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Supplementary Information

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Safety assessment of *Synechococcus* sp. PCC 7002 biomass:

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genotoxicity, acute and subchronic toxicity studies

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Table S1 Composition of *Synechococcus* sp. PCC 7002 biomass

| Pigments | g/100g dry weight | Amino acids | (% of total amino acids) |
|------------------------|---------------------------|----------------------------|---------------------------------|
| Phycocyanin | 15.81 | Threonine | 2.87 |
| Allophycocyanin | 4.77 | Valine | 3.04 |
| Phycoerythrin | 1.77 | Isoleucine | 2.43 |
| Chlorophyll a | 1.02 | Leucine | 4.52 |
| Carotenoid | 0.92 | Lysine | 2.76 |
| Minerals | mg/100g dry weight | Phenylalanine + Tyrosine | 4.50 |
| Ca | 1388.54 | Methionine + Cysteine | 0.96 |
| Cu | 2.23 | Essential Amino Acids | 21.1 |
| Fe | 25.95 | Essential amino acid index | 39.5 |
| K | 1882.29 | | |
| Mg | 825.94 | Fatty acids | (% of total fatty acids) |
| Na | 2280.21 | Palmitic (C16:0) | 59.04 |
| P | 720.83 | Stearic (C18:0) | 1.29 |
| Se (10 ⁻³) | 8.12 | Arachidonic (C20:0) | 0.61 |
| Zn | 15.98 | Total | 60.94 |
| Vitamins | mg/100g dry weight | Palmitoleic (C16:1) | 0.85 |
| VE | 12.33 | Oleic (C18:1) | 24.90 |
| VK ₁ | 0.24 | Eicosenoic (C20:1) | 7.54 |
| VB ₁ | 1.53 | Total | 33.29 |
| VB ₂ | 2.89 | Linoleic (C18:2) | 3.75 |
| VB ₃ | 10.57 | Eicosadienoic (C20:2) | 1.85 |
| VB ₆ | 0.78 | Total | 5.60 |

Table S2 Revertants in the absence or presence of S9 mix in the second Ames test

| | TA97a | | TA98 | | TA100 | | TA102 | | TA1535 | |
|------------------|------------|------------|-------------|------------|------------|------------|-----------|-------------|-----------|-----------|
| | +S9 | -S9 | +S9 | -S9 | +S9 | -S9 | +S9 | -S9 | +S9 | -S9 |
| Control | 115±7 | 106±7 | 32±3 | 34±4 | 99±6 | 96±5 | 302±16 | 271±18 | 19±2 | 17±2 |
| DMSO | 106±6 | 100±12 | 36±8 | 31±4 | 109±13 | 100±4 | 296±7 | 266±15 | 18±3 | 16±4 |
| Sterile water | 113±8 | 111±4 | 38±3 | 33±2 | 102±4 | 99±10 | 305±21 | 285±20 | 19±3 | 20±2 |
| SynB-1 | 113±8 | 110±15 | 35±2 | 32±2 | 104±8 | 102±10 | 304±26 | 288±13 | 20±3 | 17±4 |
| SynB-2 | 111±8 | 105±13 | 34±3 | 34±2 | 114±7 | 109±15 | 292±13 | 281±15 | 18±2 | 22±3 |
| SynB-3 | 136±9 | 103±7 | 32±3 | 30±4 | 116±12 | 124±11 | 301±12 | 273±7 | 20±5 | 20±2 |
| SynB-4 | 134±14 | 119±6 | 34±5 | 31±4 | 114±11 | 112±5 | 284±14 | 278±17 | 18±2 | 19±3 |
| SynB-5 | 124±5 | 105±7 | 36±3 | 33±2 | 126±6 | 123±12 | 312±3 | 264±22 | 20±2 | 16±4 |
| Dexon | | 1131±82*** | | 1260±68*** | | | | 1058±111*** | | |
| NaN ₃ | | | | | | 1051±81*** | | | | 394±23*** |
| 2-AF | 1245±57*** | | 1106±109*** | | 1267±92*** | | | | | |
| 1,8-DHAQ | | | | | | | 785±78*** | | | |
| CP | | | | | | | | | 194±22*** | |

9 Data were expressed as means ± standard deviations (n = 8). ***Significantly different from the sterile
10 water control group, $P < 0.001$. SynB-1, SynB-2, SynB-3, SynB-4 and SynB-5 denote groups treated
11 with *Synechococcus* sp. PCC 7002 biomass at dosages of 62 µg/plate, 185 µg/plate, 556 µg/plate, 1667
12 µg/plate and 5000 µg/plate, respectively.

