increased. Based on the fact that DEHP slowly migrates from polymer products during their complete lifetime, both humans and the environment are constantly exposed to DEHP [62]. Its physicochemical properties are given in Table 5.

According to Annex I of Council Directive 67/548/EEC [63], DEHP is classified as toxic to reproduction (Category 2; R60-61).

Table 5. Physicochemical characteristics of DEHP

IUPAC Name	Bis (2-ethylhexyl) phthalate	
CAS Number	117-81-7	
Molecular formular	$C_{24}H_{38}O_4$	
Molecular weight	390.56 g/mol	
Physico-chemical properties	Physical state:	Colourless oily liquid
	melting point	-55°C
	boiling point:	385°C at 1013hPa
	relative density	0.98 g/cm³ at 20°C
	vapor pressure	0.000034 PA at 20°C
	water solubility	3 µg/l at 20°C
	Partition coefficient n-octanol/water	7.5
Structural formula		

Toxicokinetics of DEHP

The DEHP exposure occurs through oral, inhalation, dermal or intravenous pathways. The main exposure source is intake of food which is DEHP-contaminated either by general environmental pollution or by contact with DEHP containing materials, DEHP handling and preparation processes.

After oral uptake, DEHP is rapidly absorbed and metabolized in the gastrointestinal tract. DEHP is quickly hydrolyzed to mono (2-ethylhexyl) phthalate (MEHP -primary metabolite) and 2-eythalhexanol (2-EH) by lipases in the small intestine. In the liver, MEHP is further modified by several side-chain oxidation and hydroxylation reactions. A toxicokinetic study conducted by Koch et al.[13] showed that after 24 hours 67 % of the applied DEHP dose was excreted as five of the major metabolites: 2-ethyl-5-hydroxy-hexylphthalate (5OH-MEHP; 23.3 %), 2-ethyl-5-carboxy-pentylphthalate (5cx-MEPP; 18.5 %), 2-ethyl-5-oxy-hexylphthalate (5 oxo-MEHP; 15 %), mono (2-ethylhexyl) phthalate (MEHP; 5.9 %) and 2-(carboxymethyl)-hexylphthalate (2cx-MMHP; 4.2 %). On the second day only 3.8 % of DEHP was excreted as 2cx-MMHP (1.6 %), 5cx-MEPP (1.2 %), 5OH-MEHP (0.6 %) and 5 oxo-MEHP (0.4 %). Further it was shown that the secondary metabolites have longer elimination half-life time and a later concentration maximum as the monoester MEHP. The long half-life elimination qualifies 5cx-MEPP and 2cx-MMHP (12-15 hours and 24 hours) as biomarkers to measure time-weighted exposure, while 5OH-MEHP and 5oxo-MEHP (10 hours) represent more a short-term exposure to DEHP. A dose dependency in metabolism and excretion was not observed. A graphic scheme is shown in Figure 4.

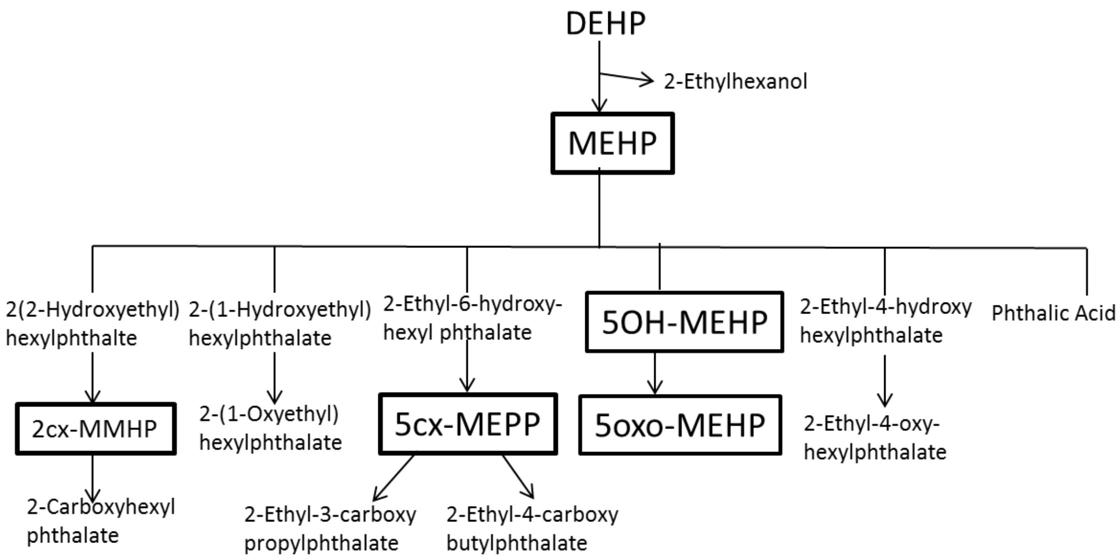


Figure 4. Schematic view of the DEHP metabolism (according to Koch et al. [64]), the framed metabolites are qualified biomarkers.

Health effects

The acute toxicity of DEHP is very low. The oral LD_{50} ranged between >20,000 mg/kg b.w. (rats) and > 10,000 mg/kg b.w. (mice). DEHP is not reported to be mutagenic or carcinogenic. In several repeat dose toxicity studies (orally administered), it was shown that DEHP induces toxicity to testes and kidneys. In rodent studies, DEHP affected fertility and reproduction in both sexes and also influenced development in the offspring. Further, it was shown that DEHP-induced testicular toxicity causes less harm to a sexually mature animal than to a developing and prepubertal animal. The primary metabolite (MEHP) is suspected to be the active metabolite, which affects testes and reproduction. The main target of DEHP (MEHP induced testicular toxicity) are the Sertoli cells and Leydig cells. Further, it decreases the capacity of the follicle stimulating hormone (FSH) in Sertoli cells, zinc and the testosterone levels in the testes. Low zinc levels in testes enhance the susceptibility to gonadotoxic effects in male rodents. Based on limited human data, the observed NOAEL for testicular effects of 4.8 mg/kg/d in animal studies was considered to be relevant for humans as well. Besides testicular effects, some research indicates that oral dosing of DEHP cause hypo-oestrogenic anovulatory and polycystic ovaries in adult female rats. An alteration in the oestrus cycle with resulting changed testosterone and oestradiol concentration in ovary cells was observed after DEHP administration to female rats. Developmental toxicity has been observed in several studies, the rats male pups

showed DEHP-induced malformations, e.g. demasculinisation or reduced AGD by inhibiting fetal testosterone production. In vitro as well as in vivo studies indicate that DEHP interferes with the endocrine function and might have antiandrogen effects. Therefore DEHP can influence sexual differentiation [15].

Besides reprotoxicity, DEHP might influence the thyroid hormone system. An inverse association between MEHP and free T4 and total T3 was observed. More data are needed to determine the importance of such an association [43].

From an epidemiological point of view, a significant association was found with a high concentration of DEHP in children's bedroom dust and diagnosed asthma [45].

1I1I5I4 Diisononyl phthalate (DINP)

There are three different kinds of DINP, which are also produced in different ways. The first DINP (CAS 68515-48-0) is made by the "polygas" process, the second one (CAS 28553-12-0) is n-butene based and the third one (CAS 28552-12-0) is produced n- and iso-butene based. DINP 1 is a mix of esters of the o-phthalic acid with C8-C10 alkyl alcohols of different chain lengths and branching distribution, whereas DINP 2 has only isomeric C9 alcohols in the ester chain. Since 1995, the production of DINP 3 (CAS 28552-12-0) has stopped. Based on the different chemical structures, the three DINPs may have different physicochemical and toxicological characteristics (the general ones are shown in Table 6) [65, 66].

In 1994, 107,200 t of DINP were produced in the European Union. Because of low toxicity, DINP is replacing DEHP and therefore the production of DINP has increased in the last two decades. Ninety-five% of the DINP produced is used as a plasticizer in PVC products, the other five % are mainly used in polymer products (e.g. rubbers).

Table 6. Physicochemical characteristics of DINP [67]

IUPAC Name	1,2-Benzenedicarboxylic acid, di-C_{8-10} branched alkyl esters, C_9 rich and di-“isononyl”phthalate	
CAS Number	685515-48-0 and 28553-12-0	
Molecular formula	$C_{8+2x}H_{6+4x}O_4$ with x=8 to 10 (x=9 as main constitution) → $C_{26}H_{42}O_4$	
Molecular weight	420.6 (average) g/mol	
Physio-chemical properties	Physical state:	Oily viscous liquid
	melting point	Ca. -50°C
	boiling point:	>400°C
	relative density	ca.0.975 at 20°C
	vapor pressure	$6*10^{-5}$ Pa at 20°C
	water solubility	0.6 µg/l at 20°C
	Partition coefficient n-octanol/water	8.8
Structural formula	[67] [68]	

Toxicokinetics of DINP

The routes of DINP exposure are mainly oral through consumer products, food or toys. The dermal or inhalation pathway is very limited. The dermal uptake of DINP is very slow (4 % of the dose within 7 days).

After the oral uptake, DINP is rapidly hydrolyzed to MINP in the gastro-intestinal tract (GIT) and then absorbed. MINP undergoes an oxidative metabolism by the ω-oxidation (oxidation of the terminal carbon atom of the side chain) or ω-1 oxidation (oxidation of the penultimate carbon atom of the side chain) pathway to form secondary metabolites with hydroxyl-, oxo- and carboxyl- functional groups (7OH-MINP, 7oxo-MINP and 7cx-MINP) [12] (see Figure 5). The distribution compartments are the liver, kidney and blood but DINP does not accumulate in the body. In a toxicokinetic study conducted by Koch et al. [69], 43.6 % of the applied dose was excreted mainly as OH-MINP (20.2 %), carboxy-MINP (10.7 %), oxo-MINP (10.6 %) and MINP (2.1 %) within 48 hours. The estimated half-life time is between three to five hours within the first elimination phase of 24 hours post dose. In rodents, a limited absorption in a high dose range was observed [11].

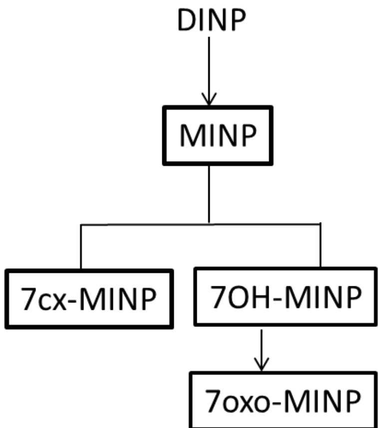


Figure 5. Schematic view of the DINP metabolism (4 methyl-octyl side chain) [69], DINP: Di-isnonylphthalate, MINP: mono (4 methyloctyl) phthalate, 7OH-MINP: -mono-(4-methyl-7-hydroxyoctyl) phthalate (7OH-MMeOP); 7-oxo-MINP: mono (4-methyl-7oxooctyl) phthalate (7oxo-MMeOP); 7cx-MINP: mono (4-methyl-7-carboxy-heptyl) phthalate (7-carboxy-MMeHP).

Health effects

DINP has a low acute toxicity and showed no irritant effects on skin, eyes or the respiratory system. Neither mutagenic nor carcinogenic effects were observed. In repeated-dose toxicity studies in rats or mice, no effects on testes weight, estrogen activity and developmental malformation were observed, but biochemical changes in liver (increased asparate-aminotransferase (AST) and alanine aminotransferase (ALT) and an increased liver weight. Only one study showed a reduced mice testis weight after a high dose of DINP (5.7 mg/kg/d). The EU did not classify DINP[14].

1I2 Dust

In industrial countries, humans spend 60 to 90% of their time indoors, either at home, in offices or in transportation facilities. The indoor environment is a significant source of pollutants which are found in various products like detergents, furniture, flooring and wall covering material. In addition, indoor activities such as smoking and cooking as well as the frequency of air exchange can influence the level of pollutants in the indoor environment. The greater amount of time spent indoors, limited room ventilation, and slower chemical degradation rates lead to higher indoor pollutant exposure compared to outdoors. Analyzing indoor dust can give information about the presence of, and exposure to, pollutants in indoor environments. House dust is linked to adverse health effects like asthma and allergies because it is a transport

medium for allergens, heavy metals, semi- and non-volatile chemical substances, e.g. phthalates or polybrominated diphenyl ethers [70-74]. Dust is characterized as indoor particles that have settled on the surface of objects, as well as floors and carpeting. It also includes soil particles or other organic matter that has been tracked or blown into the indoor environment from outside [75]. Dust composition differs not only between indoors and outdoors, it also varies between the kitchen, living room and bathroom of a dwelling [76]. Hawley et al. [25] estimated that the average amount of dust on an indoor surface is approximately 560 mg/m². Dust contains human and animal skin fragments and hair, paper fibers, glass wool, textile fibers, organic, inorganic and metal particles, in descending order [73]. The particle size of dust caused by abrasion from furniture and everyday objects ranges from 0.001 to 1 mm [77].

1I2I1 Phthalates in dust

As a consequence of their ubiquitous usage, phthalates are one of the most frequently occurring compounds in house dust. Phthalates are released as vapor from the flooring material containing PVC which is then absorbed by particles in indoor air.

Phthalates like DEP, DnBP and DiBP tend to exist as gases and are therefore more frequently found in indoor air, whereas DEHP, DINP and BBzP, being less volatile, are more common in house dust [78]. Based on the lack of degradation or dissipation of the organic pollutants which are bound to dust, organic pollutants have a high exposure potential [79]. The composition of house dust is non-homogeneous and the phthalate concentrations are highly variable. It has been shown that phthalate accumulations strongly depend on particle size distribution in house dust [30]. Wang et al. [80] investigated the accumulation rate of phthalates in different particle sizes < 63 µm, 63 - 100 µm, 100 - 280 µm and 280 - 2,000 µm; the highest rates were found at < 63 µm and 63 - 100 µm. Bioaccessibility decreased by increasing particle size. Particles with <63 µm are associated with a higher health risk for humans than particles with diameter of 280 - 2000 µm. Furthermore, it was shown that particles < 250 µm have the highest capacity to be collected by the human hand [29].

Table 7 gives an overview over the phthalate concentration in house dust.

Bornehag et al. [81] measured the phthalate content in dust in relation to building types in 390 homes in Sweden. The phthalate most frequently and with the highest levels of concentration detected in dust was DEHP, followed by DnBP and BBzP. In this study it was shown that flooring materials influence the phthalate concentration. Apartments with PVC flooring have a higher DEHP and BBzP content in dust compared to apartments with no PVC. In addition to PVC, there are other sources which influence the DEHP background concentration because the average DEHP content in dust in PVC flooring apartments was found to be around 0.7 mg/g, whereas in dwellings containing no PVC, the DEHP content was 0.55 mg/g. In contrast, this effect was not observed for BBzP. In other studies published by Fromme et al. [47] did not observe this effect. Hwang et al. [79] investigated the exposure of endocrine disrupting chemicals, like phthalates, polybrominated diphenyl ethers and polychlorinated biphenyls in dust samples from 10 apartments and one community hall in California, USA. This study revealed that DEHP was the most frequently detected pollutant in all dust samples and its concentration was 104-7630 µg/g which is two to six times higher compared to other pollutants.

DnBP is associated with compressed wood floors because DnBP is often used as a gloss agent in plastic additives or paint for such floors. Furthermore, an association between the frequency of cleaning the floor and DnBP was found, based on the fact that DnBP is often a component in cleaning products [82].

Table 7. Phthalate concentration in dust [mg/kg]

Source	BBzP			DEHP			DnBP			DINP		
	MED	Min-Max	95th P	MED	Min-Max	95th P	MED	Min-Max	95th P	MED	Min-Max	95th P
Fromme et al,**2013 n= 63 [47]**	6	0.1-348	93	888	99-10086	7616	21	2-266	95	302	30-7091	2955
Bamai et al **2013 n=128 [82]**	2	1.0-139		1110	213-7090	-	16.6	2.0-1670	-	139	11.9-2100	-
Wang et al.,**2013 (Hong Kong) n=20, [80]**	4.28	0.61-81.9	-	528	96.8-2190	-	4.8	0.25-17.4	-	-	-	-
Blanchard et al. **2014, n= 30 [78]**	8.5	-	-	289	-	-	11.9	-	-	130	-	-
Langer et al. **2010 n=497, [83]**	3.7	0.7-285	-	210	12.7-6611	-	15	0.18-253	-	-	-	-
Guo et al. **2011 (China), n=75 [84]**	0.2	n.g-12.0	-	228	9.9-8400	-	20.1	1.5-1160	-	-	-	-
Guo et al. **2011 (USA) n=33,** [83]	21.2	3.6-393	-	304	37.2-9650	-	13.1	4.5-94.5	-	-	-	-
Larsson et al. 2017,**(Sweden) n=100**,**[53]**	8.7	-	110	290	-	1900	21	-	140	380	-	3400
Abb et al. 2009 **(Germany)** n=30 **[72]**	15.2	-	-	604	-	-	29	-	-	129	-	-

All dust samples were sieved <63µm except of Blanchard et.al. (100µm), Guo et al. (2mm) from vacuum cleaning bags; Fromme et al. [47], Langer et al.[83], Bamai et al [82] Larsson et al. [53] used filters.

1I2I2 Exposure through dust

House dust is suspected to be an important exposure pathway to environmental pollutants. Especially young children ingest a high amount of dust via a high hand to mouth or object to mouth frequency. Because of their lower body weight, children have a relative high dust uptake, which can cause a potential health risk [85].

In general there are three different approaches to estimate the exposure through dust.

1. Tracer element methodology: tracer as heavy metals as e.g. lead in dust, are used to quantify the amount of dust intake. Ideally the tracer does not be metabolized and excreted via feces or urine and it's only found in high concentration in dust. Estimated dust uptake ranged between 26 - 470 mg/d.
2. Biokinetic model comparison methodology: This method is used to compare biomarkers in urine or blood from the toxicant with exposure pathways as diet, air, dust and soil. With data from the literature the dust uptake is expected to be 100 mg/d.
3. Active pattern methodology: It is a combination of analyzing the frequencies of hand to mouth and object mouth activity and time spend outdoor and indoors. Estimated dust and soil uptake of 10-1000 mg/d [85].

Based on the literature and model data's, the US-EPA estimated that the average uptake of dust ranged between 30 to 60 mg/d. Infants (six weeks to < one year) and adults have an unintended oral uptake of 30 mg, while toddler and children (one year to six years and six to twenty-one years) ingest 60 mg dust per day. For the age group three to six years, an upper percentile of 100 mg dust per day was defined [75, 85].

The Danish authorities [58] predicted the DnBP ingestion on a daily basis to be around 2.3 μg/kg b.w. in summer (50 mg of dust ingestion/day) and 4.1 μg/kg b.w. in winter (100 mg of dust per day).

Depending of the phthalate and its concentration in dust, oral exposure ranges between 0.5-21 μg/kg per day (details are shown in Table 8) [86].

Table 8. Phthalate intake through dust ingestion (reviewed by Oomen etal. [86])

Phthalate	Mean ingestion rate [µg/kg/d]		Maximum ingestion rate [µg/kg/d]	
	Adult	Child	Adult	Child
BBzP	0.23	2.1	32	304
DEHP	2.3	21	29	270
DIDP	0.052	0.49	0.05	0.49
DINP	0.12	1.2	0.12	1.2
DiBP	0.060	0.56	0.06	0.56
DnBP	0.16	1.5	3.9	36

Kang et al. [87] investigated the oral bioaccessibility[1] in dust by an *in vitro* digestion test. It was shown that the bioaccessibility of DEHP, BBzP and DnBP ranged between 10-15 %. By simulating the gastric and intestinal conditions, Wang et al. [80] published lower bioaccessibility levels as Kang et al. (2.2 - 12.6 % for DiBP, BBzP, DEHP, DIDP). Additionally, a higher bioaccessibility of DMP (15.5 %) at 63 µm dust size fraction compared to 0.14 % at 280 - 2000 µm was observed.

In a bioavailability study conducted by Freeman et al. [90], it was shown that arsenic in dust and soil is 3.5 to 5 times less bioavailable than arsenic solved in a solution. It indicates that there is an association with the n-octanol/water coefficient (K_{OW}) and bioavailability or bioaccessibility. The higher the K_{OW} value, the lower the oral bioaccessibility [91].

Several biomonitoring studies investigated the correlation between children phthalate body burden and the phthalate concentration in dust. Fromme et al. [47] measured the phthalate concentration in air and dust from German day care centers and phthalate metabolite concentration in 663 samples of children's urine after attending the investigated day care centers. A significant correlation of phthalates concentration in dust and concentrations of metabolites in urine was only found at the bivariate analysis level. Another study conducted by Fromme et al. [92] reported the occurrence of phthalates in indoor air and dust from 133 apartments and daycare centers in Berlin, Germany.

[1] Bioaccessability is the amount of a tracer that is soluble or accessible in the gastrointestinal environment but not necessarily available for assimilation, whereas bioavailabiliy means that, in addition to being accessible in the gastrointestinal environment, the ingested contaminant can reach the blood circulation and exert toxic effects. 88. Turner, A., *Bioaccessibility of Trace Metals in Household Dust A2 - Nriagu, J.O*, in *Encyclopedia of Environmental Health* 2011, Elsevier: Burlington. p. 317-322. 89. Yuswir, N.S., et al., *Bioavailability of heavy metals using in vitro digestion model: a state of present knowledge.* Rev Environ Health, 2013. **28**(4): p. 181-7.

They found that an unintended intake of dust (assumption of 100 mg dust per day) of young children could contribute 25 % of the total DEHP body burden.

In a comparative assessment of phthalate exposure through house dust in China and the USA conducted by Gou et al. [84], the results indicated that house dust intake accounts for less than 2.2 % of DiBP and DnBP and two to five percent of DEHP intake in China, whereas the intake rates in the USA were higher (1 – 16 % for DiBP and DnBP, 3 – 21 % for BBzP and 10 – 58 % for DEHP).

Langer et al. [48] observed significant correlations between DEP, DnBP, DiBP and BBzP in dust and their metabolites in children’s urine. For DEHP, there was no significant correlation with its observed metabolites.

It is still unclear how much the dust uptake contributes to phthalate exposure. The knowledge of bioavailability of phthalates in dust is very limited. Therefore the bioavailability of dust is often assumed to be 100 %. However, by this hypothesis, children could exceed the TDI through dust ingestion, but the results of human biomonitoring studies indicated that this is normally not the case.

1I3 The aim of the study

This study is conducted to examine the bioavailability of phthalates in house dust after oral administration to the model organism, the pig. In this study, we focused on phthalates which are highly present in dust and where biomonitoring data showed that the human is highly exposed to.

The aim of the study is to:

- Determine if dust is a potential source of phthalate exposure, especially for toddlers and infants;
- Investigate if the bioavailability of plasticizer differs in house dust and food matrices.

2I Material and Methods

In this *in vivo* bioavailability study of certain phthalates[2] in house dust, eight piglets (5-6 weeks old, male, 11-14 kg b.w.; one dropout) received five different dust samples and one food sample. The experiment took place from the 5th of October to the 15th of November 2015 at the Federal Institute for Risk Assessment in Berlin, Germany and was approved by the Regional Office of Health and Social Affairs (LAGeSo). The written approval for this animal experiment (Reg 0272/13) and the experiment plan (Table A1.) appears in the appendix.

2I1 Study design and sampling

The dust samples, as well as the food sample, were orally administered to the pigs in their morning feeding. The amount of dust given ranged from 432 mg to 832 mg (Table 9).

The food sample consisted of 25 µl of the 1 % ethanol-phthalate mixture. A stock solution for each phthalate was made (e.g.10 ml ethanol with 10.7 mg BBzP; 501.2 mg DEHP, 52.5 mg DINP, and 10.4 mg DnBP). Afterwards 23 µl of the BBzP stock solution, 2504.4 µl of the DEHP stock solution, 863.3 µl of the DINP stock solution and 44 µl of the DnBP stock solution were diluted in 25 ml ethanol. Table 10 gives an overview of the phthalates content in the dust samples. The chemicals which were used for the solution are listed in Table A2 in the appendix. The given dose was adjusted to the levels of the tolerable daily intake (TDI) of DEHP (50 µg/kg b.w.).

Table 9: Administered amount of dust [mg]

mg	Dust A	Dust B	Dust C	Dust D	Dust E
Pig 1	592.21	443.32	553.69	650.41	832.00
Pig 2	600.9	457.53	535.31	670.39	825.31
Pig 3	560.91	456.36	544.86	691.00	753.06
Pig 4	565.07	445.57	620.32	684.67	735.25
Pig 5	569.09	451.38	550.34	667.06	763.00
Pig 6	566.42	478.53	528.76	654.67	786.57
Pig 7	592.58	432.34	541.02	662.76	742.20

[2] Butylbenzyl phthalate (BBzP), Di-n-butylphthalate (DnBP), Diethylhexylphthalte (DEHP) and Diisononyl phthalate (DiNP)

Table 10. Overview of phthalate concentration in the administered dust and food sample

[mg/kg]	Dust A	Dust B	Dust C	Dust D	Dust E	Food
BBzP	30	180	50	4	20	19
DEHP	6900	8700	7000	5800	4800	2500
DnBP	70	50	240	50	10	31
DINP	1100	470	1700	2100	4300	857

For an eventual background exposure, a urine sample (control urine) was collected before the dust/food sample was given. After the oral administration, the urine was collected over a period of 38 h. The volume of the collected urine was noted and a maximum of 100 ml of the excreted sample was refilled in a polyethylene cup and stored at -20 °C until further analysis and measurement.

To avoid any bias, the order of the dust sample varied between the piglets. Also, before, during and after an experiment, to the extent possible, any contact with phthalates was avoided. After each single experiment, a three day washout period was followed to avoid any influence from the previous experiment. Figure 6 shows the experiment setting and a detailed description of the single experiment procedure is given in Table 11.

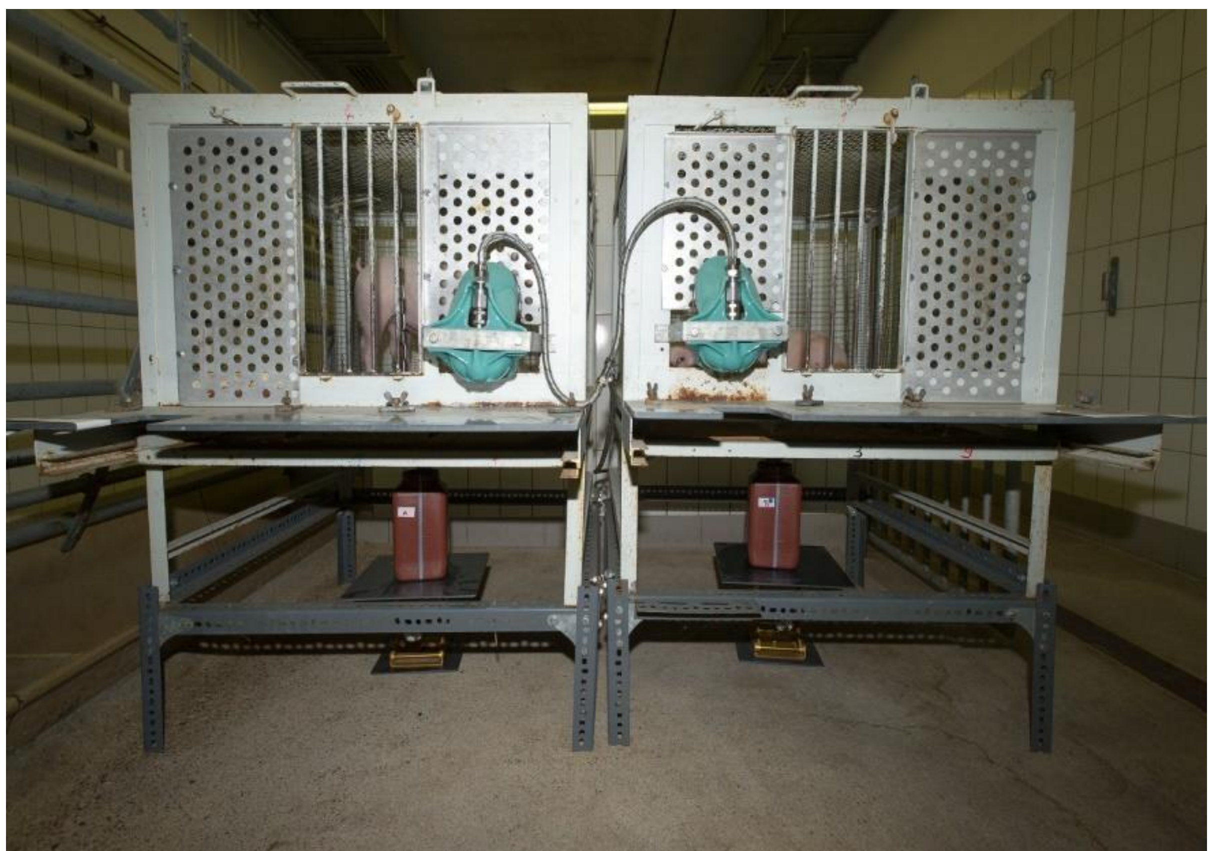


Figure 6. Metabolite cages with urine collecting vessels.

Table 11. Detailed schedule of a single oral experiment

Day	Sample no	Time	
Day 1		22:00	Piglets were put in the metabolite cages
Day 2	1	06:00	Urine sampling and sample administration
Day 2	2	07:00	Urine sampling
Day 2	3	08:00	Urine sampling
Day 2	4	09:00	Urine sampling
Day 2	5	10:00	Urine sampling
Day 2	6	11:00	Urine sampling
Day 2	7	12:00	Urine sampling
Day 2	8	13:00	Urine sampling
Day 2	9	14:00	Urine sampling
Day 2	10	15:00	Urine sampling
Day 2	11	16:00	Urine sampling
Day 2	12	17:00	Urine sampling
Day 2	13	18:00	Urine sampling
Day 2	14	20:00	Urine sampling
Day 2	15	22:00	Urine sampling
Day 3	16	06:00	Urine sampling
Day 3	17	10:00	Urine sampling
Day 3	18	14:00	Urine sampling
Day 3	19	18:00	Urine sampling

Day 3	20	20:00	Urine sampling
Day 3		20:00	Piglets left the metabolite cages

2I2 Pig keeping

The 6 week old piglets (Landschwein x Edelschwein, F1 generation x JSR Top Vital Pietrein) had access to water ad libitum and received 900 g of cooked potatoes (class: princess, from a local farmer, Germany) twice a day (6 am and 4 pm). During their three day wash-out period, 250 ml of Fresubin© (Fresenius Kabi, Germany) was added to their morning feed ration to avoid a nutrition deficiency. Two piglets shared a pigpen, the floor of which was covered with straw. The temperature in the daylight pigpen was regulated at 26 °C. The piglets were weighed regularly to verify their condition. During the total experiment period, they gained four to five kilograms each.

2I3 Analytical methods

2I3I1 Analysis of phthalates in dust

The dust sample originated from another study "Phthalates in German daycare centers: Occurrence in air and dust and the excretions of their metabolites by Children (LUPE3)" and were collected in several day care centers in Bavaria, Berlin and North-Rhine-Westphalia between November 2011 and May 2012 [47]. The five dust samples with the highest levels of phthalates were chosen for this project. The dust[3] from the vacuum bags of child daycare centers were sieved through a 63 µm sieve and stored in glass jars covered with aluminum foil. For analyzing the phthalate concentration, 100 - 150 mg of the sieved dust were distributed into glass vials and spiked with internal standard DMP-d4, DBP-d4, BBP-d4 and DEHP-d4. Subsequently, it was sonicated for 15 min and transferred to centrifuge tubes. The glass vials were washed with 5 ml MTBE and then centrifuged (3076 x g, 15 min, + 5 °C). The supernatant was refilled in brown glass screw cap vials (PTFE-silicone washer) for 15 min (3076 x g +5°C) then subsequently decanted in brown glass

[3] The dust originated from the bag of the vacuum cleaner from the child day care center. Those dust samples gives a representative overview of the general indoor phthalate exposure in the child day care centers.

screw cap vials (PTFE-silicone-washer). For quality measurement, control blank samples were prepared. The samples were analyzed by a gas chromatographic system with a mass selective detector in electron impact (EI) mode (Shimadzu GC-MS QP2010 with 30m/0.25mm ID/0.25 µm Phenomenex Zebron ZB-5 ms). The limit of detection (LOD) and limit of quantitation (LOQ) for DEHP was 0.3 ng/g and 1 ng/g, for DnBP 0.2 ng/g and 1 ng/g, for DINP 3 ng/g and 7 ng/g, and for BBzP 0.1 ng/g and 1 ng/g [47].

2I3I2 Phthalate metabolite analysis in urine

The phthalate metabolites were quantified with an accredited analytical method (QSP-O-1616-02) as previously published by Völkel et al. [93].

Sample preparation

200 µl of the thawed room temperature urine sample were mixed with 55 µl of ammonium acetate buffer (1M, pH 6.5, Riedl-de Haën), 10 ng of an internal standard mix (1 ng/µl), and 5 µl of β-glucuronidase (Type 2 H-2 from Helix promoatia, Sigma). For the enzymatic hydrolysis, the samples were placed in a thermomixer for 1.5h (500 l/min, 37 °C). After the enzymatic hydrolysis, 250 µl acetonitrile (LC-MS Grade, Fischer Chemicals) were added. The samples were centrifuged for 15 min at 20,800 x g to eliminate potential depositions. The sample solution was then decanted in a vial and filled up with 480 µl of 0.5 % formic acid (Roth, Germany).

For quality control, spiked samples consisted of 980 µl of control urine and 20 µl of native Standard mix. Two hundred µl of spiked urine were taken out and processed like the regular urine sample. In addition to the regular samples, the urine samples taken in the third and fourth hour after administration were diluted with purified water in a ratio of 1:10. The concentration values from diluted and undiluted samples were compared. Both values showed similar values. All samples were prepared as duplicates and each batch included a spiked urine sample (as well in duplicate) to control measurement quality.

Analytical methods, quantitation and chemicals

50 µl of the sample were injected into a high-performance liquid chromatography (HPLC) system (UlitMate 3000), using a column switching unit), where the separation occurred, which is coupled with an AB Sciex Q Trap 5500 tandem mass

spectrometer, where the mass detection was performed. The instrumental analytical method is listed in Table 12.

Table 12 Detail description of the used phthalate LC-method

Trap column		**25 µm, 20x2.1mm ID (Oasis HLB, Waters Oasis)**			
	Mobile phase	HPLC gradient water with 0.1% formic acid (Solvent A) &acentonitrile), with 0.1% formic acid (Solvent B)			
	Injection	50 µl			
	Time	0 - 2 min	2.1 - 16 min	16.1 - 20min	20.1 - 22 min
	Flowrate	1ml	0 ml	1ml	1ml
	Isocratic (Solvent B)	10%	10%	100%	10%
Analytical collumn		3µm, 150x3mm (Luna Phenyl-Hexyl, Phenomenex)			
	Mobile phase	HPLC gradient water with 0.1% formic acid (Solvent A) &acentonitrile with 0.1% formic acid (Solvent B)			
	Time	0 - 2 min	2 - 13 min	13.1 - 20 min	20.1 - 22 min
	Flowrate	0.4 ml	0.4 ml	0.4 ml	0.4 ml
	Gradient (Solvent B)	35%	35-65%	100%	35%

At the quadruple mass spectrometer, the following settings were used to detect the investigated analytes: a negative ion mode with curtain gas (N_2) 45 psi, nebulizer gas 55 psi, turbo gas 60 psi, heated gas temperature 600 °C, ion spray voltage -4000 V, dwell time 30 ms. Data quantitation was set in the multiple reaction monitoring (MRM) mode. The settings for the qualifier and quantifier ions, as well as the list of reference and internal standards are shown in the appendix in Table A3 and Table A4.

For quantification of the phthalate metabolites, a calibration curve with a known amount of metabolite concentration (0.5, 1, 2, 5, 10, 20, 50, 100, 200 and 400 pg/µl) was used (Figure 7). The limits of quantitation (LOQ) were 0.5 µg/l for oxo-MINP, oxo-MEHP, MODMOP, 5cx-MEPP; 1 µg/l for 7OH-MINP, 5OH-MEHP, MEHP; 1.3 µg/l for MHDMOP, 7cx-MINP, 1.5 µg/l for MINP; 2.5 µg/l for MnBP, MBZP and 5 µg/l for 2cx-MMHPP.

Figure 8 shows a chromatogram of all investigated metabolites phthalate metabolites.

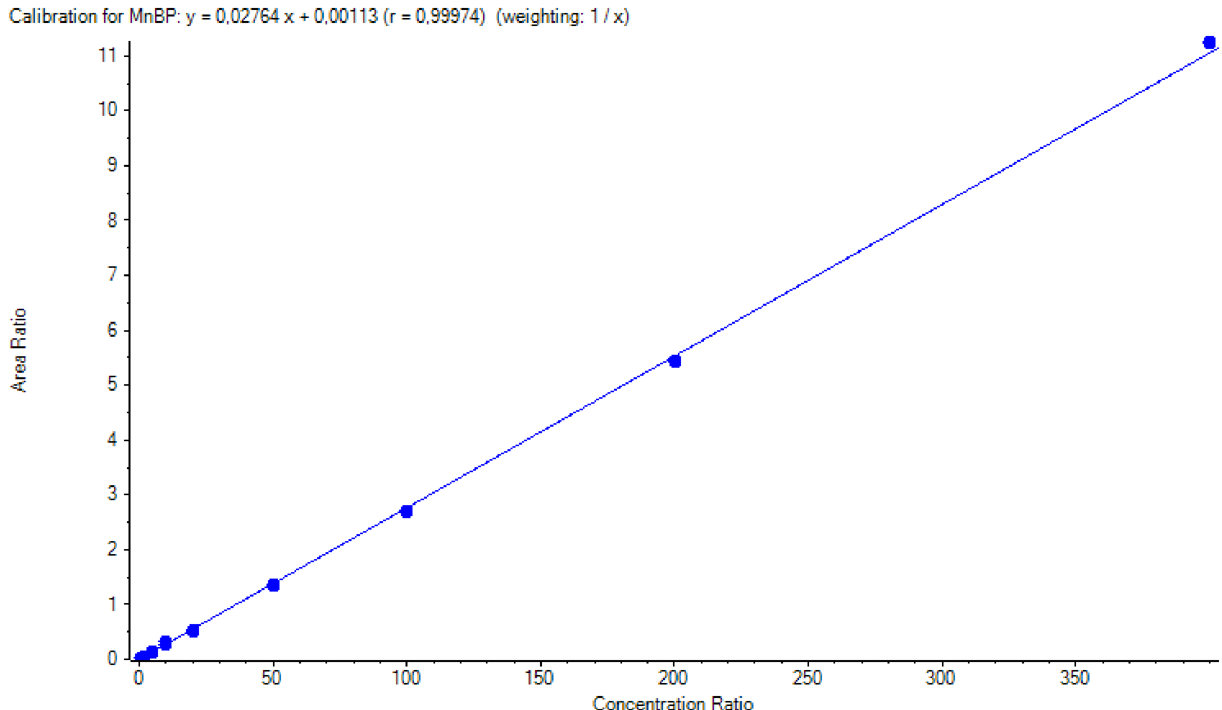


Figure 7. Example of calibration curve of MnBP.

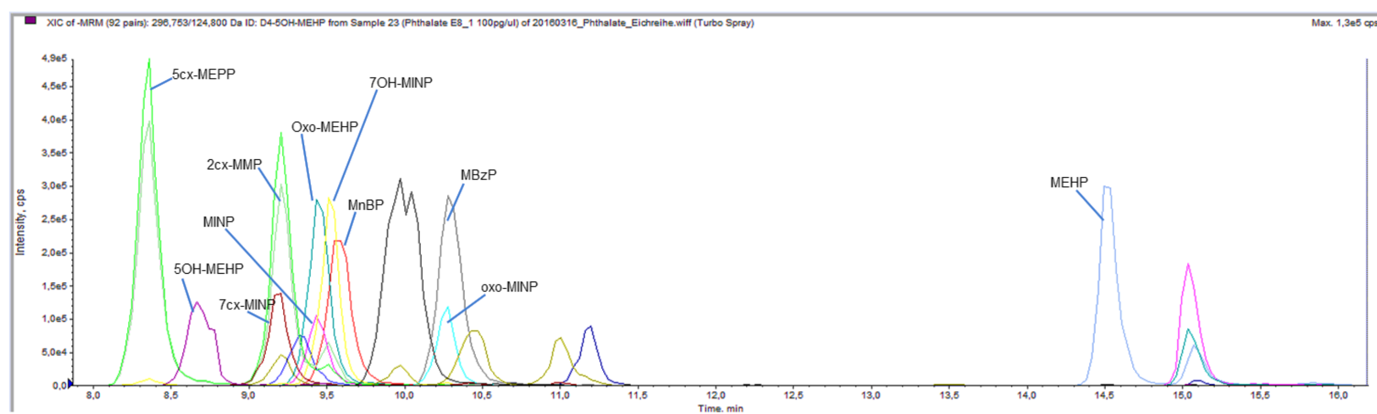


Figure 8. Chromatogram of phthalate metabolites [100 pg/µl].

2I3I4 Additional control measurements in indoor air

An indoor air sample (3.6 l/min, 1.32 m³ over 6 h) of both pigpens was collected once on a glass fiber filter and additionally on polyurethane foam by using a GGP sampler. The analytical separation of phthalates was performed by gas chromatography with subsequent detection by electron impact mass spectrometry in accordance with VDI guideline 4301-6 (2012). The indoor air phthalate concentration in the pigpens ranges between 50-59 ng/m³ (details are shown in Table 13).

The measurement and analysis were performed by the Department of Environmental Health Protection from the Berlin-Brandenburg State Laboratory.

Table 13. Phthalate indoor air concentration

	LOD [ng/m^3]	Pigpen A [ng/m^3]	Pigpen B [ng/m^3]
DnBP	10	50	59
BBzP	10	<	<
DEHP	50	<	55
DiNP	50	<	<

<: below LOD

2I3I5 Additional control measurements in animal food

To avoid further exposure to plasticizers, the potatoes and Fresubin were analyzed with an accredited method (PA 1.605) by the Fraunhofer Institute for Process Engineering and Packaging IVV. The method is described briefly as follows: For the phthalate determination, 10 g of potatoes were extracted by acetone (dest.) and n-hexane. Deuterium labeled standards (D_4-DEHP and D_4-DBP) were added. The supernatant was analyzed by GC-MS (Shimadzu QP5000; SIM-mode; Column ZB-50, 30 m x 0.25 µm; Temperature: 80 °C/1min - 10 °C/min – 300 °C/17 min). The results are shown in Table 14.

Table 14. Determination of phthalate concentration in food

	Potatoe sample	Fresubin
BBzP	< LOD (< 5 ng/g)	< LOD (< 10 ng/g)
DnBP	< LOD (< 2 ng/g)	< LOD (< 20 ng/g)
DEHP	< LOD (< 5 ng/g)	< LOD (< 20 ng/g)
DINP	< LOD (< 5 ng/g)	< LOD (< 600 ng/g)

2I4 Statistical analysis

The evaluation of urine analysis was performed with MultiQuant 2.1.1 (AB SCIEX) and for calculations and statistics Microsoft Excel 2010 and SPSS 19 (IBM) were used.

The phthalate values were normally distributed (Kolmogorov–Smirnov–Test, Dust: n=35; BBzP: p= 0.539; DEHP: p=0.275; DINP: p=0.142; DnBP: p= 0.708, Food: n=7; BBzP: p=0.684; DEHP: p=0.997, DINP: p=0.762; DnBP: p=0.918), therefore for

group comparisons (dust uptake versus dietary uptake), a T-test for independent samples was used.

Calculation of the phthalate uptake using urinary metabolite concentration

To reach the absolute concentration [µg], the measured concentration of the urine samples [ng/ml] was multiplied by the corresponding excreted urine volume [ml] and then divided by 1000. Subsequently, each absolute concentration was added to the other urine samples. The sum of the metabolite [µg] was then divided by the molecular weight [g/mol] of the metabolite. The excreted metabolite [µmol] was divided by the administered amount from the parent compound [µmol]. The sum was multiplied by 100 to calculate the percentage dose uptake [%]. The complete dose uptake, from dose application to 24 hours post dose and 24 hours to 38 hours post dose, was calculated. The molecular weight of the phthalates and their metabolites are given in Table 15.

Table 15. Molecular weight of phthalates and their metabolites

Phthalate	g/mol	Metabolite	g/mol
BBzP	312.37	MBzP	256.25
DEHP	390.56	MEHP	278.34
		5OH-MEHP	294.34
		oxo-MEHP	292.32
		5cx-MEPP	308.30
		2cx-MMHP	308.32
DINP	418.62	MINP	292.38
		oxo-MINP	306.35
		7OH-MINP	308.37
		7cx-MINP	322.35
DnBP	278.35	MnBP	222.23

Toxicokinetic calculations

The elimination half-life time ($t_{½}$) of the metabolites was estimated by using the following equation

$$t_{½} = Ln(2) * ke$$

k_{e}= elimination constants

It was observed that after 24 hours post dose, the excretion of the metabolites increased again. Therefore the half life time was calculated for each elimination period (first half life time: C_{max} to 16 hours post dose, second half life time: 24 to 38 hours). The urine sample with the highest concentration (C_{max}) is defined as t_{max}.

3I Results

Mainly no metabolites were detected in the control urine samples or only a concentration (< 1.5 µg/l) was detected. The control urine samples were not used for any calculation. Quality control samples were in good agreement with the expected concentration of 20 pg/µl (recovery was between 80 - 110 %). The coefficient of variation or the duplicate urine samples was below 15 %.

3I1 Excretion of plasticizers in the dust experiment

The mean administered dose of plasticizers in dust ranged from 2.7 µg (BBzP) to 4057 µg (DEHP) respectively (Table 16). For quality control, a reference dust was measured (SRM dust). The recovery in this dust sample was between 80 - 100 %. Additionally the dust samples were diluted (1:100), the diluted and undiluted concentration were in good agreement. The dust samples were analyzed in 2014 and 2016. Both measurements resulted in the same amount of concentration.

Table 16. The mean and standard deviation (SD) of the administered dose in µg

	BBzP	DEHP	DnBP	DINP
Dust A	17.3 ± 0.5	3989.4 ± 113.1	40.5 ± 1.1	636 ± 18
Dust B	84 ± 7.2	4056.7 ± 346.2	23.3 ± 2	219.2 ± 18.7
Dust C	27.7 ± 1.5	3874.3 ± 214.8	132.8 ± 7.4	941 ± 52.2
Dust D	2.7 ± 0.1	3878.5 ± 86.2	33.4 ± 0.7	1404.3 ± 31.2
Dust E	15.5 ± 0.8	3728.5 ± 187.7	7.7 ± 0.4	3340 ± 168

For low molecular weighted phthalates, the mean urinary excretion of the applied dose in dust measured 27.7 ± 17.6 % for BBzP and 52.2 ± 18 % for DnBP. The mean for high molecular weighted phthalates as DEHP is 43 ± 11 % and for DINP 47 ± 26 %, respectively. As shown in Figure 9, the mean excretion of BBzP, DnBP, DEHP and DINP for dust sample A was 22.5 ± 7 %, 55 ± 19 %, 44 ± 8.5 %, 75 ± 21 %; for dust sample B: 28 ± 12 % (BBzP), 42 ± 14 % (DEHP), 58 ± 12 % (DnBP); for dust sample C: 25 ± 13 % (BBzP), 45 ± 16 % (DEHP), 52 ± 28 % (DINP), 39 ± 15 % (DnBP), for dust sample D: 9.6 % (BBzP), 39 ± 6 % (DEHP), 37 ± 9 % (DINP) 46 ± 15 % (DnBP), for dust sample E: 54 ± 10 % (BBzP), 44 ± 6 % (DEHP), 23 ± 5 % (DINP), 69 ± 21 % (DnBP). Table 17 shows the urinary excretion for each plasticizer and dust sample in every single pig. Because of conflicting results in dust sample B,

DINP was rejected. For the same reason, the DnBP measurement was excluded for pigs 2, 4 and 5 in dust sample E.

