Supporting Information

Incorporating Exogenous and Endogenous Exposures into Dietary Risk Assessment of Nitrates and Nitrites in Vegetables: A Probabilistic Integrated Toxicokinetic Modeling Approach

Yi-Jun Lin,[†,‡,*] Cheng-Jih Cheng,[†] Jein-Wen Chen,[§] Zhoumeng Lin[‡,*]

[†]Institute of Food Safety and Health Risk Assessment, National Yang-Ming University, Taipei 11221, Taiwan

[‡]Institute of Computational Comparative Medicine (ICCM), Department of Anatomy and Physiology, College of Veterinary Medicine, Kansas State University, Manhattan, Kansas 66506

[§]Super Micro Mass Research & Technology Center, Cheng Shiu University, Kaohsiung, 83347, Taiwan

*Corresponding author at: Institute of Computational Comparative Medicine (ICCM), Department of Anatomy and Physiology, College of Veterinary Medicine, Kansas State University, 1800 Denison Avenue, P200 Mosier Hall, Manhattan, Kansas 66506, USA. E-mail address: zhoumeng@ksu.edu. Phone: +1-785-532-4087. Fax: +1-785-532-4557. Institute of Food Safety and Health Risk Assessment, National Yang-Ming University, Taipei 11221, Taiwan. E-mail address: yijunlin@ym.edu.tw. Phone: +886-2-2826-7000. Ext: 5245. Fax: +886-2-2823-6381.

Table of Contents

Section 1: Model Sensitivity Analysis

A sensitivity analysis was performed to allow the limited human data to be used to optimize only parameters that were most sensitive to the available data in humans after exposure to a single oral dose of 324 mg of sodium nitrite.[1] Each of the original parameters available from Zeilmaker et al.[2] (Table S4) was increased by 1%, and the corresponding area under the curve (AUC) of plasma concentrations of nitrates and nitrites, and AUCs of blood concentrations of methemoglobin (MetHb) were computed. In Table S4, mean and point values were used to calculate normalized sensitivity coefficients (NSCs) as follows:[3]

$$\mathrm{NSC} = \frac{(a-b)/b}{(c-d)d}, \tag{1}$$

where a is the AUC with 1% increased parameter value, b is the AUC with the original parameter value, c is the parameter value increased by 1%, and d is the original parameter value. Parameters with |NSCs| ≥ 0.1 were considered sensitive[4] and were therefore selected for model optimization.

Section 2: Model Evaluation

The optimized model was used to generate the simulations of nitrate and nitrite levels in plasma and then the simulation results were compared with the independent datasets using human subjects[5,6] that have not been used in the model optimization. Lambers et al.[5] investigated the nitrate concentrations in plasma from 12 human volunteers (BW = 75 ± 10.5 kg) given 300 g of spinach with 564 mg of nitrates, beetroot with 643 mg of nitrates, and lettuce with 1013 mg of nitrates. Hunault et al.[6] measured nitrate and nitrite concentrations in plasma from 9 human volunteers (BW = 66.8 ± 3.4 kg) given an aqueous nitrite solution with dose ranging from 290 to 380 mg (323.3 ± 33.2 mg).

The mean absolute percentage error (MAPE) was used to evaluate the model performance by comparing plasma concentrations of nitrate and nitrite between predictions and observed data. The MAPE can be calculated as:

$$\mathrm{MAPE} = \frac{1}{N}\sum_{n=1}^{N}\frac{\left|C_{o,n} - C_{m,n}\right|}{C_{o,n}} \times 100\%, \qquad (2)$$

where N denotes the number of observations, $C_{o,n}$ is the observed experimental data, and $C_{m,n}$ is the modeled result corresponding to data point n.

