

Long Abstract

Metabolomics approaches for biomarker discovery for toxicant exposure of di-isononyl phthalates (DINPs) using liquid chromatography-high resolution mass spectrometry (LC-HRMS)

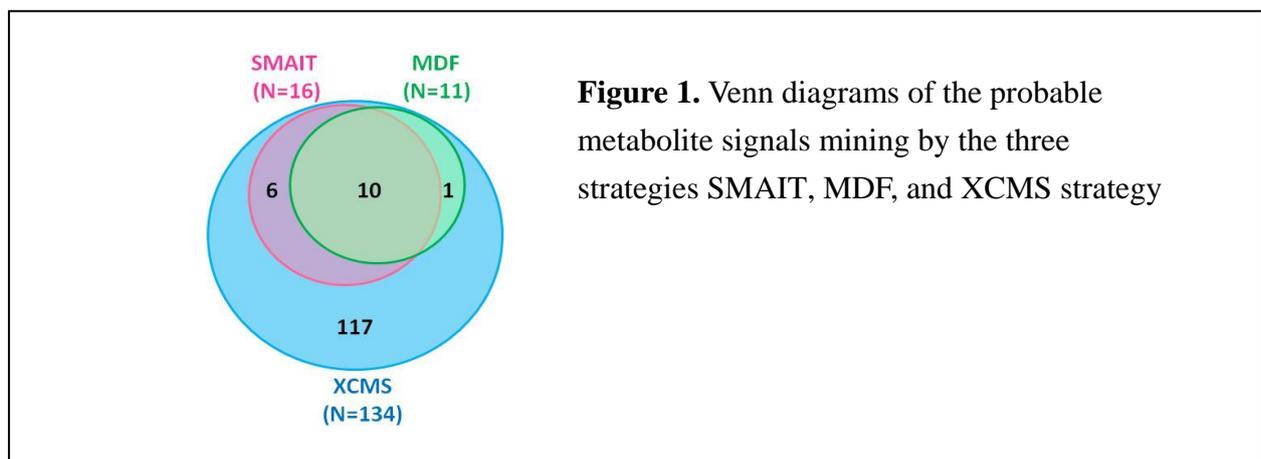
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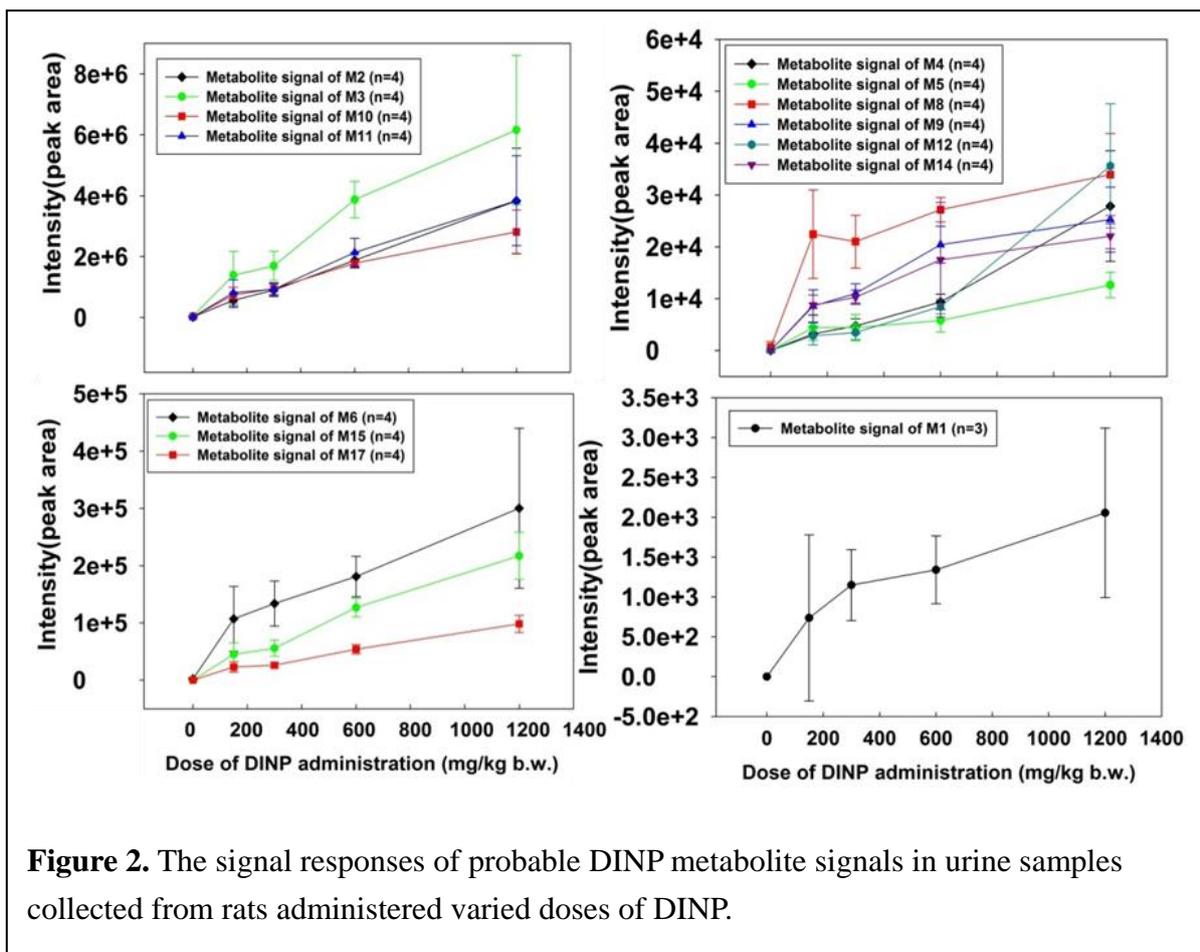
Di-isononyl phthalate (DINPs) is a kind of phthalates which are widely used in plastics, building materials, toys, and personal care products. Exposure assessment of DINPs in human is of interest because of the potential adverse health effects of DINP. However, our knowledge on DINP metabolism remains limited and previously known DINP metabolites in urine amount to only 1/3 fraction of excretion on a molar basis.¹ Recently, we have developed a statistical process, signal mining algorithm with isotope tracing (SMAIT)², which has efficiently filtered 7 probable DINPs metabolite signals out of the 8867 peaks in an LC-MS data generated by a quadrupole-time of flight mass spectrometer.³ In the present study, we explored three metabolomics approaches for DINPs exposure marker discovery using the LTQ/Orbitrap high resolution mass spectrometry and multiple post-acquisition data processing techniques, including SMAIT, mass defect filtering (MDF), and on-line web-based software XCMS.

