Toxicology and Applied Pharmacology 460 (2023) 116377

Contents lists available at ScienceDirect

Toxicology and Applied Pharmacology

journal homepage: www.elsevier.com/locate/taap

Benzo[*a*]pyrene toxicokinetics in humans following dietary supplementation with 3,3′-diindolylmethane (DIM) or Brussels sprouts

Monica L. Vermillion Maier [a,b,g,*], Lisbeth K. Siddens [a,b], Jamie M. Pennington [a,b], Sandra L. Uesugi [a], Susan C. Tilton [b,c], Emily A. Vertel [a,b], Kim A. Anderson [b,c], Lane G. Tidwell [b], Ted J. Ognibene [d], Kenneth W. Turteltaub [d,e], Jordan N. Smith [c,f], David E. Williams [a,b,c]

[a] *Linus Pauling Institute, Oregon State University, Corvallis, OR 97331, USA*
[b] *Department of Environmental and Molecular Toxicology, Oregon State University, Corvallis, OR 97331, USA*
[c] *NIEHS Superfund Research Program, Oregon State University, Corvallis, OR 97331, USA*
[d] *Center for Accelerator Mass Spectrometry, Lawrence Livermore National Laboratory, Livermore, CA 94550, USA*
[e] *Biology and Biotechnology Research Division, Lawrence Livermore National Laboratory, Livermore, CA 94550, USA*
[f] *Chemical Biology and Exposure Science, Pacific Northwest National Laboratory, Richland, WA 99354, USA*
[g] *Department of Environmental and Molecular Toxicology, ALS 1007, Oregon State University, Corvallis, OR 97331, USA*

A R T I C L E I N F O

Editor: Lawrence Lash

Keywords:
Benzo[*a*]pyrene
Brussels sprouts
3,3'-diindolylmethane
Human carcinogen dosing
Accelerator mass spectrometry
Toxicokinetics

A B S T R A C T

Utilizing the atto-zeptomole sensitivity of UPLC-accelerator mass spectrometry (UPLC-AMS), we previously demonstrated significant first-pass metabolism following escalating (25–250 ng) oral micro-dosing in humans of [^{14}C]-benzo[*a*]pyrene ([^{14}C]-BaP). The present study examines the potential for supplementation with Brussels sprouts (BS) or 3,3′-diindolylmethane (DIM) to alter plasma levels of [^{14}C]-BaP and metabolites over a 48-h period following micro-dosing with 50 ng (5.4 nCi) [^{14}C]-BaP. Volunteers were dosed with [^{14}C]-BaP following fourteen days on a cruciferous vegetable restricted diet, or the same diet supplemented for seven days with 50 g of BS or 300 mg of BR-DIM® prior to dosing. BS or DIM reduced total [^{14}C] recovered from plasma by 56–67% relative to non-intervention. Dietary supplementation with DIM markedly increased T_{max} and reduced C_{max} for [^{14}C]-BaP indicative of slower absorption. Both dietary treatments significantly reduced C_{max} values of four downstream BaP metabolites, consistent with delaying BaP absorption. Dietary treatments also appeared to reduce the $T_{1/2}$ and the plasma $AUC_{(0,\infty)}$ for Unknown Metabolite C, indicating some effect in accelerating clearance of this metabolite. Toxicokinetic constants for other metabolites followed the pattern for [^{14}C]-BaP (metabolite profiles remained relatively consistent) and non-compartmental analysis did not indicate other significant alterations. Significant amounts of metabolites in plasma were at the bay region of [^{14}C]-BaP irrespective of treatment. Although the number of subjects and large interindividual variation are limitations of this study, it represents the first human trial showing dietary intervention altering toxicokinetics of a defined dose of a known human carcinogen.

Abbreviations: AFB_1, aflatoxin B_1; AMS, accelerator mass spectrometry; AHR, aryl hydrocarbon receptor; AUC, area under the curve; ATSDR, Agency for Toxic Substances and Disease Registry; BaP, benzo[*a*]pyrene; BaP-7,8-DHD, (±)-*trans*-benzo[*a*]pyrene-dihydrodiol; BaP-DHDE, BaP-7,8-DHD-9,10-epoxide; BMI, body mass index; BR-DIM, BioResponse 3,3′diindolylmethane; C_{max}, maximum concentration in plasma; CHL, chlorophyllin; CYP, cytochrome P-450; DBC, dibenzo[*def,p*]chrysene; DIM, 3,3′-diindolylmethane; FDA IND, US Food and Drug Administration Investigational New Drug; HCC, hepatocellular carcinoma; IARC, International Agency for Research on Cancer; IPCS, International Programme on Chemical Safety; IQ, 2-amino-3-methylimidazo[4,5-*f*]quinolone; IRB, Institutional Review Board; JEFCA, Joint FAO/WHO Expert Committee on Food Additives; LLNL, Lawrence Livermore National Laboratory; LOD, limit of detection; LOQ, limit of quantitation; MeIQx, 2-amino-3,8-dimethylimidazo[4,5-*b*]quinoxaline; NPL, National Priorities List; N^2-dG, nitrogen at the 2 position of deoxyguanosine; OSU, Oregon State University; PAH, polycyclic aromatic hydrocarbon; PBMC, peripheral blood mononucleated cell; PBPK, physiologically based pharmacokinetics; PhIP, 2-amino-3-methyl-6-phenylimidazo[4,5-*b*]pyridine; RPF, Relative Potency Factor; T_{max}, time at C_{max}; UPLC, ultra-pressure liquid chromatography (Waters); US EPA, United States Environmental Protection Agency.

* Corresponding author at: Department of Environmental and Molecular Toxicology, ALS 1007, Oregon State University, Corvallis, OR 97331, USA
E-mail addresses: monica.maier@oregonstate.edu (M.L. Vermillion Maier), beth.siddens@oregonstate.edu (L.K. Siddens), jamie.pennington@oregonstate.edu (J.M. Pennington), sandra.uesugi@oregonstate.edu (S.L. Uesugi), susan.tilton@oregonstate.edu (S.C. Tilton), vertele@oregonstate.edu (E.A. Vertel), kim.anderson@oregonstate.edu (K.A. Anderson), lane.tidwell@oregonstate.edu (L.G. Tidwell), ognibene1@llnl.gov (T.J. Ognibene), turteltaub2@llnl.gov (K.W. Turteltaub), jordan.smith@pnnl.gov (J.N. Smith), david.williams@oregonstate.edu (D.E. Williams).

https://doi.org/10.1016/j.taap.2023.116377
Received 1 November 2022; Received in revised form 5 January 2023; Accepted 9 January 2023
Available online 13 January 2023
0041-008X/

1. Introduction

1.1. Benzo[a]pyrene (BaP) formation and carcinogenicity

Polycyclic aromatic hydrocarbons (PAHs) are produced from incomplete combustion of organic materials with common sources being coal, petroleum, wood, and tobacco (ATSDR, 1995; IARC, 1983, 2010; US EPA, 2017). Individual PAHs (BaP and benzo[*b*]fluoranthene) and PAH mixtures make up three of the top ten chemicals of concern on the ATSDR Substance Priority List and are drivers of remediation at a number of EPA Superfund sites (ATSDR, 2019). BaP is designated as a group 1 known human carcinogen (IARC, 1983) and, based on a large dataset of carcinogenicity in preclinical models, was proposed (suspended as policy in 2019) as the reference PAH for risk assessment of environmental mixtures in the EPA's Relative Potency Factor (RPF) approach to cancer risk assessment (US EPA, 2010).

Human exposure to BaP is primarily ($\geq$ 90%) through the diet (Bansal and Kim, 2015; Domingo and Nadal, 2015; Kazerouni et al., 2001; Sampaio et al., 2021) and is estimated at 250–700 ng/day for a non-smoking individual in the US (JEFCA, 2005). Serum analysis of BaP in non-smoking Canadian women showed levels of 220 $\pm$ 150 pg/mL (Neal et al., 2008). Pleil et al. (2010) found much lower levels in plasma (median of 4 pg/mL) from non-smoking students in the US. In a recent study employing UPLC- accelerator mass spectrometry (UPLC-AMS), a micro-dose of 250 ng of [^{14}C]-BaP produced a C_{max} of 8.1 $\pm$ 7.6 fg/mL plasma (Maier et al., 2021a). The carcinogenicity of BaP is driven primarily by cytochrome P450 (CYP)-oxygenation (Fig. 1) at what is commonly referred to as the "bay region" (Crowell et al., 2014; Shimada, 2006; Shimada and Fujii-Kuriyama, 2004; Uppstad et al., 2010). CYPs in the 1 family first form the 7,8-epoxide (*endo* or *exo*) followed by hydrolysis to two *trans*-7,8-dihydrodiols (7,8-DHD). A second epoxygenation produces four enantiomers of the ultimate carcinogenic 7,8-DHD-9,10-epoxide (DHDE) with the (+)-*anti*-DHDE being the most potent mutagen (Conney et al., 1994; Shimada and Fujii-Kuriyama, 2004). BaP-DHDE alkylates DNA primarily at the N^2 position of guanine and, in the absence of nucleotide excision repair, results in G to T transversion mutations and cancer (Kapitulnik et al., 1978; Osborne et al., 1976; Wood et al., 1977). BaP-DHDE DNA adducts, as well as the 7,8,9,10-tetraol DHDE hydrolysis products, are used as biomarkers of BaP exposure and/or cancer risk (Lodovici et al., 2004; Mensing et al., 2005; Perera et al., 2005; Villalta et al., 2017).

1.2. Brussels sprouts and DIM as chemopreventive agents

Brussels sprouts are a rich source of the glucosinolate, glucobrassicin, that when hydrolyzed by plant or microbial myrosinase results in production of indole-3-carbinol (I3C) (Hayes et al., 2008; Williams, 2021). I3C is an effective dietary chemopreventive agent in numerous preclinical cancer models and has been the focus of numerous clinical trials (reviewed in Williams, 2021). The efficacy of I3C is due to the production of acid-condensation products in the gut, primarily DIM that is the major, if not sole, I3C acid condensation product detected in systemic circulation (Anderton et al., 2004a; Bjeldanes et al., 1991; Sepkovic et al., 2001) and does not undergo further acid conjugation

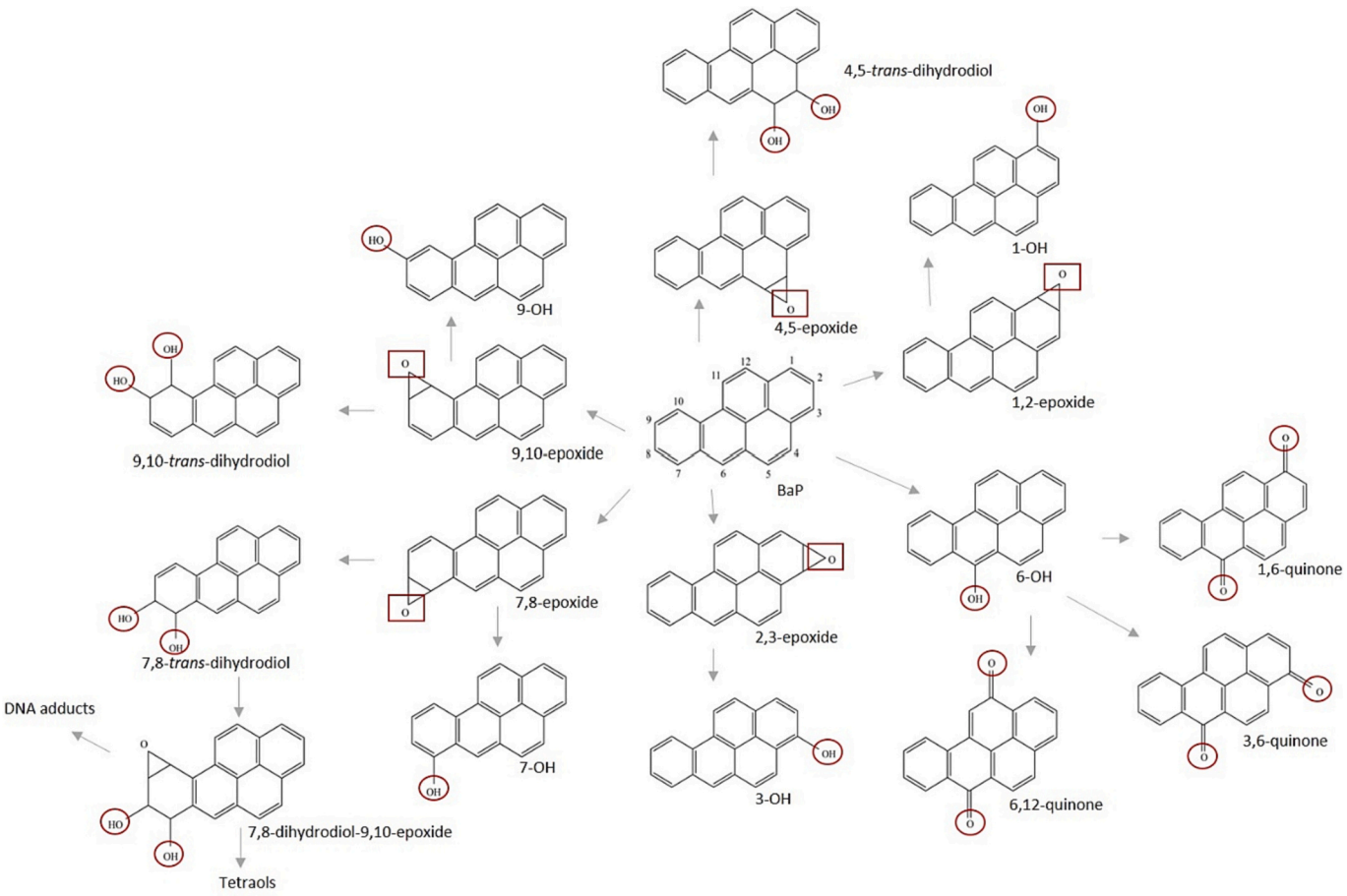

Fig. 1. Phase 1 and phase 2 metabolism of benzo[*a*]pyrene. CYPs 1A1, 1A2, or 1B1 form the epoxide (2 potential isomers) followed by hydrolysis by epoxide hydrolase to dihydrodiols (2 potential isomers). A second epoxygenation produces four enantiomers including 7,8-DHD-9,10-epoxide (DHDE). Quinones are formed through a spontaneous redox reaction following the initial phenol formation. Sites of potential glucuronidation or sulfation represented with circles and potential sites of glutathione conjugation shown with squares.

reactions (Anderton et al., 2004b; Reed et al., 2008). Urinary levels of DIM are reliable biomarkers for the consumption of Brussels sprouts (Fujioka et al., 2016). Our laboratory has recently characterized mono- and di-hydroxylated DIM metabolites along with their sulfate and glucuronide conjugates in human plasma and urine (Maier et al., 2021b) following the dosing regimen in this study.

