# *In vitro* Phase I and Phase II metabolism of bisphenol S by liquid chomatography coupled to quadrupole time-of-flight mass spectrometry

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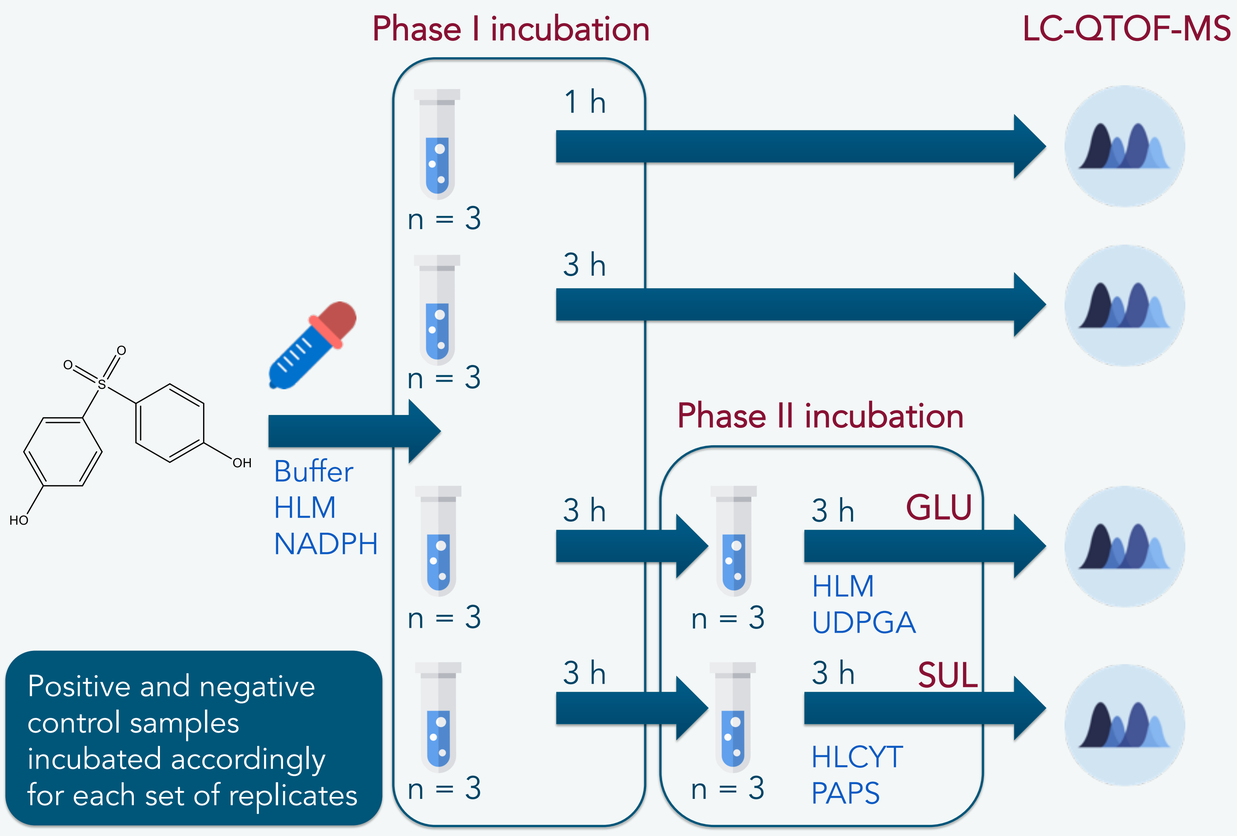
## Introduction

Bisphenol S (BPS) is increasingly used as a substitute for bisphenol A in recent industrial applications. Despite its frequent use, limited information is available on the human metabolism of BPS. Hence, current biomonitoring studies rely on the measurement of BPS itself, leading to a potential underestimation of the exposure to this emerging contaminant[1,2].

## Aims

- To investigate the human *in vitro* metabolic pathway of BPS using human liver microsomes (HLM) and cytosol (HLCYT) fractions
- To propose potential biomarkers to contribute to a reliable assessment of BPS exposure in future biomonitoring studies

## *In vitro* assay




## Data analysis

### Suspect screening

**MassHunter**

- Database generated by literature research
- Find by Formula algorithm
- Not present in negative control
- Present in 2/3 replicates

### Untargeted screening

**MZmine**

- Detected masses, built chromatograms, deconvoluted, deisotoped
- Aligned features

**R**

- Calculated fold-change (FC) between samples and controls
- Calculated p-value of t-test between samples and controls
- Identified features with FC > 10 and p < 0.05