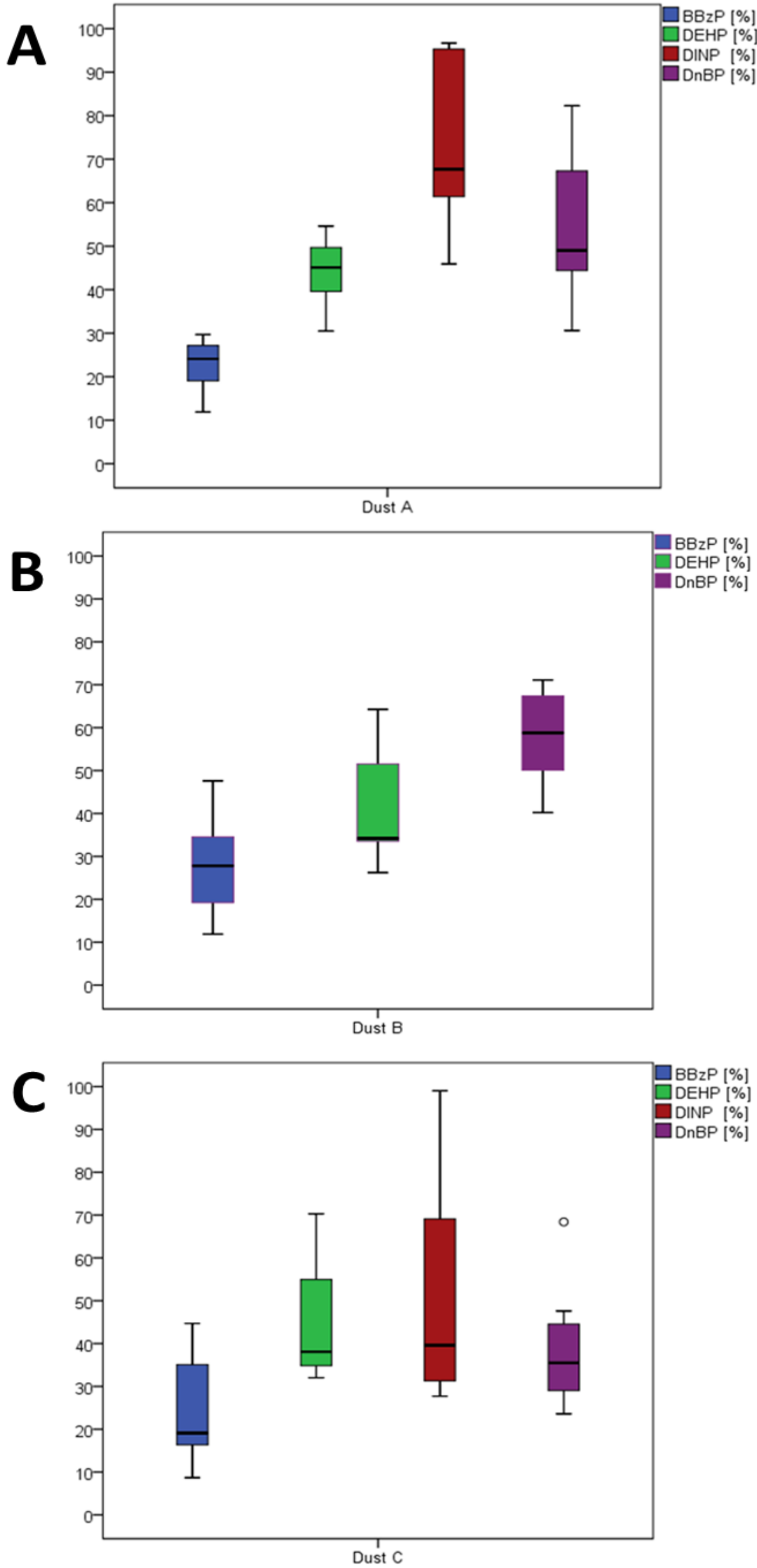


Figure 9. Excretion of phthalates in relation to the applied dose in %.

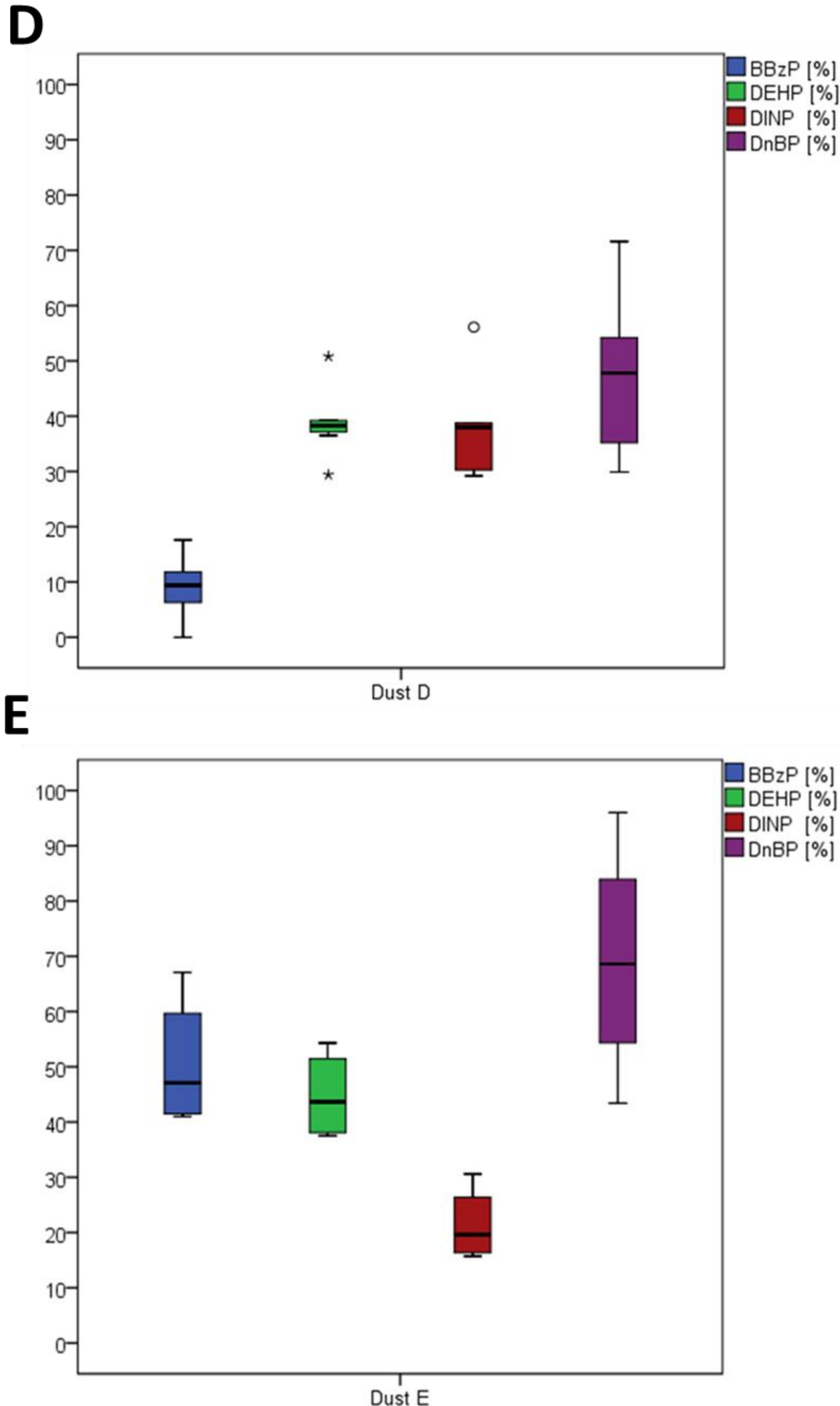


Figure 9. Excretion of phthalates in relation to the applied dose in %.

Table 17. The sum of phthalate metabolite excreted in urine (in % of the applied dose of each dust sample)

		BBzP	DEHP	DINP	DnBP
Dust A	Pig 1	23.9	49.6	93.9	49.0
	Pig 2	24.1	44.0	96.7	30.6
	Pig 3	25.3	35.2	60.5	75.6
	Pig 4	29.7	54.6	62.3	82.3
	Pig 5	14.2	45.1	67.7	59.0
	Pig 6	11.9	30.5	45.9	48.2
	Pig 7	29.0	49.8	96.7	82.6
Mean±SD		22.5 ± 7	44 ± 8.5	75 ± 21	55 ± 19
Dust B	Pig 1	39.2	48.0	-	71.0
	Pig 2	27.8	34.2	-	71.1
	Pig 3	22.6	33.9	-	40.2
	Pig 4	29.9	64.25	-	56.5
	Pig 5	11.9	33.2	-	43.7
	Pig 6	47.6	55.1	-	63.7
	Pig 7	15.9	26.2	-	58.8
Mean±SD		28 ± 12	42 ± 14		58 ± 12
Dust C	Pig 1	8.7	32.0	29.2	23.6
	Pig 2	28.5	41.1	57.9	47.6
	Pig 3	44.7	68.8	99.0	41.5
	Pig 4	14.5	32.5	27.7	30.6
	Pig 5	18.2	37.2	33.4	27.4
	Pig 6	19.1	38.1	39.6	35.5
	Pig 7	41.7	70.3	80.3	74.5
Mean±SD		25 ± 13	45 ± 16	52 ± 28	39 ± 15
Dust D	Pig 1	9.4	36.5	30.4	30.0
	Pig 2	11.2	39.1	38.0	49.4
	Pig 3	17.6	29.4	30.1	47.8
	Pig 4	8.0	39.3	29.2	31.6
	Pig 5	4.6	38.3	38.7	59.0
	Pig 6	-	37.8	38.8	38.8
	Pig 7	12.4	50.8	56.1	71.6
Mean±SD		9 ± 6	39 ± 6	37 ± 9	46 ± 15
Dust E	Pig 1	52.5	48.6	22.2	71.9
	Pig 2	60.2	50.2	29.7	-
	Pig 3	42.0	38.7	17.0	65.3
	Pig 4	55.0	39.6	22.6	-
	Pig 5	60.0	42.8	22.8	-
	Pig 6	67.1	57.3	30.6	96.0
	Pig 7	41.0	37.5	15.7	43.4
Mean±SD		54 ± 10	44 ± 6	23 ± 5	69 ± 21

3I2 Excretion of phthalates in the food experiment

The food sample contained 19 µg BBzP, 2500 µg DEHP, 31 µg DnBP and 857 µg DINP. The solution was afterwards analyzed by gas chromatograph to confirm the concentration.

As given in detail in Table 18 and Figure 10, the mean excreted amount of the applied dose in urine is 37 ± 23 % of BBzP, 53 ± 15 % of DEHP, 43 ± 12.5 % of DINP and 39 ± 16 % of DnBP. Similar to dust, pig 3 was left out for DnBP and BBzP. Pig 2 was excluded for DnBP, respectively.

Table 18. The sum of phthalate metabolites excreted in urine (in % of the applied dose of the food sample)

	BBzP	DEHP	DINP	DnBP
Pig E	69.2	61.3	50.8	61.2
Pig F	30.0	78.6	53.7	-
Pig G	-	59.0	56.6	-
Pig H	63.5	47.0	26.9	50.6
Pig I	19.1	37.7	27.7	32.6
Pig K	24.5	53.8	48.8	23.5
Pig L	17.8	36.3	35.8	26.2
Mean ±SD	37±23	53±15	43±12.5	39±16

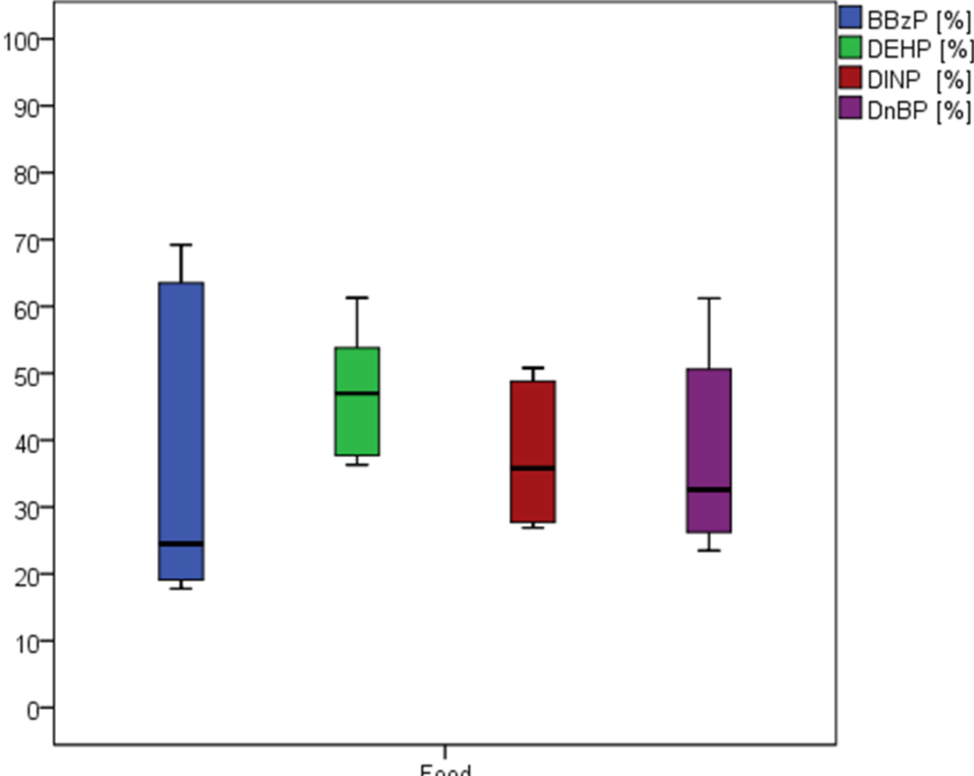


Figure 10. Excretion of phthalates in relation to the applied food sample in %.

3I3 Comparison the urine excretion of phthalates in dust and food

No significant differences between the quantities of plasticizers excreted in urine in the two experiments (dust and food ingestion) were observed (BBzP: p = 0.262; DEHP p = 0.358; DINP p = 0.055 and DnBP: p = 0.764). By testing the individual metabolites, only MEHP showed a slight significance (p = 0.05) (MBzP: p = 0.263; 5OH-MEHP: p = 0.839; 5oxo-MEHP: p = 0.08; 5cx-MEPP: p = 0.394; MINP: p = 0.021; oxo-MINP: p = 0.064; OH-MINP: p = 0.415; cx-MINP: p = 0.660 and MnBP: p =0.531). Table 19 and Figure 11 provide a detailed overview of the metabolite excretion of each pig in relation to the applied dose in dust and food.

Table 19. Mean ± SD of metabolite excretion in related to the applied dose in dust and food [%]

DEHP	Pig	MEHP		5OH-MEHP		oxo-MEHP		5cx-MEP	
		Dust	Food	Dust	Food	Dust	Food	Dust	Food
	1	9.0 ± 3.4	15.6	12.0 ± 2.5	17.7	8.3 ± 1.7	12.2	13.2 ± 1.7	15.8
	2	7.6 ± 1.8	13.0	9.0 ± 2.5	9.4	8.7 ± 0.9	18	16.4 ± 1.9	38.3
	3	9.0 ± 3.1	12.8	10.7 ± 5.0	12.0	9.0 ± 3.0	15.1	12.3 ± 5.0	19.1
	4	6.8 ± 2.5	6.5	11.5 ± 6.0	8.8	7.7 ± 1.5	7.7	20.0 ± 5.4	24
	5	4.8 ± 2.0	3.3	5.3 ± 1.6	5.8	9.0 ± 1.0	6.8	20.2 ± 2.4	21.8
	6	5.2 ± 2.4	4.7	9.5 ± 3.0	13.9	9.1 ± 4.2	10.5	19.5 ± 3.5	24.7
	7	7.4 ± 4.2	2.8	7.6 ± 2.8	8.3	12.1 ± 4.0	6.7	17.3 ± 4.8	18.5
	Total	7.1 ± 3.0	8.4±5.3	9.4 ± 4.0	10.8±4	9.2 ± 2.8	11±4.3	17.3 ± 4.8	23.2 ± 7.4
DINP		**MINP**		**oxo-MINP**		**OH-MINP**		**cx-MINP**	
		Dust	Food	Dust	Food	Dust	Food	Dust	Food
	1	34.0 ± 34.5	36.8	1.5 ± 0.7	2	3.5 ± 2.2	6.3	4.7 ± 1.5	5.7
	2	45.6 ± 32.0	41.5	3.6 ± 0.7	2.6	1.1 ± 0.6	3.2	5.2 ± 2.0	6.4
	3	44.8 ± 35.6	48.6	2.6 ± 0.4	3	1.2 ± 0.5	1.6	3.0 ± 1.3	3.4
	4	26.5 ± 19.0	20.3	2.2 ± 1.0	1.1	3.8 ± 1.3	3	2.8 ± 1.2	2.4
	5	32.0 ± 20.3	22	3.5 ± 1.6	3	2.4 ± 0.7	1.3	2.8 ± 1.0	1.5
	6	28.0 ± 10.6	36.6	3.0 ± 1.7	2.5	4.0 ± 1.8	5.8	3.4 ± 1.7	4.0
	7	50.3 ± 34.7	28.2	3.8 ± 2.4	2.3	2.0 ± 0.9	1	6.0 ± 3.0	4.4
	Total	37.3 ± 26.4	33.4±10.4	2.9 ± 1.4	2.3±0.6	2.6 ± 1.6	3.2±2.1	4.0 ± 2.0	4.0±1.7
BBzP, DnBP		**MBzP**		**MnBP**					
		Dust	Food	Dust	Food				
	1	26.7 ± 19.0	70	48.0 ± 24.0	57				
	2	30.4 ± 18.0	30	48.5 ± 17	-				
	3	30.4 ± 12.0	-	48.9 ± 15	-				
	4	27.4 ± 18.0	63.5	47.7± 20.3	47.8				
	5	21.8 ± 21.8	19	41.0 ± 10.5	33				
	6	29.0 ± 27.5	24.5	51.5 ± 28.4	23.5				
	7	28.0 ± 13.8	17.8	53.6 ± 15.0	26.2				
	Total	27.7 ± 17.7	37.3 ± 23	48.7 ±18.0	37.4±14.4				

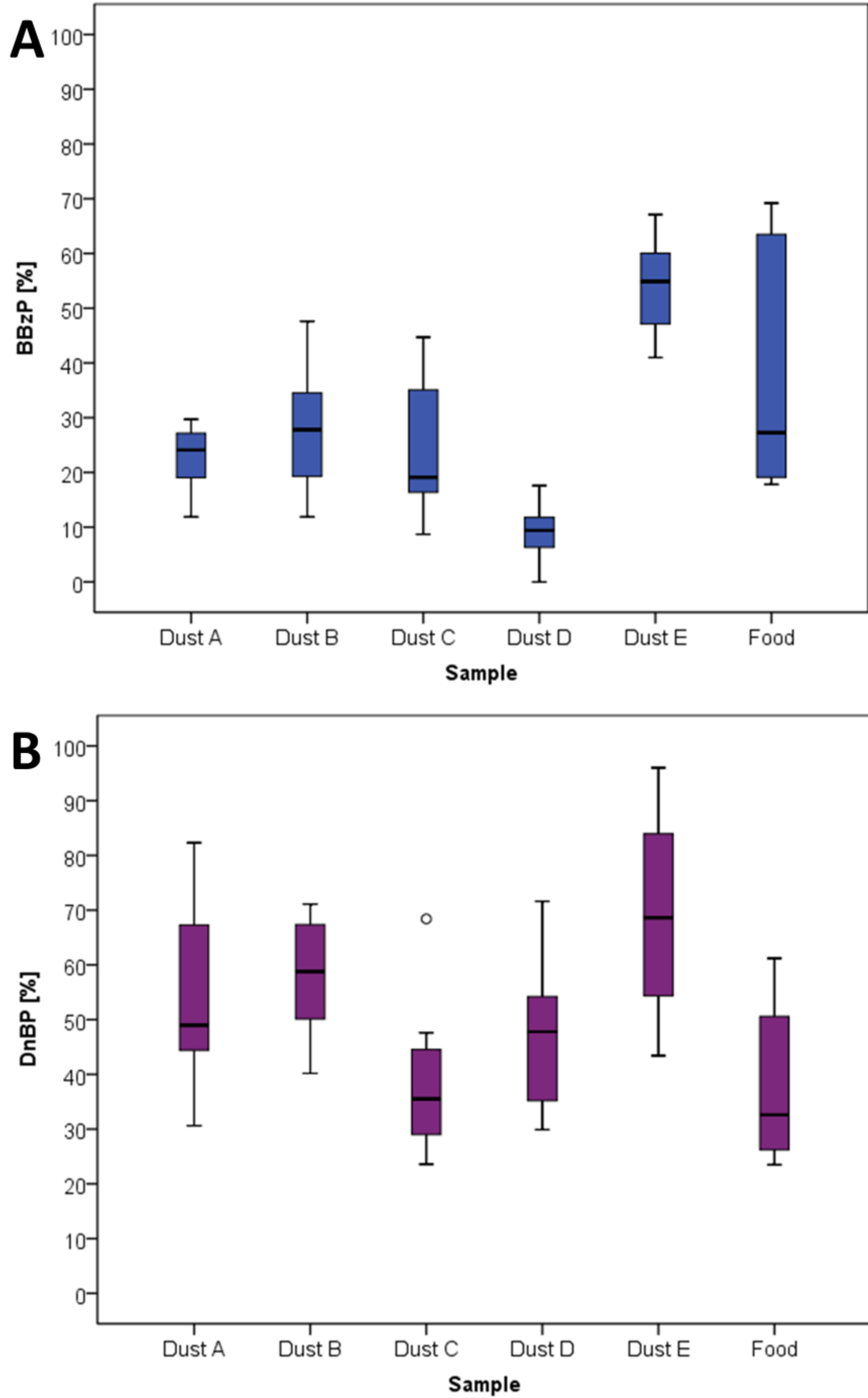


Figure 11. Percentage excretion of the sum of (A) BBzP-, (B) DnBP-, (C) DEHP- and (D) DINP-metabolite in relation to the applied dose of dust.

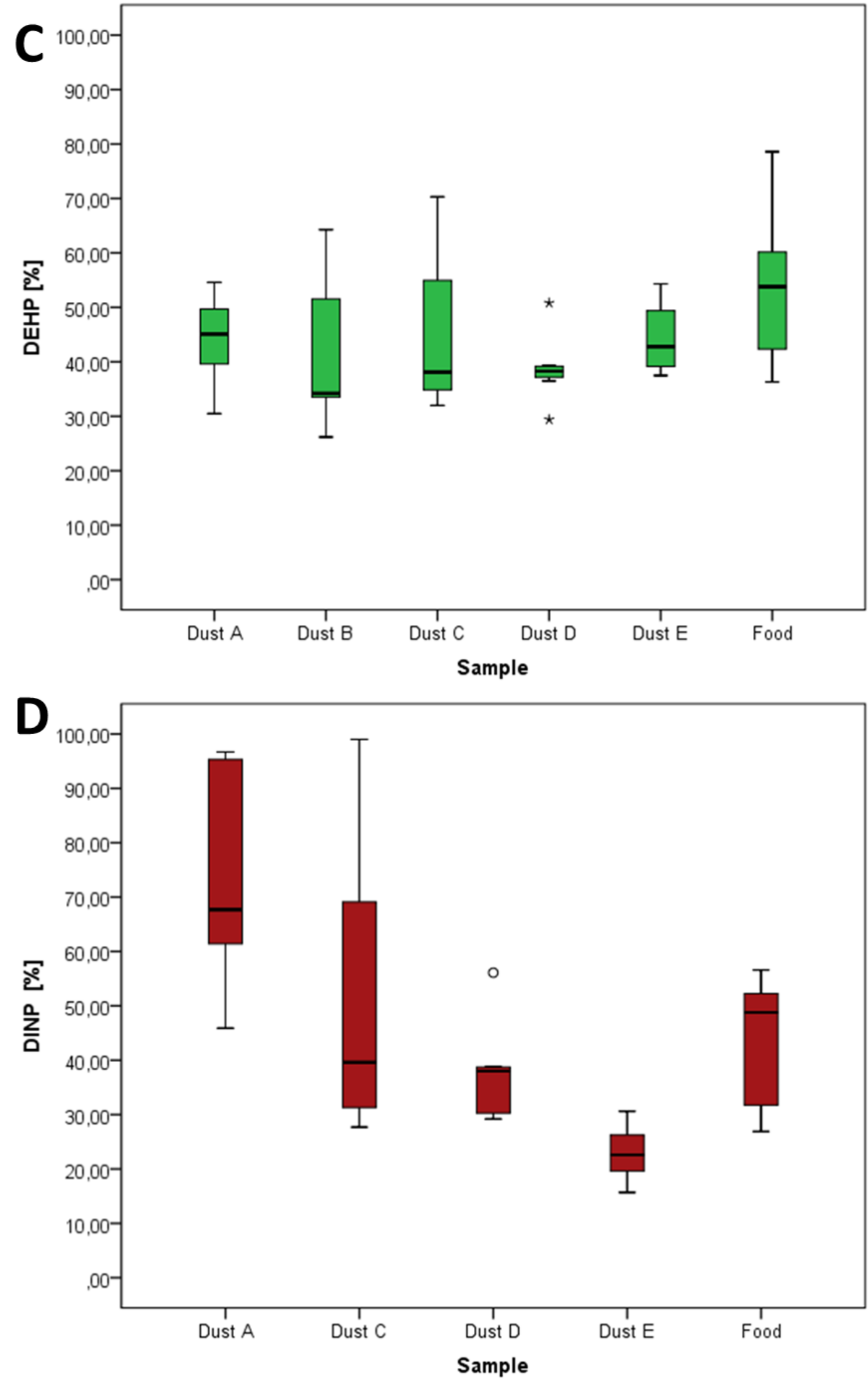


Figure 11. Percentage excretion of the sum of (A) BBzP-, (B) DnBP-, (C) DEHP- and (D) DINP-metabolites in relation to the applied dose of dust.

3I4 Toxicokinetics of phthalates

An example of the time course of the urinary excretions is given in Figure 12 (dust sample) and Figure 13 (food sample). The time course for each pig and applied sample is given in the appendix. In the dust samples, the metabolites of DEHP, DINP, BBzP and DnBP had the highest concentration within the first five hours after application. In the food sample, the highest concentration for DEHP and DINP was observed 24 hours post dose, the LMW phthalates had their highest concentration within the first five hours. The metabolism of phthalates can underlie a two-phase elimination. The initial increase of metabolite excretion is observed after three to five hours, followed then by a decrease. A second increase occurs normally 24 hours post dose. Because of this, the half-life time was calculated for both peak times. A detailed description of the toxicokinetic is given in the appendix (Section AI2).

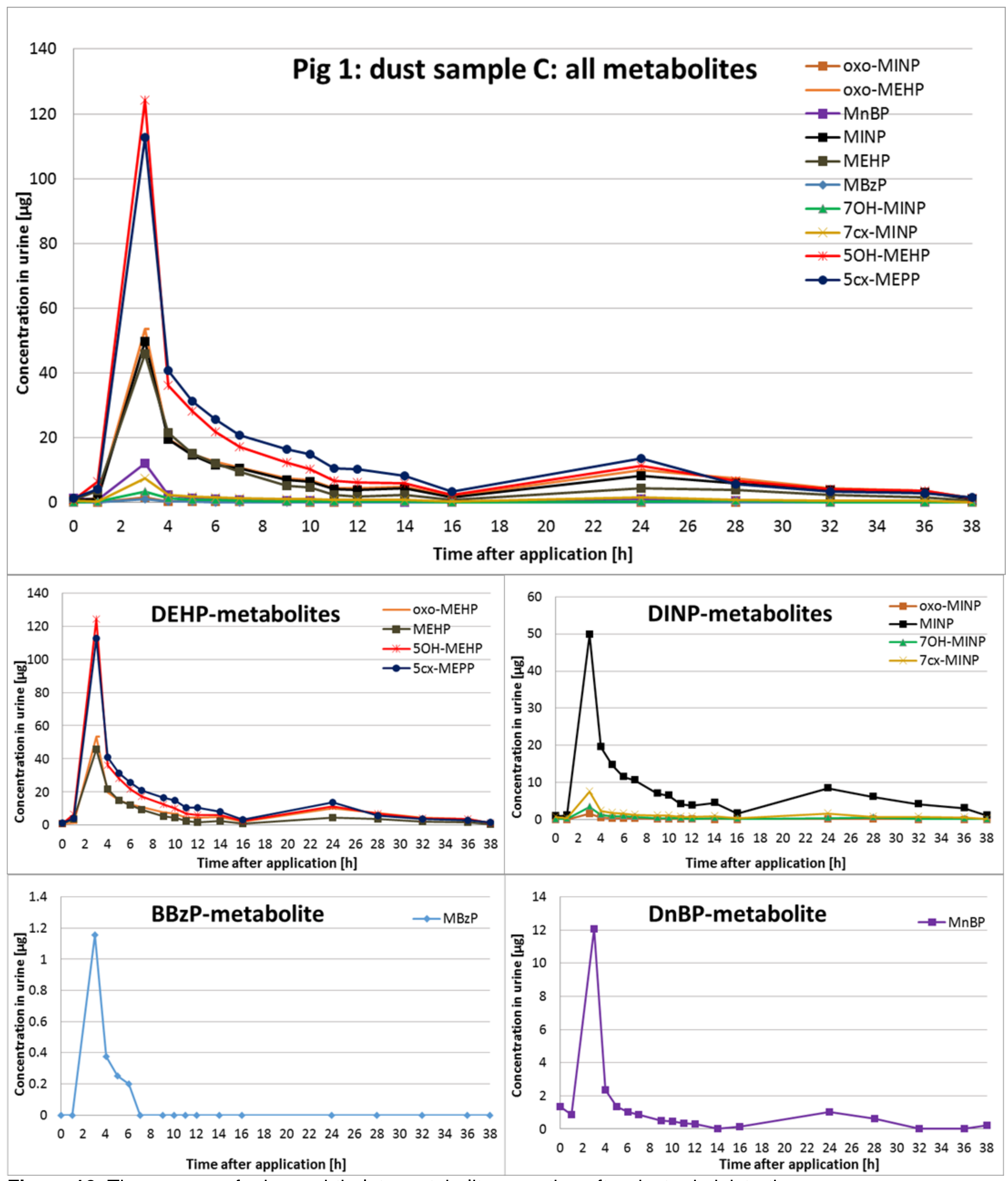


Figure 12. Time course of urinary phthalate metabolite excretion after dust administration.

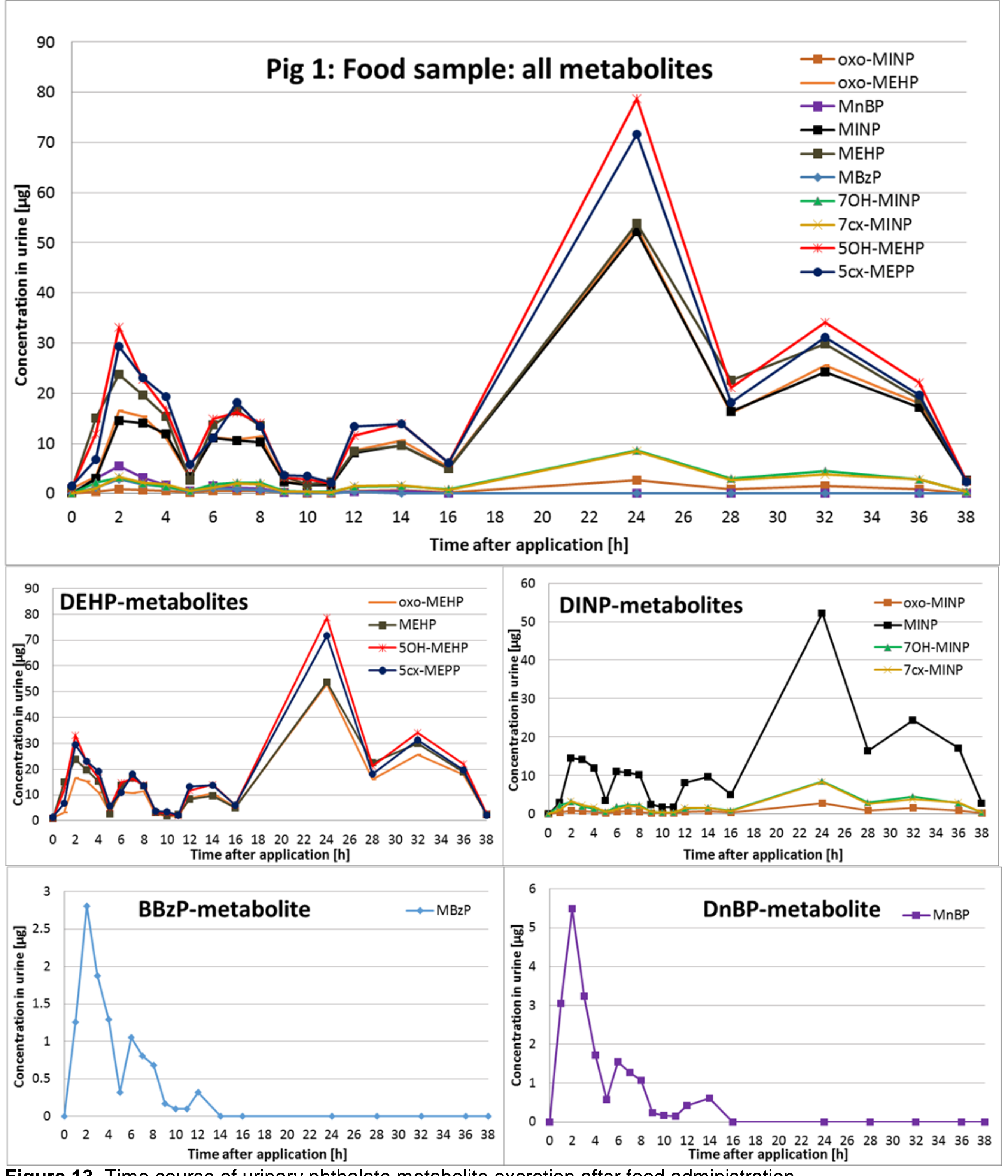


Figure 13. Time course of urinary phthalate metabolite excretion after food administration.

3I4I1 Butyl benzyl phthalate

Dust:

The urinary concentration maximum (c_{max}) occurred 3 ± 0.8 hours post dose. The estimated half-life time of MBzP was about 5 ± 4.3 hours. Within the first 24 hours, 27 ± 17 % of the applied BBzP dose was excreted as MBzP. Between 24 and 38 hours, only 0.1 ± 0.3 % of the dose was eliminated.

Food:

The maximum concentration in urine was observed after 4.2 ± 3 hours post dose. The half-life time was about 4 ± 2.3 hours. 45 ± 37.8 % of the dose was eliminated within the first 24 hours. On the second day no MBzP was detectable in the urine.

3I4I2 Di-n-butyl phthalate

Dust:

The maximum concentration was reached after 2.8 ± 1 hours post dose. The first estimated half-life time was around 3.3 ± 3.3 hours, whereas 24 hours later the second half life time was reached within 6.2 ± 5 hours. During the first 24 hours, 46 ± 17.6 % of the given dose was eliminated via urine, while 24-38 hours post dose only 2.7 ± 3 % of the given dose was detected.

Food:

After 2.8 ± 0.4 hours post dose, the urinary maximum concentration was reached. The half-life time was estimated at 3.2 ± 2.1 hours. The second half-life time (24 hours post dose) was 3.8 ± 1.2 hours. 34.3 ± 17 % of the absorbed dose was metabolized and eliminated within in the first 24 hours. On the second day only 1.3 ± 2 % of the dose was detected in urine.

3I4I3 Bis (2-etylhexyl) phthalate

Dust

T_{max} were estimated for MEHP, 5OH-MEHP, oxo-MEHP and 5cx-MEPP as 3 ± 1 hours, 2.7 ± 1 hours, 3.6 ± 1.3 hours and 5 ± 5 hours. 4.2 ± 2 hours (MEHP), 4.7 ± 2hours (5OH-MEHP), 6.3 ± 3.8 hours (oxo-MEHP) and 5.3 ± 2.7 hours (5cx-MEPP) were determined as the half-life time in the first excretion phase. The second excretion period was 24 hours post dosing, the mean half-life time was in decreasing order 5.6 ± 3 hours (MEHP), 5 ± 1.8 hours (5OH-MEHP), 5.2 ± 2 hours (oxo-MEHP), 5.1 ± 2 hours (5cx-MEPP). Within the first 24 hours, 40.2 ± 10.2 % from the DEHP

dose were excreted as 16.3 ± 4.6 % 5cx-MEPP, 8.7 ± 3.8 % 5OH-MEHP, 8.4 ± 2.6 % oxo-MEHP and 6.7 ± 3 % MEHP. After 24 hours, 2.7 ± 1 % DEHP dose was found in urine. It was mainly excreted as 1 ± 0.4 % 5cx-MEPP followed by 0.7 ± 0.3 % oxo-MEHP, 0.6 ± 0.4 % 5OH-MEHP and 0.4 ± 0.2 % MEHP.

Food:

After 24 hours, the maximum concentration was reached. The elimination half-life was 5.4 ± 1.4 hours for MEHP, 4.6 ± 1 hours for 5cx-MEPP, 6 ± 1.6 hours for oxo-MEHP and 5.2 ± 1.3 hours for 5OH-MEHP. Within the first 24 hours, 38.6 ± 7.4 % DEHP of the applied dose was mainly excreted as 5cx-MEPP (18 ± 5.3 %), oxo-MEHP (7.6 ± 2.6 %), 5OH-MEHP (7 ± 4.2 %) and MEHP (6 ± 3.6 %). 24-38 hours after the sample administration, 13.5 ± 5.2 % DEHP was found in urine (5.3 ± 2.2 % 5cx-MEPP, 3.3 ± 1.8 % oxo-MEHP, 2.4 ± 1.7 % MEHP and 2.4 ± 1.6 % 5OH-MEHP).

To sum up, for all pigs administered the dust and food samples, the metabolites were excreted in the following order of abundance: monoester < hydroxyl < oxo < carboxy. Figure 14 illustrates the measured amount of the DEHP metabolites excreted in both dust and food samples.

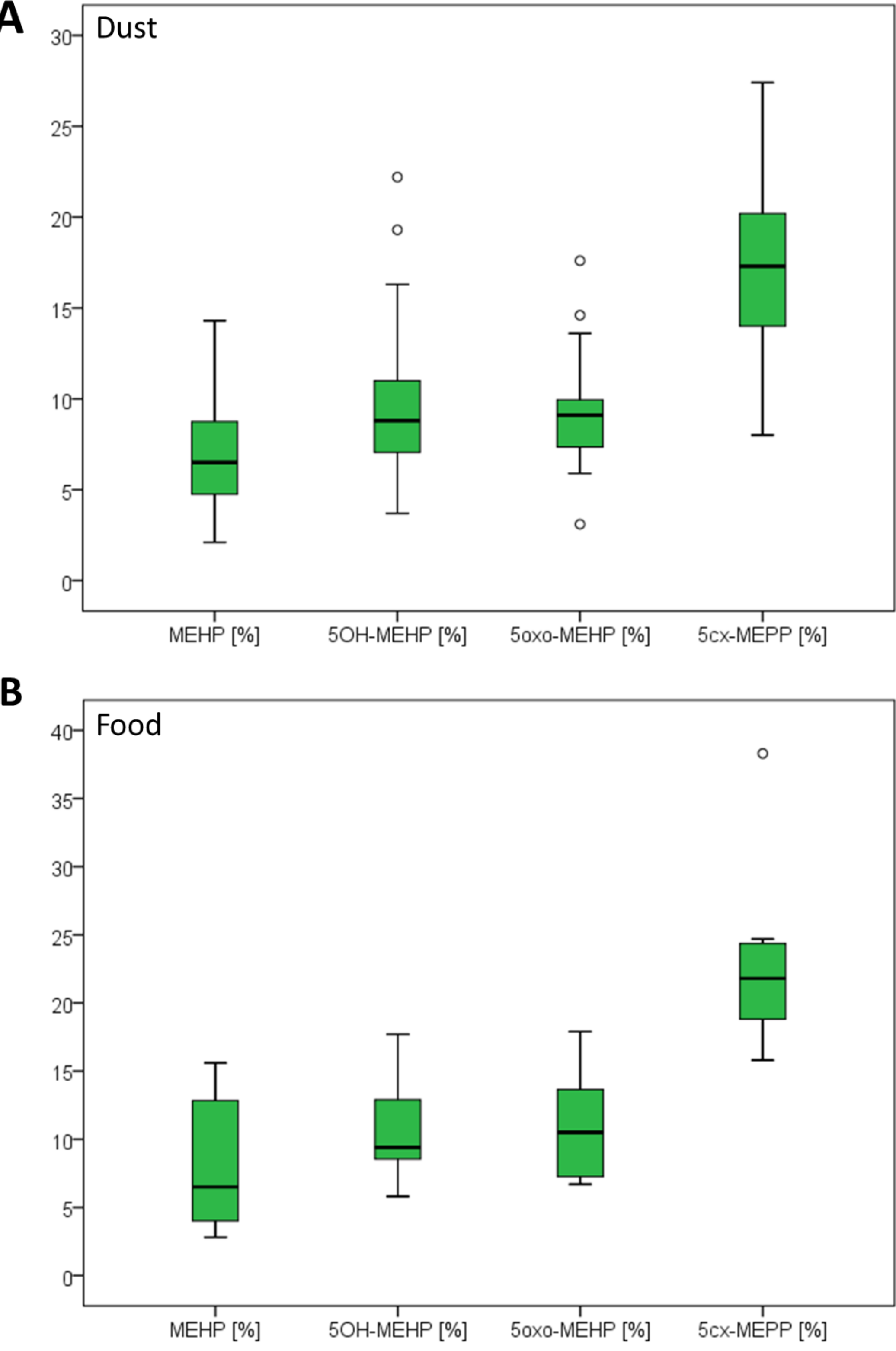


Figure 14. **A)** DEHP-metabolites in relation to the given dose in dust samples **B)** The excreted DEHP-metabolites in relation to the applied dose in food.

3I4I4 Diisononyl phthalate

Dust:

DINP metabolites reached their mean maximum concentration at 4.2 ± 4 hours (MINP) 5.5 ± 6.6 hours (oxo-MINP), 4 ± 4.2 hours (7OH-MINP) and 4.8 ± 5.6 hours (cx-MINP) after dose application.

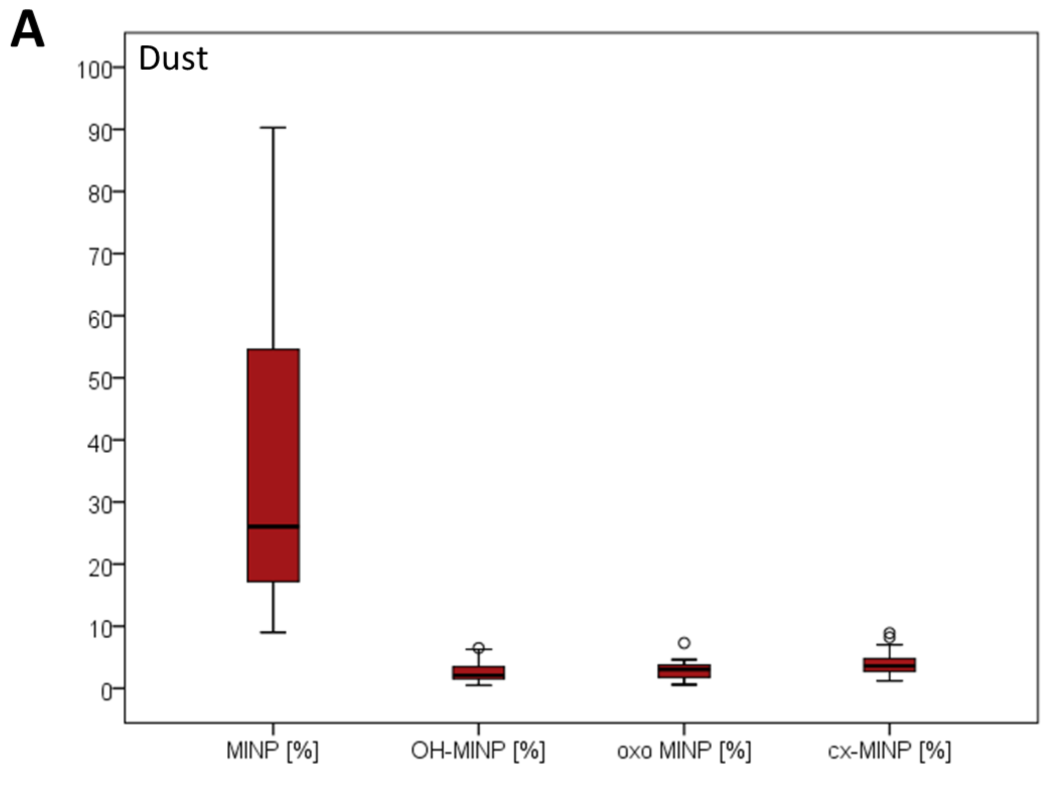
The estimated halt-life time for the first elimination phase was similar for all metabolites: 5.3 ± 2 hours (MINP), 6.6 ± 4 hours (oxo-MINP), 5.6 ± 3 hours (7OH-MINP) and 5.8 ± 3.4 hours (7cx-MINP). The second elimination phase occurred 24 hours post dose. The half-life time was estimated at 5.3 ± 2 hours for MINP, 6.6 ± 4 hours for oxo-MINP, 5.5 ± 3 hours for 7OH-MINP and 5.8 ± 3.4 hours for 7cx-MINP. During the first 24 hours, 43.4 ± 24 % of the dose was excreted as 34 ± 25 % MINP, 3.7 ± 2 % 7cx-MINP, 2.7 ± 2.3 % 7OH-MINP and 3 ± 1.6 % oxo-MINP. After 24 hours to 38 hours, only 3.6 ± 2.5 % DINP as 3 ± 2.4 % MINP, 0.27 ± 0.25 % oxo-MINP, 0.24 ± 0.2 % 7cx-MINP and 0.23 ± 0.3 % 7OH-MINP was excreted.

Food:

The maximum concentration for MINP and oxo-MINP was reached 24 hours after sample administration. The t_{max} for 7OH-MINP and oxo-MINP occurred after 24.5 ± 1.5 hours and 25.7 ± 4.5 hours, respectively. The half-life time for excreted MINP was about 6 ± 1.6 hours, for oxo-MINP about 7.3 ± 3.5 hours, for 7OH-MINP about 7.5 ± 3.7 hours and for 7cx-MINP about 5.2 ± 2.8 hours.

36.8 ± 14.8 % of the DINP dose was excreted in the first 24 hours mainly as MINP (30.3 ± 15.1 %), followed by 7cx-MINP (2.8 ± 1.3 %), 7OH-MINP (2.1 ± 1.5 %) and oxo-MINP (1.5 ± 0.4 %). On the second day, 15.3 ± 6.3 % DINP (12.3 ± 6 % MINP, 1.1 ± 0.5 % 7cx-MINP, 1 ± 0.73 % 7OH-MINP and 0.8 ± 0.3 % oxo-MINP) was eliminated by urine.

