U.S. FOOD & DRUG ADMINISTRATION

Human Foods Program
Office of Chemistry and Toxicology

Transcriptomic point-of-departure derivation for hemp extract and four major cannabinoids using a human iPSC-derived hepatotoxicity model

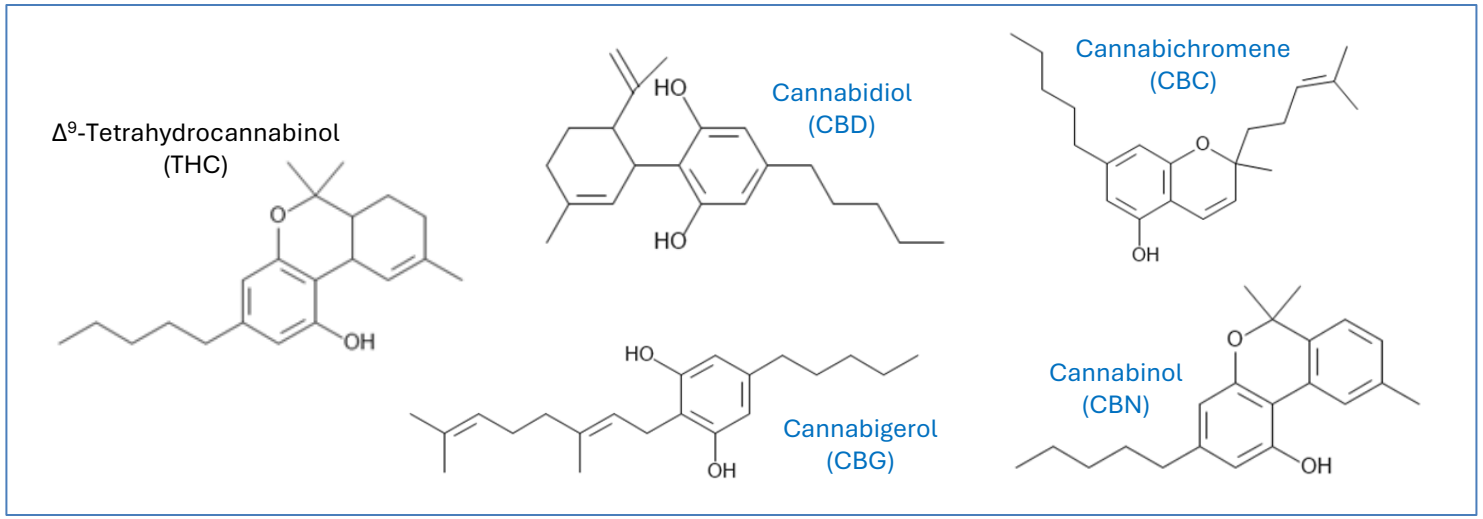
Xiugong Gao, Ph.D.

ASCCT Webinar: Advancing Cannabis Safety Assessments with New Approach Methodologies

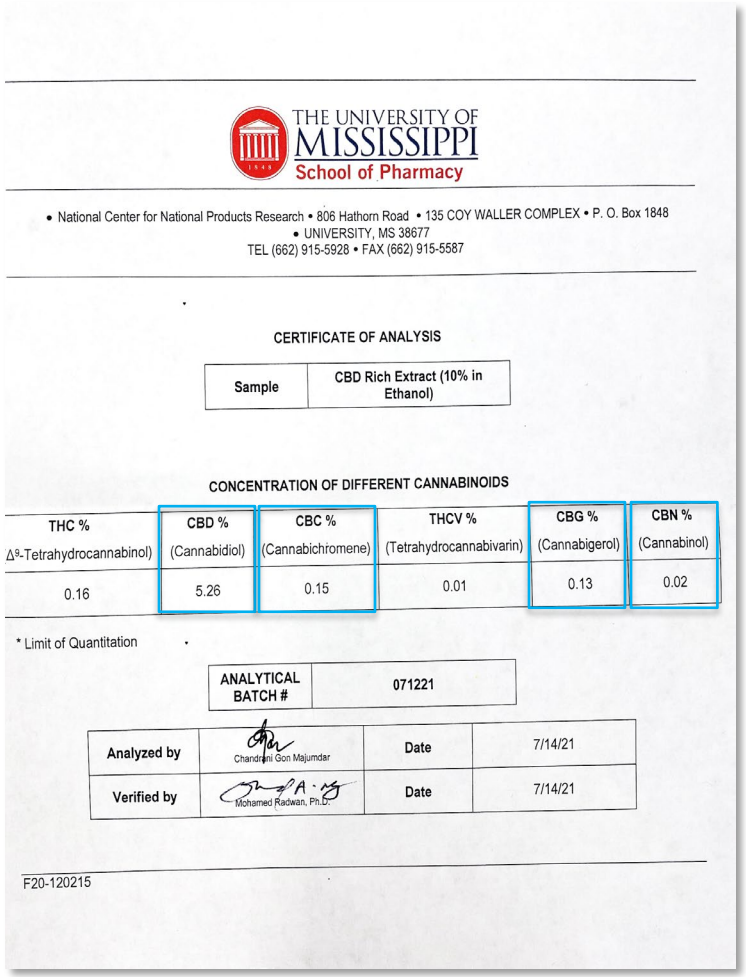
Friday, February 27, 2026

Hemp and Cannabinoids: Some Relevant Information

- Hemp is a class of botanical cultivars of the *Cannabis sativa* L. plant with ≤0.3% by dry weight of Δ^9-THC, grown specifically for industrial or medicinal use.
- 500+ compounds have been found in *Cannabis,* including 120+ (phyto)cannabinoids, non-cannabinoid phenols, flavonoids, terpenes, alkaloids, and others.
- Most cannabinoids are C21 terpenophenolic compounds primarily produced and isolated from resins of the female cannabis plants.



Composition of a CBD-rich hemp extract used in the study



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CERTIFICATE OF ANALYSIS

Sample	CBD Rich Extract (10% in Ethanol)

CONCENTRATION OF DIFFERENT CANNABINOIDS

THC % (Δ9-Tetrahydrocannabinol)	CBD % (Cannabidiol)	CBC % (Cannabichromene)	THCV % (Tetrahydrocannabivarin)	CBG % (Cannabigerol)	CBN % (Cannabinol)
0.16	5.26	0.15	0.01	0.13	0.02

* Limit of Quantitation

ANALYTICAL BATCH #	071221

Analyzed by	Chandrani Gon Majumdar	Date	7/14/21
Verified by	Mohamed Radwan, Ph.D.	Date	7/14/21

F20-120215

CBD Hepatotoxicity: Clinical Trials

- FDA has only approved Epidiolex, which contains purified CBD from hemp, to treat seizures associated with Lennox-Gastaut syndrome, Dravet syndrome, and tuberous sclerosis complex.
- In clinical trials for Epidiolex, 5-20% of patients taking the drug experienced elevated liver enzymes, with a few were withdrawn due to severe elevations of ALT/AST (>3 × upper limit of normal range, ULN).

FDA approved Epidiolex for the treatment of two rare and severe forms of epilepsy on June 25, 2018

CBD Hepatotoxicity: Early Studies

Animal Studies

- Marx et al. 2018 (90-day rats)
- Dziwenka et al. 2020 (90-day rats)
- EMA 2019 (26-week rats)
- Ewing et al. 2019 (10-day mice)
- EMA 2019 (90-day mice)
- EMA 2019 (39-week dogs)
- Vaughn et al. 2020 (28-day dogs)
- Rosenkrantz et al. 1981 (90-day monkeys)

Human Studies (healthy participants)

- Watkins et al. 2021 (27-day)
- Taylor et al. 2020 (4-week)
- Crippa et al. 2021(4-week)

» Animal studies with rats, mice, dogs and rhesus monkeys consistently showed increases in liver weights, hypertrophy of liver cells, and increases in liver enzymes or bilirubin. The pattern was different in different species and with different CBD preparations.

» Clinical studies with healthy volunteers showed increases in liver enzymes ALT and AST, and in some cases also ALP and GGT.

» A point of departure (POD) could not be established from the (then) available studies.

A report by European Food Safety Authority (EFSA)

STATEMENT

EFSA Journal

ADOPTED: 26 April 2022

doi: 10.2903/j.efsa.2022.7322

Statement on safety of cannabidiol as a novel food: data gaps and uncertainties

EFSA Panel on Nutrition, Novel Foods and Food Allergens (NDA), Dominique Turck, Torsten Bohn, Jacqueline Castenmiller, Stefaan De Henauw, Karen Ildico Hirsch-Ernst, Alexandre Maciuk, Inge Mangelsdorf, Harry J McArdle, Androniki Naska, Carmen Pelaez, Kristina Pentieva, Alfonso Siani, Frank Thies, Sophia Tsabouri, Marco Vinceti, Francesco Cubadda, Thomas Frenzel, Marina Heinonen, Rosangela Marchelli, Monika Neuhäuser-Berthold, Morten Poulsen, Miguel Prieto Maradona, Josef Rudolf Schlatter, Viviana Trezza, Henk van Loveren, Océane Albert, Céline Dumas, Andrea Germini, Wolfgang Gelbmann, Georges Kass, Eirini Kouloura, Estefania Noriega Fernandez, Annamaria Rossi and Helle Katrine Knutsen

Abstract

The European Commission has determined that cannabidiol (CBD) can be considered as a novel food (NF), and currently, 19 applications are under assessment at EFSA. While assessing these, it has become clear that there are knowledge gaps that need to be addressed before a conclusion on the safety of CBD can be reached. Consequently, EFSA has issued this statement, summarising the state of knowledge on the safety of CBD consumption and highlighting areas where more data are needed. Literature searches for both animal and human studies have been conducted to identify safety concerns. Many human studies have been carried out with Epidyolex®, a CBD drug authorised to treat refractory epilepsies. In the context of medical conditions, adverse effects are tolerated if the benefit outweighs the adverse effect. This is, however, not acceptable when considering CBD as a NF. Furthermore, most of the human data referred to in the CBD applications investigated the efficacy of Epidyolex (or CBD) at therapeutic doses. No NOAEL could be identified from these studies. Given the complexity and importance of CBD receptors and pathways, interactions need to be taken into account when considering CBD as a NF. The effects on drug metabolism need to be clarified. Toxicokinetics in different matrices, the half-life and accumulation need to be examined. The effect of CBD on liver, gastrointestinal tract, endocrine system, nervous system and on psychological function needs to be clarified. Studies in animals show significant reproductive toxicity, and the extent to which this occurs in humans generally and in women of child-bearing age specifically needs to be assessed. Considering the significant uncertainties and data gaps, the Panel concludes that the safety of CBD as a NF cannot currently be established.

