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OF ALBERTA**



TMIC

The **Metabolomics** Innovation Centre

GC×GC-TOFMS metabolomics and exposomics for studying the impact of fetal and neonatal cannabis exposures

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Cannabis sativa L.

- Flowering plant.
- Main psychoactive is tetrahydrocannabinol (THC).
- Over 500 secondary metabolites identified in cannabis.
- Many chemical components are unidentified.

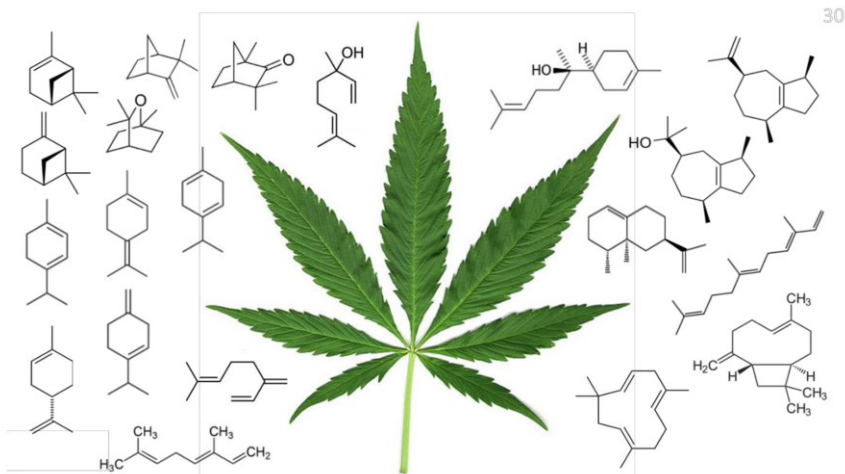
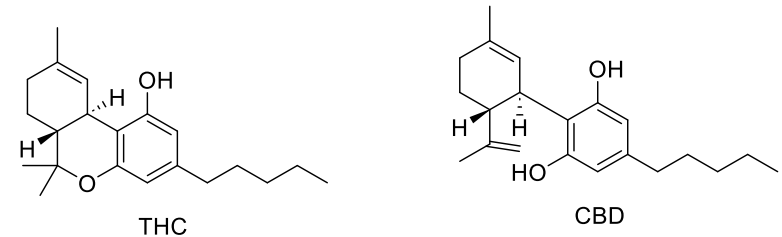


Photo: Metabolites 2020, 10(7), 276



Photo: Britannica

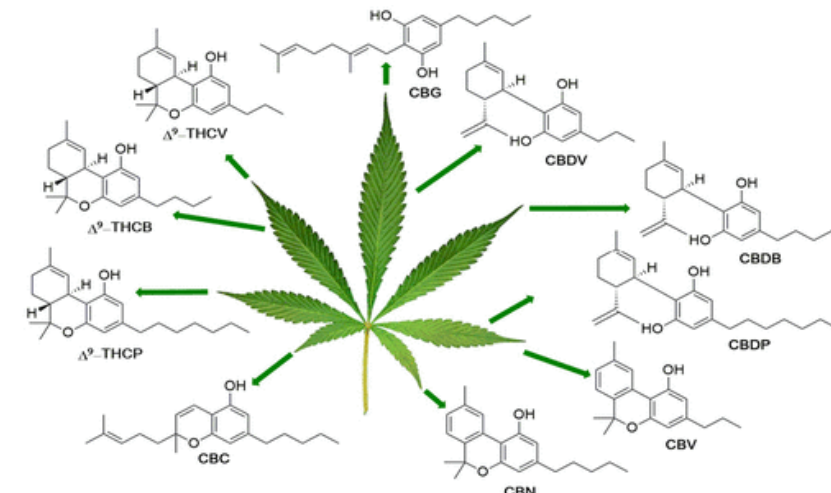


Photo: J. Nat. Prod. 2021, 84, 1, 142–160

Recreational cannabis use in Canada

- Canada legalized recreational cannabis in 2018.
- Long history of accepted use.
- Variety of products, sold through cannabis stores.
- Over 1 in 3 Canadians used cannabis in the past year (2024; StatCan).
- \$10.8 billion (CAD) market size (2023; StatCan).