In conclusion, for dust samples, the metabolites were excreted in the order of abundance: hydroxyl < oxo < carboxy< monoester. For the food sample, the order of abundance differed: oxo< hydroxyl< carboxy< monoester. Figure 15 illustrates the amount of the DINP metabolites excreted in the samples of dust (Fig 15. A) and food (Fig. 15. B), respectively.



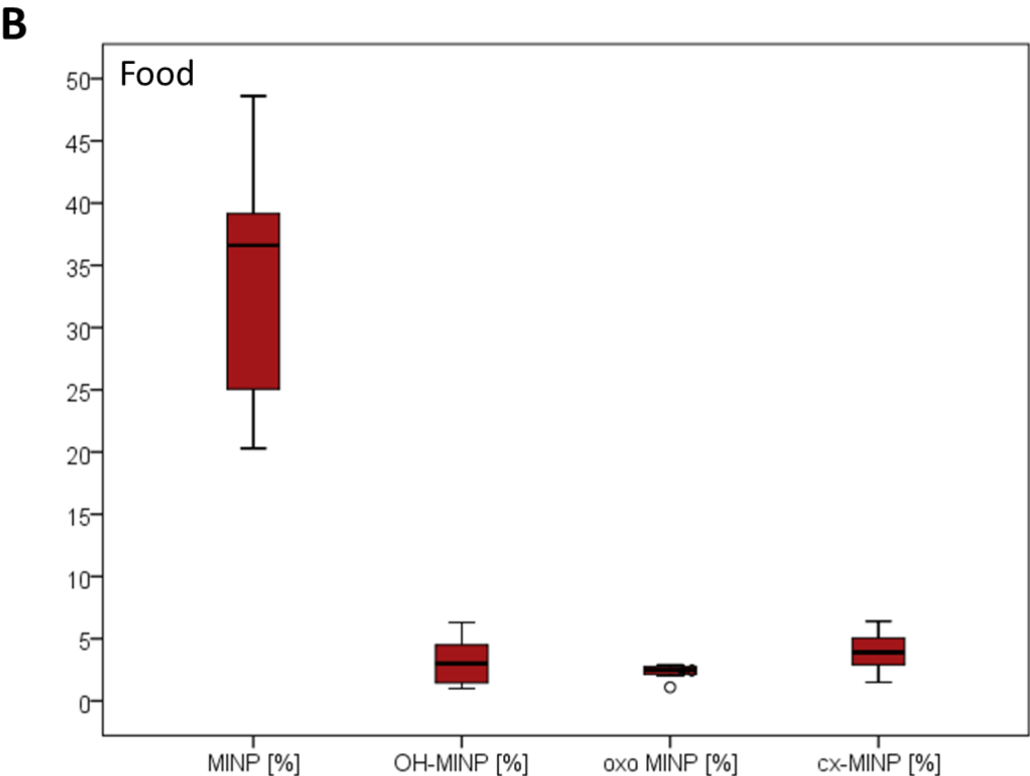


Figure 15. **A)** The sum of the excreted DINP-metabolites in relation to the applied dust samples **B)** The DINP metabolites in the food sample.

4I Discussion

Oral bioavailability is defined as the fraction of an ingested contaminant in a certain carrier matrix which reaches systemic circulation. Oral bioavailability is influenced by three different points:

1. The contaminant is released from the matrix during digestion in the gastrointestinal tract. This step is also known as bioaccessibility
2. The absorption of the bioaccessible fraction by the intestinal epithelium.
3. The metabolism of the contaminant in the intestine and liver

The bioaccessibility is mainly affected by the matrix, whereas the absorption rate and metabolism are influenced by the chemical properties of the contaminant [94].

4I1 Bioavailability of phthalates in dust

The mean bioavailability of the applied dose in dust was 27.7 ± 17.6 %, 52.2 ± 18 %, 43 ± 11 % and 47 ± 26 % for BBzP, DnBP, DEHP and DINP respectively. In this study, our collecting time was limited to 38 hours. We were still able to detected metabolites in the last urine sample and have to assume that the excretion of the applied phthalates was not completed. This might lead to a minor loss in bioavailability data. The applied dose was orientated to the TDI of DEHP. Every dust sample was applied with a similar DEHP concentration but the amount of dust varied. In consideration of intra-individuality, the bioavailability of DEHP has a low variation and seems to have a good reproducibility. It is assumed that this observation obtains as well for the other investigated phthalates. It is also noticeable that the bioavailability of DEHP and DINP is fairly similar (median: DEHP 39.3 %, DINP: 38.3 %). We also observed a saturation process: the higher the applied DINP dose, the lower the bioavailability. In dust sample A, a mean of 636 ± 18 µg of DINP was administrated with a remarkably high bioavailability of 75 ± 21% compare to dust sample E with an applied dose of 3340 ± 168 µg of DINP and bioavailability of 23 ± 5 %. The applied amount of dust varies between 0.5 g (dust A) and 0.78 g (dust E). To clarify if the concentration or the amount of dust underlies a saturation process dust sample A and C were compared. Dust sample A and dust sample C were administered in a similar amount (0.54 g) and the concentration varies from 636 ±18 µg (dust A) to 941 ± 52.2 µg (dust C). The bioavailability of DINP was determined for dust sample A of 75 ± 21 % and for dust sample C of 52 ± 28 %. Those results might

indicate that the saturation process depends on the applied concentration and not on the given amount of matrix. These findings compare favorably with Mckee et al. [11], who also observed a decreased absorption rate by an increasing orally administered dose in rats. Forty-nine % of the given DINP dose (50 mg/kg) was observed, whereas there was only an uptake of 39 % of the 500 mg/kg DINP dose. We did not observe this effect in other phthalates.

Our results showed a higher bioavailability than in vitro digestions test. In an *in vitro* digestion test a bioaccessibility of 2.24 - 12.6 % for DnBP, BBzP, DEHP, and DINP in dust was observed [80]. In another *in vitro* digestion test, slightly higher values were noted (10 – 15 % for BBzP, DnBP, DEHP and DINP) [87].

Both *in vitro* digestion tests used dust particles with a diameter of 63 µm, where the highest bioaccessibility is suspected. In this study, the same particle size was used, therefore an influence of the particle size fraction can be excluded.

As shown in this study, as well as in the *in vitro* digestion test, bioavailability varies according to the phthalate under investigation.

The *in vitro* digestion test is often used as a method to investigate bioavailability; it enables similar conditions for an experiment series and reduces inter-individual variances. Bioaccessibility is influenced in any case by natural physiological conditions like transfer time, pH, enzyme production and diffusion barrier. Changes in those parameters influence the results of *in vitro* digestions tests [95]. Additionally, in *in vitro* digestion tests, only the bioaccessibility of a compound can be determined but, e.g. the absorption rate cannot be included, which is an important influencing factor of bioavailability.

In a comparison study of five different *in vitro* digestion tests, a wide range in the bioaccessibility of heavy metals in three different soil samples was observed (e.g. Arsenic : 6 - 95 %, 1 – 19 % and 10 – 59 %). It was concluded that the pH value is probably the reason of the differences in the bioaccessibility. Increased bioaccessibility through the presence of food components (in this case milk powder) was also tested. Compared to the different pH values, the presence of food components has a minor influence [91]. In this study the dust sample was applied

with a portion of food which may lead to a slight increase of bioaccessibility as compared with dust uptake by e.g. putting a dust covered toy in their mouth.

The different results between this study and the *in vitro* digestion tests indicate that the chosen parameters for the *in vitro* test (pH, acid mixture, residual of time, ratio between matrix and liquid.) might not match the simulation of digestion, which could lead to an underestimation of the bioavailability. At the current state of knowledge, *in vitro* digestion tests cannot accurately simulate the bioaccessibility of human digestion (*in vivo*). Thus, the results of an *in vitro* digestion test should be interpreted with caution and considered only as indicative values.

In an arsenic bioavailability study conducted by Freeman et al. [96], cynomolgus monkeys received a dust, soil and soluble sodium arsenate sample via a gelatin capsule gavage and an intravenous injection. The mean absolute bioavailability (corrected by the intravenous injection) for house dust was 19 % and for the soluble arsenic was 68 %. These results suggest that the compounds in matrix dust have a reduced health risk based on its reduced bioavailability. In this study, it was not possible to calculate the absolute bioavailability. The results of the intravenous injection were not trustworthy because of difficulties in administration and therefore excluded from this study.

Additionally, arsenic and phthalates have completely different chemical properties, which makes it difficult to compare these results. Nonetheless, it can still be demonstrated that the ingestion of dust results in a noticeable exposure to chemicals.

4I2 Bioavailability of phthalates in food sample

In our study, the mean bioavailability was 37.3 ± 23 % (BBzP), 39 ± 16 % (DnBP), 53 ± 15 % (DEHP) and 43 ± 12.5 % (DINP). In a review, Wormuth et al. [24] summarized that the bioavailability of phthalate uptake through diet ranged between 69 – 78 % (BBzP), 64 – 73 % (DnBP), 15 – 95 % (DEHP) and 75 – 90 % (DINP). These reported levels are higher than those found in our study. Our food sample was a 1 % ethanol-phthalate mixture and potatoes. For this study, we are aware that the food sample might not be as representative as a normally prepared dish. Because of adding a solution to the sample, we might disregard the process of how much the

contaminant is released from the matrix food. However, the research focus was more on dust. In our study we could not observed a difference in the bioavailability of the matrix dust and food. Huwe et al. [70] observed similar findings in rats with polybrominated diphenyl ethers (PBDEs) in matrix dust and oil. They observed that PBDEs in dust bioconcentrate in rat fat tissue to the same or higher extent as PBDEs applied with oil. PBDEs accumulate in the body, which could influence the results of this study. The physicochemical characteristics of PBDEs are different to phthalates, which makes it difficult to compare these results. Anyway the study indicates a trend, which should be verified by further research.

4I3 Toxicokinetics of phthalates

Although the bioavailability in food and dust is similar, the toxicokinetic profile differs. In dust samples, the maximum concentration was reached three to five hours post dose and in the food sample, after three to four hours (LMW phthalates) and 24 hours for HMW phthalates. The delayed release or metabolism of the phthalates could be caused by the use of the carrier ethanol. It is noted that in other orally applied toxicokinetic studies, the highest concentration was reached shortly after administration [13, 59, 66, 69]. In any case, a biphasic metabolism was observed. The second increase was observed 24 hours after dose application. This effect can be explained by the fact that the metabolites underlie an enterohepatic circulation and there was no overnight collection of urine (eight hours break). Morning urine seems to be more highly concentrated in general. The half-life times, t_{max} and C_{max}, correspond to the results in other studies [13, 59, 66, 69, 97].

Compared to studies of humans, we observed variations of DINP metabolism. The metabolites were excreted in an order of abundance: OH-MINP < oxo-MINP < cx-MINP < MINP in dust. In food, the oxo-MINP metabolite came first and then the OH-MINP group. In a toxicokinetic study conducted by Koch et al. [69], it was reported that metabolite excretion is in the order of abundance: MINP < oxo-MINP < cx-MINP < OH-MINP. Our results indicate that piglets are slower in metabolizing DINP compared to humans. In a DEHP toxicokinetic study with rats, dogs and pigs, it was also shown that in pigs the eliminations of radioactivity were slowest [98]. Another difference was identified in the DEHP metabolism. The carboxy metabolite 5cx-

MEPP was the main excreted metabolite. In this study, the excretion was in an order of abundance of MEHP< 5OH-MEHP< 5oxo-MEHP< 5cx-MEPP whereas in general, the following order of abundance obtains: MEHP< 5 oxo-MEHO < 5cx-MEPP < 5OH-MEHP. However there is no indication that this has an effect on the bioavailability. Further research should verify if the metabolite excretion order is influenced by the pigs, which might have a different metabolism.

Additionally it should be clarified if e.g. 5cx-MEPP was also identified as the structurally analogues 5cx-MEPTP, which is a metabolite of a terephthalate (DEHTP) a structural isomer of DEHP. In a DEHTP- toxicokinetic study was shown that 13 % of the excreted 5cx-MEPTP is in shares comparable to the 5cx-MEPP [99]. The presence of DEHTP in dust might lead to an unintended background exposure which was probably measured as 5cx-MEPP of DEHP. This could lead to a slight overestimation of the bioavailability of DEHP.

4I4 Dust as an exposure source

In this experiment, we were able to show that dust is an exposure source for phthalates. The background exposure was kept as low as possible and verified by a control urine sample, therefore assuring that the main phthalate exposure was caused by the administered dust samples. Our results agree with biomonitoring and risk assessment studies where dust was already suspected to be an exposure source.

With our bioavailability data and assuming an daily dust intake of 60 mg and an average bodyweight of 13 kg, the mean intake for BBzP, DEHP, DINP and DnBP through dust would be 0.07 ± 0.09 µg/kg b.w., 12.1 ± 2.1 µg/kg b.w., 3.7 ± 0.6 µg/kg b.w. and 0.16 ± 0.13 µg/kg b.w.. This intake would contribute 0.01% (BBzP), 24.1 % (DEHP), 2.4 % (DINP) and 1.7 % (DnBP) to the TDI of each phthalate. For a cumulative risk assessment, the hazard Index (HI)[4] was calculated with an daily intake of 0.07 µg/kg/d BBzP, 12.1 µg/kg/d. DEHP, 3.7 µg/kg/d DINP and 0.16 µg/kg/d

[4] Hazard Index (HI) is the sum of the hazard quotients (HQ), which is defined as $\frac{\text{Daily intake}_{\text{subtance}}}{\text{TDI}_{\text{substance}}}$. An HI <1 is will not result in negative health effects, while an HI > 1 can pose a health risk. 100. https://www.epa.gov/national-air-toxics-assessment/nata-glossary-terms, 20.06.2017.

DnBP and their corresponding TDI's. The HI was 0.2. It shows that the phthalate exposure only through dust does not pose a health risk.

Kang et al. [87] calculated an average daily dose (ADD) of DEHP through non-dietary ingestion of house dust for preschool children in China. Under a moderate dust ingestion rate (0.05 gram per day), none of the children would exceed the reference dose (RfD) of 20 µg/kg b.w./d, but for a high dust ingestion rate (0.2 g/d), 13 % of the dust samples would result in a higher ADD than the RfD. Considering the bioaccessibility of phthalates in dust, no dust sample would result in exceeding the RfD. DnBP showed also no indication of exceeding the RfD (RfD: 100 µg/kg b.w./d). Kang et al. estimated that moderate dust intake and high dust intake contributes 28.4 % and 61.3 % to the overall DEHP exposure. 74.8 % (under moderate dust intake) and 92.2% (high dust intake) of the total DnBP exposure was contributed through dust intake. In a similar study conducted by Wang et al. [80], the daily intake of phthalates in house dust was evaluated. For toddlers, the highest phthalate exposure was calculated with 5.4 ng/kg b.w./d, 102 ng/kg b.w./d and 5800 ng/kg b.w./d for BBzP, DnBP and DEHP, respectively. Dust ingestion was identified as a major contributor for non-dietary DEHP exposure with 81.4 - 96.4 % of the total intake. By taking the dietary exposure into account, the dust ingestion would contribute 36.5 % of the DEHP exposure (calculated for adults). For the low molecular phthalate DnBP, indoor air seems to be the major contributor of the non-dietary DnBP exposure. By calculation of the ADD, 5 % of the dust samples exceed the RfD (20 µg/kg b.w./d for DEHP) by a high dust intake rate (200 mg/d). For a moderate dust intake (100 mg/d), no dust sample would exceed the RfD. In a China-USA comprehensive study, the daily dust intake for toddlers was 83.7 ng/kg b.w./d, 0.9 ng/kg b.w./d and 949 ng/kg b.w./d for DnBP, BBzP and DEHP in China, in the USA, the values were generally higher with 64.4 ng/ kg b.w./d (DnBP), 104 ng/kg b.w./d (BBzP) and 1500 ng/kg b.w./d (DEHP) [84]. Except for the DEHP value, the values are in good agreement to those reported by Wang et al. [80]. The ingestion of dust contributes only 2 – 5 % of the total DEHP and DnBP exposure in China and 1 – 16 % of the total DnBP, 3 – 21 % of the total BBzP and 10 – 58 % of the total DEHP intake in the USA. In another biomonitoring study by Fromme et al. [47], the phthalate content in air and dust from German daycare centers was measured. The phthalate exposure was identified by the excreted metabolites in 663 urine samples of children attending the investigated

daycare centers. No child exceeded the TDI values for DEHP and DnBP, only one child had a higher DINP value than the TDI and 16 children exceeded the TDI of DiBP. The daily intake was determined by back-calculation based on the amount of the urinary phthalate metabolites. The average intake was calculated with the median and the high intake with 95th percentile of urinary metabolite concentration. Those intakes were compared with the TDI. In the “high” total intake scenario, less than 50 % of the TDI was reached, except for DiBP (62 %). It also showed a significant correlation between the concentration in indoor air and dust and the excreted urinary metabolite concentration on a bivariate analysis, but using a multiple linear regression model, only indoor air was correlated with urinary metabolite concentration [47].

Bekö et al. [4] estimated the phthalate intake of children in urine through the exposure of dust ingestion, inhalation and dermal absorption. They analyzed dust and children’s urine samples and assumed a daily dust uptake of 60 mg. The daily total intake, which was estimated by the urinary metabolite concentration, was compared to the estimated daily uptake from the indoor environment (dust ingestion, inhalation and dermal absorption). DEHP had the highest intake resulting from dust ingestion. It was also shown, that 75 % and 95 % of the weekly indoor intake of BBzP and DEHP entered the body through dust ingestion, whereas for low molecular phthalates like DnBP and DiBP, 80% of the weekly indoor intake ($WI_{indoors}$) occurs via dermal absorption from air. Bekö et al. [4] assumed that indoor exposure corresponds to 2.2 % (DEHP), 13 % (DnBP) and 0.01 % (BBzP) of the TDI. In a similar study conducted by Langer et al. [48], a significant correlation between phthalates concentration in house dust (home and daycare samples) and urinary metabolite concentration in the urine of 441 Danish children (DnBP-MnBP $p<0.05$, BBzP-MBzP $p<0.001$) was found. Compared to the other studies, a correlation between DEHP metabolites and dust concentration was not observed.

All these studies concluded that dust is a contributor to phthalate exposure, especially for non-dietary exposure and for toddlers. They also indicate that indoor air is a major contributor to DnBP and DiBP exposure. All the mentioned studies were based on different amounts of dust ingestion (60-200 mg/d), which leads to a

different assumption of how much dust exposure contributes to the total phthalate exposure.

4I5 Dust exposure compared to food exposure

In several studies it was shown that the predominant intake source for DEHP intake is diet. As shown in a duplicate diet study performed by Fromme et al. [101], a significant correlation was determined between the DEHP concentration in food and the concentration in urine samples (where the DEHP intake was back-calculated). For DnBP, no correlation and for DiBP, a weak correlation was observed. An infant-based duplicate diet study showed that for DiBP and DnBP, the dietary intake only comprised about 24 – 32 % of the total intake. For BBzP, it was only 4 % of the total intake. However, for DEHP, dietary intake is the dominant contributor [102]. A risk characterization of dietary uptake by Heinemeyer et al. [62] showed that less than 1 % of German adults may exceed the tolerable daily intake of 50 µg/kg b.w. DEHP. Furthermore, they concluded that the average external dietary exposure of DEHP ranged between 3-14 µg/kg b.w./d and that 30 to 40 % of the total intake is contributed by the ingestion of dust. Wormuth et al. [24] assumed that for infants and toddlers, the main source for BBzP is dust (>70 %) and food might contribute 20 % to the total BBzP exposure. For children and adults, the main source is food (60 - 73 %) and contaminated indoor air (26 %). For infants, toddlers and children, the mouthing of soft plastics is up to 90 % responsible for DINP take up, whereas teenagers and adults are mainly exposed through dust (>30 %) and air (~30 %). The main source for DnBP exposure is food (40 – 90 %). For infants, toddlers and children, indoor air (20 - 40 %) and dust (10 %) are additional exposure sources. Female teenagers and adults are exposed by personal care products too (15 – 50 %). Food is the most important source for DEHP (50 – 98 %), for infants and toddlers, dust (>35 %) and toys (8 – 9 %) also contribute to the DEHP exposure. DINP is used as a substitution for DEHP. It is expected that exposure pathways are becoming more similar, thus food will also become the major exposure source for DINP.

Reviewed by Oomen et al. [86], the DEHP exposure through food and water is almost 3 - 16 µg/kg b.w./d for adults and 12-26 µg/kg b.w./d for children. Compared

to the TDI value of 50 µg/kg b.w., a child has a share of 24-52 % of the TDI through this pathway.

It has been assumed that through infant formula consumption, newborns and infants are exposed to 2.4 - 1.8 µg/kg b.w./d DINP [30]. Several human biomonitoring studies confirm that the main phthalate exposure takes place through ingestion. A duplicate diet study conducted by Fromme et al. [102] showed that the DEHP concentration in highly contaminated food lay around 4.7 µg/kg b.w. (95th percentile) and the high intake from biomonitoring data was 4.9 µg/kg b.w.. Koch et al. [103] tested in a 48 hour fasting study the phthalate exposure using excretion data in urine. The high molecular weight phthalates DEHP, DINP and DIDP showed a fast elimination rate and remained at a low level, whereas the low molecular weight phthalates DMP, DEP, BBzP, DnBP and DiBP had a rising and declining concentration in urine. These results indicated that the main exposure of high molecular weight phthalates occurs by food ingestion, whereas the others seem to have another exposure source, like personal care products or through indoor air and dust.

4I6 Phthalate in dust as a health risk?

In the last years, the toxicokinetic properties, sources and health effects of phthalates have been intensively investigated. In general, the average body burden of phthalates does not exceed the TDI value. A decreasing urinary phthalate concentration in recent years indicates a declining exposure to some phthalates. Nonetheless, especially children continue to show high urinary phthalate levels compared to adults. Children have a high food and water requirements per unit of body mass, an increased hand to mouth activity, a higher ventilation rate and unintended dust ingestion rate than adults. It is suspected that dust is an additional but minor exposure source for children. Our findings support this hypothesis. However, the question of phthalate exposure through dust posing a potential health risk still remains open. As mentioned above, several risk assessment studies concluded, that the ingestion of dust does not lead to an intake that exceeds TDI or RfD values. It was also observed that from daily intake calculations (including dust and dietary intake), children should have exceeded the TDI, but the urinary

concentration showed no higher values than the TDI for one phthalate or a combination of several phthalates. Additionally, in most of risk assessments, a bioavailability of 100% was assumed. Our study indicates that the bioavailability of phthalates in dust is less than 60%. In consideration of an estimated intake of 60 mg of dust per day, it can be concluded that dust is an additional phthalate exposure source, but it is still a minor contributor to the total exposure (0.01 % of the TDI_{BBzP}, 24.1 % of the TDI_{DEHP}, 2.4 % TDI_{DINP}, and 1.7 % of the TDI_{DnBP}). Another important consideration is that only through the ingestion of dust, the hazard index was at 0.2, which also means that no negative health effects should be expected. Especially for the HMW phthalates such as DEHP and DINP, the dietary uptake is still the main exposure source for all age groups. On the contrary, for the LMW phthalates such as BBzP, DnBP and DiBP, it shows that the indoor environment, including dust, has an influence on the total exposure, but without a negative health effect.

5I Conclusion and future perspectives

According to the US-EPA, toddlers have an unintended daily uptake of approximately 60 mg dust which contains a variety of pollutants. Especially phthalates are present in high amounts and could pose a potential health risk. Currently, there is no information about the bioavailability of phthalates in the matrix dust. Our results show that the bioavailability of phthalates is much higher (~30 – 60 %) compared to *in vitro* digestion tests (~10 – 20 %). Furthermore, it is noticeable that the bioavailability of DEHP, for example, does not vary within the dust samples. A dose dependence uptake of DINP was observed. In other phthalates, we did not find this effect. Further research is needed to understand the underlying mechanism of the dose dependence DINP uptake. Moreover it should be clarified if this effect also occurs in humans and how much does it influence the DINP exposure.

Biomonitoring studies indicate that children are highly exposed to phthalates but they do not normally exceed the TDI. In addition to diet and toys, dust could be an additional source of plasticizers for toddlers. However, because the limited amount of dust uptake does not normally lead to children exceeding the TDI, phthalate exposure through dust does not pose a health risk for children.

The next step of research should be focused on determining the actual amount of ingested dust. On dust uptake, the quantity ingested has a higher influence on the exposure of phthalates than their bioavailability. In this study we focused only on bioavailability, thus there was only one test using a higher amount of dust where we noticed an increase of the phthalate exposure. But under realistic situations, where a child ingests approximately 60 mg dust during the day, it might contribute to a constant background exposure similar to indoor air without noticing an increase or decrease of urinary phthalate excretion. Further research is still needed in regard to how much indoor air contributes to the phthalate exposure, especially DnBP and DiBP. Additionally, further research should also address the bioavailability of other contaminants in dust, to determine whether dust poses a general exposure source or just in the case of phthalates.

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7I Appendix

AI Appedix

AI1 Material and Methods

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Ihre Zeichen und Nachrichten vom	Gesch.-Z.: Bitte bei Antwort angeben	Tel.-Durchwahl/Fax	Datum	Org.-Einheit/Ansprechpartner
I C 1 – 0272/13 13.03.2014	84-3855-01-7890679	- 1948	15.07.2014	FG 84/ Dr. Spolders

Antrag auf Genehmigung des Tierversuchsvorhabens Reg 0272/13

Hier: Ihr Schreiben vom 13.03.2014

Sehr geehrte Damen und Herren,

anbei beantworten wir Ihnen die Nachfragen zu dem Versuchsvorhaben „Bioverfügbarkeit von Weichmachern im Hausstaub und Nahrungsmitteln nach oraler Zufuhr im Modellorganismus Schwein – Reg 0272/13“.

Bitte beraten Sie unseren Antrag erneut in der Tierversuchskommission.

Mit freundlichen Grüßen

im Auftrag

Dr. Markus Spolders

Anlage
84-3855-01-7890679

15.07.2014

Mit Ihrem Schreiben vom 13.03.2014 (I C 1 Reg 0272/13) baten Sie um die Beantwortung weiterer Fragen. Da sich einige Fragen hinsichtlich ihres Inhalts ähneln, möchten wir unserem Antwortschreiben einige allgemeine Ausführungen zum Hintergrund des geplanten Tierversuchsvorhabens voranstellen, die möglicherweise bisher nicht eindeutig von uns formuliert wurden:

Grundsätzlich soll in dem beantragten Tierversuch die Bioverfügbarkeit von Phthalaten aus Hausstaub und Nahrungsmitteln ermittelt werden. Dazu sollen real in Staubproben und Nahrungsmitteln vorkommende Gemische von sieben Phthalaten an wachsende Schweine verfüttert werden. Mit diesem Vorgehen sollen auch mögliche Wechselwirkungen der einzelnen Phthalate miteinander im Hinblick auf die Bioverfügbarkeit berücksichtigt werden. Aufgrund des Vorhandenseins mehrerer Phthalate in den Staub- und Nahrungsmittelproben kann die Bioverfügbarkeit einzelner Metabolite durch die Anwesenheit anderer Metabolite beeinflusst werden.

Fragen zur Zusammensetzung der Stäube sowie Dosierung der Phthalate (Fragen 1, 2, 3, 6 und 7):
Die Staubproben, die für den Versuch herangezogen werden, entstammen einer Studie, in der in Kindertagesstätten die Phthalatkonzentrationen in Staubproben ermittelt wurden. Die Phthalatkonzentrationen der ausgewählten Hausstäube sind demnach bekannt (Tabelle 1).

Tabelle 1: Phthalatkonzentrationen in den ausgewählten Staubproben

Phthalate im Staub (mg/kg)	Staub 1	Staub 2	Staub 3	Staub 4	Staub 5
BBzP	9	94	28	3	4
DEHP	7646	6977	5632	5451	8036
DIDP	133	57	22	32	20
DINP	257	346	168	1474	292
DiBP	175	40	226	20	10
DnBP	70	23	58	13	11
DINCH	224	1643	141	150	456

Die Staubproben werden zusätzlich vor der Verabreichung nochmals untersucht, um sicher zu stellen, dass die vorher ermittelten Konzentrationen und damit auch die zugeführte Dosis tatsächlich zum Zeitpunkt der Fütterung vorliegen. Dies stellt eine unabdingbare Maßnahme der Qualitätssicherung dar.

Die Staubproben weisen ein reales Gemisch aus Phthalaten mit unterschiedlichen Konzentrationen auf. Da der Mensch in der Realität immer Weichmachergemischen ausgesetzt ist, ist diese Vorgehensweise sinnvoll. Diese realen Hausstaubproben werden dem Schwein in einer Gelatinekapsel verabreicht. In den Urinproben werden dann die Phthalatmetabolite (siehe Tabelle 2) einzeln, wie bereits im Erstantrag (Seite 8) beschrieben, mittels LC/MS-MS nachgewiesen. Somit messen wir in den Urinproben die einzelnen

Phthalatmetabolite und können aufgrund der Kenntnisse aus der oralen Aufnahme Aussagen zur Bioverfügbarkeit der untersuchten Phthalate treffen. Aufgrund der äußerst kurzen Halbwertszeit werden die Phthalate nahezu vollständig als primäre und sekundäre Metabolite mit dem Urin ausgeschieden. Vor diesem Hintergrund kann, bei bekannter Dosis, bei derartigen Substanzen die Bioverfügbarkeit aus dem Verhältnis der nach oraler und intravenöser Zufuhr ausgeschiedenen Substanzmenge berechnet werden. Ein Zeitverlauf der Konzentrationen im Blut erübrigt sich somit und wird zur Schonung der Tiere nicht durchgeführt. Urin ist ein geeigneter Biomarker, um die Phthalatbelastung des Menschen hinreichend feststellen zu können.

Tabelle 2: Phthalate und die zu untersuchenden Metabolite im Urin

Phthalat Ausgangsverbindung	*Primäre und sekundäre Metabolite*
BBzP	MBzP
DEHP	MEHP, 5-oxo-MEHP, 5-OH-MEHP, 2-cx-MMHP, 5-cx-MEPP
DIDP	oxo-MiDP, OH-MIDP, cx-MiDP
DINP	MINP, 7-OH-MiNP,7-oxo-MINP,7-cx-MINP
DiBP	MiBP
DnBP	MnBP
DINCH	MINCH, OH-MINCH, cx-MINCH, oxo-MICH, CHDA

Wie auf den Seiten 3-5 des Erstantrages dargestellt, handelt es sich bei den Phthalaten um Stoffe, die insbesondere aufgrund ihrer hormonellen Wirkung gesundheitlich bedenklich sind. Da der Mensch diesen Substanzen gegenüber in hohen Konzentrationen exponiert ist, sind Maßnahmen der Risikoreduktion dringend erforderlich. Die Ergebnisse des geplanten Versuchs sollen einen wesentlichen Beitrag liefern, den Menschen vor möglichen gesundheitlichen Beeinträchtigungen durch die Aufnahme von Phthalaten über Hausstaub und Nahrungsmittel zu schützen. Dies gilt insbesondere für die sensible Gruppe der Kleinkinder, die aufgrund ihrer Fortbewegungsart (Krabbeln auf dem Boden) und der Unterschiede in der Toxikokinetik und Toxikodynamik von Schadstoffen (intensive Organentwicklung) in besonderem Maße zu schützen sind.
Maßnahmen zur Risikominimierung erscheinen aber nur dann sinnvoll, wenn die Bedeutung der einzelnen Belastungsquellen auch valide eingeschätzt werden kann. Da die Bioverfügbarkeit der zu untersuchenden Phthalate bei der Aufnahme aus Hausstaub bzw. Nahrungsmitteln bisher unbekannt ist, fehlen zur Risikoabschätzung und zum weiteren Risikomanagement wichtige Basisdaten. Unsere wissenschaftliche Hypothese ist, dass die Bioverfügbarkeit von Phthalaten aus Hausstaub und Nahrungsmitteln deutlich geringer als 100 % ist. Damit würde die Bedeutung der Aufnahme von Phthalaten über Hausstaub derzeit in der Risikobewertung überschätzt (Annahme einer Bioverfügbarkeit von 100 %). Ob dies auch wirklich zutrifft, kann erst durch das beantragte Experiment bestätigt oder abgelehnt werden.

Anhand der vorliegenden Ergebnisse der Staubproben ist geplant, den Tieren eine einmalige orale Gabe einer bestimmten an den TDI angepassten Menge Staub (1,2 bis 1,8 g; Tabelle 3) zu geben. Aufgrund der unterschiedlichen Phthalatkonzentrationen in den fünf Staubproben wurde die zu fütternde Menge an Staub individuell berechnet. Diese einmali-

ge Dosis orientiert sich an der duldbaren täglichen Aufnahmemenge für den Menschen (TDI-Wert) unter Berücksichtigung einer angenommenen Bioverfügbarkeit von 20 % (Annahme von Kang et al. 2012).

Wir orientieren uns bei der Dosierung am TDI, da uns der gesundheitliche Schutz der Schweine wichtig erscheint und er einen geeigneten Richtwert für die Dosisgabe darstellt. In dem beantragten Versuch geht es darum, eine vergleichbare Dosis an Phthalaten sowohl über Staub, Nahrungsmittel als auch i.v. den Schweinen zu verabreichen, um die Bioverfügbarkeit der Phthalate über die unterschiedlichen Eintrittspfade zu bestimmen. Die verabreichte Menge an Staub und Nahrungsmitteln ist dabei nur das Mittel zum Zweck, den Tieren eine bestimmte Dosis an Phthalaten zuzuführen.
Für die meisten zu prüfenden Phthalate liegt die Dosierung, bei einer angenommenen Bioverfügbarkeit von 20 %, deutlich unterhalb der TDI-Werte (bei Annahme eines Schweins von 20 kg Körpergewicht). Der TDI beschreibt die Dosis, die lebenslang zugeführt werden kann ohne dass gesundheitliche Beeinträchtigungen zu erwarten sind (Tabelle 3). Eine Ausnahme stellt das DEHP dar, welches durchaus in einer Menge oberhalb des TDIs für den Menschen aufgenommen würde. Da es sich aber um eine einmalige Dosis handelt und in der Literatur Tierexperimente mit Dosen von 10 mg DEHP/kg Körpergewicht über mehrere Wochen täglich ohne gesundheitliche Folgen verabreicht wurde, würden wir auch bei einer möglichen Bioverfügbarkeit von 100 % und der daraus resultierenden Überschreitung des TDIs keine unerwünschten gesundheitlichen Folgen bei den Schweinen erwarten. Aus den Ergebnissen des Tierversuchs kann letztlich auf verschiedene Expositionsszenarien bei Kindern (unterschiedliche Staubaufnahmemengen) zurück gerechnet werden.

Tabelle 3: Dosierung der einzelnen Phthalate anhand der ausgewählten Staubproben und TDI-Werte (µg/20 kg Tier und Tag)

Phthalate	Dosis µg					TDI µg
	Staubprobe 1 [1.3g]	Staubprobe 2 [1.4g]	Staubprobe 3 [1.7g]	Staubprobe 4 [1.8g]	Staubprobe 5 [1.2g]	
BBzP	11.7	134.2	47.6	5.4	4.8	10 000
DEHP	9940	9767.8	9574.4	9811.8	9643.2	1000
DIDP	172.9	79.8	37.4	57.6	24	3000
DINP	334.1	484.4	285.6	2653.2	350.4	3000
DiBP	227.5	56	384.2	36	12	200
DnBP	91	127.4	98.6	23.4	13.2	200
DINCH	291.2	2300	239.7	270	547.2	20 000

Die Staubproben stammen aus einer Erhebung in Kindertagesstätten aus Bayern, Berlin und Nordrhein-Westfalen. Die Staubproben wurden wie folgt gesammelt: Mit einem ALK-Abello Probenahmekopf, der als Aufsatz auf einen handelsüblichen Staubsauger gesteckt wurde, wurde eine Querschnittsprobe vom Fußboden des Gruppenraumes über einen Zeitraum von ca. 5-10 Minuten bei geringer Leistung gesaugt. Zusätzlich wurden noch Staubsaugerbeutel aus den Kindertagesstätten gesammelt. Der Inhalt der Staubsaugerbeutel wurde dann mithilfe eines Siebes auf 63 µm durchgesiebt, um so eine Probe zu erhalten. Die Maschengröße des Siebes wurde so gewählt, da Untersuchungen zeigten, dass Phthalate eine sehr hohe Akkumulationsrate bei dieser Partikelgröße aufweisen. Anders als im ursprünglichen Antrag beschrieben, werden nur Staubproben (insgesamt fünf) aus Kinder-

tagesstätten an die Schweine verfüttert. Diese fünf Staubproben wurden ausgewählt, da sie einen sehr hohen Gehalt an Phthalaten aufweisen und daher eine weitere Untersuchung erfolgen sollte.

Die gewählte Anzahl an Staubproben ist auf das notwendige erforderliche Minimum beschränkt, welches jedoch erlaubt statistisch aussagekräftige Ergebnisse zur Bioverfügbarkeit unterschiedlicher Phthalate für die zukünftige Berücksichtigung der Bioverfügbarkeit in der Risikobewertung zu erhalten. Um die tatsächliche Bioverfügbarkeit von Phthalaten aus Hausstaub bestimmen zu können, bedarf es der Verfütterung von „realen“ Hausstaubproben, denen Kinder möglicherweise tatsächlich auch ausgesetzt sein könnten.

Ein weiteres Ziel des beantragten Tierversuchs ist es, die zugeführte Phthalatmenge über Staubproben und über Nahrungsmittel gleich hoch anzusetzen, um die Ergebnisse dahingehend vergleichen zu können, inwieweit die Matrix „Staub“ oder „Nahrungsmittel“ einen Einfluss auf die Höhe der Bioverfügbarkeit der Phthalate haben könnte.

Die Dosis an Phthalaten, die i.v. verabreicht werden, entspricht der Menge und der Mischung von Phthalaten, die sich an vorkommenden Staub- und Nahrungsmittelproben orientiert. Die Bioverfügbarkeit aus der i.v. Verabreichung (100%) dient als Bezugsbasis für die Messungen der Bioverfügbarkeit aus der oralen Zufuhr aus Staub bzw. Nahrungsmitteln. Die einzelnen Phthalate werden in einer Kochsalzlösung zusammengemischt und verabreicht.

Bei der Verabreichung der Phthalate als „Nahrungsmittel“ werden Proben herangezogen, die im Zuge einer Duplikatstudie gewonnen wurden. Das Ziel der Studie war, einerseits eine Quantifizierung der täglichen Aufnahme von Phthalaten durch die Nahrung und zum anderen ein Vergleich der Phthalatkonzentrationen in Lebensmitteln mit der Ausscheidung von Phthalaten und deren Metabolite im Urin vorzunehmen. Die Phthalatkonzentrationen der zu untersuchenden Nahrungsmittelduplikate sind bekannt (Tabelle 4) und werden vor Beginn des Fütterungsversuches erneut bestimmt. Es werden geeignete Duplikate herausgesucht, welche sich an der oben beschriebenen verabreichten Dosis von Phthalaten im Hausstaub und in der i.v.- Lösung orientieren. Die Konzentration der Phthalate wird durch die Menge an Nahrungsmittelprobe, die dem Schwein oral zugeführt wird, sichergestellt. Bei den zu verfütternden Nahrungsmittelproben ist nicht mit einer gesundheitlichen Beeinträchtigung der Schweine zu rechnen. Diese Lebensmittel wurden bereits vom Menschen ohne gesundheitliche Folgen verzehrt. Duplikate dieser verzehrten Lebensmittel dienen als Matrix für die Verfütterung an die Schweine.

Tabelle 4: Phthalatkonzentration in Nahrungsmittelduplikaten (Fromme et al. 2013)

Phthalate	Konzentration [ng/g f.w]
BBzP	0,3 - 9
DEHP	4,3 - 131
DIDP	Nicht nachweisbar
DINP	Nicht nachweisbar
DiBP	1,2 - 163
DnBP	0,1 – 17,2
DINCH	nicht gemessen

Durch dieses Versuchsvorgehen ist ein Vergleich der unterschiedlichen Aufnahmepfade von Phthalaten im Hinblick auf die Bioverfügbarkeit möglich. Die den Tieren zugeführte Dosis an Phthalaten muss für alle Eintragspfade (Staub, Nahrungsmittel, i.v.) vergleichbar sein. Das Schwein entweder eine Staub- oder eine Nahrungsmittelprobe einmal täglich, zusätzlich hat es während des gesamten Versuchs Zugang zu Wasser und Mastfutter zur freien Aufnahme.

Zusätzlich machen wir folgende ergänzende Ausführungen zu Ihren weiteren Fragen:

4. **Im Vorversuch wollen Sie den Tieren Nahrung mit einem „hohen Gehalt an Weichmachern“ verabreichen. Sie haben nicht erläutert, was ein hoher Gehalt ist. Wie ist die Zusammensetzung? Welche Konzentrationen entsprechen einem „hohen Gehalt“ im Gegensatz zu den anderen geplanten Dosierungen?**

Mit einem „hohen“ Gehalt ist diejenige Dosis gemeint, die der duldbaren täglichen Aufnahmemenge für den Menschen entspricht (Tabelle 5):

Tabelle 5: Täglich duldbare Aufnahmemenge (TDI) der einzelnen Phthalate für den Menschen

Phthalate	TDI [µg/d/kg bw]
BBzP	500
DEHP	50
DIDP	150
DINP	150
DiBP	10
DnBP	10
DINCH	1000

Bei diesen Dosierungen ist nach der Definition eines TDI nicht mit gesundheitlichen Beeinträchtigungen der Tiere zu rechnen.

5. **Der Vorversuch soll nach Ihrer Aussage zur Frage 8a) dazu dienen, grundlegende toxikokinetische Daten zu ermitteln. Das lässt sich aus Ihren Angaben nicht ableiten? Welche grundlegenden toxikokinetischen Daten können Sie mit diesem Versuch ermitteln? Bitte erläutern Sie das detailliert.**

Mit dem Vorversuch kann der zeitliche Verlauf der Ausscheidung der Metabolite ermittelt werden. Er dient dazu sicherzustellen, dass im Urin alle Metabolite valide nachgewiesen werden können. Des Weiteren werden Erkenntnisse gewonnen, wie lange der Urin gesammelt werden muss. Zudem dient der Vorversuch dazu, die Harnsammelperiode im Hauptversuch zu erproben, um damit die Bewegungseinschränkung der Tiere im Hauptversuch auf einen möglichst minimal erforderlichen Zeitrahmen zu begrenzen, um das Ausscheidungsverhalten der verschiedenen Phthalate abbilden zu können.

8. **Bezüglich des Verbleibs der Schweine stimmen Sie zu, dass eine Tötung zur Ermittlung der Ergebnisse nicht erforderlich ist. Sie wollen „vor dem Versuchsende“ prüfen, wie die Tiere weiter verwendet werden können. Diese Prüfung soll vor Versuchsbeginn bzw. im Rahmen der Beantragung erfolgen. Über den Verbleib der Tiere sind entsprechende Angaben zu machen. Zu berücksichtigen sind hier die §§ 10 und 28 der Tierschutz-Versuchstierverordnung.**

Nach dem Tierkörperbeseitigungsgesetz dürfen Tiere, die als Versuchstiere verwendet wurden (Kategorie 1), nicht in die Lebensmittelkette gelangen und müssen unschädlich beseitigt werden. Daher werden die Schweine entweder direkt nach dem Versuchsende oder nach einer weiteren Haltung auf dem Versuchsgut des BfR an eine Einrichtung (z.B. FU Berlin, HU Berlin, Charite) abgegeben, mit der das BfR sehr intensive Kooperationen pflegt. Dort werden die Schweine zu Aus-/Fort- und Weiterbildungszwecke weiter genutzt werden.

9. **Bezüglich der Möglichkeit, die Bioverfügbarkeit der Phthalate aus Stäuben in einem in vitro Test zu untersuchen hatte ich auch gefragt, welche Recherchen Sie vorgenommen haben, um ggf. geeignete in vitro Tests oder andere Möglichkeiten der Risikobewertung ohne Tierversuche zu ermitteln. Hier steht die Antwort noch aus.**

Der Entscheidung für ein in vivo Experiment ging eine umfangreiche Literaturrecherche voraus wie im ursprünglichen Antrag dargestellt. Geeignete in vitro Verfahren liegen allenfalls für Einzelaspekte, wie z.B. Messungen von Verdauungsvorgängen vor. Der Prozess der Resorption ist nicht durch in vitro Tests hinreichend abbildbar, so dass sie für die Fragestellung des beantragten Versuchsvorhabens zur Resorptionsverfügbarkeit eines Stoffes nicht ausreichend sind. In einer Vergleichsstudie von Oomen et al. (2002) wurden z.B. fünf verschiedene in vitro Verdauungstests geprüft. Die Ergebnisse in Bezug auf die Resorptionsverfügbarkeit von Kontaminanten in drei getesteten Bodenproben differierten dabei erheblich. Die verwendeten Methoden unterschieden sich u.a. im pH-Wert der Verdauungssäfte, in der Verweildauer der Verdauungssaftlösung sowie im Verhältnis zwischen Bodenprobe und Verdauungssaft. Bislang ist noch nicht geklärt, welcher dieser Schritte die Resorptionsverfügbarkeit beeinflusst. Des Weiteren wurde bisher noch nicht verifiziert, welche Methode zur Abbildung der Vorgänge beim Menschen am besten geeignet ist. Daher wird für die zu untersuchende Fragestellung ein in vitro Verdauungstest als nicht Ziel führend eingeschätzt.

10. **Auch wurde die Frage nicht beantwortet, wie Sie die Aussichten beurteilen, entsprechende Tests weiter zu entwickeln, anstatt ein Tiermodell zu etablieren. Diese Diskussion ist aus tierschutzrechtlicher Sicht unbedingt zu führen.**

Es gibt Bereiche, in denen Alternativen zum Tierexperiment entwickelt werden können bzw. bereits entwickelt worden sind. Je komplexer jedoch die wissenschaftliche Fragestellung ist, desto schwieriger wird die Entwicklung eines geeigneten Modells sein. Die Aufnahme, der Metabolismus und die Ausscheidung eines Fremdstoffes stellen in diesem Sinne sicherlich außerordentlich komplexe Prozesse dar. Ob es in der Zukunft gelingt, hierfür valide Modelle zu etablieren, bleibt abzuwarten. Bisher liegen sie nicht vor. Daher können hierzu von unserer Seite keine weiterführenden Aussagen getroffen werden.

11. Bezüglich der Vorversuche haben Sie die Versuchsdauer verkürzt. Nicht beantwortet wurde die Frage nach der Möglichkeit der weiteren Verwendung dieser Schweine im Hauptversuch. Da auch die Tiere im Hauptversuch wiederholt eingesetzt werden, ist nicht erkennbar, warum die Tiere aus dem Vorversuch nicht im Hauptversuch weiter eingesetzt werden könnten. Bitte nehmen Sie dazu Stellung.

Die Ergebnisse des Vorversuchs sollen dazu dienen, um gegebenenfalls im Hauptversuch entsprechende Anpassungen im Versuchsdesign vornehmen zu können. Da zwischen dem Vor- und dem Hauptversuch eine ausreichende Zeitspanne für die Auswertungen und Schlussfolgerungen aus dem Vorversuch eingeplant werden, um die bestmöglichen Bedingungen für den Hauptversuch zu ermöglichen, würden die Versuchstiere aus dem Vorversuch bei Beginn des Hauptversuchs bereits zu alt sein. In diesem Fall könnten sie nicht mehr als „Modell" für die Bedingungen des Kleinkindes herangezogen werden.