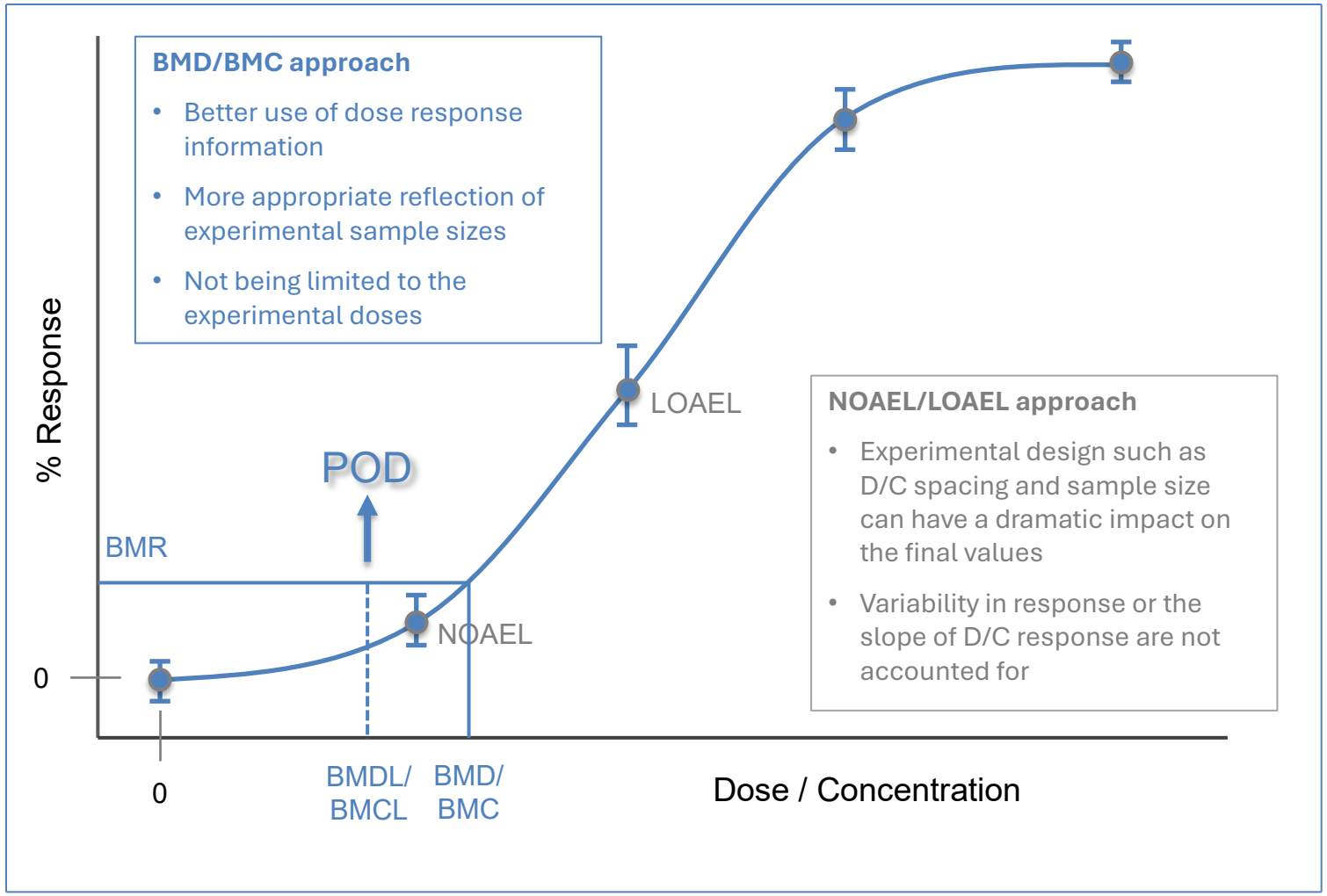
Keywords: Cannabidiol, Novel Food, safety, data gaps

Requestor: European Commission

... studies are necessary both in humans and in experimental animals that enable the identification of a point of departure ...

NDA et al. EFSA J. 2022; 20(6):e07322.

Point of Departure (POD): What is it, and what is it for?



» **Point of departure** (**POD**): the transition point between a safe, low dose and the dose that starts to cause adverse effect. Traditionally, NOAEL (or LOAEL) was used. More recently, BMD(L) is used.

» **Reference dose** (**RfD**): an estimate of a daily oral or dermal exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime.

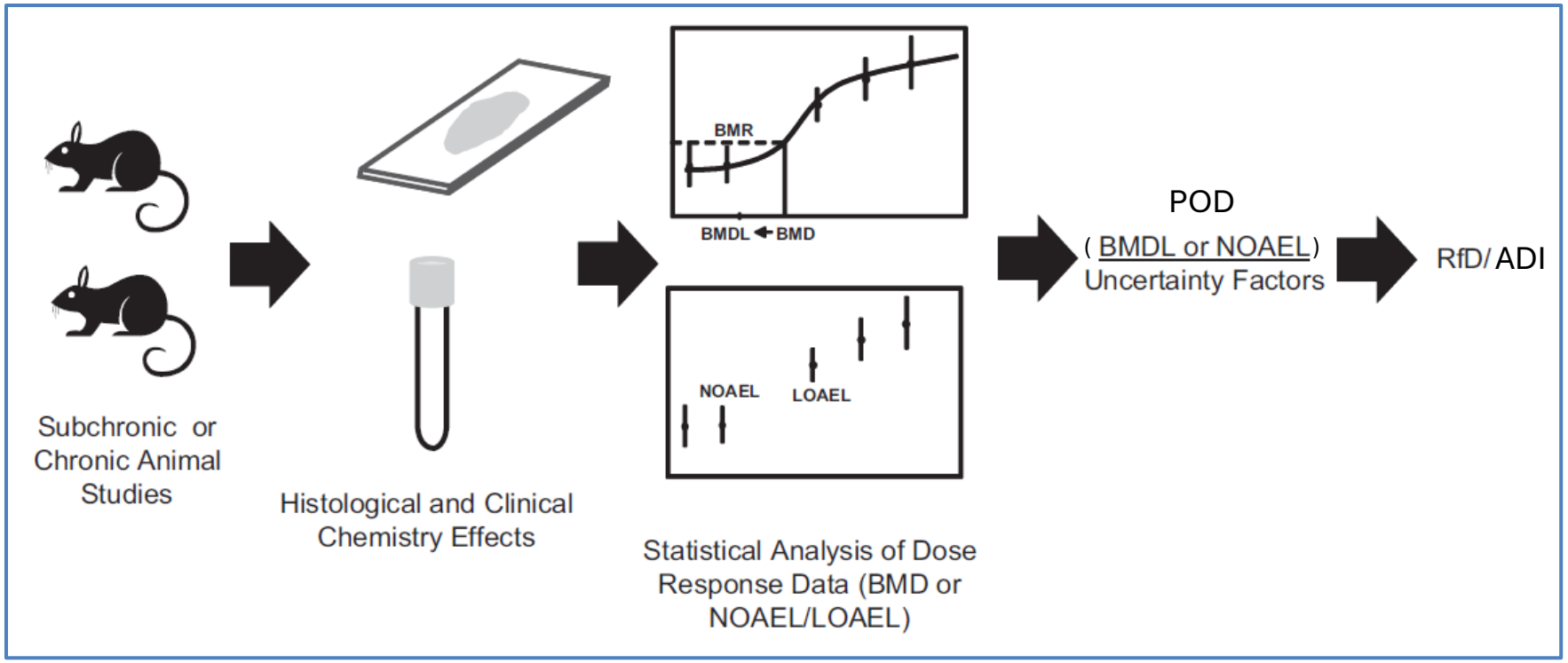
» **Acceptable daily intake** (**ADI**): the amount of a chemical to which a person can be exposed on a daily basis over an extended period of time (usually a lifetime) without suffering a deleterious effect.

$$RfD \text{ or } ADI = \frac{Point\ of\ Departure\ (POD)}{UF1 * UF2 * UF3 * UF4 * MF}$$

UF1: human population variability (10)
UF2: animal to human extrapolation (10)
UF3: sub-chronic to chronic (10, default 1)
UF4: using LOAEL instead of NOAEL or BMD (10, default 1)
MF: additional modifying factors such data quality (0 < MF <=10)

Source: ChemSafetyPRO

Traditional Animal Studies: apical POD



- Long durations:
  - Subchronic: 1–3 months
  - Chronic: 6–12+ months
- Large numbers of animals
- Need inter-species extrapolation

Adapted from: Thomase RS et al, *Mutat Res* 2012; 746:135-143.