1.3. AMS and micro-dosing of humans with carcinogens

The extreme sensitivity of AMS allows for micro-dosing of humans with [^{14}C]-labeled carcinogens with *de minimus* risk (Brown et al., 2006; Dueker et al., 2011; Enright et al., 2016). AMS has been utilized to assess covalent binding to DNA following dosing of humans with [^{14}C]-labeled PAHs, heterocyclic amines (IQ, MeIQ, PhiP) from cooked meat (Malfatti et al., 1999; Turteltaub et al., 1999), as well as aflatoxin B_1 (AFB_1) (Cupid et al., 2004). Jubert et al. (2009) demonstrated that co-administration of chlorophyll or chlorophyllin (CHL) was effective in reducing the plasma C_{max} and AUC of [^{14}C]-AFB_1eq in human participants using total [^{14}C] via AMS-graphite analysis. This study provided mechanistic support for results from a clinical trial in a region of China (Qidong Province) with an extremely high incidence of hepatocellular carcinoma (HCC). Administration of CHL tablets to participants reduced the AFB_1-DNA adduct repair product, aflatoxin-N^7-guanine, a biomarker of AFB_1 exposure and cancer risk (Egner et al., 2001). The development of a UPLC interface for AMS (Thomas et al., 2011) now allows for the determination of the metabolic profile and toxicokinetics of procarcinogens requiring metabolic activation.

1.4. UPLC-AMS in determination of [^{14}C]-BaP toxicokinetics and metabolite profiles

Our initial studies employed graphite AMS (total [^{14}C] only) in the micro-dosing of humans with [^{14}C]-PAHs utilized dibenz[*def,p*]chrysene (DBC) followed by BaP (Madeen et al., 2015, 2016). UPLC-AMS allowed for characterization of the metabolic profile and toxicokinetics of [^{14}C]-BaP as a function of dose (25–250 ng). It was somewhat surprising to observe the extent of metabolism (90–97% in plasma) following oral administration of [^{14}C]-BaP over this dose range (Maier et al., 2021a). The 7,8,9,10-tetraols, as well as 7,8- and 9,10-DHD and the 9-phenol (bay region metabolites), were the predominant metabolites. In the present study we describe the impact of seven days of supplementation with Brussels sprouts or BR-DIM® on the metabolic profile and pharmacokinetics of a subsequent dose of 50 ng [^{14}C]-BaP.

2. Material and methods

2.1. Human subjects

2.1.1. Recruitment, enrollment, and pre-dosing protocol

This study was conducted in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki). Seven subjects (6 males and 1 female) ranging from 26 to 65 years old with BMIs of 23.8–33.4 (Table 1) were recruited and enrolled under the guidelines of an FDA IND (#117175) and IRBs at both Oregon State University (OSU; protocols 8233 and 8789) and Lawrence Livermore National Laboratory (LLNL; Protocol #2017–008). Exclusion criteria can be found at ClinicalTrials.gov (NCT03802721) and Madeen et al. (2019). Volunteers were given a list of foods from which to abstain (e.g. charcoal-broiled meats and smoked meats or cheeses) for two weeks prior to and during the study. They were also instructed to avoid cruciferous vegetables and certain condiments over this same period (Supplemental Table 1). In order to estimate BaP consumption, all foods and non-water beverages were recorded in a food diary that spanned 3 days prior to and throughout the 48-h study cycle. Volunteers also completed the Arizona Cruciferous Vegetable Food Frequency Questionnaire (ACVFFQ) to assess their usual intake of cruciferous vegetables and condiments in the previous three months (Thomson et al., 2007, 2016) (Supplemental Table 2). A three-week minimum separation between doses was established as a washout period with the requirement that all three doses were administered in less than one year (Table 1).

2.1.2. Dietary intervention with Brussels sprouts or DIM

Brussels sprouts were provided precooked, frozen, in seven individually vacuum-sealed packets. Volunteers consumed either Brussels sprouts (50 g, thawed, warmed, and seasoned as desired) or two capsules (300 mg total) of BioResponse DIM® 150 (45.3 mg DIM/capsule) daily with dinner for seven days prior to the 48-h study cycle. Based on self-report diaries and empty return vials, compliance was 100%. Volunteers also consumed two BR-DIM® capsules at the start of the 48-h toxicokinetic study during the DIM arm of the study. Prior to consumption of BR-DIM® capsules and/or [^{14}C]-BaP, 25 mL of blood and spot urine were collected as a baseline for background DIM. This plasma was also used to obtain background levels of 64 different PAHs by GC–MS/MS as previously described (Maier et al., 2021a). Subjects fasted overnight (10−12*h*) prior to the initiation of the 48-h study of [^{14}C]-BaP toxicokinetics.

2.1.3. Micro-dosing with [^{14}C]-BaP, timed blood collection, and preparation of plasma

Participants were given a food-grade capsule containing 50 ng of [^{14}C]-BaP (5.4 nCi) with 200–250 mL water. Quality control with respect to the purity of [^{14}C]-BaP as well as preparation and quality control of the capsule was approved by FDA (IND117175) and has been previously described (Hummel et al., 2018). Fasting continued for 2 h following dosing after which participants were provided a breakfast. Blood was drawn into heparinized tubes prior to the study (time 0, 25 mL) and 0.25, 0.5, 1, 1.5, 2, 3, and 4 h post-dose (10 mL) using an indwelling catheter. Later blood collections at 8, 24, and 48 h post-dose (10 mL) were done by straight needle stick. Blood was centrifuged (rcf ~ 1000 for 10 min) immediately following the draw and held at 4 °C until extraction.

Table 1
Demographics of participants and dosing dates.

Volunteer	Age	Sex	BMI[a]	Race	Hispanic	Dosing Date (& days since initial dose)		Dosed BS DIM
BaP021	27	M	24.3	White	N	9–10-19	(+56)	(+28)
BaP022	65	M	31.8	White	N	7–25-18	(+285)	(+201)
BaP025	44	M	28.0	White	N	7–23-18	(+259)	(+231)
BaP028	49	M	28.0	White	N	3–18-19	(+259)	(+217)
BaP031	59	F	33.4	White	N	1–22-19	(+126)	(+96)
BaP041	26	M	23.8	Black/AA	N	11–12-19	(+104)	(+62)
BaP042	43	M	37.1	White	N	10–15-19	(+105)	(+64)

[a] BMI listed is at the time of initial dosing of [^{14}C]-BaP. The BMIs at the time of dosing following Brussels sprouts and DIM were BaP021, 24.8, 24.7; BaP022, 32.8, 32.6; BaP025, 26.0, 26.3; BaP028, 28.5, 28.0; BaP031, 33.6, 33.9; BaP041, 23.8, 23.0; BaP042, 37.7, 36.0.

2.2. Extraction of plasma and UPLC-AMS at Lawrence Livermore National Laboratory (LLNL)

2.2.1. Plasma extraction

All plasma was extracted within 2 h of collection with established methods (Crowell et al., 2011; Hummel et al., 2018). Plasma aliquots (1.5 mL) were acidified and vortexed thrice with ethyl acetate (1.5 mL) containing K_2SO_4. Ethyl acetate extracts of each sample were pooled, evaporated to dryness under nitrogen, reconstituted with 100 μL ethyl acetate and shipped on dry ice to the BioAMS Center at LLNL. Plasma aliquots used to determine pre-dosing levels of PAHs were stored at −80 °C until GC–MS/MS analysis by the Analytical Chemistry Core of the Oregon State University Superfund Research Program (Maier et al., 2021a).

2.2.2. UPLC-AMS separation, detection, and identification

Just prior to UPLC-AMS analysis, samples were taken to dryness under vacuum and reconstituted with 50 μL acetonitrile. Samples were maintained in the autosampler of a Waters Acquity UPLC H-Class System at 20 °C, the column at 28 °C, and injection volume set at 10 μL. Elution of [^{14}C]-BaP and metabolites was achieved over 20 min with a Waters Acquity UPLC BEH C_{18} 1.7 μm 2.1 × 50 mm column equipped with a guard column (Vanguard HSS PFP 1.8 μm 2.1 × 5 mm) and a gradient of 0.3% formic acid in water (A) and PEG-free acetonitrile (B) at 0.25 mL/min (0.0 min, 70:30 A:B; 0.1 min, 60:40; 10.0 min, 54:46; 10.1 min, 43:57; 14.3 min, to 16.3 min, 0:100; 16.4 min to 20 min 70:30). [^{14}C]-BaP metabolites were identified by retention time using analytical standards obtained from Oregon State University Superfund Program PAH repository (http://limsweb.science.oregonstate.edu/). Elution times of metabolites and parent compound included in the analytical standard are given in Supplemental Table 3. The eluate first went through a PDA detector and then was split; 0.12 mL/min (48%) with deposition onto a moving wire interface for AMS and the remainder sent to waste. The eluate was carried by a periodically indented nickel wire through a drying oven to evaporate solvent followed by a combustion oven to convert analytes to [^{14}C]-CO_2, allowing transfer to the CO_2-gas-accepting ion source of the 250 kV AMS (Ognibene et al., 2015; Thomas et al., 2011). ^{13}C and ^{14}C have known isotope ratios allowing for quantification of [^{14}C]-BaP and metabolites. Accuracy (1–3%), precision (expressed as a coefficient of variation of 1–6%), and sensitivity expressed as limit of detection (LOD) of 1 attomole of [^{14}C] (0.58 fg for [^{14}C]-BaP) have been established (Keck et al., 2010; Ognibene et al., 2019). The LOQ (0.60 fg/mL) was established with plasma blanks from each individual and at elution times for each compound quantified (Maier et al., 2021a).

AMS measurements of ^{12}C, ^{13}C, and ^{14}C in biological samples vary by source article, individual, and sample type (Keck et al., 2010; Salehpour et al., 2008). AMS counts individual ^{14}C atoms in relation to ^{12}C and/or ^{13}C ion currents with extremely high sensitivity, and any variation in background signal impacts the degree of confidence in LOQ. To increase confidence, a matrix-matched LOQ was established using the standard deviation of the signal in the samples from each participant taken just prior to dosing with [^{14}C]-BaP. By time-matching signal to noise ratios from blanks and study samples, the LOQ is matched to individual peaks of interest and compared to background noise. This results in an LOQ equal to 10-x the signal standard deviation in blanks (at the respective elution times).

Due to the unavailability of radiolabeled BaP metabolite standards, chromatography and metabolite identification cannot be confirmed with AMS directly. In order to use BaP metabolite standards with known retention times to identify metabolites, the UPLC eluate went to a PDA detector and a moving wire interface for AMS analysis; however, not all peaks seen using UPLC-AMS could be identified using the analytical standard mix. Unknown A eluted at the beginning of the chromatography run (0.48 min) and is likely conjugated metabolites. BaP phenols, dihydrodiols, dihydrodiol-epoxides, and quinones are all subject to conjugation (glucuronides, sulfates, and glutathione) and would be the most polar BaP metabolites in plasma. Based on retention times, Unknowns B (3.16 min) and C (4.85 min) are possibly additional dihydrodiols. Unknown D (5.39 min) is either a late eluting dihydrodiol or a quinone, while Unknown E (9.18) is likely a quinone. The peak identified as the 3-phenol may also contain small amounts of the 7- and/or 1-OH-BaP phenols as these minor hydroxylated metabolites tend to co-elute. As reported previously (Maier et al., 2021a) no PAHs, including BaP (LOD, 240 pg/mL), classified as known or probable human carcinogens, were detected in plasma from any participant by GC–MS/MS prior to micro-dosing with [^{14}C]-BaP.

2.3. Toxicokinetic analysis

Time-course concentrations of [^{14}C]-BaP, individual BaP metabolites, and a sum of BaP metabolites were evaluated using non-compartmental analyses. Area under the curve (AUC) of concentrations in plasma from time zero to the last measured time point and extrapolated to infinity (using last 3 time points) were calculated using the trapezoidal rule (Gibaldi and Perrier, 1982). Mean residence times were calculated as a ratio of AUC under the 1st moment curve extrapolated to infinity to AUC extrapolated to infinity. Non-compartmental half-lives were calculated as the product of mean resident times with the natural log of two. Linear regression models were used to evaluate impact of BMI on non-compartmental pharmacokinetic parameters from participants receiving [^{14}C]-BaP only. We used linear mixed effects models to examine the relationship between non-compartmental BaP and BaP metabolite pharmacokinetic parameters maximum concentration (C_{max}), AUC to last time point ($AUC_{(0,48)}$), AUC to infinity ($AUC_{(0,\infty)}$), half-life ($T_{1/2}$), clearance (Cl), and volume of distribution (V_d) and effects of DIM and Brussels sprouts supplementation. In order to account for repeated measures, linear mixed effects models set treatment effects (i.e. BaP only, DIM, or Brussels sprouts) as fixed effects with random intercepts for subjects. *P*-values were obtained using the likelihood ratio tests of the full model with the effect in question against the model without the effect in question. The non-parametric Friedman Rank Sum Test was used to evaluate treatment effects on T_{max} (Supplemental Table 4). We analyzed non-detect data using two assumptions consistent with maximum upper and lower biases. First, we assumed non-detects were 0, providing a maximum lower bias to the data. Second, we assumed non-detects were approximate limits of detection (0.6 fg BaP/mL), providing the maximal upper bias. We acknowledge that the reality is somewhere in between these two assumptions. Major overall conclusions did not change when using both assumptions, and we reported *p*-values assuming non-detects were 0 in the text and provide both p-values in the supplemental information (Supplemental Table 5). Software used to statistically analyze data was "R: A language and environment for statistical computing" Version 4.0.3 (R Foundation for Statistical Computing, Vienna, Austria). Linear mixed effects models were fit using the lme4 package (Bates et al., 2015).

