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Circulating phthalates during critical illness in children are associated with long-term attention deficit: a study of a development and a validation cohort

Verstraete S, M.D.* [1], Vanhorebeek I, Ph.D.* [1], Covaci A, Ph.D. [2], Güiza F, Ph.D. [1], Malarvannan G, Ph.D. [2], Jorens PG, M.D., Ph.D. [2,3], Van den Berghe G, M.D., Ph.D. [1]

[1]Clinical Division and Laboratory of Intensive Care Medicine, Department of Cellular and Molecular Medicine, KU Leuven, Leuven, Belgium; [2]Toxicology Center, University of Antwerp, Antwerp, Belgium; [3]Department of Critical Care Medicine, Antwerp University Hospital, University of Antwerp, Edegem, Belgium. * contributed equally

Address correspondence to: Greet Van den Berghe, Clinical Division and Laboratory of Intensive Care Medicine, KU Leuven, Herestraat 49, B-3000 Leuven, Belgium. Phone: 32-16-34-40-21; Fax: 32-16-34-40-15; E-mail: greet.vandenberghe@med.kuleuven.be.

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Abstract

Background Environmental phthalate exposure has been associated with attention deficit disorders in children. We hypothesized that in children treated in the pediatric intensive care unit (PICU), phthalates leaching from indwelling medical devices contribute to their long-term attention deficit.

Methods Plasma concentrations of di(2-ethylhexyl)phthalate (DEHP) metabolites were quantified in 100 healthy children and in 449 children who had been treated in PICU and were neurocognitively tested 4 years later. In a development patient cohort (N=228), a multivariable bootstrap study identified the stable thresholds of exposure to circulating DEHP metabolites above which there was an independent association with worse neurocognitive outcome. Subsequently, in a second patient cohort (N=221), the observed independent associations were validated.

Results Plasma concentrations of DEHP metabolites, that were virtually undetectable [0.029(0.027-0.031) μmol/l] in healthy children, were 3.93(3.53-4.32) μmol/l in critically ill children upon PICU admission (P<0.001). Plasma DEHP metabolite concentrations decreased rapidly but remained 17-times elevated until PICU discharge (P<0.001). After adjusting for baseline risk factors and duration of PICU stay, and further for PICU complications and treatments, exceeding the potentially harmful threshold for exposure to circulating DEHP metabolites was independently associated with the attention deficit (all P≤0.008) and impaired motor coordination (all P≤0.02). The association with the attention deficit was confirmed in the validation cohort (all P≤0.01). This phthalate exposure effect explained half of the attention deficit in post-PICU patients.

Conclusions Iatrogenic exposure to DEHP metabolites during intensive care was independently and robustly associated with the important attention deficit observed in children 4 years after critical illness.

Trial registration at ClinicalTrials.gov: NCT00214916

Introduction

Children who have been critically ill at a young age suffer from a substantial neurocognitive deficit when assessed years after admission to the pediatric intensive care unit (PICU).[1-6] This neurocognitive legacy of pediatric critical illness comprises, besides lower scores for intelligence, visual-motor integration, memory and behavior, also an important deficit in executive functions, motor coordination and attention.[1] The degree of this impairment is not negligible as for example the intelligence deficit is 15 IQ points and the attention deficit is about 40% larger than that reported for children suffering from attention deficit disorders.[1,7] Until recently, this neurocognitive legacy was considered to be the consequence of the illness and thus not modifiable. However, recent studies support the notion that it can be, at least partially, diminished by altering medical care. For example, changing the surgical technique for correction of congenital heart defects and preventing hyperglycemia during intensive care have shown to reduce the neurocognitive impairment.[1,8-10] Nevertheless, the largest part of the neurocognitive legacy of critical illness in children remains unexplained.[11]

Intensive medical care relies heavily on indwelling medical devices, miniaturized for use in small children and made more soft and pliable by incorporating chemical plastic softeners such as phthalates. Phthalates are not chemically bound to these devices and thus can gradually leach during use.[12] One of the most widespread phthalates, di(2-ethylhexyl)phthalate (DEHP), has been banned from children's toys but is currently still used to soften medical devices. When DEHP leaches from the plastic into the circulation, it is hydrolyzed to mono(2-ethylhexyl) phthalate (MEHP). Further metabolization of MEHP occurs mainly in the liver and includes 6-hydroxylation, with formation of 2-ethyl-5-carboxy-pentylphthalate (5cx-MEPP), and 5-hydroxylation with formation of 2-ethyl-5-hydroxy-hexylphthalate (5OH-MEHP) and subsequent conversion to 2-ethyl-5-oxo-hexylphthalate (5oxo-MEHP).[13] These metabolites are eliminated via the urine.[13] A few small observational studies have reported high urinary

levels of DEHP metabolites in premature neonates inferred to originate from indwelling medical devices.[14-16]

In recent years, concerns have been expressed about potential toxicity for fertility and development of phthalate exposure via the food chain and via long-term hemodialysis, long-term blood transfusions and repeated use of extracorporeal oxygenation in children.[17-20] Results from *in vitro* studies, studies in animal models and observational reports in humans suggest that chronic exposure to DEHP may have deleterious effects on neurocognitive development.[21-26] Specifically, a toxic effect on dopaminergic neurons was suggested to contribute to an increased incidence of attention deficit disorders in young children exposed to environmental phthalates.[27,28] However, it is unclear whether any phthalate leaching from routinely used medical devices during the short time that young children are being treated in PICUs plays a causal role in the neurocognitive legacy of pediatric critical illness.

We hypothesized that high circulating phthalate levels are present in critically ill children treated in the PICU, contributing to the attention deficit and possibly also other aspects of the neurocognitive legacy documented at 4 years follow-up. To test this hypothesis, concentrations of DEHP metabolites were quantified in plasma samples obtained during PICU stay from critically ill children who were tested for neurocognitive development 4 years later[1,29] and compared with healthy children. In a development cohort, stable potentially harmful threshold levels of exposure to circulating DEHP metabolites were first identified with a multivariable bootstrap approach. Subsequently, the observed independent associations between phthalate exposure during PICU stay and the neurocognitive test results were validated in a second cohort.

Methods

Study population: a development and a validation cohort

In this study, 449 critically ill infants and children (aged 0–16 years upon PICU admission) who had been enrolled in a randomized controlled trial on blood glucose control in PICU,[29] of whom plasma was available and who underwent neurocognitive testing at 4 years follow-up,[1] were included. For the full study protocols we refer to the original articles. For comparison with plasma concentrations of DEHP metabolites resulting from environmental exposure, 100 demographically matched healthy children were sampled immediately after IV catheterization for minor elective surgery. The study was approved by the institutional ethical review board (ML2586). Written informed consent was obtained from the parents or legal guardians and/or the child when 18 years or older at follow-up.

Given the inevitably observational study design, we *a priori* decided to chronologically divide the study population in two. This was done to first identify any independent associations between exposure to circulating DEHP metabolites during PICU stay and the neurocognitive test results 4 years later in a development cohort and to subsequently validate the findings in a second cohort (see statistical analyses).

