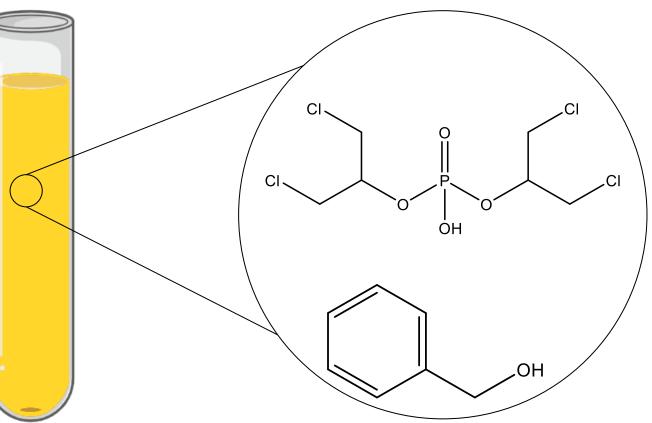
Comprehensive suspect screening for the identification of contaminants of emerging concern in urine of Flemish adolescents by LC-HRMS

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INTRODUCTION

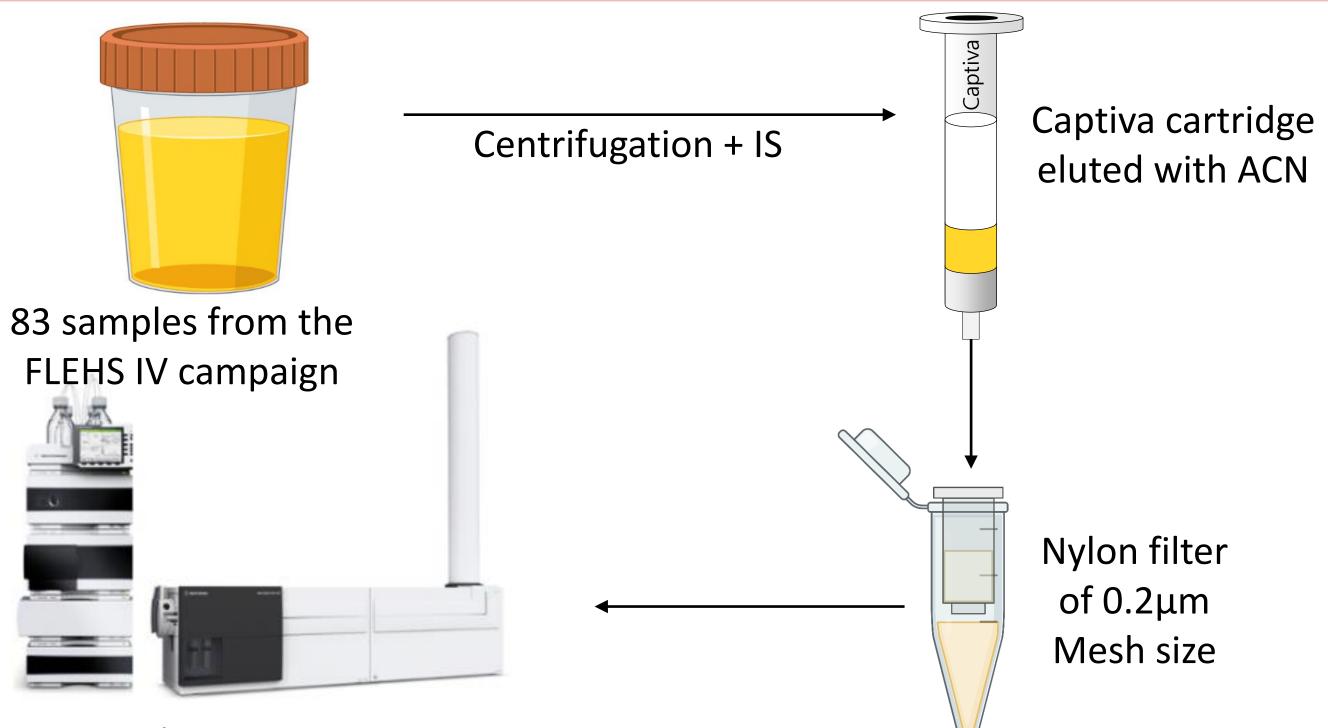
83 urine samples of Flemish adolescents **predicted metabolization reactions**, such The increasing human exposure to were collected in the frame of the FLEHS contaminants of emerging concern (CECs) as hydroxylation, glucuronidation and cannot be fully assessed by targeted **IV study**. Samples were divided in a high methylation. biomonitoring methods alone. and a low exposure group based on the In this study, 68 compounds were Suspect screening approaches allow exposure of 45 known **tentatively identified** at a confidence level overall the simultaneous detection of a high contaminants.¹ Samples were analyzed (CL) of 3 or better, as proposed by number of CECs and/or their predicted using a previously developed method by Schymanski et al.³, with most of the Caballero-Casero et al.² metabolites leading to a comprehensive detected compounds not included in assessment of human exposure to these The applied suspect list contained a current biomonitoring programs.