3. Results

3.1. Dietary crucifer consumption by participants

Volunteers were instructed to restrict intake of cruciferous vegetables (Supplemental Table 1) for two weeks prior to administration of supplements and during the 48 h period following [^{14}C]-BaP dosing. In addition, the Arizona Cruciferous Vegetable Food Frequency Questionnaire (Thomson et al., 2007) was conducted to estimate crucifer consumption over the three-month period preceding the study (Supplemental Table 2). A wide range of cruciferous vegetable consumption (64 ± 40 g/day with a range of 4–127 g/day) was observed for the participants in this study. For comparison, the average daily consumption in the US is 25–30 g/day (IARC, 2004). For participants in this study, total glucosinolate consumption is estimated at 54 ± 34 mg/day

(range 8–105 mg/day) based on reported crucifer intake. The US daily average intake is estimate at 21–25 mg/day (IARC, 2004).

3.2. Consumption of BaP estimated from diet diaries

Volunteers were provided a list of foods highest in BaP to avoid for two weeks prior to initiation of the study and throughout the study as previously described (Maier et al., 2021b). Daily dietary consumption of BaP was estimated over three days pre-study as well as during the study. The average low estimates for the three pre-dosing periods were 206 ± 79, 201 ± 52, and 187 ± 114 ng/day with a range of 75–384 ng/day (Table 2). The high intake estimates for the three pre-dosing periods were 256 ± 83, 283 ± 82, and 262 ± 170 ng/day with a range of 80–556 ng/day. These values are near or below the range for the daily dietary intake for a non-smoking individual in the U.S of 250–700 ng (JEFCA, 2005). The low and high estimates of daily BaP intake for the non-supplemented group during the 2-day study was 408 ± 287 ng and 514 ± 378 ng, respectively. During the study period, following a week of Brussels sprout supplementation, the low and high BaP daily intake estimates were 253 ± 134 ng and 324 ± 155 ng, respectively. Following a week of DIM supplements the low and high BaP daily intake estimates were 201 ± 83 and 273 ± 113 ng, respectively (Table 2).

3.3. Background levels of PAHs in plasma

As previously described, GC–MS/MS analysis capable of quantifying 64 PAHs was employed to analyze levels in plasma for all individuals prior to each micro-dosing with [^{14}C]-BaP. A complete description of the method and results can be found in Maier et al. (2021a) and results for this study given in Table 3. Naphthalene was present at quantifiable levels in plasma from all individuals over all three arms of the study. The levels and frequency of naphthalene and alkylated naphthalenes were in the range and frequency of our previous study, as was fluorene (Maier et al., 2021a). Phenanthrene, pyrene, and 1-methylpyrene were similar with respect to previous plasma levels, but the percentage of detects for both phenanthrene and pyrene was higher in this study (50% versus 18% of samples, and 68% compared to 25%, respectively). As before, the PAH found at the highest level was perylene (18–24 ng/mL), but it was only present in 3/22 samples. Retene, a PAH especially abundant in smoke from wildfires, was present in all but one sample at levels of 5.3 ± 2.2 ng/mL (range < LOQ-11.8 ng/mL) compared to our previous study (8.8 ± 10.7 with 100% frequency). In addition to the high levels, frequency of detection and presence in wildfire smoke, retene is of interest given it appears effective in activation of AHR signaling and AHR-dependent toxicity in zebrafish (Geier et al., 2018). Retene is not capable of being metabolized to a DHDE, but human HepG2 cells produce toxic mono- and di-ortho quinones (redox cycling), probably through CYP1A1 and epoxide hydrolase production of DHDs followed by aldo-keto reductase oxidation to catechols (Huang et al., 2017).

3.4. Toxicokinetics of [^{14}C] BaP in plasma

Following an oral dose of 50 ng [^{14}C]-BaP, the seven participants displayed similar time course patterns of [^{14}C]-BaP in plasma (Fig. 2). Maximum concentrations (C_{max}) of [^{14}C]-BaP in blood peaked at 3.69 ± 4.12 fg/mL (mean ± SD) at a median time of 1.25 h. [^{14}C]-BaP $T_{1/2}$ in blood was 3.69 ± 3.80 h (mean ± SD) and only one blood sample had detectable BaP at 48 h. No statistically significant relationships among C_{max}, $AUC_{(0,48\ h)}$, $AUC_{(0,\infty)}$, $T_{1/2}$, clearance, or V_d were observed as a function of BMI or age ($p \geq 0.35$).

Supplementation for one week with DIM significantly altered [^{14}C]-BaP toxicokinetics in plasma of human participants. Treatment of DIM significantly reduced [^{14}C]-BaP C_{max} 5-fold ($p = 0.048$) and significantly increased T_{max} 9-fold ($p = 0.033$) compared to no treatment (Fig. 2, Table 4). Effects of Brussel sprouts on BaP C_{max} and T_{max} were not statically significant. BaP AUCs, $T_{1/2}$, V_d, and clearance were not significantly affected by dietary treatments ($p \geq 0.29$). These changes provide evidence that DIM treatment delayed BaP absorption (reduced C_{max} and increased T_{max}).

3.5. Toxicokinetics of [^{14}C] BaP metabolites in plasma over 48 h

The toxicokinetics for the sum of all [^{14}C]-BaP metabolites in plasma (Fig. 3) followed concentric patterns in all three arms of the study (Fig. 4-6, Table 4). Although not statistically significant, C_{max} of the total metabolites from [^{14}C]-BaP and summed bay region metabolites from plasma of non-supplemented subjects appeared higher than those given dietary supplements ($p = 0.057$ and 0.065, respectively). Total [^{14}C]-BaP metabolites made up 96.1–100% of the total [^{14}C] in plasma even at the earliest sampling time, indicating rapid and extensive metabolism of BaP (Fig. 3). As in our previous study (Maier et al., 2021a) the T_{max} ranged between 1 and 2 h and little or no [^{14}C] remained in plasma 24–48 h post-dose. Total metabolite AUCs, $T_{1/2}$, and T_{max} values were not significantly affected by dietary treatment ($p \geq 0.11$).

Early eluting peaks, indicative of more water-soluble metabolites, predominate (Fig. 4). We have posited that Unknown A (no reference standard available for any of the metabolites labeled as unknowns) likely represents highly polar conjugates such as glucuronides, sulfates, or glutathione (Maier et al., 2021a). Tetraols are a mix of isomers of 7,8,9,10-tetraol formed following hydrolysis of the four enantiomers of

Table 2
Estimated average low and high dietary BaP consumption prior to and during study as estimated by self-reported food logs (ng/day)[a].

Volunteer	Pre-dosing[b]		Post- dosing[b]		Pre-dosing Brussels sprouts[c]		Post-Brussels sprouts after dosing[c]		Pre-dosing DIM[d]		Post-DIM after dosing[d]		Average ± SD Low	Average ± SD High
	L	H	L	H	L	H	L	H	L	H	L	H		
021	189	200	130	165	151	179	167	173	78	80	121	169	139 ± 39	161 ± 42
022	138	208	247	325	154	196	462	538	107	168	128	187	206 ± 134	270 ± 142
025	331	393	274	368	220	305	151	228	194	356	315	438	248 ± 071	348 ± 73
028	141	222	783	1215	273	389	208	449	384	556	291	408	347 ± 229	540 ± 348
031	208	289	829	876	253	381	128	161	276	356	244	312	323 ± 253	396 ± 247
041	148	178	572	663	211	283	420	462	75	108	121	190	258 ± 196	314 ± 210
042	170	193	343	387	147	250	236	258	195	207	184	205	213 ± 70	250 ± 72
Average ± SD	206 79	256 83	408 287	514 378	201 52	283 82	253 134	324 155	187 114	262 170	201 83	273 113		

[a] The source of the low (L) and high (H) estimates in foods was taken from Jakszyn et al., 2004; Kazerouni et al., 2001; EFSA, 2008; Wu and Yu, 2012; Zelinkova and Wenzl, 2015
The "pre-"is the 3 days prior to [^{14}C]-BaP dosing and the estimates for columns labeled "post-"are from the 2-days post dosing.
[b] Non-supplemented.
[c] Brussels sprout arm of the study.
[d] The DIM arm of the study.

Table 3
Background levels of PAHs for each subject and treatment group[a].

Subject/ Following Cycle[a]	Naphthalene[b, c]	1-Methylnaphthalene	2-Methylnaphthalene	1,6- and 2,6-Dimethylnaphthalene	2-Ethylnaphthalene	Phenanthrene	2-Methylpheneanthrene	Fluorene	Pyrene	1-Methylpyrene	Perylene	Retene
021/BaP only	1.44	–	0.20	–	–	0.64	–	0.39	0.60	0.72	–	9.19
021/BS	0.63	0.16	0.39	–	–	0.57	0.51	0.16	0.71	0.64	–	6.68
021/DIM	0.59	0.19	0.55	0.45	0.25	0.44	–	0.12	0.67	0.48	–	5.90
022/BaP only	1.45	–	0.99	–	–	–	–	0.53	–	0.32	–	3.05
022/BS	0.23	–	–	–	–	–	–	–	1.20	–	19.2	6.85
022/DIM	0.39	–	–	–	–	0.34	–	–	–	0.77	–	11.8
025/BaP only	0.38	–	–	–	–	–	–	0.40	–	–	–	1.01
025/BS	0.14	–	–	–	–	–	–	–	0.16	–	–	–
025/DIM	0.48	–	–	–	–	–	–	–	–	0.38	–	4.62
028/BaP only	0.67	–	–	–	–	–	–	–	–	0.34	–	4.64
028/BS	0.45	0.17	0.63	0.51	–	–	0.30	0.14	0.44	0.43	–	4.22
028/DIM	0.43	–	0.65	0.55	–	0.38	0.42	0.15	0.62	0.49	–	5.66
031/BaP only	0.88	–	–	–	–	–	–	–	–	0.35	–	4.38
031/BS	0.20	–	–	–	–	–	–	–	0.87	–	18.1	5.50
031/DIM	0.26	–	–	–	–	–	–	–	0.62	–	24.1	6.00
041/BaP only	0.58	0.20	0.28	0.46	–	0.38	0.47	0.16	0.52	0.55	–	5.80
041/BS	0.32	–	0.32	0.43	–	0.58	0.62	0.16	0.88	0.51	–	5.34
041/DIM	0.34	–	0.24	–	–	0.55	0.51	0.18	0.93	0.58	–	6.32
042/BaP only	0.37	–	0.55	0.53	–	0.55	0.43	0.14	0.58	0.45	–	4.10
042/BS	0.37	–	1.08	–	–	0.97	0.30	–	0.59	0.40	–	3.10
042/DIM	0.31	0.14	0.57	0.52	–	0.69	0.48	0.17	0.91	0.57	–	5.60

[a] 64 PAHs were analyzed in plasm from blood taken just prior to the dosing of [^{14}C]-BaP; BaP only, BS and DIM are the non-supplemented, Brussels sprouts, and DIM arms of the study.
[b] Results are in ng/mL of plasma.
[c] Only the PAHs above the LOQ for at least one occurrence are tabulated and no BaP or any other PAH classified by IARC as a known or probable human carcinogen was present above the LOQ.

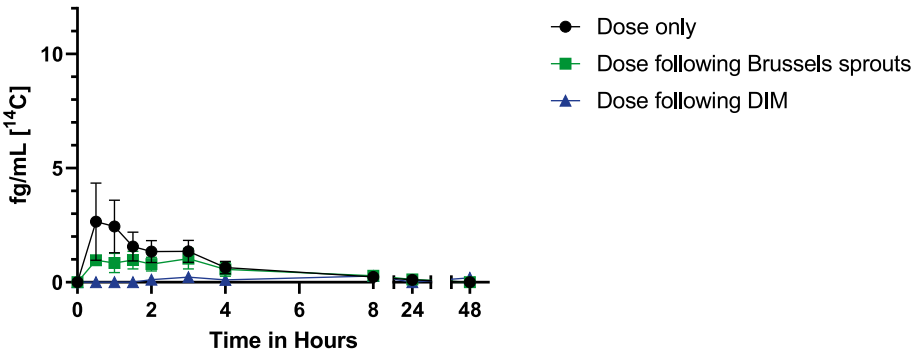

Fig. 2. Time course of [^{14}C]-benzo[*a*]pyrene in plasma from seven individuals over 48 h. The mean plasma levels (fg/mL) ± SE of [^{14}C] for parent BaP is shown with no supplementation (black circles), one week of Brussels sprouts (green squares), or one week of DIM (blue triangles). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 4
Summary of pharmacokinetic constants for [^{14}C]-BaP[a].