Measurement of plasma concentrations of the phthalate metabolites, identification of phthalate-leaching devices and definition of "phthalate exposure" during PICU stay

Blood samples were taken upon PICU admission and daily at 6 a.m. until discharge, with plasma stored at -80°C. *Plasma concentrations* of the 4 DEHP metabolites (MEHP, 5OH-MEHP, 5cx-MEPP, and 5oxo-MEHP) were quantified with high-performance liquid-chromatography tandem mass-spectrometry (Agilent 6410 triple Quad LCMS; Agilent, Santa Clara, California),[30] as described in the Supplementary-Appendix (Methods_S1, Table_S1). Individual standards of phthalate metabolites and corresponding ^{13}C-labeled standards were obtained from Cambridge Isotope Laboratories, Inc.

(Andover, MA). Concentrations below the limit of quantification (2 ng/ml) were assigned a value of 1.9 ng/ml. Concentrations were measured in ng/ml and converted to µmol/l for statistical analysis. Quality assurance and control measures are given in the Supplementary-Appendix (Table_S1).

Leaching experiments excluded any contamination with DEHP from the containers used to collect and store the plasma. Leaching experiments were also performed to assess which of the indwelling devices used in these patients were actively leaching DEHP when placed in DEHP-free saline for 18h at room temperature.[31] The number and type of indwelling medical devices used in every child during 24h preceding sampling was noted.

We assumed that plasma concentrations of DEHP metabolites would be highest upon PICU admission and lowest on the last PICU day. This assumption was based on the fact that many patients were admitted after exposure to large bore extracorporeal circuits for surgery,[32] and on the number of indwelling medical devices typically present during PICU stay, taking into account the half-life of the metabolites (between 5-15h).[13] Plasma concentrations of the DEHP metabolites were measured on these 2 time points for all patients. To confirm the inferred time course, plasma concentrations were also measured on day 1, 2, and 3 in a subset of 25 children from the development cohort. The available evidence suggests that both the concentration of circulating DEHP metabolites to which the brain is exposed and the duration of this exposure determine any eventual long-term neurocognitive harm[21,26] within a concept of "threshold" toxicity.[17] Therefore, we *a priori* decided to consider the product of the last day plasma concentration and the duration of PICU stay as that exposure to circulating phthalates during PICU stay that was minimally present for each patient, further referred to as the phthalate *"exposure".* This was done for the sum of all DEHP metabolites, for each of the individual DEHP metabolites and for the sum of the 3 MEHP metabolites.[13] The latter was considered necessary to exclude bias originating from any artifactual *ex vivo* hydrolysis of DEHP.[13]

Neurocognitive testing

Internationally recognized and validated tests with adequate normative data were used to quantify performance for a broad range of neurocognitive functions.[1] The neuropsychological test battery comprised age range-specific tests measuring attention, motor coordination, inhibitory control and cognitive flexibility (Amsterdam Neuropsychological Tasks), general intellectual functioning (Wechsler Intelligence Quotient scales), visual-motor integration (Beery-Buktenica Developmental Test), verbal and visual-spatial learning and memory (Children's Memory Scale), and behavior (Child Behavior Checklist) (Supplementary-Appendix Methods_S2).[1] These tests have been extensively used to quantify neurocognitive development in various pediatric populations.[33,34]

Statistical Analyses

Data are presented as means and 95% confidence intervals, medians and interquartile ranges or as numbers and proportions. *Differences between plasma concentrations of DEHP metabolites* in critically ill and healthy children, and among patients with increasing numbers of indwelling medical devices, were compared with Wilcoxon signed-rank test. The latter analysis was subsequently corrected for markers of liver and kidney function.

The primary study aim was to assess, in a multivariable regression analysis adjusting for other risk factors, the presence of an independent association between "exposure" to the total circulating DEHP metabolites during PICU stay and the attention deficit 4 years later, in both the development *and* the validation patient cohort. Secondary study aims were similar analyses for the other neurocognitive outcomes, and for the individual DEHP metabolites and the sum of MEHP metabolites.

First, in the development cohort, each "exposure"/concentration value was evaluated independently as a *potential harmful threshold for neurocognitive development*. This was done with

multivariable regression analyses, adjusted for the following risk factors (Supplementary-Appendix Methods_S3): age at PICU admission, gender, race, geographic and linguistic origin, history of malignancy or diabetes, prior to PICU admission presence of a syndrome or illness *a priori* defined as affecting neurocognitive development, socioeconomic status of the parents, type and severity of the critical illness, blood glucose management and the duration of PICU stay. The stability of the identified thresholds was evaluated via 400 bootstrap resamplings per neurocognitive outcome, as further described in the Supplementary-Appendix (Methods_S3, Figures_S1-S2). Each child received a dichotomized label according to whether or not these potentially harmful thresholds were exceeded. *Second*, the *significance and effect sizes of exceeding the identified potentially harmful thresholds* of phthalate "exposure" in the multivariable regression models, adjusted for other risk factors as described above, were determined for the development cohort and repeated for the validation cohort. *Third, as a sensitivity analysis,* the multivariable models were further adjusted for complications occurring during PICU stay, including new infections, duration of antibiotic and corticosteroid use, organ failure as reflected by peak levels of plasma creatinine and bilirubin, the duration of hemodynamic/ventilatory support, treatment with hypnotics and cumulative doses of benzodiazepines and opioids.[35] In addition, it was also assessed whether any of the documented independent associations with the attention deficit observed for "exposure" to the circulating DEHP metabolites in the validation cohort were also present for the raw plasma concentrations.

Statistical analyses were performed with use of Matlab 2014b® (The MathWorks, Natick, MA) and JMP® version 11.0.0 (SAS Institute, Inc, Cary, NC). Two-sided P-values of 0.05 or less were considered to indicate statistical significance. No corrections for multiple comparisons were done.

Results

Demographic and medical characteristics and neurocognitive test results of patients in the development and validation cohorts and of healthy children are presented in Table_1 and Supplementary-Appendix Table_S2.

In healthy children, total DEHP metabolites in plasma were virtually undetectable [0.029 (0.027-0.031) μmol/l], with just detectable levels of some individual DEHP metabolites in only 15 children. In the development and validation patient cohorts, mean plasma concentrations of DEHP metabolites were 3.93 (3.53-4.32) μmol/l upon PICU admission, and thus 136-times higher than in healthy children ($P<0.001$) (Figure_1A,B), decreasing subsequently with time (Figure_1A). The lowest plasma concentrations of the total DEHP metabolites were found on the last PICU day (Figure_1A,B), which were still 17-times higher than in healthy children ($P<0.001$).

During the 24h preceding PICU discharge, children were instrumented with 1-12 indwelling medical devices, of which up to 5 were intravascular. All these indwelling devices and/or the essential accessories for using them (Supplementary-Appendix_Table_S3) were shown to leach DEHP *ex vivo*. Plasma concentrations of DEHP metabolites were always elevated irrespective of the number of indwelling medical devices and significantly increased by reduced kidney function (Supplementary-Appendix_Figure_S3A).