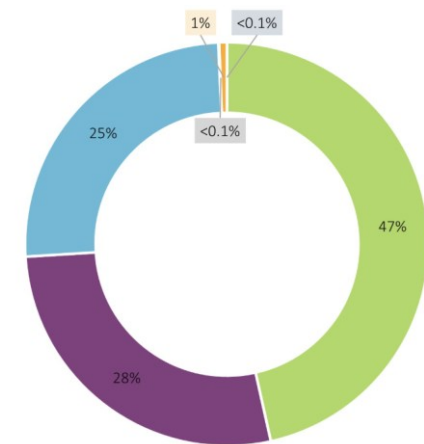
Photo: C&EN



Photo: Global News



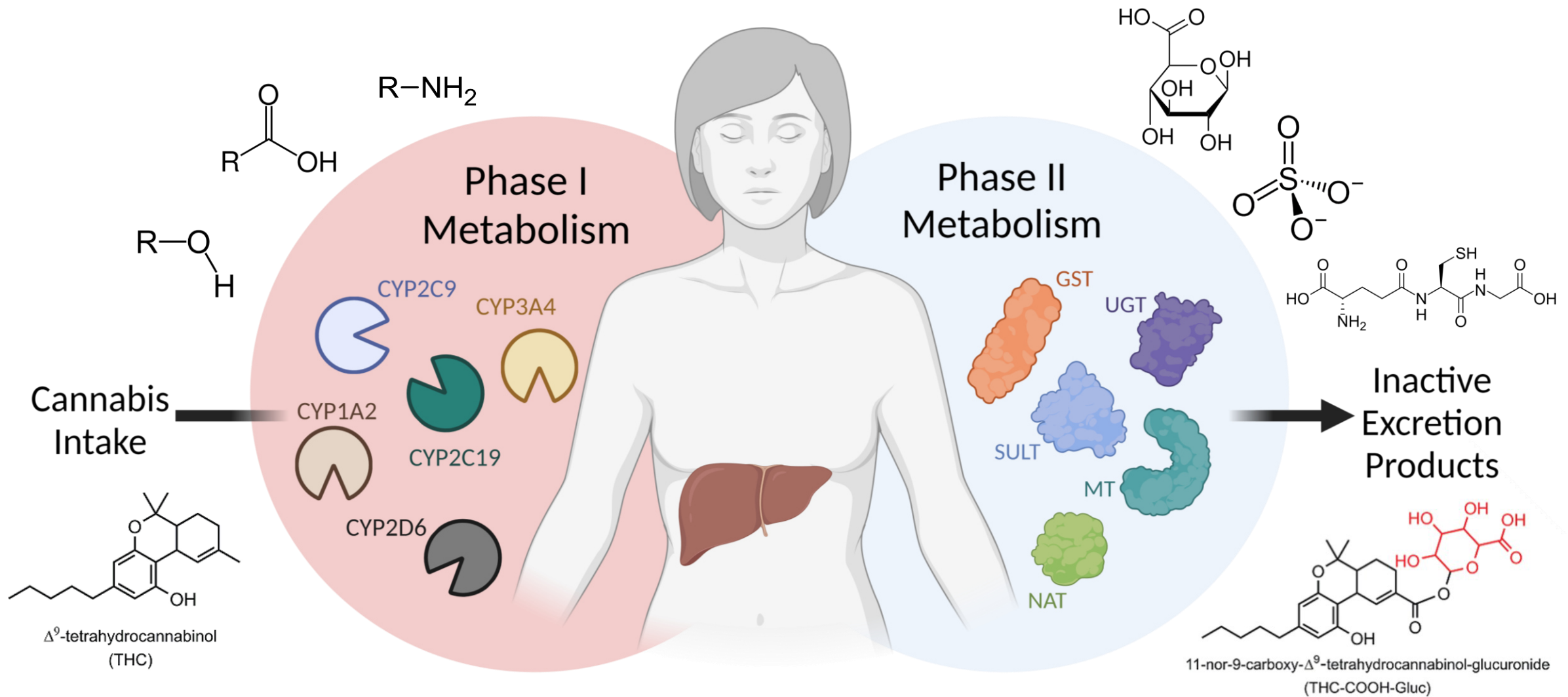
Photo: Wikipedia



Plot: Statistics Canada

- Dried cannabis
- Edible cannabis
- Cannabis Extracts
- Seeds
- Topicals
- Vegetative cannabis plant

Cannabis metabolism



Prenatal cannabis use



5.0% of pregnant women used cannabis daily or weekly¹



Anxiety is the most common reason (33.3%), then nausea (22.2%), and sleep (22.2%)¹



Between 2002 and 2014, cannabis use increased 62%²



Perception that cannabis does no harm has increased 3-fold from 2005 to 2015²

[1] [https://www.jogc.com/article/S1701-2163\(20\)30157-2/abstract](https://www.jogc.com/article/S1701-2163(20)30157-2/abstract)

[2] <https://pubmed.ncbi.nlm.nih.gov/30552948/>

Figures: Gwen Nguyen

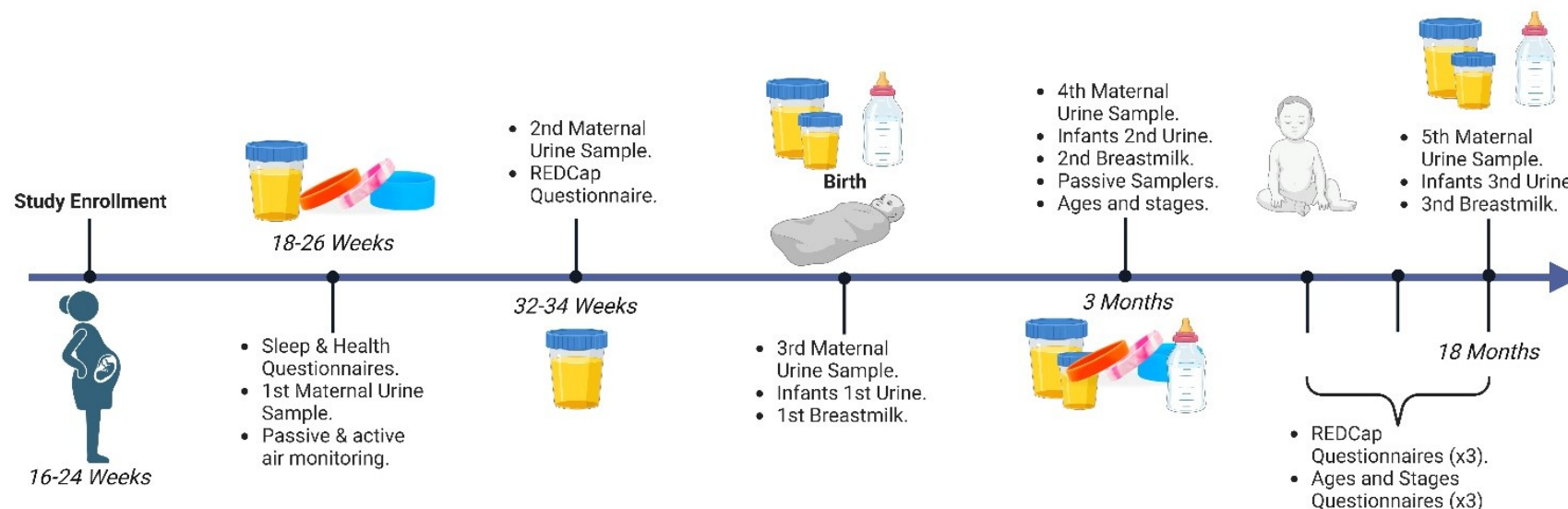
Prenatal cannabis use: harmful?

- Current research examines cannabis use during perinatal period.
 - Decreased birth weight.
 - Higher chance of preterm birth.
 - Conflicting results on neurodevelopmental outcomes.
- Problems with cannabis research:
 - What is a cannabis dose?
 - How do we measure cannabis exposure?
 - Each administration route is different.
 - Products are far from homogenous.
 - First vs. second hand exposures?
- Further work is needed so families can make informed decisions.



Healthy baby brains

- *How do prenatal cannabis exposures influence physiology, metabolism, and infant development?*
- Recruiting ~ 400 expecting mothers from Edmonton region, half reporting *some* cannabis exposure.
- Interdisciplinary study, involving physicians, psychologists, and analytical chemists.
- Urine and breast milk untargeted metabolomics by GC×GC-TOFMS.
- Passive air sampling wrist bands to measure exposomes of mother and infant.