### Identification

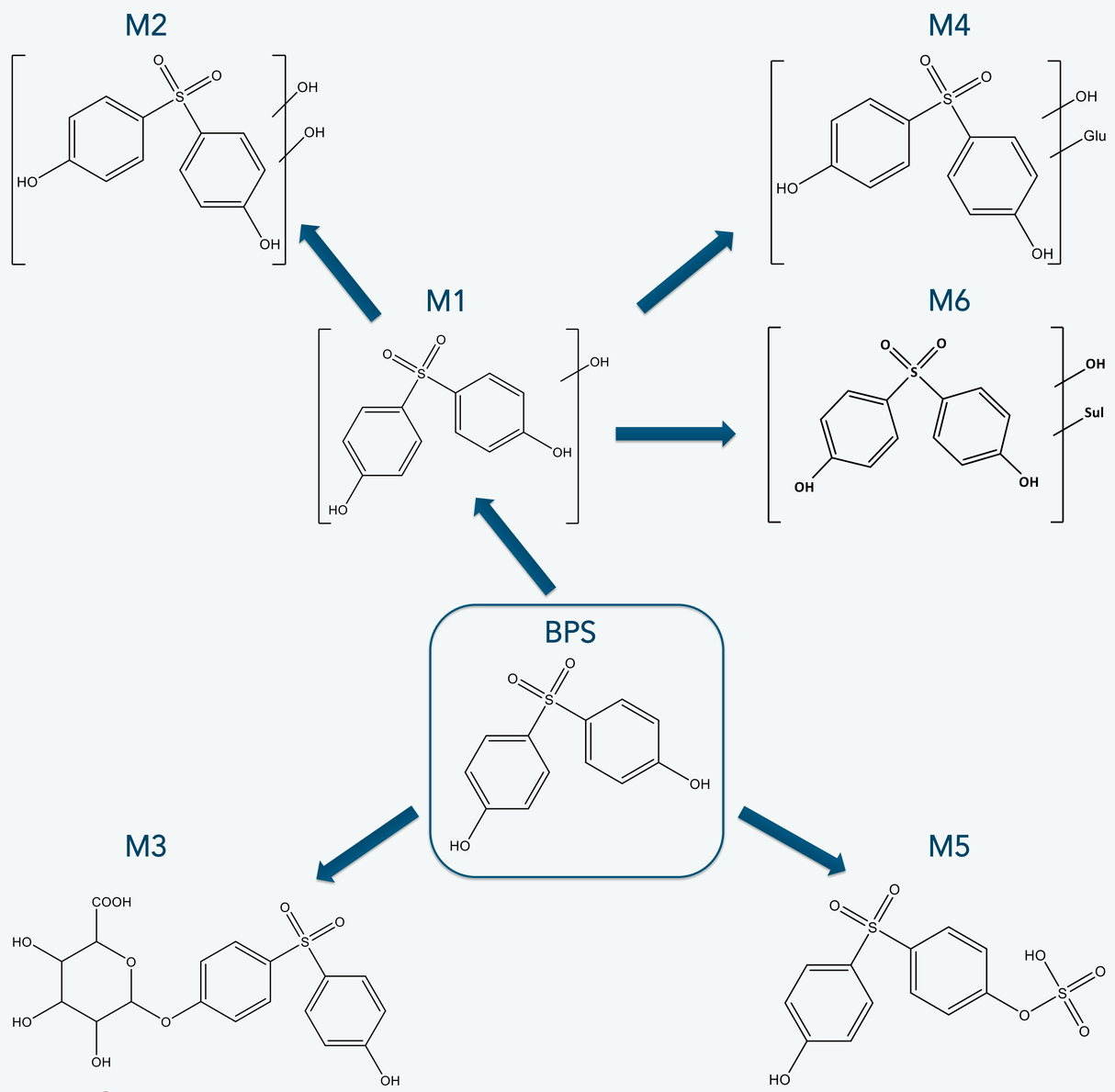
a) Maximal Δ 10 ppm for MS
b) Maximal Δ 25 ppm for MS/MS
c) Isotope pattern scoring ≥ 70%
d) DBE values match structure

All identified metabolites were detected using both data-analysis workflows

## Biotransformation of BPS

- Two Phase I metabolites (hydroxylation), four Phase II metabolites (conjugation)
- M1 (hydroxy-BPS) is the major Phase I *in vitro* metabolite
- **M2** (dihydroxy-BPS) **elucidated for the first time**
- Formation of M3 (BPS-glucuronide) and M5 (BPS-sulfate) in accordance with other human *in vitro* metabolism studies
- **M4** (hydroxy-BPS-glucuronide) and **M6** (hydroxy-BPS-sulfate) **elucidated for the first time**
- M4 and M6 both gave rise to two peaks at different RT; two isomers suggesting conjugation can occur on different hydroxyl groups
- Hydroxy-BPS and its glucuronidated and sulfated conjugates should be considered as potential additional biomarkers

## Proposed *in vitro* metabolic pathway




## Identified metabolites

| ID | Tentative formula | RT [min] | Error [ppm] | Confirmation | Confidence level | Phase I | Phase II GLU | Phase II SUL |
|---|---|---|---|---|---|---|---|---|
| M1 | $C_{12}H_{10}O_5S$ | 7.42 | -6.79 | MS/MS | L3 | X | X | X |
| M2 | $C_{12}H_{10}O_6S$ | 5.95 | -8.18 | MS/MS | L3 | X | | |
| M3 | $C_{18}H_{18}O_{10}S$ | 3.91 | -2.35 | Anal. ref. std. | L1 | | X | |
| M4-A | $C_{18}H_{18}O_{11}S$ | 5.92 | 0.68 | MS/MS | L3 | | X | |
| M4-B | $C_{18}H_{18}O_{11}S$ | 3.71 | 1.13 | MS1 + isotope | L4 | | X | |
| M5 | $C_{12}H_{10}O_7S_2$ | 6.62 | -9.73 | MS/MS | L2b | | | X |
| M6-A | $C_{12}H_{10}O_8S_2$ | 6.22 | 8.70 | MS/MS | L3 | | | X |
| M6-B | $C_{12}H_{10}O_8S_2$ | 5.93 | 8.41 | MS/MS | L3 | | | X |

## References

1. Gramec Skledar, D. *et al.*, *Environ. Toxicol. Pharmacol.* 47 (2016): 182-199.
2. Oh, J. *et al.*, *Environment International* 112 (2018): 127-133.

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