Transcriptomic POD (tPOD) for Risk Assessment

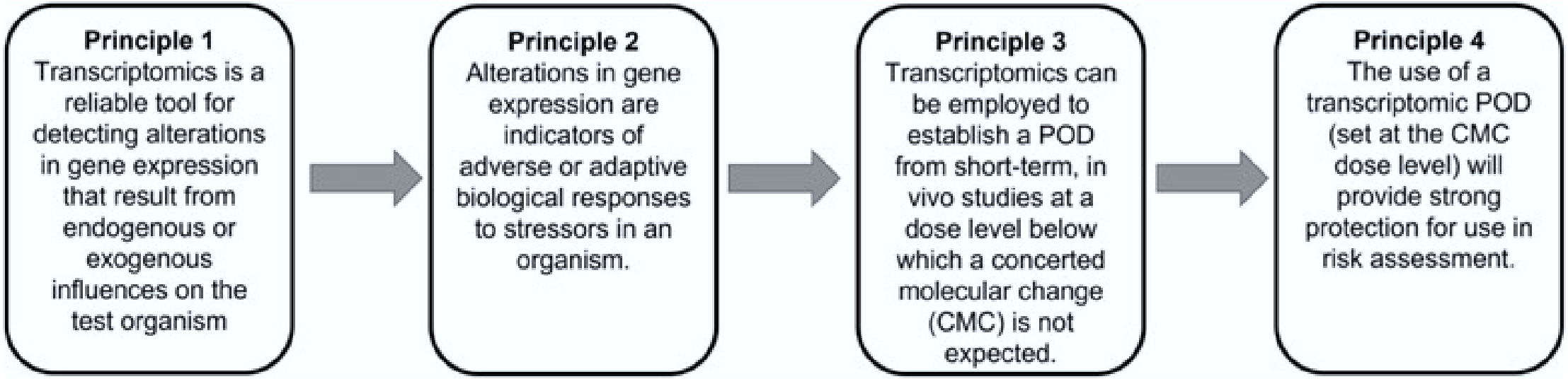
- Implementation of *pathway agnostic* tPODs in risk assessment to refine and replace the current apical endpoint-based regulatory toxicity testing paradigm

FORUM

A Transformative Vision for an Omics-Based Regulatory Chemical Testing Paradigm

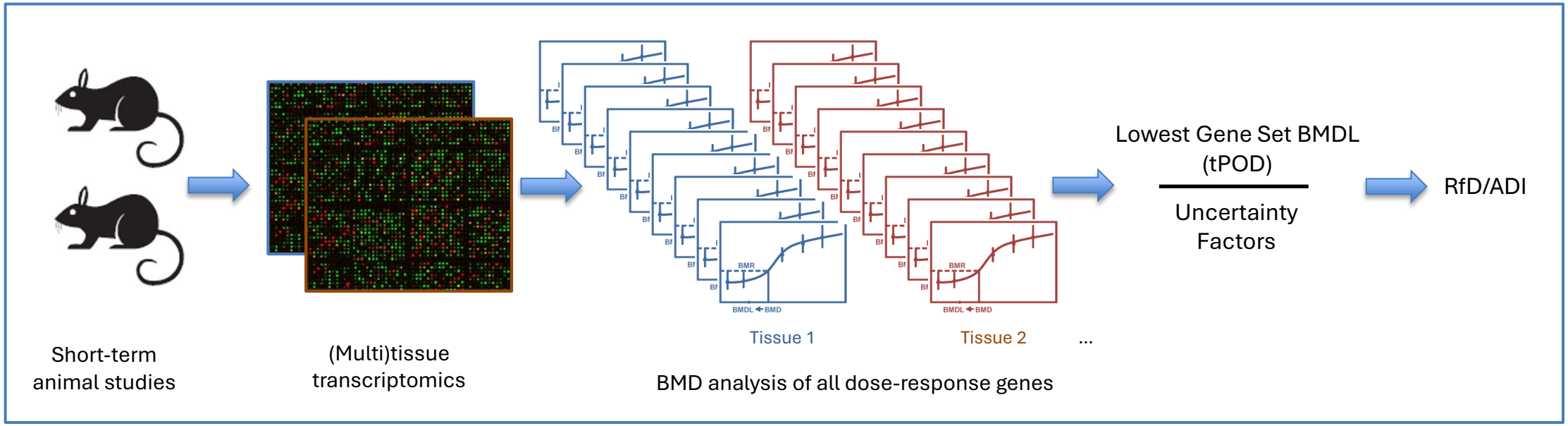
Kamin J. Johnson,* Scott S. Auerbach,† Tina Stevens,‡ Tara S. Barton-Maclaren,§ Eduardo Costa,* Richard A. Currie,¶ Deidre Dalmas Wilk,‖ Saddef Haq,‖ Julia E. Rager,‖ Anthony J. F. Reardon,§ Leah Wehmas,# Andrew Williams,§ Jason O'Brien,** Carole Yauk,†† Jessica L. LaRocca,* and Syril Pettit‖1

Foundational Principles:



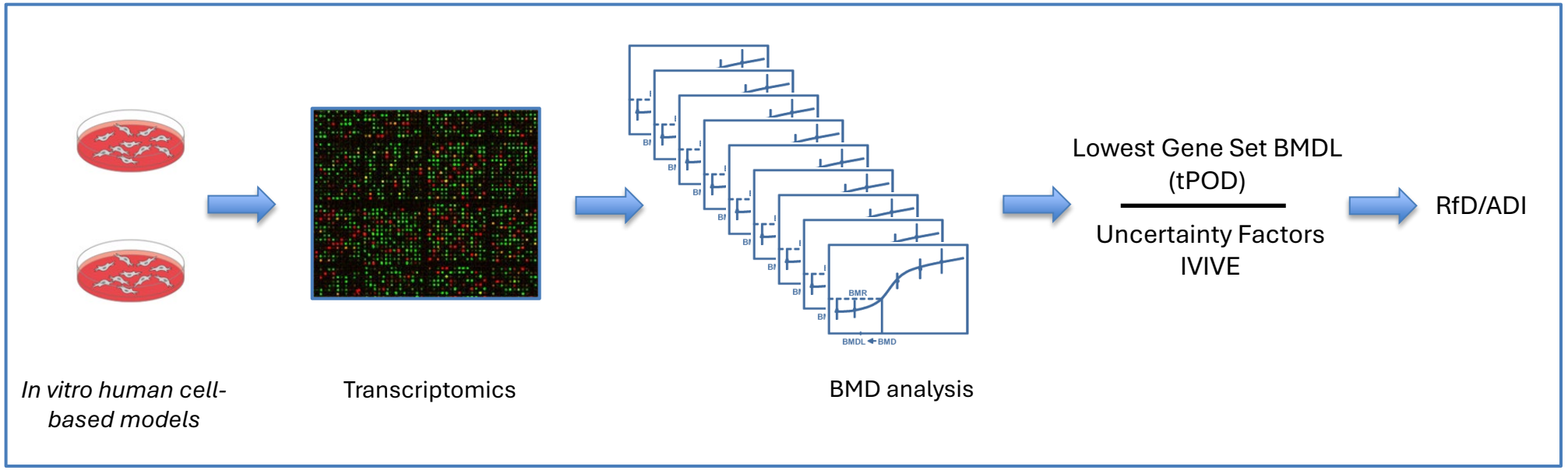
Johnson KJ et al. *Toxicol Sci* 2022; 190: 127–132.

In Vivo Transcriptomic Studies: tPOD



- Significantly reduces experimental duration and number of animals used for the study
- Animal study
- Need inter-species extrapolation

In Vitro Transcriptomic Studies: tPOD

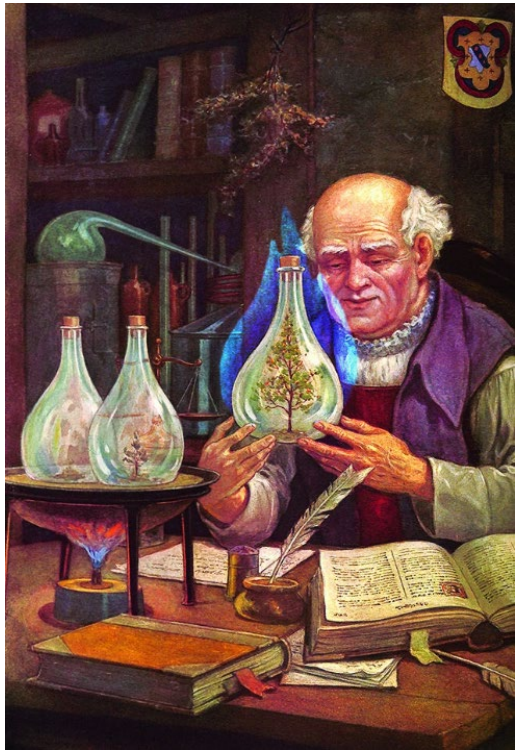


- No need to use animals – NAM compatible
- No need for inter-species extrapolation
- Need *in vitro* to *in vivo* extrapolation (IVIVE)

Objectives

To use a toxicogenomics approach to investigate liver toxicity of hemp extract and four major constituent cannabinoids (CBD, CBC, CBG, and CBN) in a human *in vitro* hepatotoxicity model to:

- Derive tPOD values
- Explore mechanisms of action

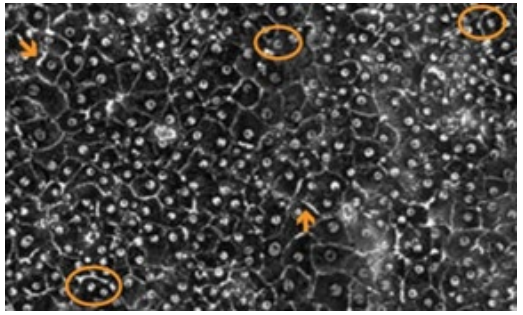


All things are poison and nothing is without poison; only the dose makes a thing not a poison.
- Paracelsus (1493 – 1541)

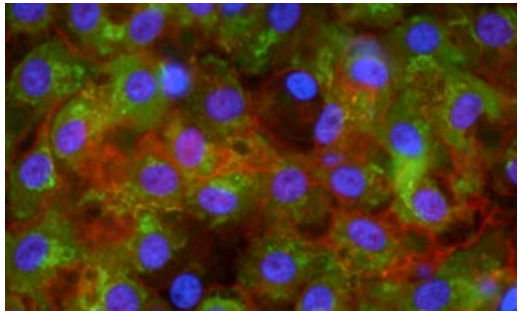
In Vitro Hepatotoxicity Model

» Hepatocytes derived from human induced pluripotent stem cells (iPSCs) – *iCell Hepatocytes 2.0* (iHep) from Fujifilm Cellular Dynamics, Inc. (FCDI)

