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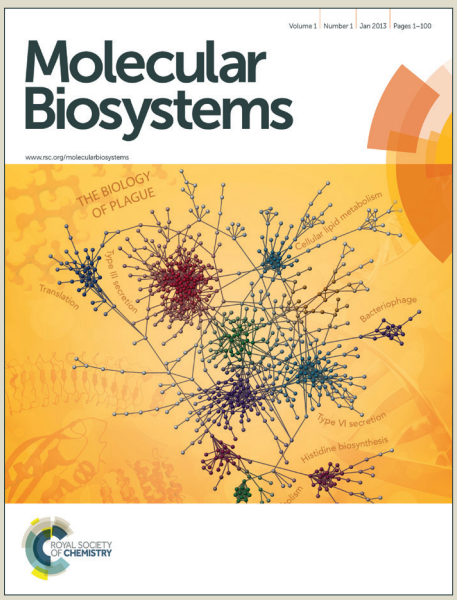

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The toxicity of acute exposure of T-2 toxin evaluated by metabonomics technique

Qianfen Wan[1,5], Guangyao Wu[2], Qinghua He[1,6], Huiri Tang[1,4], Yulan Wang[1,3,*]

1. *Key Laboratory of Magnetic Resonance in Biological Systems, State Key Laboratory of Magnetic Resonance and Atomic and Molecular Physics, Wuhan Centre for Magnetic Resonance, Wuhan Institute of Physics and Mathematics, Chinese Academy of Sciences, Wuhan 430071, P. R. China*
2. *Zhongnan Hospital, Wuhan University, Wuhan, 430072, P. R. China*
3. *Collaborative Innovation Center for Diagnosis and Treatment of Infectious Diseases, Hangzhou 310058, China*
4. *State Key Laboratory of Genetic Engineering, Biospectroscopy and Metabolomics, School of Life Sciences, Fudan University, Shanghai 200433, China*
5. *Graduate School of the Chinese Academy of Sciences, Beijing 100049, P. R. China*
6. *Present address: College of Chemistry and Chemical Engineering, Shenzhen University, Shenzhen 518060*

**To whom the correspondences should be addressed: E-mail:yulan.wang@wipm.ac.cn, Fax: +86-27-87199291, Tel.: +86-27-87197143*

Abstract

T-2 toxin is a common contaminant in grains and animal feedstuff, which becomes an increasing threat to human and animal health due to its high toxicity. Investigating the systemic effects of T-2 toxin is important to evaluate the toxicity and facilitate assessment of food safety. In our investigation, rats were treated with a single dose of T-2 toxin at dosage levels of 0, 0.5, 2.0 and 4.0 mg/kg body weight via gavage. The metabolic profiles of body fluids and multiple organs were obtained by NMR spectroscopy and analyzed by multivariate data analysis methods. The results show that low and moderate doses of T-2 toxin only influenced the urinary metabonomes, while a high dose of T-2 toxin induced metabolic alterations in urine and multiple organs. These changes include alterations in the levels of membrane metabolites, TCA cycle intermediates, a range of amino acids, nucleic acids, nucleosides and nucleotides. T-2 toxin exposure impaired spleen function, causing immunotoxicity, and inhibited protein and DNA biosynthesis. In addition, T-2 toxin also caused oxidative stress and disturbance in energy metabolism and gut microbiome. Our work provided a comprehensive insight into T-2 toxicity and revealed the great potential of metabonomics in assessing the impact of a toxic compound.

Keywords: T-2 toxin, metabonomics, nuclear magnetic resonance (NMR), in vivo toxicity

Introduction

T-2 toxin is a secondary metabolite produced by fusarium species and presents in cereal grains and feedstuff, which poses a great threat to animal and human health and causes considerable economic burden.[1,2] It is a type-A trichothecene mycotoxin that contains a double bond between C9 and C10, an epoxy group between C12 and C13, and variable numbers of acetoxy groups (supplementary Figure S1). Animals infected by T-2 toxin show a large range of pathological signs including growth retardation,[3] oral lesions,[4] gastrointestinal bleeding,[5] and depression of the immune response.[6] Actively dividing cells are more susceptible to T-2 toxin, and the gastrointestinal tract and immune system have been regarded as the target organs.[7]

To date, *in vitro* and *in vivo* studies on T-2 toxicity have mainly focused on single metabolic pathways or organs in one experiment using traditional biochemical or molecular biology methods. These studies show that T-2 toxin can interact with the cell membrane[8] and enter cells, where it binds to the 60S ribosomal subunit and inhibits protein synthesis.[9,10] In addition, T-2 toxin causes oxidative stress and stimulates lipid peroxidation.[11-14] Furthermore, it also impairs mitochondrial function by inhibiting the electron transport chain[15] and induces apoptosis by activating various signaling mediators, such as MAPKs and caspases.[16, 17] More recently, alternations in gene expression induced by T-2 toxin were reported in yeast; these altered genes are associated with oxidative stress, lipid and amino acid metabolism.[18] Systemic metabolic profiling of the T-2 toxicity in animals has not previously been reported. Therefore, a holistic investigation of the systemic metabolic effects of T-2 toxin on a whole living bio-system is required to better understand the overall toxicity of this molecule.

Metabonomics, a well-established technology based on ^{1}H NMR spectroscopy or HPLC-MS techniques assisted with multivariate statistical analysis,[19,20] provides an effective method for evaluating the metabolic responses of living organisms to physiological and pathological stresses. Owing to its efficient and non-invasive characteristics, metabonomics profiling has been successfully applied to different fields, such as drug toxicity evaluations,[21-23] as well as the studies of nutritional intervention[24,25] and mammalian-parasite interactions.[26,27] Our group has previously reported the metabolic effects of aflatoxin B1,[28] mequindox,[29] CCl_4[30] and cyadox[31] on living organisms using a NMR-based metabonomics approach.