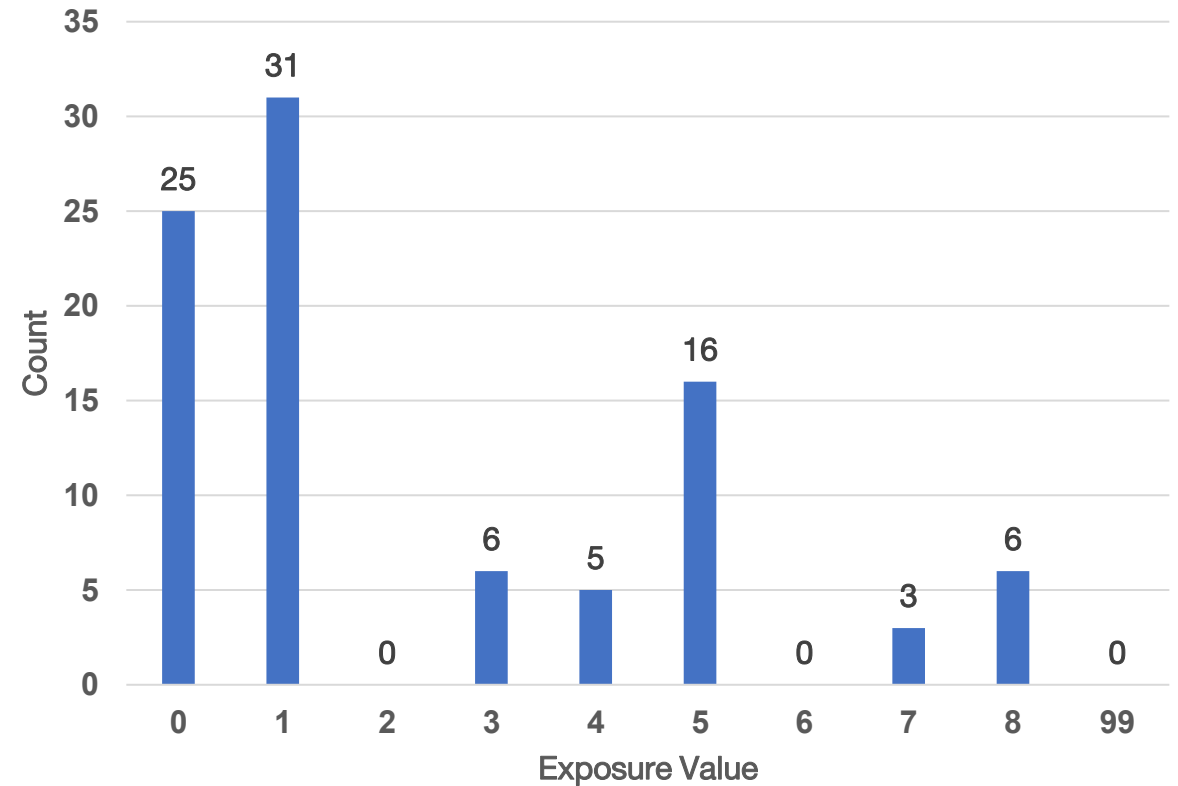
GC×GC-TOFMS untargeted metabolomics

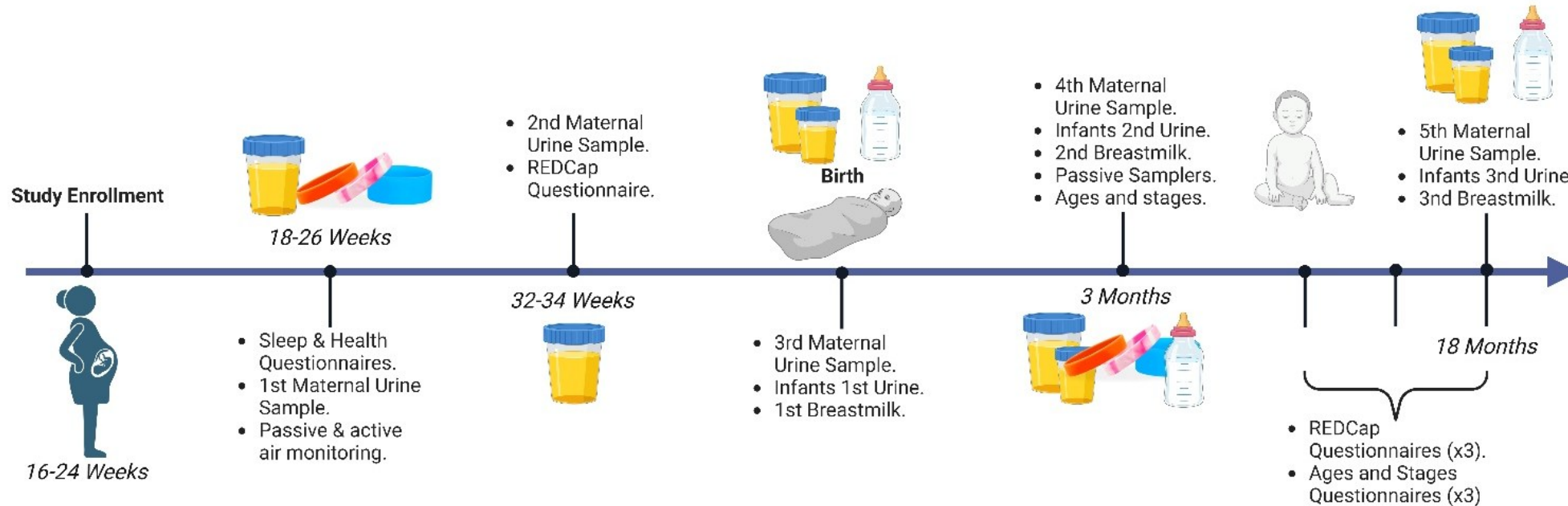
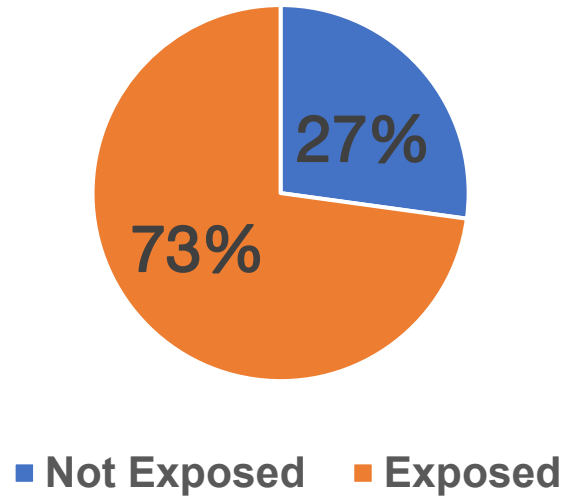
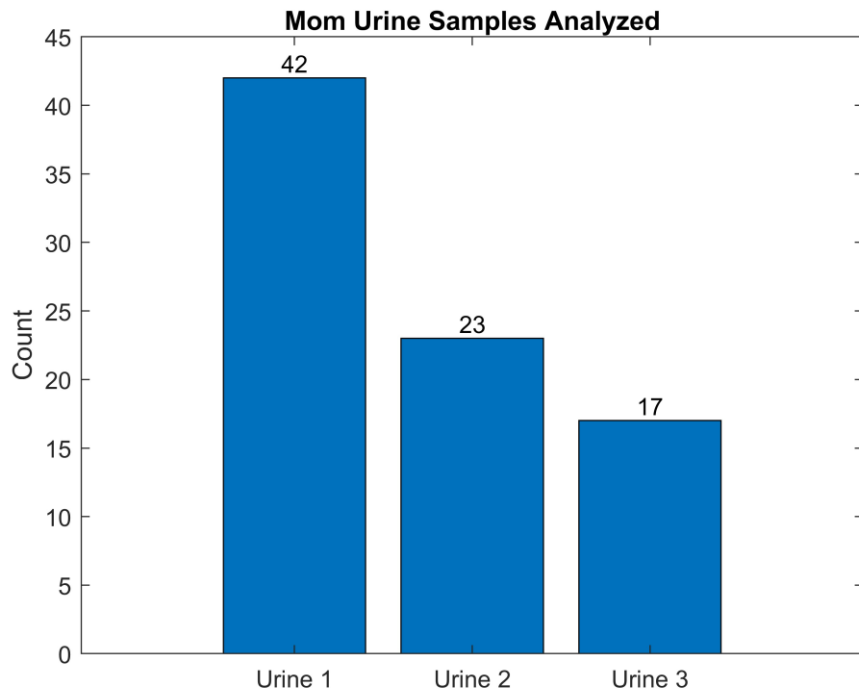
- LECO BT for untargeted metabolomics.
- 92 samples analyzed.
- Methoximation followed by trimethyl silylation.
- Cryogenic modulation.
- First Dimension: Rxi-5SilMS.
- Second Dimension: Rtx-200MS.
- Data processed in ChromaTOF 5 and aligned with a prototype software package (LECO).