The multivariable bootstrap analysis of the development cohort identified all stable threshold levels of exposure to circulating DEHP metabolites as potentially harmful for neurocognitive development (Supplementary-Appendix_Table_S4, Figure_2). These potentially harmful thresholds were between the 25th percentile and the median of the distribution (Table_2).

The multivariable linear regression analyses of the development cohort, adjusted for baseline risk factors and duration of PICU stay, revealed that exceeding the potentially harmful threshold for

“exposure” to circulating DEHP metabolites was independently and highly significantly associated only with the attention deficit and with the impaired motor coordination (Table_2, Supplementary-Appendix_Table_S5). The independent association with the attention deficit, and part of the independent association with impaired motor coordination, were reproduced in the validation cohort (Table_3, Supplementary-Appendix Table_S5). Similar findings were obtained for the “exposure” to the sum of the 3 circulating MEHP metabolites, to MEHP and most robustly, to 5oxo-MEHP (Table_2-3, Supplementary-Appendix_Table_S5). These independent associations remained highly significant after a further adjustment for the occurrence of new infections, organ failure, the use of vital treatments during PICU stay and for treatment with hypnotics, benzodiazepines and opioids (Table_2-3, Supplementary-Appendix Table_S5). The calculated estimates, reflecting the size of the effects on attention of exceeding the potentially harmful threshold, were the equivalent of about 50% of the differences between post-PICU patients and healthy children (Supplementary-Appendix_Table_S6).

The sensitivity analyses performed on the raw data of the plasma concentrations revealed that the briefly, but substantially, elevated plasma concentrations of DEHP metabolites on the PICU admission day were not significantly associated with the attention deficit or any of the other neurocognitive test results (data not shown). However, on the last PICU day, the sum of the plasma concentrations of the 3 MEHP metabolites was also significantly associated only with the attention deficit (Supplementary-Appendix_Table_S7-S9). This potentially harmful threshold could be reached even with minimal instrumentation of the patients (Supplementary-Appendix_Figure_S3B).

Discussion

Critically ill children revealed very high circulating levels of DEHP metabolites throughout PICU stay, whereas these were virtually undetectable in healthy children. All indwelling medical devices and/or essential accessories showed to actively leach DEHP. Exceeding the potentially harmful threshold of exposure to circulating DEHP metabolites, identified by a multivariable bootstrap study of the development cohort, was found to be independently associated with the attention deficit and to a lesser extent with the motor coordination deficit documented 4 years after PICU admission. These independent associations were largely confirmed in the validation cohort, even after further adjustment for PICU morbidities and treatments. This phthalate exposure effect explained about half of the attention deficit in post-PICU patients . By comparison with the attention deficit that has been reported for children with attention deficit disorders, the effect size of phthalate exposure during pediatric critical illness was large.[7]

As hypothesized, a large part of the attention deficit in post-PICU patients could be explained by exceeding a threshold of exposure to circulating DEHP metabolites, composed of the minimal plasma concentrations and the time in PICU that these concentrations were present, and not by the extremely high plasma concentrations that were only briefly present upon PICU admission. This further supports the notion that phthalates leaching from a single run of extracorporeal support in children may not be a concern. However, these data are in line with experimental studies suggesting that both level and duration of exposure to phthalates determine their toxicity.[17,21,27] The potentially harmful threshold could be reached even with minimal indwelling instrumentation of the children and thus, with the currently used material, appears unavoidable. Although the daily amount of phthalates that leached from these routinely used medical devices may well have been below the previously identified minimal dose that may evoke toxicity to the gonads[19,20], alterations in kidney and liver function may have augmented

circulating levels and increased the risk of exceeding a threshold that is potentially harmful to early life brain development.

It was striking that the phthalate exposure specifically affected attention and to a lesser extent motor coordination, but not the other aspects of the neurocognitive legacy. This corroborates epidemiological studies[23,36,37] suggesting a rather selective toxic effect of DEHP exposure on the biological substrate of the attention function. Attention is determined by neuronal networks involving connections between cerebellum, thalamus and frontoparietal cortex.[38] The exact brain areas vulnerable to the neurodevelopmental toxicity of phthalates related to the attention deficit remain unclear. Degeneration of midbrain dopaminergic nuclei and an effect on dopamine transporters may play a role[39] as aberrations in the dopaminergic system are involved in the pathophysiology of attention deficit disorders.[40]

As compared with previous epidemiological studies, this study has some strengths. First, the timing and duration of the iatrogenic exposure to circulating phthalates related to the indwelling devices was accurately defined. Second, more than one measurement was done per child, reducing the risk of inaccurate classification of exposure as may occur with single spot urine samples.[18] Third, the current study used a development and a validation cohort, the latter to reduce the risk of observations by chance. The strikingly similar results in these two cohorts specifically for the association between phthalate exposure and the attention deficit strongly supports that these associations are reproducible and biologically meaningful. Fourth, highly standardized tests were used to quantify attentional performance and other neurocognitive developmental functions whereas other studies drew conclusions based only on responses to questionnaires provided by teachers, parents or guardians.[37]

This study also has limitations, most importantly that it was not a randomized controlled intervention trial and hence cannot provide proof of causality. However, unlike in animal models, deliberate random exposure of humans to potentially toxic phthalates is not possible for obvious ethical reasons. There is some evidence for causality generated via studies in animal models.[28] The

reproducibility of the current results in two patient cohorts, adjusted for dominant premorbid predestinators of neurocognition as well as for the severity of the critical illness and its treatments, underscores the robustness of the findings. One can only speculate about underlying molecular mechanisms in which epigenetic alterations may play a role.[18]

In conclusion, a robust and reproducible independent association was found between the currently inevitable, iatrogenic exposure to circulating phthalates during treatment in intensive care and the attention deficit observed 4 years after pediatric critical illness.

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Author contributions

GVdB, SV, IV and PGJ designed the study. SV, GM and AC gathered the data. SV, IV, FG and GVdB analyzed and vouch for the data. SV, IV and GVdB wrote the first draft, reviewed and approved by all authors and all authors jointly decided to publish. No confidentiality agreements existed between sponsors and authors/institutions.

References

1. Mesotten D, Gielen M, Sterken C, et al. Neurocognitive development of children 4 years after critical illness and treatment with tight glucose control: a randomized controlled trial. JAMA 2012;308:1641-50.

2. Hovels-Gurich HH, Seghaye MC, Sigler M, et al. Neurodevelopmental outcome related to cerebral risk factors in children after neonatal arterial switch operation. Ann Thorac Surg 2001;71:881-8.

3. Hovels-Gurich HH, Konrad K, Skorzenski D, Herpertz-Dahlmann B, Messmer BJ, Seghaye MC. Attentional dysfunction in children after corrective cardiac surgery in infancy. Ann Thorac Surg 2007;83:1425-30.

4. Miatton M, De Wolf D, Francois K, Thiery E, Vingerhoets G. Neuropsychological performance in school-aged children with surgically corrected congenital heart disease. J Pediatr 2007;151:73-8, 8 e1.