Section 3: Supplementary Figures

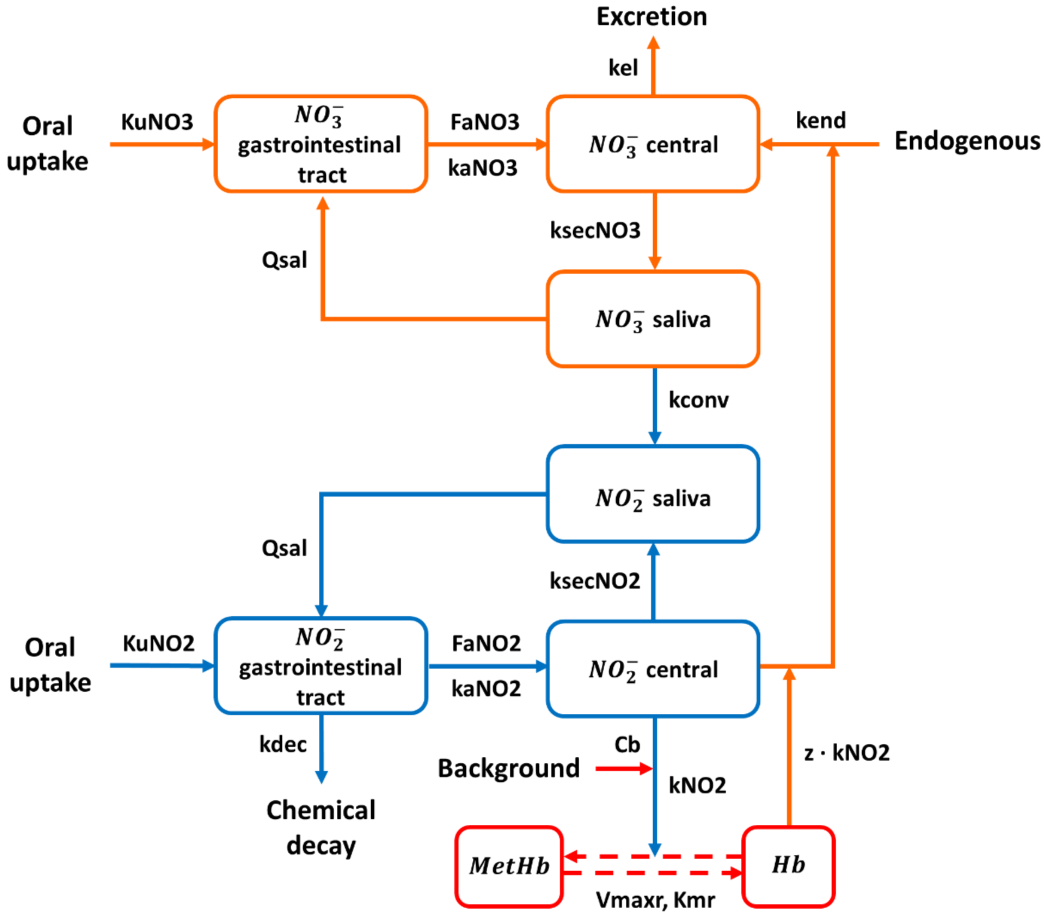

Figure S1. Structure of the human toxicokinetic model of nitrates and nitrites.[2]

For the kinetics of nitrates: (i) nitrates enter the gastrointestinal (GI) tract by oral intake of food and then transfer to the central distribution compartment (plasma); (ii) nitrates enter the central compartment also by endogenous nitrate synthesis and by conversion of nitrites to nitrates in the blood; (iii) elimination of nitrates from the central compartment occurs by secretion into saliva and by a lumped elimination rate for metabolism and urinary pathways; and (iv) salivary nitrates are removed by either reduction to nitrites or transfer to GI tract. For the kinetics of nitrites: (i) salivary nitrites undergo transfer to GI tract, where they can be absorbed into the central compartment or may chemically decay; (ii) nitrites also enter the GI tract by oral uptake of food and then transfer to the central compartment; and (iii) elimination of nitrites from the central compartment occurs by secretion into saliva and by interaction with hemoglobin (Hb) to produce nitrates that are returned to blood or methemoglobin (MetHb) that can be reconverted to Hb by the erythrocytes' MetHb reductase activity. Definitions of model parameters refer to Table S4 below.