METHODS

Data analysis

Sample processing



LC/ESI-HRMS

Column: InfinityLab Poroshell 120 EC-C18: 100 mm x 3.7 mm, 2.7 μm Acquisition Mode: Auto MS/MS

Mobile phases ESI+: $(A) H_2O + 0.1\% FA$ (B) Methanol + 0.1% FA **Mobile phases ESI-:** $(A) H_2O + 5 \text{ mM NH}_4Ac$ (B) Methanol+ 5 mM NH_4Ac

Raw dataset RT stability – mass accuracy – stable signal intensities QA/QC Batch recursive feature extraction Peak picking • Peak height > 2,000 counts Deconvolution Mass window = 20 ppm; RT window = 0.3 min Match score > 70 PCA analysis Filtering • Fold change (FC) analysis: FC > 5 between samples and procedural blanks Suspect list with > 12,500 compounds Identification Mass tolerance < 7ppm • Isotope abundance score > 80; overall ident. score > 75 Manual MS² spectra NO **CL 4** checking acquired? ¥ YES **RT difference** Ref. std. YES **CL 1** available? < 0.2 min? ↓ NO Match with Library spectra YES library > available? 70%? YES NO NO Only one possible **CL 2** Match with *in silico* candidate? fragm. or fragments NO explained by structure of NO **CL 4** suspect? YES **CL 3**