Cannabis exposure reporting

Value	Label
0	Never used and partner does not use
1	Used in past but not in past 12 months. Partner not using
2	Never used but partner uses
3	Used in past but not in past 12 months. Partner using
4	Any use in the past 12 months. Partner not using
5	Any use in the past 12 months and Partner using.
6	Any use in the past 30 days
7	Frequent (1-4 days per week)
8	Daily or almost daily use (5-7 days per week)
99	No response

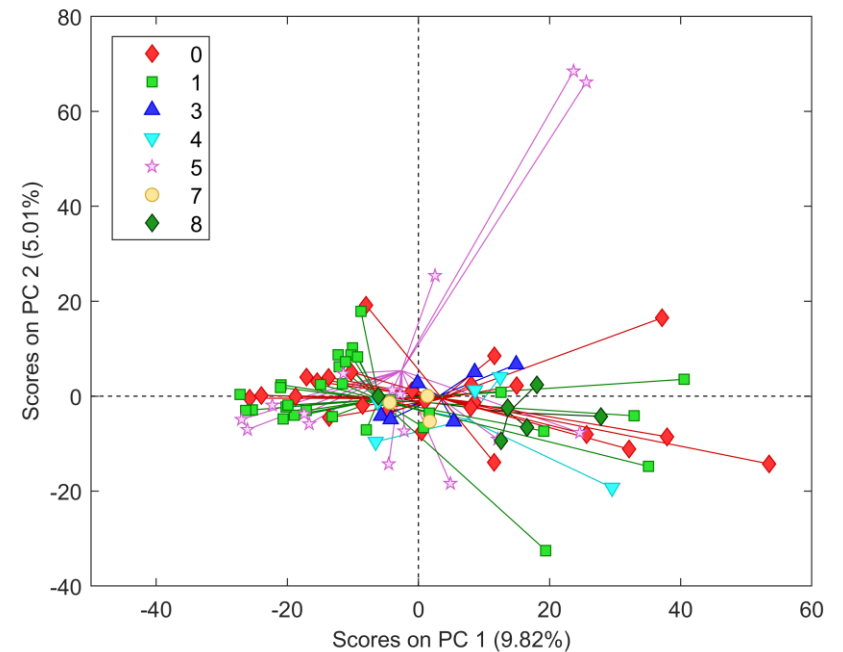
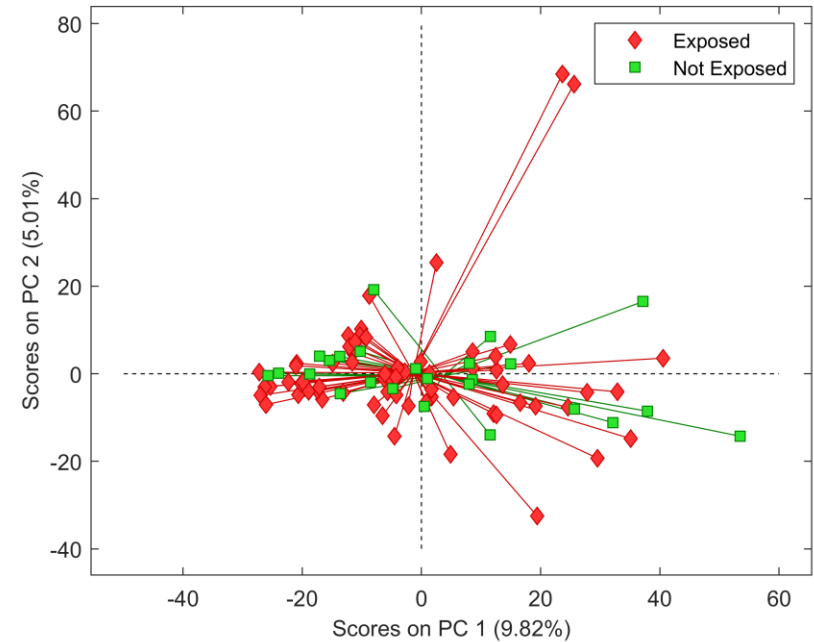




Metabolomics results

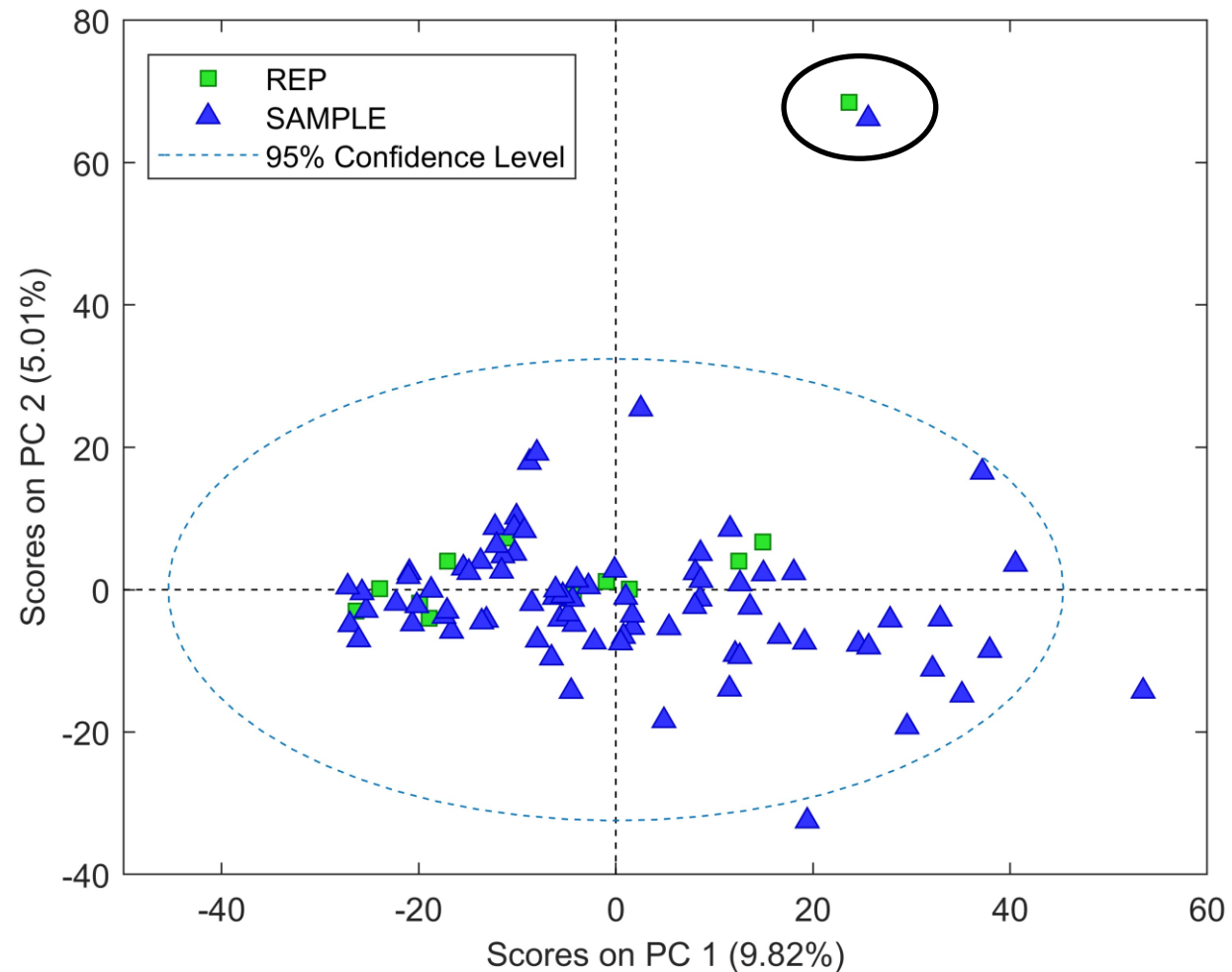
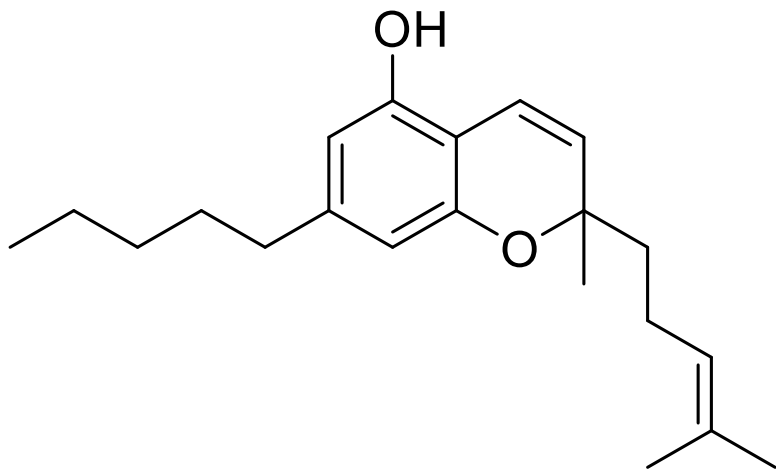
- 3356 metabolites detected.
- No clear separation between two groups.
- $p = 0.2160$, $F = 1.0952$, $R^2 = 0.0120$.
- No trend observed with exposure labels.

