# Contents

# Foreword

The project ”DAP metabolites in Danish Children and Women” was conducted in 2014-2015 as part of the project “Pesticide Measurements in urine - establishment of analysis method and analysis of urine samples from Danish mothers and children collected as part of DEMOCHOPHES ” financed by the Danish Environmental Agency.

This report describes the concentrations of six dialkylphosphate metabolites (DAPs) used as biomarkers for organophosphate exposure. The DAPs have been measured in Danish school children and mothers participating in the European project DEMOCOPHES co-funded by the LIFE+ programme of EU (GD environment – LIFE09 ENV/BE/000410) and the Danish Environmental Agency, the Danish Veterinary and Food Administration and the Danish Health and Medicines Authority.

The project is a collaboration between Environmental Medicine, Institute of Public Health at University of Southern Denmark and section of Environmental Health at the Institute of Public Health, University of Copenhagen. The samples were analyses by the Flemish Institute for Technological Research NV (VITO) in Belgium in collaboration with professor Greet Schoeters.

We would like to thank the families participating in DEMOCOPHES and the collaborating schools taking part in the recruiting process.

# Abbreviations

C, creatinine
COPHES, Consortium to perform human biomonitoring on a European scale
DAP, dialkyl phosphate
DEAP, diethyl alkylphosphates
DEDTP, Diethyl dithiophosphate
DEMOCOPHES, Demonstration of a study to coordinate and perform human biomonitoring on a European scale
DEP, Diethyl phosphate
DETP, Diethyl thiophosphate
DMAP, dimethyl alkylphosphates
DMDTP, Dimethyl dithiophosphate
DMP, Dimethyl phosphate
DMTP, Dimethyl thiophosphate
GM, geometric mean
LOD, limit of detection
NAAP, paracetamol
NHANES, National Health and Nutrition Examination Survey (US)
OP, organophosphate
PFBBr, pentafluorobenzylchloride
POPs, Persistent organic pollutants
SES, socioeconomic status
2.4-DCP, 2,4-dichlorophenol
2.5-DCP, 2,5-dichlorophenol
2-PP, 2-phenylphenol.

# Summary and conclusion

**Background**
The use of organophosphate insecticides (OPs) in agriculture and as biocides in Denmark is very restricted due to their relatively high acute toxicity and neurotoxic effects. However, the population might still be exposed by ingestion of imported food items. Exposure to organophosphates can be estimated by measurements of dialkylphosphate (DAP) metabolites and in studies from the US urinary concentrations of DAPs have been associated with adverse neurobehavioural outcomes. Children with increased OP exposure may have higher risk of ADHD, and prenatal OP exposure was reported to be associated with structural brain anomalies and neurobehavioral deficits at school age. Urinary concentrations of DAP metabolites have only been measured in the Danish population in one study previously.

**Objectives**
The objectives of the present study was to investigate the urinary concentration of six unspecific DAP metabolites, which are commonly used as biomarkers for organophosphate exposure to pesticides such as dichlorvos, fenthion, dimethoat, malathion and chlorpyrifos and to compare the found concentrations with one previous Danish study and concentrations measured in other countries.

**Methods**
The urinary concentrations of Dimethyl phosphate (DMP), Dimethyl thiophosphate (DMTP), Dimethyl dithiophosphate (DMDTP), Diethyl phosphate (DEP), Diethyl thiophosphate (DETP), Diethyl dithiophosphate (DEDTP) were analyzed in 144 Danish school children and 145 mothers. The study persons were part of a large EU pilot project called DEMOCOPHES (Demonstration of a study to coordinate and perform human biomonitoring on a European scale), with focus on harmonization of human biomonitoring in Europe and which was ongoing in Europe from 2010 to 2012. In Denmark mother child pairs were recruited from an urban and a rural area and urine, hair and blood samples were collected from September to December 2011 The urine samples from the biobank of DEMOCOPHES in Denmark were used for the DAP analyses in the present study.