RESULTS AND DISCUSSION

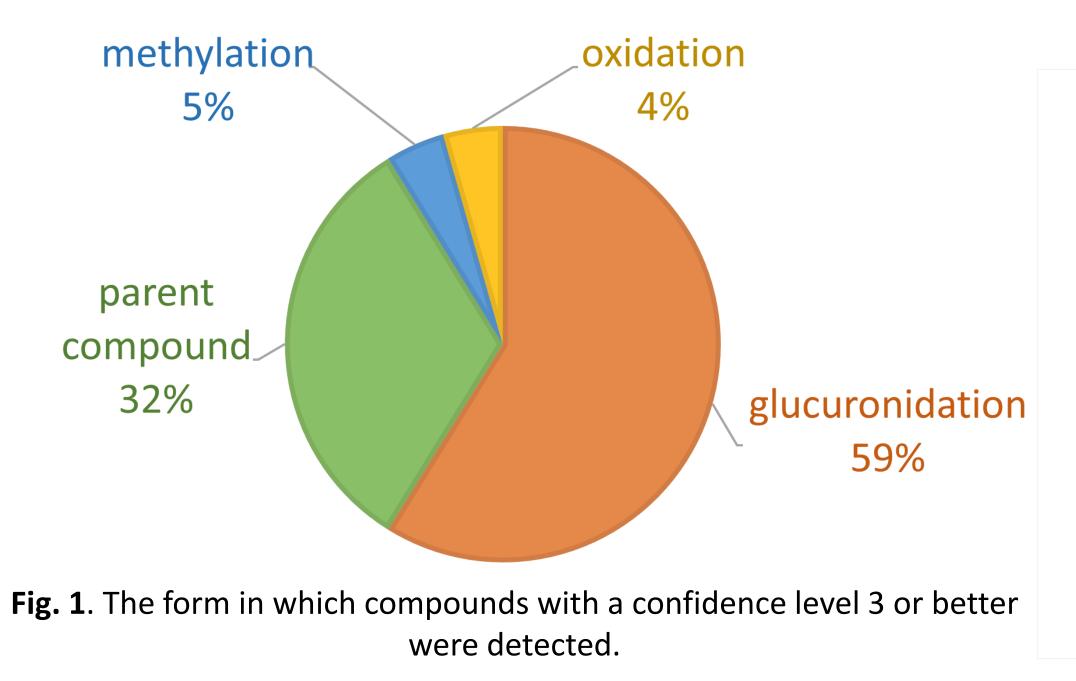


Table. 1. The compounds detected at a confidence level of 2. DF: detection frequency, PCP; personal care products, PFR; phosphate flame retardant

| Name | Formula | Compound Class | DF [%] | | | | | |
|------------------------|----------|------------------------|--------------------------|--|--|--|--|--|
| L-/D-Pantothenate | C9H17NO5 | Personal care procucts | 68.7 (CL 2); 16.9 (CL 4) | | | | | |
| 4-hydroxy-benzaldehyde | C7H6O2 | Personal care procucts | 36.1 (CL 2); 49.4 (CL 4) | | | | | |
| Catechol | C6H6O2 | Personal care procucts | 100 (CL 2) | | | | | |

- \rightarrow 13 compounds were identified at CL2
- \rightarrow The majority of compounds was detected in their glucuronidated form
- \rightarrow A higher number compounds was detected in the high exposure group as opposed to the low exposure group but only for female participants (p < 0.01).