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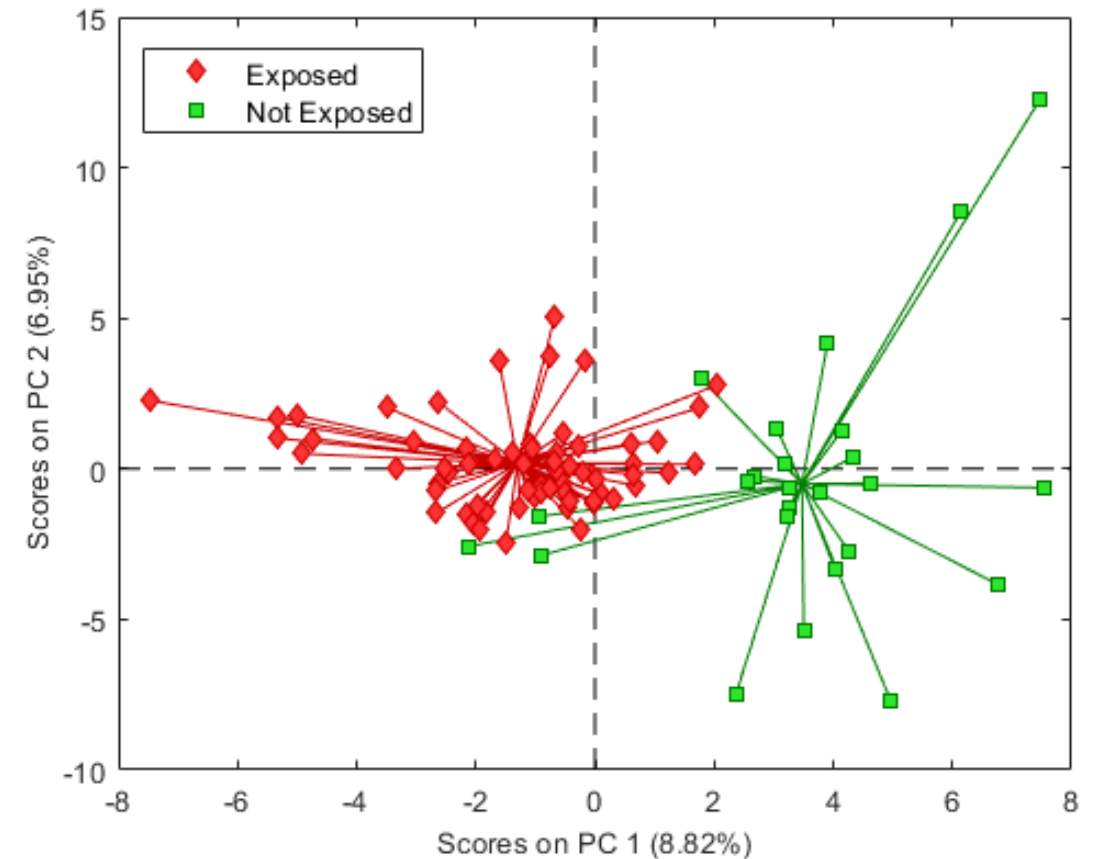
Metabolomics results

- One sample and its technical replicate projects far from other samples.
- Cannabichromene (CBC) correlated with these samples.
- Other are unidentified features.



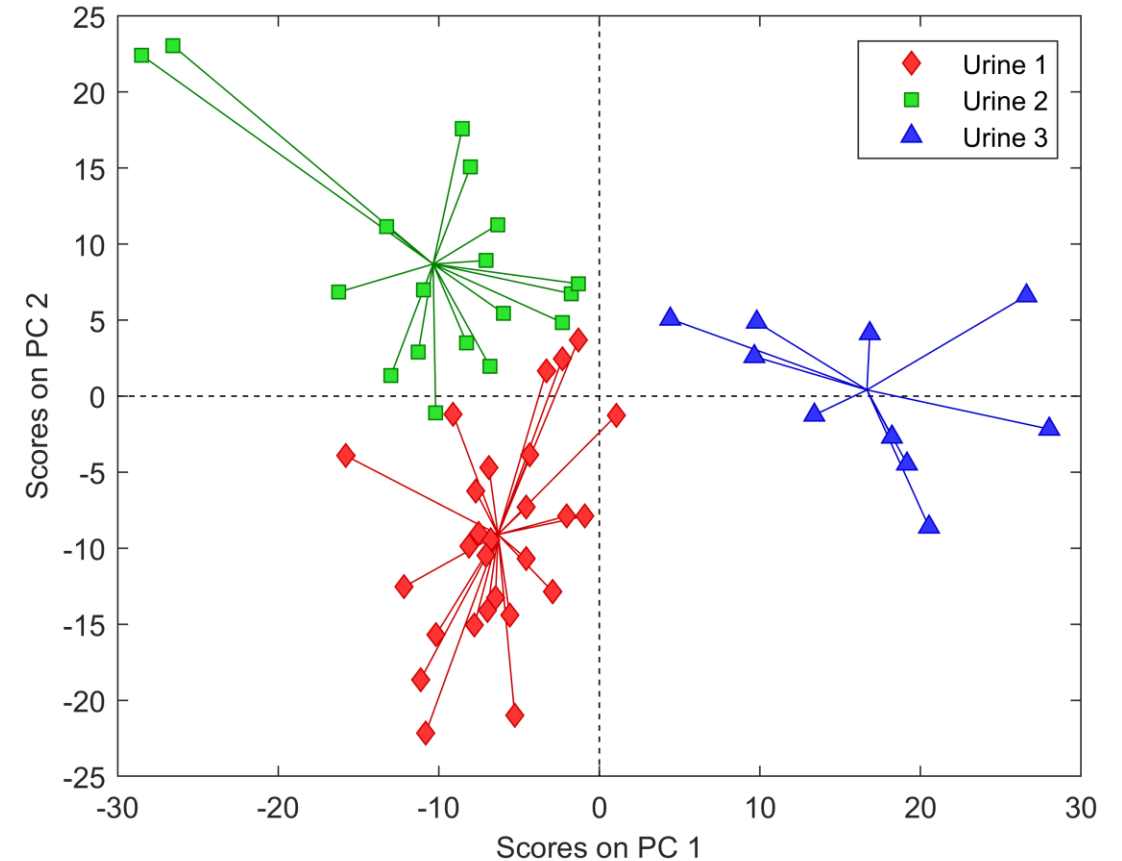
Feature selection results

- Feature selection by cluster resolution found 95 variables.
- $p = 0.00001$, $F = 5.2970$, $R^2 = 0.0556$.
- 7 correlated with exposed, 88 correlated with not exposed.
- None of the variables are cannabinoids or their metabolites.
- Half of selected variables are unidentified features.