Cobblestone morphology, binucleation, and bile canaliculi formation

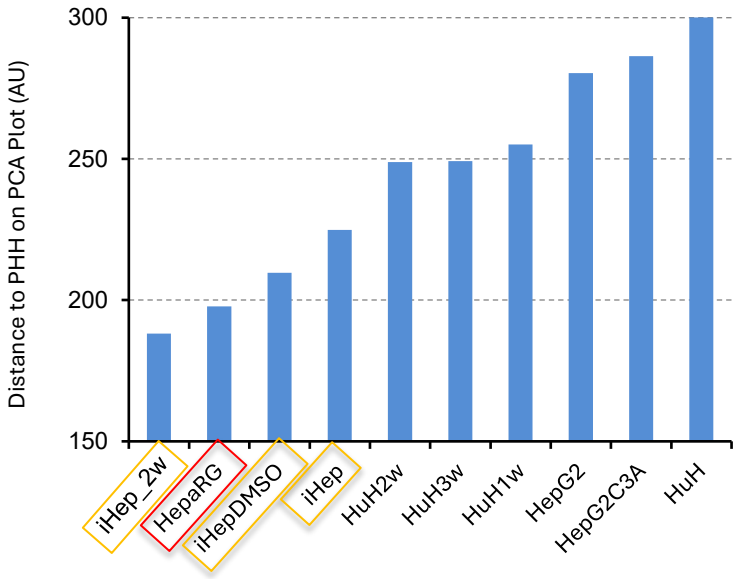


Expression of major hepatic makers



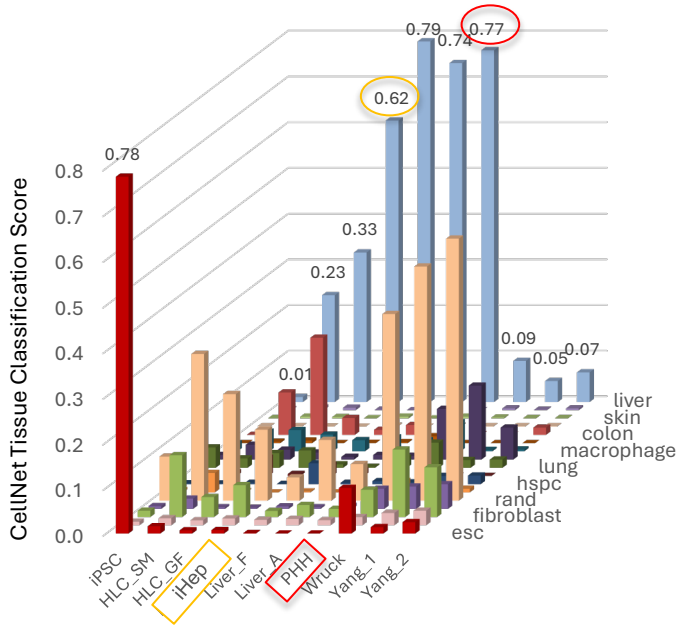
Source: FCDI

iHep displayed transcriptomic similarity to HepaRG; more similar to PHH than other hepatoma cell lines.



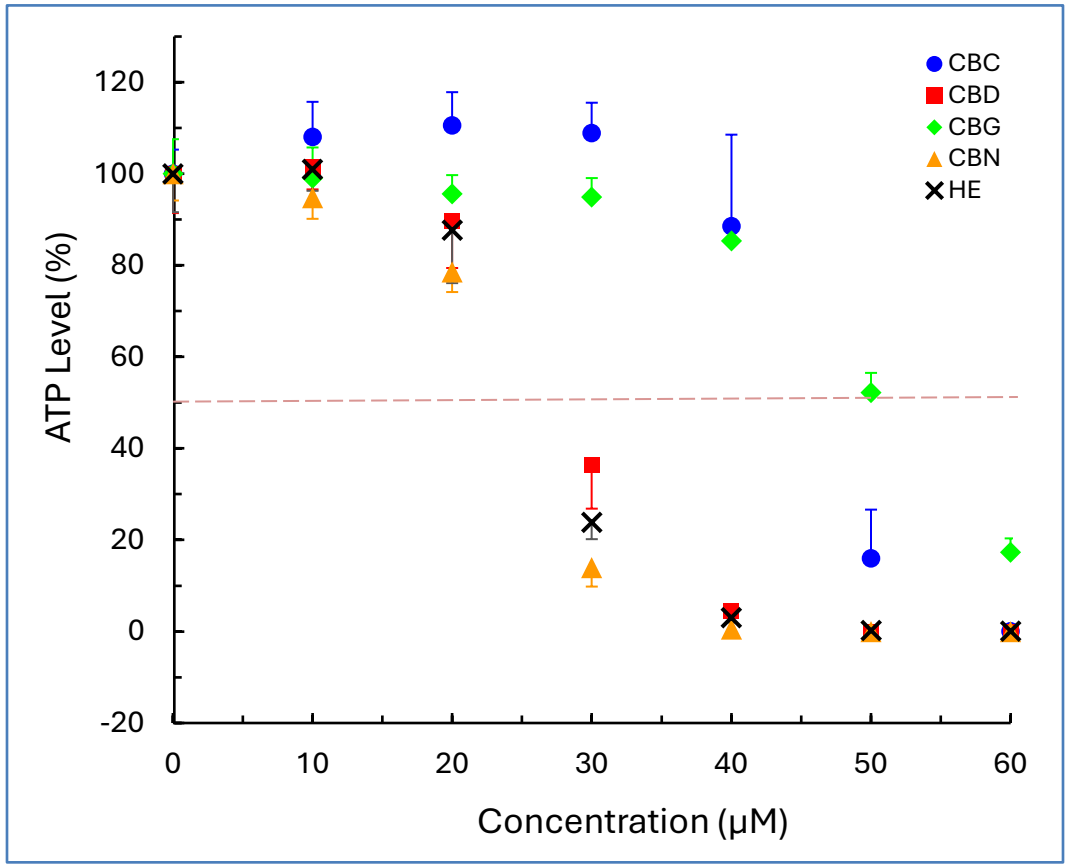
Gao X & Liu Y. *Cell Biol Toxicol* 2017; 33(4): 407-421.

CellNet tissue (liver) classification showed iHep with >80% similarity to PHH



Gao X et al. *Stem Cell Res Ther* 2020; 11(1): 393.

Cytotoxicity of Cannabinoids and Hemp Extract (HE)



	IC_{50} (μM)	IC_{10} (μM)
CBN	24.0	17.5
CBD	27.7	20.2
CBC	44.5	39.8
CBG	52.5	37.1
HE	25.8	19.4

Concentrations (μM) used for the transcriptomics study

0, 0.1, 0.5, 1.0, 5.0, 10, 20

0, 0.1, 0.5, 1.0, 5.0, 10, 20

0, 0.1, 0.5, 1.0, 5.0, 10, 20, 40

0, 0.1, 0.5, 1.0, 5.0, 10, 20, 40

0, 0.1, 0.5, 1.0, 5.0, 10, 20

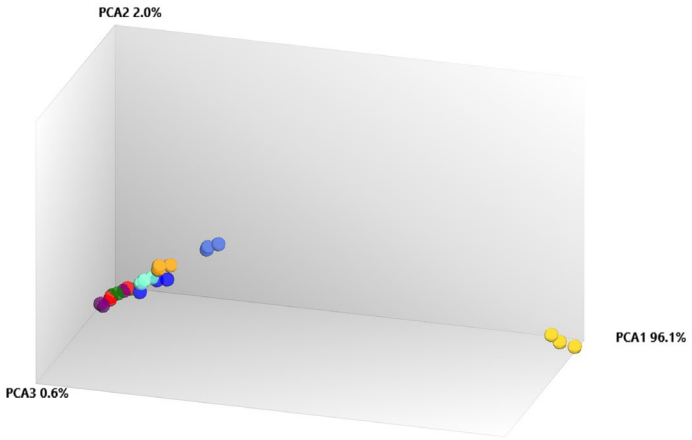
cytotoxicity potency ranking:
CBN > CBD > CBC > CBG

highest [C] for transcriptomics study

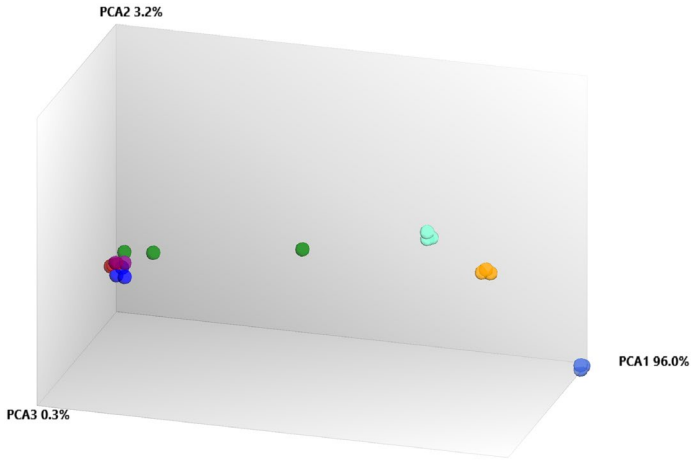
Gao X et al, *Toxicology* 2024; 506:153885.