	Dose								
Parameter[b]	BaP only			BS			DIM		
C_{max} (fg BaP/mL)[c]	3.69	±	4.12	1.67	±	1.03	**0.68**	**±**	**0.51**
T_{max} (h)[d]	1.58	±	1.16	1.14	±	0.90	**14.00**	**±**	**19.17**
AUC (fg BaP/mL × h)	10.93	±	9.19	9.34	±	10.02	5.51	±	6.17
$AUC_{(0,\infty)}$ (fg BaP/mL × h)	10.93	±	9.19	9.34	±	10.02	5.49	±	6.15
$T_{1/2}$ (h)	3.69	±	3.80	3.86	±	4.62	9.75	±	13.25
Cl (L/h)[e]	14,836.59	±	26,620.71	51,409.16	±	80,206.38	22,025.01	±	33,131.07
V_d (L)[e]	48,883.70	±	78,019.06	54,224.57	±	44,889.28	106,380.48	±	88,815.96

[a] The values shown are the mean ± standard deviation of all participants.
[b] Toxicokinetic measures that were significantly different are shown in **bold** as determined by the Freidman test (T_{max} only), or the likelihood ratio tests of mixed effect models.
[c] DIM significantly reduced [^{14}C]-BaP C_{max} ($p = 0.048$).
[d] DIM significantly increased [^{14}C]-BaP T_{max} ($p = 0.034$).
[e] Note: BaP was through oral administration and bioavailability has not been determined in this study.

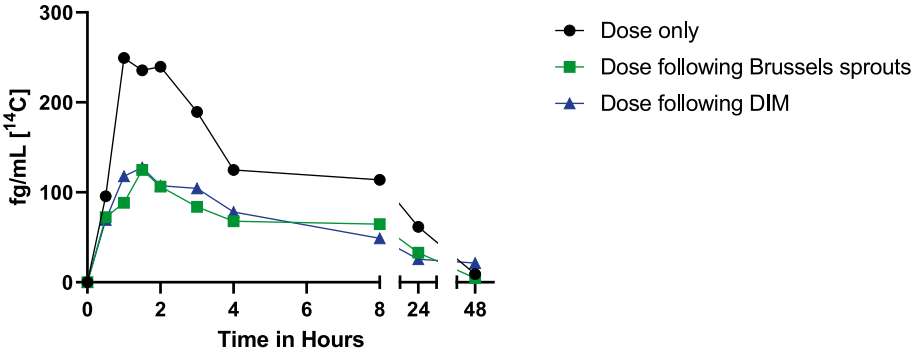

Fig. 3. Time course of the sum of total metabolites over 48 h. The total [^{14}C] plasma levels (fg/mL) of the combined twelve metabolites from seven participants is shown with no supplementation (black circles), one week of Brussels sprouts (green squares), or one week of DIM (blue triangles). Standard error of the sum too low to be visualized. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

the carcinogenic metabolite BaP-7,8-DHD-9,10-E. The 9,10- and 7,8-DHDs are the next to elute flanked by 3 unknowns (Fig. 5) which may be a diol (unknown B), additional diols or quinones (unknowns C and D) followed by the 1,6- and 3,6-quinones, the 9- and 3-phenols (Fig. 6) and finally parent BaP (Supplemental Table 3).

Dietary treatments caused significant changes to a number of non-compartmental pharmacokinetic parameters of certain metabolites. C_{max} of 4 different metabolites (Unknown A, 9,10-DHD, Unknown D, and 3,6-dione) were significantly reduced by DIM and/or Brussel sprout treatments ($p \leq 0.033$, Table 5). These observed downstream effects are consistent with dietary treatments delaying BaP absorption as observed with DIM effects on BaP concentrations in plasma. Interestingly, 3,6-dione was not detected following either dietary supplement. Unknown metabolite E was only detected following DIM treatment. 9-phenol was not detected following DIM treatment. Brussels sprouts significantly reduced the $T_{1/2}$ of Unknown C ($p = 0.022$), and while not significant, DIM trended in a similar pattern ($p = 0.17$). Both dietary treatments significantly reduced the $AUC_{(0,\infty)}$ of Unknown C ($p = 0.015$,). Brussels sprouts significantly reduced AUC and $AUC_{(0,\infty)}$ of Unknown D ($p \leq 0.037$, Table 5). These observed patterns of non-compartmental toxicokinetic parameters of metabolites are consistent with dietary treatment effects on [^{14}C]-BaP absorption with resulting downstream

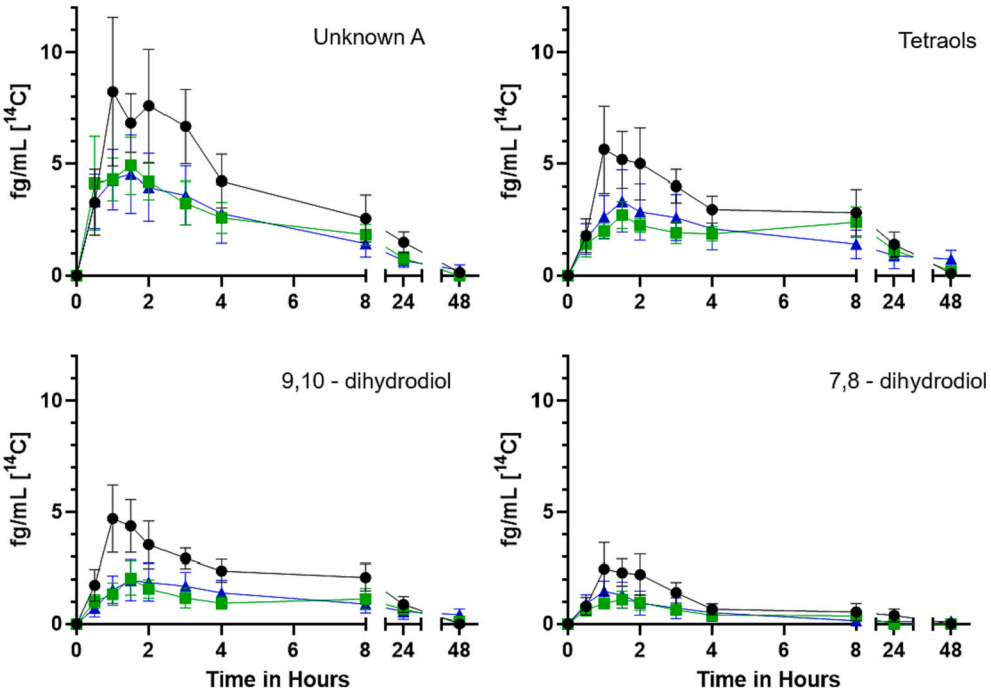

Fig. 4. Time course of the [^{14}C] plasma metabolites unknown A, tetraols, 9,10- and 7,8-dihydrodiols over 48 h from seven participants. The mean plasma levels (fg/mL) ± SE of [^{14}C] for the (upper left) unknown peak A (likely conjugates), (upper right) mix of 7,8,9,10-tetraol isomers, (lower left) 9,10- and (lower right) 7,8-dihydrodiols are shown with no supplementation (black circles), one week of Brussels sprouts (green squares), or one week of DIM (blue triangles). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

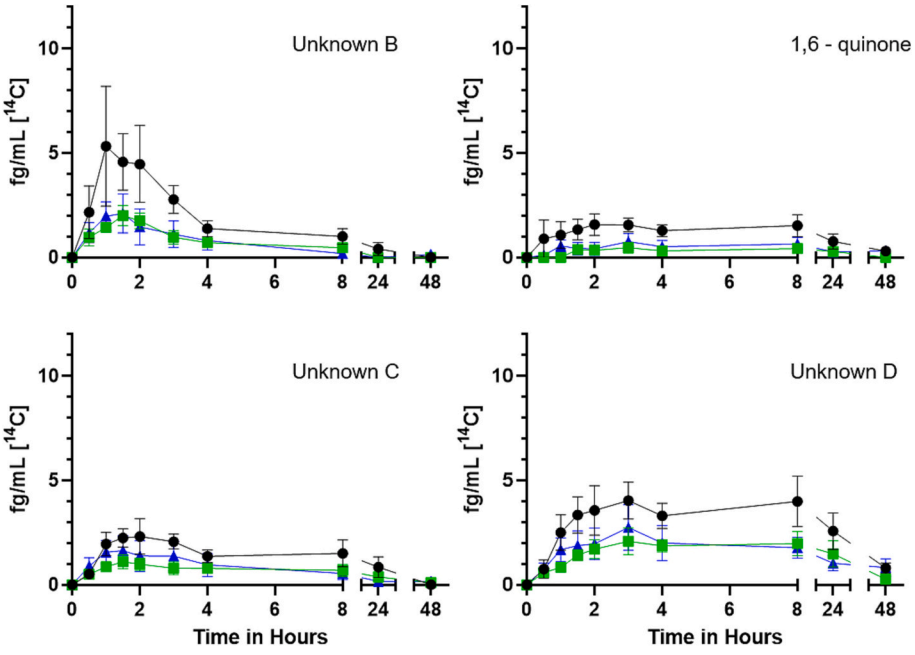

Fig. 5. Time course of the [^{14}C] representing individual plasma metabolites unknown B, C and D as well as 1,6-quinone over 48 h from seven participants. The mean plasma levels (fg/mL) ± SE of [^{14}C] for the (upper left) unknown peak B, 1,6-quinone (upper right) unknown peak C, and (lower center) unknown D are shown with no supplementation (black circles), one week of Brussels sprouts (green squares), or one week of DIM (blue triangles). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

impacts on metabolites.

When examined individually, three participants (BaP022, BaP025 and BaP028) exhibited a marked reduction in the C_{max} of 7,8-DHD (immediate precursor of BaP-7,8-DHD-9,10-E) plus the 7,8,9,10-tetraols (hydrolysis product of the 4 enantiomers of BaP-7,8-DHD-9,10-E) following a week of supplementation with either Brussels sprouts or DIM (Fig. 7). One individual (BaP031, the lone female) showed increased formation of 7,8-DHD and tetraols following supplementation with DIM. The remaining three individuals showed little or no change. Individuals with the large decreases were the most robust producers of the precursor (7,8-DHD) and product (tetraols) of the ultimate BaP carcinogenic metabolite prior to supplementation.

4. Discussion

The atto-zeptomole sensitivity of AMS allows for micro-dosing of humans with known [^{14}C]-labeled carcinogens with *de minimus* risk (Enright et al., 2016; Hah et al., 2009). Initial micro-dosing studies with [^{14}C]-chemical carcinogens examined covalent DNA damage utilizing a graphite technique that quantified total [^{14}C] without speciation (Farmer et al., 2005). Development of a UPLC interface allows for identification (based on co-elution with standards) of metabolites along with the parent carcinogen (Thomas et al., 2011). Our group has used both approaches to examine the toxicokinetics of the PAHs DBC and BaP (Madeen et al., 2015, 2016, 2019; Hummel et al., 2018). The results

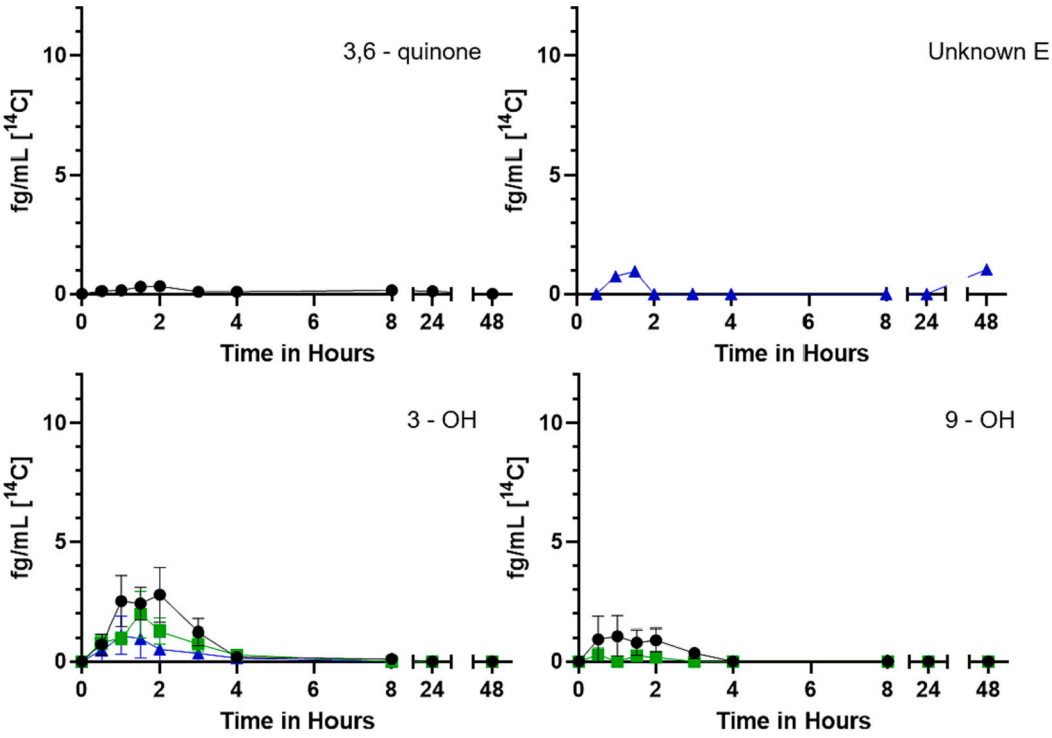

Fig. 6. Time course of the [^{14}C] representing individual plasma 3,6-quinone, unknown E, and phenol metabolites over 48 h from seven participants. The mean plasma levels (fg/mL) ± SE of [^{14}C] for the (upper left) 3,6-quinone, (upper right) unknown E, (lower left) 3-phenol and (lower right) 9-phenol are shown with no supplementation (black circles), one week of Brussels sprouts (green squares), or one week of DIM (blue triangles). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

with graphite (DBC_{eq} and BaP_{eq}) fit the PBPK model previously developed for rodents (Crowell et al., 2011; Pande et al., 2021) supporting its use in extrapolation to humans. The FDA (Kinders et al., 2007) has recognized the power of micro-dosing in Phase 0 protocols in part due to results such as from Lappin and Garner (2008) in which 15 of 18 drugs exhibited linear pharmacokinetics, when micro-dosed, not significantly different than therapeutic doses and consistent with our results to date with micro-dosing of PAHs. The potential value of AMS in cancer chemoprevention studies was discussed by Kensler and Groopman (2009) in conjuncture with the study by Jubert et al. (2009). As in the present study with BaP and DIM or Brussels sprouts, co-administration of chlorophyllin or purified chlorophylls to humans dosed with [^{14}C]-aflatoxin B_1 (AFB_1) reduced the k_a, C_{max} and AUC (graphite analysis for total [^{14}C]). The mechanism was postulated to be complexation (pi-pi stacking) of chlorophylls and CHL with AFB_1 (observed in vitro and in preclinical models) inhibiting intestinal absorption. This mechanism is unlikely to apply in our study as all groups were fasted overnight prior to dosing with [^{14}C]-BaP. In the DIM group, a final dose was given concurrent with [^{14}C]-BaP administration, but there is no evidence of complexation of DIM with BaP. In a previous study to determine if coadministration of a complex mixture of PAHs would impact [^{14}C]-BaP toxicokinetics, we fed participants 22 or 125 g of smoked salmon containing a complex mixture of PAHs (Confederated Tribes of the Umatilla Indian Reservation with 10-x the [^{14}C]-BaP dose as BaP_{eq}) or 180 g of commercially obtained canned salmon containing low levels of PAHs. The k_a, $C_{max,}$ and AUC of [^{14}C]-BaP were markedly reduced, but the effect was due to the quantity of salmon consumed and not the presence or absence of PAHs (Hummel et al., 2018). The impact of DIM or Brussels sprouts on kinetic constants could not be due to differences in stomach contents as all groups were fasted overnight and not given food until two h post [^{14}C]-BaP dose.