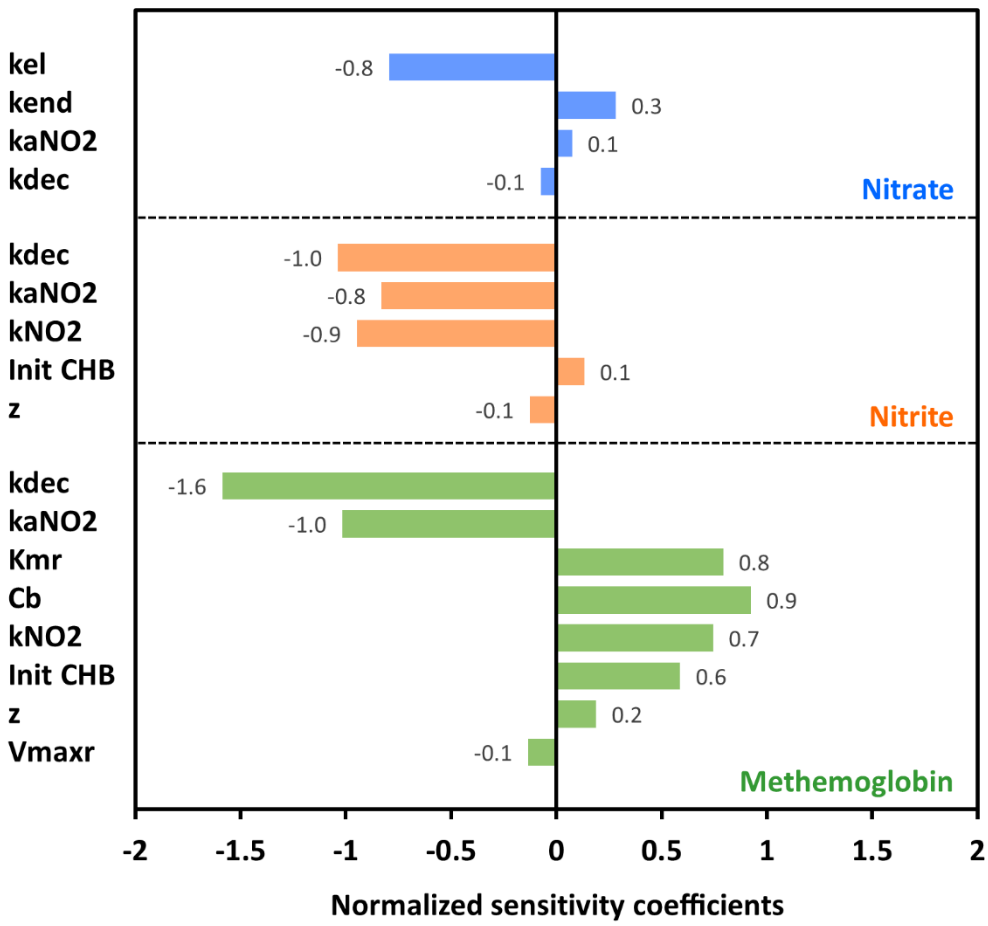

Figure S2. Normalized sensitivity coefficients (NSCs) of parameters using the area under the curves for concentrations of nitrates and nitrites in plasma and of methemoglobin in the blood in human volunteers exposed to a single oral dose of 324 mg of sodium nitrite. Definitions of model parameters listed on the y axis refer to Table S4 below.

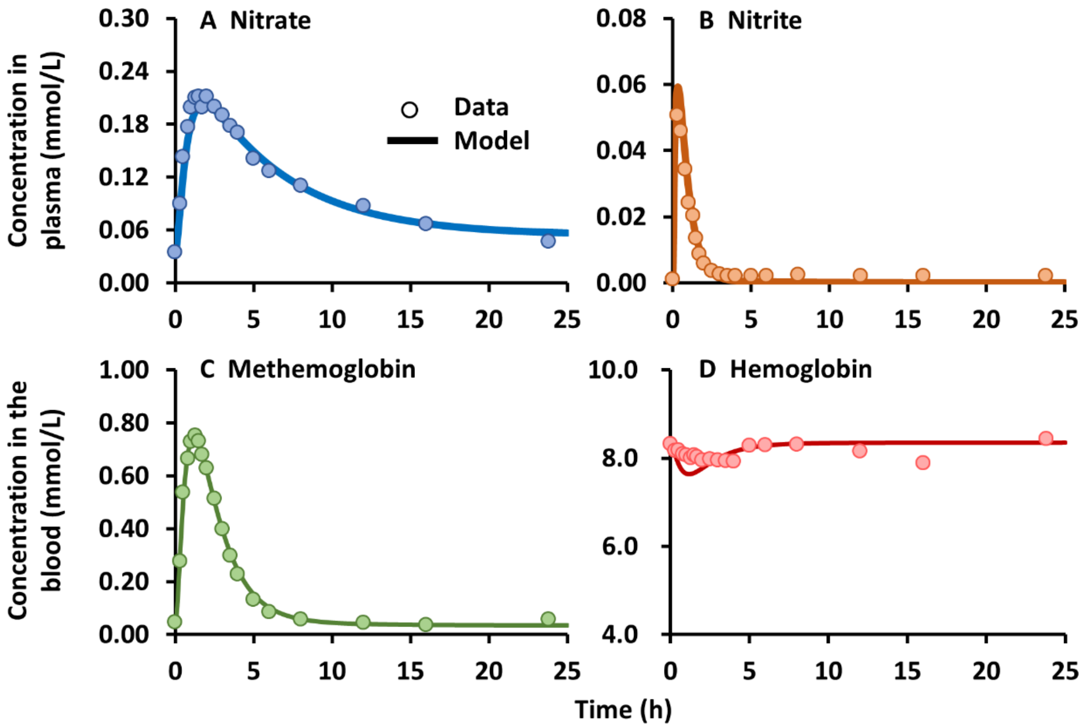

Figure S3. Model optimization results. Comparisons of the simulated versus observed concentrations of (A) nitrates and (B) nitrites in the plasma, and of (C) methemoglobin and (D) hemoglobin in the blood in the human study following a single oral intake of 324 mg of sodium nitrite.[1]

Section 4: Supplementary Tables

Table S1. List of core foods, mean consumption rate, and consumers percentage for nitrates and nitrites in vegetables in Taiwan.

Core food (CF)	Vegetables	Mean consumption rate (CR, g/day)	Consumers percentage (CP, %)
1. Wrapped vegetables			
01	Cabbage	39.0	37.0%
02	Chinese cabbage	8.37	8.9%
03	Cauliflower and broccoli	6.65	10.0%
04	Other wrapped vegetables	0.34	1.5%
2. Leafy vegetables			
05	Sweet potato vines	11.5	8.2%
06	Chinese mustard green	8.91	8.5%
07	Cruciferae leafy vegetable	23.2	26.1%
08	Asteraceae leafy vegetable	12.3	11.4%
09	Spinach	6.21	6.6%
10	Celery	2.21	11.7%
11	Amaranth	2.63	2.8%
12	Chayote vine	0.82	1.6%
13	Scallion and Leek	3.90	30.0%
14	Other leafy vegetables	2.12	5.4%
3. Fruit vegetables			
15	Tomato	5.49	8.2%
16	Sweet pepper	1.69	3.5%
17	Other fruit vegetables	2.62	3.6%
4. Legumes			
18	Chinese long beans	1.10	3.8%
19	String bean	0.76	2.0%
20	Snow peas	0.74	7.2%
21	Bean sprouts	3.74	11.6%
22	Other legumes	0.48	1.8%