Concentration-response Transcriptomic Changes: PCA Plot

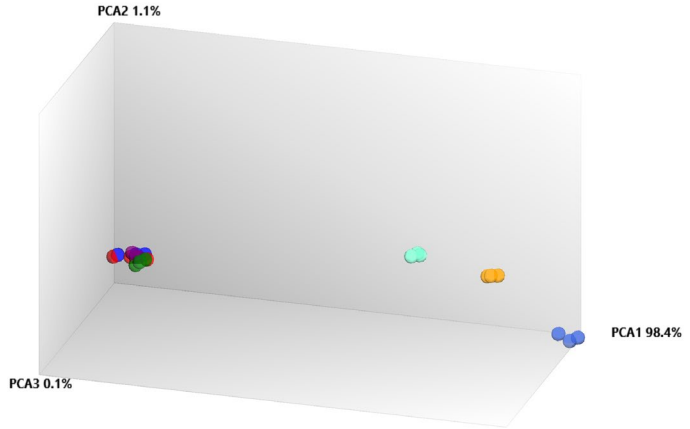
CBC



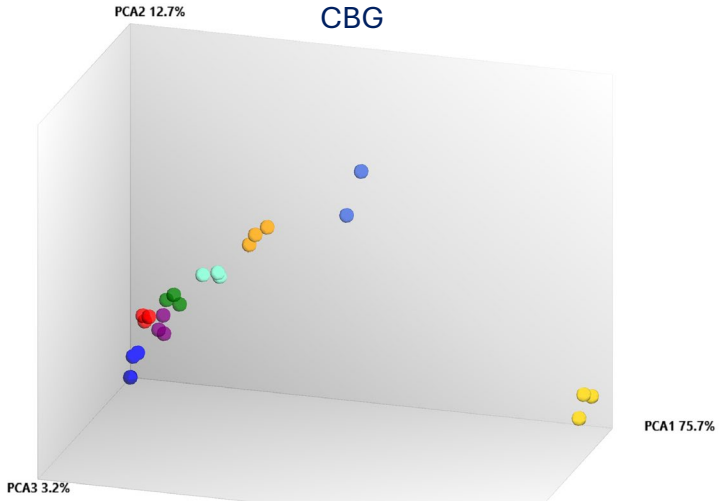
CBD



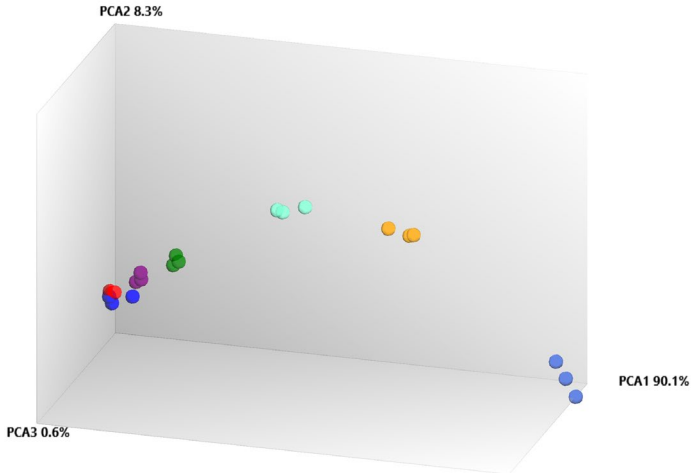
HE



CBG



CBN

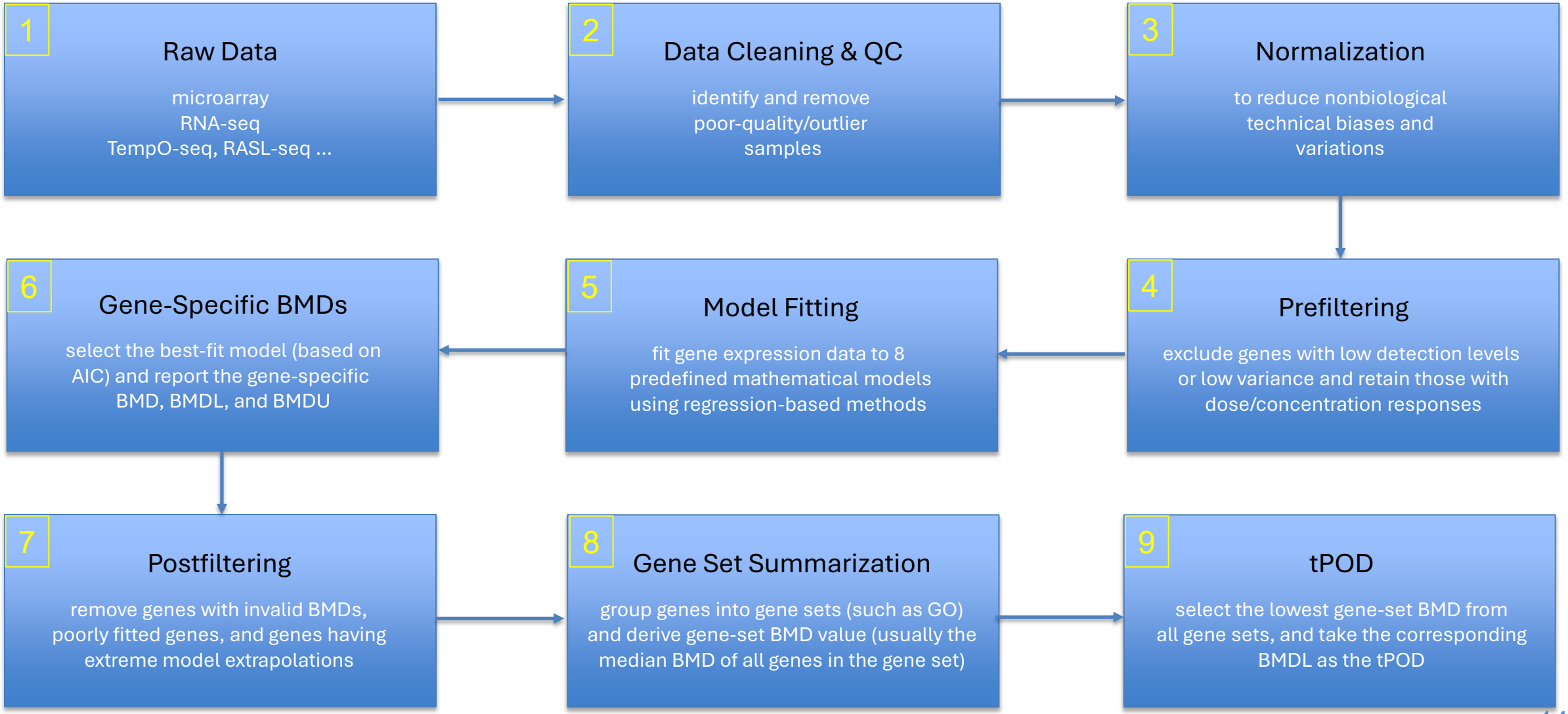


Conc. (μM)

- 0.0
- 0.1
- 0.5
- 1.0
- 5.0
- 10
- 20
- 40

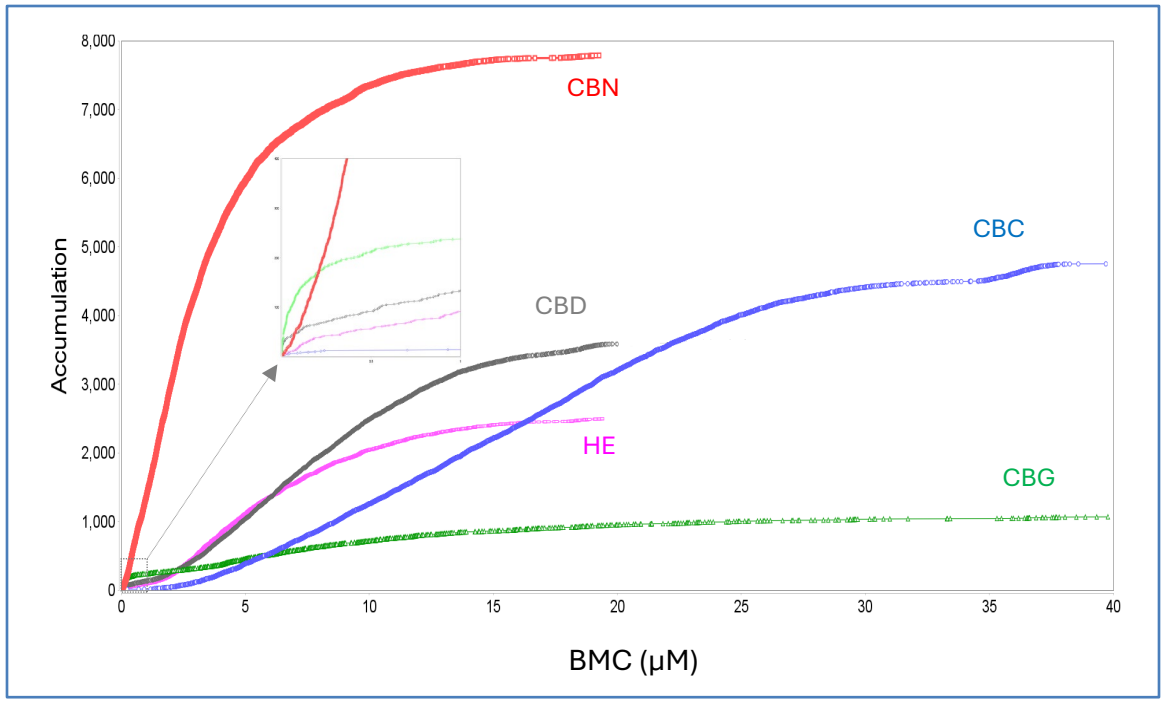
Gao X et al. *Toxicology* 2024; 506:153885.

Bioinformatics Workflow Using *BMDExpress*

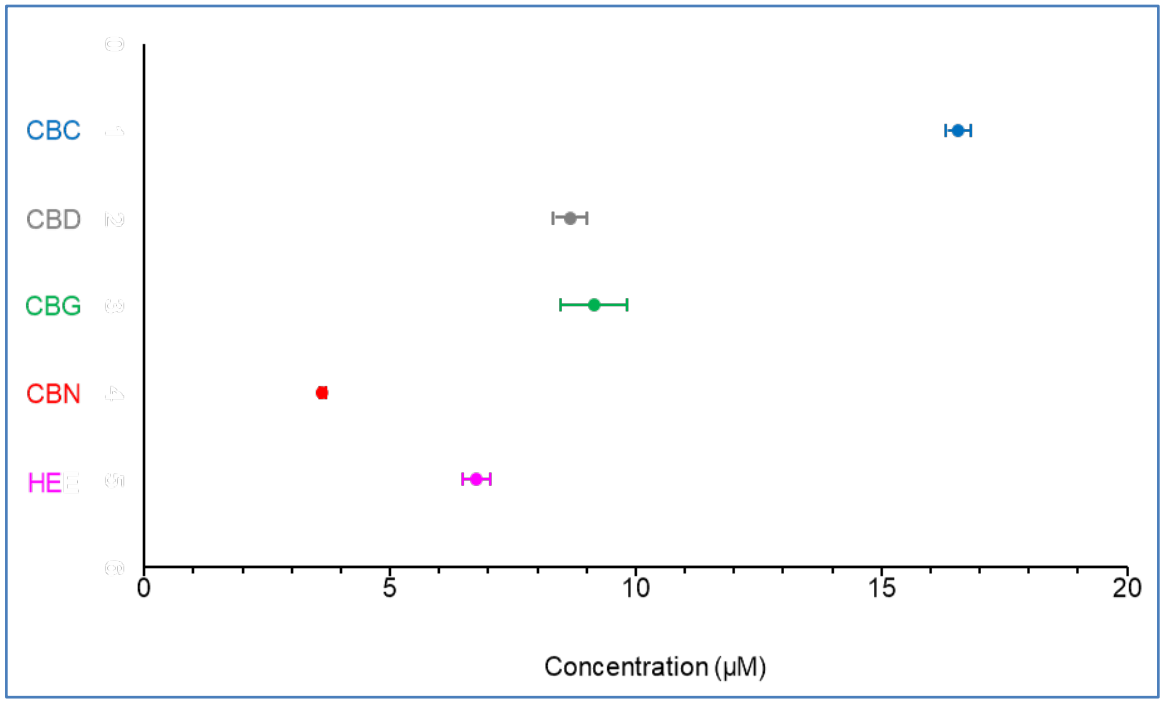


Benchmark Concentration (BMC) Modeling

BMC Accumulation Plot



Median Overall BMC (w/ 95% confidence intervals)



$$\textit{transcriptomic potency} = \frac{\textit{\# modeled genes (probesets)}}{\textit{highest concentration * median BMC}}$$