**Results**
At least one of the six DAP metabolites was detected in more than 90%, and four metabolites was detected in more than 30%, of both children and mothers. There was a tendency of higher DAP concentrations in children compared to their mothers. Furthermore, there was a tendency of higher concentrations in younger mothers and in children from families with higher socioeconomic status. The exposure source of the organophosphates in this study was difficult to determine as the study was not initially designed for this purpose. DAP concentrations were generally lower in participants from the rural compared to the urban area and the concentrations of the methylated DAPs were lower in children who often were eating homegrown fruit and vegetables, though only statistically significant for DMP. The levels of total DAP and DMAPs were lower in the investigated population than in Danish children investigated in 2007-08 indicating a decline in the total OP exposure level. However, the concentration of DEAPs was not similarly reduced. In general, the DAP levels were comparable with concentrations measured in other European countries in recent years, but the levels of total DAPs and DEAPs were higher than levels found in the US.

The concentration of the individual DAP metabolites were significantly correlated with each other in both mothers and children and in the mothers they were also significantly correlated with other chemicals associated with pesticide exposure (2.4-DCP, 2.5-DCP and 2-PP) measured in the same urine sample residues.

**Conclusion**

The findings of relatively high detection frequency of the DAP metabolites DEP, DETP, DMP and DMTP in a Danish group of children and their mothers, clearly indicate that there is still a widespread exposure to organophosphate pesticides in the Danish population, even though there have been major restrictions on their use in agriculture. The concentrations of the metabolites DEP and DETP found in the children and women of the present study are in line with what was found in Europe and previous measurements in Denmark. The methylated metabolites seem to have decreased in Denmark compared to previous measurements in 2007-08 and levels found in other European countries before 2007. However, the levels of total DAPs and DEAPs in the present study, as well as the levels found in other European countries, are higher than concentrations found in the biomonitoring program NHANES in the US, indicating higher exposure to some organophosphates in Europe. As the exposure to OPs has been associated with adverse health effects in some studies from the US population, there may also be a risk of adverse effects in Europe and Denmark, as we have found even higher levels. Although we do not know the specific organophosphates responsible for the relatively high concentrations of DEAPs in Denmark, chlorpyrifos is likely to be an important contributor since it is often found in samples of imported fruit and vegetables, which is considered the main exposure source for OPs in Denmark. Thus, further studies of potential adverse health effects related to organophosphate exposure in European populations are needed. Also identification of the main dietary exposure sources and related OPs is warranted in order to introduce adequate measures to reduce the exposure - especially for vulnerable population groups as children and pregnant women.

# Resume og konklusion

**Baggrund**
Brugen af organofosfat-insekticider i landbruget og som biocider i Danmark er meget restriktiv på grund af deres relativt høje akutte toksicitet og neurotoksiske effekter. Den danske befolkning kan dog stadig være eksponeret for organofosfater via indtagelse af importerede fødevarer. Man kan estimere befolkningens udsættelse for organofosfater ved at måle dialkylfosfat (DAP) metabolitter i urinen. I undersøgelser fra USA er der fundet sammenhæng mellem urinkoncentrationen af DAP og negativ påvirkning af nervesystemets udvikling. Børn med højere organofosfateksponering synes at have øget risiko for at udvikle ADHD, og prænatal organofosfateksponering er fundet associeret med strukturelle ændringer i hjernen og adfærdsmæssige vanskeligheder i skolealderen. Koncentrationen af DAP-metabolitter er kun blevet målt i ét studie tidligere i Danmark.

**Formål**
Formålet med dette studie var at måle urinkoncentrationen af seks uspecifikke DAP-metabolitter, som bliver brugt som biomarkører for eksponering for organofosfat-insekticider som dichlorvos, fenthion, dimethoat, malathion and chlorpyrifos. Formålet var desuden at sammenligne de målte koncentrationer med koncentrationer fra et tidligere studie lavet i Danmark, samt koncentrationer målt i andre lande.