Core food (CF)	Vegetables	Mean consumption rate (CR, g/day)	Consumers percentage (CP, %)
5. Cucurbits			
23	Loofah	5.59	5.2%
24	Bitter melon	3.05	3.2%
25	Baby cucumber	3.08	12.6%
26	White gourd	2.96	4.3%
27	Cucumber	2.10	4.3%
28	Bottle gourd	3.25	2.9%
29	Other cucurbits	2.05	2.3%
6. Tuber and starchy vegetables			
30	Carrot and daikon radish	17.0	48.8%
31	Bamboo shoot	7.67	18.8%
32	Onion	4.56	11.9%
33	Sweet potato	4.67	6.7%
34	Taro	1.10	2.1%
35	Potato	4.81	8.4%
36	Other starchy vegetables	4.01	5.8%
7. Fungus			
37	Shiitake mushrooms	3.92	14.2%
38	String mushrooms	1.80	5.5%
39	Chinese fungus	2.15	10.0%
40	King oyster mushroom	1.10	3.2%
41	Other mushrooms	0.60	2.3%
8. Aquatic vegetables			
42	Kelp	2.50	9.3%
43	Other aquatic vegetables	1.19	4.0%
44	Aquatic vegetable products	0.09	0.5%
9. Other vegetables			
45	Other vegetables	5.70	7.0%

Note: Grayscale indicates that CFs were selected for the shortened list through the following criteria: (1) mean CR > 5 g/day and CP > 5% or (2) CP > 10%.

Table S2. Consumption rate (mean ± SD) of 21 vegetable core foods by age populations.

Vegetables	Consumption rate (g/day)				
	Preschoolers (n = 523)	Children (n = 1324)	Teenagers (n = 2546)	Adult (n = 2039)	Elderly (n = 893)
Cabbage	18.0 ± 40.5	34.0 ± 65.1	37.6 ± 71.4	48.3 ± 93.3	46.7 ± 99.3
Chinese cabbage	3.04 ± 13.8	6.13 ± 27.8	7.60 ± 32.0	10.7 ± 47.9	12.9 ± 64.3
Cauliflower and broccoli	2.61 ± 13.0	6.37 ± 23.2	6.68 ± 26.8	7.28 ± 27.4	8.89 ± 35.3
Sweet potato vines	3.39 ± 16.9	5.41 ± 25.9	4.04 ± 25.7	18.6 ± 69.9	32.0 ± 101
Chinese mustard green	3.34 ± 16.0	6.70 ± 28.0	6.45 ± 27.7	11.8 ± 45.9	16.7 ± 55.9
Cruciferous leafy vegetable	10.7 ± 32.1	22.2 ± 48.7	22.1 ± 51.7	26.5 ± 72.3	30.4 ± 123
Asteraceae leafy vegetable	3.97 ± 16.9	6.54 ± 28.4	6.19 ± 28.6	22.2 ± 82.1	22.3 ± 85.9
Spinach	3.38 ± 16.4	4.09 ± 22.3	4.12 ± 20.9	8.70 ± 40.4	11.4 ± 52.4
Celery	1.09 ± 6.97	1.56 ± 7.25	1.38 ± 7.85	2.58 ± 14.3	2.42 ± 12.4
Scallion and Leek	1.17 ± 5.04	3.04 ± 9.78	3.77 ± 12.8	5.70 ± 23.7	3.77 ± 20.3
Tomato	3.40 ± 19.1	3.81 ± 17.2	3.85 ± 25.1	9.17 ± 41.6	6.08 ± 37.8
Bean sprouts	2.00 ± 8.39	3.71 ± 13.0	3.83 ± 13.5	4.87 ± 19.9	2.39 ± 13.4
Loofah	4.45 ± 28.8	4.30 ± 27.2	1.92 ± 14.8	6.86 ± 37.7	15.2 ± 73.6
Baby cucumber	1.30 ± 6.74	3.65 ± 16.1	3.72 ± 12.7	2.83 ± 20.0	2.40 ± 25.5
Carrot and daikon radish	12.2 ± 25.9	17.9 ± 29.7	17.8 ± 35.4	16.8 ± 49.0	17.3 ± 63.9
Bamboo shoot	4.18 ± 16.0	4.48 ± 15.9	6.02 ± 24.1	11.3 ± 32.5	11.5 ± 34.6
Onion	2.32 ± 10.2	4.69 ± 16.1	5.93 ± 22.4	3.87 ± 20.8	3.91 ± 30.5
Sweet potato	2.96 ± 21.9	3.26 ± 18.3	2.71 ± 25.3	6.48 ± 39.0	9.18 ± 32.6
Potato	1.79 ± 9.36	6.47 ± 23.3	6.85 ± 31.1	2.13 ± 13.7	5.18 ± 78.3
Shiitake mushrooms	4.63 ± 13.8	4.62 ± 27.9	2.84 ± 13.2	4.89 ± 20.1	3.15 ± 16.1
Chinese fungus	1.67 ± 8.24	2.11 ± 7.51	2.31 ± 12.9	2.29 ± 12.9	1.85 ± 14.0

Note: Consumption rates were calculated based on the food consumption database available from the Nutrition and Health Survey in Taiwan (NAHSIT).