5. Sarrechia I, De Wolf D, Miatton M, et al. Neurodevelopment and behavior after transcatheter versus surgical closure of secundum type atrial septal defect. J Pediatr 2015;166:31-8.

6. Wray J, Pot-Mees C, Zeitlin H, Radley-Smith R, Yacoub M. Cognitive function and behavioural status in paediatric heart and heart-lung transplant recipients: the Harefield experience. BMJ 1994;309:837-41.

7. Kalff AC, De Sonneville LM, Hurks PP, et al. Speed, speed variability, and accuracy of information processing in 5 to 6-year-old children at risk of ADHD. J Int Neuropsychol Soc 2005;11:173-83.

8. Bellinger DC, Jonas RA, Rappaport LA, et al. Developmental and neurologic status of children after heart surgery with hypothermic circulatory arrest or low-flow cardiopulmonary bypass. N Engl J Med 1995;332:549-55.

9. Bellinger DC, Wypij D, Kuban KC, et al. Developmental and neurological status of children at 4 years of age after heart surgery with hypothermic circulatory arrest or low-flow cardiopulmonary bypass. Circulation 1999;100:526-32.

10. Bellinger DC, Wypij D, Rivkin MJ, et al. Adolescents with d-transposition of the great arteries corrected with the arterial switch procedure: neuropsychological assessment and structural brain imaging. Circulation 2011;124:1361-9.

11. Tasker RC. Pediatric critical care, glycemic control, and hypoglycemia: what is the real target? JAMA 2012;308:1687-8.

12. Kastner J, Cooper DG, Maric M, Dodd P, Yargeau V. Aqueous leaching of di-2-ethylhexyl phthalate and "green" plasticizers from poly(vinyl chloride). Sci Total Environ 2012;432:357-64.

13. Koch HM, Preuss R, Angerer J. Di(2-ethylhexyl)phthalate (DEHP): human metabolism and internal exposure-- an update and latest results. Int J Androl 2006;29:155-65.

14. Green R, Hauser R, Calafat AM, et al. Use of di(2-ethylhexyl) phthalate-containing medical products and urinary levels of mono(2-ethylhexyl) phthalate in neonatal intensive care unit infants. Environ Health Perspect 2005;113:1222-5.

15. Weuve J, Sanchez BN, Calafat AM, et al. Exposure to phthalates in neonatal intensive care unit infants: urinary concentrations of monoesters and oxidative metabolites. Environ Health Perspect 2006;114:1424-31.

16. Frederiksen H, Kuiri-Hanninen T, Main KM, Dunkel L, Sankilampi U. A longitudinal study of urinary phthalate excretion in 58 full-term and 67 preterm infants from birth through 14 months. Environ Health Perspect 2014;122:998-1005.

17. Gore AC. Editorial: an international riposte to naysayers of endocrine-disrupting chemicals. Endocrinology 2013;154:3955-6.

18. Schug TT, Blawas AM, Gray K, Heindel JJ, Lawler CP. Elucidating the links between endocrine disruptors and neurodevelopment. Endocrinology 2015:en20141734.

19. Safety assessment of di(2-ethylhexyl)phthalate (DEHP) released from PVC medical devices. Center for Devices and Radiological Health. US Food and Drug Administration. 2001.

20. European Union Risk Assessment Report Bis(2-Ethylhexyl) Phthalate (DEHP), CAS-No. 117-81-7 Vol. 80; EUR 23384EN; Office for Official Publications of the European Communities: Luxembourg, 2008.

21. Chen T, Yang W, Li Y, Chen X, Xu S. Mono-(2-ethylhexyl) phthalate impairs neurodevelopment: inhibition of proliferation and promotion of differentiation in PC12 cells. Toxicol Lett 2011;201:34-41.

22. Tanaka T. Reproductive and neurobehavioural toxicity study of bis(2-ethylhexyl) phthalate (DEHP) administered to mice in the diet. Food Chem Toxicol 2002;40:1499-506.

23. Engel SM, Miodovnik A, Canfield RL, et al. Prenatal phthalate exposure is associated with childhood behavior and executive functioning. Environ Health Perspect 2010;118:565-71.

24. Whyatt RM, Liu X, Rauh VA, et al. Maternal prenatal urinary phthalate metabolite concentrations and child mental, psychomotor, and behavioral development at 3 years of age. Environ Health Perspect 2012;120:290-5.

25. Polanska K, Ligocka D, Sobala W, Hanke W. Phthalate exposure and child development: the Polish Mother and Child Cohort Study. Early Hum Dev 2014;90:477-85.

26. Cho SC, Bhang SY, Hong YC, et al. Relationship between environmental phthalate exposure and the intelligence of school-age children. Environ Health Perspect 2010;118:1027-32.

27. Park S, Lee JM, Kim JW, et al. Association between phthalates and externalizing behaviors and cortical thickness in children with attention deficit hyperactivity disorder. Psychol Med 2014:1-12.

28. Masuo Y, Ishido M, Morita M, Oka S. Effects of neonatal treatment with 6-hydroxydopamine and endocrine disruptors on motor activity and gene expression in rats. Neural Plast 2004;11:59-76.

29. Vlasselaers D, Milants I, Desmet L, et al. Intensive insulin therapy for patients in paediatric intensive care: a prospective, randomised controlled study. Lancet 2009;373:547-56.

30. Dirtu AC, Geens T, Dirinck E, et al. Phthalate metabolites in obese individuals undergoing weight loss: Urinary levels and estimation of the phthalates daily intake. Environ Int 2013;59:344-53.

31. Hanawa T, Muramatsu E, Asakawa K, et al. Investigation of the release behavior of diethylhexyl phthalate from the polyvinyl-chloride tubing for intravenous administration. Int J Pharm 2000;210:109-15.

32. Huygh J, Clotman K, Malarvannan G, et al. Considerable exposure to the endocrine disrupting chemicals phthalates and bisphenol-A in intensive care unit (ICU) patients. Environ Int 2015;In press.

33. Anderson V, Catroppa C, Morse S, Haritou F, Rosenfeld JV. Intellectual outcome from preschool traumatic brain injury: a 5-year prospective, longitudinal study. Pediatrics 2009;124:e1064-71.

34. Amant F, Van Calsteren K, Halaska MJ, et al. Long-term cognitive and cardiac outcomes after prenatal exposure to chemotherapy in children aged 18 months or older: an observational study. Lancet Oncol 2012;13:256-64.

35. Rappaport BA, Suresh S, Hertz S, Evers AS, Orser BA. Anesthetic neurotoxicity--clinical implications of animal models. N Engl J Med 2015;372:796-7.

36. Kim BN, Cho SC, Kim Y, et al. Phthalates exposure and attention-deficit/hyperactivity disorder in school-age children. Biol Psychiatry 2009;66:958-63.

37. Chopra V, Harley K, Lahiff M, Eskenazi B. Association between phthalates and attention deficit disorder and learning disability in U.S. children, 6-15 years. Environ Res 2014;128:64-9.