12. Die ethische Vertretbarkeit der beantragten Tierversuche ist nach wie vor unzureichend dargelegt. Welche Erkenntnisse erwarten Sie mit den Tierversuchen gewinnen zu können? Was soll mit den Werten, die für die Ferkel gewonnen werden, im Hinblick auf Kleinkinder passieren? Welche Wertigkeit haben die erhofften Erkenntnisse im Hinblick auf die Risikobewertung von Stäuben für Kleinkinder? Welchen konkreten Nutzen können Ihre Erkenntnisse bringen? Diese Fragen müssen hier noch einmal diskutiert und den Schmerzen, Leiden und Schäden, die den Versuchstieren zugefügt werden gegenübergestellt werden. Rechtfertigen die erwarteten Erkenntnisse die Schmerzen, Leiden und Schäden der Versuchstiere? Das ist wissenschaftlich begründet darzulegen.

Aus dem beantragten Tierversuchsvorhaben erwarten wir wesentliche Erkenntnisse über die Höhe der Bioverfügbarkeit von Phthalaten im Hausstaub und in Nahrungsmitteln. Ohne diese Daten kann die gesamte Exposition des Menschen über die unterschiedlichen Expositionspfade nicht abgeschätzt werden. Kleinkinder stellen eine besonders schützenswerte Gruppe der Bevölkerung dar. Daher besteht nicht nur ein wissenschaftliches, sondern auch ein generelles Interesse, sie möglichst umfassend vor möglichen gesundheitlichen Risiken zu schützen. Es ist bekannt, dass Kleinkinder (aufgrund ihrer Art der Fortbewegung) wesentlich mehr Staub (ca.100 mg/Tag) als Erwachsene aufnehmen. Für eine fundierte wissenschaftliche Risikobewertung ist es daher unerlässlich zu wissen wie bioverfügbar die Phthalate im Staub bzw. in der Nahrung sind. Der Nutzen dieses Versuchsdesigns besteht aus Sicht des gesundheitlichen Verbraucherschutzes darin, eindeutige Aussagen darüber treffen zu können, ob Phthalate aus Hausstaub, auch im Vergleich zu Nahrungsmitteln, vergleichsweise gut bioverfügbar sind und damit wesentlich zur Gesamtexposition des Menschen beitragen können. Dies stellt eine unabdingbare Vorraussetzung dar, um geeignete Maßnahmen zu ergreifen, den Menschen vor möglichen gesundheitlichen Gefahren durch die Aufnahme von Phthalaten über Hausstaub zu schützen.

Literatur

Oomen, A.G., et al., *Comparison of five in vitro digestion models to study the bioaccessibility of soil contaminants.* Environ Sci Technol, 2002. **36**(15): p. 3326-34.

Fromme, H., et al., *Phthalate and di-(2-ethylhexyl) adipate (DEHA) intake by German infants based on the results of a duplicate diet study and biomonitoring data (INES 2).* Food Chem Toxicol, 2013. **53**: p. 272-80.

Abkürzungsverzeichnis:

BBzP= Bezylbutyl Phthalat
DEHP= Di-2-ehtylhexyl Phthalat
DIDP= Di-isodecyl Phthalat
DINP= Di-isononyl Phthalat
DiBP= Di-isobutyl Phthalat
DnBP= Di-n-butyl Phthalat
DINCH= Di(isononyl)cyclohexane-1,2-dicarboxylat
MBzP = Mono-benzyl phthalat (metabolit von BBzP)
MEHP = Mono (2-ethylhexyl) Phthalat (Metabolit von DEHP)
5-oxo-MEHP = Mono-(2-ethyl-5-oxohexyl) Phthalat (Metabolit von DEHP)
5-OH-MEHP = Mono-(2-ethyl-5-hydroxyhexyl) pPthalat (Metabolit von DEHP)
2-cx-MMHP = Mono(2-carboxymethylhexyl) Phthalat (Metabolit von DEHP)
5-cx-MEPP= Mono(2- ethyl-5-carboxypentyl) Phthalat (Metabolit von DEHP)
oxo-MiDP= Mono(4,5-dimethyl-7-oxooctyl) Phthalat (Metabolit von DIDP)
OH-MIDP = Mono(4,5-dimethyl-7-hydroxyoctyl) Phthalat (Metabolit von DIDP)
cx-MiDP = Mono(4,5-dimethyl-7carboxy –heptyl) Phthalat (Metabolit von DIDP)
MINP = Mono isononyl phthalate (Metabolit von DINP)
7-OH-MiNP = Mono(4-methyl-7-hydroxy-octyl) Phthalat (Metabolit von DINP)
7-oxo-MINP= Mono(4-methyl-7-oxo-octyl) Phthalat (Metabolit von DINP)
7-cx-MINP= Mono(4-methyl-7-carboxy-heptyl) Phthalat (Metabolit von DINP)
MiBP= Mono-isobutyl Phthalat (Metabolit von DiBP
MnBP = Mono n-butyl Phthalat (Metabolit von DnBP)
MINCH= cyclohexane-1,2-dicarboxylic monoisononyl ester (Metabolit von DINCH)
OH-MINCH= cyclohexan-1,2-dicarboxyl mono hydroxyisononyl ester (Metabolit von DINCH)
cx-MINCH= cyclohexan-1,2-dicarboxylic mono carboxyisooctyl ester (Metabolit von DINCH)
oxo-MICH = cyclohexan-1,2-dicarboxylic mono oxoisononyl ester (Metabolit von DINCH
CHDA = cyclohexan-1,2-dicarboxyk säure (Metabolit von DINCH)

Table A1. Experiment timetable. Letters are dust samples, numbers are pigs

05.10.2015 **8pm** Pigs: 1,2,3 and 4 into the metabolite cage	**06.10.2015** **6am-10pm** B-1 C-2 D-3 E-4	**07.10.2015** **6am-8pm** B-1 C-2 D-3 E-4	**08.10.2015** **8pm Pigs**: 5,6 and 7 into the metabolite cage	**09.10.2015** **6am-10pm** E-5 C-6 B-7	**10.10.2015** **6am-8pm** E-5 C-6 B-7	**11.10.2015** **8pm Pigs:** 1,2,3 and 4 into the metabolite cage
12.10.2015 **6am-10pm** A-1 B-2 E-3 C-4	**13.10.2015** **6am-8pm** A-1 B-2 E-3 C-4	**14.10.2015** **8pm** Pigs: 5,6 and 7 into the metabolite cage	**15.10.2015** **6am-10pm** C-5 A-6 E-7	**16.10.2015** **6am-8pm** C-5 A-6 E-7	**17.10.2015** **8pm** Pigs: 1,2,3 and 4 into the metabolite cage	**18.10.2015** **6am-10pm** C-1 E-2 B-3 A-4
19.10.2015 **6am-8pm** C-1 E-2 B-3 A-4	**20.10.2015** **8pm** Pigs: 5,6 and 7 into the metabolite cage	**21.10.2015** **6am-10pm** D-5 B-6 A-7	**22.10.2015** **6am-8pm** D-5 B-6 A-7	**23.10.2015** **8pm** Pigs: 1,2,3 and 4 into the metabolite cage	**24.10.2015** **6am-10pm** D-1 A-2 C-3 B-4	**25.10.2015** **6am-8pm** D-1 A-2 C-3 B-4
26.10.2015 **8pm** Pigs: 5,6 and 7 into the metabolite cage	**27.10.2015** **6am-10pm** Food sample for Pig 5,6 and 7	**28.10.2015** **6am-8pm** Food sample for Pig 5,6 and 7	**29.10.2015** **8pm** Pigs: 1,2,3 and 4 into the metabolite cage	**30.10.2015** **6am-10pm** Food sample for Pig 1,2,3 and 4	**31.10.2015** **6am-8pm** Food sample for Pig 1,2,3 and 4	**1.11.2015** **8pm** Pigs: 5,6 and 7 into the metabolite cage
02.11.2015 **6am-10pm** A-5 D-6 C-7	**03.11.2015** **6am-8pm** A-5 D-6 C-7	**04.11.2015** **8pm** Pigs: 1,2,3 and 4 into the metabolite cage	**05.11.2015** **6am-10pm** E-1 D-2 A-3 D-4	**06.11.2015** **6am-8pm** E-1 D-2 A-3 D-4	07.11.2015	**08.11.2015** **8pm** Pigs: 5,6 and 7 into the metabolite cage
09.11.2015 **6am-10pm** IV-Injection For Pigs 5,6 and 7	**10.11.2015** **6am-8pm** IV-Injection For Pigs 5,6 and 7	**11.11.2015** **6am-10pm** IV-Injection For Pig 1,2,3 and 4	**12.11.2015** **6am-8pm** IV-Injection For Pig 1,2,3 and 4	**13.11.2015** **8pm** Pigs: 5,6,7 into the metabolite cage	**14.11.2015** **6am-10pm** B-5 E-6 D-7	**15.11.2015** **6am-8pm** B-5 E-6 D-7

Table A2. Used Standards for food sample

Plasticizer	Brand	CAS-Number
BBP	Fluka	85-68-7
DEHP	Fluka	117-81-7
DnBP	Fluka	84-74-2
DINP	Fluka	28553-12-0

Table A3. Transition settings of the investigated and labeled phthalate metabolites RT: retention time; DP: declustering potential; EP: entrance Potential; CEP: collision cell entrance potential; CE: Collision energy

RT	Group	Q1 Mass [Da]	Q3 Mass [Da]	ID	DP	EP	CEP	CE
8.3	5cx-MEPP	307	113	Qual.	-15	-10	-12.71	-40
			159	Quant.				-22
		311	113	D4 lab. Qual.	-15	-10	-12.86	-36
			159	D4 lab. Quant.				-18
8.6	5-OH-MEHP	293	121	Quant.	-30	-10	-12.21	-28
			145	Qual.				-20
		297	125	D4 lab. Quant.	-30	-10	-12.35	-22
			145	D4 lab. Qual.				-24
9.1	7cx-MMeHP	321	121	Qual.	-35	-10	-13.22	-30
			173	Quant.				-18
		325	125	D4 lab. Qual.	-35	-10	-13.36	-32
			173	D4 lab. Quant.				-18
9.1	2cx-MMHP	307	113	Qual.	-10	-10	-12.71	-42
			159	Quant.				-14
		311	113	D4 lab. Qual.	-10	-10	-12.86	-36
			159	D4 lab. Quant.				-16
9.2	MiBP	221	77	Quant.	-20	-10	-9.62	-24
			134	Qual.				-20
		225	81	D4 lab. Quant.	-20	-10	-9.76	-28
			138	D4 lab. Qual.				-20
9.2	5-oxo-MEHP	291	121	Quant.	-25	-10	-12.14	-22
			143	Qual.				-22
		295	124	13C4 lab. Quant.	-25	-10	-12.28	-22
			143	13C4 lab. Qual.				-20
9.3	MnBP	221	77	Quant.	-25	-10	-9.62	-26
			121	Qual.				-20
		225	71	D4 lab. Qual.	-25	-10	-9.76	-20
			79	D4 lab. Quant.				-28
9.4	7OH-MMeOP	307	77	Qual.	-25	-10	-12.71	-46
			121	Quant.				-32
		311	81	D4 lab. Qual.	-25	-10	-12.86	-44

			125	D4 LAB. QUANT.				-28
9.9	MCDMHP	335	121	QUAL.	-20	-10	-13.72	-30
			187	QUANT.				-28
		339	125	D4 LAB. QUAL.	-20	-10	-13.87	-40
			187	D4 LAB. QUANT.				-20
10.1	MBzP	255	77	QUAL.	-20	-10	-10.84	-34
			107	QUANT.				-18
		259	77	13C4 LAB. QUAL.	-20	-10	-10.98	-36
			107	13C4 LAB.QUANT.				-20
10.1	7OXO-MINP	305	77	QUAL.	-25	-10	-12.64	-42
			121	QUANT.				-26
		309	81	D4 LAB. QUAL.	-25	-10	-12.79	-42
			125	D4 LAB. QUANT.				-22
9.8 - 10.8	MHDMOP	321	77	QUAL.	-45	-10	-13.22	-48
			121	QUANT.				-30
		325	81	D4 LAB. QUAL.	-45	-10	-13.36	-50
			125	D4 LAB. QUANT.				-26
11.0	MODMOP	319	77	QUAL.	-30	-10	-13.15	-52
			121	QUANT.				-22
		323	81	D4 LAB. QUAL.	-30	-10	-13.29	-44
			125	D4 LAB. QUANT.				-32
14.3	MEHP	277	127	QUAL.	-25	-10	-11.63	-22
			134	QUANT.				-24
		281	127	13C4 LAB. QUAL.	-25	-10	-11.78	-30
			137	13C4 LAB.QUANT.				-22
14.8	MINP	291	77	QUANT.	-30	-10	-12.13	-38
			139	QUAL.				-28
		295	79	13C4 LAB.QUANT.	-30	-10	-12.28	-38
			141	13C4 LAB. QUAL.				-24

Table A4. List of the used standards

Native standard	Internal standard
MEHP[a]	^{13}C2-MEHP[a]
5OH-MEHP[a]	D4-5-OH-MEHP[b]
5 oxo–MEHP[a]	^{13}C4-5-oxo-MEHP[a]
MBzP[a]	^{13}C2- MBzP[a]
MnBP[a]	^{13}C2-MnBP[a]
MINP[a]	^{13}C2-MINP[a]
2cx-MMHP[b]	D4-2cx-MMHP[b]
5cx-MEPP[b]	D4-5cx-MEPP[b]
OH-MINP[b]	D4-7OH-MINP[b]
oxo-MINP[b]	D4-oxo-MINP[b]
cx-MINP[b]	D4-cx-MINP[b]

a)Campridge Isotope, b)Biochemisches Institute für Umweltcarginogene

AI2 Results

The detailed description of toxicokinetics of phthalates

AI2I1 Butyl benzyl phthalate

Pig 1

Dust:

The urinary concentration maximum occurred 3.5 ± 0.7 hours post dose. The estimated half-life time of MBzP was about 7.8 ± 9.1 hours.

Within the first 24 hours, 26.6 ± 18.6 % of applied BBzP dose was excreted as MBzP. Between 24 to 38 hours only 0.2 ± 0.5 % of the Dose was eliminated.

Food:

Two hours after dose administration, the maximum concentration was observed in urine. The half-life time was about 2.4 hours. 69.2 % of the applied dose was eliminated within the first 24 hours. On the second day no MBzP was detectable in the urine.

Pig 2

Dust:

MBzP reached its maximum concentration 2.2 ± 0.5 hours post dose. The average $t_{½}$ was about 5 ± 3.6 hours. On the first day, 30.3 ± 18.1 % of the applied BBzP dose was eliminated as MBzP. On the second day, only 0.08 ± 0.17 % of dose was found in urine.

Food:

The concentration maximum was reached three hours post dose. Based on the excretion profile, it was not possible to calculate the half-life. The uptake and metabolism took place in the first 24 hours where 30 % of the BBzP dose was excreted

Pig 3

Dust:

The concentration maxima was reached 3.2 ± 1 hours post dose. The estimated half-life time was about 2.8 ± 0.5 hours. On the first sampling day, 30.2 ± 12.3 % and on the second day 0.3 ± 0.6 % of the applied BBzP dose was detected.

Food:

It was more than the applied dose excreted in urine.

Pig 4

Dust:

The urinary concentration maximum was observed 2.5 ± 0.6 hours post dose. $t_{½}$ was determined about 6.4 ± 6.6 hours. 27.4 ± 18.1 % of the applied dose was completely excreted on the first day.

Food:

The maximum concentration was reached three hours post dose. 4.4 hours was determined as elimination half-life time. In Sum 63.4 % of the BBzP dose was completely excreted within 24 hours as MBzP.

Pig 5

Dust:

T_{max} was observed 3 ± 1.1 hours post dose and the half-life time was about 3.2 ± 0.6 hours. In total, 18.2 ± 14.4 % of the dose was completely eliminated during the first day.

Food:

Within three hours, the maximum concentration was reached. The calculated half-life time was 2.2 hours. Similar to dust, the complete 0.2 % of the applied BBzP dose was excreted within 24 hours.

Pig 6

Dust:

MBzP reached its urinary C_{max} at 2.7 ± 1 hours after dose. The $t_{½}$ was estimated as 7.1 ± 3.5 hours. 29 ± 27.4 % of the given dose was excreted in the first 24 hours. From 24 hours to 38 hours 0.1 ± 0.2 % of the given dose was detected in urine.

Food:

After ten hours the maximum concentration was reached. The half-life time was estimated with 8.4 hours. 24.5 % of the applied dose was completely excreted on the first day, on the second day no metabolites were detected.

Pig 7

Dust:

C_{max} was estimated after three hours post dose. The elimination half-life was determined around 3.4 ± 1.6 hours. During the first 24 hours, 28 ± 13.6 % of the given dose was detected.

Food:

Similar to the other piglets, C_{max} was noticed after three hours post dose and t ½ was after 3.9 hours. The complete absorbed dose of 17.8 % was excreted within 24 hours.

AI2I2 Di-n-butyl phthalate

Pig 1

Dust:

The maximum concentration was reached after 2.8 ± 0.8 hours post dose. The first estimated half-life time was around 3.3 ± 1.8 hours, whereas 24 hours later the second half life time was reached with 7.3 ± 4.8 hours.

During the first 24 hours, 45 ± 21 % of the given dose was eliminated via urine, while 24 - 38 hours post dose only 3.2 ± 3 % of applied dose was detected.

Food:

After two hours post dose the urinary maximum concentration was reached. The half-life time was estimated for 1.8 hours. The second half-life time (24 hours post dose) was 3.8 hours. The complete absorbed dose of 56.8 % was metabolized and eliminated within in the first 24 hours.

Pig 2

Dust:

The highest concentration was 2.5 ± 0.6 hours post dose noticed. During the first elimination period, the half-life time was about 6.6 ± 7.6 hours, while at the second elimination phase, $t_{½}$ was 6.3 ± 8.8 hours. The second elimination period occurred 11 to 36 hours post dose (see table A5).

43.7 ± 16.1 % of the applied dose was excreted in the first 24 hours. On the second day, 4.6 ± 5.9 % was detected.

Food:

More than 100 % of the applied dose was found

Pig 3

Dust:

The concentration maxima was 2.6 ± 1.5 hours post dose noticed. $t_{½}$ of the first elimination period was 1.9 ± 0.2 hours, while the second $t_{½}$ was estimated with 2.6 ± 0.2 hours and occurred 9 - 24 hours post dose (for details see table X).

Within the first 24 hours, 45.7 ± 16.7 % of the given dose was eliminated. 24 - 38 hours post dose, 3.1 ± 2.3 % of the applied dose was found in urine.

Food:

The uptake was more than the given dose

Pig 4

Dust:

It took 2.2 ± 0.5 hours after dose administration to reach the maximum concentration. $t_{½}$ of the first elimination phase was 1.9 ± 0.4 hours, the second elimination half-life time was 7.5 ± 9.2 hours and occurred 12 - 14 hours post dose

On the first day, 46.6 ± 19.4 % of the applied dose was excreted, whereas on the second day, only 1.1 ± 1.5 % respectively.

Food:

The maximum concentration was reached three hours post dose. The elimination half-life time was determined with four hours.

47.8 % of the dose was absorbed and metabolized and completely excreted within 24 hours post dose.

Pig 5

Dust:

The maximum concentration was 3.2 ± 0.9 hours post dose observed. During the first elimination, the half-life time was determined at 2.5 ± 0.6 hours, the second $t_{½}$ was calculated with 6.8 ± 4.3 hours (12-24 hours post dose).

38.6 ± 10.7 % of the dose was determined in the first 24 hours. Only 2.2 ± 2.4 % was excreted on the second day, respectively.

Food:

Three hours post dose, the maximum of concentration was reached. The half-life time amounted 2.8 hours.

21.3 % of the applied Dose was excreted in the first 24 hours as MnBP. After 24 hours 2.4 % of the applied dose was eliminated.

Pig 6

Dust:

The concentration maximum was observed 3.4 ± 1.7 hours post dose. $t_{½}$ for the first excretion phase was about 4.4 ± 1.6 hours, the second $t_{½}$ was 7.6 ± 2 hours (11 - 24 hours post dose)

50.5 ± 29 % of the applied dose was detected in 24 hours post dose. 0.9 ± 1.3 % was found on the second day.

Food:

The C_{max} was determined three hours after dose administration. The estimated elimination half –life time was 8.7 hours. Within 24 hours, 23.5 % of the applied dose was detected in urine.

Pig 7

Dust:

2.7 ± 0.5 hours post dose, the highest concentration was measured. During the first elimination phase, the half-life time was estimated around 2.1 ± 1.4 hours, the second above 5.6 ± 2.2 hours (24 h post dose).

49.7 ± 13.4 % of the administrated dose was found in urine as MnBP. On the second day, only 3.8 ± 2.9 % was detected.

Food:

Same as dust samples, the concentration maximum was reached three hours post dose. The first and second $t_{½}$ was determined with 5.6 hours and 5 hours.

22 % of the applied dose was detected in urine samples 24 hours post dose. 24 hours to 38 hours post dose, only 4.2 % of the applied dose was identify and excreted as MnBP.

A detail description of the toxicokinetic parameters of MBzP and MnBP, concentration maximum (C_{max}) and it's time appearance (t_{max}), half-life time ($t_{½}$) in the first excretion and second period (second t_{max} [h] + second $t_{½}$) are given in Table A5.

Table A5. Detail description of kinetic parameters of BBzP and DnBP metabolism [C_{max} in µg, t_{max} and $t_{½}$ in hour].

Dust	Pig	MBzP				MnBP			
		c_{max}	t_{max}	$t_{½}$		c_{max}	t_{max}	$t_{½}$	
A	**1**	0.7		2		3.4	2	4	24+5
	2	0.9	2	6		3.1	2		
	3	0.9	3	2		5.5	3	2	24+3
	4	1.4	2	2		9	2	2	14+1
	5	0.5	4	4		3	4	4	
	6	0.3	2	5		2.5	2	4	
	7	1.6	3	2		4.9	3	2	
B	**1**	4.7	4	18.4		2.08	4	5.7	24+15.1
	2	4.6	3	10		3	3	4	24+2
	3	3.6	2	3		1.8	2	2	
	4	4.6	2	16		2.8	2	5	
	5	1.8	4	3		1.6	4	2	
	6	5	4	8	24+1	2.7	4	5	11+9
	7	4.3	3	3		3	3	4	
C	**1**	1.1	-	-	-	12.1	3	2	24+5
	2	2.1	2	2		14.7	2	18	36+0.5
	3	2.6	5	3		1.8	5	2	12+3
	4	1.2	3	2		11	3	2	12+14
	5	0.9	2	4		5.6	2	3	24+4
	6	0.9	2	5		6.3	2	3	28+6
	7	2.6	3	3		18.7	3	2	24+7
D	**1**	0.1	-	-	-	1.8	2	2	14+3
	2	0.2	3	-	-	4.7	3	3	
	3	0.1	2	-	-	1.8	1	4	
	4	0.2	2	-	-	2.8	2	2	
	5	0.1.	12	-	-	4.4	3	2	12+9
	6	0	-	-		1.4	6	2	14+5
	7	0.3		-		4.7	2	4	24+7
E	**1**	2.3	3	3		1.8	3	2	
	2	2.7	2	2		1.9	2	2	11+2
	3	1.3	3	3		0.9	2	2	
	4	1.6	3	5		1	32	2	
	5	2.2	2	3		2.1	2	5	
	6	4.4	3	5		2.6	3	2	
	7	1.4		6		0.6	3	-	-
Food	**1**	2.8	2	2.3		5.5	2	2.9	
	2	2.9	3	-		13	32	2	
	3	4	4	3		6.2	4	2	24+7
	4	2.1	3	4		2.8	3	4	
	5	1.2	3	2		3.3	3	3	
	6	0.6	10	8		0.9	3	9	
	7	0.6	3	4		1	3	5	

AI2I3 DEHP

Pig 1

Dust:

MEHP and oxo-MEHP reached their maximum concentration after 3.4 ± 0.5 hours, 5OH-MEHP and 5cx-MEPP had their t_{max} at 2.8 ± 0.8 hours. During the first elimination period, MEHP has a half-life time of 5 ± 2.4 hours, followed by 5cx-MEPP (5.2 ± 1 hours), 5OH-MEHP (5.3 ± 2.8 hours) and oxo-MEHP (7.7 ± 4.8 hours). In the second elimination phase, which normally occurred 24 hours after drug administration, the estimated mean half-life time for MEHP, oxo-MEHP, 5OH-MEHP and 5cx-MEPP was 4.4 ± 1 hours, 4.7 ± 0.7 hours, 4.6 ± 0.7 hours and 4.1 ± 0.5 hours. Within the first 24 hours 40 ± 7.5 % of the applied DEHP dose was excreted as 12.6 ± 1.6 % 5cx-MEPP), 11.5 ± 2.3 % 5OH-MEHP, 7.6 ± 1.6 % oxo-MEHP, 8.4 ± 3.33 % MEHP. Between the 24 hours to 38 hours post dose, 2.3 % of the applied Dose was excreted as 0.7 ± 0.2 % oxo-MEHP, 0.7 ± 0.3 % 5OH-MEHP, 0.6 ± 0.2 % 5cx-MEPP, 0.5 ± 0.3 % MEHP.

Food

All metabolites reached their maximum concentration after 24 hours and have an elimination half –life time of four hours. Within the first 24 hours, 46 % of DEHP dose was excreted as 11.5 % MEHP, 8.9 % oxo-MEHP, 13.4 % 5OH-MEHP, 12.2 % 5cx-MEPP. On the second day 15.3 % DEHP was eliminated as 4.1 % MEHP, 3.3 % oxo-MEHP, 4.2 % 5OH-MEHP and 3.6 % 5cx-MEPP.

Pig 2

Dust:

The mean T_{max} for MEHP and 5OH-MEHP were determined after 2.4 ± 0.5 hours and for oxo-MEHP and 5cx-MEPP after 3 ± 1 hours after dust sample application.

The mean half-life times for the first elimination period was estimated as 2.8 ± 0.9 hours (MEHP), 3.5 ± 1.7 hours (5OH-MEHP), 5 ± 2.5 hours (oxo-MEHP), 6.1 ± 3.4 hours (5cx-MEPP). During the second elimination, which normally was after 24 hours, the mean half-life time was determined around 9.1 ± 3 hours for the primary metabolite MEHP, the secondary metabolite had a shorter half life time with 4.4 ± 0.8

hours (5OH-MEHP), 4.7 ± 1 hours (5cx-MEPP) and 4.8 ± 1.1 hours (oxo-MEHP), respectively.

During the first 24 hours, 40 ± 6 % of the applied DEHP-Dose was eliminated as 15.8 ± 2 % 5cx-MEPP, 8.3 ± 0.8 % oxo-MEHP, 7.2 ± 1.6 % MEHP, 8.6 ± 2.5 % 5OH-MEHP. After 24 hours only 1.8 ± 0.4 % of the administrated DEHP dose were eliminated as 0.6 ± 0.07 % 5cx-MEPP, 0.4 ± 0.08 % oxo-MEHP and 5OH-MEHP and 0.4 ± 0.3 % MEHP.

Food:

The maximum concentration was determined 24 hours after dose application and the elimination half-life times ranged around three hours. 48.5 % of the DEHP dose was excreted in the first 24 hours. The main metabolite was 5cx-MEPP with 28.6 % followed by 11.6 % oxo-MEHP, 8.1 % MEHP and only 0.06 % 5OH-MEHP. In the sampling period 24 hours to 38 hours post dose: 20.8 % DEHP (9.7 % 5cx-MEPP, 6.3 % oxo-MEHP, 4.8 % MEHP, 0.03 % 5OH-MEHP) was eliminated by urine.

Pig 3

Dust:

MEHP, oxo-MEHP and 5cx-MEPP reached their mean maximum concentration after 4.4 ± 1.3 hours post dose, while 5OH-MEHP had its t_{max} after 3 ± 1.2 hours.

In the first elimination period, the mean half-life time at 4 ± 2.5 hours 5cx-MEPP, 5.7 ± 3.3 hours 5OH-MEHP, 6 ± 3.7 hours MEHP to 6.3 ± 3.3 hours oxo-MEHP. After 24 hours, the second elimination phase occurred with a mean half-life of 3.75 ± 3.7 hours for MEHP, 5.9 ± 3.6 hours for 5OH-MEHP, 7.4 ± 2.4 hours for 5cx-MEPP and 7.7 ± 3.6 hours for oxo-MEHP. On the first day 38 ± 14.3 % of the DEHP dose were excreted in form of MEHP (8.4 ± 3 %), oxo-MEHP (8.2 ± 2.7 %), 5OH-MEHP (9.7 ± 4.5 %) and 5cx-MEPP (11.5 ± 4.5 %). After 24 hours, only 3.3 ± 1.6 % of the DEHP dose was excreted as 0.9 ± 0.4 % 5cx-MEPP, 0.9 ± 0.5 % oxo-MEHP and OH-MEHP and 0.6 ± 0.2 % MEHP.

Food:

Twenty-four hours after dose application, all metabolites reached their maximum concentration. The half-life time for MEHP, oxo-MEHP, 5OH-MEHP and 5cx-MEPP was estimated as 5.4 hours, 6 hours, 5.4 hours and 4.5 hours. 41 % of the applied DEHP-dose was excreted within the first 24 hours (14.1 % 5cx-MEPP, 10.2 % oxo-

MEHP, 9 % MEHP and 8.1 % 5OH-MEHP). In the sampling period from 24 to 38 hours only 17.5 % of DEHP dose was eliminated by urine (5 % 5cx-MEPP and oxo-MEHP, 3.8 % MEHP, 3.7 % 5OH-MEHP).

Pig 4

Dust:

T_{max} for MEHP and 5OH-MEHP were estimated as 2.4 ± 0.5 hours and three hours for oxo-MEHP. For 5cx-MEPP, the mean of t_{max} was determined as 11.2 ± 11.7 hours.

During, the first elimination period the half-life time was estimated for MEHP as 4.5 ± 1.6 hours, 5cx-MEPP as 5.2 ± 1.4 hours, 5OH-MEHP as 5.3 ± 2 hours and oxo-MEHP as 7 ± 2.6 hours. At the second elimination period (24 hours after dust application), the half-life time ranged between 5.3 ± 1.7 hours (5cx-MEPP), 5.6 ± 1.8 hours (oxo-MEHP), 5.7 ± 2.6 hours (5OH-MEHP) and 6.5 ± 3.5 hours (MEHP).

18.7 ± 5.2 % 5cx-MEPP, 10.8 ± 5.6 % 5OH-MEHP, 7.2 ±1.4 % oxo-MEHP, 6.5 ± 2.4 % MEHP were excrete. 43.2 ± 12.2 % of the DEHP-doses was eliminated in the first 24 hours. On the second collecting day 2.8 ± 1.1 % of the applied DEHP dose was excreted as 1.1 ± 0.4 % 5cx-MEPP, 0.7 ± 0.5 % 5OH-MEHP, 0.6 ± 0.2 % oxo-MEHP, 0.3 ± 0.1 % MEHP.

Food

The maximum concentration was determined 24 hours after sample administration. The elimination half-life of six hours for MEHP, 6.3 hours for oxo-MEHP, 5.5 hours for 5OH-MEHP and 5.3 hours for 5cx-MEPP was determined. On the first day, 18.4 % 5cx-MEPP, 6.7 % 5OH-MEHP, 5.7 % oxo-MEHP, 5.1 % MEHP, in total 36 % of the applied DEHP dose was eliminated. On the second day only 11 % of applied DEHP dose was found in urine (5.6 % 5cx-MEPP, 2 % 5OH-MEHP and oxo-MEHP, 1.4 % MEHP)

Pig 5

Dust:

T_{max} (mean) were estimated for MEHP, 5OH-MEHP, oxo-MEHP and 5cx-MEPP as 2.4 ± 0.5 hours, 3.2 ± 1.6 hours, 4.2 ± 1.8 hours and 5 ± 1 hours.

3.2 ± 0.3 hours (MEHP), 4 ± 0.9 hours (5OH-MEHP), 4.3 ± 0.8 hours (oxo-MEHP) and 4.6 ± 1.3 hours (5cx-MEPP) were determined as the mean half-life time in the first excretion phase. The second excretion period was 24 hours post dose, the mean half-life time was in decreasing order 7 ± 2.7 hours (MEHP), 4.8 ± 1 hours (5OH-MEHP), 4.6 ± 0.8 hours (oxo-MEHP), 4.5 ± 0.8 hours (5cx-MEPP). Within the first 24 hours, 35.1 % from DEHP dose were excreted as 19 ± 2.9 % 5cx-MEPP, 8.1 ± 0.82 % oxo-MEHP, 4.6 ± 1.1 % 5OH-MEHP and 4.3 ± 1.5 % MEHP. After 24 hours 2.1 % DEHP dose was found in urine. It was mainly excreted as 0.9±0.2 % 5cx-MEPP followed by 0.5 ± 0.1 % oxo-MEHP, 0.2 ± 0.06 % 5OH-MEHP, 0.19 ± 0.04 % MEHP.

Food:

After 24 hours, the maximum concentration was reached. The elimination half-life was 5.3 hours for MEHP and 5cx-MEPP, eight hours for oxo-MEHP, 6.2 hours for 5OH-MEHP. Within the first 24 hours, 29 % DEHP of the applied dose was mainly excreted as 5cx-MEPP (17.2 %), oxo-MEHP (5 %), 5OH-MEHP (4.5 %) and MEHP (2.4 %). 24 - 38 hours after the sample administration 8.6 % DEHP was found in urine (4.6 % 5cx-MEPP, 1.8 % oxo-MEHP, 1.3 % 5OH-MEHP and 0.9 % MEHP).

Pig 6

Dust:

The mean maximum concentration for metabolites MEHP and 5OH-MEHP was 2.6 ± 0.9 hours, 4 ± 2.3 hours for oxo-MEHP and 4.4 ± 2 hours for 5cx-MEPP.

In the first elimination period, the mean half-life of MEHP, oxo-MEHP, 5OH-MEHP, 5cx-MEPP were predicted as 4.3 ± 2.1hours, 8 ± 7.5 hours, 5.1 ± 1.8 hours and 7.1 ± 4.8 hours.

During the second elimination period (24 hours after dose application), the half-life time of MEHP, oxo-MEHP, 5OH-MEHP, 5cx-MEPP were determined as 4.2 ± 0.4 hours, 4.3 ± 0.8 hours, 4 ± 0.6 hours and 4 ± 0.4 hours. During the first 24 hours, 34.7 % DEHP of the applied dose were eliminated. In Detail: 18 ± 3.6 % 5cx-MEPP, 8.4 ± 3.9 % oxo-MEHP, 8.7 ± 2.9 % 5OH-MEHP and 4.8 ± 2.4 % MEHP. After 24 hours, 1.4 ± 0.5 % 5cx-MEPP, 0.7 ± 0.4 % oxo-MEHP, 0.7 ± 0.3 % 5OH-MEHP, 0.3 ± 0.1 % MEHP. To sum it up, on the second day only 3.2 ± 0.9 % DEHP of the applied Dose was measured in urine.

Food:

After 24 hours, t_{max} for all metabolites was reached. The elimination half-life time was 5.8 hours, 6.5 hours, 5.7 hours and 4.8 hours for MEHP, oxo-MEHP, 5OH-MEHP and 5cx-MEPP. Within the first 24 hours, the elimination of the applied DEHP dose was 38.7 % (18.8 % 5cx-MEPP, 9.9 % 5OH-MEHP, 6.7 % oxo-MEHP, 3.3 % MEHP). After 24 to 38 hours, 15 % was excreted (5.8 % 5cx-MEPP, 4.1 % 5OH-MEHP, 3.7 % oxo-MEHP, 1.4 % MEHP)

Pig 7

Dust:

The mean maximum concentration were found at three hours for 5cx-MEPP and oxo-MEHP, 2.6 ±0.5 hours for 5OH-MEHP and for 2.8 ±0.4 hours MEHP after dust application

The median half-life was in increasing order 3.5 ± 1.1 hours (MEHP), 4.3 ± 1.7 hours (5OH-MEHP), 5 ± 2.5 hours (5cx-MEPP) and as 5.6 ± 2.6 hours (oxo-MEHP) with the highest half life time. After 24 hours the second elimination period was determined. The mean half-life times ranged between: 4.3 ± 2 hours, 4.7 ± 1.4 hours, 4.8 ± 1.4 hours and 6.2 ± 3.2 hours for MEHP, 5OH-MEHP, oxo-MEHP and 5cx-MEPP. Within the first 24 hours, 44 ± 15.8 % of the DEHP dose was eliminated as 19 ± 5.5 % 5cx-MEPP, 11 ± 4 % oxo-MEHP, 7 ± 2.6 % 5OH-MEHP. On the second day, 3.5 % DEHP were mainly excreted as 5cx-MEPP (1.2 ± 0.4 %), followed by oxo-MEHP (1 ± 0.4 %), 5OH-MEHP (0.5 ± 0.2 %) and MEHP (0.3 ± 0.1 %).

Food:

Similar to the other piglets, t_{max} was estimated 24 hours after dose administration. In increasing order the elimination half-life ranged from 5.5 hours, 6.6 hours, 6.8 hours to 7.8 hours for 5cx-MEPP, 5OH-MEHP, oxo-MEHP and MEHP. On the first sampling day, 30 % of DEHP dose as 15.8 % 5cx-MEPP, 6.8% 5OH-MEHP, 5.4 % oxo-MEHP and 2.2 % MEHP). On the second day only 6 % of the applied dose was excreted as mainly 5cx-MEPP (2.6 %) followed by 5OH-MEHP (1.4 %), oxo-MEHP (1.3 %) and MEHP (0.6 %).

A detail description of the toxicokinetic parameters, concentration maximum (C_{max}) and it's time appearance (t_{max}), half-life time ($t_{½}$) in the first excretion and second period (24 hours plus second $t_{½}$) are given in Table A6.

Table A6. Detail description of kinetic parameters of DEHP metabolism [C_{max} in µg, t_{max} and $t_{½}$ in hours].

Dust	Pig	MEHP				oxo-MEHP				5OH-MEHP				5cx-MEPP			
		c_{max}	t_{max}	$t_{½}$		c_{max}	t_{max}	$t_{½}$		c_{max}	t_{max}	$t_{½}$		c_{max}	t_{max}	$t_{½}$	
A	**1**	42.3	3	5	24+4	43.2	3	7	24+4	88.3	2	5	24+4	67	2	5	24+4
	2	60.1	2	4	14+12	47	2	7	24+6	59.8	2	5	24+6	85.6	4	9	24+6
	3	52.1	3	2	24+10	44.8	3	4	24+8	38.2	3	3	24+8	69	3	3	24+9
	4	87.6	2	3	24+12	54.1	3	5	24+8	64.5	2	4	24+7	113.7	3	6	24+6
	5	34	3	3	24+4	48	4	3	24+4	24	3	4	24+4	122.5	4	4	24+3
	6	17	2	5	24+4	28.2	3	7	24+4	34.3	2	4	24+4	62.6	3	6	24+4
	7	49.2	3	3	32+2	98.2	3	4	24+5	72	3	3	24+5	154.2	3	3	24+4
B	**1**	43.3	4	8.8	24+5	47.5	4	16	24+5	61.9	4	10	24+5.3	73.8	4	6	24+4
	2	34.6	3	4	14+11	36.7	3	8	24+4	47.2	3	5	24+4	45.8	3	10	24+5
	3	31.8	6	12	36+1	24	6	5	24+11	50.9	2	11	36+1	51.2	6	4	24+9
	4	47.3	2	5	24+6	33.7	3	10	24+6	100.9	2	8	24+5	130	24	4	
	5	12.7	2	4	24+6	35.1	6	4	24+5	18.5	2	5	24+5	101.5	6	4	24+5
	6	31.6	2	8	24+4	55.6	4	20	24+4	58.6	2	8	24+4	94.6	4	14	24+4
	7	37.6	3	3	24+6	49.6	3	4	24+5	53.2	3	3	24+5	78.1	3	3	24+4
C	**1**	45.9	3	2	24+6	53.5	3	4	24+5	124.3	3	3	24+5	112.7	3	3	24+5
	2	72.7	2	2	12+8	57.7	2	2	12+6	86.6	2	1	24+4	27.4	2	3	24+4
	3	88.5	5	5	24+5	68.4	5	12	24+4	95.7	2	3	12+10	114.4	5	3	12+7
	4	26.8	3	6	24+3	37.8	3	7	24+3	45.5	3	7	24+3	100.8	24	2	
	5	30.6	2	3	24+11	33.7	6	5	24+6	15.3	6	5	24+6	88.5	6	6	24+6
	6	25.2	2	2	28+4	43.7	8	1	28+3	37.2	2	3	28+3	79.8	8	1	24+4
	7	103	3	3	24+7	106.1	3	4	24+6	75	3	3	24+7	152.2	3	4	24+5
D	**1**	27.3	4	4	24+3	29.8	4	5	24+4	46.7	2	4	24+4	59.1	2	5	24+3
	2	49	3	2	14+10	72.3	4	4	24+4	73	3	3	24+4	137.4	4	4	24+5
	3	36.2	5	7	36+1	33.4	5	4	14+11	35.7	5	6	24+8	44.9	5	1	14+8
	4	41.7	2	3	24+5	31.5	3	5	24+3	55.6	2	3	24+4	91.6	3	4	24+4
	5	33.4	3	3	24+6	59.3	3	4	24+4	56.1	3	3	24+4	124.6	4	3	24+4
	6	15.4	4	3	24+4	18.7	2	6	11+5	34.4	4	4	24+4	96.6	4	5	24+3
	7	53.8	2	4	24+4	60.8	2	7	24+4	52.7	2	5	24+4	89.9	2	3	
E	**1**	86.5	3	4	24+4	58.7	3	6	24+5	93.2	3	4	24+5	110.4	3	6	24+4
	2	78	2	2	24+4	76.3	4	4	24+4	121.2	2	3	24+4	136.7	2	4	24+4
	3	37.5	3	4	28+3	32.5	3	7	24+4	44.9	3	5	24+4	43	3	8	24+3
	4	33.3	3	6	24+6	39	3	9	24+7	57.7	3	4	12+10	80.5	2	5	12+8
	5	45	2	3	24+6	45.1	2	5	24+4	50.2	2	3	24+5	84.1	5	5	24+4
	6	100	3	4	24+5	113	3	6	24+5	141.2	3	5	24+5	137	3	9	24+4

	7	24.9	3	5	28+3	48.1	3	10	28+3	27	2	7	28+3	70.9	3	9	12+11
Food	**1**	53.8	24	4		53	24	4.5		78.8	24	4		71.6	24	4	
	2	69.9	24	3		101.,1	24	3		53.7	24	3		267.7	24	3	
	3	47.5	24	5		69.5	24	6		31.8	24	5		107.8	24	4.5	
	4	17.2	24	6		31.8	24	6		36.3	24	5.5		106.5	24	5	
	5	11.2	24	5		19.6	24	8		16	24	6		76.5	24	5	
	6	15	24	6		38.1	24	6.5		55	24	6		111.5	24	5	
	7	6.8	24	8		19.3	24	7		21.2	24	7		50	24	5.5	

AI2I4 Diisononyl phthalate

Pig 1

Dust:

All metabolites of DINP reached their mean maximum concentration at 3.2 ± 0.5 hours (MINP and Oxo-MINP) and 2.7 ± 0.5 hours (7OH-MINP and cx-MINP) post dose.

The estimated half-life time for the first elimination phase was similar for all metabolites: 5.6 ± 1.7 hours (MINP), 5.7 ± 1.5 hours (oxo-MINP), 5.1 ± 1.5 hours (7OH-MINP), 6 ± 1.3 hours (7cx-MINP). The second elimination phase occurred 24 hours post dose. The half-life time was estimated for MINP (4.7 ± 0.7 hours), 4 ± 0.3 hours oxo-MINP, 3.8 ± 0.8 hours 7OH-MINP, 4.5 ± 0.6 hours 7cx-MINP. During the first 24 hours, 40 ± 31 % of the Dose was excreted as 32 ± 32 % MINP, 4.4 ± 1.4 % 7cx-MINP, 3.4 ± 2 % 7OH-MINP and 1.5 ± 0.6 % oxo-MINP. After 24 hours to 38 hours only 6.4 % DINP as 5 ± 5.5 % MINP, 0.6 ± 0.3 % 7cx-MINP, 0.4 ± 0.2 % 7OH-MINP and 0.2 ± 0.1 % oxo-MINP was excreted.

Food:

The maximum concentration was reached 24 hours after sample administration. MINP was excreted with a half-life time of 4.5 hours, oxo-MINP about 4.3 hours, 7OH-MINP about 4.4 hours and 7cx-MINP about 4.3 hours.

37 % of the DINP dose was excreted in the first 24 hours mainly as MINP (26.7 %) followed by 7OH-MINP (4.6 %), 7cx-MINP (4.2 %) and 1.4 % (oxo-MINP). On the second day, 13.8 % DINP (10.1 % MINP, 1.7 % 7OH-MINP, 1.4 % 7cx-MINP, 0.6 % oxo-MINP) was eliminated by urine.

Pig 2

Dust:

The primary metabolite MINP had the mean maximum concentration at 3.3 ± 1.1 hours post dose. The secondary metabolites oxo-MINP and 7OH-MINP reached their maximum concentration after 2.3 ± 0.6 hours. The mean of t_{max} for 7cx-MINP was 2.7 ± 1 hours after dust application. The half-life time during the first elimination phase was estimated for MINP about 4.2 ± 2.1 hours, 5.4 ± 3.7 hours for oxo-MINP, 2.8 ± 0.8 hours for 7OH-MINP, 5.5 ± 4.8 hours for 7cx-MINP. The second elimination phase occurred 11 - 24 hours post dose with the following half-life time: 4.4 ± 0.9

hours for MINP, 3.7 ± 2.1 for oxo-MINP, 21.4 ± 14.2 hours for 7 OH-MINP, 6.6 ± 6 hours for 7cx-MINP. 53 ± 28 % of the applied dose were excreted within 24 hours as 43 ± 30 % MINP, 5 ± 2 % 7cx-MINP, 3.5 ± 0.6 % oxo-MINP, 1.1 ± 0.5 % 7OH-MINP. On the second day only 2.6 ± 1.8 % DINP as 2.4 ± 2 % MINP, 0.1 ± 0.11 % 7cx-MINP, 0.1 ± 0.04 % oxo-MINP, 0.01 ± 0.01 % 7OH-MINP were excreted.

Food:

After 24 hours the maximum concentration was reached. The half-life was determined as 3.1 hours (MINP); three hours (oxo-MINP and 7cx-MINP) and 3.3 hours (7OH-MINP). Within the first 24 hours, 35 % of the DINP dose as 27 % MINP, 4.5 % 7cx-MINP, 2 % 7OH-MINP, 1.6 % oxo-MINP whereas on the second day 18.4 % DINP (14.1 % MINP, 2 % 7cx-MINP, 1.2 % 7OH-MINP, 1 % oxo-MINP) was excreted.