Table S3. Body weight of age populations in Taiwan.

Age population	Body weight (kg)			
	Mean	SD	Min	Max
Preschoolers (n = 523)	19.0	4.58	10.2	45.6
Children (n = 1324)	38.6	12.4	16.3	96.6
Teenagers (n = 2546)	57.8	13.4	29.4	129
Adult (n = 2039)	63.7	10.1	37.2	122
Elderly (n = 893)	60.3	8.55	31.2	107

Note: Body weights were obtained from the NAHSIT database.

Table S4. Original parameter values for the human TK model used for the sensitivity analysis and model optimization.

Parameter	Symbol	Unit	Values
Physiological parameters			
Volume of the saliva compartment	VS	L	0.001[a]
Volume of blood (fraction of BW)	Vb	uintless	0.079[b]
Salivary flow rate	Qsal	L/h	0.069 (0.042–0.120)[a]
Body weight	BW	kg	67[c]
Nitrate parameters			
Dietary intake of nitrates	KuNO3	mmol/h	0[c]
Gastrointestinal absorption fraction	FaNO3	uintless	1[a]
Gastrointestinal absorption rate constant	kaNO3	/h	5.35[a]
Rate constant of endogenous nitrate synthesis	kend	mmol/h	0.109[a]
Overall elimination rate constant of nitrates from the central compartment	kel	/h	0.14 ± 0.01[a]
Nitrate blood-to-saliva secretion rate	ksecNO3	/h	0.045 ± 0.003[a]
Conversion rate of nitrates to nitrites in saliva	kconv	/h	19.95 ± 1.75[a]
Volume of the central nitrate distribution compartment (fraction of BW)	FVNO3	uintless	0.30 (0.29–0.33)[a]
Nitrite parameters			
Dietary intake of nitrate (a single oral dose)	KuNO2	mmol/h	324 mg[c]
Gastrointestinal absorption rate constant	kaNO2	/h	5.35[a]
Gastrointestinal absorption fraction	FaNO2	uintless	1[a]
Nitrite blood-to-saliva secretion rate	ksecNO2	/h	0.045 ± 0.003[a]
Rate constant of nitrite gastrointestinal decay to other products	kdec	/h	0.67[a]
Volume of the central nitrate distribution compartment (fraction of BW)	FVNO2	uintless	0.65 ± 0.03[a]
Hemoglobin/methemoglobin parameters			
Nitrite reaction rate constant with hemoglobin	kNO2	/mM/h	4.23 ± 0.15[a]
Methemoglobin reductase maximum metabolic rate	Vmaxr	mM/h	1.00 ± 0.07[a]
Michaelis-Menten constant of methemoglobin reductase activity	Kmr	mM	0.124 ± 0.0018[a]
Stoichiometric constant for regeneration of nitrates from methemoglobin	z	uintless	0.5 ± 0.01[a]
Background concentration of hemoglobin oxidizing reactants in the blood	Cb	mM	0.00569[a]
Background concentration of hemoglobin in the blood	init CHB	mM	8.3234[a]
Background concentration of methemoglobin concentration in the blood	init CMetHg	mM	0.046[a]

[a]Adopted from Zeilmaker et al.[2] [b]Adopted from Brown et al.[7] [c]Adopted from Kortboyer et al.[1] The value of 324 mg was converted to dietary intake rate of nitrates in the unit of mmol/h.

Table S5. Dietary intake estimates of nitrates and nitrites for different age populations.

Age population	Dietary intake (DI)				
	Mean	SD	2.5th percentile	50th percentile	97.5th percentile
Nitrate					
Preschoolers	2.52	4.68	0.29	1.37	11.9
Children	2.36	3.99	0.29	1.34	10.5
Teenagers	1.57	2.61	0.19	0.90	7.14
Adult	2.11	3.52	0.32	1.28	8.60
Elderly	2.66	6.03	0.33	1.49	11.6
Nitrite					
Preschoolers	0.0151	0.0300	0.003	0.010	0.056
Children	0.0134	0.0216	0.003	0.009	0.047
Teenagers	0.0087	0.0104	0.002	0.006	0.031
Adult	0.0131	0.0324	0.003	0.009	0.045
Elderly	0.0160	0.0267	0.003	0.010	0.062

Unit: mg/kg bw per day

Table S6. Predicted values of internal plasma dose equivalent to the acceptable daily intake (ADI) and plasma dose of dietary intake for nitrates and nitrites.