The development of high-resolution mass spectrometry (HRMS) instrumentation such as FT ion cyclotron resonance and Orbitrap, along with new data processing techniques, has

improved the quality and productivity of metabolite identification process. We used LTQ/Orbitrap high resolution mass spectrometry coupled with three data processing methods, including SMAIT, MDF, and XCMS, to perform mass spectral signal filtering in a complex LC-HRMS data obtained from liver enzyme incubation for DINP metabolites. As shown in the venn diagrams (Figure 1), there are total of 134 probable DINP metabolite signals filtered by the three strategies. Among the 134 signals, 10 were found by all three strategies. A rat model was used to validate the 17 probable metabolite signals filtered either by SMAIT or MDF as DINP exposure markers.

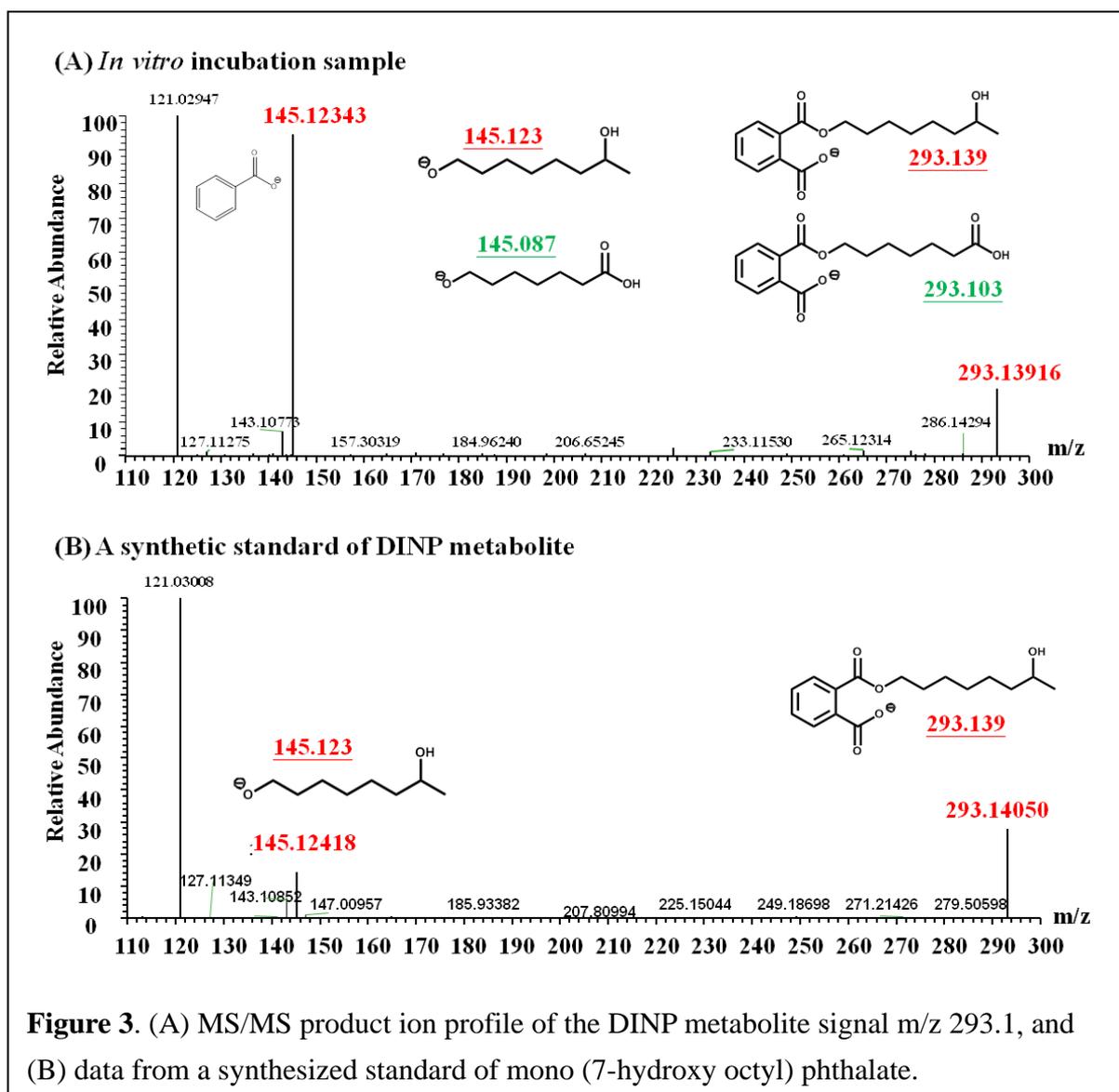


The rat urine samples were collected from the rats administered with several doses of commercial DINP-2 or corn oil (carrier). Using the revealed retention times, m/z values, and MS/MS data of the 17 probable metabolite signals obtained from liver enzyme incubation, these probable metabolites could be detected in rat urine samples using UPLC-LTQ/Orbitrap. Out of the 17 probable metabolite signals, 14 signals were validated as effective exposure markers because of the established dose-response relationship (as shown in Figure 2). Among the 14 DINP exposure-related signals, 8 have not been reported in the prior literature.



The chemical structure of the probable metabolite can be elucidated by MS/MS product ion profile and accurate mass measurement. In a previous report, the DINP metabolite signal m/z 293.1 was identified as mono(carbonyl isoheptyl) phthalate (m/z 293.103)⁴. However, we hypothesized that it could be mono(hydroxyl isoheptyl) phthalate (m/z 293.139) as suggested by accurate mass measurement and relative isotopic abundance (Figure 3). The hypothesis was further supported by the data derived from a synthesized standard.

In conclusion, the metabolomics platform can efficiently and systematically filter probable metabolite signals from a complex LC-HRMS dataset for toxic exposure marker discovery.



References

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