CBN (5.12) > CBD (1.00) > CBC (0.34) > CBG (0.14)

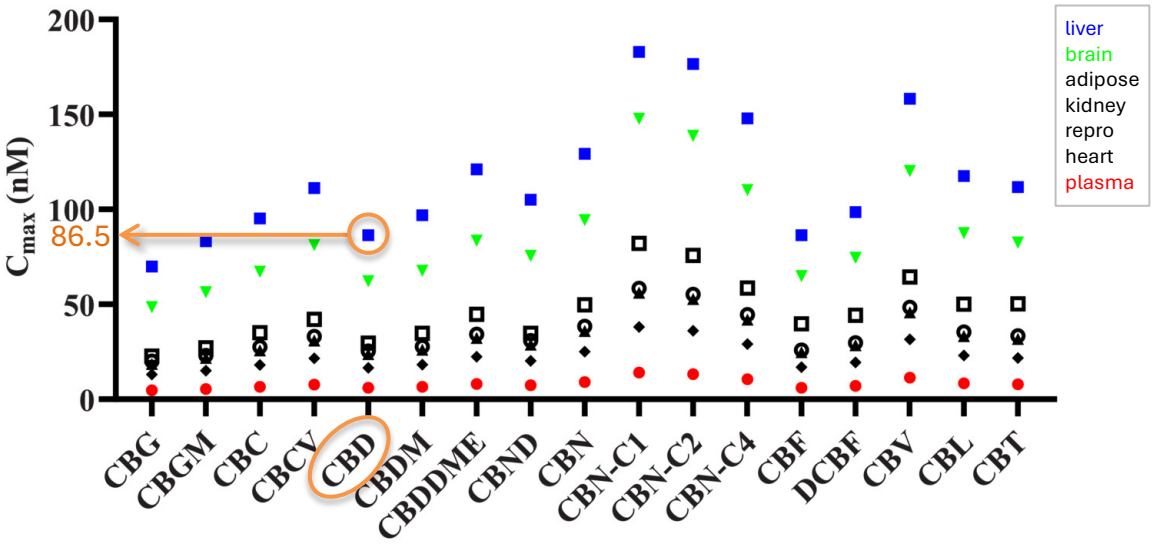
Gao X et al. *Toxicology* 2024; 506:153885.

tPOD Values – How reliable Are They?

Compound		tPOD (μM)
CBC	Pathway Agnostic	1.346
CBD		0.106
CBG		0.046
CBN		0.141
Hemp extract		0.094

ADI
0.49 mg/kg-bw

PBPK modeling and simulation of cannabinoids in human plasma and tissues following 0.4 mg/kg-bw oral administration



Liu Y & Sprando RL. *J Appl Toxicol* 2022; 43: 589-598.

Cannabidiol safety considerations: Development of a potential acceptable daily intake value and recommended upper intake limits for dietary supplement use

Rayetta G. Henderson [a,*], Melissa Vincent [b], Brianna N. Rivera [c], Marcel O. Bonn-Miller [d,e], Candace Doepker [b]

ABSTRACT

Consumer use of hemp-derived products continues to rise, underscoring the need to establish evidence-based safety guidance. The present study sought to develop recommendations for oral upper intake limits of cannabidiol (CBD) isolate. Sufficiently robust and reliable data for this purpose were identified from published human clinical trials and guideline-compliant toxicity studies in animal models. Based on the metrics used in this assessment, a potential Acceptable Daily Intake (ADI) value of 0.43 mg/kg-bw/d (e.g., 30 mg/d for 70-kg adult) was determined for the general population based on liver effects in human studies. This value applies to the most sensitive subpopulations, including children, over a lifetime of exposure and from all sources, including food. For dietary supplements with adequate product labeling intended for use by healthy adults only, a potential Upper Intake Limit (UL) of 70 mg/d was determined based on reproductive effects in animals. For healthy adults, except those trying to conceive, or currently pregnant or lactating, a conservative dietary supplement UL of 100 mg/d was identified based on liver effects; however, as the target population excludes individuals at risk for liver injury, an alternative dietary supplement UL of 160 mg/d for this population can also be considered.

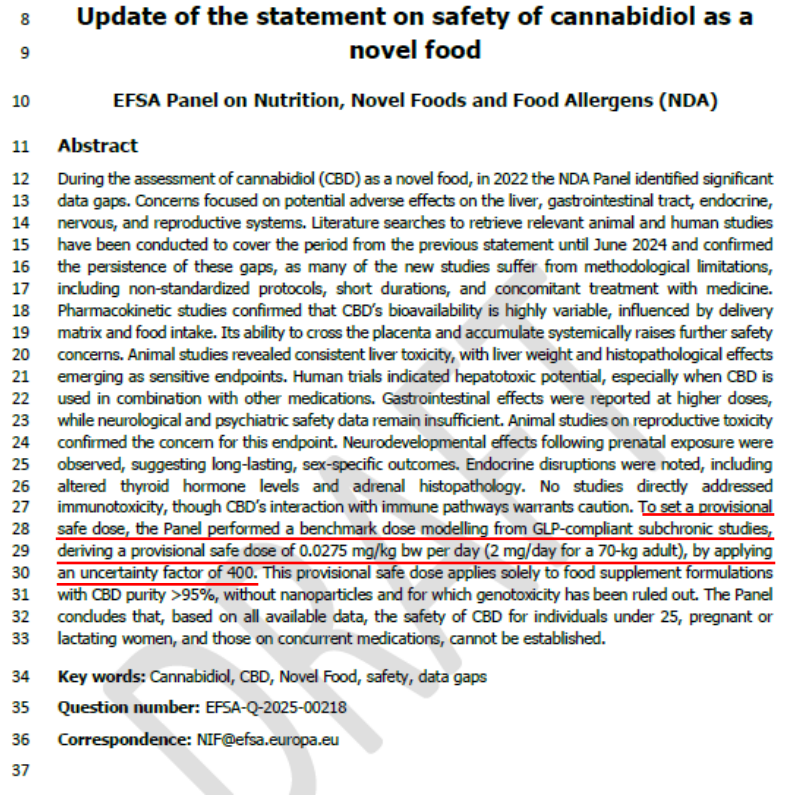
Henderson RG et al. *Reg Toxicol Pharmacol* 2023; 144: 105482

EFSA Proposed ADI of 2 mg/day for CBD

- In September 2025, EFSA released its updated safety assessment on CBD (as a Novel Food), concluding that a daily intake of >2 mg CBD/person could not currently be considered safe for the general population.
- Old UK Limit (Pre-2023): 70 mg/day; New UK Limit (Post-2023): 10 mg/day

Key Points of EFSA's New Stance:

- *Proposed Limit*: 2 mg daily for an average adult (70 kg) – 0.0275 mg/kg bw.
- *Method*: BMD modeling of 5 GLP-compliant 90-day subchronic studies in rats, applying an uncertainty factor of 400.
- *Reasoning*: Persistent data gaps in toxicology, particularly liver, gastrointestinal, nervous, endocrine, and reproductive systems.
- *Vulnerable Groups*: Safety cannot be established for those under 25, pregnant/lactating individuals, or those taking other medicines.



Update of the statement on safety of cannabidiol as a novel food

EFSA Panel on Nutrition, Novel Foods and Food Allergens (NDA)

Abstract

During the assessment of cannabidiol (CBD) as a novel food, in 2022 the NDA Panel identified significant data gaps. Concerns focused on potential adverse effects on the liver, gastrointestinal tract, endocrine, nervous, and reproductive systems. Literature searches to retrieve relevant animal and human studies have been conducted to cover the period from the previous statement until June 2024 and confirmed the persistence of these gaps, as many of the new studies suffer from methodological limitations, including non-standardized protocols, short durations, and concomitant treatment with medicine. Pharmacokinetic studies confirmed that CBD's bioavailability is highly variable, influenced by delivery matrix and food intake. Its ability to cross the placenta and accumulate systemically raises further safety concerns. Animal studies revealed consistent liver toxicity, with liver weight and histopathological effects emerging as sensitive endpoints. Human trials indicated hepatotoxic potential, especially when CBD is used in combination with other medications. Gastrointestinal effects were reported at higher doses, while neurological and psychiatric safety data remain insufficient. Animal studies on reproductive toxicity confirmed the concern for this endpoint. Neurodevelopmental effects following prenatal exposure were observed, suggesting long-lasting, sex-specific outcomes. Endocrine disruptions were noted, including altered thyroid hormone levels and adrenal histopathology. No studies directly addressed immunotoxicity, though CBD's interaction with immune pathways warrants caution. To set a provisional safe dose, the Panel performed a benchmark dose modelling from GLP-compliant subchronic studies, deriving a provisional safe dose of 0.0275 mg/kg bw per day (2 mg/day for a 70-kg adult), by applying an uncertainty factor of 400. This provisional safe dose applies solely to food supplement formulations with CBD purity >95%, without nanoparticles and for which genotoxicity has been ruled out. The Panel concludes that, based on all available data, the safety of CBD for individuals under 25, pregnant or lactating women, and those on concurrent medications, cannot be established.