In this study, we used a NMR-based metabonomics strategy to analyze systemic endogenous metabolic changes in rat urine, liver, stomach, spleen and thymus, in responses to a single dose of acute T-2 toxin exposure. The objective of this investigation was to explore the underlying toxicity mechanisms of T-2 toxin, which could be important for food safety assessment.

Experimental Methods

Animal Experiment and Sample Collection

The experiment was performed according to the Chinese guidelines for animal welfare and experimental protocol. Forty-eight SPF female Wistar rats (aged 6 weeks, weighted 159 ± 9 g) were purchased from the Center for Disease Control (Hubei, China) and housed in an environmentally controlled facility (22-24 °C, 40-60% relative humidity, 12 h light-dark cycle) at Wuhan Institute of Physics and Mathematics (Hubei, China). All rats had access to food and water. After two weeks of acclimation, rats were randomly divided into four groups of twelve rats in each group: a control group (C, vehicle: 4% ethanol), low-dose group (L, 0.5 mg/kg body weight), moderate-dose group (M, 2.0 mg/kg body weight) and high-dose group (H, 4.0 mg/kg body weight). T-2 toxin was dissolved in 4% ethanol-water and administrated to the dosed rats with a single dose via gavage. Urine samples were collected on 1 day prior to dose, and 8h, 16h post-dose and then on a daily basis until 7 days post-dose. The bodyweight of each rat was monitored at both the start and end of the trial.

At 7 days post-dose with T-2 toxin, all rats were anesthetized under isoflurane after a 12 h fasting. Blood samples were taken from the orbital plexus of each rat and were packed into two Eppendorf tubes with or without the addition of sodium-heparin. The blood samples were kept on ice for several minutes before centrifugation at 1610×*g* for 10 minutes to obtain plasma and serum samples for NMR and clinical biochemistry analysis respectively. Rats were then sacrificed by decapitation. Liver, spleen, stomach and thymus were immediately excised from each rat and each tissue was divided into two portions, half of which were fixed in 10% formalin solution for histopathological evaluation and the other half were snap-frozen in liquid nitrogen and stored at -80 °C until subsequent NMR analysis.

Histopathology and Clinical Biochemistry

To investigate the histopathological changes, the fixed stomach, liver, and thymus tissues were embedded in wax blocks, sectioned, stained with hematoxylin and eosin (H&E), and evaluated under microscopic observation.

A range of clinical biochemistry parameters were measured, including alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatinine (CREA), total protein (TP), albumin (ALB), triglycerides (TG), glucose (GLC), alkaline phosphatase (ALKP) and total cholesterol (CHOL). The data was expressed as mean ± standard deviation (SD) for each group. One-way analysis of variance (one-way ANOVA) was used to evaluate the significant discrimination between control and treatment groups at 5% statistical significance level.

Sample Preparation and NMR Spectroscopy

Spectra of plasma and extracts of kidney tissues obtained from T-2 toxin-dosed and control rats showed no significant differences, hence no further details are given here.

Urine samples (550 μL) were mixed with D_2O phosphate buffer (55 μL, pD 7.4, 1.5 M K_2HPO_4/NaH_2PO_4)[32] containing 0.05% sodium 3-trimethylsilyl-1-[2,2,3,3-$^2D_4$] propionate (TSP) and 0.1% NaN_3 and followed with vortexing and centrifugation (16090 ×*g*, 4 °C). A total of 550 μL of supernatants were transferred into 5 mm NMR tube for subsequent NMR analysis.

Approximately 50 mg of liver, spleen, stomach and thymus tissues were separately extracted with 600 μL cooled methanol/water (2:1) using a Qiagen tissue-lyzer (Retsch GmBH, Germany) at room temperature at 20 Hz for 90 s. The homogenate mixtures were sonicated for 5 cycles consisting of 1 min sonication and 1 min break in an ice bath. After centrifugation (16090 ×*g*, 4 °C) for 10 min, the 500 μL of supernatants were transferred into a 2 mL Eppendorf tube, while the insoluble residues were further extracted twice using the above procedure. The supernatants from three extracts were combined and after removing methanol under vacuum, samples were lyophilized. The obtained powder was reconstituted in 600 μL phosphate buffer for NMR analysis (pD 7.4, K_2HPO_4/NaH_2PO_4 0.15 M, 50%D_2O).

All ^{1}H NMR spectra were acquired at 298 K on a Bruker Avance III 600 MHz NMR spectrometer (operating at 600.13 MHz for ^{1}H) equipped with an inverse cryogenic probe using a standard solvent-suppressed one-dimensional pulse sequence [recycle delay (RD)-90°-3μs-90°-t_m-90°-acquisition]. A total of 32 K data points and 64 transients were collected for each spectrum with a spectral width of 20 ppm. To suppress the water signal, a low-power presaturation pulse was used to irradiate the water resonance during RD (2 s) and t_m (80 ms). For resonance assignment purposes, a set of 2D spectra including ^{1}H J-resolved spectroscopy (JRES), ^{1}H–^{1}H correlation spectroscopy (COSY), ^{1}H–^{1}H total correlation spectroscopy (TOCSY), ^{1}H–^{13}C heteronuclear single quantum correlation (HSQC), and ^{1}H–^{13}C heteronuclear multiple bond correlation (HMBC) were recordedfor selected samples of urine and extracts of spleen, thymus, stomach and liver tissues, as described previously.[33,34]