**Metoder**
Urin koncentrationen af Dimethyl phosphate (DMP), Dimethyl thiophosphate (DMTP), Dimethyl dithiophosphate (DMDTP), Diethyl phosphate (DEP), Diethyl thiophosphate (DETP og Diethyl dithiophosphate (DEDTP) blev analyseret i 144 danske skolebørn og 145 mødre. Forsøgspersonerne var en del af et større EU pilotprojekt omhandlende harmonisering af human biomonitering i Europa kaldet DEMOCOPHES (Demonstration of a study to coordinate and perform human biomonitoring on a European scale). Projektet kørte i Europa fra 2010 til 2012. Mor-barn par blev rekrutteret i Danmark i to områder, som henholdsvis repræsenterede et urbant og et ruralt område. Urin, hår og blodprøver blev indsamlet mellem september og december 2011. Urinprøver fra den indsamlede biobank fra den danske del af DEMOCOPHES blev brugt til analyse af DAP metabolitterne i dette studie.

**Resultater**
Mindst en af de seks DAP metabolitter kunne måles i mere end 90% af forsøgspersonerne og fire af metabolitterne blev detekteret i mere end 30 % af både børn og mødre. Der var en tendens til højere DAP-koncentrationer i børnene sammenlignet med deres mødre. Desuden var der en tendens til højere koncentrationer i de yngste grupper af børn og mødre, samt i børn fra familier med højere socioøkonomisk status. Kilder til eksponeringen for organofosfater var svær at identificere ud fra dette studie, eftersom DEMOCOPHES projektet ikke initialt var designet til at undersøge udsættelsen for disse stoffer. DAP-koncentrationerne var generelt højere i deltagere fra byområdet sammenlignet med deltagere fra det mere landlige område og koncentrationen af de methylerede DAP'er (DMAPs) var højere i børn, der ikke spiste hjemmedyrkede fødevarer særlig ofte, dette var dog kun signifikant for DMP. Det samlede niveau af DAP-metabolitter og DMAP-metabolitter var lavere i dette studie sammenlignet med danske børn undersøgt i 2007-08, hvilket indikerer at der er sket et samlet fald i organofosfat-eksponeringen i Danmark. Koncentrationen af de ethylerede DAP'er (DEAPs) ser dog ikke ud til at være reduceret. Generelt var DAP koncentrationerne sammenlignelige med målinger fra andre Europæiske lande, men det samlede niveau af alle DAP-metabolitter og DEAP-metabolitter var højere end koncentrationer fundet i USA i

# 6. References


Andersen HR, Wohlfahrt-Veje C, Debes F, Nielsen F, Jensen TK, Grandjean P, et al. 2012. Langtidseffekter af prænatal pesticideksponering. (Bekæmpelsesmiddelforskning fra Miljøstyrelsen ).

Attfield KR, Hughes MD, Spengler JD, Lu C. 2014. Within- and between-child variation in repeated urinary pesticide metabolite measurements over a 1-year period. Environ Health Perspect 122:201-206.

Babina K, Dollard M, Pilotto L, Edwards JW. 2012. Environmental exposure to organophosphorus and pyrethroid pesticides in south australian preschool children: A cross sectional study. Environ Int 48:109-120.

Baranski M, Srednicka-Tober D, Volakakis N, Seal C, Sanderson R, Stewart GB, et al. 2014. Higher antioxidant and lower cadmium concentrations and lower incidence of pesticide residues in organically grown crops: A systematic literature review and meta-analyses. The British journal of nutrition:1-18.

Barr DB, Bravo R, Weerasekera G, Caltabiano LM, Whitehead RD, Jr., Olsson AO, et al. 2004. Concentrations of dialkyl phosphate metabolites of organophosphorus pesticides in the u.S. Population. Environ Health Perspect 112:186-200.