Key words: Cannabidiol, CBD, Novel Food, safety, data gaps

Question number: EFSA-Q-2025-00218

Correspondence: NIF@efsa.europa.eu

Functional Comparison Across the Four Cannabinoids

Canonical Pathways	CBC	CBN	CBD	CBG
Semaphorin Neuronal Repulsive Signaling Pathway	1.512	2.496	1.069	1.633
FXR/RXR Activation	1.511	2.333	2.03	0.775
Cholecystokinin/Gastrin-mediated Signaling	1.147	1.387	2.138	1
Erythropoietin Signaling Pathway	1.89	1.342	2.041	0.302
LPS/IL-1 Mediated Inhibition of RXR Function	2.746	1.508	1	0
Thrombin Signaling	1.3	2	1.698	0
Warburg Effect Signaling Pathway	0.667	2.041	1.671	0
NRF2-mediated Oxidative Stress Response	1.606	2.837	1.606	n/a
mTOR Signaling	1.342	1.886	2	n/a
Gα12/13 Signaling	2.065	1.5	1.291	n/a
Regulation of Cellular Mechanics by Calpain Protease	2.333	0.816	1.633	n/a
Immunogenic Cell Death Signaling Pathway	2.333	0.632	1.342	n/a
Angiopoietin Signaling	2.53	1	0.632	n/a
14-3-3-mediated Signaling	2	1.414	0.707	n/a
PI3K/AKT Signaling	2.5	2	1.508	-0.816
HER-2 Signaling in Breast Cancer	1.89	2.558	1.4	-1.89
Oxytocin Signaling Pathway	0.801	2.2	0.174	-0.577
Glioma Invasiveness Signaling	0.655	2.53	0.333	-1
Proteasomal PSMD10 Signaling Pathway	1.606	2.5	0.258	-2.121
Hepatic Cholestasis	2.021	1.219	0.164	-1.265
Role of Tissue Factor in Cancer	1.151	0.898	1.061	-2.121
Thyroid Cancer Signaling	0.775	1.667	0.258	-2.236
Gap Junction Signaling	1.131	2.921	0	-2.5
Actin Nucleation by ARP-WASP Complex	1.265	2	0	n/a
Role of MAPK Signaling in Promoting the Pathogenesis of Influenza	1.633	0	1.387	2
Unfolded protein response	1	-0.632	2.646	2
Protein Sorting Signaling Pathway	2.268	-1.069	2.065	0
Ceramide Signaling	2	0	1.134	n/a
GADD45 Signaling	0.333	-1.633	0.632	2
Neutrophil Extracellular Trap Signaling Pathway	2.1	0.426	-0.209	-1
Endocannabinoid Cancer Inhibition Pathway	-0.756	-1.387	0.2	2.309
NF-κB Activation by Viruses	1.291	0.707	0	-2
Virus Entry via Endocytic Pathways	1.5	2.333	-1.291	-2.646
RAC Signaling	1.147	1.265	-0.577	-2
Integrin Signaling	0.928	2.183	-1.177	-2.333
IL-8 Signaling	1.715	0.655	-0.18	-2.673
Chronic Myeloid Leukemia Signaling	0.295	1.234	0.174	-2.309
Tumor Microenvironment Pathway	1.061	1.342	-0.73	-2.714
NAD Signaling Pathway	-1.342	-2.183	0.243	1.134
Hepatitis B Chronic Liver Pathogenesis Signaling Pathway	0.539	0.218	-1	-2.111
Pulmonary Healing Signaling Pathway	1.061	0.775	-0.898	-3.317
PAK Signaling	0.775	0	-1.155	-2
Molecular Mechanisms of Cancer	0.862	1.068	-0.632	-4.004
Epithelial Membrane Protein Signaling Pathway	0.343	-0.218	-0.845	-2.333
S100 Family Signaling Pathway	0.422	0.156	-0.717	-2.921
Caveolar-mediated Endocytosis Signaling	0.707	0.447	-1.941	-2.449
SPINK1 General Cancer Pathway	-1.069	0.447	-2.714	n/a
HEY1 Signaling Pathway	-0.5	0.832	-2.132	-2.449
Leukocyte Extravasation Signaling	0.6	-0.535	-1.789	-2.828
Pulmonary Fibrosis Idiopathic Signaling Pathway	-0.525	0.822	-1.857	-3.53
Sertoli Cell-Sertoli Cell Junction Signaling	-1.109	0.73	-2.694	-2.84
Regulation of the Epithelial Mesenchymal Transition by Growth Factors Pathway	-0.174	0	-0.928	-3
Role of IL-17A in Psoriasis	n/a	-1	-2	-2
Sirtuin Signaling Pathway	-1.372	-3.053	-0.378	1.508
Multiple Sclerosis Signaling Pathway	-1.976	-2.449	-1.225	0.302
DHCR24 Signaling Pathway	-2.897	-2.043	-2.449	0.632
GABAergic Receptor Signaling Pathway (Enhanced)	-1.414	-2.065	-1.886	0
LXR/RXR Activation	-2.335	-2.4	-1.606	0
Intrinsic Prothrombin Activation Pathway	-3.051	-2.828	-2.449	n/a
Colorectal Cancer Metastasis Signaling	-0.539	-0.243	-0.756	-2.121
Irritable Bowel Syndrome Signaling Pathway	-0.762	-0.6	-2.121	-1
Cardiac Hypertrophy Signaling (Enhanced)	-0.372	-1.093	-1.414	-2
Autism Signaling Pathway	-0.98	-1.061	-2.058	-1.387
Cyclophilin Signaling Pathway	-1.265	-0.816	-1.043	-2.673
IL-17A Signaling in Fibroblasts	-0.775	-2.333	-1	-2
Xenobiotic Metabolism PXR Signaling Pathway	-2.117	-1.528	-2.183	-0.447
Parkinson's Signaling Pathway	-2.064	-1.521	-2.197	-0.5
Pathogen Induced Cytokine Storm Signaling Pathway	-2.101	-2.058	-1.206	-0.943
Xenobiotic Metabolism CAR Signaling Pathway	-2.646	-1	-2.236	-0.447
Kinetochore Metaphase Signaling Pathway	-1.508	-1.134	-1.698	-2.236
Nuclear Cytoskeleton Signaling Pathway	-0.365	-0.229	-3.024	-3.5
Role of JAK2 in Hormone-like Cytokine Signaling	-1.732	-2.714	-1.291	-2
Atherosclerosis Signaling	-1.961	-2.5	-2.138	-1.667

Nuclear Receptor	CBC	CBN	CBD	CBG
RARA	-3.592	-2.453	-4.52	-1.01
ESR1	-0.44	-0.924	-1.75	-2.272
NR4A1	-0.799	-0.923	-2.479	-0.494
NR5A2	-2.158	-0.613	-1.277	-0.186
RXRG	-2.111	-1.342	-0.816	0
NR4A3	-1.714	-2.602	-1.723	0.022
PPARD	-2.625	-3.311	-2.011	1.174
PPARG	-1.893	-1.715	0.442	2.952
THRB	-0.691	-1.564	0.191	2.781
PPARA	-1.49	1.293	0.175	2.067
NR1D1	-0.458	0.485	1.18	2.813
NR1I2	-0.153	0.83	2.24	1.35
NR1H4	-0.412	0.48	1.15	3.431
NR0B1	2.463	1.213	1.378	n/a
PGR	1.996	1.673	3.281	-0.2

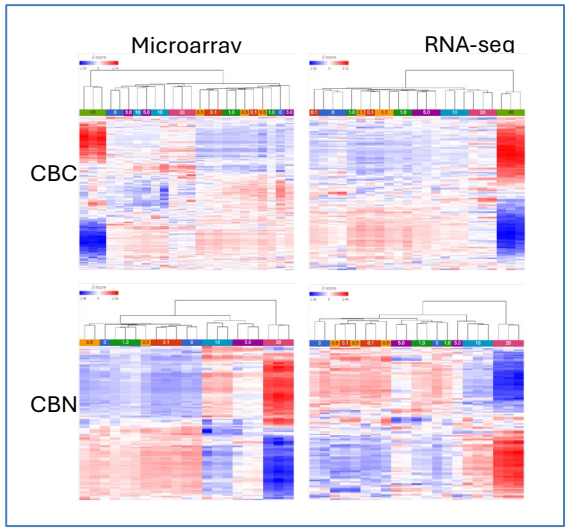
Diseases and Disorders	CBC	CBN	CBD	CBG
Invasive cancer	4.01	3.419	1.408	-3.273
Metastasis	3.912	3.389	1.547	-2.996
Advanced malignant tumor	3.885	3.323	1.508	-2.915
Cancer of cells	3.725	2.661	1.809	-2.537
Metastasis of cells	3.659	2.899	1.123	-2.985
Neoplasia of tumor cell lines	3.654	2.464	1.216	-2.93
Cell transformation	3.837	2.137	1.041	-3.078
Metastasis of tumor cell lines	3.461	2.777	0.992	-2.828
Neoplasia of cells	3.416	1.932	0.983	-2.681
Invasive tumor	4.053	3.42	1.46	n/a

Tox Functions	CBC	CBN	CBD	CBG
Hepatic steatosis	3.017	3.925	0.627	0.224
Liver tumor	2.248	1.983	1.275	-0.623
Activation of hepatic stellate cells	1.341	-0.563	-1.976	-2.138
Development of liver tumor	1.153	2.664	1.207	-0.78
Steatohepatitis	1.849	2.563	-0.508	-0.128
Development of hepatocellular carcinoma	0.758	2.406	0.82	-0.861
Liver Hypoplasia	n/a	-1.48	-2.562	n/a
Liver cancer	0.83	2.001	0.326	-0.495

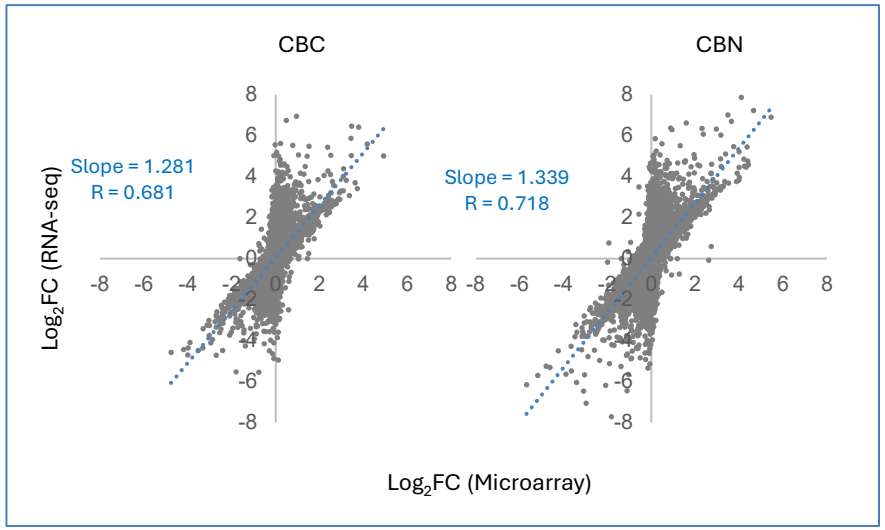
- Each compound was found to impact a unique list of canonical pathways, upstream regulators, diseases and disorders, toxicity functions, and networks with distinctive activation/inhibition patterns.
- All the four cannabinoids were predicted to affect metabolism and to have some beneficial effects on cardiovascular disease but adverse effects on the neural system.
- Similar to (but more potently than) CBD, CBC and CBN displayed liver toxicity and the potential to cause cancer, while CBG protected from these adverse effects.
- Further studies are necessitated to confirm these results and to fully understand the mechanisms of action of the different cannabinoids.