38. Fan J, McCandliss BD, Fossella J, Flombaum JI, Posner MI. The activation of attentional networks. Neuroimage 2005;26:471-9.

39. Tanida T, Warita K, Ishihara K, et al. Fetal and neonatal exposure to three typical environmental chemicals with different mechanisms of action: mixed exposure to phenol, phthalate, and dioxin cancels the effects of sole exposure on mouse midbrain dopaminergic nuclei. Toxicol Lett 2009;189:40-7.

40. Krause J. SPECT and PET of the dopamine transporter in attention-deficit/hyperactivity disorder. Expert Rev Neurother 2008;8:611-25.

Tables

Table 1. Demographics and patient PICU outcomes

	Development cohort N=228	Validation cohort N=221	Healthy children N=100
Baseline characteristics			
Male gender, no. (%)	123 (54.0)	133 (60.2)	58 (58.0)
Age at sampling (years), mean (95% CI)	3.4 (2.9-4.0)	3.3 (2.7-3.8)	4.2 (3.5-4.9)
Height at sampling (%), mean (95% CI) *	45.6 (41.6-49.6)	44.4 (40.4-48.4)	47.5 (40.4-54.7)
Weight at sampling (%), mean (95% CI) *	44.4 (40.4-48.5)	41.6 (37.7-45.5)	52.7 (46.6-58.8)
Caucasian race, no. (%)	219 (96.1)	200 (90.5)	93 (93.0)
Exclusively European, no. (%)	210 (92.1)	192 (86.9)	
Exclusively Dutch language, no. (%)	185 (81.1)	175 (79.2)	
Socioeconomic status, mean (95% CI)	35 (33-37)	35 (33-37)	
Pre-existing syndrome, no. (%)	58 (25.4)	66 (29.9)	
History of malignancy, no. (%)	5 (2.2)	8 (3.6)	
History of diabetes, no. (%)	2 (0.9)	0 (0.0)	
Randomization to strict glycemic control, no. (%)	105 (46.1)	112 (50.7)	
Diagnostic category, no. (%)			
Cardiac surgery for congenital heart defects	178 (78.1)	183 (82.8)	
Complicated/high risk surgery or trauma	25 (11.0)	16 (7.2)	
Neurological medical disorders	5 (2.2)	3 (1.4)	
Infectious medical diseases	9 (4.0)	4 (1.8)	
Other medical disorders	8 (3.5)	10 (4.5)	
Solid organ transplants	3 (1.3)	5 (2.3)	
PeLOD score first 24 hrs, mean (95% CI)	9 (8-10)	10 (9-11)	
PICU Outcomes			
ICU stay (days), mean (95% CI)	5.1 (4.3-5.8)	4.9 (4.0-5.8)	
Duration mechanical ventilation (days), mean (95% CI)	3.6 (3.0-4.2)	3.6 (2.9-4.4)	
Peak creatinine (mg/dl), mean (95% CI)	0.65 (0.62-0.68)	0.66 (0.59-0.73)	
Peak bilirubine (mg/dl), mean (95% CI)	1.42 (1.16-1.67)	1.43 (1.06-1.80)	
Hemodynamic support (days), mean (95% CI)	3.1 (2.6-3.6)	3.0 (2.4-3.6)	
Corticosteroid treatment (days), mean (95% CI)	0.3 (0.1-0.4)	0.8 (0.3-1.4)	
Infection, no. (%)	68 (29.8)	62 (28.1)	
Antibiotics (days), mean (95% CI)	3.3 (2.8-3.8)	3.6 (2.9-4.3)	

* Height and weight, expressed as percentiles of population norms, were calculated with the anthropometric calculators for normal children, based on the World Health Organization Growth Charts for Canada (version 2015/02/24), and for children with syndromes known to affect height and weight (version 2014/09/25) (http://www.bcchildrens.ca/Services/SpecializedPediatrics/EndocrinologyDiabetesUnit/ForProfessionals/AnthropometricCalculators.htm). Patients and healthy children were not matched for height and weight, as a growth delay is part of the long-term legacy of critical illness in children. CI: confidence interval; PeLOD score: pediatric logistic organ dysfunction score.

Table 2. Multivariable linear regression analyses determining significant and independent associations between exposure to a potentially harmful threshold of circulating phthalate metabolites and neurocognitive functions in the development cohort (n=228)

			Model adjusted for baseline characteristics and duration of PICU stay			Model further adjusted for in-PICU complications and treatments		
Phthalate exposure†	**Neurocognitive functions affected, as determined by a multivariable bootstrap approach‡**	**Potentially harmful threshold (µmol/l ×days)£@**	**R^2 of the model**	**Estimate [95% CI]**	**P value**	**R^2 of the model**	**Estimate [95% CI]**	**P value**
Total DEHP*	**Attention and motor coordination**							
0.78	*Alertness (msec)§*							
(0.34-2.66)	Reaction time dominant hand	>0.551	0.57	78.26 [37.75;118.76]	<0.001	0.59	80.05 [35.68;124.42]	<0.001
(µmol/l×days)	Reaction time nondominant hand	>0.551	0.55	64.94 [27.61;102.27]	<0.001	0.59	70.93 [31.04;110.82]	<0.001
¥	Reaction time overall	>0.551	0.60	71.44 [35.39;107.49]	<0.001	0.62	75.15 [36.21;114.09]	<0.001
	Inconsistency of alertness (msec)§§							
	SD reaction time dominant hand	>0.551	0.51	55.81 [21.17;90.46]	<0.001	0.51	51.78 [13.49;90.07]	0.008
	SD reaction time nondominant hand	>0.551	0.43	43.16 [9.84;76.47]	0.01	0.47	47.94 [12.25;83.64]	0.008
	SD reaction time overall	>0.551	0.55	53.91 [24.03;83.79]	<0.001	0.56	53.69 [21.06;86.32]	0.001
	Motor coordination (No. of taps in 10s)§§§							
	No. of unimanual taps							
	Dominant hand	>0.551	0.76	-1.10 [-2.20;-0.01]	0.04	0.76	-1.34 [-2.54;-0.14]	0.02
	Nondominant hand	>0.551	0.76	-1.31 [-2.32;-0.29]	0.01	0.77	-1.23 [-2.32;-0.15]	0.02
	No. of valid synchronous taps	>0.551	0.66	-2.19 [-3.40;-0.97]	<0.001	0.66	-2.35 [-3.67;-1.03]	<0.001
MEHP Metabolites**	**Attention and motor coordination**							
	Alertness (msec)§							
0.47	Reaction time dominant hand	>0.365	0.58	84.05 [41.57;126.52]	<0.001	0.59	84.35 [37.70;131.01]	<0.001
(0.22-1.80)	Reaction time nondominant hand	>0.365	0.55	67.94 [28.72;107.17]	<0.001	0.58	70.91 [28.84;112.98]	0.001
(µmol/l×days)	Reaction time overall	>0.365	0.60	76.07 [38.25;113.89]	<0.001	0.62	77.57 [36.56;118.58]	<0.001
¥	*Inconsistency of alertness (msec)§§*							
	SD reaction time dominant hand	>0.365	0.51	61.87 [25.59;98.14]	<0.001	0.52	59.73 [19.62;99.83]	0.003
	SD reaction time nondominant hand	>0.365	0.44	50.73 [15.88;85.57]	0.004	0.47	50.48 [12.96;88.01]	0.008
	SD reaction time overall	>0.365	0.56	61.78 [30.60;92.96]	<0.001	0.57	59.89 [25.70;94.08]	<0.001
	Motor coordination (No. of taps in 10s)§§§							
	No. of valid synchronous taps	>0.365	0.65	-2.05 [-3.33;-0.77]	0.001	0.66	-2.03 [-3.43;-0.63]	0.004
MEHP	**Attention and motor coordination**							
0.23	*Alertness (msec)§*							
(0.10-0.68)	Reaction time dominant hand	>0.157	0.56	58.79 [18.29;99.28]	0.004	0.57	58.25 [14.88;101.62]	0.008
(µmol/l×days)	Reaction time nondominant hand	>0.157	0.55	56.24 [19.25;93.24]	0.003	0.58	57.99 [19.20;96.79]	0.003
¥	Reaction time overall	>0.157	0.59	57.19 [21.24;93.15]	0.002	0.61	57.68 [19.64;95.73]	0.003
	Motor coordination (No. of taps in 10s)§§§							
	No. of valid synchronous taps	>0.157	0.66	-2.53 [-3.72;-1.34]	<0.001	0.67	-2.70 [-3.96;-1.44]	<0.001