Pig 3

Dust:

The mean of t_{max} was for all DINP-Metabolites 4 ± 1.1 hours after dose application. The first half-life time for the metabolites was 6.5 ± 2.6 hours for MINP, 6.1 ± 3 hours for oxo-MINP, 4.7 ± 2.4 hours for 7OH-MINP and 4.6 ± 2.3 hours for 7cx-MINP. The second elimination phase was determined 10 to 24 hours poste dose, the half-life times were for MINP at 6 ± 2 hours, oxo-MINP: 6.5 ± 1.9 hours, 7OH-MINP: 6.7 ±3 hours, 7cx-MINP: 7.7 ± 3.3 hours.

Within the first 24 hours 46.7 ± 32 % of the applied Dose were excreted as 40.5 ± 31.4 % MINP, 2.7 ± 1.1 % 7cx-MINP, 2.4 ± 0.4 % oxo MINP, 1.1 ± 0.4 % 7OH-MINP. 24 - 38 hours after the dust application only 5 ± 4.5 % DINP were excreted as 4.3 ± 4.3 % MINP, 0.2 ± 0.15 % 7cx-MINP, 0.3 ± 0.1 % oxo-MINP

Food:

T_{max} was noticed 24 hours post dose. The half-life time was estimated 5.8 hours for MINP, 6.4 hours for 7OH-MINP, 6.3 hours for 7cx-MINP and 7 hours for oxo-MINP. During the first 24 hours 37 % of the applied DINP dose was excreted as 33 % MINP, 2.2 % 7x-MINP, 1.8 % oxo-MINP and 0.9 % 7OH-MINP. On the second day 18.8 % DINP was eliminated (15.8 % MINP, 1.2 % 7cx-MINP, 1.1 % oxo-MINP, 0.7 % 7OH-MINP).

Pig 4

Dust:

Three hours after dose application t_{max} of MINP was reached. In the case of oxo-MINP the mean t_{max} was: 7.7 ± 11 hours, 3 ± 1.4 hours for 7OH-MINP and 3.2 ± 1.2 hours for 7cx-MINP. The first half-life time was estimated as 5.8 ± 1.7 hours for MINP, 4.3 ± 1.1 hours for oxo-MINP, 4.6 ± 1.5 hours for 7OH-MINP and 6.2 ± 3.8 hours 7cx-MINP. The second half was determined 14 to 24 hours post dose and was calculated as: 5.4 ± 2 hours for MINP, 6 ± 3 hours for oxo-MINP, 5.3 ± 2.9 hours for 7OH-MINP, 5 ± 2.4 for 7cx-MINP.

In the first 24 hours, 30.5 ± 20.4 % of the administrated dose were excreted as 22 ± 21 % MINP, 3.6 ± 1.3 % 7OH-MINP, 2.7 ± 1.2 % 7cx-MINP and 2 ± 1 % oxo-MINP. In the collection period of 24-38 hours only 2 ± 1.1 % DINP were eliminated as 1.6 ± 1.4 % MINP, 0.13 ± 0.1 % oxo-MINP, 0.16 ± 0.13 % 7OH-MINP, 0.13 ± 0.1 % 7cx-MINP.

Food:

The highest concentration was observed 24 hours after dose. The eliminated half-life varies from 5.8 hours (7cx-MINP), 6.1 hours (MINP), 7.6 hours (oxo-MINP) to 8.2 hours (7OH-MINP).

67.5 % of the DINP dose were excreted within 24 hours (62.6% MINP, 2.2 % 7OH-MINP, 1.8 % 7cx-MINP, 0.8 % oxo-MINP), whereas on the second day 24.3 % of the applied dose were eliminated as 22.6 % MINP, 0.8 % 7OH-MINP, 0.6 % 7cx-MINP and 0.3 % oxo-MINP.

Pig 5

Dust:

The highest concentration was determined for all metabolites at 3.7 ± 1.7 hours post dose.

The elimination half-life time was calculated for MINP, oxo-MINP, 7OH-MINP and 7cx-MINP with: 4.1 ± 0.7 hours, 8.4 ± 3.7 hours, 8.3 ± 2.6 hours and 5.7 ± 1.3 hours respectively. 24 hours post dose, the second elimination half-life time was determined with 4.7 ± 1 hours, 3.3 ± 0.5 hours, 6.6 ± 6.8 hours and 2.4 ± 1.1 hours for MINP, oxo-MINP, 7 OH-MINP and 7cx-MINP.

In the first 24 hours, 37 ± 19 % of the dose were excreted as 29 ± 20 % MINP, 3.1 ± 1.4 % oxo-MINP, 2.6 ± 1 % 7cx- and 2.1 ± 0.5 % 7OH-MINP. After 24 hours 2.2 ± 1.3 % of the applied dose were excreted as 1.8 ± 1.3 % MINP, 0.2 ± 0.1 % oxo-MINP, 0.15 ± 0.04 % 7OH-MINP and 0.05 ± 0.04 % 7cx-MINP.

Food:

The maxima of MINP, oxo-MINP, 7OH-MINP and 7cx-MINP were reached after 24 hours post dose. The half-life was determined as followed 7.8 hours (MINP), 14 hours (oxo-MINP), 15 hours (7OH-MINP) and 8.6 hours (7cx-MINP).

20 % of the applied DINP dose was eliminated within the first sampling day: In detail: 16.2 % MINP, 1.9 % oxo-MINP, 1 % 7cx-MINP and 0.8 % 7OH-MINP. On the second sampling day 7.7 % DINP was eliminated (5.7 % MINP, 1 % oxo-MINP, and 0.5 % 7cx-MINP and 7OH-MINP.

Pig 6

Dust:

Compare to other pigs, t_{max} was delayed 9.5 ± 10 hours for MINP and oxo-MINP. 7OH-MINP and 7cx- MINP had their t_{max} at 9.2 ±10 hours. The first estimated half-life for MINP, oxo-MINP, 7OH-MINP and 7cx-MINP was 4.7 ± 2.3 hours, 6.1 ± 4.7 hours, 4.6 ± 2.2 hours and 4.9 ± 3 hours respectively.

The second half-life time appeared 24 - 28 hours post dose, with 4 ± 0.8 hours, 4.5 ± 0.4 hours, 4.2 ± 0.8 hours, and 4.6 ± 1 hours for MINP, oxo-MINP, 7 OH-MINP and 7cx MINP.

Within in the first 24 hours, 36 ± 7.3 % DINP were excreted as 25 ± 10 % MINP, 4.2 ± 2.7 % oxo-MINP, 3.6 ± 1.6 % 7OH-MINP and 3.1 ± 1.3 % 7cx-MINP. On the second day 4.5 ± 2 % DINP were eliminated as 3 ± 1.3 % MINP, 0.6 ± 0.4 % oxo-MINP, 0.48 ± 0.3 % 7OH- MINP and 0.35 ± 0.31 % 7cx-MINP.

Food:

The highest concentration for MINP and oxo-MINP was determined at 24 hours, for 7OH-MINP 28 hours and for 7cx-MINP 36 hours post dose.

The elimination half-life time was calculated for MINP, oxo-MINP, 7OH-MINP and 7cx-MINP 6.1 hours, 7.8 hours, 7.3 hours and 0.7 hours respectively

Within the first 24 hours, 31.4 % of the applied DINP dose was excreted mainly as 24 % MINP, followed by 3.5 % 7OH-MINP, 2.4 % 7cx-MINP and 1.5 % oxo-MINP, from

24 hours to 38 hours 17 % DINP was found in urine (12.5 % MINP, 2.3 % 7OH-MINP, 1.4 % 7cx-MINP and 1 % oxo-MINP.

Pig 7

Dust:

MINP reached their maximum concentration after three hours, similar to 7OH-MINP (2.6 ± 0.5 hours). Oxo-MINP and 7cx-MINP had their t_{max} at 8.25 ± 10.5 hours post dose. The first estimated half-life was 6 ± 2.6 hours for MINP, 9.5 ± 8 hours for oxo-MINP, 8.5 ± 6.2 hours for 7OH-MINP and 8.2 ± 7 hours for 7cx-MINP.

The second half-life (24 - 28 hours post dose) was estimated as 4.7 ± 1.4 hours for MINP), 5 ± 1.5 hours for oxo-MINP, 5 ± 1.8 hours for 7-OH-MINP and 4.2 hours for 7cx-MINP.

On the first day 59 ± 28 % DINP was eliminated by urine as 46 ± 32 % MINP, 5.6 ± 2.7% 7cx-MINP, 3.5 ± 2.1 % oxo-MINP and 4.2 ± 4.9 % 7OH-MINP. On the second day, 5.5 ± 2.2 % DINP of the administrated dose were found in urine. The main metabolite was 4 ± 2.8 % MINP followed by 0.4 ± 0.3 % oxo-MINP, 0.4 ± 0.3% 7cx-MINP, 0.5 ± 0.2 % 7OH-MINP.

Food:

After 24 hours the maximum concentration was reached. The half-life was determined for 7.4 hours MINP, 7.5 hours oxo-MINP, 8 hours 7cx-MINP, 8.2 hours 7OH-MINP.

22.7 % MINP, 3.5 % 7cx-MINP, 1.8 % oxo-MINP and 0.8 % 7OH-MINP (∑28.8 % of the applied DINP dose) were eliminated in the first 24 hours. Only 7 % of the DINP dose was found in urine as 5.4 % MINP, 0.8 % 7cx-MINP, 0.5 % oxo-MINP, 0.2 % 7OH-MINP on the second day

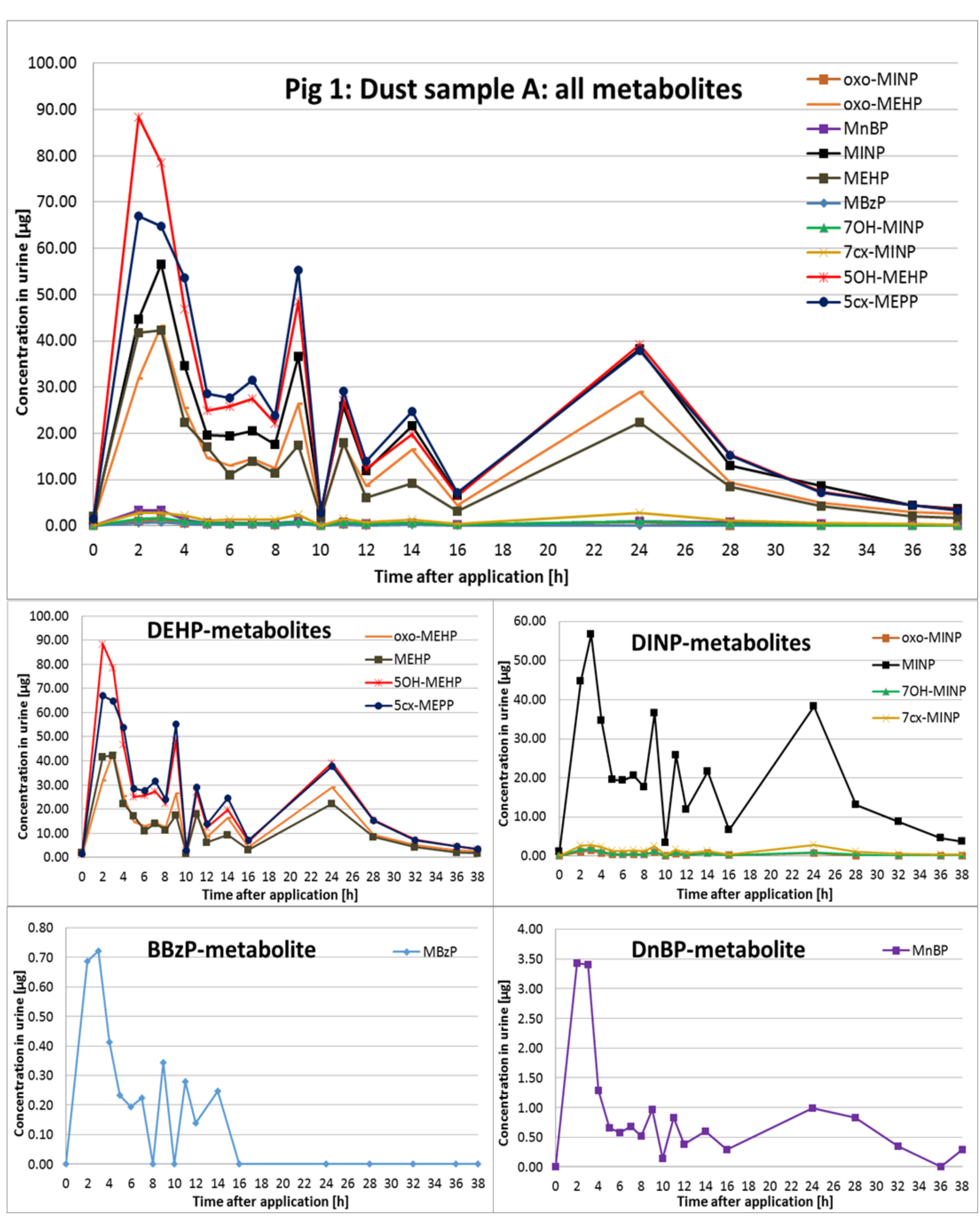
A detail description of the toxicokinetic parameters, concentration maximum (C_{max}) and it's time appearance (t_{max}), half-life time ($t_{½}$) in the first excretion and second period (24 hours plus second $t_{½}$) are given in Table A7.

The following figures reperesent the toxicokinetic time course of every pig with each dust sample and food sample.

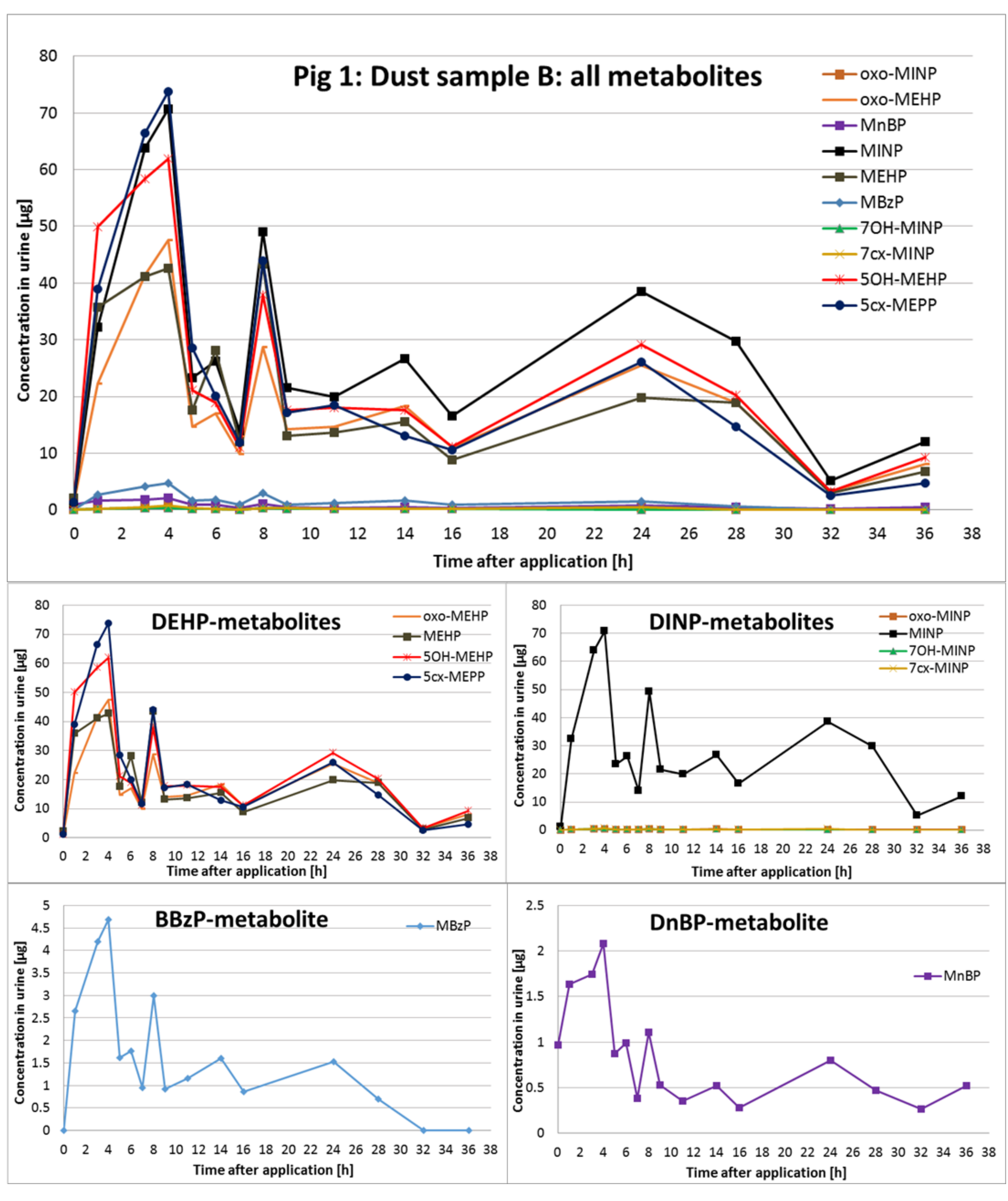
Table A7. Detail description of kinetic parameters of DINP metabolism [C_{max} in µg; t_{max} and $t_{½}$ in hour]

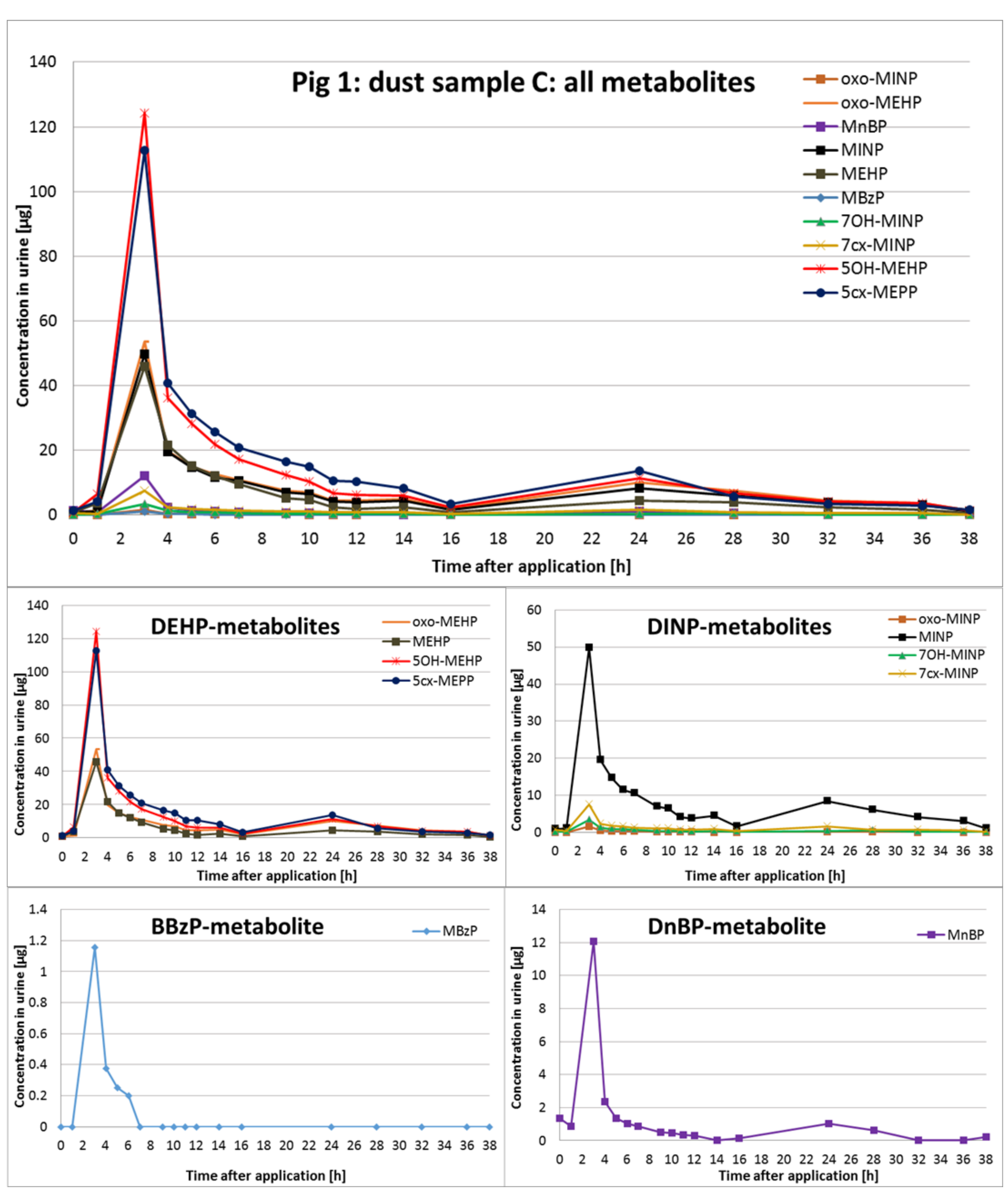
Dust	Pig	MINP				Oxo-MINP				7OH-MINP				7cx-MINP			
		c_{max}	t_{max}	$t_{½}$		c_{max}	t_{max}	$t_{½}$		c_{max}	t_{max}	$t_{½}$		c_{max}	t_{max}	$t_{½}$	
A	**1**	56.6	3	7	24+4	1.4	3	4	-	1.7	3	7	24+3	2.8	3	7	24+4.6
	2	64.6	2	7	24+6	2.1	2	11	24+2	0.7	2	4	11+7	2.8	2	13	14+15
	3	41	3	4	24+7	1.3	3	7	24+7	0.6	3	7	10+5	1.8	3	5	24+7
	4	45.1	3	5	24+8	1	2	11		3.4	2	3	14+7	2.5	5	11	
	5	45.8	4	3	24+4	2.3	4	5	24+4	1.1	4	7	24+17	1.2	4	18	
	6	25	3	7	24+4	1	3	12	24+5	1.8	2	7	24+4	1.3	2	6	24+4
	7	99.1	3	4	24+5	3	3	5	24+5	1.3	3	-		4.6	3	4	24+4
C	**1**	49.8	3	3	24+5	1.6	3	3		3.4	3	3	24+5	7.5	3	4	24+5
	2	86.6	2	2	24+4	4.5	2	2	12+6	1	2	3		5.3	2	2	24+2
	3	97.2	5	10	24+4	3.3	5	8	16+7	1.1	5	3	12+6	5	5	4	12+11
	4	31.7	3	7	24+3	1.7	24	2		3.1	5	7	24+2	1.6	3	12	24+2
	5	25.3	6	5	24+6	1.1	6	14	24+2	1.5	6	12	24+3	1.9	6	6	24+2
	6	34.3	8	2	28+3	1.5	8	2	28+4	3.6	8	2	28+3	2.7	8	2	24+6
	7	99.3	3	4	24+6	5.1	3	4	24+6	3.4	3	4	24+7	14	3	4	26+5
D	**1**	24	4	5	24+4	3.1	4	6	24+4	7	2	5	24+4	10	2	6	24+4
	2	63.5	4	4	24+4	10	3	5	24+4	5	3	4	24+3	18	3	4	24+5
	3	40.6	5	6	24+8	4.2	5	2	14+8	2.6	5	2	14+12	3.3	5	2	14+6
	4	28	3	4	24+4	4.7	2	5	24+3	11	2	4	24+4	6.5	5	4	24+5
	5	64.5	3	4	24+4	7.2	3	6	24+3	4.4	3	6	24+3	9	3	4	24+2
	6	33.1	24	4		7	24	5		6	24	5		8.2	24	4	
	7	56.6	3	8	24+4	11.5	24	4		5.4	2	5	24+4	17.7	24	4	
E	**1**	46.7	3	6	24+5	8.8	3	7	24+4	42.7	3	5	24+4	33.3	3	6	24+4
	2	122.2	4	4	24+4	24	2	4	24+4	1.9	2	3	14+14	10.1	4	3	24+4
	3	36.3	3	6	24+4	8.3	3	8	24+4	6.1	3	7	24+4	12.7	3	7	24+4
	4	45.8	3	7	24+6	12	3	5	12+9	20.4	3	4	12+8	11.6	3	5	12+8
	5	48.1	2	4	24+4	17	2	9	24+4	14.2	2	8	24+4	11.8	2	6	24+4
	6	116.5	3	5	24+5	31.8	3	6	24+5	65.8	3	5	24+5	38.3	3	7	24+4
	7	37.6	3	9	28+3	7.1	3	19	28+3	8.8	3	16	28+4	11.7	3	16	28+3
Food	**1**	52.2	24	4		2.7	24	4		8.5	24	4		8.4	24	4	
	2	72.2	24	3		4.8	24	3		5.9	24	3		14.6	24	3	
	3	68.8	24	6		4.5	24	7		2.4	24	6		5.6	24	6	
	4	27	24	6		1.4	24	8		3.1	24	8		3.7	24	6	
	5	19.7	24	8		2.8	24	14		1.2	24	15		1.9	24	9	
	6	47.1	24	6		3.1	24	8		7.1	28	7		6.5	36	1	

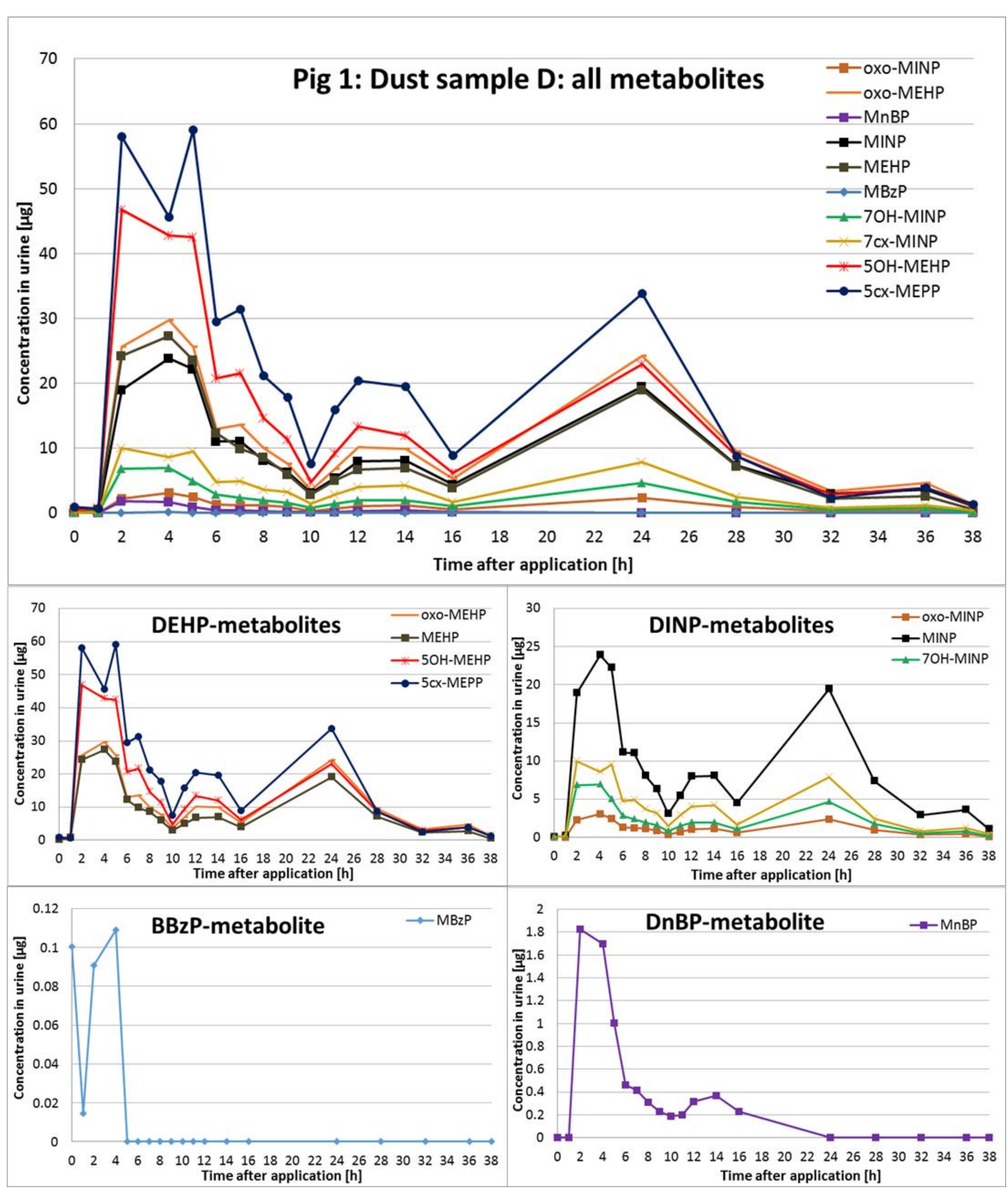
	7	23	24	7	2.2	24	7	0.8	24	8	3.8	24	8

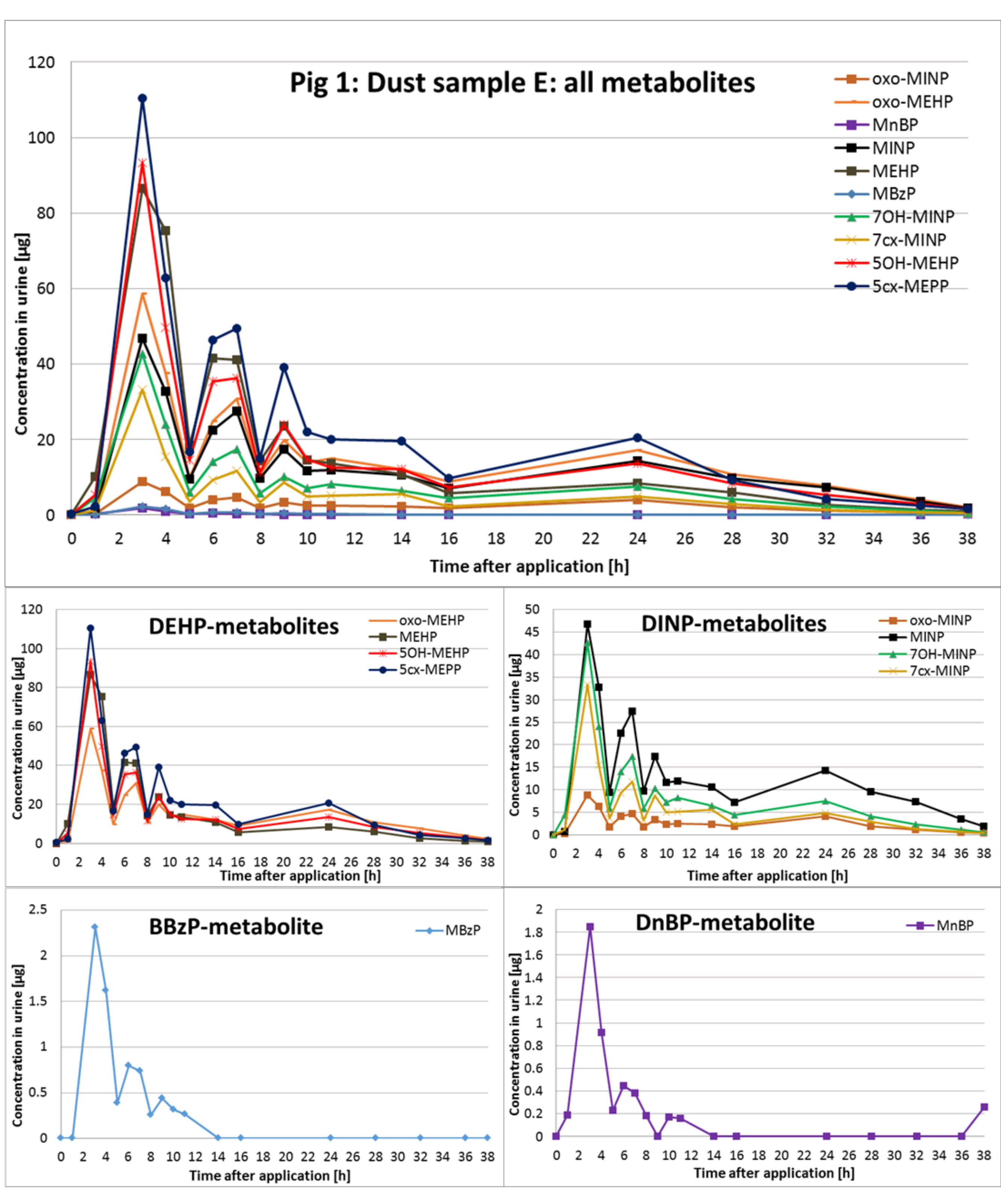


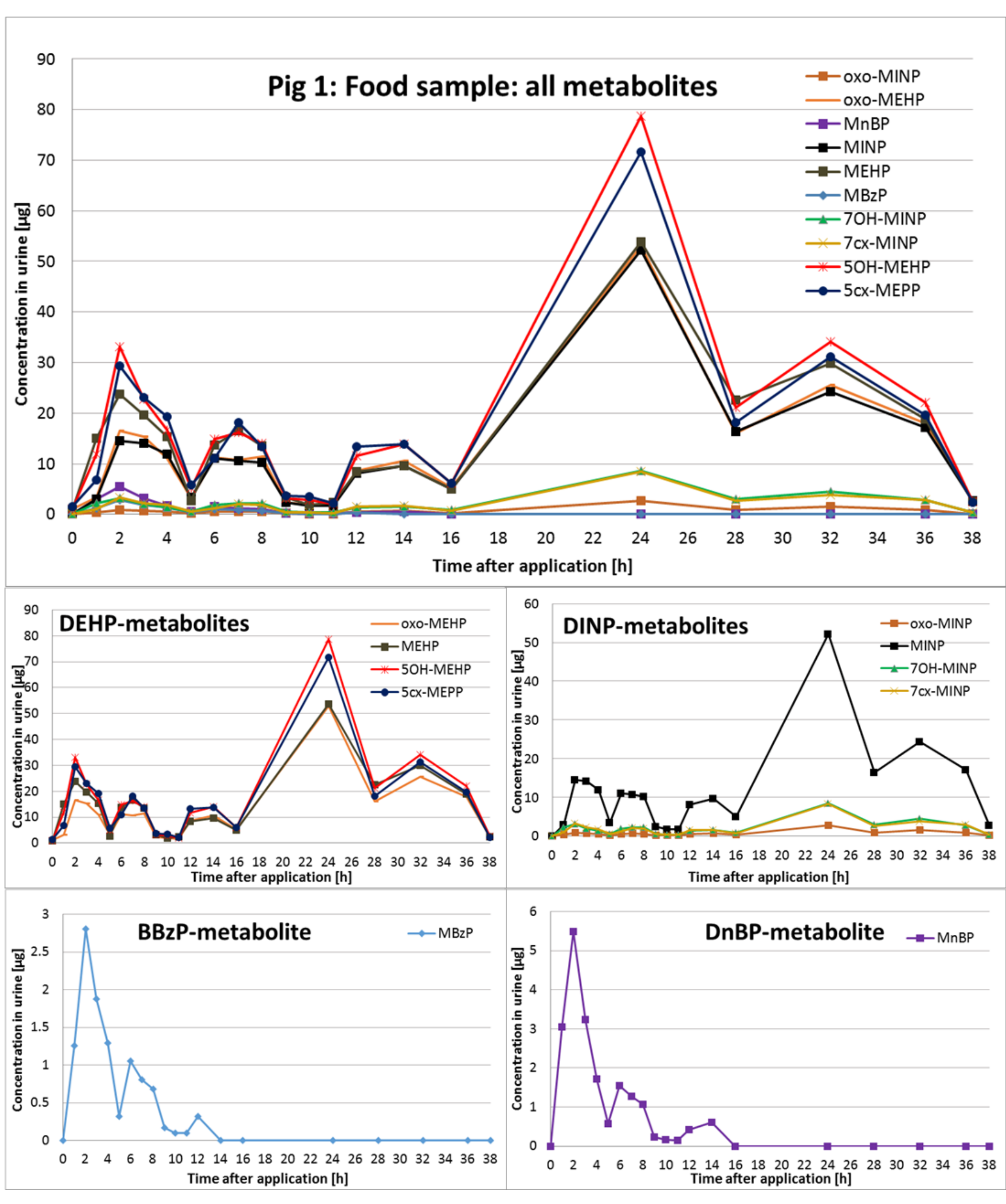
Pig 1: Dust sample A: all metabolites
oxo-MINP
oxo-MEHP
MnBP
MINP
MEHP
MBzP
7OH-MINP
7cx-MINP
5OH-MEHP
5cx-MEPP
Concentration in urine [µg]
Time after application [h]
DEHP-metabolites
DINP-metabolites
BBzP-metabolite
DnBP-metabolite

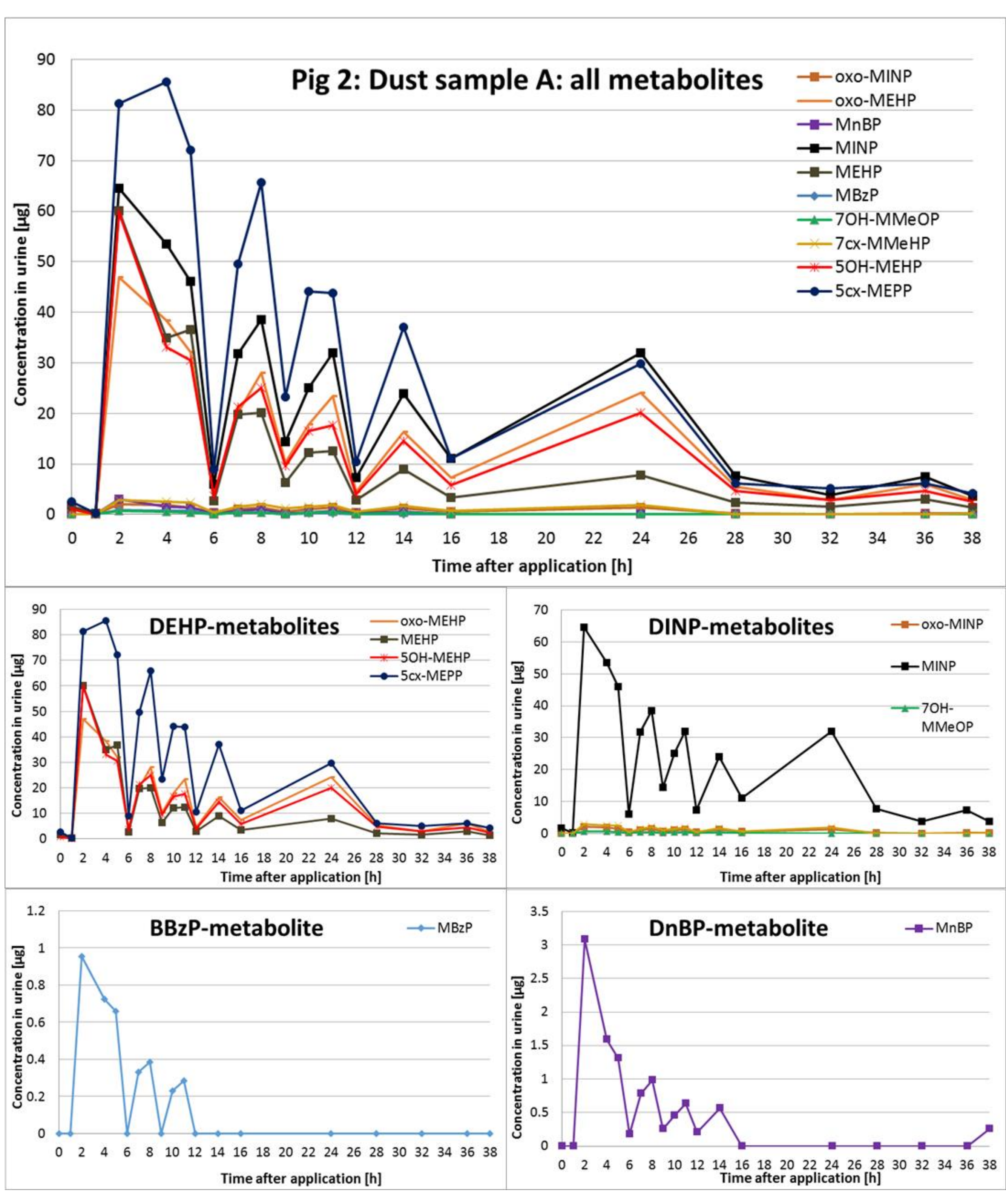

Pig 1: Dust sample B: all metabolites
oxo-MINP
oxo-MEHP
MnBP
MINP
MEHP
MBzP
7OH-MINP
7cx-MINP
5OH-MEHP
5cx-MEPP
Concentration in urine [µg]
Time after application [h]
DEHP-metabolites
DINP-metabolites
BBzP-metabolite
DnBP-metabolite

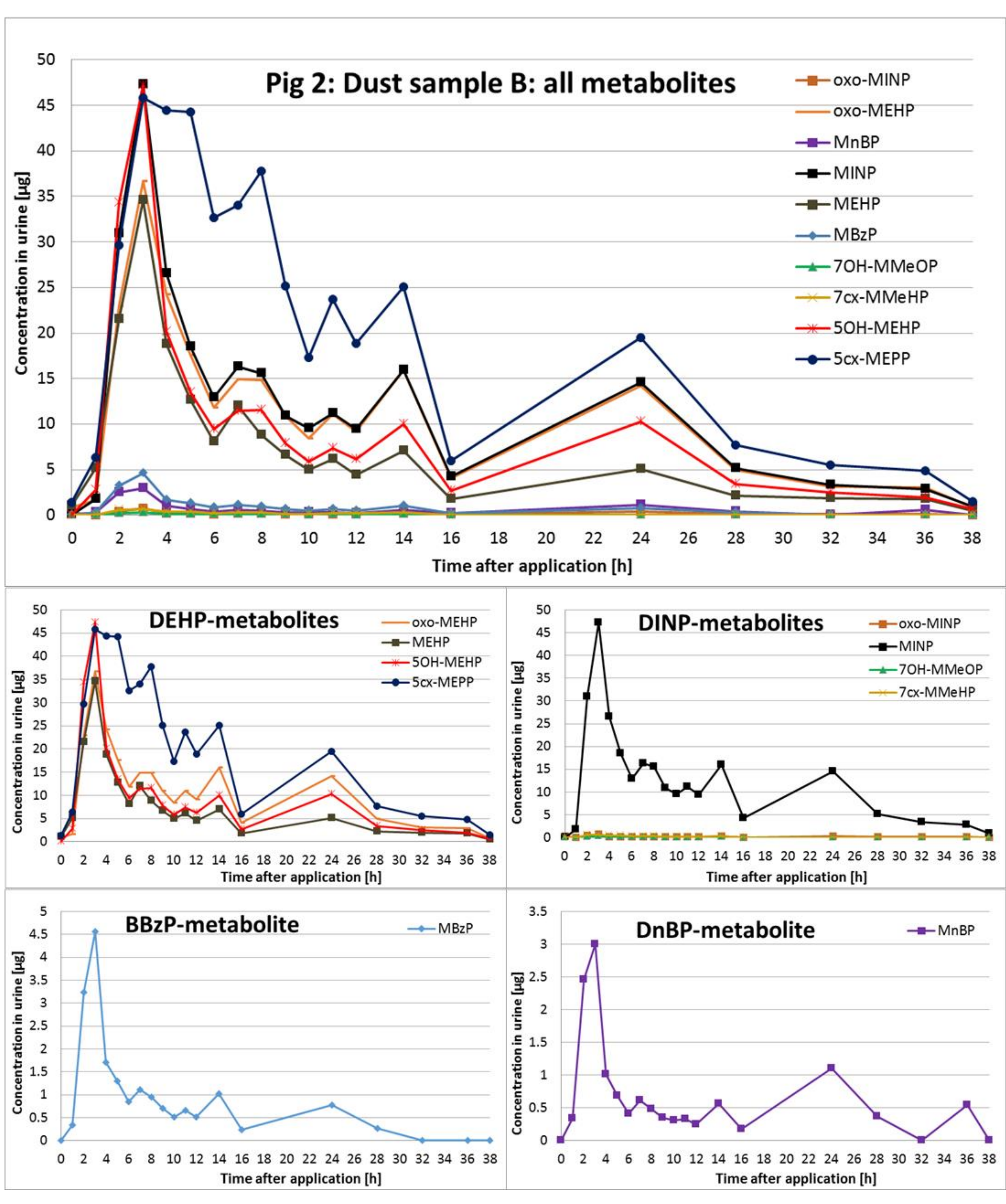

Pig 1: dust sample C: all metabolites
oxo-MINP
oxo-MEHP
MnBP
MINP
MEHP
MBzP
7OH-MINP
7cx-MINP
5OH-MEHP
5cx-MEPP
Concentration in urine [µg]
Time after application [h]
DEHP-metabolites
DINP-metabolites
BBzP-metabolite
DnBP-metabolite


Pig 1: Dust sample D: all metabolites
oxo-MINP
oxo-MEHP
MnBP
MINP
MEHP
MBzP
7OH-MINP
7cx-MINP
5OH-MEHP
5cx-MEPP
Concentration in urine [µg]
Time after application [h]
DEHP-metabolites
DINP-metabolites
BBzP-metabolite
DnBP-metabolite

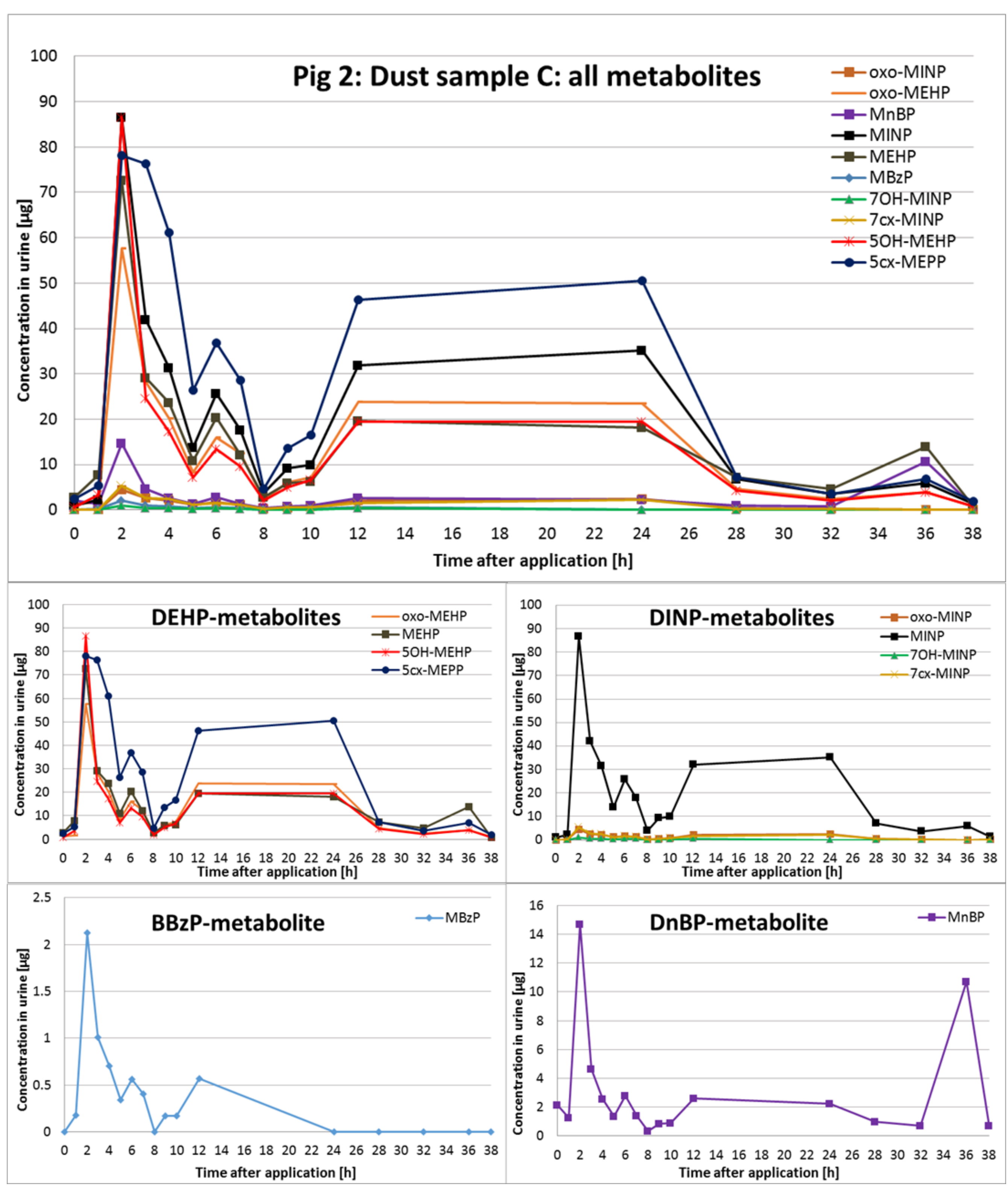

Pig 1: Dust sample E: all metabolites
oxo-MINP
oxo-MEHP
MnBP
MINP
MEHP
MBzP
7OH-MINP
7cx-MINP
5OH-MEHP
5cx-MEPP
Concentration in urine [µg]
Time after application [h]
DEHP-metabolites
DINP-metabolites
BBzP-metabolite
DnBP-metabolite