Cruciferous vegetables and DIM have been thoroughly studied in preclinical models and have been the subject of numerous clinical trials (Ambrosone and Tang, 2009; Higdon et al., 2007; Kim and Park, 2009; Minich and Bland, 2007; van Poppel et al., 1999; Williams, 2021). In addition to I3C, the immediate precursor of DIM, cruciferous vegetables contain other cancer chemopreventive agents such as sulforaphane, fiber, and antioxidants supporting the assertion that the whole food would be superior to isolated bioactives such as I3C, DIM, and sulforaphane (Ambrosone and Tang, 2009; Higdon et al., 2007; Kim and Park, 2009; Minich and Bland, 2007; van Poppel et al., 1999; Williams, 2021). The overall impact of crucifer supplementation in human cancer prevention is positive, but not impressively so. A key factor is simply dose. Brussels sprouts are a rich source of glucobrassicin, the precursor of I3C and subsequently DIM, but one would need to consume over 10 kg (Shorey et al., 2013) to achieve the manufacturer's daily-recommended dose of 300 mg (BR-DIM®, 90.6 mg DIM).

The mechanisms attributed to the chemopreventive properties of cruciferous vegetables, I3C, and DIM are numerous. One widely studied mechanism involves binding to and activating the aryl hydrocarbon receptor (AHR). Induction of phase 1 xenobiotic metabolizing enzymes such as cytochromes P450 (CYPs) and phase 2 enzymes such as glutathione-S-transferases (GSTs), UDP-glucuronosyl transferases (UGTs), and sulfotransferase (SULTs) would reduce metabolic activation of procarcinogens and enhance formation and excretion of non-toxic conjugates (Bjeldanes et al., 1991; Bradfield and Bjeldanes, 1987; Fahey and Kensler, 2007; Higdon et al., 2007; Lampe et al., 2000; Navarro et al., 2009; Peterson et al., 2009; Pool-Zobel et al., 2005; Stresser et al., 1994; Williams, 2021; Wu et al., 2015). Enzyme induction is unlikely to explain the altered toxicokinetics in this study given that we did not see increased [^{14}C]-BaP metabolites including conjugates.

The results from this study are consistent with our previous AMS studies with DBC and BaP in that a large interindividual variability is apparent. A previous study employing graphite AMS (total [^{14}C] representing parent BaP plus metabolites) and repeated dosing of the same individual resulted in relatively small variations between dosings with no relationship to the length of the interval between dosing. In the present study, individuals (BaP022, 025 and 028) with the greatest

Table 5
Summary of pharmacokinetic constants for [^{14}C]-BaP individual metabolites.[a]

Compound	Following	C^{d}_{max} (fg BaP/mL)			T_{max} (h)			AUC[g] (fg BaP/mL × h)			$AUC_{(0,\infty)}$[e] (fg BaP/mL × h)			$T^{f}_{1/2}$ (h)		
Unknown A	BaP only	10.63	±	8.59	1.5	±	0.7	88.26	±	67.48	**99.38**	±	**70.48**	14.46	±	17.83
	BS	6.28	±	4.85	1.3	±	1.0	47.63	±	28.47	47.63	±	28.47	6.52	±	3.89
	DIM	**5.07**	±	**4.24**	1.4	±	0.8	49.33	±	37.09	49.31	±	37.10	9.26	±	8.65
Tetraols	BaP only	6.89	±	5.06	1.9	±	1.1	78.31	±	65.08	80.88	±	65.40	8.93	±	5.23
	BS	3.65	±	1.10	7.8	±	7.8	58.50	±	30.23	52.01	±	27.26	7.95	±	3.26
	DIM	4.13	±	3.26	18.1	±	22.0	54.06	±	50.93	53.23	±	59.27	10.68	±	10.77
9,10-dihydrodiol	BaP only	5.61	±	4.10	2.1	±	1.3	54.86	±	41.68	54.86	±	41.68	6.04	±	3.06
	BS	**2.22**	±	**0.35**	2.3	±	2.7	29.56	±	17.48	29.55	±	17.47	10.00	±	6.14
	DIM	2.64	±	2.04	18.2	±	21.9	33.57	±	30.42	30.94	±	32.46	9.78	±	11.45
Unknown B	BaP only	6.68	±	7.38	1.4	±	0.3	32.98	±	37.79	32.98	±	37.79	3.98	±	2.69
	BS	2.15	±	0.88	1.4	±	0.8	9.64	±	7.01	9.64	±	7.01	2.62	±	1.50
	DIM	2.46	±	2.25	4.7	±	9.2	9.98	±	12.84	9.97	±	12.84	5.52	±	10.42
7,8-dihydrodiol	BaP only	3.06	±	2.94	1.6	±	0.7	20.13	±	30.87	20.13	±	30.87	3.66	±	3.87
	BS	1.51	±	0.60	1.2	±	0.5	6.47	±	5.55	6.47	±	5.55	2.73	±	1.74
	DIM	1.69	±	1.46	1.1	±	0.4	8.96	±	10.31	8.95	±	10.30	8.34	±	11.65
1,6-quinone	BaP only	2.82	±	2.08	2.9	±	2.5	41.25	±	47.16	41.25	±	47.16	5.80	±	3.94
	BS	1.45	±	0.39	6.9	±	8.1	18.14	±	13.02	18.14	±	13.01	7.26	±	7.10
	DIM	1.95	±	1.79	9.2	±	19.0	17.01	±	18.35	17.00	±	18.35	8.38	±	10.87
Unknown C	BaP only	5.16	±	3.43	4.4	±	2.5	118.92	±	89.90	160.14	±	107.74	26.26	±	23.18
	BS	3.12	±	1.57	10.2	±	9.8	59.86	±	34.51	**55.77**	±	**35.89**	**8.39**	±	**4.64**
	DIM	3.24	±	2.69	18.4	±	21.8	59.17	±	33.64	**63.91**	±	**47.84**	13.84	±	7.06
Unknown D	BaP only	2.56	±	2.07	4.3	±	3.1	41.17	±	36.08	43.11	±	44.22	10.47	±	3.86
	BS	**0.77**	±	**0.64**	13.4	±	9.9	**11.09**	±	**9.74**	**11.09**	±	**9.74**	9.56	±	6.20
	DIM	1.20	±	0.89	20.9	±	22.7	18.16	±	13.33	18.08	±	13.34	13.95	±	11.23
3,6-quinone[c]	BaP only	**0.48**	±	**0.48**	3.1	±	3.3	4.45	±	10.64	4.45	±	10.64	3.61	±	4.94
Unknown E[c]	DIM	**0.39**	±	**0.49**	16.8	±	27.0	1.89	±	4.62	1.88	±	4.60	11.67	±	18.71
9-OH[c]	BaP only	1.49	±	2.32	1.3	±	0.6	2.17	±	3.65	2.17	±	3.65	1.22	±	0.35
	BS	0.17	±	0.46	2.0	±	–	0.13	±	0.35	0.13	±	0.35	1.39	±	–
3-OH	BaP only	3.68	±	3.38	1.5	±	0.4	7.41	±	7.51	7.41	±	7.51	1.53	±	0.64
	BS	1.69	±	1.02	1.2	±	0.5	2.65	±	1.79	2.65	±	1.79	1.16	±	0.53
	DIM	1.12	±	2.17	1.3	±	0.4	2.22	±	5.19	2.22	±	5.19	1.15	±	0.49

aThe values shown are the mean ± standard deviation of all participants.
bToxicokinetic constants that were significantly different are shown in **bold** as determined by the Freidman test (T_{max} only), or the likelihood ratio tests of mixed effect models.
c, d3,6-quinone was not seen following either of the supplemented diets (C_{max} was significantly different, $p = 0.011$), Unknown E was only seen following DIM supplementation (C_{max} was significantly different, p = 0.037), and 9-OH was not seen following DIM supplementation.
dC_{max} Unknown A ($p = 0.024$) was significantly reduced following DIM supplementation; Brussels sprouts (9,10-dihydrodiol, $p = 0.031$; Unknown D, $p = 0.030$).
e$AUC_{(0,\infty)}$ of Unknown C was significantly reduced following both Brussels sprouts (p = 0.024) and DIM ($p = 0.040$) supplementation, as was Unknown A (full model $p = 0.041$), and Unknown D by Brussels sprouts ($p = 0.037$).
f$T_{1/2}$ was significantly reduced following Brussels sprouts (Unknown C, $p = 0.037$).
gAUC was significantly reduced following Brussels sprouts (Unknown D, $p = 0.019$).

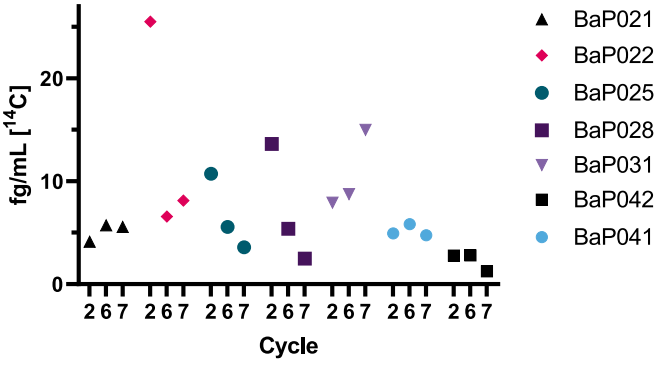

Fig. 7. Impact of supplementation on metabolism of the bay region of [^{14}C]-BaP. Sum of the [^{14}C] representing C_{max} (fg/mL plasma) for 7,8,9,10-tetraols and 7,8-dihydrodiol for each individual before (Cycle 2), or after one week of supplementation with Brussels sprouts (Cycle 6) or DIM (Cycle 7).

reduction in total [^{14}C] and bioactivation (bay region metabolites) were also those with the longest interval between dosings (201–237 days compared to 28–96 days). The intervals between DIM and Brussels sprout [^{14}C]-BaP dosing were much shorter (28–48 days). If the interval length were important, this would be consistent with the similarity in BaP toxicokinetics in those groups.

Inter-individual variability of BaP metabolism is a function of genetics, environment, and gene-environment interactions. Our study design attempted to minimize the environmental impact with dietary restrictions, food diaries, and rigorous quality control in the preparation and administration of the [^{14}C]-BaP dose as well as sample collection, extraction, and analysis. Based on the dietary questionnaire, the mean daily BaP consumption during the three pre-dosing cycles exhibited low estimates of 189 ± 78, 201 ± 52, and 187 ± 114 ng/day and highs of 240 ± 76, 283 ± 82, and 262 ± 170 ng/day which fall in the low range of the JEFCA (2005) estimate (250–700 ng/day) for non-smokers in the US. During the 48 h of the three studies the mean for the low estimates of daily dietary BaP were 454 ± 275, 253 ± 134, and 201 ± 83 with high estimates of 571 ± 369, 324 ± 155, and 273 ± 113 ng/day. The enzymes involved in phase 1 and phase 2 metabolism of BaP, as well as transporters, exhibit genetic polymorphisms and would be expected to be a large contributor to intraindividual variability in BaP toxicokinetics. The participant with the greatest production of 7,8-DHD and 7,8,9,10-tetraols (BaP022) is homozygous for the CYP1B1*3 and GSTM1*0 (null) alleles and a CYP1A2 genotype (163C > A; 5347C > T) (data not shown) associated with ultrarapid metabolism, all of which would be consistent with enhanced production of these bay region metabolites.

Previous work has shown that urinary levels of DIM are reliable biomarkers for the consumption of Brussels sprouts despite reported

issues of participant compliance due to the high volume of raw Brussels sprouts required (Fujioka et al., 2016). In an effort to have presumed urine levels of DIM be within the range of those shown to be reliable biomarkers and encourage volunteer compliance, 50 g Brussels sprouts were consumed, thawed, warmed, and seasoned as desired. Glucosinolates and subsequently glucobrassicin concentrations in crucifers vary by cultivar, and are effected by harvesting, storage, and cooking methods, potentially altering downstream concentrations of I3C and DIM (McNaughton and Marks, 2003). Brussel sprout glucobrassicin levels were not quantified in this study, however, in order to control for variation in the Brussels sprouts between participants all provided Brussels sprouts purchased were the same brand and lot number, then combined, weighed, and stored together in individual vacuum pouches. Following the Brussels sprout regimen in this study, DIM and DIM metabolites were not quantifiable in participants fasted overnight, consistent with previous work (Fujioka et al., 2016).