5cx-MEPP	**Memory**								
0.39	*Verbal-auditory*								
(0.17-1.24)		Working memory, repeating							
(µmol/l×days)		backward (up to 19 numbers)§§§§	>0.379	0.34	-0.95 [-1.67;-0.22]	0.01	0.41	-0.80 [-1.65;0.06]	0.06
¥									
5oxo-MEHP	**Attention and motor coordination**								
0.03	*Alertness (msec)§*								
(0.01-0.12)		Reaction time dominant hand	>0.031	0.57	82.72 [34.77;130.66]	<0.001	0.58	89.04 [36.96;141.12]	0.001
(µmol/l×days)		Reaction time nondominant hand	>0.031	0.57	92.93 [50.07;135.79]	<0.001	0.60	106.16 [61.02;151.31]	<0.001
¥		Reaction time overall	>0.031	0.60	87.12 [44.88;129.37]	<0.001	0.63	97.19 [52.05;142.32]	<0.001
	Inconsistency of alertness (msec)§§								
		SD reaction time dominant hand	>0.031	0.51	64.82 [24.07;105.56]	0.002	0.51	63.61 [18.91;108.32]	0.005
		SD reaction time nondominant hand	>0.031	0.44	56.30 [17.62;94.97]	0.004	0.48	68.53 [27.60;109.45]	0.001
		SD reaction time overall	>0.031	0.55	62.34 [27.19;97.50]	<0.001	0.57	67.51 [29.51;105.51]	<0.001
	Motor coordination (No. of taps in 10s)§§§								
		No. of unimanual taps							
		Dominant hand	>0.031	0.76	-1.89 [-3.17;-0.61]	0.004	0.76	-2.10 [-3.49;-0.71]	0.003
		No. of valid synchronous taps	>0.024	0.64	-1.76 [-3.12;-0.41]	0.01	0.65	-1.91 [-3.40;-0.42]	0.01

† "Exposure" was defined as the product of the last day concentrations in µmol/l and the number of days exposed in PICU.

‡ Only the neurocognitive functions that were significantly associated with the phthalate exposures as identified by bootstrapping are listed.

£: identified via bootstrap resampling.

@ threshold values are rounded to three decimals.

* Exposure to the sum of MEHP and its metabolites 5cx-MEPP, 5oxo-MEHP and 5OH-MEHP.

** Exposure to the sum of 5cx-MEPP, 5oxo-MEHP and 5OH-MEHP.

¥ Distribution of metabolite exposures within the patient cohort (µmol/l ×days). Data are median (interquartile range).

§ For reaction times, higher scores reflect worse performance. Mean scores for healthy children are 558 (95% CI 521-595) for the dominant hand reaction time, 562 (95% CI 524-600) for the nondominant hand reaction time and 560 (95% CI 524-600) for the overall reaction time.[1]

§§ For inconsistency of alertness, higher scores reflect worse performance. Mean scores for healthy children are 259 (95% CI 229-290) for the dominant hand, 262 (95% CI 232-292) for the nondominant hand and 275 (95% CI 246-304) for the overall SD, standard deviation.[1]

§§§ For motor coordination, higher scores reflect better performance. Mean scores for healthy children are 36 (95% CI 34-38) for dominant hand unimanual taps, 32 (95% CI 30-33) for nondominant hand unimanual taps and 22 (95% CI 20-24) for valid synchronous taps.[1]

§§§§ For numbers within verbal-auditory memory, higher scores reflect better performance. Mean scores for healthy children are 11 (95% CI 10-11) for working memory (repeating numbers backward).[1]

Table 3. Multivariable linear regression analyses determining significant and independent associations between exposure to a potentially harmful threshold of circulating phthalate metabolites and neurocognitive functions in the validation cohort (n=221)