NMR Data Processing and Multivariate Data Analysis

An exponential window function with a 1 Hz line broadening factor was applied prior to Fourier transformation. ^{1}H NMR spectra were manually corrected for phase and baseline distortions and referenced to TSP (δ 0.0) using Topspin 2.0 (Bruker). The spectra region of δ 0.5-9.0 for tissue extracts (spleen, thymus, and stomach) and δ

0.5-9.4 for liver extracts were uniformly reduced into small bins of 0.002 ppm width, whereas the spectral region of δ 0.5-10 for urine were reduced into small bins of 0.004 ppm width using the AMIX software package (V3.9.5, Bruker Biospin). To eliminate the effects of imperfect water presaturation, the region near the water signal for each spectrum was discarded. Subsequently, to compensate for differences in concentration between different samples, urine spectra were normalized to the total sum of spectral intensity, whereas spectra of tissue extracts were normalized to the fresh weight of tissue prior to multivariate data analysis.

Multivariate data analysis was performed using Simca-P 11.0 software (Umetrics, Sweden). Principal component analysis (PCA) of mean-centered data was performed to show an overview and identify possible outliers within the dataset. Orthogonal projection to latent structure discriminant analysis (OPLS-DA) of the data scaled to unit variance was subsequently carried out to maximize the separation between rats dosed with T-2 toxin and the control rats.[35] Simultaneously, the standard seven-fold cross-validation method and permutation test (permutation number = 200) were used to validate the model. In order to facilitate the interpretation of the result of OPLS-DA model, the loadings from the OPLS-DA were back-transformed by multiplying their respective standard deviations and plotted with a color coded correlation coefficient values in MATLAB.[36] The color represents the significance of the metabolite on class discrimination, with red representing higher significancethan blue. Correlation coefficient was set based on discrimination significance ($p < 0.05$).

Results

Clinical Measurement of Dosed Animals

Rats dosed with T-2 toxin presented lethargy and dry ruffled fur at 8 hour post-dose. Rats in the high-dosed group developed diarrhea, exudative dermatitis around the mouth and nose, and five rats in the high-dosed group died during the study period.

We analyzed the effects of T-2 toxin on bodyweight and growth rate of the rats in the dosed group (Table S1 and Figure S2). The bodyweight of rats in all groups increased throughout the 7-days experimental period, with the rats in T-2 dosed group growing much slower than the rats in the control group. This indicated that T-2 toxin suppressed the growth of rats and that the degree of suppression depends on the dosage of the toxin administered. Serum clinical biochemistry analysis (supplementary Table S2) showed that the serum of low-dosed rats contains higher levels of ALB and lower levels of CHOL than controls. The serum of the moderate-dosed rats presented higher levels of ALB and glucose than controls, whereas the serum of the high-dosed rats contained higher level of

ALKP than controls. Histological examinations showed that T-2 toxin causes no apparent pathological changes in the thymus. However, the stomach from high T-2 dosed rats showed basal cell hyperplasia in the gastric mucosa, while the liver in the high dose group showed signs of steatosis (Figure S3).

Metabolite Assignments Using ^{1}H NMR Spectroscopy

Typical ^{1}H NMR spectra of urine (U) and tissue extracts of spleen (SP), thymus (T), stomach (S) and liver (L) obtained from a control rat (U2, SP2, T2, S2, L2) and a rat treated with high dose of T-2 toxin (U1, SP1, T1, S1, L1) are shown in Figure 1. A total of 64 metabolites are assigned with the aid of literature data[30] and confirmed by a series of 2D NMR spectra. The detailed NMR signals assigned to a particular ^{1}H resonance together with their multiplicity are listed in Table S3.

The urinary spectra are dominated by organic acids, amines, gut microbial-host co-metabolites such as hippurate, phenylacetylglycine (PAG), *p*-cresol glucuronide and *p*-cresol sulfate. Visual inspection of urinary spectra reveals that T-2 toxin causes marked metabolic perturbations including changes in gut microbial-host co-metabolites and TCA cycle intermediates such as succinate and citrate.

The spectra of spleen, thymus, stomach and liver tissue extracts display signals from carboxylic acids, amino acids, glucose, choline metabolites and nucleotide metabolites. Visual inspection of the spectra suggests that rats treated with high-level of T-2 toxin contain higher levels of glycerophosphocholine (GPC) in the liver, higher levels of valine and tyrosine in the stomach, and higher levels of AMP but lower level of valine and xanthine in the spleen.

T-2 toxin-Induced Metabolic Changes in Urine

PCA scores plot (supplementary Figure S4) showed the clear separation between control rats and T-2 toxin dosed rats at 8 h post-dose. Moreover, urinary metabolic profiles of T-2 toxin dosed rats rapidly deviate from the control in the PC1 direction (Figure 2), which is particularly true for the moderate and high levels dosed groups. In addition, the recovery of this deviation is dose dependent: the metabolic profiles in the low-level dosed group display a recovery 8h following toxin administration, whereas those of the moderate-level dosed group recover one day post-dose. In the high-level dosed group, the recovery is achieved three days after dosing.