Becker K, Seiwert M, Casteleyn L, Joas R, Joas A, Biot P, et al. 2014. A systematic approach for designing a hbm pilot study for europe. International Journal of Hygiene and Environmental Health 217:312-322.

Berman T, Goldsmith R, Goen T, Spungen J, Novack L, Levine H, et al. 2013. Urinary concentrations of organophosphate pesticide metabolites in adults in israel: Demographic and dietary predictors. Environ Int 60:183-189.

Bomhard EM, Brendler-Schwaab SY, Freyberger A, Herbold BA, Leser KH, Richter M. 2002. O-phenylphenol and its sodium and potassium salts: A toxicological assessment. Critical reviews in toxicology 32:551-625.

Bouchard MF, Bellinger DC, Wright RO, Weisskopf MG. 2010. Attention-deficit/hyperactivity disorder and urinary metabolites of organophosphate pesticides. Pediatrics 125:e1270-1277.
Bouchard MF, Chevrier J, Harley KG, Kogut K, Vedar M, Calderon N, et al. 2011. Prenatal exposure to organophosphate pesticides and iq in 7-year-old children. Environ Health Perspect 119:1189-1195.

Bradman A, Kogut K, Eisen EA, Jewell NP, Quiros-Alcala L, Castorina R, et al. 2013. Variability of organophosphorous pesticide metabolite levels in spot and 24-hr urine samples collected from young children during 1 week. Environ Health Perspect 121:118-124.

Bradman A, Quiros-Alcala L, Castorina R, Aguilar Schall R, Camacho J, Holland NT, et al. 2015. Effect of organic diet intervention on pesticide exposures in young children living in low-income urban and agricultural communities. Environ Health Perspect.

Grandjean P, Nielsen GD, Jorgensen PJ, Horder M. 1992. Reference intervals for trace elements in blood: Significance of risk factors. Scandinavian journal of clinical and laboratory investigation 52:321-337.

Grandjean P, Landrigan PJ. 2014. Neurobehavioural effects of developmental toxicity. Lancet neurology 13:330-338.

Groth MV, Fagt S, Brondsted L. 2001. Social determinants of dietary habits in denmark. European journal of clinical nutrition 55:959-966.

Haines DA, Murray J. 2012. Human biomonitoring of environmental chemicals--early results of the 2007-2009 canadian health measures survey for males and females. Int J Hyg Environ Health 215:133-137.

Handal AJ, Hund L, Paez M, Bear S, Greenberg C, Fenske RA, et al. 2015. Characterization of pesticide exposure in a sample of pregnant women in ecuador. Archives of environmental contamination and toxicology.

Harari R, Julvez J, Murata K, Barr D, Bellinger DC, Debes F, et al. 2010. Neurobehavioral deficits and increased blood pressure in school-age children prenatally exposed to pesticides. Environ Health Perspect 118:890-896.

Heudorf U, Angerer J, Drexler H. 2004. Current internal exposure to pesticides in children and adolescents in germany: Urinary levels of metabolites of pyrethroid and organophosphorus insecticides. IntArchOccupEnviron Health 77:67-72.

Joas R, Casteleyn L, Biot P, Kolossa-Gehring M, Castano A, Angerer J, et al. 2012. Harmonised human biomonitoring in europe: Activities towards an eu hbm framework. International Journal of Hygiene and Environmental Health 215:172-175.

Levin ED, Timofeeva OA, Yang L, Petro A, Ryde IT, Wrench N, et al. 2010. Early postnatal parathion exposure in rats causes sex-selective cognitive impairment and neurotransmitter defects which emerge in aging. BehavBrain Res 208:319-327.

Lewis RC, Cantonwine DE, Anzalota Del Toro LV, Calafat AM, Valentin-Blasini L, Davis MD, et al. 2014. Urinary biomarkers of exposure to insecticides, herbicides, and one insect repellent among pregnant women in puerto rico. Environ Health 13:97.