Dose in plasma	Age population				
	Preschoolers	Children	Teenagers	Adults	Elderly
Internal plasma dose equivalent to the ADI ($IDE_{ADI\mathit{a}}$)					
Nitrate	0.153 (0.0051)	0.155 (0.0057)	0.161 (0.0062)	0.164 (0.0057)	0.162 (0.0056)
Nitrite	5.75×10^{-4} (8.85×10^{-5})	5.81×10^{-4} (8.83×10^{-5})	5.85×10^{-4} (8.69×10^{-5})	5.94×10^{-4} (8.61×10^{-5})	5.87×10^{-4} (8.95×10^{-5})
Internal plasma dose of dietary intake ($ID_{DI\mathit{a}}$)					
Nitrate	0.169 (0.058)	0.114 (0.049)	0.079 (0.025)	0.084 (0.038)	0.092 (0.073)
Nitrite	4.54×10^{-4} (1.78×10^{-4})	3.09×10^{-4} (1.43×10^{-4})	2.15×10^{-4} (7.99×10^{-5})	2.27×10^{-4} (1.10×10^{-4})	2.56×10^{-4} (2.04×10^{-4})

Note: Values were expressed as mean (SD). The internal plasma dose represents simulated steady-state plasma concentration at 48 h after exposure to nitrate and/or nitrite, using the validated human TK model. Unit: mmol/L.

Table S7. Exceedance risks assessed by external does and internal dose metrics for different age populations.

Chemical	Exceedance risk				
	Preschoolers	Children	Teenagers	Adults	Elderly
Probability of %ADI_{EXa} > 100% based on external dose					
Nitrate	0.179	0.150	0.080	0.124	0.162
Nitrite	0.034	0.012	0.007	0.010	0.024
Probability of %ADI_{EXa} > 200% based on external dose					
Nitrate	0.072	0.057	0.033	0.047	0.058
Probability of %ADI_{INa} > 100% based on internal dose					
Nitrate	0.553	0.164	0.008	0.040	0.127
Nitrite	0.228	0.064	0.003	0.015	0.067

Table S8. Nitrate and nitrite concentrations in vegetables by regions of Taiwan.

Chemical	Concentration (mg/kg) in different regions				*p*-value
	North	Central	South	East	
Nitrate	454 ± 616	153 ± 188	224 ± 282	329 ± 495	0.754
Nitrite	2.45 ± 1.44	2.61 ± 1.43	2.49 ± 1.03	4.04 ± 8.78	0.940

Note: Values were expressed as mean ± SD. Because the concentrations did not follow a normal distribution, the non-parametric Kruskal-Wallis test was used to test for statistical differences in nitrate and nitrite concentration levels among the four regions of Taiwan. The statistical analysis was conducted using R software (version 3.6.1, 2019; R Development Core Team, http://www.R-project.org).