Variations of urinary metabolic profiles induced by T-2 toxin are obtained by OPLS-DA comparisons of data from dosed rats with those of control rats at matched time points and a typical example is shown in Figure 3. The time dependence of metabolic variation of the three groups is displayed in Figure 4 and Table S4. Here, the red colored

metabolites denote increased levels in the dosed rats compared to the controls, whereas the blue colored metabolites denote decreased levels. Exposure to moderate and high doses of T-2 toxin induced similar urinary metabolic alteration. Both dose levels caused a continuous increase in the urinary levels of *p*-cresol glucuronide, *p*-cresol sulfate and phenylacetylglycine and a decrease in the levels of 1-methylnicotinamide (MNA), trigonelline, dimethylglycine (DMG) and hippurate. The levels of taurine were increased at early time points but decreased later. The urinary levels of TCA cycle intermediates were decreased in the high dose group but increased in the moderate dose group.

T-2 toxin-Induced Metabolic Changes in Multiple Organs

In order to further evaluate the effects of T-2 toxin on organs, OPLS-DA models are constructed to compare metabolic profiles of extracts from spleen, thymus, liver and stomach obtained from dosed rats to those obtained from control rats (Figure 5). The results suggest that the low-dose of T-2 toxin exposure has no significant influences on the metabonomes of all the organs studied here and moderate dosage only affects the metabonomes of the stomach, whereas high dose exposure causes marked effects on all organs studied. In the spleen, high dose of T-2 exposure induces increase in the levels of betaine, succinate, fumarate and nucleotides (AMP, IMP and UMP) accompanied with reduction of PC, PE, nicotinamide and a range of amino acids and nucleotides. The thymus obtained from high-level dosed rats contains higher levels of uridine and GSSG and lower levels of PE as compared to the control rats. The high level of T-2 toxin exposure induces a significant reduction in the levels of fumarate, lysine and alanine and increases in NAD and GPC in the liver. Moderate-dose of T-2 toxin exposure increases the levels of alanine, hypoxanthine and tyrosine in the stomach, whereas further increased levels of a range of amino acids (phenylalanine, tyrosine, glycine, methionine and valine), 3-hydroxybutyrate and nucleotides (adenosine, hypoxanthine, xanthine and uracil) are noted in the stomach of rats exposed to high levels of T-2 toxin.

Discussion

T-2 toxin has been a focus of many studies due to its widespread existence as a natural food contaminant and its potential threat to the health of humans and farm animals. Recently, this molecule was shown to induce several cytotoxic effects, including induction of oxidative stress, inhibition of protein, DNA and RNA synthesis, disruption of cell membrane structure, and impairment of mitochondrial functions. To explore this toxicity further, we analyzed the metabolic profiles of urine and multiple organs of rats exposed to T-2 toxin utilizing a metabonomic

approach. The results showed that exposure to low (0.5 mg/kg) and moderate (2 mg/kg) doses of T-2 toxin caused slight metabolic perturbations mainly in the urinary profiles (Figure 4 and Table S4). However, high levels of T-2 toxin exposure induced significant metabolic changes in the urine and multiple organs (Table 1 and Figure 4, 5).

One of the most pronounced metabolic changes induced by T-2 exposure is the depletion in the levels of a range of amino acids in the spleen (Table 1 and Figure 5). The spleen plays a role in the recycling of hemoglobin from senescent erythrocytes to generate free amino acids; hence spleen is rich in amino acids (Figure 1). The depletion of amino acids in the spleen of T-2 exposed rats may imply an impairment of spleen function. This would in turn be expected to lead to immunotoxicity in response to T-2 exposure. This has indeed been observed previously as T-2 ingestion suppressed synthesis of antibodies towards sheep red blood cells, dinitrophenyl-bovine serum albumin[37] and horse globulin, together with reduced leukocyte counts and hematotoxicity.[38] Disturbances of spleen osmolarity could contribute to these effects and is suggested by the increase in the level of betaine, an organic osmolyte.[39] Having said this, T-2 toxin is known to inhibit protein synthesis due to its strong affinity for peptidyltransferase,[9] and since the toxin was administrated into rats via gavage, the stomach is expected to be affected the most. Indeed, we observed increased levels of valine, methionine, phenylalanine, tyrosine and glycine in the stomach of treated animals. In addition, T-2 toxin was reported to damage DNA[40,41] and inhibit DNA synthesis in mice after 7 days of treatment,[42] which would inevitably cause the alterations in nucleic acids, nucleosides and nucleotides observed in organs from T-2 exposed rats. Furthermore, owing to its lipophilicity, T-2 toxin can disrupt the integrity and function of cellular membranes leading to cytotoxicity.[8] Consistent with this notion, we observed a decrease in the levels of phosphocholine (PC) in the spleen and phosphoethanolamine (PE) in both spleen and thymus and the elevation of glycerophosphocholine (GPC) in the liver of treated rats.

We also observed the elevation of GSSG and 3-hydroxybutyrate. 3-hydroxybutyrate is a product of fatty acid peroxidation, in which free radicals are produced. The observation of elevated GSSG clearly indicate that T-2 promotes an anti-oxidative response in the host, supporting that free radicals are being produced as a consequence of T-2 toxin exposure. Data in support of this hypothesis can be found in the literature regarding the association between T-2 and free radical production. For example, Chaudhari et.al. reported that T-2 toxin induced oxidative damage and changes in the gene expression profiles of antioxidant enzymes in mice liver.[14] Rizzo et.al. reported that T-2 toxin and DON stimulated lipid peroxidation in animal liver via the production of free radicals and the use of selenium, ascorbic acid and α-tocopherol, could provide protection against oxidative damage.[13] Hoehler et.al. also found that T-2 toxin stimulated lipid peroxidation via the generation of hydroxyl radicals in yeast.[43] In addition to the changes of 3-hydroxybutyrate and GSSG, we noted depletion in urinary 1-methylnicotinate and

1-methylnicotinamide (methylated metabolites of niacin (vitamin B3) and nicotinamide, respectively) during the conversion of *S*-adenosyl-methionine to *S*-adenosyl-homocysteine during cysteine biosynthesis (Figure 6). The decrease in levels of 1-methylnicotinamide and 1-methylnicotinate may be related to oxidative stress via the methionine cycle pathway. The concentration of *S*-adenosyl-methionine, a necessary substrate for 1-methylnicotinate and 1-methylnicotinamide synthesis, was depleted due to regenerate glutathione stores through the transsulfuration pathway. All of these results suggest that T-2 toxin induced oxidative stress in the rats exposed to T2.