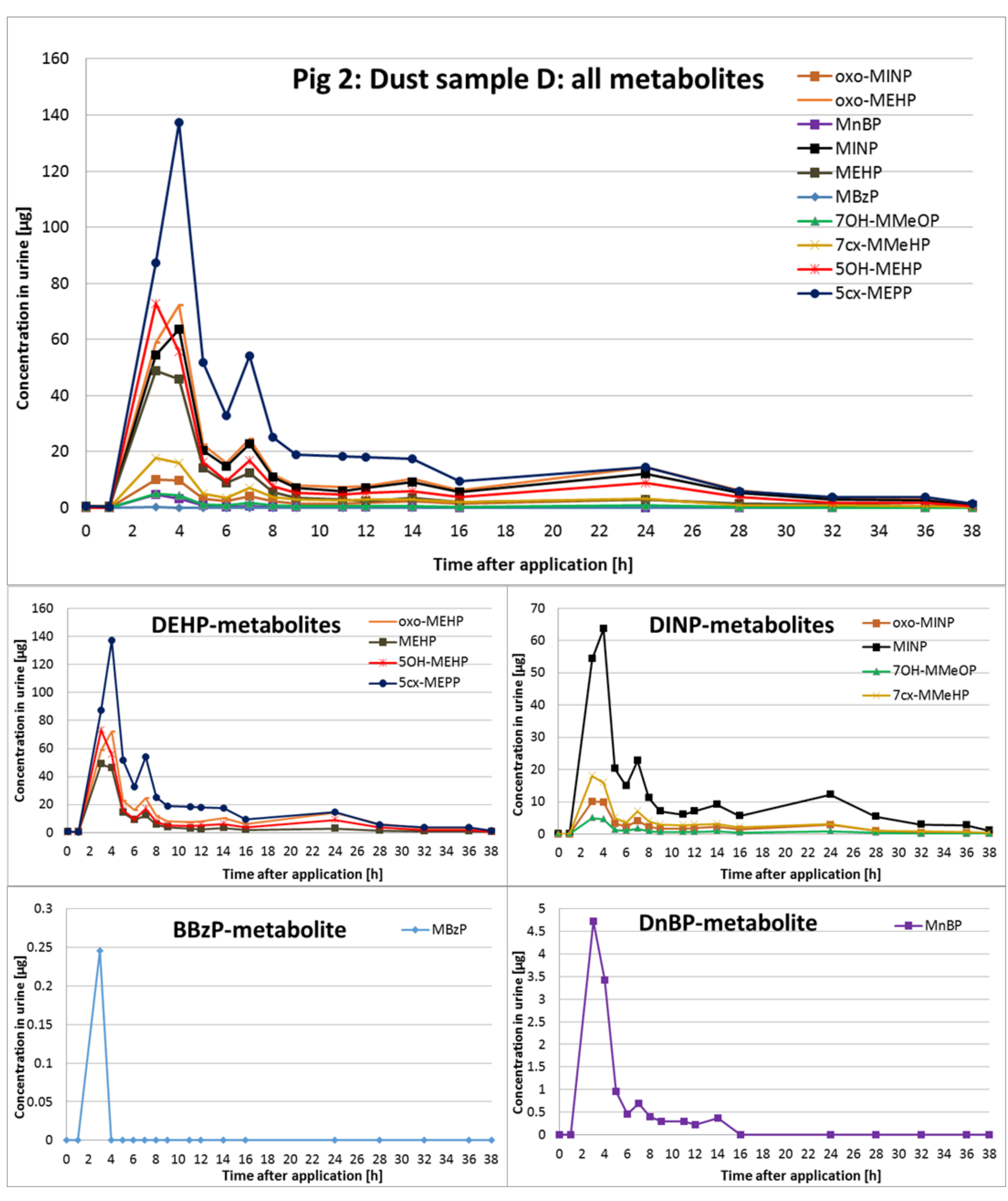

Pig 1: Food sample: all metabolites
oxo-MINP
oxo-MEHP
MnBP
MINP
MEHP
MBzP
7OH-MINP
7cx-MINP
5OH-MEHP
5cx-MEPP
Concentration in urine [µg]
Time after application [h]
DEHP-metabolites
DINP-metabolites
BBzP-metabolite
DnBP-metabolite

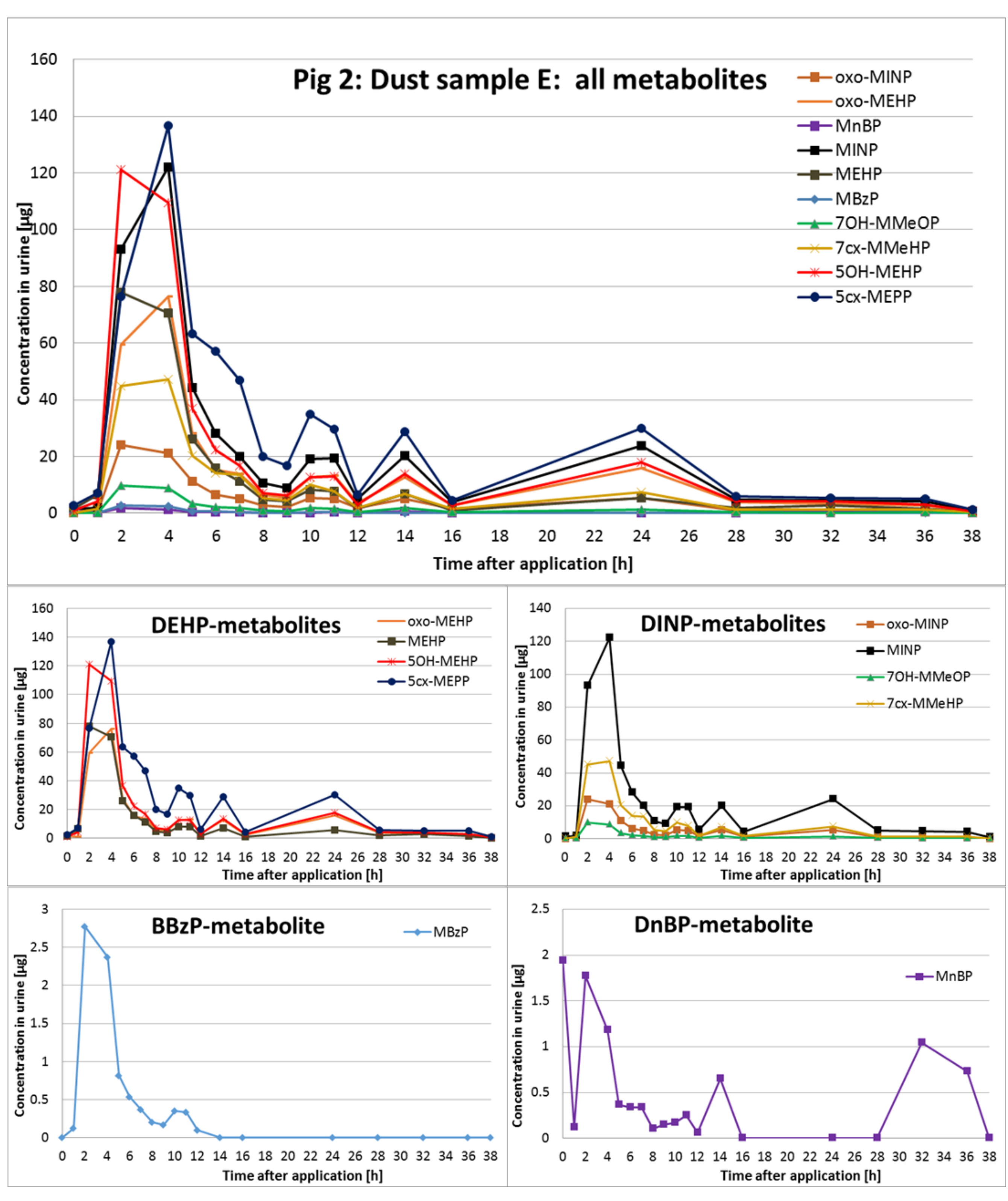

Pig 2: Dust sample A: all metabolites
oxo-MINP
oxo-MEHP
MnBP
MINP
MEHP
MBzP
7OH-MMeOP
7cx-MMeHP
5OH-MEHP
5cx-MEPP
Concentration in urine [µg]
Time after application [h]
DEHP-metabolites
DINP-metabolites
BBzP-metabolite
DnBP-metabolite



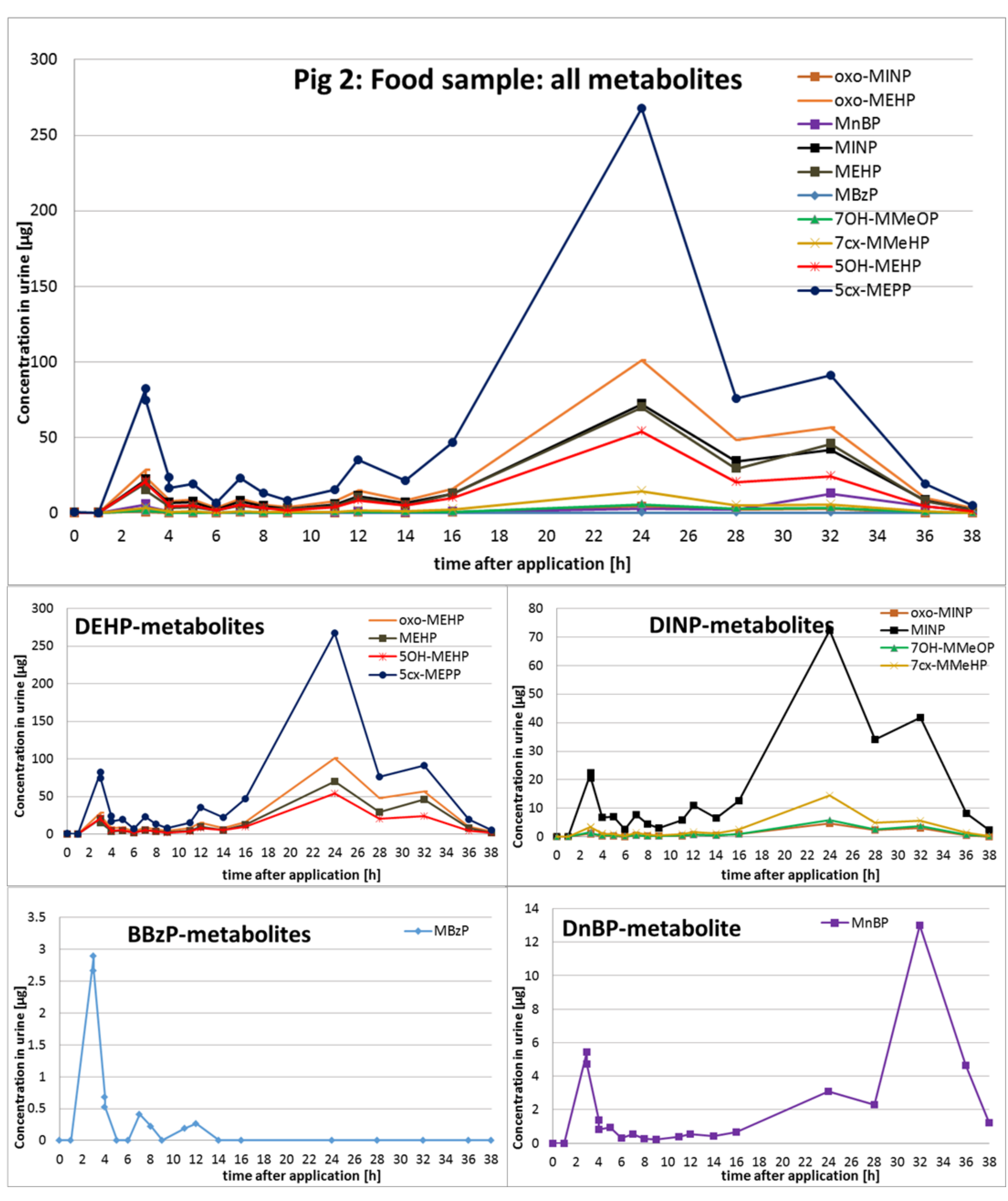
Pig 2: Dust sample B: all metabolites
oxo-MINP
oxo-MEHP
MnBP
MINP
MEHP
MBzP
7OH-MMeOP
7cx-MMeHP
5OH-MEHP
5cx-MEPP
Concentration in urine [µg]
Time after application [h]
DEHP-metabolites
DINP-metabolites
BBzP-metabolite
DnBP-metabolite


Pig 2: Dust sample C: all metabolites
Concentration in urine [µg]
Time after application [h]
oxo-MINP
oxo-MEHP
MnBP
MINP
MEHP
MBzP
7OH-MINP
7cx-MINP
5OH-MEHP
5cx-MEPP
DEHP-metabolites
DINP-metabolites
BBzP-metabolite
DnBP-metabolite

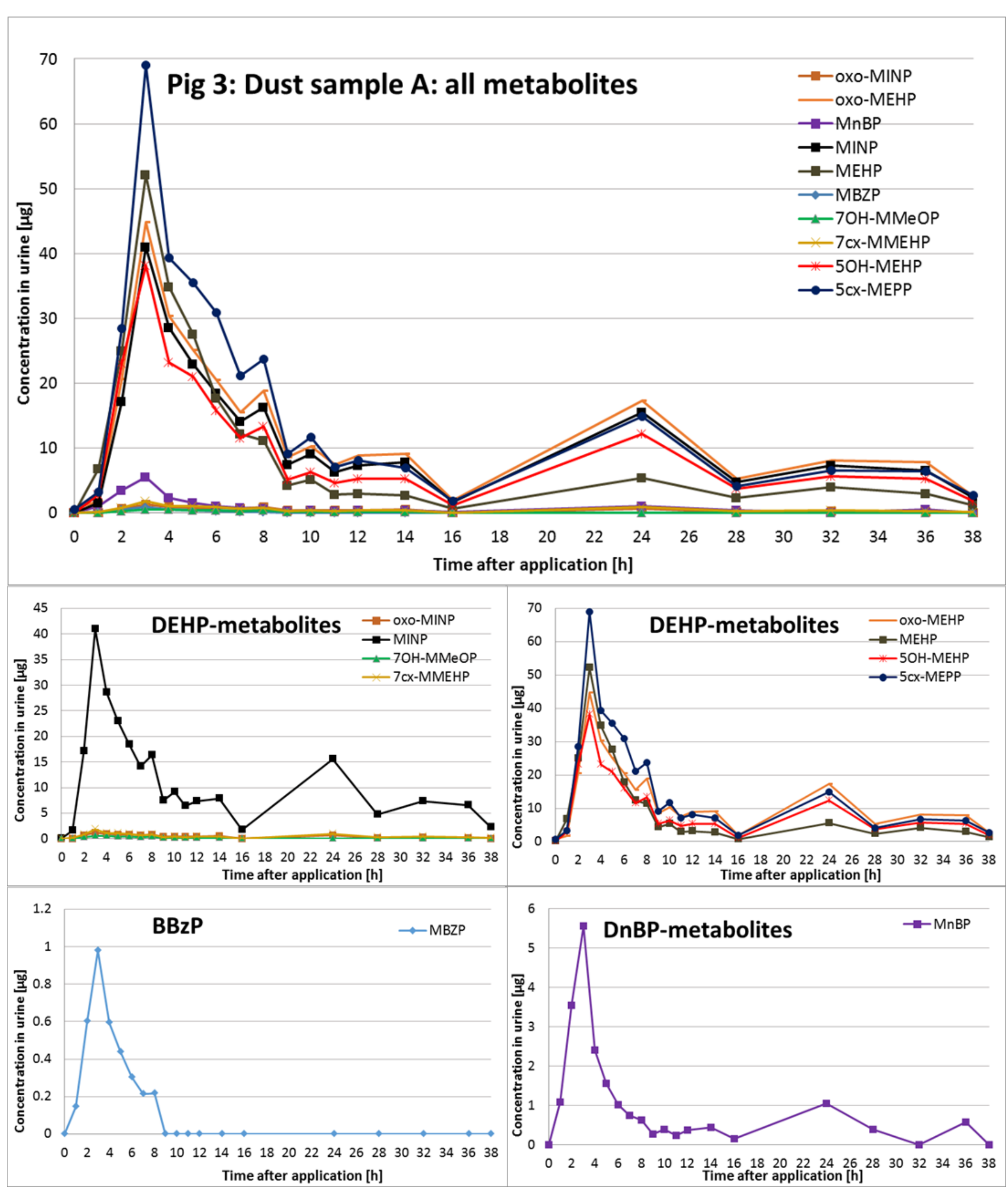

Pig 2: Dust sample D: all metabolites
oxo-MINP
oxo-MEHP
MnBP
MINP
MEHP
MBzP
7OH-MMeOP
7cx-MMeHP
5OH-MEHP
5cx-MEPP
Concentration in urine [µg]
Time after application [h]
DEHP-metabolites
DINP-metabolites
BBzP-metabolite
DnBP-metabolite

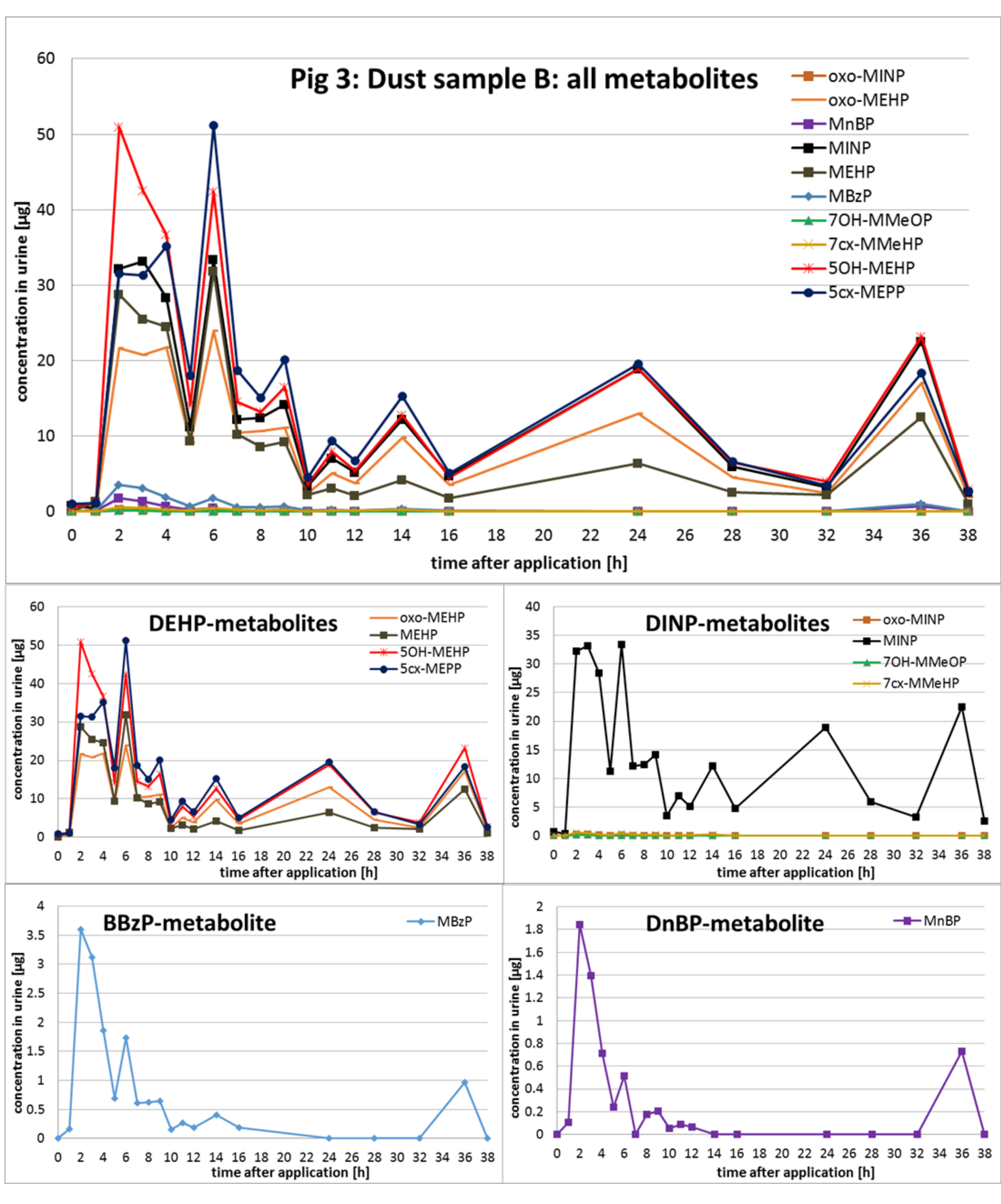


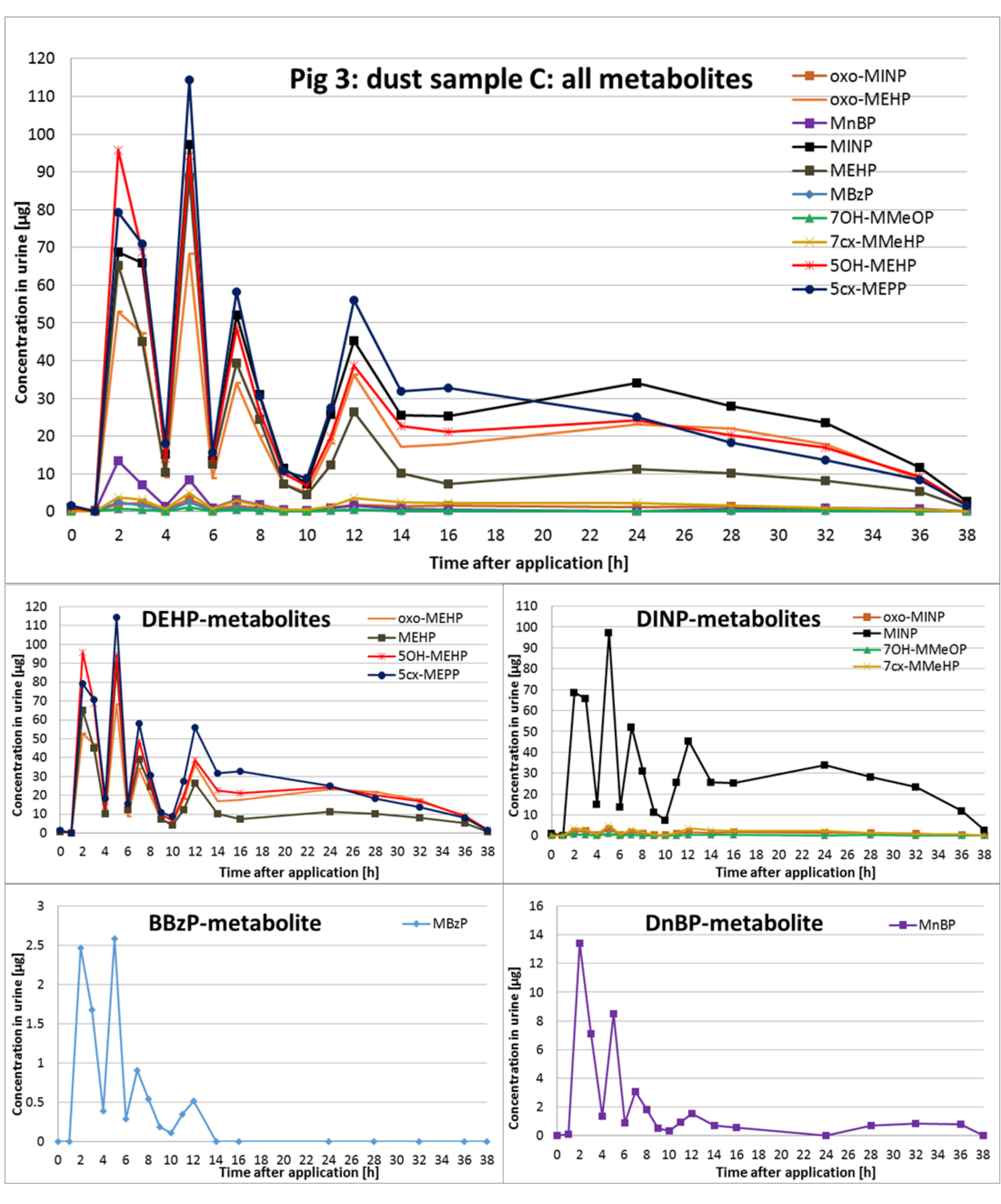
Pig 2: Dust sample E: all metabolites
oxo-MINP
oxo-MEHP
MnBP
MINP
MEHP
MBzP
7OH-MMeOP
7cx-MMeHP
5OH-MEHP
5cx-MEPP
Concentration in urine [µg]
Time after application [h]
DEHP-metabolites
oxo-MEHP
MEHP
5OH-MEHP
5cx-MEPP
DINP-metabolites
oxo-MINP
MINP
7OH-MMeOP
7cx-MMeHP
BBzP-metabolite
MBzP
DnBP-metabolite
MnBP

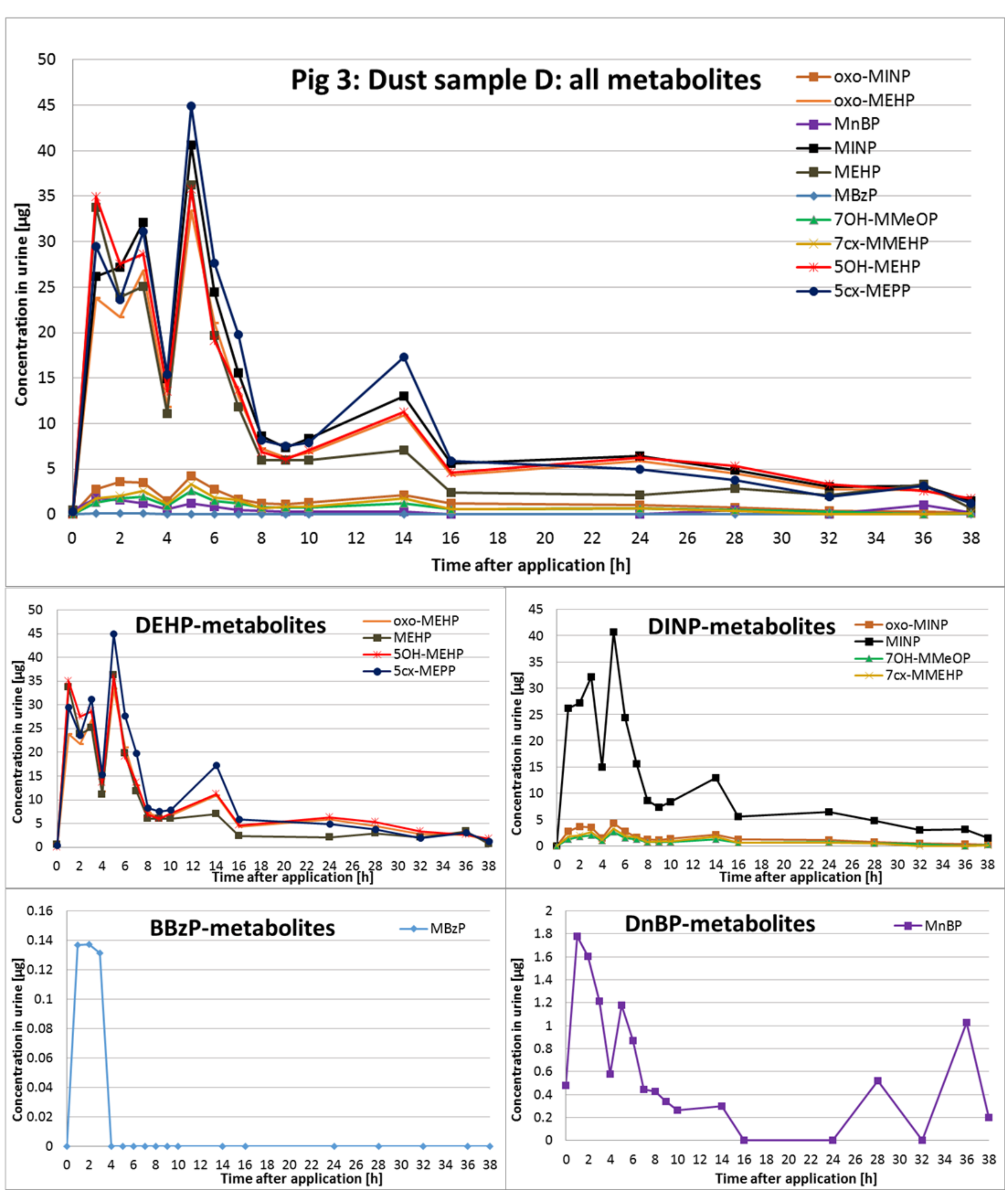


Pig 2: Food sample: all metabolites
oxo-MINP
oxo-MEHP
MnBP
MINP
MEHP
MBzP
7OH-MMeOP
7cx-MMeHP
5OH-MEHP
5cx-MEPP
Concentration in urine [µg]
time after application [h]
DEHP-metabolites
DINP-metabolites
BBzP-metabolites
DnBP-metabolite

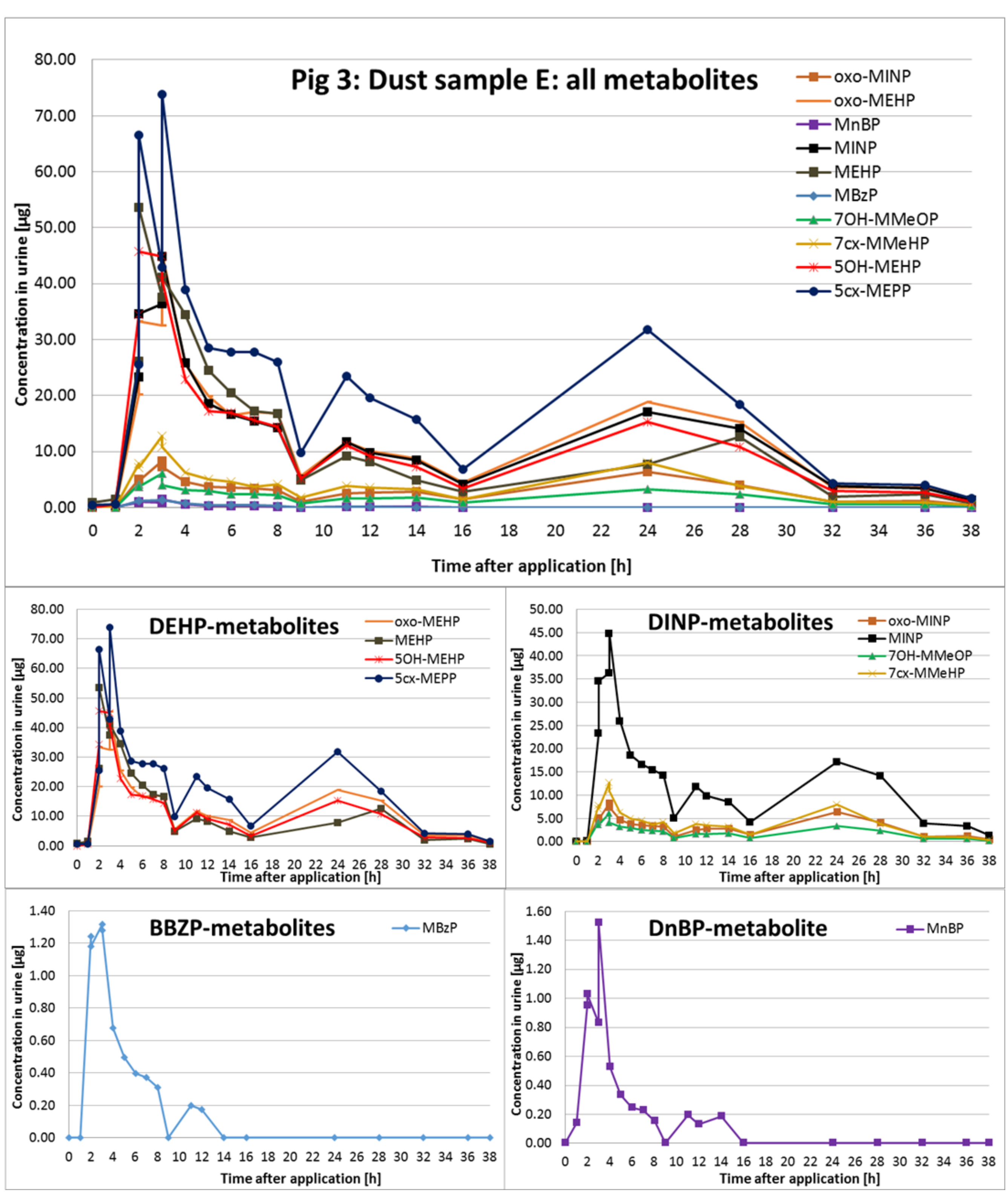

Pig 3: Dust sample A: all metabolites
Concentration in urine [µg]
Time after application [h]
oxo-MINP
oxo-MEHP
MnBP
MINP
MEHP
MBZP
7OH-MMeOP
7cx-MMEHP
5OH-MEHP
5cx-MEPP
DEHP-metabolites
BBzP
DnBP-metabolites

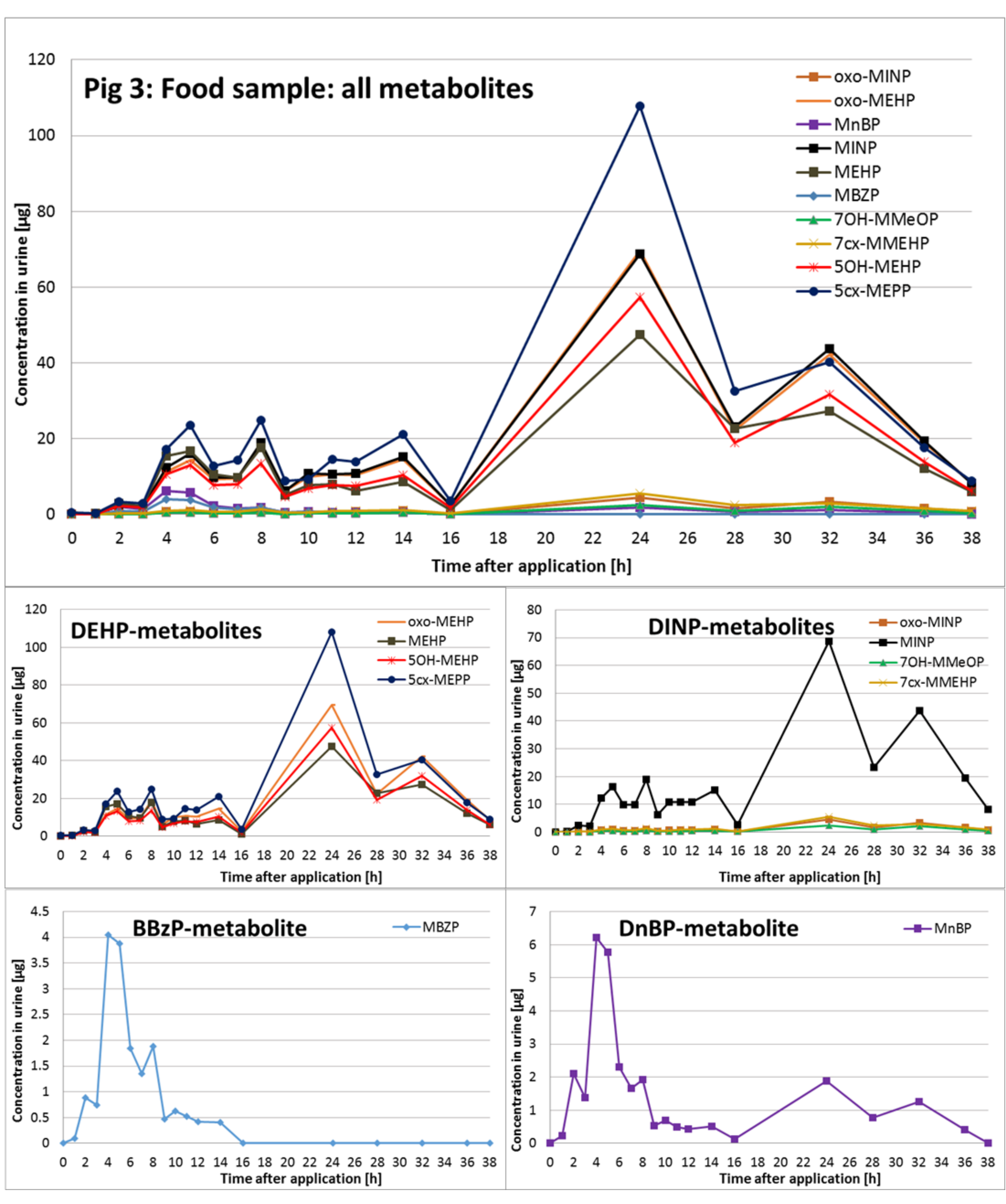

Pig 3: Dust sample B: all metabolites
oxo-MINP
oxo-MEHP
MnBP
MINP
MEHP
MBzP
7OH-MMeOP
7cx-MMeHP
5OH-MEHP
5cx-MEPP
concentration in urine [µg]
time after application [h]
DEHP-metabolites
DINP-metabolites
BBzP-metabolite
DnBP-metabolite


Pig 3: dust sample C: all metabolites
oxo-MINP
oxo-MEHP
MnBP
MINP
MEHP
MBzP
7OH-MMeOP
7cx-MMeHP
5OH-MEHP
5cx-MEPP
Concentration in urine [µg]
Time after application [h]
DEHP-metabolites
DINP-metabolites
BBzP-metabolite
DnBP-metabolite

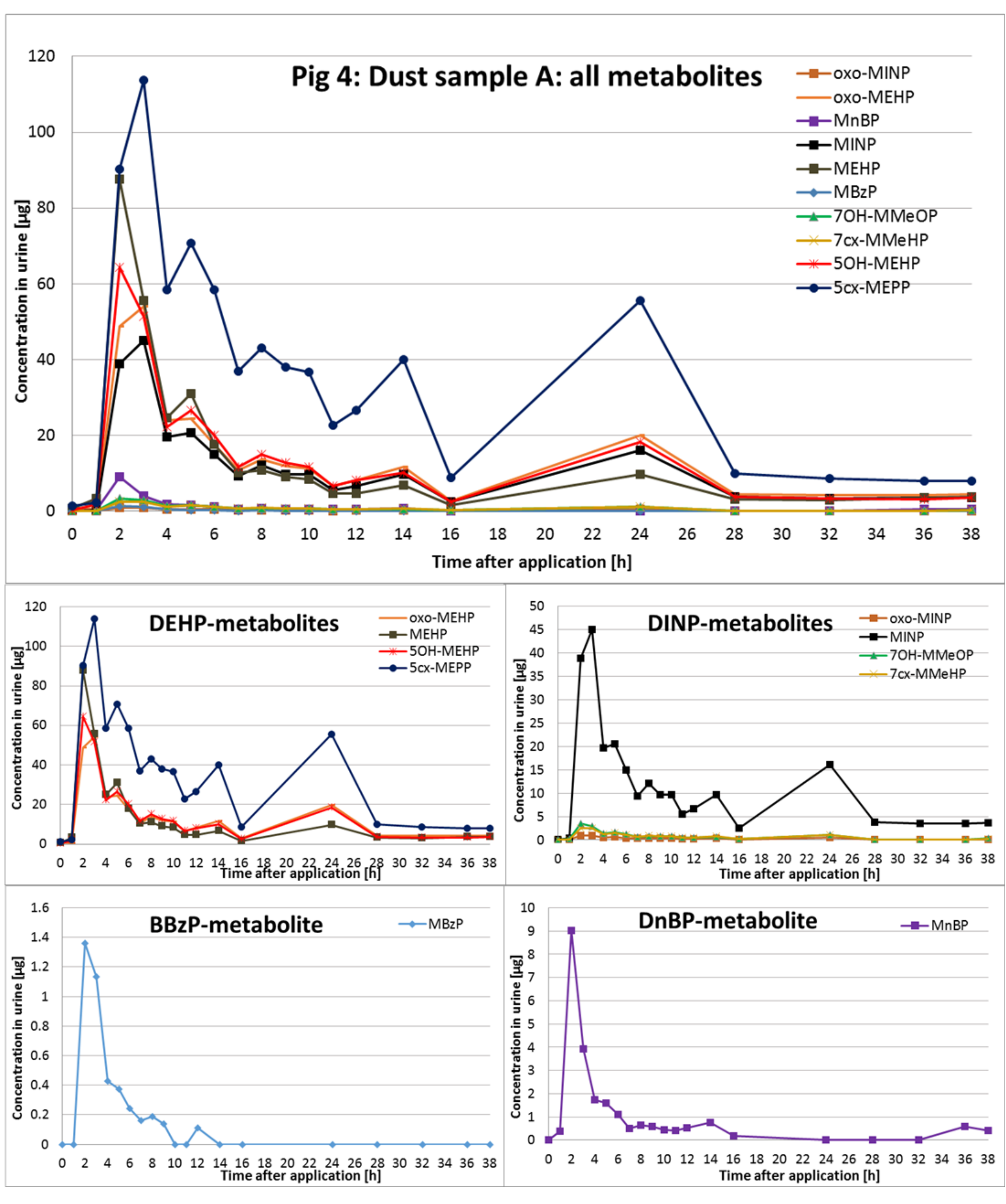


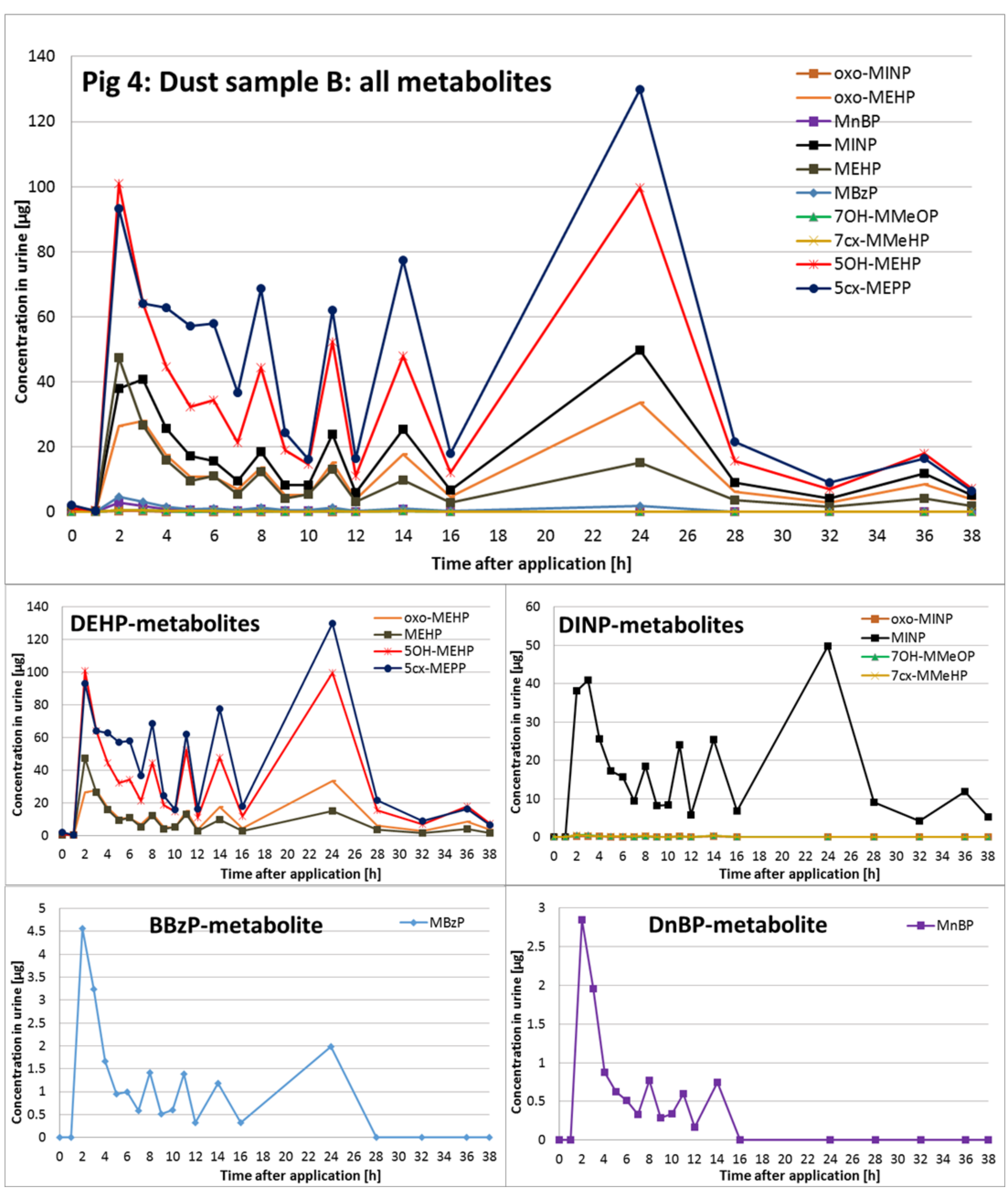
Pig 3: Dust sample D: all metabolites
oxo-MINP
oxo-MEHP
MnBP
MINP
MEHP
MBzP
7OH-MMeOP
7cx-MMEHP
5OH-MEHP
5cx-MEPP
Concentration in urine [µg]
Time after application [h]
DEHP-metabolites
DINP-metabolites
BBzP-metabolites
DnBP-metabolites