The final dose of DIM was administered to fasted participants just prior to administration of [^{14}C]-BaP. We adopted this strategy to ensure we would be able to observe effects due to direct inhibition as well as enzyme induction. Our recent study found a $T_{1/2}$ in plasma of 4.3 ± 2.5 h in agreement with pharmacokinetic studies of DIM in humans (Maier et al., 2021a; Reed et al., 2008). Although parent DIM was not detected in participants fasted overnight, substantial amounts of the sulfate and glucuronide conjugates were present (Maier et al., 2021b). These results confirm that little or no DIM would be present in plasma the morning of [^{14}C]-BaP dosing. Our laboratory and others have shown DIM to be a non-specific inhibitor of multiple rodent and human CYPs with K_is in the low μM range (Parkin et al., 2008; Schiering et al., 2017; Stresser et al., 1995; Williams, 2021). If the liver/plasma ratio is similar to mice (Anderton et al., 2004a, 2004b), based on the C_{max} we observed (~0.4 μM), estimated liver concentrations of DIM in this study would be 3–4 μM and in the range expected to inhibit human CYPs 1A1, 1A2, and 3A4 (Williams, 2021). In addition, we would expect that if CYP inhibition were responsible for the alteration of toxicokinetics, there would be a corresponding increase in unmetabolized BaP. The yield of bay region metabolites (bioactivation) remained constant (32–34%) across all groups, again arguing against DIM inhibition of CYP-dependent BaP metabolism as the mechanism involved as is the observation that the C_{max} for metabolites follow the same pattern as for parent BaP C_{max} while Cl_{int} is not altered. The markedly reduced T_{max} and C_{max} for BaP and reduced C_{max} of four downstream BaP metabolites for [^{14}C]-BaP are indicative of slower absorption. Overall, both Brussels sprouts and more so Dim appear to reduce the overall absorption of BaP, however the variability between participants, and subsequent pharmacokinetic parameters, does not allow for adequate statistical evidence. DIM is known to enhance intestinal barrier integrity and alter xenobiotic transporters which could explain the reduction in overall [^{14}C] with no impact on metabolite profiles (Kim et al., 2019; Metidji et al., 2018; Penta et al., 2021; Schiering et al., 2017). The issue with any of the mechanisms for DIM discussed above is that it does not account for the similar effects observed following one week of dietary supplementation with Brussels sprouts.

This study demonstrated that a whole food and dietary supplement, described as effective dietary chemopreventives in numerous preclinical cancer models, showed promise in this small human clinical trial. Validation of AMS micro-dosing studies for establishing absorption, metabolism, distribution and excretion (ADME) in drug development (CREAM study, Lappin et al., 2006) prompted the FDA to approve a Phase 0 approach saving pharmaceutical companies time and money needed to conduct a typical Phase 1 trial (FDA, 2008). In drug development, phase 0 trials can also be used for establishing a lead candidate (Kinders et al., 2007). Kensler and Groopman (2009), in proposing the use of AMS in chemoprevention studies, suggested the term "reverse phase 0" for human trials involving micro-dosing with carcinogens to evaluate chemopreventive agents. The atto- to zepto-mole sensitivity allows analysis of chemopreventive agent-dependent alterations in carcinogen toxicokinetics in vivo in humans (as was done in this study). The exquisite sensitivity of UPLC-AMS also allows for detection of carcinogen-DNA adducts (and their identity) with a sensitivity (1 adduct in 10^{11}–10^{12} bp) 10,000-fold greater than conventional LC-MS/MS adductions (Brown et al., 2006). Comparison of DNA adduction (an important tool in cancer chemoprevention studies) following treatment with MeIQx to humans and rodents in vivo showed a 10-x greater level of adduction per unit dose in humans (Turteltaub et al., 1997, 1999) highlighting the perspective as discussed by Seymour (2009) that "The Best Model for Humans is Humans". The current cost/sample is about $500 for UPLC-AMS. The low number (4–8) of participants needed (Penner et al., 2012) and use of a single dose followed over 2–3 days (rather than multiple dosing over months) results in a cost of about $9000 per participant for a complete time-course of plasma and urine analysis. A 250 kV AMS is currently 2–3-times the cost of a conventional triple quadrupole mass spectrometer and the cost and availability of AMS instrumentation continues to decrease. For all of these reasons, the use of UPLC-AMS in "reverse phase 0" human clinical trials has a great deal of potential utility and should be an important tool in cancer chemoprevention. Although this study could not definitely establish a mechanism of chemoprevention for DIM or Brussels sprouts, we were able to eliminate alteration of metabolism of [^{14}C]-BaP. Future studies could examine potential mechanisms related to absorption and DIM or Brussels sprout impact on the microbiome.

5. Conclusions

This study employed UPLC-AMS to assess the potential for the whole food Brussels sprouts or DIM, a dietary supplement that is a major bioactive and known cancer chemopreventive agent in preclinical models, to alter the toxicokinetics of a known human dietary carcinogen. Although not uniformly statistically significant, due to a large interindividual variability among the seven subjects, one week of supplementation with either Brussels sprouts or DIM did significantly reduce the C_{max} for parent BaP as well as four of the eleven metabolites observed. Dietary treatments also appeared to reduce the $T_{1/2}$ and the plasma $AUC_{(0,\infty)}$ for Unknown Metabolite C, indicating some effect in accelerating clearance of this metabolite. This reduction occurred with little change in the overall metabolite profile and no change in the relative oxygenation at the bay region of BaP. Our toxicokinetic analysis suggest DIM and Brussels sprout supplementation reduced the rate but not the extent of BaP absorption from the gut. Although DIM is a known inhibitor of CYPs, the observation that the C_{max} for metabolites followed the same pattern as BaP and overall Cl rates for these metabolites was not significantly impacted, argues against DIM inhibition of CYP-dependent BaP metabolism as the mechanism. This is the first study in humans documenting the impact of a whole food or dietary supplement on the toxicokinetics, including metabolic profiles, of a known dose of an IARC group 1 human carcinogen.

Author contributions

All authors have given approval to the final version of the manuscript.

Funding sources

This study was funded by Public Health Service NIH grants P42ES016465, R01ES028600, T32ES007060, and P30ES030287. Work performed in part at the National User Resource for Biological Accelerator Mass Spectrometry, which is operated at LLNL under the auspices of the US Department of Energy under contract DE-AC52-07NA27344. The User Resource is supported by the National Institutes of Health (NIH), National Institute of General Medical Sciences (NIGMS) under grant R24GM137748.

CRediT authorship contribution statement

Monica L. Vermillion Maier: Data curation, Formal analysis, Investigation, Methodology, Validation, Visualization, Writing – original draft, Writing – review & editing. **Lisbeth K. Siddens:** Data curation, Investigation, Methodology, Supervision, Validation, Writing – review & editing. **Jamie M. Pennington:** Data curation, Investigation, Writing – review & editing. **Sandra L. Uesugi:** Data curation, Methodology, Project administration, Resources, Supervision, Writing – review & editing. **Susan C. Tilton:** Methodology, Resources, Supervision, Writing – review & editing. **Emily A. Vertel:** Data curation. **Kim A. Anderson:** Data curation, Formal analysis, Methodology, Resources, Validation. **Lane G. Tidwell:** Formal analysis, Writing – review & editing. **Ted J. Ognibene:** Formal analysis, Methodology, Resources, Software, Validation, Writing – review & editing. **Kenneth W. Turteltaub:** Project administration, Resources. **Jordan N. Smith:** Formal analysis, Methodology, Software, Visualization, Writing – original draft, Writing – review & editing. **David E. Williams:** Conceptualization, Funding acquisition, Investigation, Project administration, Resources, Supervision, Validation, Writing – original draft, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgements

The authors would like to thank the participants who volunteered to be part of this study. Special thanks are also given to Carolyn Kinzie, Danny Chen, and Peter Scruggs for their contributions with sample extractions and preparation.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.taap.2023.116377.

References

Ambrosone, C.B., Tang, L., 2009. Cruciferous vegetable intake and cancer prevention: role of nutrigenetics. Cancer Prev. Res. 2, 298–300. https://doi.org/10.1158/1940-6207.CAPR-09-0037.

Anderton, M.J., Manson, M.M., Verschoyle, R.D., Gescher, A., Lamb, J.H., Farmer, P.B., Steward, W.P., Williams, M.L., 2004a. Pharmacokinetics and tissue disposition of indole-3-carbinol and its acid condensation products after oral administration to mice. Clin. Cancer Res. 10, 5233–5241. https://doi.org/10.1158/1078-0432.CCR-04-0163.

Anderton, M.J., Manson, M.M., Verschoyle, R., Gescher, A., Steward, W.P., Williams, M. L., Mager, D.E., 2004b. Physiological modeling of formulated and crystalline 3,3′-diindolylmethane pharmacokinetics following oral administration in mice. Drug Metabol. Disp. 32, 632–638. https://doi.org/10.1124/dmd.32.6.632.

ATSDR, 1995. Agency for Toxic Substances and Disease Registry. Toxicological Profile for Polycyclic Aromatic Hydrocarbons, pp. 1–487. https://wwwn.cdc.gov/TSP/ToxProfiles/ToxProfiles.aspx?id=122&tid=25 (accessed June 15, 2022).

ATSDR, 2019. https://www.atsdr.cdc.gov/spl/index.html (accessed June 15, 2022).

Bansal, V., Kim, K.-H., 2015. Review of PAH contamination in food products and their health hazards. Environ. Int. 84, 26–38. https://doi.org/10.1016/j.envint.2015.06.016.

Bates, D., Maechler, M., Bolker, B., Walker, S., 2015. Fitting linear mixed-effects models using lme4. J. Stat. Softw. 67, 1–48. https://doi.org/10.18637/jss.v067.i01.

Bjeldanes, L.F., Kim, J.-Y., Grose, K.R., Bartholomew, J.C., Bradfield, C.A., 1991. Aromatic hydrocarbon responsiveness-receptor agonists generated from indole-3-carbinol in vitro and in vivo: comparisons with 2,3,7,8-tetrachlorodibenzo-p-dioxin. Proc. Natl. Acad. Sci. U. S. A. 88, 9543–9547. https://doi.org/10.1073/pnas.88.21.9543.

Bradfield, C.A., Bjeldanes, L.F., 1987. Structure-activity relationships of dietary indoles: a proposed mechanism of action as modifiers of xenobiotic metabolism. J. Toxicol. Environ. Health 21, 311–323. https://doi.org/10.1080/15287398709531021.

Brown, K., Tompkins, E.M., White, I.N., 2006. Applications of accelerator mass spectrometry for pharmacological and toxicological research. Mass Spectrom. Rev. 25, 127–145. https://doi.org/10.1002/mas.20059.

Conney, A.H., Chang, R.L., Jerina, D.M., Wei, S.-J.C., 1994. Studies on the metabolism of benzo[a]pyrene and dose-dependent differences in the mutagenic profile of its ultimate carcinogenic metabolite. Drug Metab. Rev. 26, 125–163. https://doi.org/10.3109/03602539409029788.

Crowell, S.R., Amin, S.G., Anderson, K.A., Krishnegowda, G., Sharma, A.K., Soelberg, J. J., Williams, D.E., Corley, R.A., 2011. Preliminary physiologically based pharmacokinetic models for benzo[a]pyrene and dibenzo[def,p]chrysene in rodents. Toxicol. Appl. Pharmacol. 257, 365–376. https://doi.org/10.1016/j.taap.2011.09.020.

Crowell, S.R., Hanson-Drury, S., Williams, D.E., Corley, R.A., 2014. In vitro metabolism of benzo[a]pyrene and dibenzo[def,p]chrysene in rodent and human hepatic microsomes. Toxicol. Lett. 228, 48–55. https://doi.org/10.1016/j.toxlet.2014.04.004.

Cupid, B.C., Lightfoot, T.J., Russell, D., Gant, S.J., Turner, P.C., Dingley, K.H., Curtis, K. D., Leveson, S.H., Turteltaub, K.W., Garner, R.C., 2004. The formation of AFB(1)-macromolecular adducts in rats and humans at dietary levels of exposure. Fd. Chem. Toxicol. 42, 559–569. https://doi.org/10.1016/j.fct.2003.10.015.

Domingo, J.L., Nadal, M., 2015. Human dietary exposure to polycyclic aromatic hydrocarbons: a review of the scientific literature. Fd. Chem. Toxicol. 86, 144–153. https://doi.org/10.1016/j.fct.2015.10.002.

Dueker, S.R., Vuong, L.T., Lohstroh, P.N., Giacomo, J.A., Vogel, J.S., 2011. Quantifying exploratory low dose compounds in humans with AMS. Adv. Drug Deliv. Rev. 63, 518–531. https://doi.org/10.1016/j.addr.2010.10.009.

Egner, P.A., Wang, J.B., Zhu, Y.R., Zhang, B.C., Wu, Y., Zhang, Q.N., Qian, G.S., Kuang, S. Y., Gange, S.J., Jacobson, L.P., Helzlsouer, K.J., Bailey, G.S., Groopman, J.D., Kensler, T.W., 2001. Chlorophyllin intervention reduces aflatoxin-DNA adducts in individuals at high risk for liver cancer. Proc. Natl. Acad. Sci. U. S. A. 98, 14601–14606. https://doi.org/10.1073/pnas.251536898.

Enright, H.A., Malfatti, M.A., Zimmermann, M., Ognibene, T., Henderson, P., Turteltaub, K.W., 2016. Use of accelerator mass spectrometry in human health and molecular toxicology. Chem. Res. Toxicol. 29, 1976–1986. https://doi.org/10.1021/acs.chemrestox.6b00234.