T-2 toxin caused the depletion of urinary succinate, citrate and fumarate in the liver, accompanied with the increase in NAD in high levels of T-2 exposed rat, suggesting that T-2 suppressed the rate of the tricarboxylic acid (TCA) cycle (Figure 6). Previous research showed that T-2 toxin can impair mitochondrial function through the inhibition of succinate dehydrogenase activity and the promotion of mitochondrial NADH dehydrogenase activity.[15,44] The intestinal absorption of monosaccharide in rats was inhibited after T-2 toxin treatment, which further contributed to the changes in energy metabolism.[45] This is also in agreement with the reduced growth rate observed in rats dosed with T-2 toxin (Figure S2).

The increase in phenylacetylglycine, *p*-cresol glucuronide and *p*-cresol sulfate accompanied with the decrease in hippurate, all observed in the urine from T-2 exposed rats suggested that T-2 toxin disturbed the gut microbial ecosystem. However, impairment of hepatic microsomal cytochrome P-450 (CYP-450) cannot be ruled out. Indeed, the aromatic amino acids, phenylalanine and tyrosine, are firstly converted into phenylacetate and *p*-cresol via the action of the gut microbiota[46], then phenylacetate and *p*-cresol conjugate with glycine and glucuronide to form phenylacetylglycine and *p*-cresol glucuronide in the liver and the gut mucosa.[47,48] Our findings are consistent with previous investigations of piglets exposed to T-2 toxin, where T-2 toxin uptake was shown to change almost all the gut flora and predominantly induced an increase of aerobic bacteria in the intestine.[49] A disturbance of the gut microbes was also observed in aflatoxin B1[28] and deoxynivalenol exposed animals.[50] Other studies also showed that T-2 toxin exposure decreased the activity of CYP-450[51,52] but enhanced the activity of UDP-glucuronosyl transferase,[51] which may represent an alternative mechanism for T-2 toxin action.

Conclusion

In this study, we systematically analyzed the metabolic responses of rats to a single acute dose of T-2 toxin using metabonomics profiling. The results showed that low and moderate levels of T-2 toxin caused slight metabolic alterations in the urine, while high levels of T-2 toxin induced a series of metabolic changes in the urine, spleen,

thymus, stomach and liver involving several metabolic pathways. These include oxidative stress, effects on cellular membrane, alteration in energy metabolism, inhibition of protein, DNA and RNA synthesis, and disruption of gut microbial balance. The immune system was particularly susceptible to T-2 toxin due to the impairment of spleen function. The metabolic responses of different species to long-term T-2 toxin exposure are needed to increase our understanding of the toxicity of this molecule and provide vital information for the assessment of food safety.

Acknowledgement

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Table and Figure Caption:

Table 1. High dose levels of T-2 toxin induced significantly changed metabolites in the extracts of liver, thymus, spleen and stomach.

Figure 1. Typical 600 MHz ^{1}H NMR spectra obtained from urine (U) and tissue extracts of spleen (SP), thymus (T), stomach (S) and liver (L) obtained from a control rat (U2, SP2, T2, S2, L2) and a rat treated with high dose of T-2 toxin (U1, SP1, T1, S1, L1). Keys are listed in Supplementary Table S3.

Figure 2. PCA trajectory mapping the urinary metabolic alterations of rats following treatment with 0 mg/kg.bw (black line) or low-dose (red line), moderate-dose (green line) and high-dose (purple line) of T-2 toxin. The solid symbols of each group represent the starting and ending points.

Figure 3. OPLS-DA scores (left) and coefficient plots (right) obtained from ^{1}H NMR spectra of urine. Top panel: comparison between control group (black box) and low-dosed group (red dot) at 8h post-dose; middle panel: comparison between control group and moderate-dosed group (blue diamond) at 16h post-dose; bottom panel: comparison between control group and high-dosed group (green triangle) at 2 day post-dose. Keys: 11, succinate. 13, citrate. 15, creatine. 16, creatinine. 22, taurine. 49, hippurate. 50, phenylacetylglycine. 51, 1-methylnicotinate. 52, 1-methylnicotinamide. 53, pseudouridine. 54, p-cresol glucuronide. 55, p-cresol sulfate. U1, U2: unknown.

Figure 4. Significant altered metabolites in response to low-dose (C-L), moderate-dose (C-M) and high-dose (C-H) of T-2 toxin at different time points. The red color indicates an increase of urinary metabolite in the dosed rats relative to control group and blue color indicates a decrease.

Figure 5. OPLS-DA scores (left) and coefficient plots (right) obtained from comparing^{1}H NMR spectra of spleen, thymus, liver and stomach tissue extracts of control rats (black box) with rats treated with high-dose of T-2 toxin (green triangle). Keys are shown in Table 1.