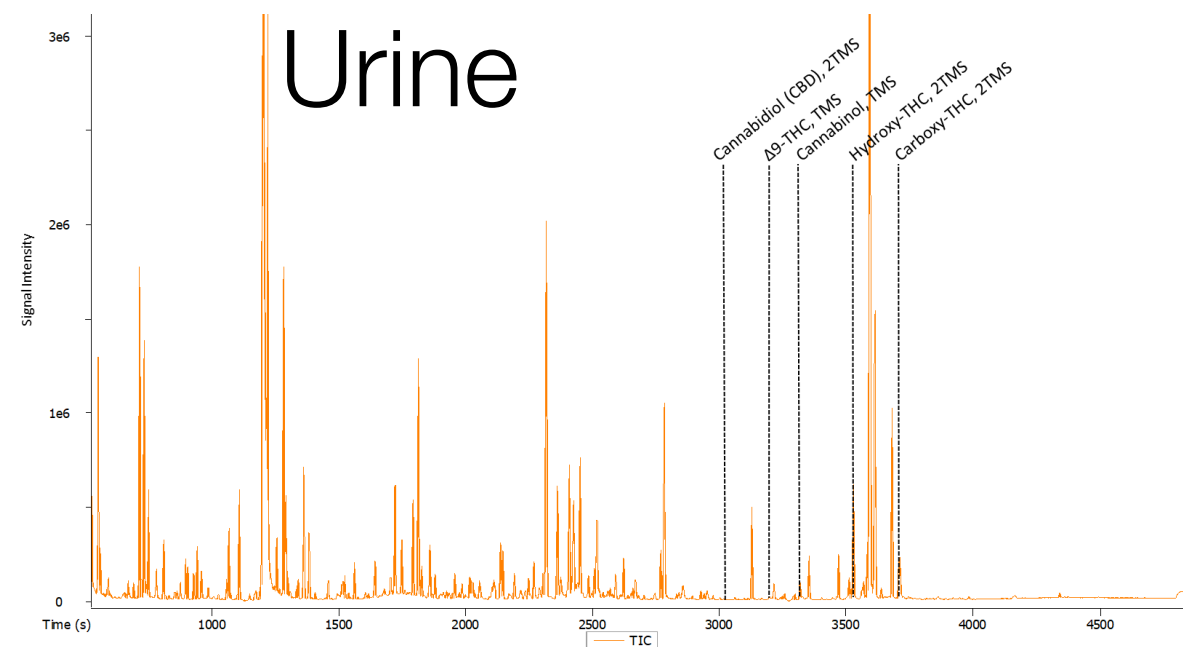
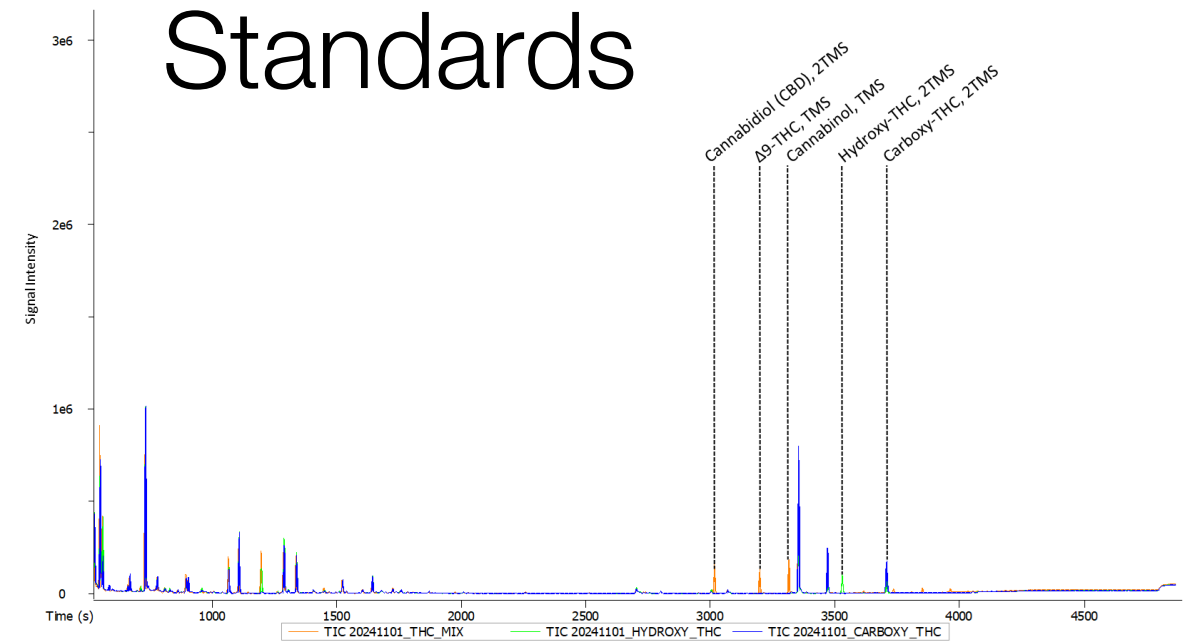
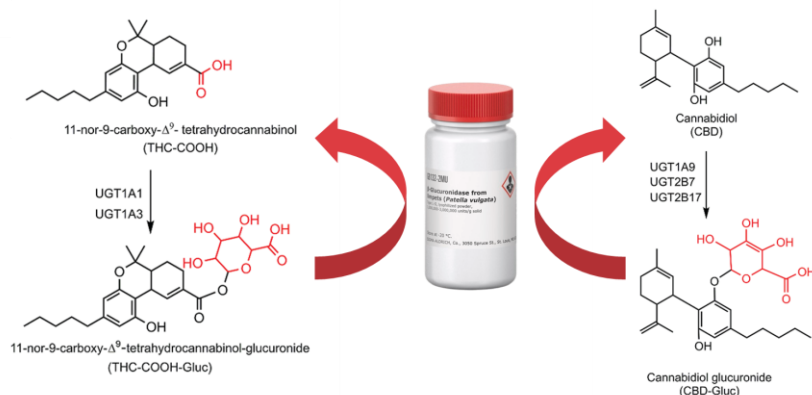
Remove low exposures?

- Removed exposures 1-3.
- Identified 70 significant variables by FS-CR.
- Some overlap with original FS-CR results.
- Majority are unidentified.
- ANOVA simultaneous component analysis (ASCA) found 'urine time' factor significant.