European Food Safety Authority (EFSA), 2008. Scientific opinion of the panel on contaminants in the food chain on a request from the European Commission on polycyclic aromatic hydrocarbons in food. EFSA J. 724, 1–114.

Fahey, J.W., Kensler, T.W., 2007. Role of dietary supplements/nutraceuticals in chemoprevention through induction of cytoprotective enzymes. Chem. Res. Toxicol. 20, 572–576. https://doi.org/10.1021/tx7000459.

Farmer, P.B., Brown, K., Tompkins, E., Emms, V.L., Jones, D.J.L., Singh, R., Phillips, D. H., 2005. DNA adducts: mass spectrometry methods and future prospects. Toxicol. Appl. Pharmacol. 207 (2 Suppl), 293–301. https://doi.org/10.1016/j.taap.2004.12.020.

FDA, 2008. Guidance for Industry: Safety Testing of Drug Metabolites. US Department of Health & Human Services, Food & Drug Administration, Center for Drug Evaluation and Research (CDER), Rockville, MD, USA.

Fujioka, N., Ransom, B.W., Carmella, S.G., Upadhyaya, P., Lindgren, B.R., Roper-Batker, A., Hatsukami, D.K., Fritz, V.A., Rohwer, C., Hecht, S.S., 2016. Harnessing the power of cruciferous vegetables: developing a biomarker for Brassica vegetable consumption using urinary 3,3′-diindolylmethane. Cancer Prev. Res. 9, 788–793. https://doi.org/10.1158/1940-6207.CAPR-16-0136.

Geier, M.C., Minick, D.J., Truong, L., Tilton, S., Pande, P., Anderson, K.A., Teeguardan, J., Tanguay, R.L., 2018. Systematic developmental neurotoxicity assessment of a representative PAH superfund mixture using zebrafish. Toxicol. Appl. Pharmacol. 354, 115–125. https://doi.org/10.1016/j.taap.2018.03.029.

Gibaldi, M., Perrier, D., 1982. Pharmacokinetics. M. Dekker, New York.

Hah, S.S., Henderson, P.T., Turteltaub, K.W., 2009. Recent advances in biomedical applications of accelerator mass spectrometry. J. Biomed. Sci. 16, 54. https://doi.org/10.1186/1423-0127-16-54.

Hayes, J.D., Kelleher, M.O., Eggleston, I.M., 2008. The cancer chemopreventive actions of phytochemicals derived from glucosinolates. Eur. J. Nutr. 47 (Suppl. 2), 73–88. https://doi.org/10.1007/s00394-008-2009-8.

Higdon, J.V., Delage, B., Williams, D.E., Dashwood, R.H., 2007. Cruciferous vegetables and human cancer risk: epidemiologic evidence and mechanistic basis. Pharmacol. Res. 55, 224–236. https://doi.org/10.1016/j.phrs.2007.01.009.

Huang, M., Mesaros, C., Hackfeld, L.C., Hodge, R.P., Zang, T., Blair, I.A., Penning, T.M., 2017. Potential metabolic activation of a representative C4-alkylated polycyclic aromatic hydrocarbon retene (1-methyl-7-isopropyl-phenanthrene) associated with the Deepwater Horizon oil spill in human hepatoma (HepG2) cells. Chem. Res. Toxicol. 30, 1093–1101. https://doi.org/10.1021/acs.chemrestox.6b00457.

Hummel, J.M., Madeen, E.P., Siddens, L.K., Uesugi, S.L., McQuistan, T., Anderson, K.A., Turteltaub, K.W., Ognibene, T.J., Bench, G., Krueger, S.K., Harris, S., Smith, J., Tilton, S.C., Baird, W.M., Williams, D.E., 2018. Pharmacokinetics of [^{14}C]-benzo[a]pyrene (BaP) in humans: impact of co-administration of smoked salmon and BaP dietary restriction. Fd. Chem. Toxicol. 115, 136–147. https://doi.org/10.1016/j.fct.2018.03.003.

IARC, 1983. IARC Working Group on the Evaluation of the Carcinogenic Risk of Chemicals to Humans. Polynuclear aromatic compounds, Part 1: Chemical, environmental and experimental data. IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans, 32. International Agency for Research on Cancer, pp. 1–453. https://publications.iarc.fr/Book-And-Report-Series/Iarc-Monog

raphs-On-The-Identification-Of-Carcinogenic-Hazards-To-Humans/Polynuclear-Aromatic-Compounds-Part-1-Chemical-Environmental-And-Experimental-Data-1983 (accessed June 16, 2022).
IARC, 2004. IARC Handbooks of Cancer Prevention, Cruciferous Vegetables, Isothiocyanates and Indoles, 9. International Agency for Research on Cancer, pp. 1–265. https://publications.iarc.fr/Book-And-Report-Series/Iarc-Handbooks-Of-Cancer-Prevention/Cruciferous-Vegetables-Isothiocyanates-And-Indoles-2004 (accessed June 14, 2022).
IARC, 2010. IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. Some non-heterocyclic polycyclic aromatic hydrocarbons and some related exposures. In: IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, 92. International Agency for Research on Cancer. https://www.ncbi.nlm.nih.gov/books/NBK321712/ (accessed June 16, 2022).
Jakszyn, P., Agudo, A., Ibáñez, R., García-Closas, R., Pera, G., Amiano, P., González, C.A., 2004. Development of a food database of nitrosamines, heterocyclic amines, and polycyclic aromatic hydrocarbons. J. Nutr. 134, 2011–2014. https://doi.org/10.1093/jn/134.8.2011.
JEFCA (Joint FAO/WHO Expert Committee on Food Additives), 2005. Sixty-fourth Meeting (JECFA/64/SC), Rome. https://www.fao.org/documents/card/en/c/f95956a6-c4ac-4843-bd48-b6a1fbe3afac/ (accessed June 16, 2022).
Jubert, C., Mata, J., Bench, G., Dashwood, R., Pereira, C., Tracewell, W., Turteltaub, K., Williams, D., Bailey, G., 2009. Effects of chlorophyll and chlorophyllin on low-dose aflatoxin B(1) pharmacokinetics in human volunteers. Cancer Prev. Res. 2, 1015–1022. https://doi.org/10.1158/1940-6207.CAPR-09-0099.
Kapitulnik, J., Wislocki, P.G., Levin, W., Yagi, H., Jerina, D.M., Conney, A.H., 1978. Tumorigenicity studies with diol-epoxides of benzo(a)pyrene which indicate that (+/−)-trans-7beta,8alpha-dihydroxy-9alpha,10alpha-epoxy-7,8,9,10-tetrahydrobenzo(a)pyrene is an ultimate carcinogen in newborn mice. Cancer Res. 38, 354–358.
Kazerouni, N., Sinha, R., Hsu, C.H., Greenberg, A., Rothman, N., 2001. Analysis of 200 food items for benzo[a]pyrene and estimation of its intake in an epidemiologic study. Fd. Chem. Toxicol. 39, 423–436. https://doi.org/10.1016/s0278-6915(00)00158-7.
Keck, B.D., Ognibene, T., Vogel, J.S., 2010. Analytical validation of accelerator mass spectrometry for pharmaceutical development. Bioanalysis 2, 469–485. https://doi.org/10.4155/bio.10.14.
Kensler, T.W., Groopman, J.D., 2009. Is it time to advance the chemoprevention of environmental carcinogenesis with microdosing trials? Cancer Prev. Res. 2, 1003–1007. https://doi.org/10.1158/1940-6207.CAPR-09-0232.
Kim, M.K., Park, J.H.Y., 2009. Conference on "multidisciplinary approaches to nutritional problems". Symposium on "nutrition and health". Cruciferous vegetable intake and the risk of human cancer: epidemiological evidence. Proc. Nutr. Soc. 68, 103–110. https://doi.org/10.1017/S0029665108008884.
Kim, J.Y., Le, T.A.N., Lee, S.Y., Song, D.G., Hong, S.C., Cha, K.H., Lee, J.W., Pan, C.H., Kang, K., 2019. 3,3′-Diindolylmethane improves intestinal permeability dysfunction in cultured human intestinal cells and the model animal *Caenorhabditis elegans*. J. Agric. Fd. Chem. 67, 9277–9285. https://doi.org/10.1021/acs.jafc.9b03039.
Kinders, R., Parchment, R.E., Ji, J., Kummar, S., Murgo, A.J., Gutierrez, M., Collins, J., Rubinstein, L., Pickeral, O., Steinberg, S.M., Yang, S., Hollingshead, M., Chen, A., Helman, L., Wiltrout, R., Simpson, M., Tomaszewski, J.E., Doroshow, J.H., 2007. Phase 0 clinical trials in cancer drug development: from FDA guidance to clinical practice. Mol. Interv. 7, 325–334. https://doi.org/10.1124/mi.7.6.9.
Lampe, J.W., Chen, C., Li, S., Prunty, J., Grate, M.T., Meehan, D.E., Barale, K.V., Dightman, D.A., Feng, Z., Potter, J.D., 2000. Modulation of human glutathione S-transferases by botanically defined vegetable diets. Cancer Epidemiol. Biomark. Prev. 9, 787–793.
Lappin, G., Garner, R.C., 2008. The utility of microdosing over the past 5 years. Expert Opin. Drug Metab. Toxicol. 4, 1499–1506. https://doi.org/10.1517/17425250802531767.
Lappin, G., Kuhnz, W., Jochemsen, R., Kneer, J., Chaudhary, A., Oosterhuis, B., Drijfhout, W.J., Rowland, M., Garner, R.C., 2006. Use of microdosing to predict pharmacokinetics at the therapeutic dose: experience with 5 drugs. Clin. Pharmacol. Ther. 80, 203–215. https://doi.org/10.1016/j.clpt.2006.05.008.
Lodovici, M., Luceri, C., Guglielmi, F., Bacci, C., Akpan, V., Fonnesu, M.L., Boddi, V., Dolara, P., 2004. Benzo(a)pyrene diolepoxide (BPDE)-DNA adduct levels in leukocytes of smokers in relation to polymorphism of CYP1A1, GSTM1, GSTP1, GSTT1, and mEH. Cancer Epidemiol. Biomark. Prev. 13, 1342–1348.
Madeen, E., Corley, R.A., Crowell, S., Turteltaub, K., Ognibene, T., Malfatti, M., McQuistan, T.J., Garrard, M., Sudakin, D., Williams, D.E., 2015. Human in vivo pharmacokinetics of [(14)C]dibenzo[def,p]chrysene by accelerator mass spectrometry following oral microdosing. Chem. Res. Toxicol. 28, 126–134. https://doi.org/10.1021/tx5003996.
Madeen, E.P., Ognibene, T.J., Corley, R.A., McQuistan, T.J., Henderson, M.C., Baird, W.M., Bench, G., Turteltaub, K.W., Williams, D.E., 2016. Human microdosing with carcinogenic polycyclic aromatic hydrocarbons: In vivo pharmacokinetics of dibenzo[def,p]chrysene and metabolites by UPLC accelerator mass spectrometry. Chem. Res. Toxicol. 29, 1641–1650. https://doi.org/10.1021/acs.chemrestox.6b00169.
Madeen, E., Siddens, L.K., Uesugi, S., McQuistan, T., Corley, R.A., Smith, J., Waters, K.M., Tilton, S.C., Anderson, K.A., Ognibene, T., Turteltaub, K., Williams, D.E., 2019. Toxicokinetics of benzo[a]pyrene in humans: extensive metabolism as determined by UPLC-accelerator mass spectrometry following oral micro-dosing. Toxicol. Appl. Pharmacol. 364, 97–105. https://doi.org/10.1016/j.taap.2018.12.010.
Maier, M.L.V., Siddens, L.K., Pennington, J.M., Uesugi, S.L., Anderson, K.A., Tidwell, L.G., Tilton, S.C., Ognibene, T.J., Turteltaub, K.W., Smith, J.N., Williams, D.E., 2021a. Benzo[a]pyrene (BaP) metabolites predominant in human plasma following escalating oral micro-dosing with [^{14}C]-BaP. Environ. Int. 159, 107045 https://doi.org/10.1016/j.envint.2021.107045.
Maier, M.L.V., Siddens, L.K., Uesugi, S.L., Choi, J., Leonard, S.W., Pennington, J.M., Tilton, S.C., Smith, J.N., Ho, E., Chow, H.H.S., Nguyen, B.D., Kolluri, S.K., Williams, D.E., 2021b. 3,3′-Diindolylmethane exhibits significant metabolism after oral dosing in humans. Drug Metab. Dispos. 49, 694–705. https://doi.org/10.1124/dmd.120.000346.
Malfatti, M.A., Kulp, K.S., Knize, M.G., Davis, C., Massengill, J.P., Williams, S., Nowell, S., MacLeod, S., Dingley, K.H., Turteltaub, K.W., Lang, N.P., Felton, J.S., 1999. The identification of [2-^{14}C]-2-amino-1-methyl-6-phenylimidazo[4,5-b] pyridine metabolites in humans. Carcinogenesis 20, 705–713. https://doi.org/10.1093/carcin/20.4.705.
McNaughton, S., Marks, G., 2003. Development of a food composition database for the estimation of dietary intakes of glucosinolates, the biologically active constituents of cruciferous vegetables. Br. J. Nutr. 90 (3), 687–697. https://doi.org/10.1079/BJN2003917.
Mensing, T., Marczynski, B., Engelhardt, B., Wilhelm, M., Preuss, R., Kappler, M., Angerer, J., Kafferlein, H.U., Scherenberg, M., Seidel, A., Brüning, T., 2005. DNA adduct formation of benzo[a]pyrene in white blood cells of workers exposed to polycyclic aromatic hydrocarbons. Int. J. Hyg. Environ. Health 208, 173–178. https://doi.org/10.1016/j.ijheh.2005.01.023.
Metidji, A., Omenetti, S., Crotta, S., Li, Y., Nye, E., Ross, E., Li, V., Maradana, M.R., Schiering, C., Stockinger, B., 2018. The environmental sensor AHR protects from inflammatory damage by maintaining intestinal stem cell homeostasis and barrier integrity. Immunity 49, 353–362. https://doi.org/10.1016/j.immuni.2018.07.010.
Minich, D.M., Bland, J.S., 2007. A review of the clinical efficacy and safety of cruciferous vegetable phytochemicals. Nutr. Rev. 65, 259–267. https://doi.org/10.1301/nr.2007.jun.259-267.
Navarro, S.L., Peterson, S., Chen, C., Makar, K.W., Schwarz, Y., King, I.B., Li, S.S., Li, L., Kestin, M., Lampe, J.W., 2009. Cruciferous vegetable feeding alters UGT1A1 activity: diet- and genotype-dependent changes in serum bilirubin in a controlled feeding trial. Cancer Prev. Res. 2, 345–352. https://doi.org/10.1158/1940-6207.CAPR-08-0178.
Neal, M.S., Zhu, J., Foster, W.G., 2008. Quantification of benzo[a]pyrene and other PAHs in the serum and follicular fluid of smokers versus non-smokers. Reprod. Toxicol. 25, 100–106. https://doi.org/10.1016/j.reprotox.2007.10.012.
Ognibene, T.J., Thomas, A.T., Daley, P.F., Bench, G., Turteltaub, K.W., 2015. An interface for the direct coupling of small liquid samples to AMS. Nucl. Inst. Methods Phys. Res. B 361, 173–177. https://doi.org/10.1016/j.nimb.2015.05.018.
Ognibene, T.J., Haack, K.W., Bench, G., Turteltaub, K.W., 2019. Trials and tribulations in the first three years in operation of the SSAMS for biomedical 14C-AMS at LLNL. Nucl. Inst. Methods Phys. Res. B 438, 166–171. https://doi.org/10.1016/j.nimb.2018.05.008.
Osborne, M.R., Beland, F.A., Harvey, R.G., Brookes, P., 1976. The reaction of (+/−)-7alpha, 8beta-dihydroxy-9beta, 10beta-epoxy-7,8,9,10-tetrahydrobenzo(a)pyrene with DNA. Int. J. Cancer 18, 362–368. https://doi.org/10.1002/ijc.2910180315.
Pande, P., Madeen, E.P., Williams, D.E., Crowell, S.R., Ognibene, T.J., Turteltaub, K.W., Corley, R.A., Smith, J.N., 2021. Translating dosimetry of dibenzo[def,p]chrysene (DBC) and metabolites across dose and species using physiologically based pharmacokinetic (PBPK) modeling. Toxicol. Appl. Pharmacol. 438, 115830 https://doi.org/10.1016/j.taap.2021.115830.
Parkin, D.R., Lu, Y., Bliss, R.L., Malejka-Giganti, D., 2008. Inhibitory effects of a dietary phytochemical 3,3′-diindolylmethane on the phenobarbital-induced hepatic CYP mRNA expression and CYP-catalyzed reactions in female rats. Fd. Chem. Toxicol. 46, 2451–2458. https://doi.org/10.1016/j.fct.2008.03.029.
Penner, N., Xu, L., Prakash, C., 2012. Radiolabeled absorption, distribution, metabolism, and excretion studies in drug development: why, when, and how? Chem. Res. Toxicol. 25, 513–531. https://doi.org/10.1021/tx300050f.
Penta, D., Mondal, P., Natesh, J., Meeran, S.M., 2021. Dietary bioactive diindolylmethane enhances the therapeutic efficacy of centchroman in breast cancer cells by regulating ABCB1/P-gp efflux transporter. J. Nutr. Biochem. 94, 108749 https://doi.org/10.1016/j.jnutbio.2021.108749.
Perera, F., Tang, D., Whyatt, R., Lederman, S.A., Jedrychowski, W., 2005. DNA damage from polycyclic aromatic hydrocarbons measured by benzo[a]pyrene-DNA adducts in mothers and newborns from Northern Manhattan, the World Trade Center Area, Poland, and China. Cancer Epidemiol. Biomark. Prev. 14, 709–714. https://doi.org/10.1158/1055-9965.EPI-04-0457.
Peterson, S., Schwarz, Y., Li, S.S., Li, L., King, I.B., Chen, C., Eaton, D.L., Potter, J.D., Lampe, J.W., 2009. CYP1A2, GSTM1, and GSTT1 polymorphisms and diet effects on CYP1A2 activity in a crossover feeding trial. Cancer Epidemiol. Biomark. Prev. 18, 3118–3125. https://doi.org/10.1158/1055-9965.EPI-09-0589.
Pleil, J.D., Stiegel, M.A., Sobus, J.R., Tabucchi, S., Ghio, A.J., Madden, M.C., 2010. Cumulative exposure assessment for trace-level polycyclic aromatic hydrocarbons (PAHs) using human blood and plasma analysis. J. Chromatogr. B 878, 1753–1760. https://doi.org/10.1016/j.jchromb.2010.04.035.
Pool-Zobel, B., Veeriah, S., Böhmer, F.D., 2005. Modulation of xenobiotic metabolising enzymes by anticarcinogens – focus on glutathione S-transferases and their role as targets of dietary chemoprevention in colorectal carcinogenesis. Mutat. Res. 591 (1–2), 74–92. https://doi.org/10.1016/j.mrfmmm.2005.04.020.
Reed, G.A., Sunega, J.M., Sullivan, D.K., Gray, J.C., Mayo, M.S., Crowell, J.A., Hurwitz, A., 2008. Single-dose pharmacokinetics and tolerability of absorption enhanced 3,3′-diindolylmethane in healthy subjects. Cancer Epidemol. Biomarkers Prev. 17, 2619–2624. https://doi.org/10.1158/1055-9965.EPI-08-0520.