Figure 6. The summary of altered metabolic pathway in Wistarrats following T-2 toxin treatment. Metabolites with red or blue color denote significant increases or decreases in T-2 toxin treated rats with respect to control rats. Metabolites with black color denote no marked change.

Supporting Information:

Table S1, Bodyweight and growth rate for control and T-2 toxin dosed rats.

Table S2, Clinical biochemistry parameters of rats exposed to different levels of T-2 toxin.

Table S3, NMR assignments of the metabolites in rat urine, thymus, stomach, spleen and liver aqueous tissue extracts.

Table S4, The significantly changed metabolites in the urine of low, moderate and high dosed rats.

Figure S1, Structure of T-2 toxin.

Figure S2, The bodyweight of control group and different levels of T-2 dosed groups at both the starting and ending of the trial as well as the growth rates of different groups.

Figure S3, Histological examinations of liver (× 200) and stomach (× 100) from control rats and the rats after 7 days high dose of T-2 toxin exposure. The liver in the high dose group showed signs of steatosis. The stomach from high T-2 dosed rats showed basal cell hyperplasia in the gastric mucosa.

Figure S4, PCA scores plot of 1D spectra of urine obtained from the control, low dose, moderate dose and high dose rats after 8 hour T-2 toxin exposure.

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Table 1: High dose levels of T-2 toxin induced significantly changed metabolites in the liver, thymus, spleen and stomach extract.

(key)	metabolites	liver R2X=0.493 Q2=0.669	thymus R2X=0.467 Q2=0.781	spleen R2X=0.504 Q2=0.718	stomach R2X=0.333 Q2=0.702
2	leucine(0.965)	-[a]	-	-0.909[b]	-
3	valine(1.051)	-	-	-0.882	0.755
4	3-hydrobutyrate(1.209)	-	-	-	0.74
7	alanine(1.495)	-0.814	-	-	-
8	lysine(1.735)	-0.754	-	-0.862	-
11	succinate(2.407)	-	-	0.897	-
12	GSSG(2.553)	-	0.93	-	-
14	aspartate(2.801)	-	-	-0.769	-
17	PC(4.169)	-	-	-0.921	-
18	GPC(4.331)	0.812	-	-	-
20	PE(3.981)	-	-0.917	-0.805	-
21	betaine(3.911)	-	-	0.724	-
22	taurine(3.423)	-	-	-0.869	-
26	methionine(2.657)	-	-	-	0.788
27	glycine(3.565)	-	-	-	0.816
36	uracil(7.555)	-	-	-	0.815
37	uridine(7.871)	-	0.824	-0.73	-
39	UMP(8.107)	-	-	0.92	-
41	adenosine(8.351)	-	-	-	0.798
42	fumarate(6.525)	-0.751	-	0.769	-
43	tyrosine(6.913)	-	-	-0.702	0.868
44	phenylalanine(7.341)	-	-	-0.911	0.75
46	hypoxanthine(8.217)	-	-	-0.868	0.872
47	xanthine(7.893)	-	-	-0.894	0.85
48	nicotinamide(8.953)	-	-	-0.759	-
61	NAD(9.347)	0.747	-	-	-
63	IMP(8.571)	-	-	0.779	-
64	AMP(8.599)			0.851	-

a: No difference between control and T-2 toxin dosed group.

b: Metabolite with positive or negative correlation coefficient indicate an increase or decrease after T-2 toxin exposure.