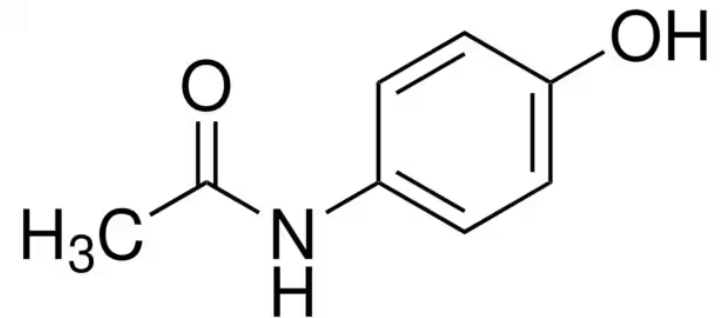
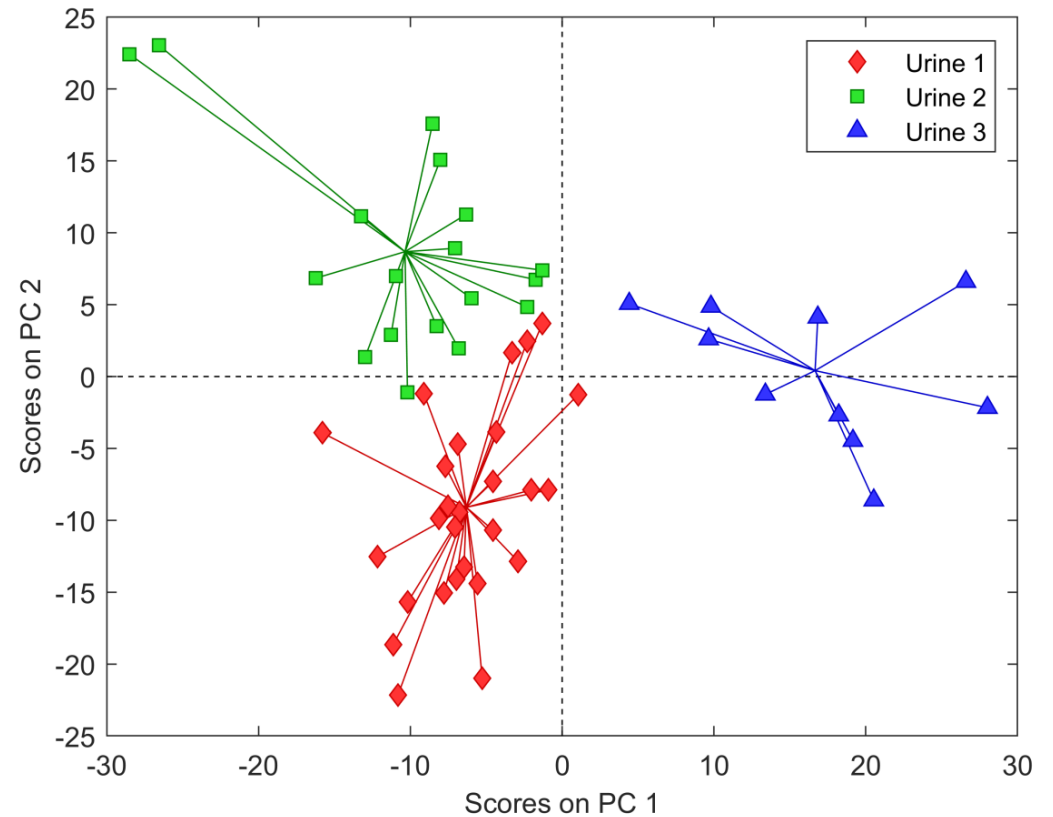
No cannabinoids?

- Cannabinoid and metabolite standards identified (high resolution TOF).
- Target analyte find from BT data negative.
- None of these common metabolites identified in exposed urines.
- Metabolites trapped in glucuronide form from phase-II metabolism.
- β -Glucuronidase assay.




Acetaminophen?

- Acetaminophen and metabolites identified in urines.
- Initially concerning to pediatrician, however only identified in urine 3.
- Urine 3 is post partum.
- Mothers consuming as post partum analgesic.



Conclusions and future directions

- First urinary metabolomics results from cannabis exposure study.
 - Differentiated metabolites between classes.
 - Many metabolites are not putatively identified.
 - Only cannabichromene identified.
 - Acetaminophen identified only in post partum urines.
- 
- The illustration shows a stylized grey silhouette of a person holding a baby. To the left is a white pill bottle with a green cap and a cannabis leaf on the label. To the right is a blue baby bottle with a pink cap and a yellow nipple. A large green cannabis leaf is positioned behind the person and baby.
- Hypothesize metabolites are (1) unidentified and/or (2) trapped in glucuronide form.
 - Develop reliable β -Glucuronidase assay for GC \times GC-TOFMS metabolomics.
 - As more samples are analyzed, investigate each urine time independently.
 - Analyze breast milk and passive samplers.

Coming July 2025...

- Starting as Assistant Professor at University of Victoria (UVic), Victoria, British Columbia.
- Research program will focus on multidimensional liquid chromatography for untargeted metabolomics.
- Environmental metabolomics, wildlife.
- Chemometrics and data.
- Chromatography.
- Recruiting grad students.
- Open to collaborate!



Funding and Support



**UNIVERSITY
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Healthy



Baby Brains

UVIC



**NSERC
CRSNG**



CIHR IRSC

Canadian Institutes of Health Research
Instituts de recherche en santé du Canada



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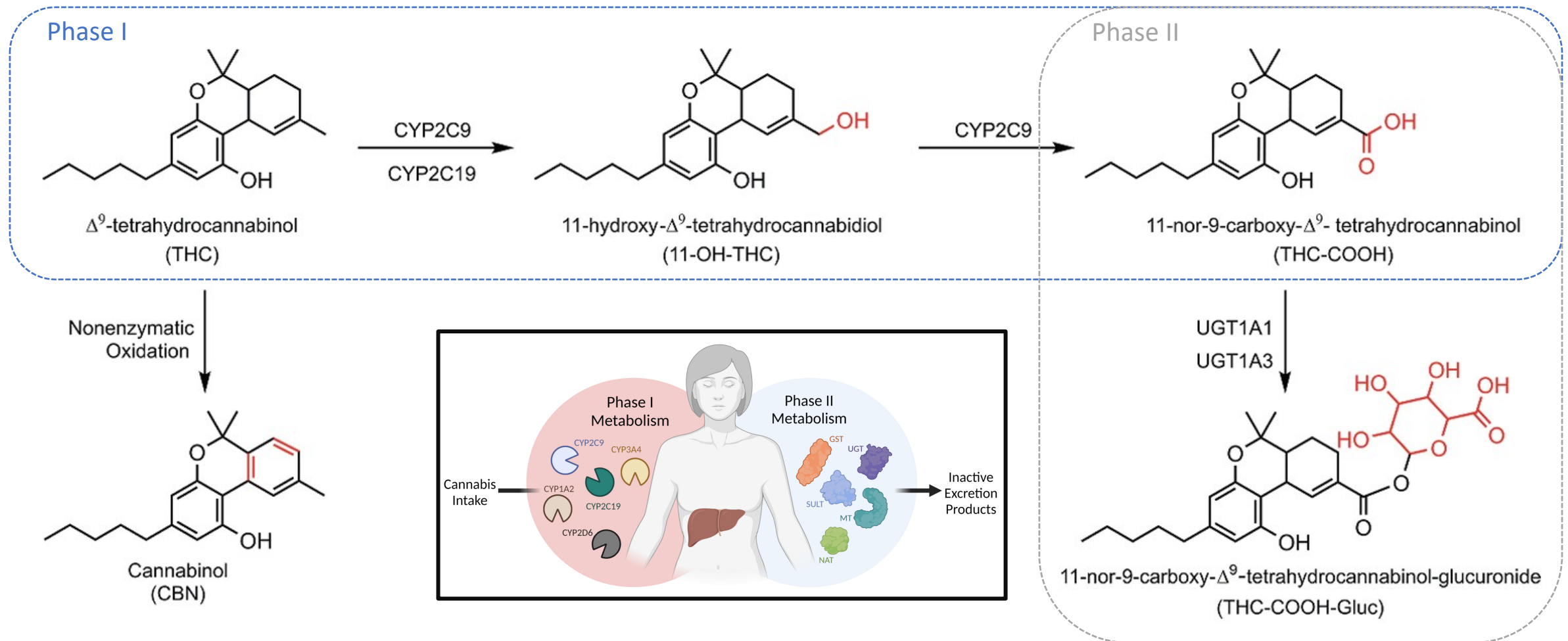