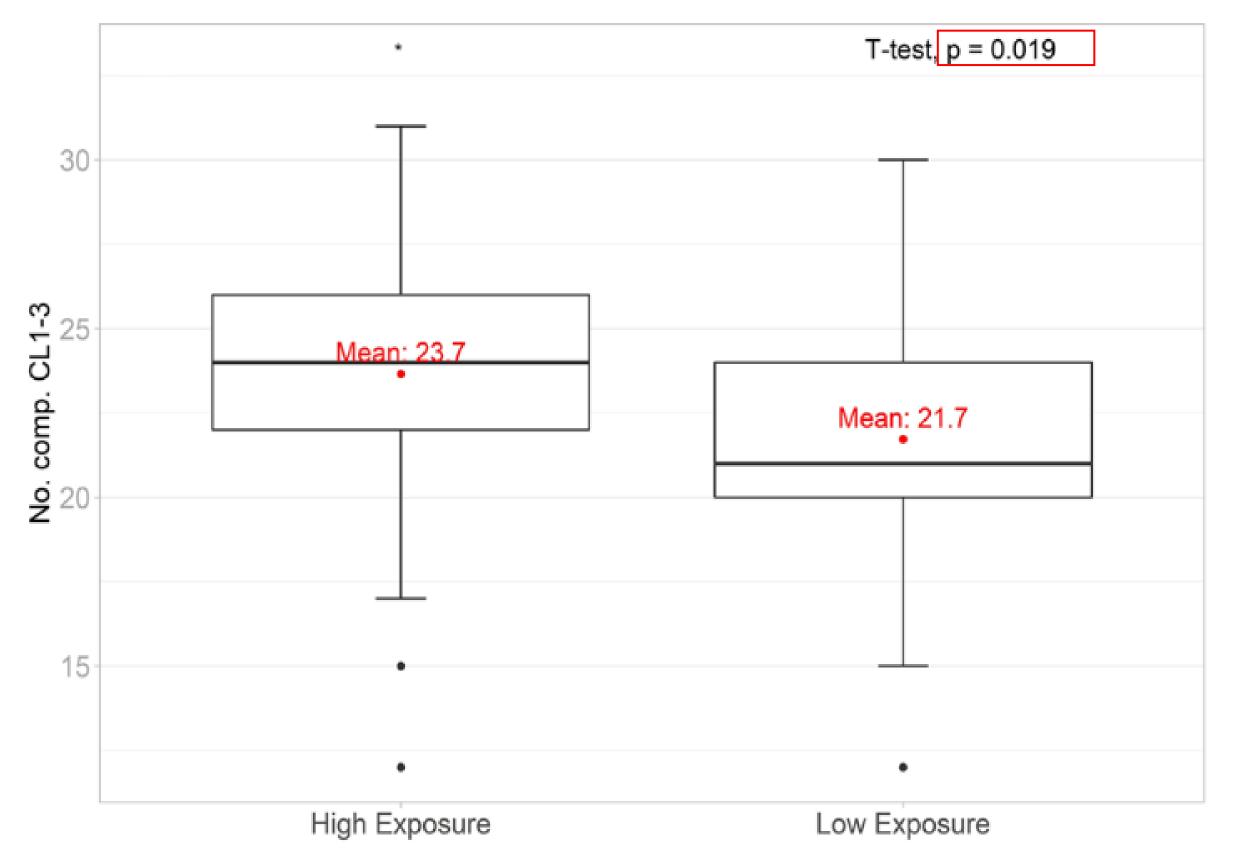


Fig. 2. Boxplots representing the number of identified compounds at CL 3 or better in the low and high exposure load groups.



| Benzyl alcohol | C7H8O | Personal care procucts | 97.6 (CL 2); 2.4 (CL 4) | | T | | | |
|-------------------------------|------------|------------------------------------|--------------------------|---|---------------|--------------|---------------|--------------|
| Diphenyl hydrogen phosphate | C12H11O4P | Organophosphate flame retardants | 43.4 (CL 2) | ကို | Mean: 24.5 | | | T |
| bis(1,3-dichloro-isopropyl) | | | | | • | | Mean: 23.1 | |
| phosphate | C6H11Cl4O4 | P Organophosphate flame retardants | 25.3 (CL 2) | np. (| | | | Mean: 22.5 |
| 2-ethylhexyl phenyl phosphate | C14H23O4P | Organophosphate flame retardants | 1.2 (CL 2) | COL | | Mean: 20.9 | | |
| Theobromine | C7H8N4O2 | Personal care procucts | 84.3 (CL 2) | <u>o</u> 20 | | | | |
| Theophylline | C7H8N4O2 | Personal care procucts | 63.9 (CL 2) | | | | | |
| 8-Hydroxy-quinoline | C9H7NO | Other | 94.0 (CL 2); 6.0 (CL 4) | 15 | • | | | |
| Phthalic anhydride | C8H4O3 | Plasticizers | 6.0 (CL 2); 74.7 (CL 4) | | | | • | |
| Riboflavin | C17H20N4O | 6 Personal care procucts | 18.1 (CL 2); 39.8 (CL 4) | | High Exposure | Low Exposure | High Exposure | Low Exposure |
| Quinoline, | | | | Fig. 3. Boxplots divided by sex representing the number of identified compounds | | | | |
| Isoquinoline | C9H7N | Other | 25.3 (CL 2); 34.9 (CL 4) | at CL 3 or better in the low and high exposure load groups. | | | | |

LITERATURE

1. Buekers, J., et al., Combined chemical exposure using exposure loads on human biomonitoring data of the 4th Flemish Environment and Health Study (FLEHS-4). Int J Hyg Environ Health, 2021. 238: p. 113849. 2. Caballero-Casero, N., et al., Identification of chemicals of emerging concern in urine of Flemish adolescents using a new suspect screening workflow for LC-QTOF-MS. Chemosphere, 2021. 280: p. 130683-130692. 3. Schymanski, E.L., et al., Identifying small molecules via high resolution mass spectrometry: communicating confidence. Environ Sci Technol, 2014. 48(4): p. 2097-8.

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