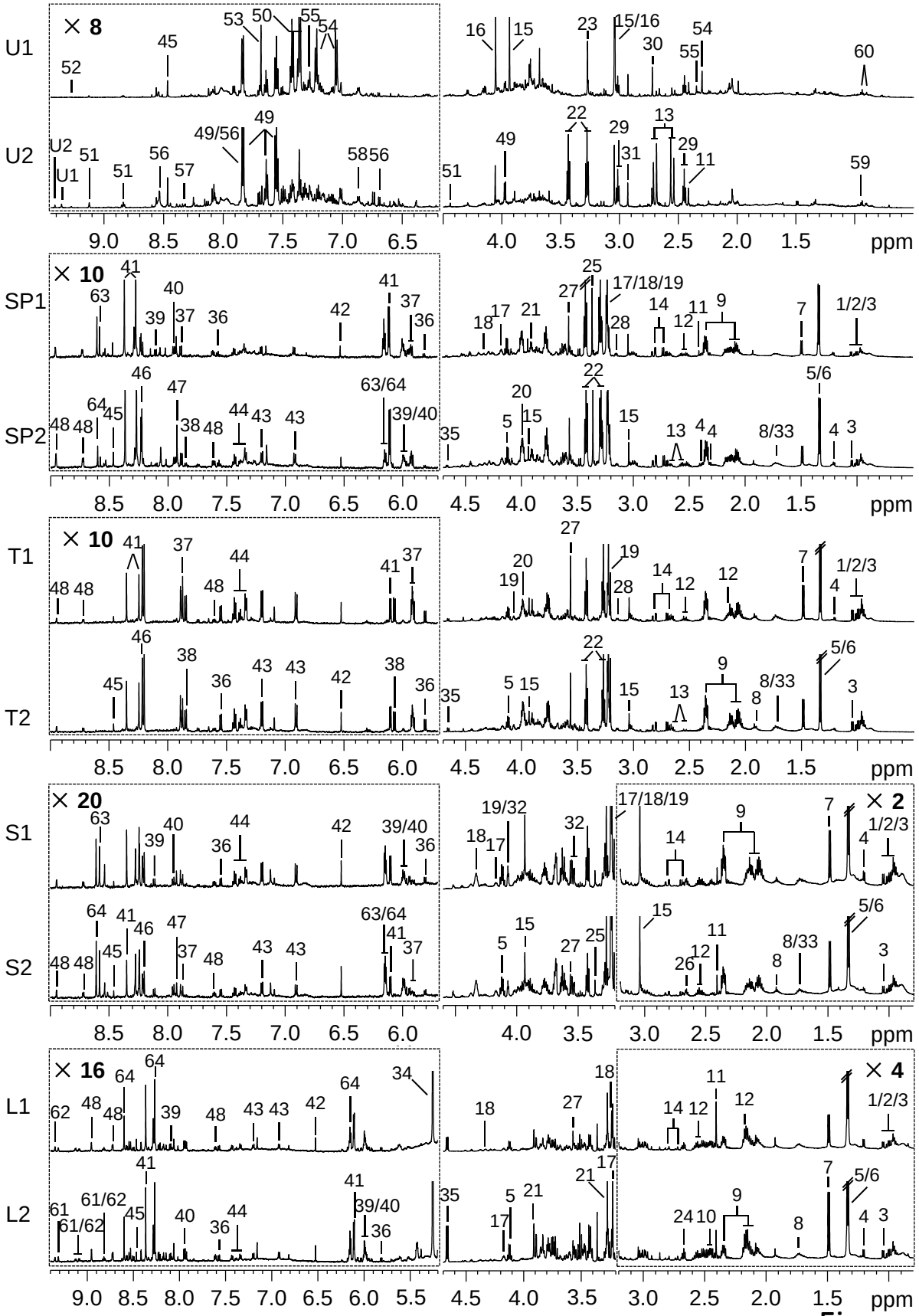

Figure 1

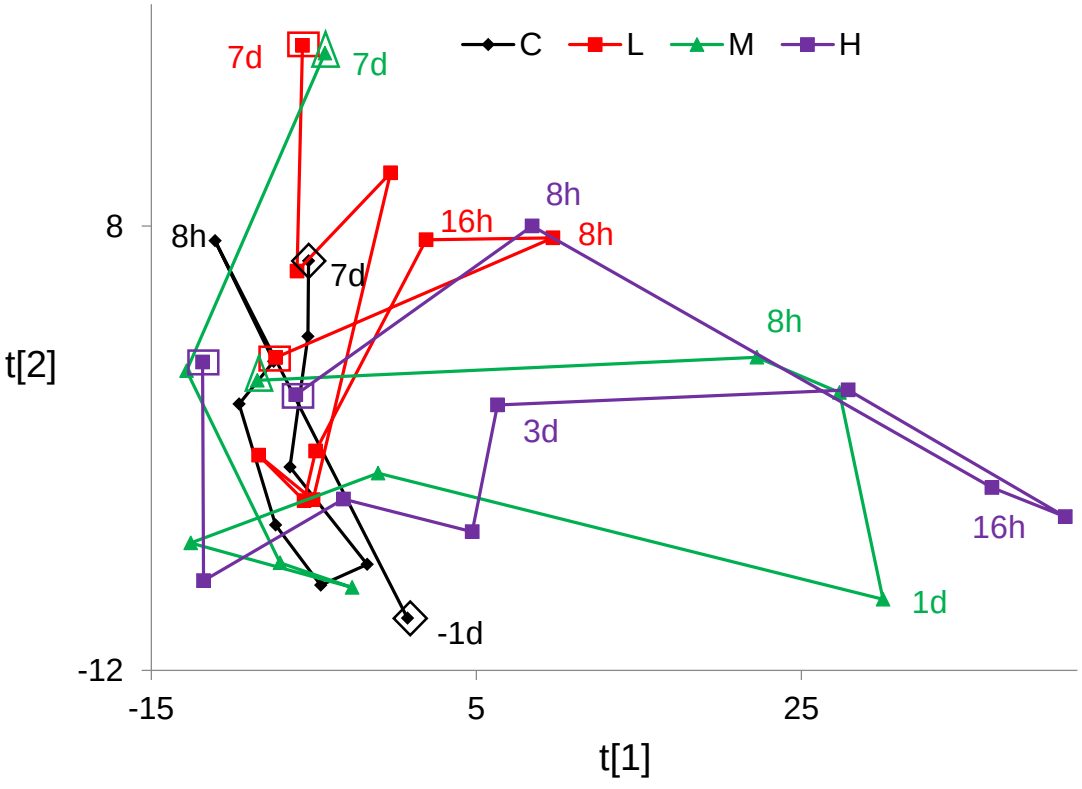

Figure 2