Mage DT, Allen RH, Kodali A. 2008. Creatinine corrections for estimating children's and adult's pesticide intake doses in equilibrium with urinary pesticide and creatinine concentrations. Journal of exposure science & environmental epidemiology 18:360-368.

Marks AR, Harley K, Bradman A, Kogut K, Barr DB, Johnson C, et al. 2010. Organophosphate pesticide exposure and attention in young mexican-american children: The chamacos study. Environ Health Perspect 118:1768-1774.

McKelvey W, Jacobson JB, Kass D, Barr DB, Davis M, Calafat AM, et al. 2013. Population-based biomonitoring of exposure to organophosphate and pyrethroid pesticides in new york city. Environ Health Perspect.

Motojyuku M, Saito T, Akieda K, Otsuka H, Yamamoto I, Inokuchi S. 2008. Determination of glyphosate, glyphosate metabolites, and glufosinate in human serum by gas chromatography-mass spectrometry. JChromatogrB AnalytTechnolBiomedLife Sci 875:509-514.

Mullins RJ, Xu S, Pereira EF, Pescrille JD, Todd SW, Mamczarz J, et al. 2015. Prenatal exposure of guinea pigs to the organophosphorus pesticide chlorpyrifos disrupts the structural and functional integrity of the brain. Neurotoxicology 48:9-20.

Munoz-Quezada MT, Lucero BA, Barr DB, Steenland K, Levy K, Ryan PB, et al. 2013. Neurodevelopmental effects in children associated with exposure to organophosphate pesticides: A systematic review. Neurotoxicology 39C:158-168.

Mørck TA, Erdmann SE, Long M, Mathiesen L, Nielsen F, Siersma VD, et al. 2014. Pcb concentrations and dioxin-like activity in blood samples from danish school children and their mothers living in urban and rural areas. Basic & clinical pharmacology & toxicology 115:134-144.

Mørck TA, Nielsen F, Nielsen JK, Jensen JF, Hansen PW, Hansen AK, et al. 2015a. The danish contribution to the european democophes project: A description of cadmium, cotinine and mercury levels in danish mother-child pairs and the perspectives of supplementary sampling and measurements. Environmental research:96-105.

Mørck TA, Nielsen F, Nielsen JK, Siersma VD, Grandjean P, Knudsen LE. 2015b. Pfas concentrations in plasma samples from danish school children and their mothers. Chemosphere 129:203-209.

Oates L, Cohen M, Braun L, Schembri A, Taskova R. 2014. Reduction in urinary organophosphate pesticide metabolites in adults after a week-long organic diet. Environ Res 132:105-111.

Oulhote Y, Bouchard MF. 2013. Urinary metabolites of organophosphate and pyrethroid pesticides and behavioral problems in canadian children. Environ Health Perspect 121:1378-1384.

Petersen A, Jensen BH, Andersen JH, Poulsen ME, Christensen T, Nielsen E. 2013. Pesticide residues, results from the period 2004-2011.Danmarks Tekniske Universitet, Fødevareinstituttet.

Quiros-Alcala L, Alkon AD, Boyce WT, Lippert S, Davis NV, Bradman A, et al. 2011. Maternal prenatal and child organophosphate pesticide exposures and children's autonomic function. Neurotoxicology 32:646-655.

Raina-Fulton R. 2014. A review of methods for the analysis of orphan and difficult pesticides: Glyphosate, glufosinate, quaternary ammonium and phenoxy acid herbicides, and dithiocarbamate and phthalimide fungicides. J AOAC Int 97:965-977.

Rauh V, Arunajadai S, Horton M, Perera F, Hoepner L, Barr DB, et al. 2011. 7-year neurodevelopmental scores and prenatal exposure to chlorpyrifos, a common agricultural pesticide. EnvironHealth Perspect.