14 **Table S3** Effect of acute administration of *Synechococcus* sp. PCC 7002 biomass on the organ
 15 coefficients

| | CN-M | CN-F | SynB-M | SynB-F |
|-----------------------------|-----------|-----------|-----------|-----------|
| Heart (*10 ⁻³) | 6.09±0.98 | 6.79±0.91 | 6.19±0.49 | 5.66±1.04 |
| Liver (*10 ⁻²) | 5.03±0.58 | 4.73±0.17 | 4.85±0.39 | 4.79±0.44 |
| Spleen (*10 ⁻³) | 4.99±1.19 | 4.87±0.43 | 5.16±1.34 | 5.72±1.62 |
| Kidney (*10 ⁻²) | 1.58±0.12 | 1.33±0.11 | 1.59±0.12 | 1.37±0.09 |

16 Data were expressed as means ± standard deviations. Abbreviation: CN-M, male mice in the control
 17 group; CN-F, female mice in the control group; SynB-M, male mice in the *Synechococcus* sp. PCC
 18 7002 biomass supplementation group; SynB-F, female mice in the *Synechococcus* sp. PCC 7002
 19 biomass supplementation group.

21 **Table S4** Effect of acute administration of *Synechococcus* sp. PCC 7002 biomass on
 22 hematological parameters

| | CN-M | CN-F | SynB-M | SynB-F |
|-----------------------|--------------|-------------|--------------|--------------|
| WBC ($10^9/L$) | 8.92±4.07 | 8.48±2.58 | 8.75±3.24 | 9.88±2.93 |
| LYM ($10^9/L$) | 7.14±3.46 | 7.22±2.14 | 7.3±2.86 | 7.12±1.65 |
| RBC ($10^{12}/L$) | 9.23±0.39 | 8.55±0.32 | 8.86±0.76 | 8.32±0.11 |
| Platelet ($10^9/L$) | 613.6±119.88 | 645.2±147.1 | 626.8±182.34 | 592.5±102.31 |
| Hb (g/L) | 149.4±8.36 | 138.6±4.11 | 136.0±13.35 | 121.3±18.54 |

23 Data were expressed as means ± standard deviations. Abbreviation: CN-M, male mice in the control
 24 group; CN-F, female mice in the control group; SynB-M, male mice in the *Synechococcus* sp. PCC
 25 7002 biomass supplementation group; SynB-F, female mice in the *Synechococcus* sp. PCC 7002
 26 biomass supplementation group.

Table S5 Urinalysis of mice in the 90-day feeding study

| □ | Dosing period (n=10) | | | □ | Recovery period (n=5) | | |
|---------------|----------------------|-----------|-----------|-----------|-----------------------|---|---------|
| | □ | Control | SynB-L | | SynB-H | □ | Control |
| Male | | | | | | | |
| UBG | - | - | - | - | - | - | |
| BLD | - | - | - | - | - | - | |
| BIL | - | - | - | - | - | - | |
| KET | - | - | - | - | - | - | |
| GLU | - | - | - | - | - | - | |
| PRO | - | - | - | - | - | - | |
| PH | 6.0-6.5 | 6.0-7.0 | 6.0-7.0 | 6.0-6.5 | 6.0-6.5 | | |
| NIT | - | - | - | - | - | | |
| LEU | - | - | - | - | - | | |
| SG | 1.03-1.06 | 1.04-1.05 | 1.04-1.05 | 1.03-1.05 | 1.03-1.05 | | |
| Vc | - | - | - | □ | - | - | |
| Female | | | | | | | |
| UBG | - | - | - | - | - | | |
| BLD | - | - | - | - | - | | |
| BIL | - | - | - | - | - | | |
| KET | - | - | - | - | - | | |
| GLU | - | - | - | - | - | | |
| PRO | - | - | - | - | - | | |
| PH | 6.0-7.0 | 6.0-7.0 | 6.0-7.0 | 6.0-7.0 | 6.0-7.0 | | |
| NIT | - | - | - | - | - | | |
| LEU | - | - | - | - | - | | |
| SG | 1.03-1.05 | 1.04-1.05 | 1.04-1.06 | 1.04-1.06 | 1.04-1.06 | | |
| Vc | - | - | - | - | - | | |

29 “-” indicates a negative testing result. Abbreviation: SynB-L and SynB-H denote mice groups
30 treated with *Synechococcus* sp. PCC 7002 biomass at dosages of 5 g/kg BW and 10 g/kg BW.