			Model adjusted for baseline characteristics and duration of PICU stay			Model further adjusted for in-PICU complications and treatments		
Phthalate exposure†	**Neurocognitive functions from the development cohort, significantly affected in validation cohort‡**	**Best threshold (µmol/l ×days)@ of development cohort**	**R^2 of the model**	**Estimate [95% CI]**	**P value**	**R^2 of the model**	**Estimate [95% CI]**	**P value**
Total DEHP*	**Attention and motor coordination**							
0.72	*Alertness (msec)§*							
(0.34-2.19)	Reaction time dominant hand	>0.551	0.54	58.30 [17.88;98.72]	0.004	0.59	61.39 [19.96;102.81]	0.003
(µmol/l×days)	Reaction time nondominant hand	>0.551	0.56	47.92 [10.58;85.27]	0.01	0.60	48.15 [9.30;87.00]	0.01
¥	Reaction time overall	>0.551	0.58	54.43 [17.44;91.43]	0.004	0.62	56.13 [18.04;94.22]	0.004
	Inconsistency of alertness (msec)§§							
	SD reaction time dominant hand	>0.551	0.45	55.76 [18.1;93.43]	0.003	0.51	58.04 [19.41;96.68]	0.003
	SD reaction time overall	>0.551	0.52	44.36 [11.54;77.17]	0.008	0.55	43.83 [9.37;78.29]	0.01
	Motor coordination (No. of taps in 10s)§§§							
	No. of unimanual taps							
	Nondominant hand	>0.551	0.63	-1.57 [-2.84;-0.30]	0.01	0.65	-1.48 [-2.81;-0.16]	0.02
MEHP metabolites**	**Attention and motor coordination**							
0.53	*Alertness (msec)§*							
(0.23-1.42)	Reaction time dominant hand	>0.365	0.54	45.05 [4.77;85.33]	0.03	0.58	45.70 [4.26;87.13]	0.03
(µmol/l×days)	Reaction time nondominant hand	>0.365	0.56	44.23 [7.25;81.21]	0.01	0.59	43.62 [5.07;82.18]	0.02
¥	Reaction time overall	>0.365	0.57	45.99 [9.21;82.76]	0.01	0.61	46.09 [8.10;84.07]	0.01
	Inconsistency of alertness (msec)§§							
	SD reaction time dominant hand	>0.365	0.45	45.46 [7.97;82.95]	0.01	0.50	47.41 [8.87;85.95]	0.01
	SD reaction time overall	>0.365	0.51	38.16 [5.58;70.74]	0.02	0.54	36.61 [2.33;70.90]	0.03
MEHP	**Attention and motor coordination**							
0.18	*Alertness (msec)§*							
(0.09-0.53)	Reaction time dominant hand	>0.157	0.54	45.57 [5.83;85.31]	0.02	0.58	43.12 [2.50;83.75]	0.03
(µmol/l×days)	Reaction time nondominant hand	>0.157	0.55	35.62 [-1.07;72.32]	0.05	0.59	32.63 [-5.36;70.62]	0.09
¥	Reaction time overall	>0.157	0.57	41.30 [4.88;77.71]	0.02	0.61	38.61 [1.24;75.98]	0.04
	Motor coordination (No. of taps in 10s)§§§							
	No. of valid synchronous taps	>0.157	0.58	-1.77 [-3.00;-0.53]	0.005	0.60	-1.53 [-2.82;-0.24]	0.02
5oxo-MEHP	**Attention and motor coordination**							
0.03	*Alertness (msec)§*							
(0.02-0.10)	Reaction time dominant hand	>0.031	0.58	100.67 [59.92;141.41]	<0.001	0.63	109.50 [68.58;150.43]	<0.001
(µmol/l×days)	Reaction time nondominant hand	>0.031	0.58	73.85 [35.51;112.20]	<0.001	0.62	81.79 [42.61;120.96]	<0.001
¥	Reaction time overall	>0.031	0.60	88.60 [51.10;126.11]	<0.001	0.65	96.65 [58.80;134.51]	<0.001
	Inconsistency of alertness (msec)§§							

SD reaction time dominant hand	>0.031	0.49	91.91 [53.8;130.03]	<0.001	0.55	92.90 [54.19;131.62]	<0.001
SD reaction time nondominant	>0.031	0.46	48.76 [11.11;86.41]	0.01	0.48	50.01 [10.27;89.76]	0.01
SD reaction time overall	>0.031	0.55	74.64 [41.32;107.96]	<0.001	0.58	76.19 [41.62;110.75]	<0.001

† "Exposure" was defined as the product of the last day concentrations in µmol/l and the number of days exposed in PICU.
‡ Neurocognitive functions, that were significantly associated with the phthalate exposure in the development cohort, were tested in the validation cohort. Only the remaining functions that were also significantly associated with the phthalate exposure in the validation cohort are listed.
@ threshold values are rounded to three decimals.
* Exposure to the sum of MEHP and its metabolites 5cx-MEPP, 5oxo-MEHP and 5-HO-MEHP.
** Exposure to the sum of 5cx-MEPP, 5oxo-MEHP and 5-HO-MEHP.
¥ Distribution of metabolite exposures within the patient cohort (µmol/l ×days). Data are median (interquartile range).
§ For reaction times, higher scores reflect worse performance. Mean scores for healthy children are 558 (95% CI 521-595) for the dominant hand reaction time, 562 (95% CI 524-600) for the nondominant hand reaction time and 560 (95% CI 524-600) for the overall reaction time.[1]
§§ For inconsistency of alertness, higher scores reflect worse performance. Mean scores for healthy children are 259 (95% CI 229-290) for the dominant hand, 262 (95% CI 232-292) for the nondominant hand and 275 (95% CI 246-304) for the overall SD, standard deviation.[1]
§§§ For motor coordination, higher scores reflect better performance. Mean scores for healthy children are 36 (95% CI 34-38) for dominant hand unimanual taps, 32 (95% CI 30-33) for nondominant hand unimanual taps and 22 (95% CI 20-24) for valid synchronous taps.[1]

Figure legends

Figure 1. Plasma phthalate concentrations in critically ill children

Panel A shows the time course of the DEHP metabolite concentrations in 25 critically ill children, with quantification of the respective concentrations upon admission to the PICU (Adm), day 1 (d1), day 2 (d2), day 3 (d3) and last day in the PICU (LD). Panel B shows the DEHP metabolite concentrations upon admission and the last PICU day for the patients in the development (N=228) and the patients in the validation cohort (N=221). Total DEHP metabolite concentrations are calculated as the sum of MEHP and its metabolites 5cx-MEPP, 5OH-MEHP and 5oxo-MEHP, total MEHP metabolite concentrations are calculated as the sum of 5cx-MEPP, 5OH-MEHP and 5oxo-MEHP. Concentrations in matched healthy children were mostly below the quantification limit of the assay and are represented by thick dark grey lines upon the x-axis of the graphs. Data of the critically ill children are presented as means and 95% confidence intervals.

Figure 2. Univariate illustration of the alertness test results for patients who did and those who did not exceed the potentially harmful thresholds of exposure to circulating phthalate levels that were identified via multivariable bootstrap analysis

Measures of alertness are presented for patients in the development and the validation cohorts who had a total circulating DEHP metabolite exposure above and below the potentially harmful threshold (T), the latter identified with multivariable bootstrap analysis of the development cohort. Similar data are shown for the exposure to the sum of the 3 circulating MEHP metabolites and to 5oxo-MEHP. Data are presented as means and 95% confidence intervals. The size of the effect of exposure to these phthalates and the level of significance were determined with multivariable analysis after corrections for other risk factors as shown in Tables 2 and 3.