Salehpour, M., Possnert, G., Bryhni, H., 2008. Subattomole sensitivity in biological accelerator mass spectrometry. Anal. Chem. 80, 3515–3521. https://doi.org/10.1021/ac800174j.

Sampaio, G.R., Guizellini, G.M., da Silva, S.A., de Almeida, A.P., Pinaffi-Langley, A.C.C., Rogero, M.M., de Camargo, A.C., Torres, E.A.F.S., 2021. Polycyclic aromatic hydrocarbons in foods: biological effects, legislation, occurrence, analytical methods, and strategies to reduce their formation. Int. J. Mol. Sci. 22, 6010. https://doi.org/10.3390/ijms22116010.

Schiering, C., Wincent, E., Metidji, A., Iseppon, A., Li, Y., Potocnik, A.J., Omenetti, S., Henderson, C.J., Wolf, C.R., Nebert, D.W., Stockinger, B., 2017. Feedback control of AHR signaling regulates intestinal immunity. Nature 542, 242–245. https://doi.org/10.1038/nature21080.

Sepkovic, D.W., Bradlow, H.L., Bell, M., 2001. Quantitative determination of 3,3′-diindolylmethane in urine of individuals receiving indole-3-carbinol. Nutr. Cancer 41, 57–63. https://doi.org/10.1080/01635581.2001.9680612.

Seymour, M., 2009. The best model for humans is human – how to accelerate early drug development safely. Altern. Lab. Anim 37 (Suppl. 1), 61–65. https://doi.org/10.1177/026119290903701S09.

Shimada, T., 2006. Xenobiotic-metabolizing enzymes involved in activation and detoxification of carcinogenic polycyclic aromatic hydrocarbons. Drug Metabol. Pharmacokinet. 21, 257–276. https://doi.org/10.2133/dmpk.21.257.

Shimada, T., Fujii-Kuriyama, Y., 2004. Metabolic activation of polycyclic aromatic hydrocarbons to carcinogens by cytochromes P450 1A1 and 1B1. Cancer Sci. 95, 1–6. https://doi.org/10.1111/j.1349-7006.2004.tb03162.x.

Shorey, L.E., Madeen, E.P., Atwell, L.L., Ho, E., Löhr, C.V., Pereira, C.B., Dashwood, R.H., Williams, D.E., 2013. Differential modulation of dibenzo[def,p]chrysene transplacental carcinogenesis: maternal diets rich in indole-3-carbinol versus sulforaphane. Toxicol. Appl. Pharmacol. 270, 60–69. https://doi.org/10.1016/j.taap.2013.02.016.

Stresser, D.M., Williams, D.E., McLellan, L.I., Harris, T.M., Bailey, G.S., 1994. Indole-3-carbinol induces a rat liver glutathione transferase subunit (Yc2) with high activity toward aflatoxin B1 exo-epoxide. Association with reduced levels of hepatic aflatoxin-DNA adducts in vivo. Drug Metab. Dispos. 22, 392–399.

Stresser, D.M., Bjeldanes, L.F., Bailey, G.S., Williams, D.E., 1995. The anticarcinogen 3,3′-diindolylmethane is an inhibitor of cytochrome P-450. J. Biochem. Toxicol. 10, 191–201. https://doi.org/10.1002/jbt.2570100403.

Thomas, A.T., Ognibene, T., Daley, P., Turteltaub, K., Radousky, H., Bench, G., 2011. Ultrahigh efficiency moving wire combustion interface for online coupling of high-performance liquid chromatography (HPLC). Anal. Chem. 83, 9413–9417. https://doi.org/10.1021/ac202013s.

Thomson, C.A., Newton, T.A., Graver, E.J., Jackson, K.A., Reid, P.M., Hartz, V.L., Cussler, E.C., Hakim, I.A., 2007. Cruciferous vegetable intake questionnaire improves cruciferous vegetable intake estimates. J. Am. Diet. Assoc. 107, 631–643. https://doi.org/10.1016/j.jada.2007.01.016.

Thomson, C.A., Ho, E., Strom, M.B., 2016. Chemopreventive properties of 3,3′-diindolylmethane in breast cancer: evidence from experimental and human studies. Nutr. Rev. 74, 432–443. https://doi.org/10.1093/nutrit/nuw010.

Turteltaub, K.W., Mauthe, R.J., Dingley, K.H., Vogel, J.S., Frantz, C.E., Garner, R.C., Shen, N., 1997. MeIQx-DNA adduct formation in rodent and human tissues at low doses. Mutat. Res. 376, 243–252. https://doi.org/10.1016/s0027-5107(97)00049-3.

Turteltaub, K.W., Dingley, K.H., Curtis, K.D., Malfatti, M.A., Turesky, R.J., Garner, R.C., Felton, J.S., Lang, N.P., 1999. Macromolecular adduct formation and metabolism of heterocyclic amines in humans and rodents at low doses. Cancer Lett. 43, 149–155. https://doi.org/10.1016/s0304-3835(99)00116-0.

Uppstad, H., Øvrebø, S., Haugen, A., Mollerup, S., 2010. Importance of CYP1A1 and CYP1B1 in bioactivation of benzo[a]pyrene in human lung cell lines. Toxicol. Lett. 192, 221–228. https://doi.org/10.1016/j.toxlet.2009.10.025.

US EPA, 2010. Development of a Relative Potency Factor (RPF) Approach for Polycyclic Aromatic Hydrocarbon (PAH) Mixtures (External Review Draft, Suspended) [WWW Document]. U. S. Environ. Prot. Agency Sci. Inventory. URL. https://cfpub.epa.gov/si/si_public_record_report.cfm?Lab=NCEA&dirEntryId=194584 (accessed June 16, 2022).

US EPA, 2017. Toxicological Review of Benzo[a]pyrene [CASRN 50–32-8]. https://cfpub.epa.gov/ncea/iris/iris_documents/documents/toxreviews/0136tr.pdf.

van Poppel, G., Verhoeven, D.T., Verhagen, H., Boldbohm, R.A., 1999. Brassica vegetables and cancer prevention. Epidemiology and mechanisms. Adv. Exp. Med. Biol. 472, 159–168. https://doi.org/10.1007/978-1-4757-3230-6_14.

Villalta, P.W., Hochalter, J.B., Hecht, S.S., 2017. Ultrasensitive high-resolution mass spectrometric analysis of a DNA adduct of the carcinogen benzo[a]pyrene in human lung. Anal. Chem. 89, 12735–12742. https://doi.org/10.1021/acs.analchem.7b02856.

Williams, D.E., 2021. Indoles derived from glucobrassicin: cancer chemoprevention by indole-3-carbinol and 3,3′-diindolylmethane. Front. Nutr. 8, 734334 https://doi.org/10.3389/fnut.2021.734334.

Wood, A.W., Chang, R.L., Levin, W., Yagi, H., Thakker, D.R., Jerina, D.M., Conney, A.H., 1977. Differences in mutagenicity of the optical enantiomers of the diastereomeric benzo[a]pyrene 7,8-diol-9,10-epoxides. Biochem. Biophys. Res. Commun. 77, 1389–1396. https://doi.org/10.1016/s0006-291x(77)80133-2.

Wu, S., Yu, W., 2012. Liquid-liquid extraction of polycyclic aromatic hydrocarbons in four different edible oils from China. Fd. Chem. 134, 597–601. https://doi.org/10.1016/j.foodchem.2012.02.155.

Wu, T.-Y., Huang, Y., Zhang, C., Su, Z.-Y., Boyanapalli, S., Khor, T.O., Wang, H., Lin, H., Gounder, M., Kagan, L., Androulakis, I.P., Kong, A.-N.T., 2015. Pharmacokinetics and pharmacodynamics of 3,3′-diindolylmethane (DIM) in regulating gene expression of phase II drug metabolizing enzymes. J. Pharmacokinet. Pharmacodyn. 42, 401–408. https://doi.org/10.1007/s10928-015-9421-5.

Zelinkova, Z., Wenzl, T., 2015. The occurrence of 16 EPA PAHs in food – a review. Polycycl. Aromat. Compd. 35, 248–284. https://doi.org/10.1080/10406638.2014.918550.