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Table S6 Hematologic parameters of mice in the 90-day feeding study

| □ | Dosing phase (n=10) | | | Recovery phase (n=5) | |
|------------------------------|---------------------|---------------|---------------|----------------------|--------------|
| | Control | SynB-L | SynB-H | Control | SynB-H |
| Male | | | | | |
| HGB (g/L) | 158.63±8.17 | 153.71±5.31 | 152.29±7.45 | 151.67±7.36 | 154.67±5.73 |
| WBC (10 ⁹ /L) | 4.65±0.79 | 5.85±0.61 | 5.71±1.36 | 4.30±0.61 | 4.26±0.64 |
| P-LCR (%) | 10.82±3.13 | 10.00±0.99 | 10.20±3.19 | 15.60±0.43 | 19.43±5.59 |
| RBC (10 ¹² /L) | 10.05±0.20 | 9.93±0.36 | 9.94±0.34 | 8.97±0.76 | 9.93±0.37 |
| HCT (%) | 51.78±1.97 | 49.83±1.98 | 50.21±2.31 | 38.63±4.42 | 53.90±1.92 |
| LY (%) | 62.70±4.45 | 67.50±6.36 | 70.61±4.91 | 57.57±25.95 | 51.90±14.73 |
| LY (10 ⁹ /L) | 2.93±0.53 | 3.91±0.20 | 4.05±1.09 | 2.24±0.63 | 3.38±1.82 |
| MCHC (g/L) | 306.32±11.07 | 308.71±5.82 | 300.29±11.61 | 318.33±12.18 | 297.67±11.84 |
| MPV (fl) | 7.18±0.55 | 6.87±0.32 | 7.04±0.62 | 7.50±0.80 | 8.43±0.71 |
| BA (%) | 0.13±0.04 | 0.09±0.06 | 0.13±0.04 | 0.17±0.09 | 0.20±0.08 |
| EO (%) | 3.44±1.12 | 3.03±0.41 | 2.29±0.93 | 3.30±0.80 | 3.77±1.44 |
| PDW (fl) | 14.91±0.15 | 14.97±0.10 | 14.91±0.13 | 15.4±0.08 | 15.2±0.08 |
| PLT (10 ⁹ /L) | 1136.2±146.56 | 984.57±116.70 | 1182.3±115.60 | 981.67±64.88 | 1093.0±58.01 |
| PCT (%) | 0.82±0.16 | 0.76±0.09 | 0.83±0.12 | 0.79±0.05 | 0.92±0.05 |
| Female | | | | | |
| HGB (g/L) | 162.43±5.93 | 159.00±5.72 | 155.00±6.44 | 155.00±4.55 | 161.00±2.16 |
| WBC (10 ⁹ /L) | 5.96±1.50 | 5.8±1.15 | 7.07±1.40 | 2.14±0.57 | 3.01±0.30 |
| P-LCR (%) | 10.91±2.11 | 12.01±2.32 | 10.39±1.96 | 31.57±10.09 | 21.47±1.49 |
| RBC (10 ¹² /L) | 10.32±0.29 | 10.15±0.24 | 10.09±0.37 | 9.10±1.01 | 9.15±1.86 |
| HCT (%) | 52.31±2.00 | 51.33±2.34 | 49.93±1.60 | 49.40±6.09 | 52.00±8.00 |
| LY (%) | 60.91±21.08 | 45.33±24.83 | 58.1±22.02 | 48.60±33.59 | 58.73±30.57 |
| LY (10 ⁹ /L) | 3.69±1.75 | 2.43±1.41 | 4.19±1.89 | 2.06±0.84 | 2.81±1.05 |
| MCHC (g/L) | 310.43±9.31 | 310.04±5.77 | 310.14±7.67 | 314.67±4.15 | 319.33±7.76 |
| MPV (fl) | 7.16±0.41 | 7.33±0.36 | 7.01±0.36 | 9.57±1.34 | 8.53±0.33 |
| BA (%) | 0.10±0.01 | 0.12±0.03 | 0.07±0.04 | 0.17±0.05 | 0.10±0.05 |
| EO (%) | 3.74±1.95 | 3.44±2.10 | 3.34±0.86 | 3.33±0.12 | 3.07±0.29 |
| PDW (fl) | 15.07±0.13 | 15.07±0.12 | 15.04±0.10 | 15.73±0.96 | 15.30±0.08 |
| PLT (10 ⁹ /L) | 1097.7±223.37 | 1018.4±216.90 | 1199.6±157.51 | 1262.3±122.42 | 1124±318.86 |
| PCT (%) | 0.79±0.19 | 0.75±0.16 | 0.85±0.15 | 1.35±0.46 | 0.96±0.28 |

33 Data were expressed as means ± standard deviations. SynB-L and SynB-H denote mice groups treated
 34 with *Synechococcus* sp. PCC 7002 biomass at dosages of 5 g/kg BW and 10 g/kg BW.