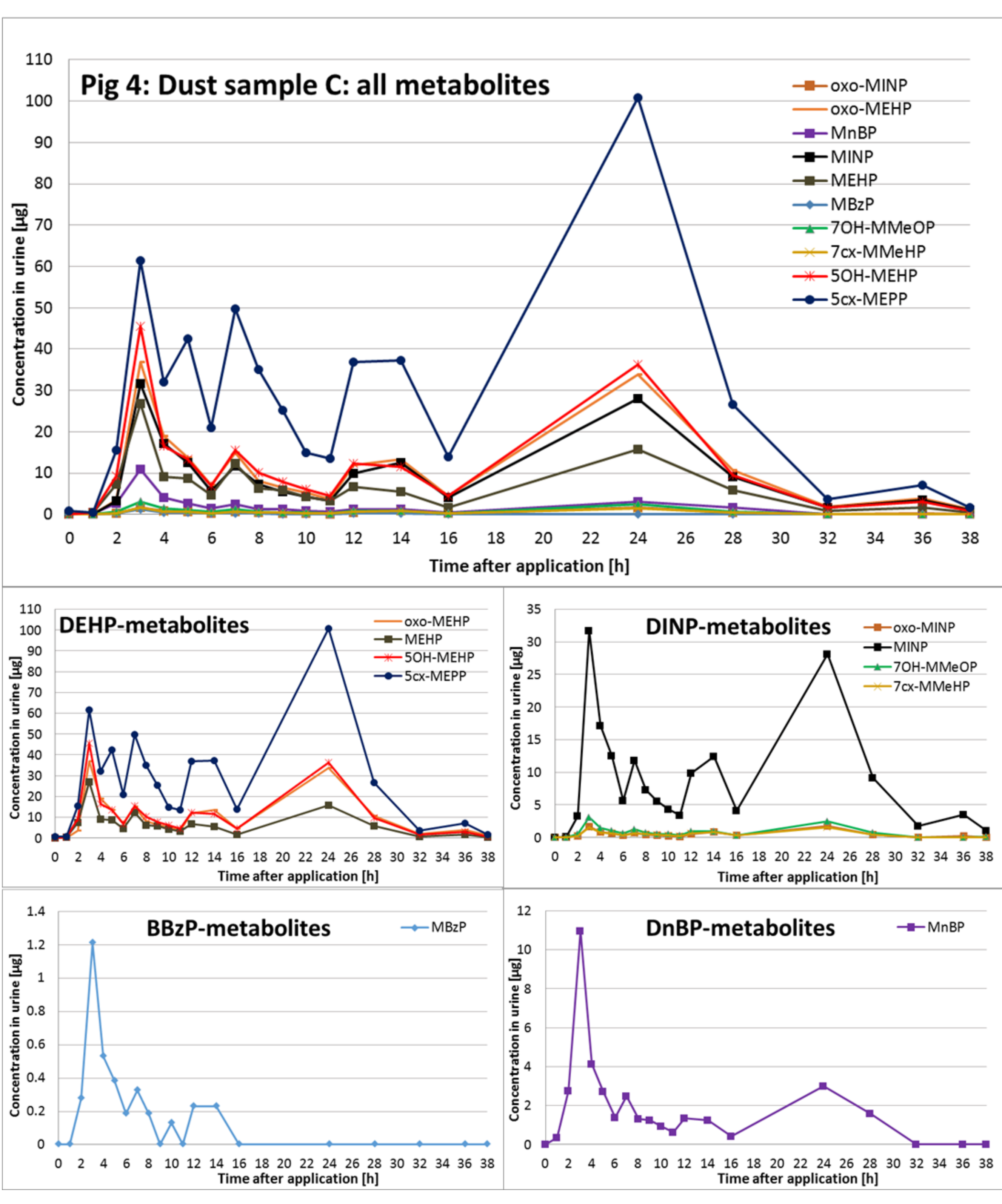

Pig 3: Dust sample E: all metabolites
oxo-MINP
oxo-MEHP
MnBP
MINP
MEHP
MBzP
7OH-MMeOP
7cx-MMeHP
5OH-MEHP
5cx-MEPP
Concentration in urine [µg]
Time after application [h]
DEHP-metabolites
DINP-metabolites
BBZP-metabolites
DnBP-metabolite



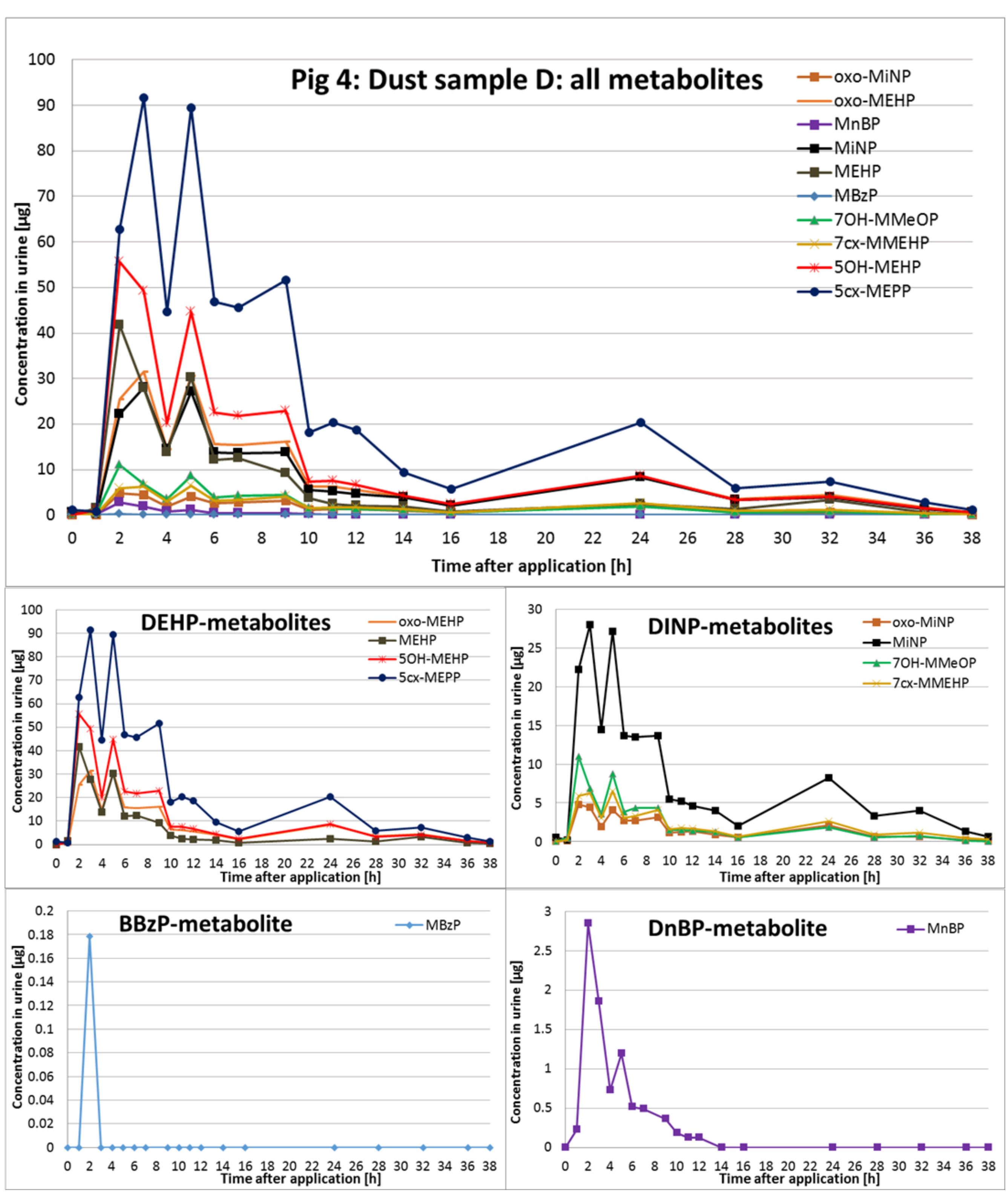
Pig 3: Food sample: all metabolites
oxo-MINP
oxo-MEHP
MnBP
MINP
MEHP
MBZP
7OH-MMeOP
7cx-MMEHP
5OH-MEHP
5cx-MEPP
Concentration in urine [µg]
Time after application [h]
DEHP-metabolites
oxo-MEHP
MEHP
5OH-MEHP
5cx-MEPP
DINP-metabolites
oxo-MINP
MINP
7OH-MMeOP
7cx-MMEHP
BBzP-metabolite
MBZP
DnBP-metabolite
MnBP

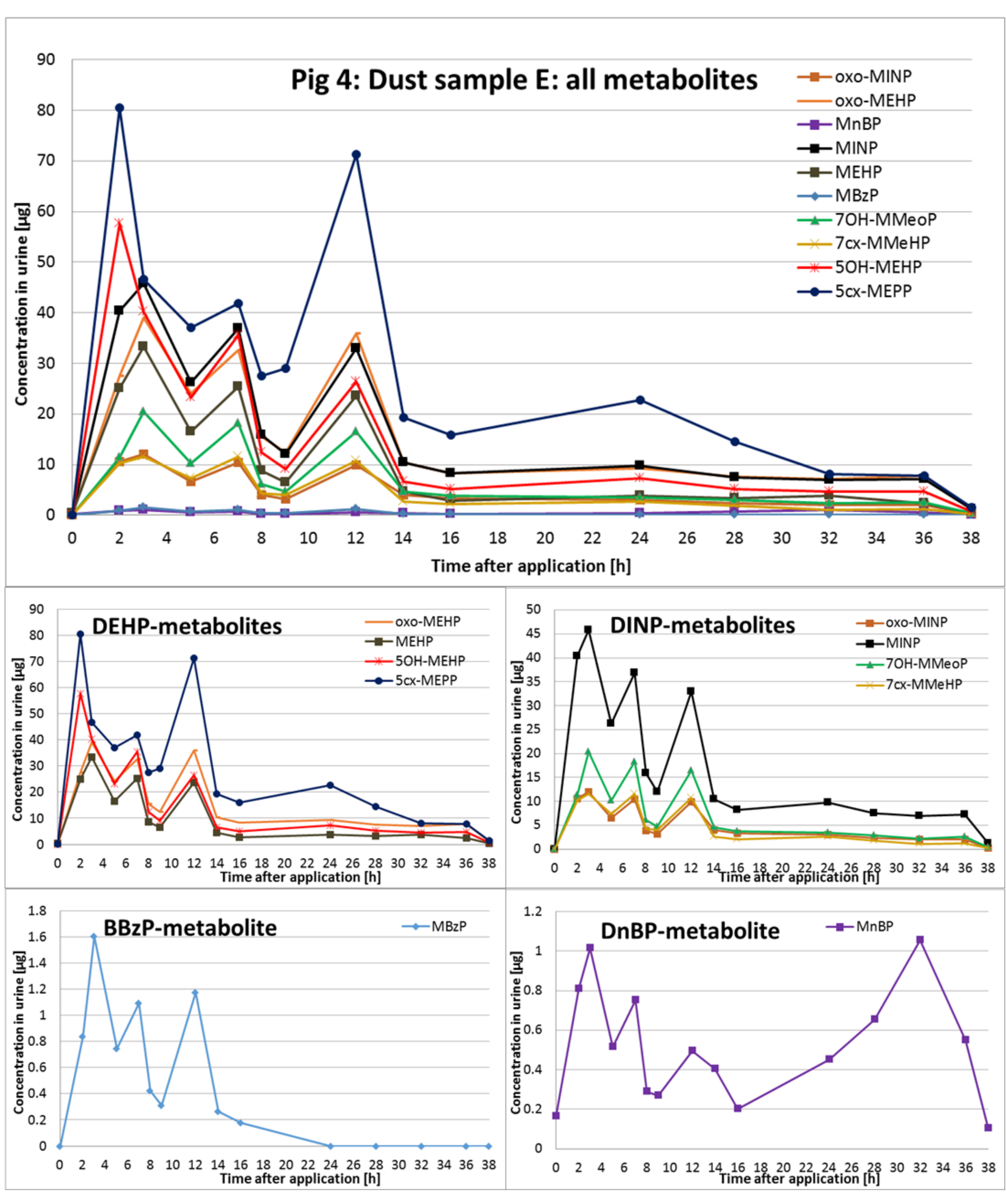

Pig 4: Dust sample A: all metabolites
oxo-MINP
oxo-MEHP
MnBP
MINP
MEHP
MBzP
7OH-MMeOP
7cx-MMeHP
5OH-MEHP
5cx-MEPP
Concentration in urine [µg]
Time after application [h]
DEHP-metabolites
DINP-metabolites
BBzP-metabolite
DnBP-metabolite

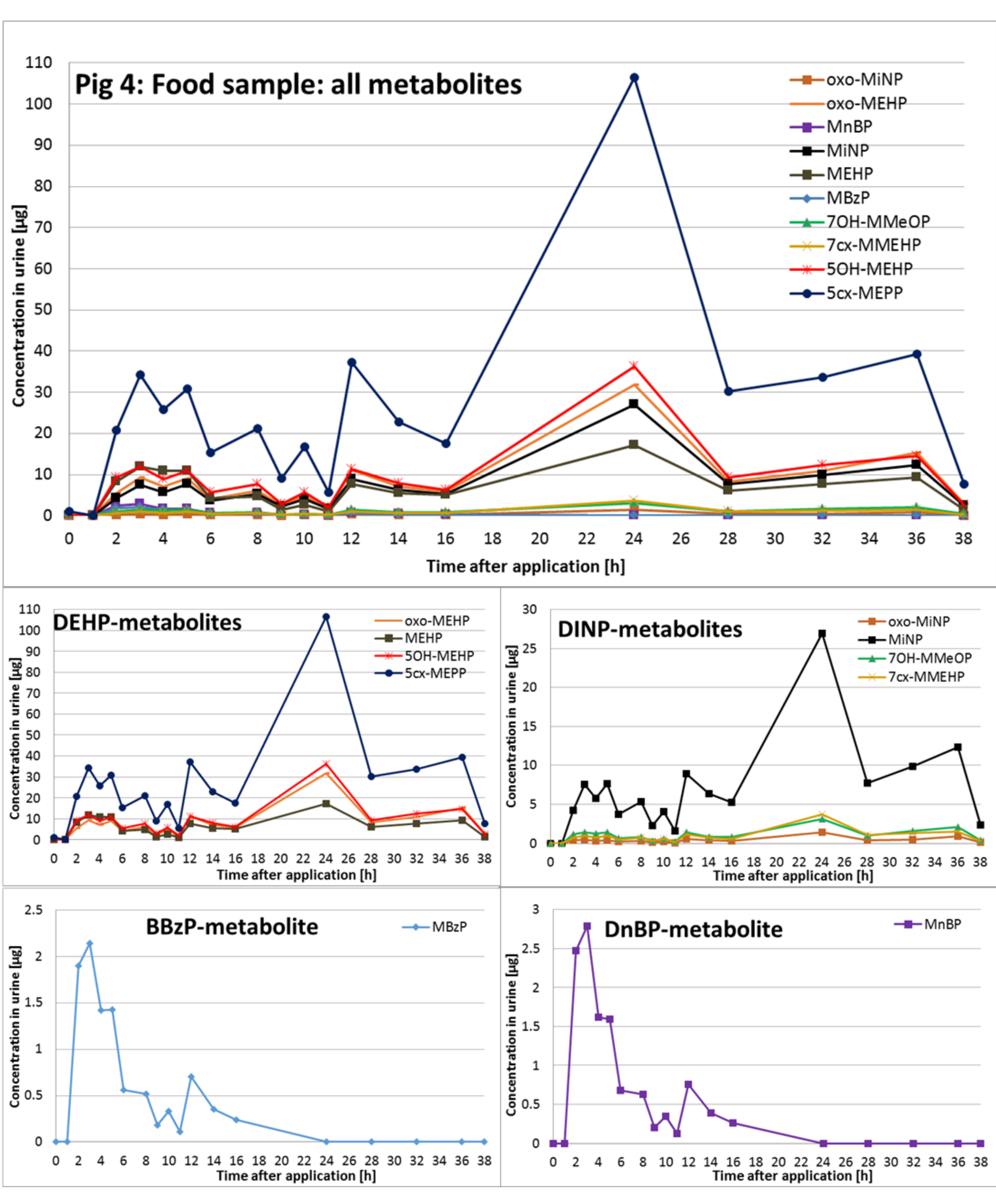

Pig 4: Dust sample B: all metabolites
oxo-MINP
oxo-MEHP
MnBP
MINP
MEHP
MBzP
7OH-MMeOP
7cx-MMeHP
5OH-MEHP
5cx-MEPP
Concentration in urine [µg]
Time after application [h]
DEHP-metabolites
DINP-metabolites
BBzP-metabolite
DnBP-metabolite



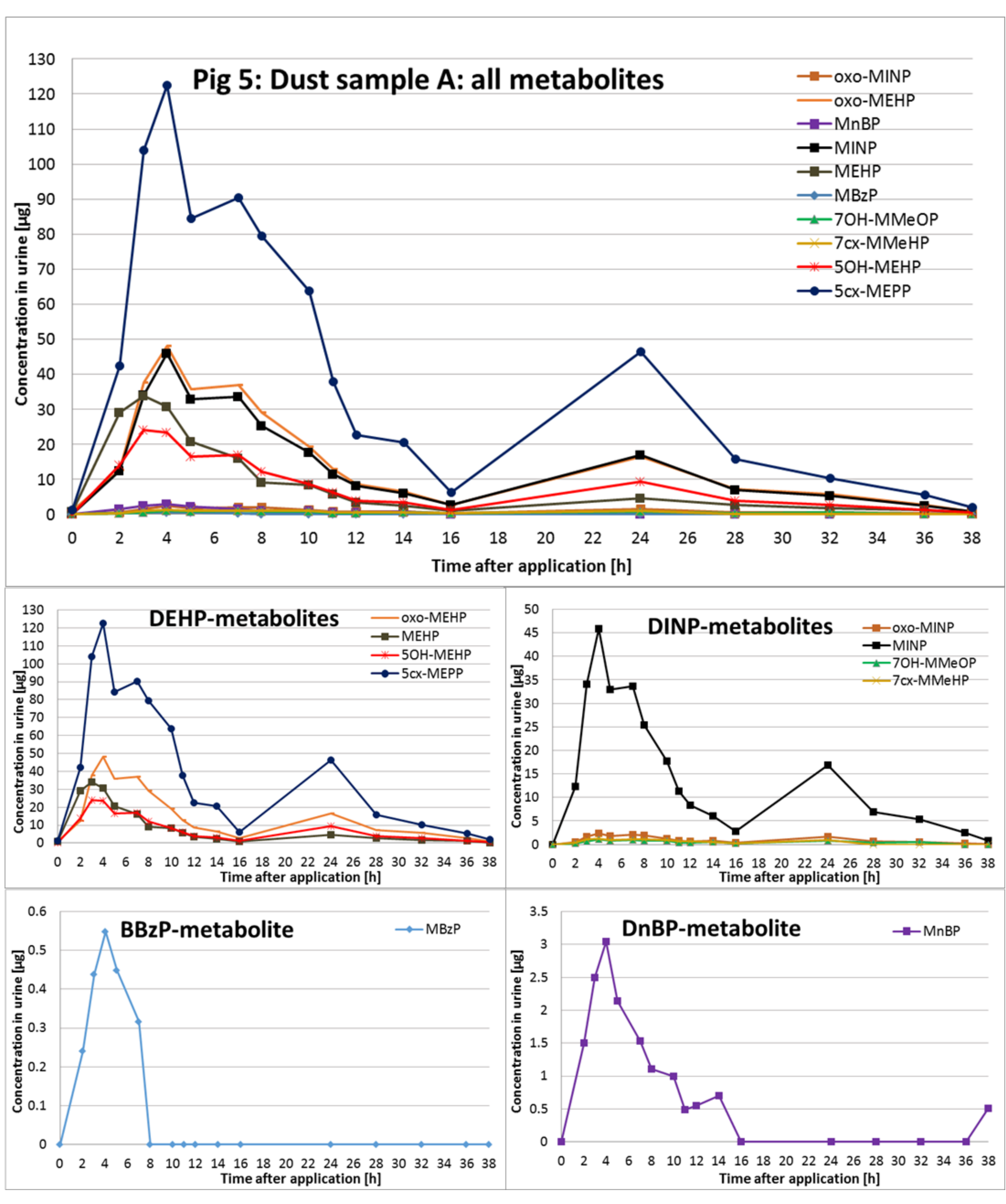
Pig 4: Dust sample C: all metabolites
oxo-MINP
oxo-MEHP
MnBP
MINP
MEHP
MBzP
7OH-MMeOP
7cx-MMeHP
5OH-MEHP
5cx-MEPP
Concentration in urine [µg]
Time after application [h]
DEHP-metabolites
DINP-metabolites
BBzP-metabolites
DnBP-metabolites

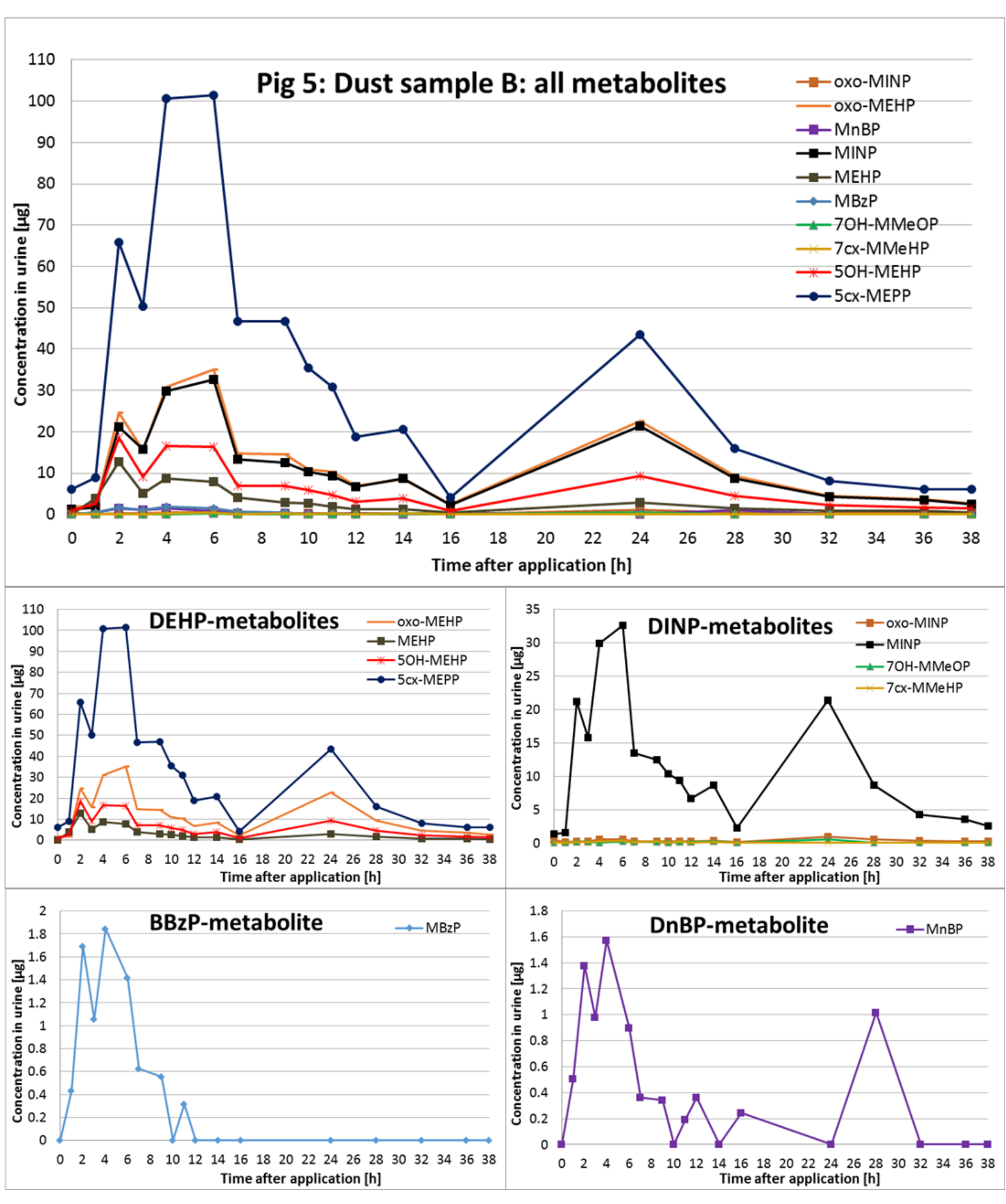

Pig 4: Dust sample D: all metabolites
oxo-MiNP
oxo-MEHP
MnBP
MiNP
MEHP
MBzP
7OH-MMeOP
7cx-MMEHP
5OH-MEHP
5cx-MEPP
Concentration in urine [µg]
Time after application [h]
DEHP-metabolites
DINP-metabolites
BBzP-metabolite
DnBP-metabolite

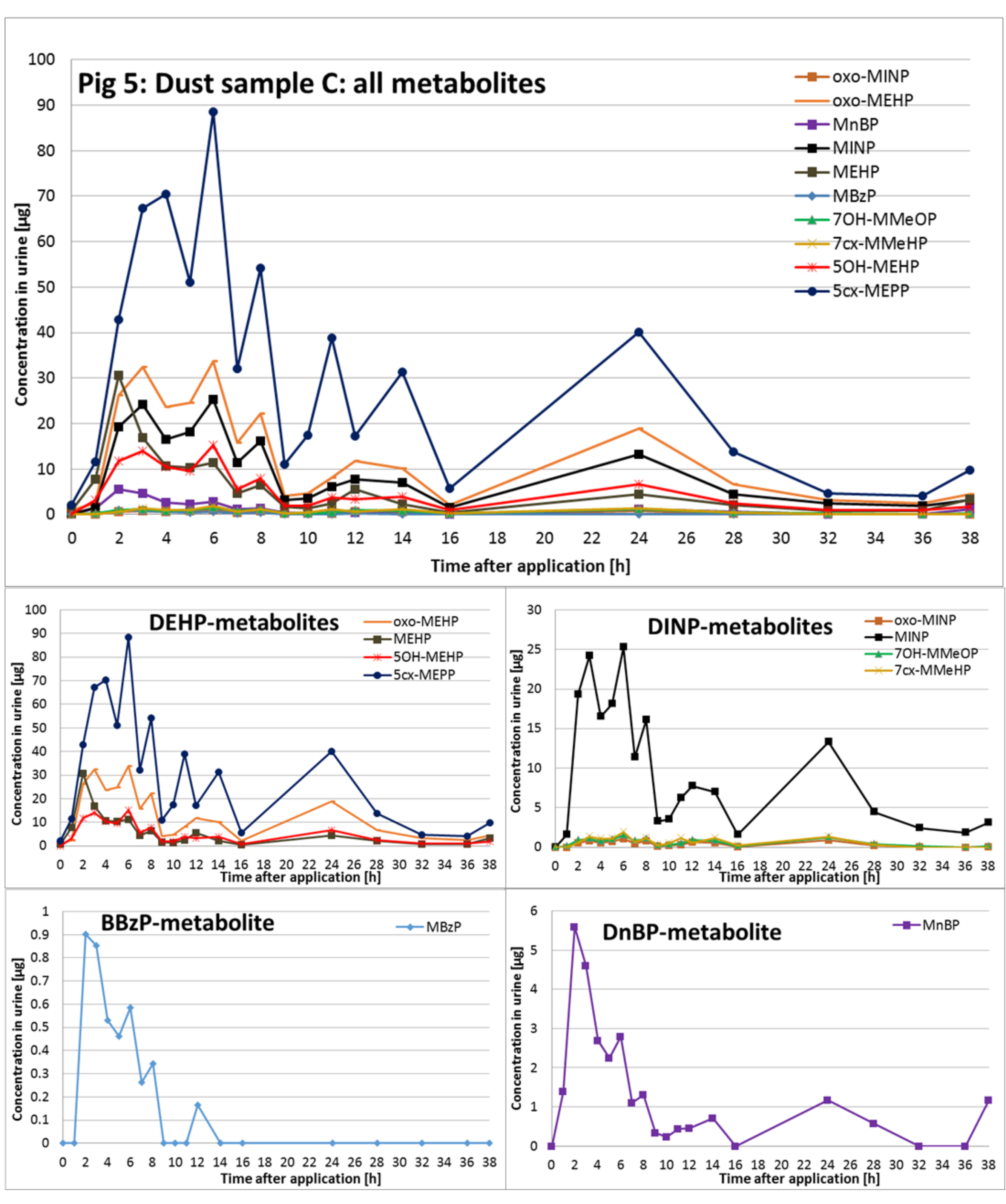

Pig 4: Dust sample E: all metabolites
oxo-MINP
oxo-MEHP
MnBP
MINP
MEHP
MBzP
7OH-MMeoP
7cx-MMeHP
5OH-MEHP
5cx-MEPP
Concentration in urine [µg]
Time after application [h]
DEHP-metabolites
DINP-metabolites
BBzP-metabolite
DnBP-metabolite



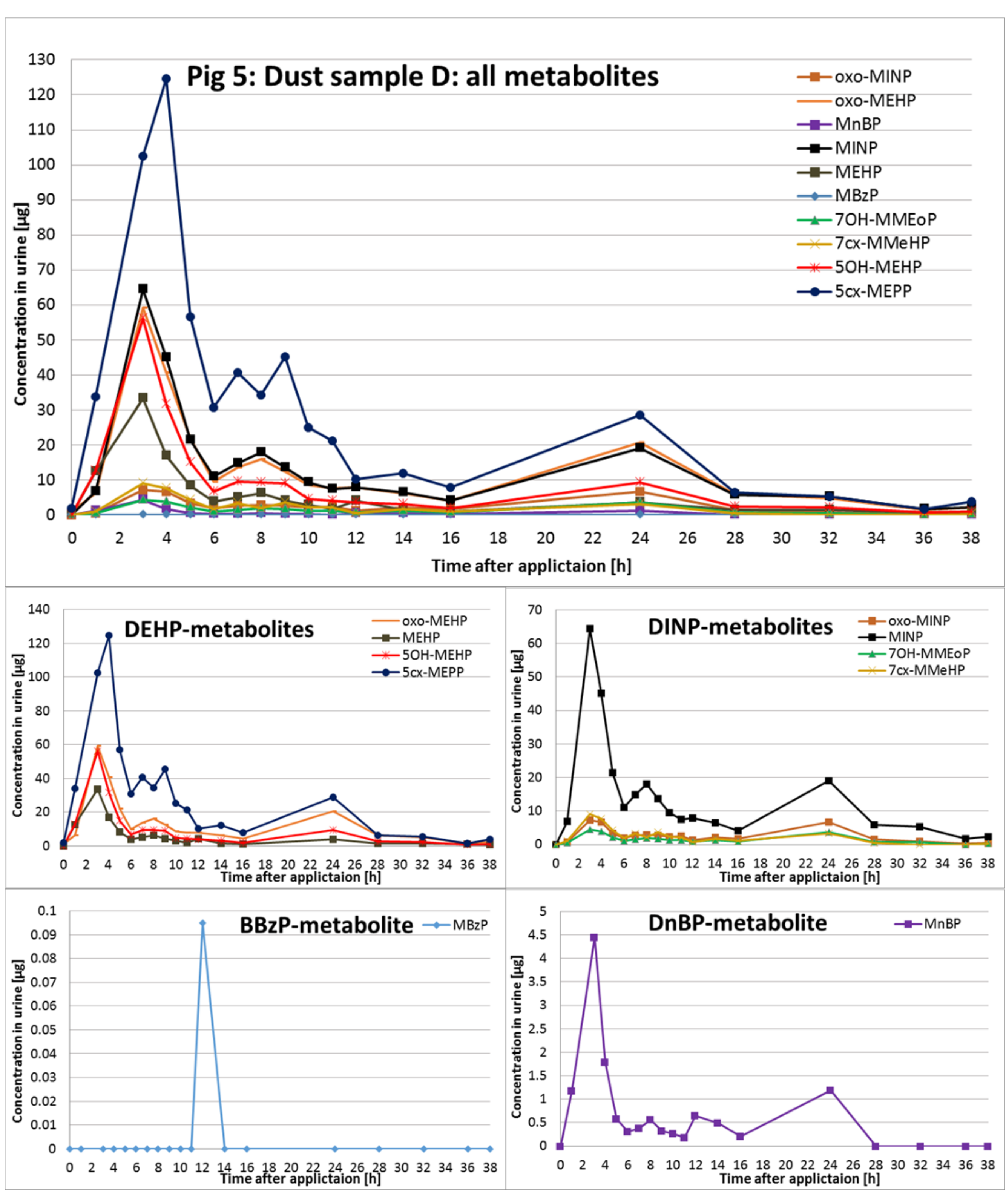
Pig 4: Food sample: all metabolites
oxo-MiNP
oxo-MEHP
MnBP
MiNP
MEHP
MBzP
7OH-MMeOP
7cx-MMEHP
5OH-MEHP
5cx-MEPP
Concentration in urine [µg]
Time after application [h]
DEHP-metabolites
DINP-metabolites
BBzP-metabolite
DnBP-metabolite

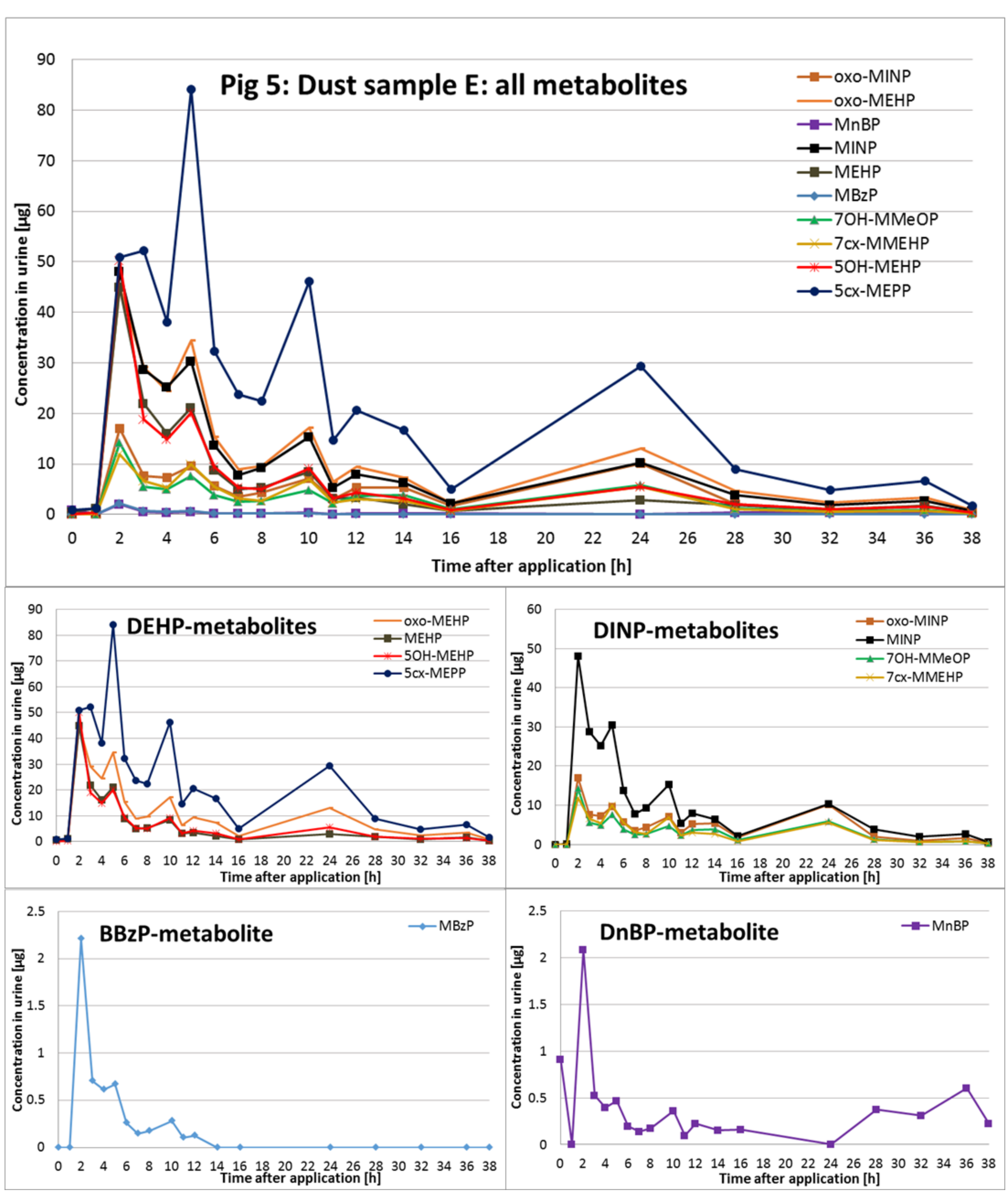

Pig 5: Dust sample A: all metabolites
oxo-MINP
oxo-MEHP
MnBP
MINP
MEHP
MBzP
7OH-MMeOP
7cx-MMeHP
5OH-MEHP
5cx-MEPP
Concentration in urine [µg]
Time after application [h]
DEHP-metabolites
DINP-metabolites
BBzP-metabolite
DnBP-metabolite


Pig 5: Dust sample B: all metabolites
Concentration in urine [µg]
Time after application [h]
oxo-MINP
oxo-MEHP
MnBP
MINP
MEHP
MBzP
7OH-MMeOP
7cx-MMeHP
5OH-MEHP
5cx-MEPP
DEHP-metabolites
DINP-metabolites
BBzP-metabolite
DnBP-metabolite

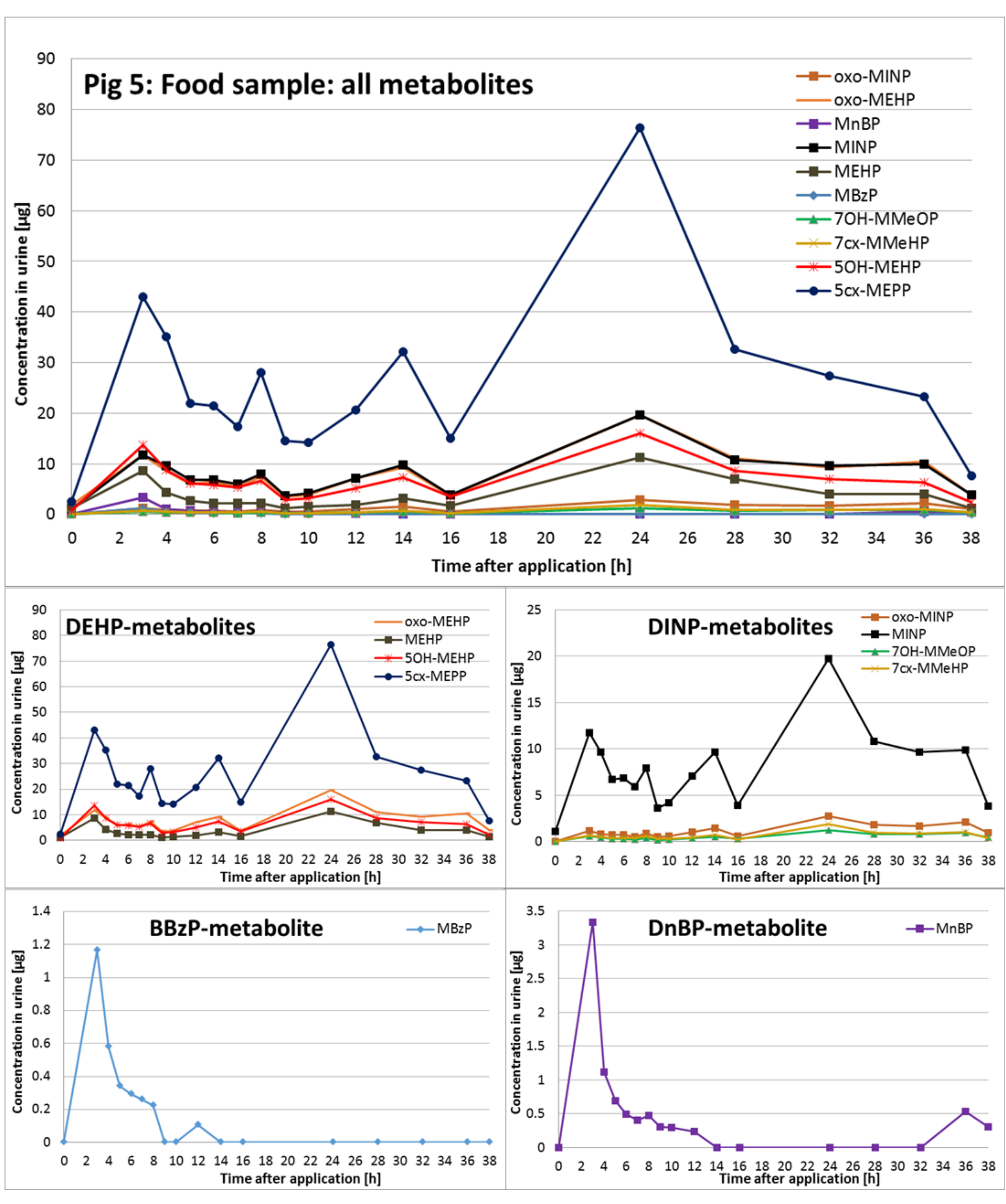


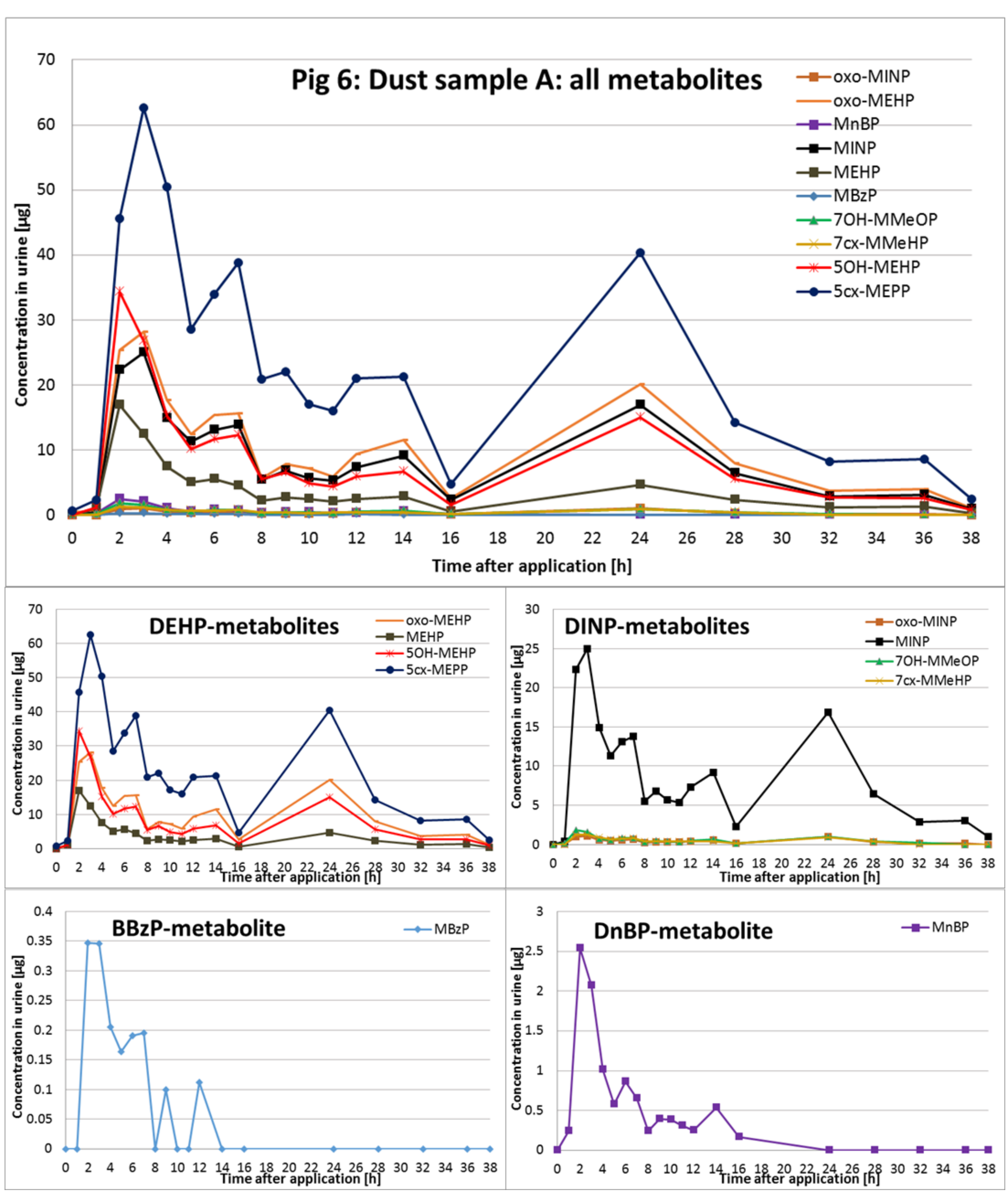
Pig 5: Dust sample C: all metabolites
oxo-MINP
oxo-MEHP
MnBP
MINP
MEHP
MBzP
7OH-MMeOP
7cx-MMeHP
5OH-MEHP
5cx-MEPP
Concentration in urine [µg]
Time after application [h]
DEHP-metabolites
DINP-metabolites
BBzP-metabolite
DnBP-metabolite

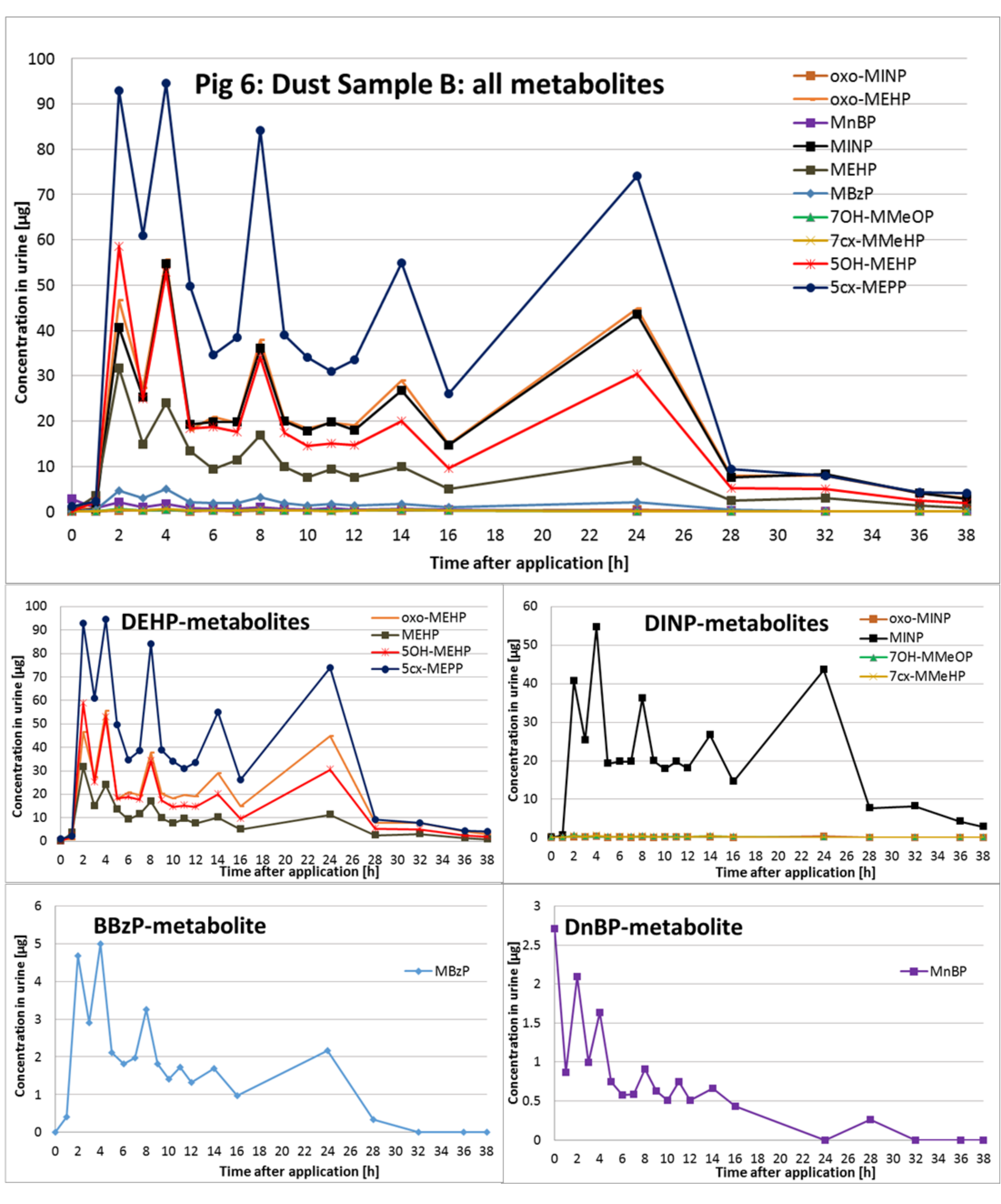

Pig 5: Dust sample D: all metabolites
oxo-MINP
oxo-MEHP
MnBP
MINP
MEHP
MBzP
7OH-MMEoP
7cx-MMeHP
5OH-MEHP
5cx-MEPP
Concentration in urine [µg]
Time after applictaion [h]
DEHP-metabolites
DINP-metabolites
BBzP-metabolite
DnBP-metabolite