Microarray *vs.* RNA-seq Comparison (for CBC and CBN)

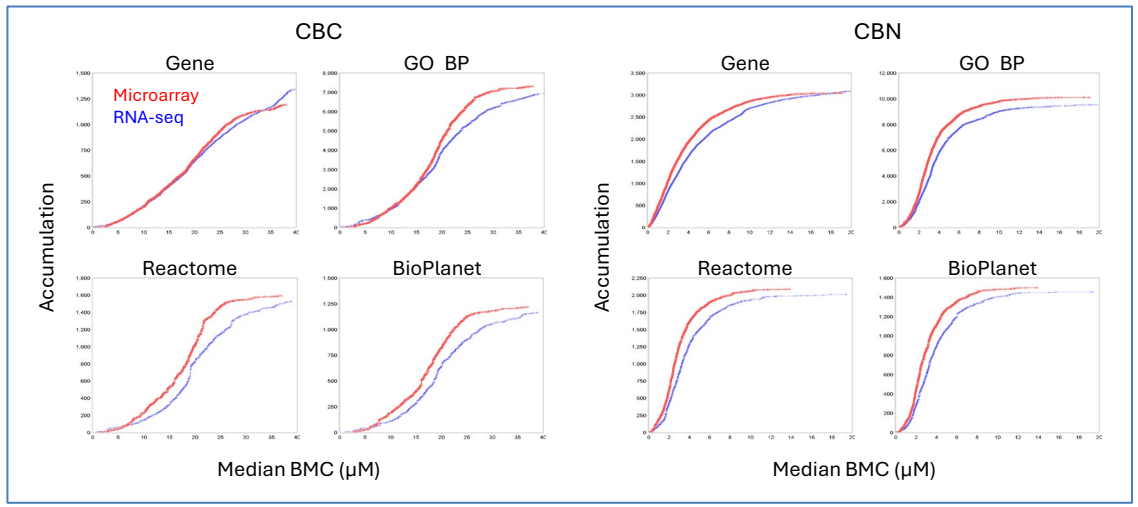
Similar overall expression patterns



Fold change (FC) correlation for the 18,376 common genes



BMC accumulation of functional categories



Very similar tPOD values were obtained between the two platforms for both CBC and CBN

Table 6 Comparison of tPoD values derived for CBC from microarray and RNA-seq*

GO/Pathway ID	GO/Pathway Name	# All Genes	# Genes	Percentage	BMC Median	BMCL Median	BMCU Median
Microarray							
GO:1901661 (5)	Quinone metabolic process	38	3	7.9	1.777	1.346	2.379
GO:0007586 (3)	Digestion	34	3	8.8	1.777	1.346	2.379
GO:0008207 (5) †	*C21-steroid hormone metabolic process*	*26*	*3*	*11.5*	*1.777*	***1.346***	*2.379*
R-HSA-156590	Glutathione conjugation	36	3	8.3	3.148	2.359	4.615
R-HSA-9818027	NFE2L2 regulating antioxidant/detoxification enzymes	17	3	17.7	3.148	2.359	4.615
1552	Glutathione conjugation	25	3	12.0	3.148	2.359	4.615
1739	Keap1-Nrf2 pathway	13	3	23.1	3.148	2.359	4.615
RNA-seq							
GO:0007586 (3)	Digestion	35	3	8.6	1.162	0.917	1.546
GO:0016137 (6)	Glycoside metabolic process	21	3	14.3	1.162	0.917	1.546
GO:0042448 (6)	Progesterone metabolic process	13	3	23.1	1.162	0.917	1.546
R-HSA-193368	Synthesis of bile acids and bile salts via 7alpha-hydroxycholesterol	24	3	12.5	1.162	0.917	1.546
848†	*Benzo(a)pyrene metabolism*	*9*	*3*	*33.3*	*1.162*	***0.917***	*1.546*

* Entry (-ies) with the lowest BMC median value in each category (GO_BP, Reactome, and BioPlanet) is (are) included. In many instances, multiple entries with equal BMC median values are included for each category. GO_BP ID starts with "GO:" followed by a number; Reactome ID starts with "R-HAS-" followed by a number; BioPlanet ID only contains a number. The numbers in the parentheses following a GO_BP ID indicate the GO level

† For multiple entries with equal lowest BMC median values, the one with the highest percentage (of genes) was selected as the tPoD defining biological process or pathway (highlighted in italic), and its BMCL median value is defined as the tPoD value (highlighted in bold)

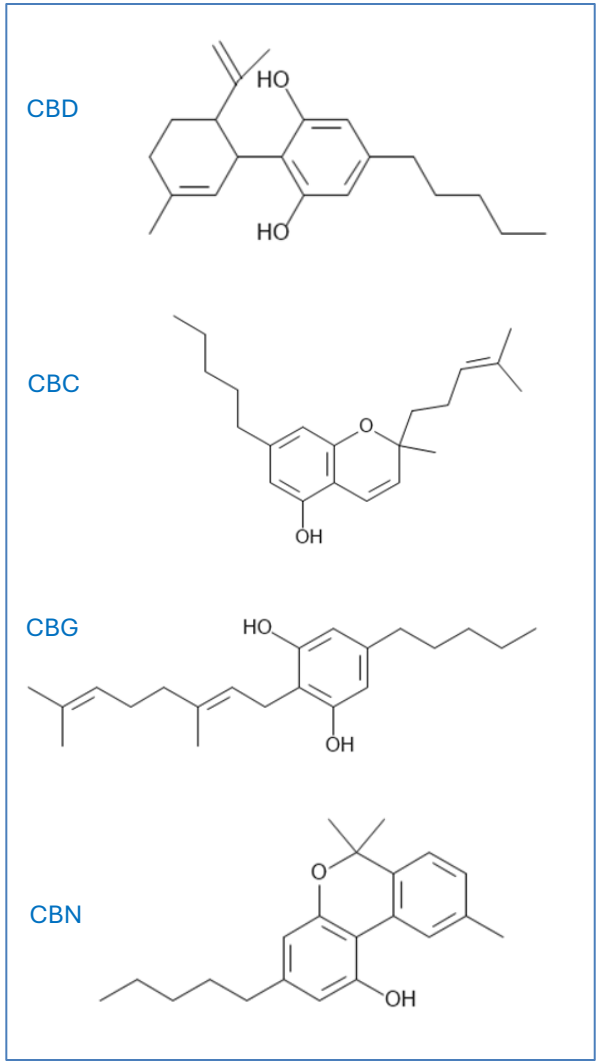
Table 7 Comparison of tPoD values derived for CBN from microarray and RNA-seq*

GO/Pathway ID	GO/Pathway Name	# All Genes	# Genes	Percentage	BMC Median	BMCL Median	BMCU Median
Microarray							
GO:0042448 (6)	Progesterone metabolic process	13	3	23.1	0.223	0.141	0.369
GO:1902644 (5)	Tertiary alcohol metabolic process	15	5	33.3	0.223	0.141	0.369
GO:0030638 (4)	Polyketide metabolic process	10	5	50.0	0.223	0.141	0.369
GO:0030647 (7)	Aminoglycoside antibiotic metabolic process	10	5	50.0	0.223	0.141	0.369
GO:0044598 (4)	Doxorubicin metabolic process	10	5	50.0	0.223	0.141	0.369
GO:0044597 (5)	Daunorubicin metabolic process	9	5	55.6	0.223	0.141	0.369
GO:0009753 (5)	Response to jasmonic acid	4	3	75.0	0.223	0.141	0.369
GO:0071395 (6) †	*Cellular response to jasmonic acid stimulus*	*4*	*3*	*75.0*	*0.223*	***0.141***	*0.369*
R-HSA-5336415	Uptake and function of diphtheria toxin	6	4	66.7	0.310	0.185	0.594
172	Acetylation and deacetylation of RelA in the nucleus	16	3	18.8	0.342	0.216	0.588
1598	NF-kappaB activation through FADD/RIP-1 pathway	12	3	25.0	0.342	0.216	0.588
RNA-seq							
GO:0032094 (4)	Response to food	24	4	16.7	0.342	0.153	0.931
R-HSA-9818027	NFE2L2 regulating antioxidant/detoxification enzymes	17	8	47.1	0.282	0.167	0.513
734†	*Nucleotide di- and triphosphate biosynthesis and interconversion*	*18*	*3*	*16.7*	*0.160*	***0.099***	*0.281*

Gao X et al, *BMC Genomics* 2025; 26(1):392.

Conclusions

- PODs have been derived for hemp extract and four major cannabinoids (CBD, CBC, CBG, and CBN) by BMC modeling of transcriptomic data from an *in vitro* hepatotoxicity model using human iPSC-derived hepatocytes.
- Transcriptomic potency (based on tPODs) of the cannabinoids was in the order of CBN > CBD > CBC > CBG, consistent with the order of their IC_{50} values.
- The tPOD value for CBD (0.106 µM) was concordant with a previously reported one derived from apical endpoints of clinical and animal studies, suggesting the validity of using tPOD data to inform human safety assessment of the cannabinoids.
- Each cannabinoid acted through a unique combination of biological processes and pathways with distinctive activation/inhibition patterns. Further studies are needed to fully understand the toxicity of these compounds.
- <u>The current work demonstrates the potential utility of transcriptomic BMC analysis as a NAM for hazard assessment of data-poor chemicals.</u>



Acknowledgements