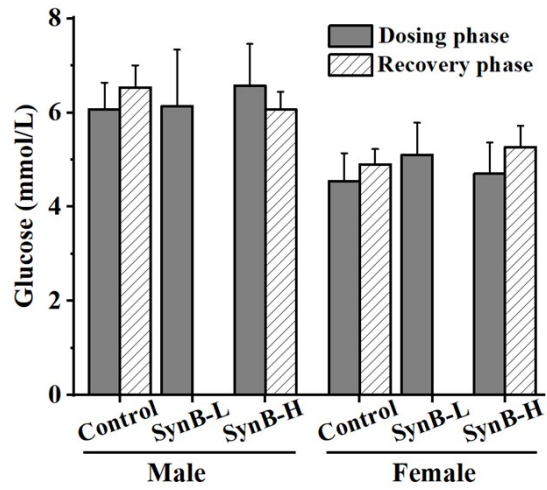
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Table S7 Organ coefficients of mice in the 90-day feeding study

| | Dosing period (n=10) | | | Recovery period (n=5) | | | |
|-----------------------------------|----------------------|-----------|-----------|-----------------------|---|-----------|-----------|
| | □ | Control | SynB-L | SynB-H | □ | Control | SynB-H |
| Male | | | | | | | |
| Testis (10 ⁻³) | | 6.23±1.35 | 6.75±1.23 | 7.03±0.84 | | 6.48±0.23 | 6.64±0.05 |
| Epididymides (10 ⁻³) | | 1.20±0.25 | 1.58±0.35 | 1.3±0.14 | | 1.54±0.14 | 1.63±0.03 |
| Liver (10 ⁻²) | | 4.1±0.29 | 3.93±0.33 | 4.06±0.22 | | 4.05±0.4 | 4.33±0.17 |
| Kidney (10 ⁻²) | | 1.55±0.18 | 1.63±0.16 | 1.51±0.16 | | 1.41±0.13 | 1.57±0.15 |
| Adrenal gland (10 ⁻⁴) | | 1.04±0.14 | 1.14±0.11 | 1.27±0.17 | | 1.63±0.14 | 1.62±0.14 |
| Spleen (10 ⁻³) | | 2.57±0.27 | 2.64±0.21 | 2.82±0.25 | | 2.32±0.17 | 2.17±0.23 |
| Heart (10 ⁻³) | | 4.53±0.47 | 5.01±0.44 | 4.21±0.67 | | 4.65±0.31 | 4.74±0.39 |
| Cerebrum (10 ⁻³) | | 6.66±0.66 | 6.97±0.58 | 7.3±0.45 | | 4.83±0.35 | 4.78±0.48 |
| Cerebellum (10 ⁻³) | | 3.17±0.32 | 3.02±0.26 | 3.49±0.40 | | 3.18±0.21 | 3.24±0.29 |
| Female | | | | | | | |
| Ovary (10 ⁻³) | | 0.91±0.15 | 0.88±0.11 | 1.08±0.14 | | 0.97±0.03 | 0.96±0.03 |
| Uterus (10 ⁻³) | | 4.7±0.89 | 4.13±1.06 | 3.94±0.76 | | 4.54±0.2 | 4.24±0.33 |
| Liver (10 ⁻²) | | 3.87±0.29 | 4.25±0.39 | 4.02±0.17 | | 4.27±0.11 | 4.38±0.22 |
| Kidney (10 ⁻²) | | 1.08±0.13 | 1.19±0.17 | 1.17±0.17 | | 1.26±0.12 | 1.21±0.12 |
| Adrenal gland (10 ⁻⁴) | | 2.36±0.43 | 2.48±0.48 | 2.73±0.28 | | 2.43±0.23 | 2.75±0.35 |
| Spleen (10 ⁻³) | | 3.39±0.41 | 3.99±1.10 | 4.11±0.86 | | 3.38±0.24 | 3.43±0.26 |
| Heart (10 ⁻³) | | 4.97±0.56 | 5.25±0.53 | 5.36±0.57 | | 5.37±0.59 | 5.1±0.56 |
| Cerebrum (10 ⁻³) | | 8.24±0.74 | 8.66±0.62 | 8.97±0.51 | | 8.64±0.33 | 8.43±0.56 |
| Cerebellum (10 ⁻³) | | 3.65±0.22 | 3.75±0.30 | 3.52±0.40 | | 3.83±0.36 | 3.91±0.29 |

38 Data were expressed as means ± standard deviations. SynB-L and SynB-H denote mice groups treated
 39 with *Synechococcus* sp. PCC 7002 biomass at dosages of 5 g/kg BW and 10 g/kg BW.



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42 **Figure S1.** The blood glucose levels of ICR mice in the 90-day feeding study. Data were
 43 expressed as means \pm standard deviations. SynB-L and SynB-H denote mice groups treated with
 44 *Synechococcus* sp. PCC 7002 biomass at dosages of 5 g/kg BW and 10 g/kg BW.