Section 5: PBPK Model Code

Note: The Berkeley Madonna model code below is an example of the TK model of nitrate and nitrite for adults. Thus, if the model is applied to other age populations, the mean, standard deviation, lower bound, upper bound values of the parameters BW, DINO3, and DINO2 need to be replaced. The relevant parameter values for BW, DINO3, and DINO2 are shown in Table S3 and Table S5, respectively. Other model parameter values used in the model code are provided in Table 1.

```
METHOD RK4

STARTTIME = 0
STOPTIME = 48
DT = 0.01
DTOUT = 0.1

{Physiological parameters}
init BW = Normal(63.69, 10.11)          ;Normally distributed body weight of adults, kg
next BW = BW                            ;Assignment of the first created value for each integration time step
limit BW >= 37.20                       ;Limit the values within the lower bound
limit BW <= 122.00                      ;Limit the values within the upper bound
Vs = 0.001                              ;Volume of the saliva compartment, L
Vb = 0.079*BW                           ;Volume of blood (fraction of BW), L
init Qsal = Normal(0.069, 0.0167)       ;Normally distributed salivary flow rate L/h
next Qsal = Qsal                        ;Assignment of Qsal
limit Qsal >= 0.042                     ;Limit the values within the lower bound
limit Qsal <= 0.12                      ;Limit the values within the upper bound

{Nitrate parameters}
KuNO3 = DINO3*BW/24/MWNO3                         ;Uptake of nitrates from vegetables, mmol/h
init DINO3 = exp(Normal(DINO3_ln, DINO3_lnsd))    ;Lognormally distributed dietary intake, mg/kg bw/day
next DINO3 = DINO3                                ;Assignment of DINO3
DINO3_ln = logn(DINO3_mean^2/(DINO3_sd^2+DINO3_mean^2)^0.5)    ;Logarithmized DINO3 (mean)
DINO3_lnsd = (logn(1+DINO3_sd^2/DINO3_mean^2))^0.5              ;Logarithmized DINO3 (SD)
DINO3_mean = 2.1046                                             ;Dietary intake DINO3 (mean)
DINO3_sd = 3.5150                                               ;Dietary intake DINO3 (SD)
MWNO3 = 62.0049                                                 ;Molecular weight of nitrates, g/mol

FaNO3 = 1                           ;Gastrointestinal absorption fraction
kaNO3 = 5.35                        ;Gastrointestinal absorption rate constant, /h
kend = 0.198006                     ;Rate constant of endogenous nitrate synthesis, mmol/h
Skel = 0.517331                     ;Overall elimination rate constant of nitrates, /h/BW^-0.25
kel = Skel*BW^-0.25                 ;Allometric overall elimination rate constant of nitrates, /h
init ksecNO3 = Normal(0.045, 0.003) ;Normally distributed nitrate blood-to-saliva secretion rate, /h
next ksecNO3 = ksecNO3              ;Assignment of ksecNO3
limit ksecNO3 >= 0.039              ;Limit the values within the lower bound
limit ksecNO3 <= 0.051              ;Limit the values within the upper bound
init kconv = Normal(19.95, 1.5)     ;Normally distributed conversion rate of nitrates to nitrites in saliva, /h
next kconv = kconv                  ;Assignment of kconv
limit kconv >= 16.981               ;Limit the values within the lower bound
limit kconv <= 22.919               ;Limit the values within the upper bound
VNO3 = FVNO3*BW                     ;Volume of the central nitrate compartment, fraction of BW, L
init FVNO3 = Normal(0.3, 0.0098)    ;Normally distributed fraction of volume of the central nitrate compartment
next FVNO3 = FVNO3                  ;Assignment of FVNO3
limit FVNO3 >= 0.29                 ;Limit the values within the lower bound
limit FVNO3 <= 0.33                 ;Limit the values within the upper bound

{Nitrite parameters}
KuNO2 = DINO2*BW/24/MWNO2                         ;Uptake of nitrites from vegetables, mmol/h
init DINO2 = exp(Normal(DINO2_ln, DINO2_lnsd))    ;Lognormally distributed dietary intake, mg/kg bw/day
next DINO2 = DINO2                                ;Assignment of DINO2
```

```
DINO2_ln = logn(DINO2_mean^2/(DINO2_sd^2+DINO2_mean^2)^0.5)     ;Logarithmized DINO2 (mean)
DINO2_lnsd = (logn(1+DINO2_sd^2/DINO2_mean^2))^0.5              ;Logarithmized DINO2 (SD)
DINO2_mean = 0.0131                                             ;Dietary intake DINO2 (mean)
DINO2_sd = 0.0324                                               ;Dietary intake DINO2 (SD)
MWNO2 = 46.006                                                  ;Molecular weight of nitrites, g/mol

FaNO2 = 1                          ;Gastrointestinal absorption fraction
SKaNO2 = 14.0004                   ;Gastrointestinal absorption rate constant, /h/BW^-0.25
kaNO2 = SKaNO2*BW^-0.25            ;Allometric gastrointestinal absorption rate constant, /h
SKdec = 1.00242                    ;Rate constant of nitrite gastrointestinal decay to other products, /h/BW^-
0.25
kdec = SKdec*BW^-0.25              ;Allometric rate constant of nitrite gastrointestinal decay to other products,
/h
init ksecNO2 = Normal(0.045, 0.003) ;Normally distributed nitrite blood-to-saliva secretion rate, /h
next ksecNO2 = ksecNO2             ;Assignment of ksecNO2
limit ksecNO2 >= 0.039             ;Limit the values within the lower bound
limit ksecNO2 >= 0.051             ;Limit the values within the upper bound
VNO2 = FVNO2*BW                    ;Volume of the central nitrite compartment, fraction of BW, L
init FVNO2 = Normal(0.65, 0.03)    ;Normally distributed fraction of volume of the central nitrite compartment
next FVNO2 = FVNO2                 ;Assignment of FVNO2
limit FVNO2 >= 0.591               ;Limit the values within the lower bound
limit FVNO2 <= 0.709               ;Limit the values within the upper bound

{Hemoglobin/methemoglobin parameters}
kNO2 = 2.70321                     ;Nitrite reaction rate constant with hemoglobin, /mM/h
SVmaxr = 123.326                   ;Methemoglobin reductase maximum metabolic rate, mM/h/BW^0.75
Vmaxr = SVmaxr*BW^0.75             ;Allometric methemoglobin reductase maximum metabolic rate, mM/h
Kmr = 4589.93                      ;Michaelis-Menten constant of methemoglobin reductase activity, mM
Cb = 8.04602e-4                    ;Background concentration of hemoglobin oxidizing reactants in blood, mM
z = 0.626123                       ;Stoichiometric constant for regeneration of nitrates from methemoglobin

{Nitrate dynamics}
;AaNO3 = Amount of nitrates in the absorption compartment
d/dt(AaNO3) = KuNO3 + Qsal*CsNO3 - FaNO3*kaNO3*AaNO3
init AaNO3 = 0

;AcNO3 = Amount of nitrates in the central compartment
;CcNO3 = Concentration of nitrates in the central compartment
;AUCCcNO3 = Area under the curve of CcNO3
d/dt(AcNO3) = FaNO3*kaNO3*AaNO3 + kend + z*kNO2*CHB*CcNO2*Vb - ksecNO3*AcNO3 - kel*AcNO3
init AcNO3 = 0
CcNO3 = AcNO3/VNO3
d/dt(AUCCcNO3) = CcNO3
init AUCCcNO3 = 0

;AsNO3 = Amount of nitrates in the salivary compartment
;CsNO3 = Concentration of nitrates in the salivary compartment
d/dt(AsNO3) = ksecNO3*AcNO3 - Qsal*CsNO3 - kconv*AsNO3
init AsNO3 = 0
CsNO3 = AsNO3/Vs

{Nitrite dynamics}
;AaNO2 = Amount of nitrites in the absorption compartment
d/dt(AaNO2) = KuNO2 + Qsal*CsNO2 - FaNO2*kaNO2*AaNO2 - kdec*AaNO2
init AaNO2 = 0

;AcNO2 = Amount of nitrites in the central compartment
;CcNO2 = Concentration of nitrites in the central compartment
;AUCCcNO2 = Area under the curve of CcNO2
d/dt(AcNO2) = FaNO2*kaNO2*AaNO2 - z*kNO2*CHB*CcNO2*Vb - ksecNO2*AcNO2
init AcNO2 = 0
CcNO2 = AcNO2/VNO2
d/dt(AUCCcNO2) = CcNO2
init AUCCcNO2 = 0

;AsNO2 = Amount of nitrites in the salivary compartment
;CsNO2 = Concentration of nitrites in the salivary compartment
```

```
d/dt(AsNO2) = ksecNO2*AcNO2 + kconv*AsNO3 - Qsal*CsNO2
init AsNO2 = 0
CsNO2 = AsNO2/Vs

{Hemoglobin and methemoglobin dynamics}
;CMetHB = Concentration of methemoglobin in the blood
;CHB = Concentration of hemoglobin in the blood
;AUCCMetHB = Area under the curve of CMetHB
d/dt(CMetHB) = kNO2*CHB*(CcNO2 + Cb) - (Vmaxr*CMetHB)/(Kmr+CMetHB)
d/dt(CHB) = (Vmaxr*CMetHB)/(Kmr+CMetHB) - (kNO2*CHB*(CcNO2 + Cb))
init CMetHB = 0.046
init CHB = 8.33752
d/dt(AUCCMetHB) = CMetHB
init AUCCMetHB = 0

{Mass balance check}
d/dt(INPUT) = KuNO3 + kend + z*kNO2*CHB*CcNO2*Vb
CALCULATION = AaNO3 + AcNO3 + AsNO3 + ExcretedNO3 + ConvNO3
d/dt(ExcretedNO3) = kel*AcNO3
d/dt(ConvNO3) = kconv*AsNO3
MASSBALANCE = INPUT - CALCULATION
init INPUT = 0
init ExcretedNO3 = 0
init ConvNO3 = 0
```