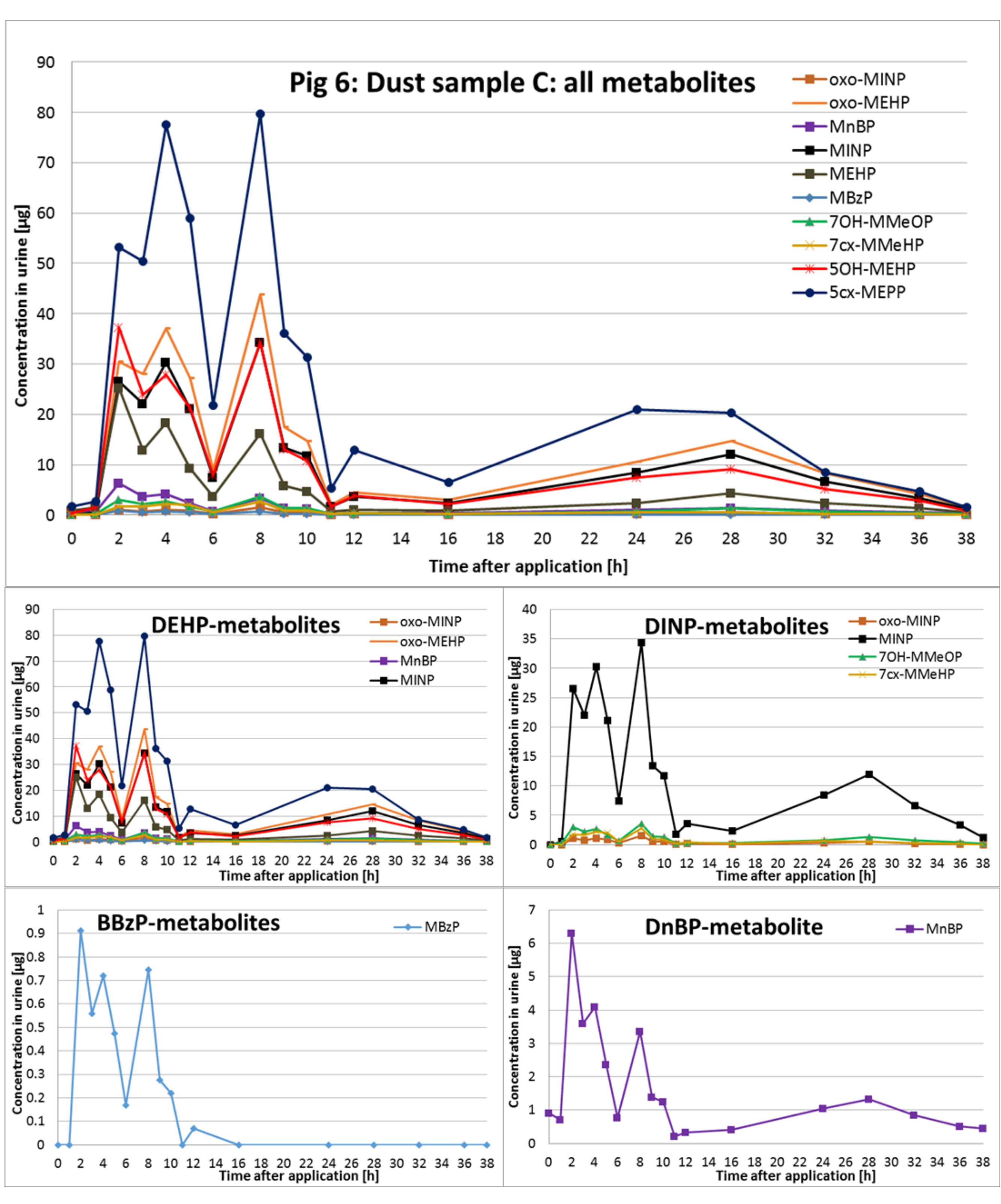


Pig 5: Dust sample E: all metabolites
oxo-MINP
oxo-MEHP
MnBP
MINP
MEHP
MBzP
7OH-MMeOP
7cx-MMEHP
5OH-MEHP
5cx-MEPP
Concentration in urine [µg]
Time after application [h]
DEHP-metabolites
oxo-MEHP
MEHP
5OH-MEHP
5cx-MEPP
DINP-metabolites
oxo-MINP
MINP
7OH-MMeOP
7cx-MMEHP
BBzP-metabolite
MBzP
DnBP-metabolite
MnBP

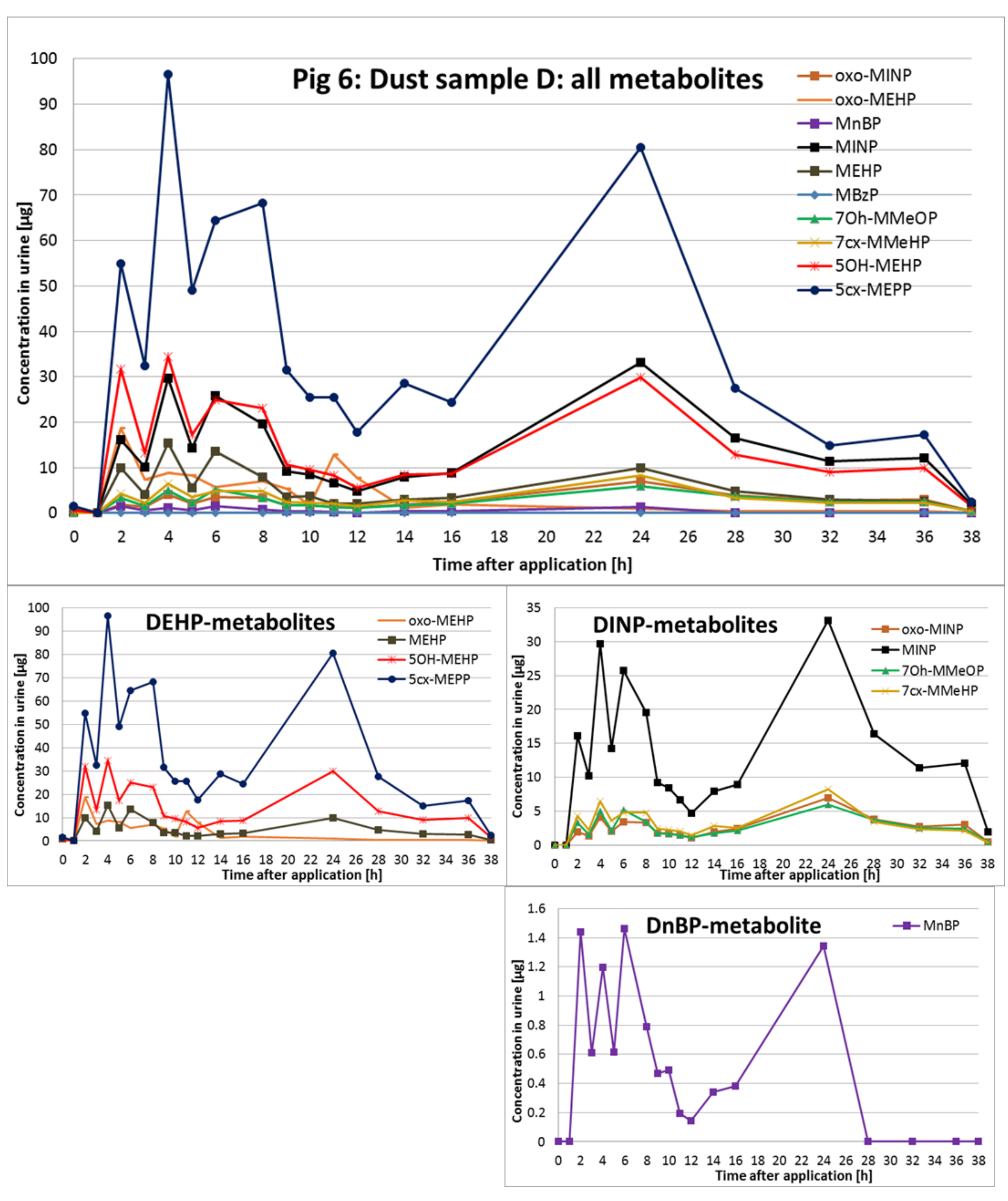

Pig 5: Food sample: all metabolites
oxo-MINP
oxo-MEHP
MnBP
MINP
MEHP
MBzP
7OH-MMeOP
7cx-MMeHP
5OH-MEHP
5cx-MEPP
Concentration in urine [µg]
Time after application [h]
DEHP-metabolites
DINP-metabolites
BBzP-metabolite
DnBP-metabolite


Pig 6: Dust sample A: all metabolites
oxo-MINP
oxo-MEHP
MnBP
MINP
MEHP
MBzP
7OH-MMeOP
7cx-MMeHP
5OH-MEHP
5cx-MEPP
Concentration in urine [µg]
Time after application [h]
DEHP-metabolites
DINP-metabolites
BBzP-metabolite
DnBP-metabolite

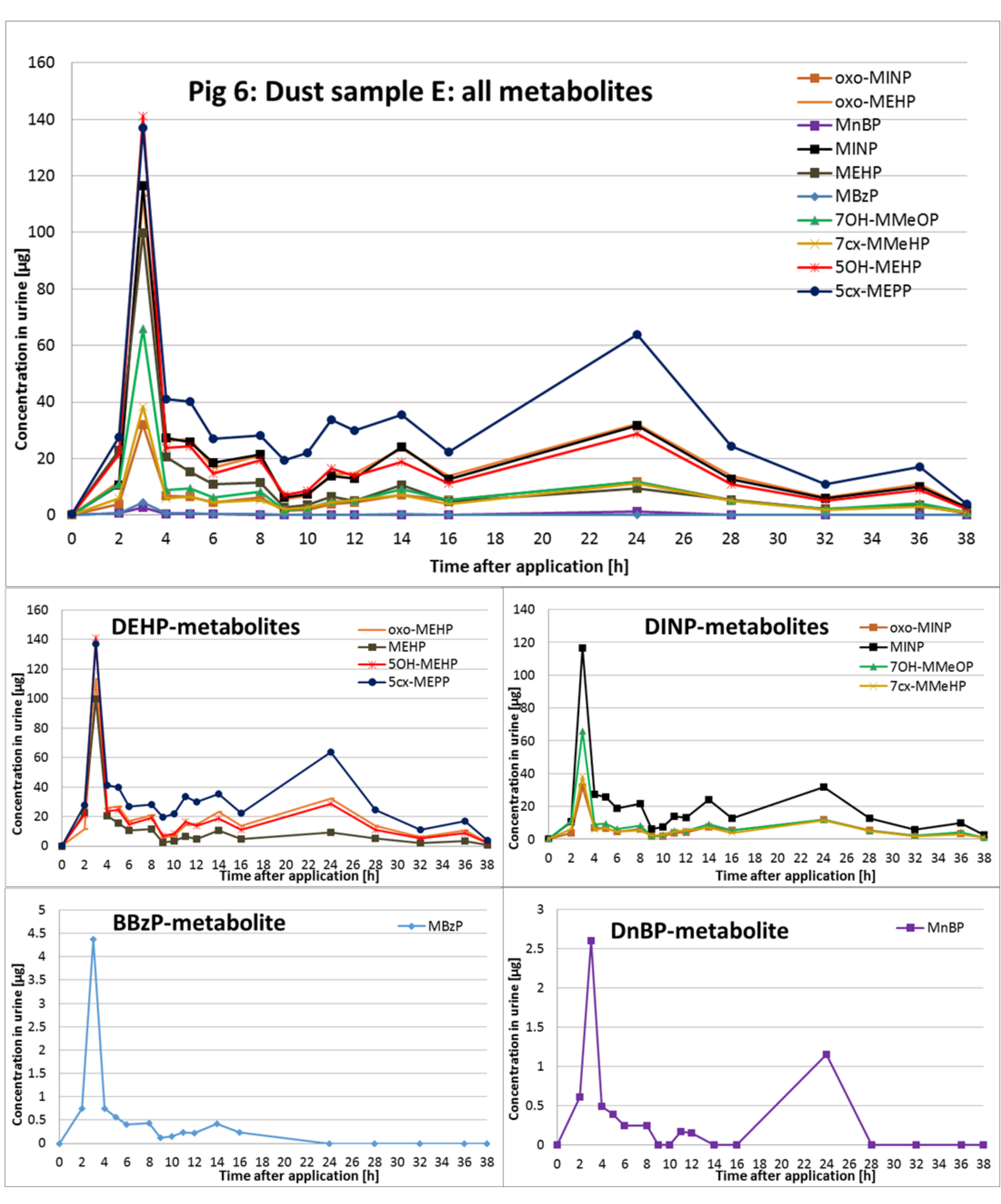

Pig 6: Dust Sample B: all metabolites
oxo-MINP
oxo-MEHP
MnBP
MINP
MEHP
MBzP
7OH-MMeOP
7cx-MMeHP
5OH-MEHP
5cx-MEPP
Concentration in urine [µg]
Time after application [h]
DEHP-metabolites
DINP-metabolites
BBzP-metabolite
DnBP-metabolite

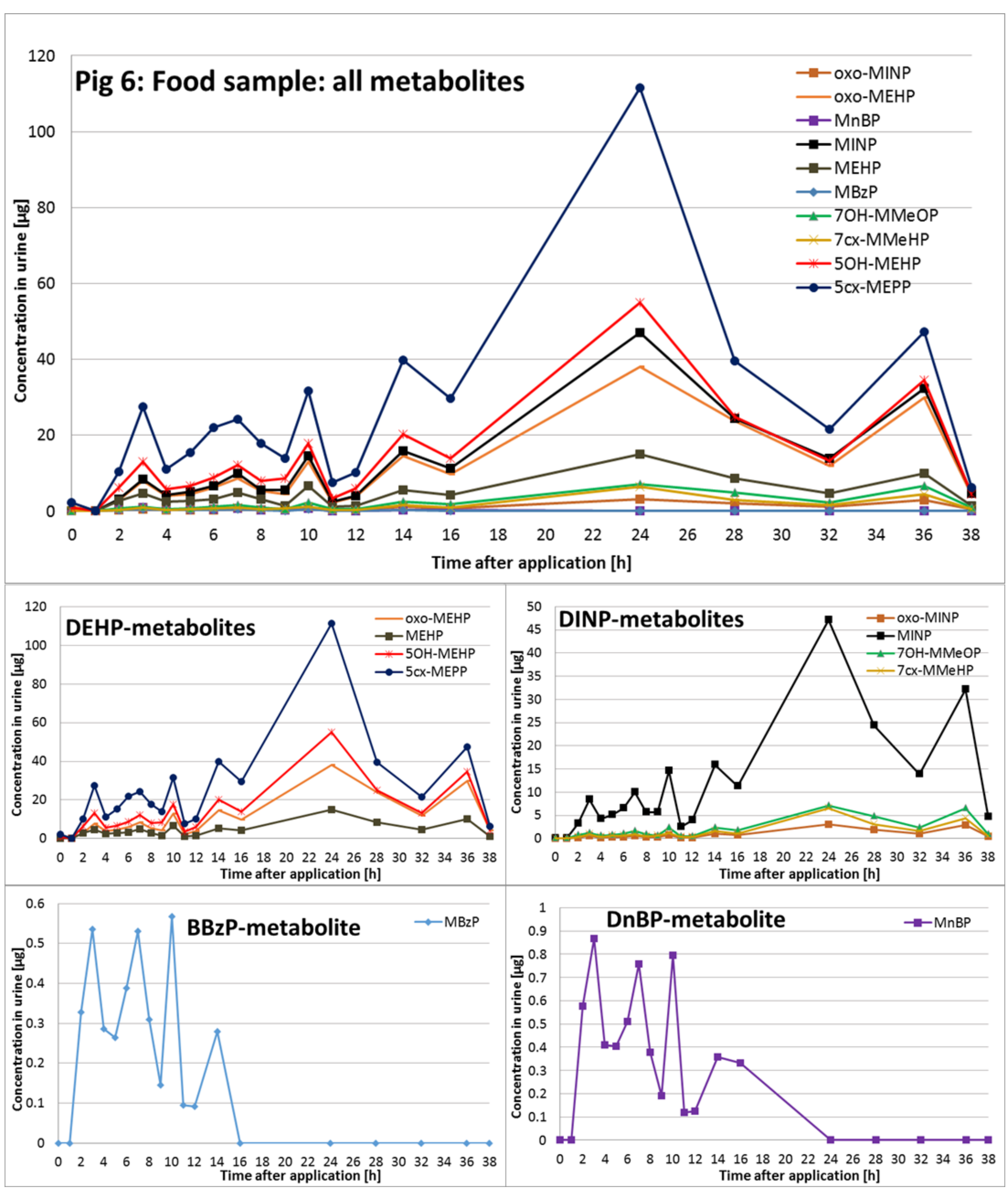


Pig 6: Dust sample C: all metabolites
oxo-MINP
oxo-MEHP
MnBP
MINP
MEHP
MBzP
7OH-MMeOP
7cx-MMeHP
5OH-MEHP
5cx-MEPP
Concentration in urine [µg]
Time after application [h]
DEHP-metabolites
DINP-metabolites
BBzP-metabolites
DnBP-metabolite

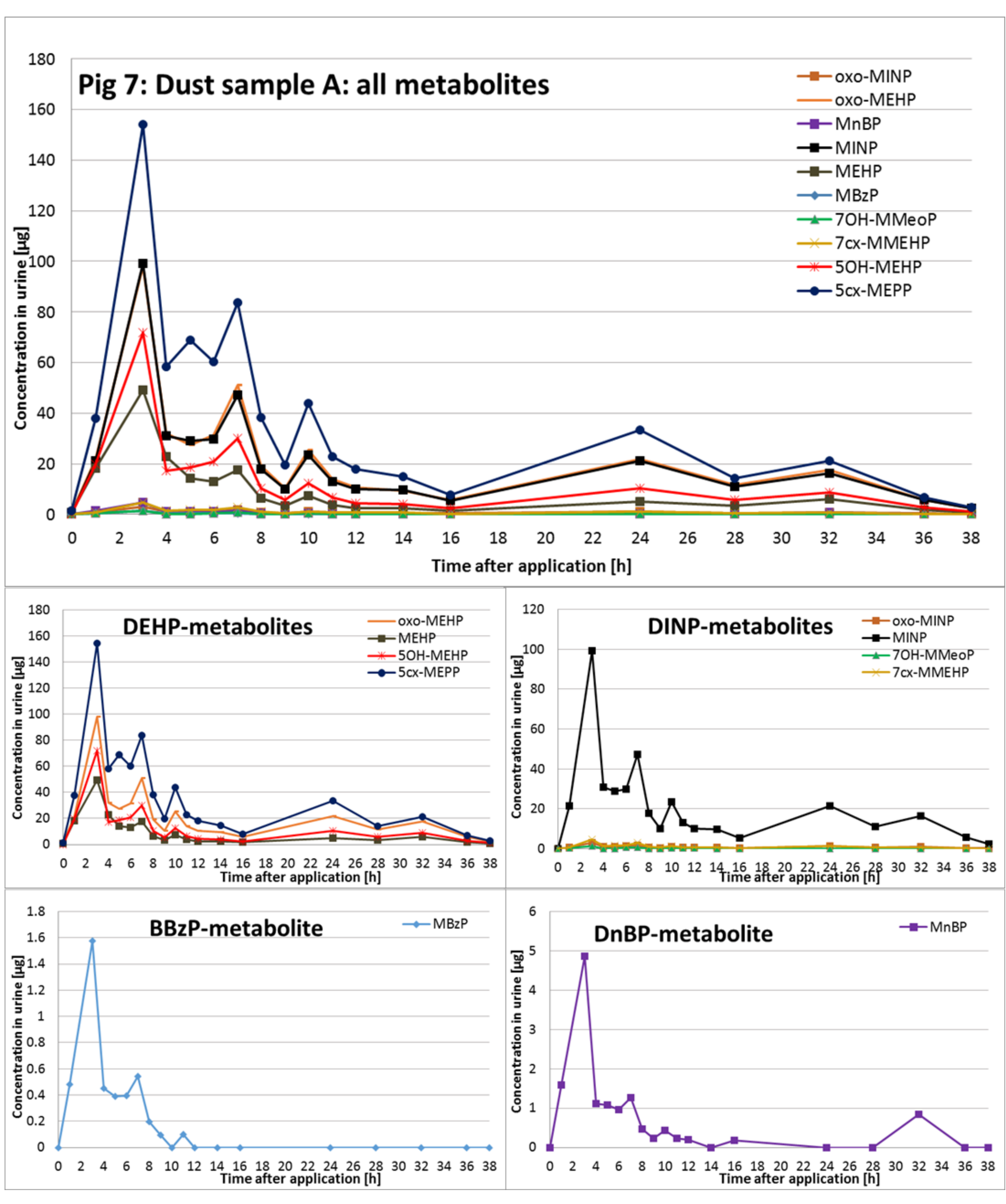


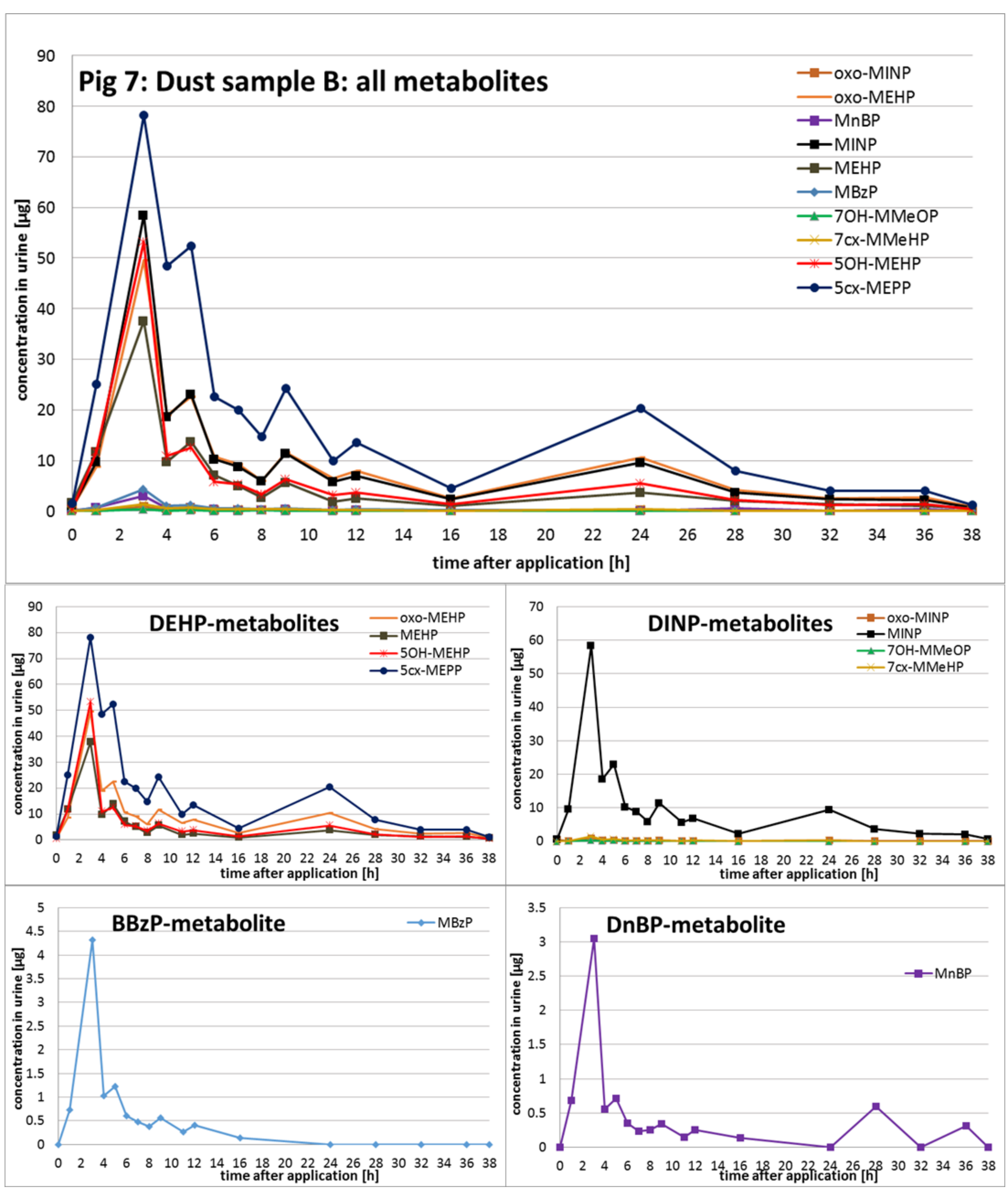
No BBzP were detected.

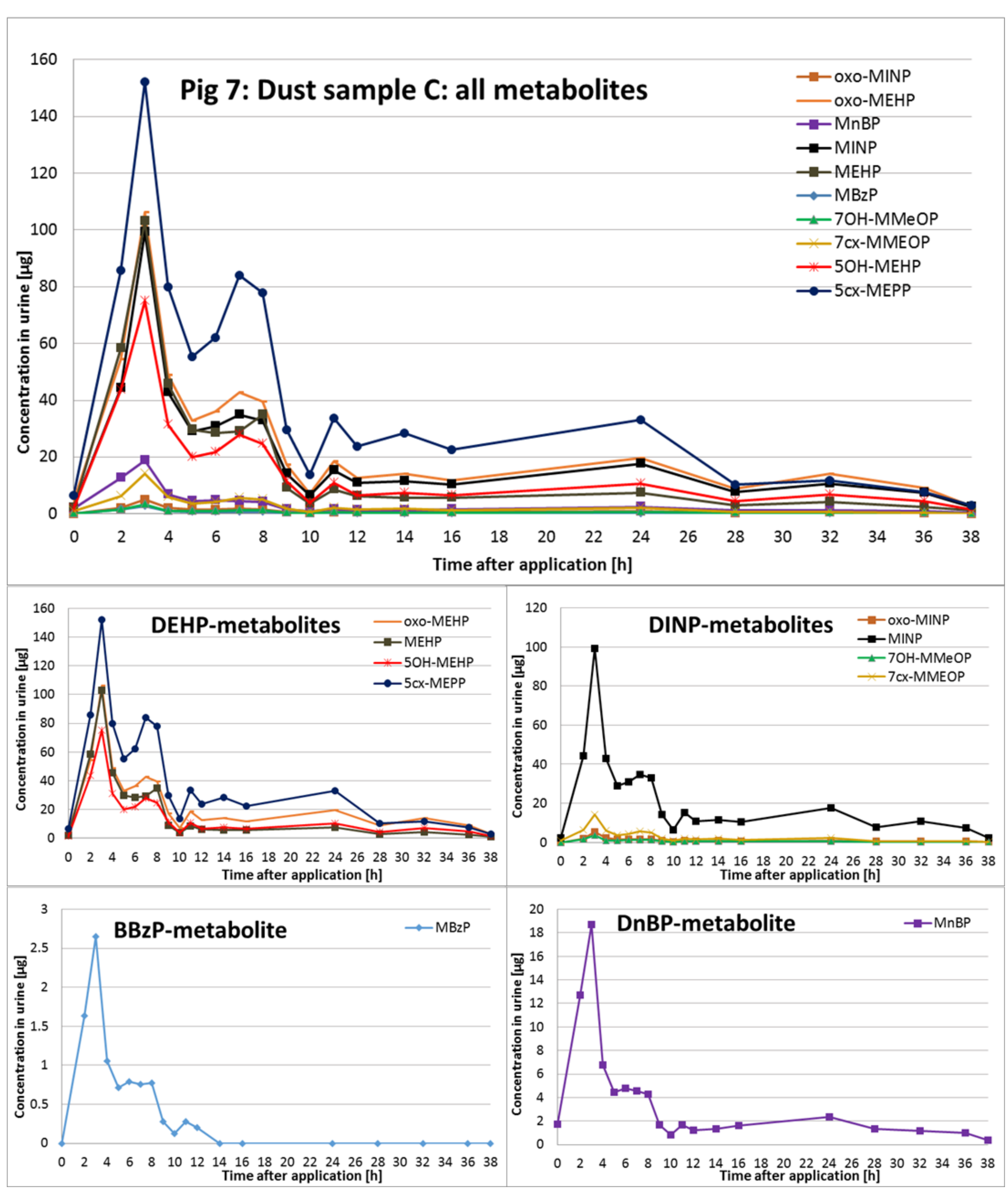

Pig 6: Dust sample E: all metabolites
oxo-MINP
oxo-MEHP
MnBP
MINP
MEHP
MBzP
7OH-MMeOP
7cx-MMeHP
5OH-MEHP
5cx-MEPP
Concentration in urine [µg]
Time after application [h]
DEHP-metabolites
DINP-metabolites
BBzP-metabolite
DnBP-metabolite



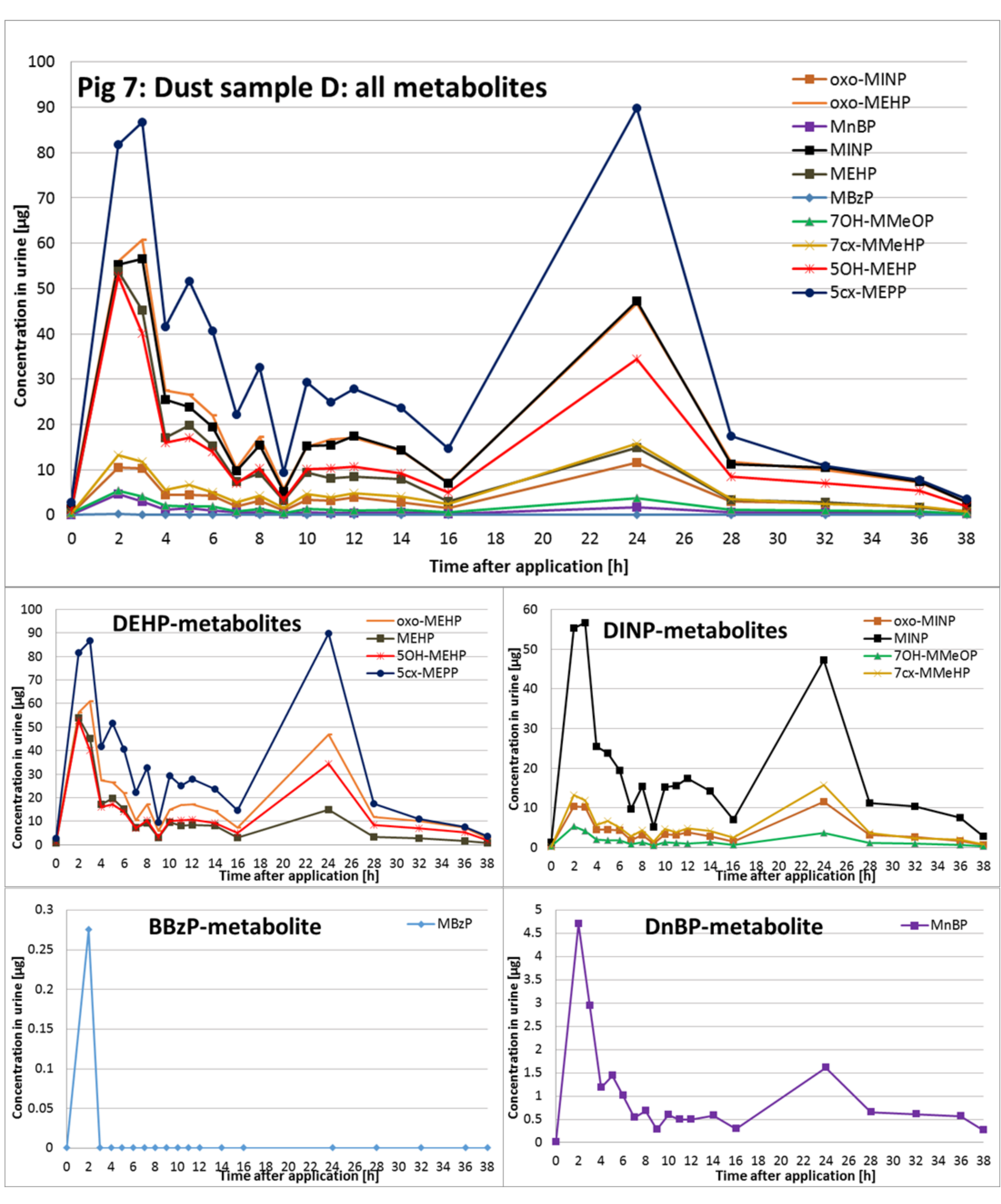
Pig 6: Food sample: all metabolites
oxo-MINP
oxo-MEHP
MnBP
MINP
MEHP
MBzP
7OH-MMeOP
7cx-MMeHP
5OH-MEHP
5cx-MEPP
Concentration in urine [µg]
Time after application [h]
DEHP-metabolites
DINP-metabolites
BBzP-metabolite
DnBP-metabolite


Pig 7: Dust sample A: all metabolites
oxo-MINP
oxo-MEHP
MnBP
MINP
MEHP
MBzP
7OH-MMeoP
7cx-MMEHP
5OH-MEHP
5cx-MEPP
Concentration in urine [µg]
Time after application [h]
DEHP-metabolites
DINP-metabolites
BBzP-metabolite
DnBP-metabolite

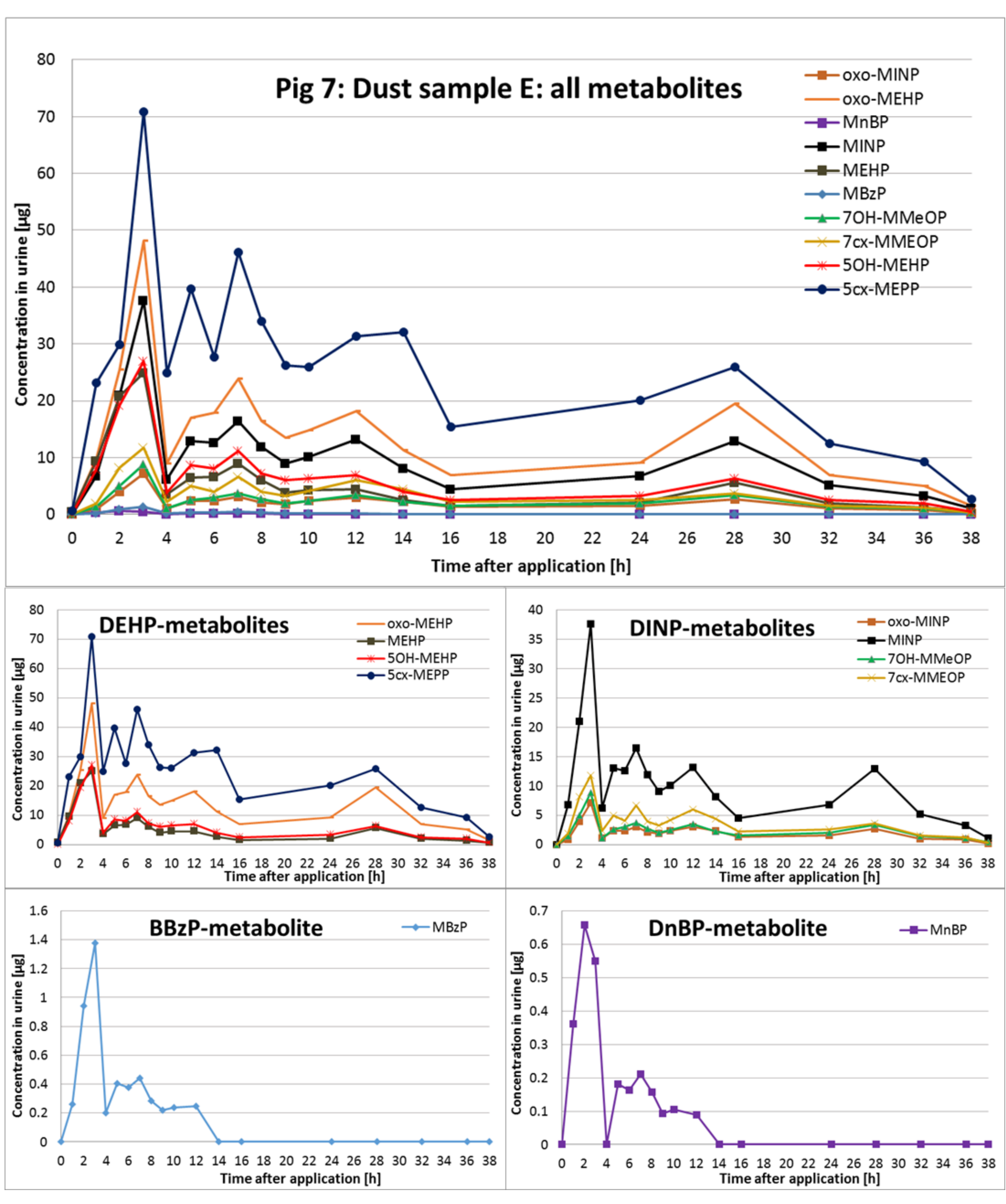

Pig 7: Dust sample B: all metabolites
oxo-MINP
oxo-MEHP
MnBP
MINP
MEHP
MBzP
7OH-MMeOP
7cx-MMeHP
5OH-MEHP
5cx-MEPP
concentration in urine [µg]
time after application [h]
DEHP-metabolites
DINP-metabolites
BBzP-metabolite
DnBP-metabolite

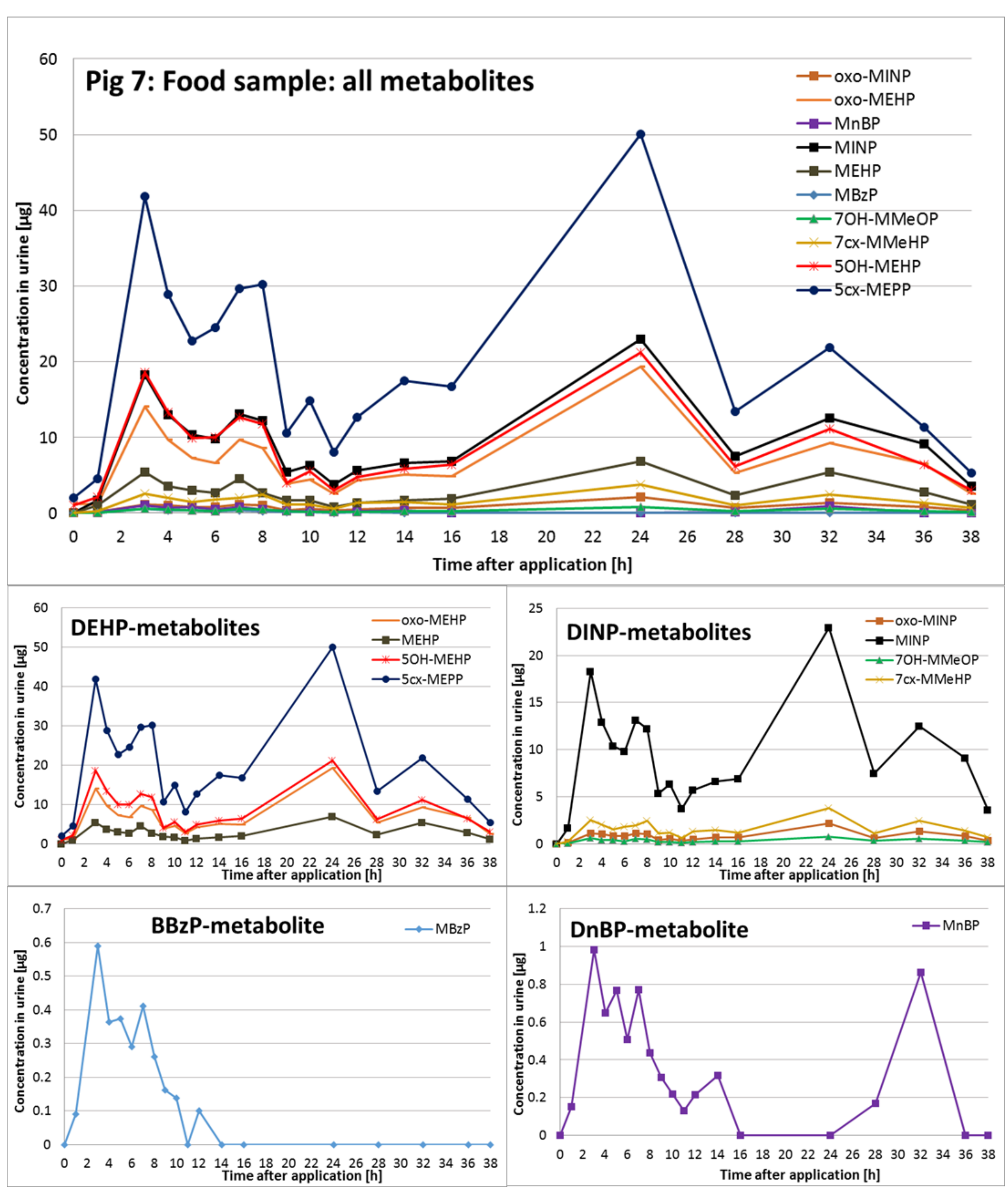


Pig 7: Dust sample C: all metabolites
oxo-MINP
oxo-MEHP
MnBP
MINP
MEHP
MBzP
7OH-MMeOP
7cx-MMEOP
5OH-MEHP
5cx-MEPP
Concentration in urine [µg]
Time after application [h]
DEHP-metabolites
DINP-metabolites
BBzP-metabolite
DnBP-metabolite



Pig 7: Dust sample D: all metabolites
oxo-MINP
oxo-MEHP
MnBP
MINP
MEHP
MBzP
7OH-MMeOP
7cx-MMeHP
5OH-MEHP
5cx-MEPP
Concentration in urine [µg]
Time after application [h]
DEHP-metabolites
DINP-metabolites
BBzP-metabolite
DnBP-metabolite


Pig 7: Dust sample E: all metabolites
oxo-MINP
oxo-MEHP
MnBP
MINP
MEHP
MBzP
7OH-MMeOP
7cx-MMEOP
5OH-MEHP
5cx-MEPP
Concentration in urine [µg]
Time after application [h]
DEHP-metabolites
DINP-metabolites
BBzP-metabolite
DnBP-metabolite


Pig 7: Food sample: all metabolites
oxo-MINP
oxo-MEHP
MnBP
MINP
MEHP
MBzP
7OH-MMeOP
7cx-MMeHP
5OH-MEHP
5cx-MEPP
Concentration in urine [µg]
Time after application [h]
DEHP-metabolites
DINP-metabolites
BBzP-metabolite
DnBP-metabolite

8I Curriculum Vitae

Persönliche Daten:

Name: MMag^{a}.rer.nat Veronika Plichta

Ausbildung:

Seit 02/2016	Weiterbildung zur Fachtoxikologin bei der Deutschen Gesellschaft für experimentelle und klinische Pharmakologie und Toxikologie (DGPT), Helmholtz Zentrum München
Seit 02/2014	Doktorarbeit am Landesamt für Gesundheit und Lebensmittelsicherheit in München, Deutschland *„Bioverfügbarkeit von Weichmachern im Hausstaub und in Nahrungsmitteln nach oraler Zufuhr“*
11/2011-11/2013	Diplomarbeiten für die Studienzweige Anthropologie und Ökologie bei Prof. Gundacker an der Medizinischen Genetik, Medizinischen Universität Wien “*The relevance of the genetic background in prenatal mercury exposure in Bratislava”* *“The influence of the diet in prenatal mercury exposure in Vienna and Bratislava”*
2008-11/2013	Diplomstudium Anthropologie (Schwerpunkt Humanökologie) an der Universität Wien
2008-08/2013	Diplomstudium Ökologie (Schwerpunkt Humanökologie) an der Universität Wien
2006-2008	Diplomstudium der Biologie an der Universität Wien
2005-2006	Diplomstudium der Molekularen Biologie an der Universität Wien
1997-2005	BG Rahlgasse Wien, Matura am 07.06.2005

Berufliche Qualifikation:

11/2012–01/2014	wissenschaftliche Mitarbeit am Projekt: ***„Mercury toxicokinetics in human term placenta: functional proof and localization of involved candidate proteins“*** Betreut von Prof. Gundacker, Medizinische Genetik, Medizinische Universität Wien
11/2011–11/2012	wissenschaftliche Mitarbeit am Projekt: „***UM-MUKI: Umweltschadstoffe in Mutter-Kind-Paaren-Belastungssituation“***

	Betreut von Prof. Gundacker, Medizinische Genetik, Medizinische Universität Wien
04/2013 04/2012	**Tutorin** im Humangenetisches Praktikum für Mediziner (Block 5) bei Prof. Neesen und Prof. Mikula, Medizinischen Universität Wien

9I Acknowledgement

An erster Stelle möchte ich mich bei Herr Prof. Fromme, Herr Priv. Doz. Dr. Wolfgang Völkel, Herr Prof. Dr. med. Nowak bedanken. Zum einen dafür, dass sie mich mit dem Thema dieser Doktorarbeit bekannt gemacht haben und zum anderen für die großartige Betreuung, Unterstützung und Geduld.

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Ganz herzlichst möchte ich mich auch bei meinem ArbeitsWg-Zimmer (Bettina Aschenbrenner, Franziska Alt und Marina Sysoltseva) für die unzähligen lustigen Momente, die den Arbeitsalltag um so vieles schöner gemacht haben, bedanken. Hierbei gilt ein besonderer Tank (© Newsletter Gewerbeaufsicht) Bettina Aschenbrenner, die mich nicht nur mit ihren unglaublich guten Weihnachtskeksen, selbst gemachten Kuchen, Marmeladen und Toffifeelikör verwöhnt hat, sondern auch für die aufbauenden Worte, Ratschläge und fachliche Expertise, die jede Hürde der Doktorarbeit nur noch halb so schlimm wirken haben lassen.

Ein ganz privater und persönlicher Dank gilt Regine Keitel für die unzähligen balkonversumpernden Gesprächsabende und für das Gefühl in München zuhause zu sein. Außerdem möchte ich Nika Pende ganz herzlichst danken, die mich gerade

kurz vor der Fertigstellung noch unterstützt hat und vermutlich einer der wenigen war die ohne Erklärung verstanden hat, wie es mir in den verschiedenen Stadien der Doktorarbeit ging.

Last but not least, möchte ich mich bei meiner Familie und Freunden für ihre Unterstützung und Verständnis der letzten 4 Jahre bedanken.