Contact:

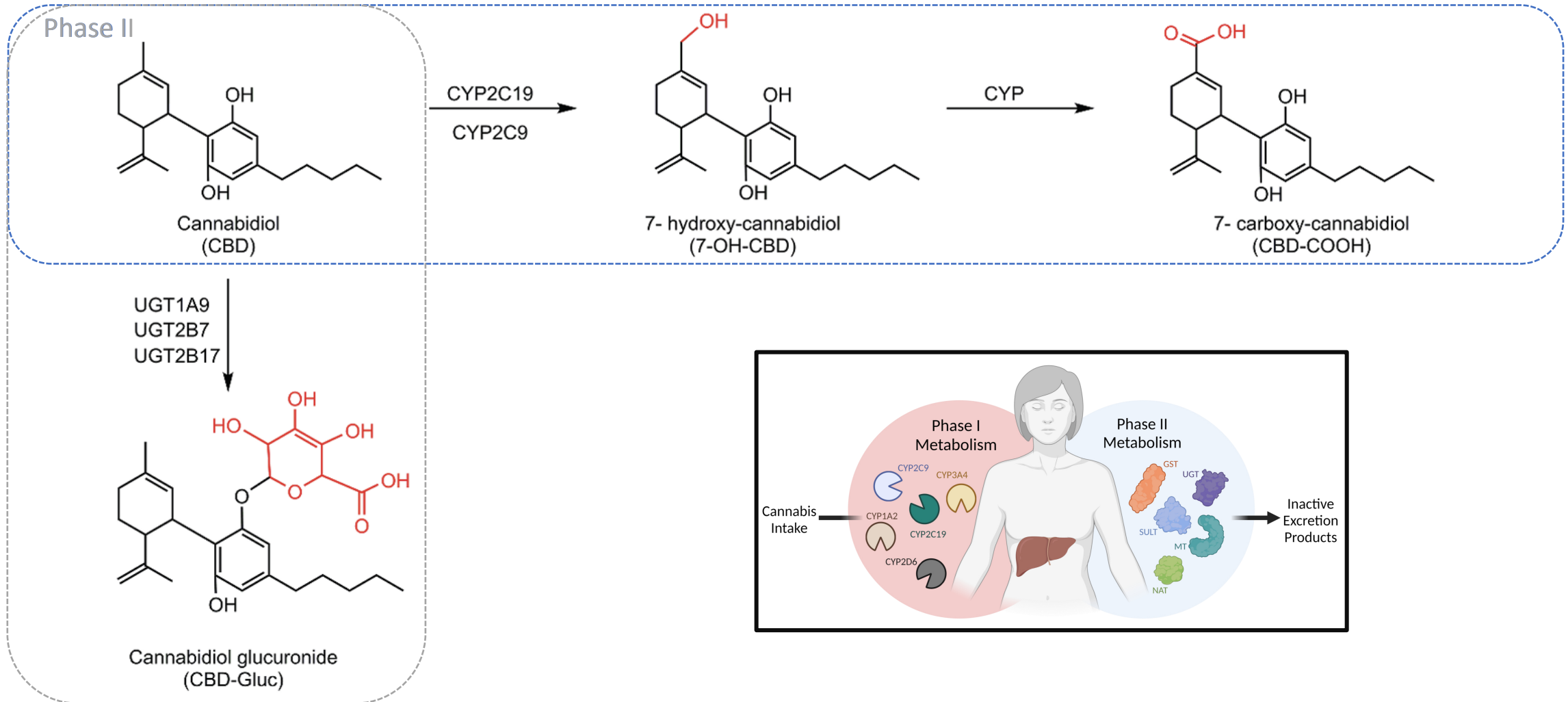
rgiebelhaus@uvic.ca



THC metabolism

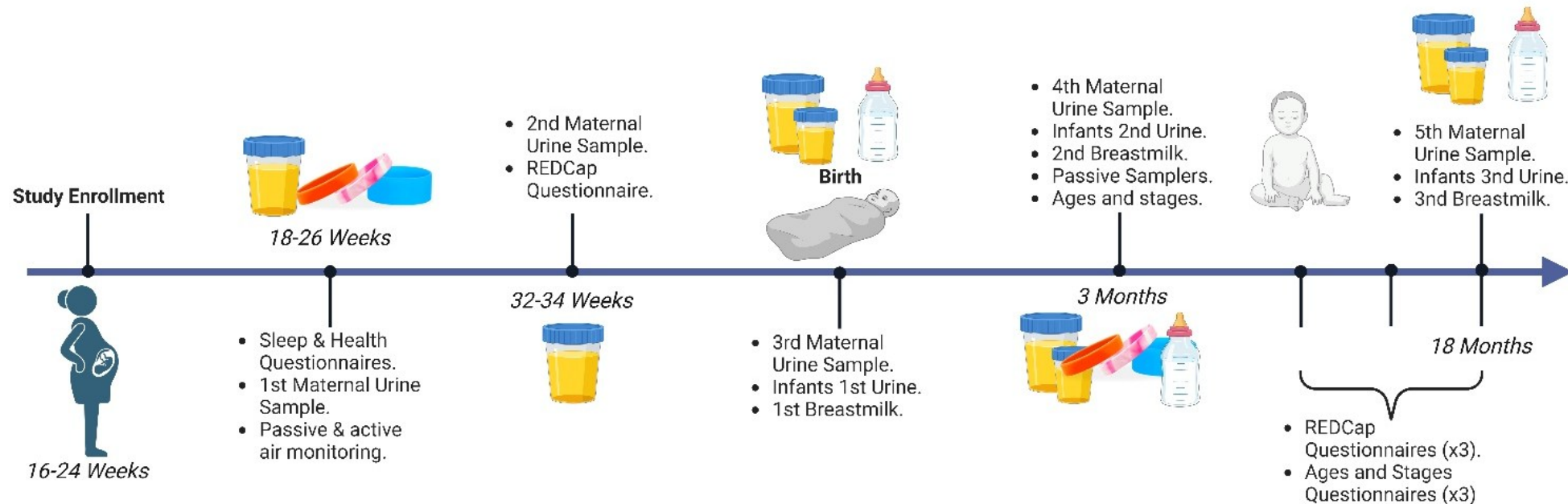


CBD metabolism



Healthy baby brains

- Mother wears Oura Ring to measure sleep and vital signs.
- Active air monitoring in house.
- Questionnaires at multiple stages for cannabis exposures and developmental milestones.



Cannabis exposure matrix

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