Rauh VA, Garfinkel R, Perera FP, Andrews HF, Hoepner L, Barr DB, et al. 2006. Impact of prenatal chlorpyrifos exposure on neurodevelopment in the first 3 years of life among inner-city children. Pediatrics 118:e1845-e1859.

Rauh VA, Perera FP, Horton MK, Whyatt RM, Bansal R, Hao X, et al. 2012. Brain anomalies in children exposed prenatally to a common organophosphate pesticide. Proceedings of the National Academy of Sciences of the United States of America 109:7871-7876.

Reiss R, Chang ET, Richardson RJ, Goodman M. 2015. A review of epidemiologic studies of low-level exposures to organophosphorus insecticides in non-occupational populations. Crit Rev Toxicol 45:531-641.

Roca M, Miralles-Marco A, Ferre J, Perez R, Yusa V. 2014. Biomonitoring exposure assessment to contemporary pesticides in a school children population of spain. Environ Res 131C:77-85.

Ross SM, McManus IC, Harrison V, Mason O. 2013. Neurobehavioral problems following low-level exposure to organophosphate pesticides: A systematic and meta-analytic review. Crit Rev Toxicol 43:21-44.

Schulz C, Wilhelm M, Heudorf U, Kolossa-Gehring M. 2012. Reprint of "update of the reference and hbm values derived by the german human biomonitoring commission". Int J Hyg Environ Health 215:150-158.

Slotkin TA, Bodwell BE, Levin ED, Seidler FJ. 2008. Neonatal exposure to low doses of diazinon: Long-term effects on neural cell development and acetylcholine systems. Environ Health Perspect 116:340-348.

Spaan S, Pronk A, Koch HM, Jusko TA, Jaddoe VW, Shaw PA, et al. 2015. Reliability of concentrations of organophosphate pesticide metabolites in serial urine specimens from pregnancy in the generation r study. Journal of Exposure Science and Environmental Epidemiology 25:286-294.

Tarbah FA, Kardel B, Pier S, Temme O, Daldrup T. 2004. Acute poisoning with phosphamidon: Determination of dimethyl phosphate (dmp) as a stable metabolite in a case of organophosphate insecticide intoxication. J Anal Toxicol 28:198-203.

Wei Y, Zhu J, Nguyen A. 2014. Urinary concentrations of dichlorophenol pesticides and obesity among adult participants in the u.S. National health and nutrition examination survey (nhanes) 2005-2008. Int J Hyg Environ Health 217:294-299.

Ye M, Beach J, Martin JW, Senthilselvan A. 2015. Associations between dietary factors and urinary concentrations of organophosphate and pyrethroid metabolites in a canadian general population. Int J Hyg Environ Health 218:616-626.

Yolton K, Xu Y, Sucharew H, Succop P, Altaye M, Popelar A, et al. 2013. Impact of low-level gestational exposure to organophosphate pesticides on neurobehavior in early infancy: A prospective study. Environ Health 12:79.

**Organophosphate metabolites in urine samples from Danish children and women**

This report describes the concentrations of six dialkylphosphate metabolites (DAPs) used as biomarkers for organophosphate exposure in Danish school children and mothers participating in the European project DEMOCOPHES.

The findings of relatively high detection frequency of DAP indicate that there is still a widespread exposure to organophosphate pesticides in the Danish population, even though there have been major restrictions on their use in agriculture. Chlorpyrifos is likely to be an important contributor to the DAP found since it is often found in samples of imported fruit and vegetables, which is considered the main exposure source for OPs in Denmark.

The concentrations of the DAPs found in the children and women of the present study are in line with what was found in Europe and previous measurements in Denmark. However, methylated metabolites seem to have decreased in Denmark compared to previous measurements in 2007-08 and levels found in other European countries before 2007.

Exposure to OPs has been associated with adverse health effects in some studies in USA, hence, there may also be a risk of adverse effects in Europe and Denmark, as we have found even higher levels of exposure.

Environmental
Protection Agency
Strandgade 29
DK-1401 København K

**www.mst.dk**