Figures

Figure 1

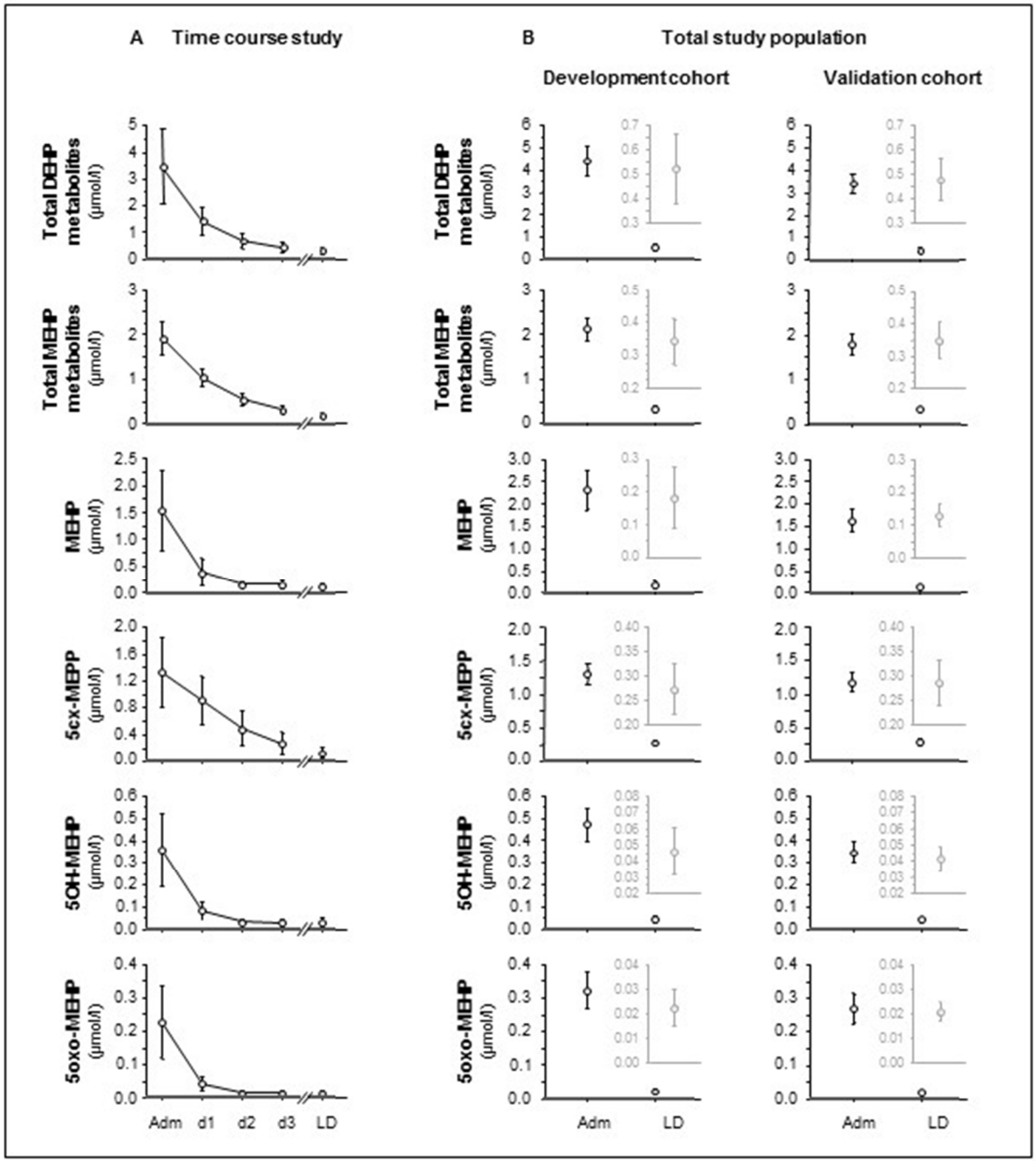
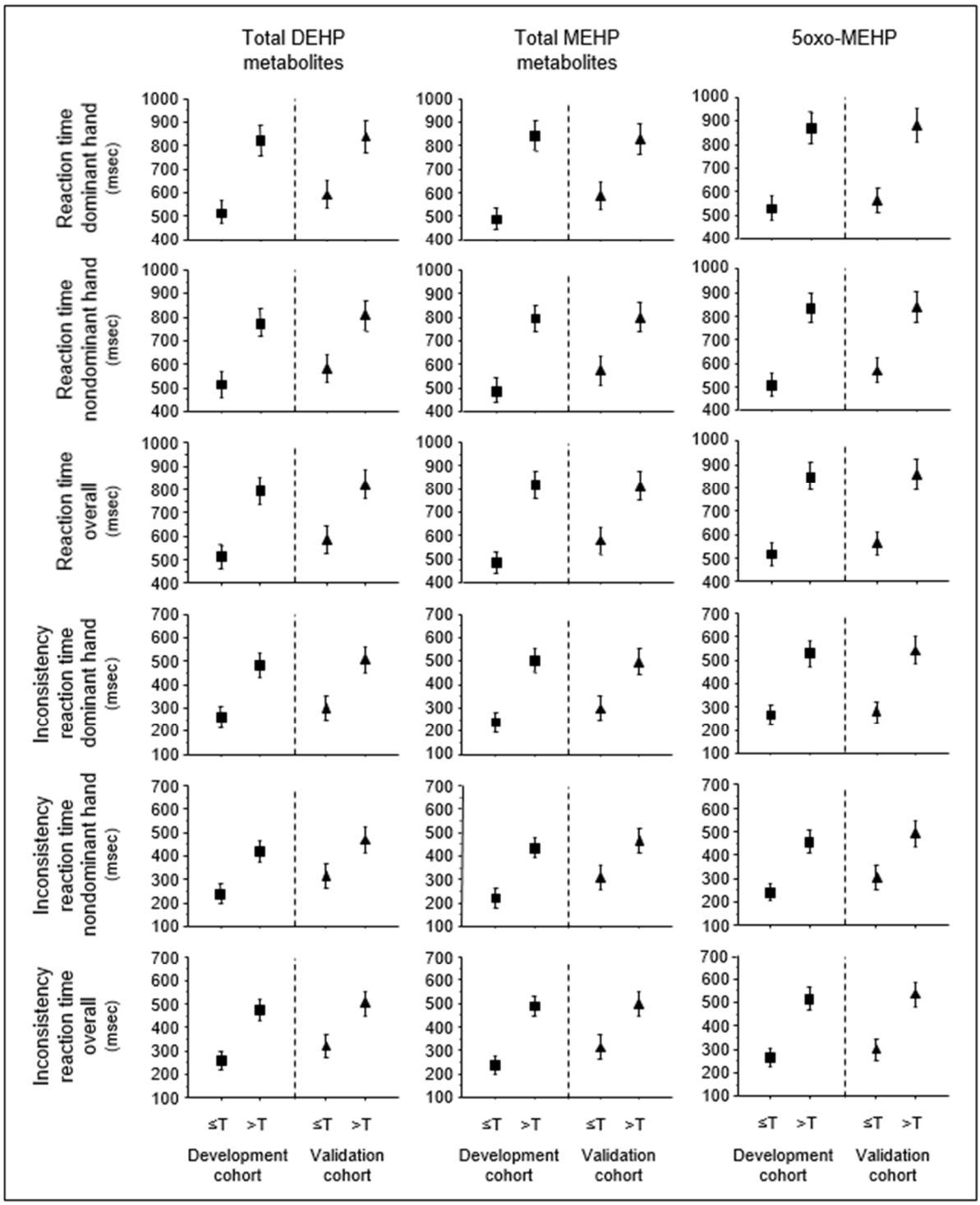


Figure 2


Total DEHP metabolites
Total MEHP metabolites
5oxo-MEHP
Reaction time dominant hand (msec)
Reaction time nondominant hand (msec)
Reaction time overall (msec)
Inconsistency reaction time dominant hand (msec)
Inconsistency reaction time nondominant hand (msec)
Inconsistency reaction time overall (msec)
≤T
>T
Development cohort
Validation cohort