References

(1) Kortboyer, J. M.; Olling, M.; Zeilmaker, M. J.; Slob, W.; Boink, A. B. T. J.; Schothorst, R. C.; Sips, A. J. A. M.; Meulenbelt, J. *The Oral Bioavailability of Sodium Nitrite Investigated in Healthy Adult Volunteers*; National Institute for Public Health and the Environment (RIVM): Bilthoven, Netherlands, 1997.

(2) Zeilmaker, M. J.; Bakker, M. I.; Schothorst, R.; Slob, W. Risk assessment of N-nitrosodimethylamine formed endogenously after fish-with-vegetable meals. *Toxicol. Sci.* **2010**, *116*, 323–335.

(3) Lin, Z.; Fisher, J. W.; Ross, M. K.; Filipov, N. M. A physiologically based pharmacokinetic model for atrazine and its main metabolites in the adult male C57BL/6 mouse. *Toxicol. Appl. Pharmacol.* **2011**, *251*, 16–31.

(4) Worley, R. R.; Yang, X.; Fisher, J. Physiologically based pharmacokinetic modeling of human exposure to perfluorooctanoic acid suggests historical non drinking-water exposures are important for predicting current serum concentrations. *Toxicol. Appl. Pharmacol.* **2017**, *330*, 9–21.

(5) Lambers, A. C.; Kortboyer, J. M.; Schothorst, R. C.; Sips, A. J. A. M.; Cleven, R. F. M. J.; Meulenbelt, J. *The Oral Bioavailability of Nitrate from Vegetables Investigated in Healthy Volunteers*; National Institute for Public Health and the Environment (RIVM): Bilthoven, Netherlands, 2000.

(6) Hunault, C. C.; van Velzen, A. G.; Sips, A. J. A. M.; Schothorst, R. C.; Meulenbelt, J. Bioavailability of sodium nitrite from an aqueous solution in healthy adults. *Toxicol. Lett.* **2009**, *190*, 48–53.

(7) Brown, R. P.; Delp, M. D.; Lindstedt, S. L.; Rhomberg, L. R.; Beliles, R. P. Physiological parameter values for physiologically based pharmacokinetic models. *Toxicol. Ind. Health* **1997**, *13*, 407–484.