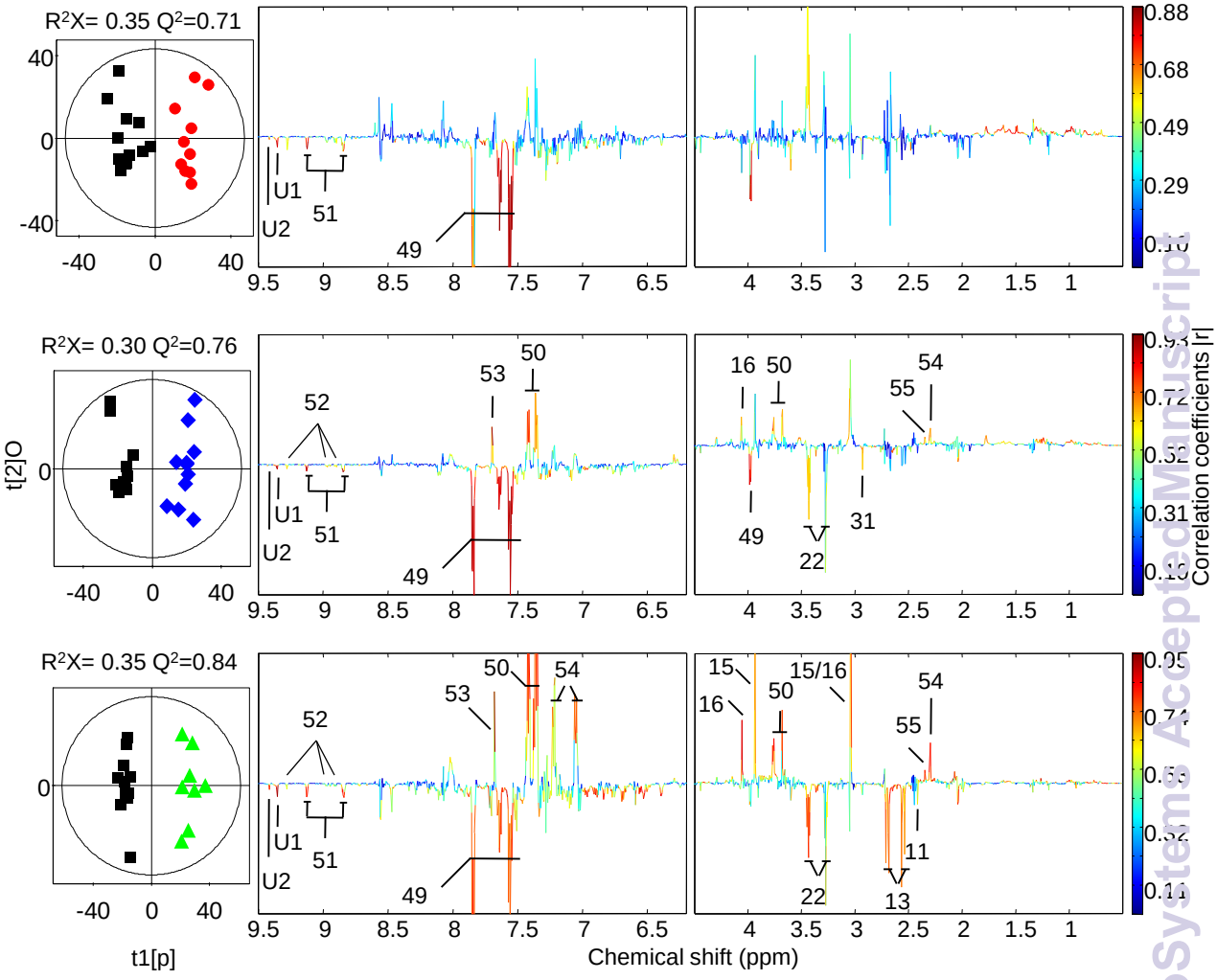

Figure 3

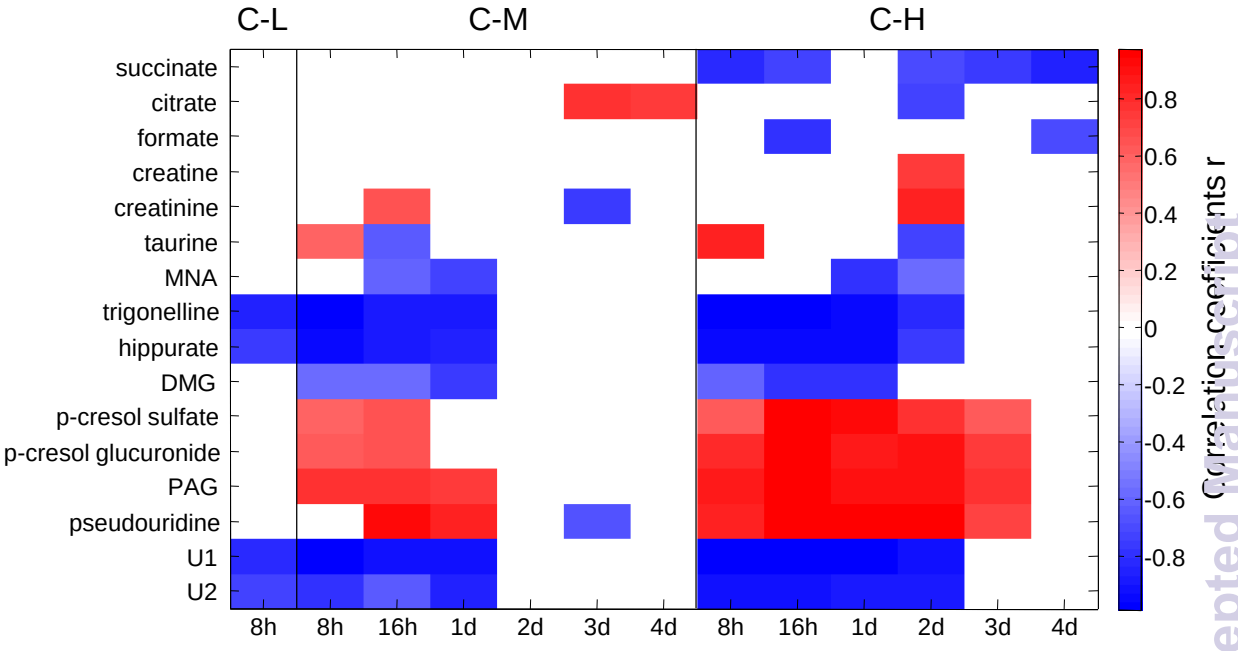

Figure 4

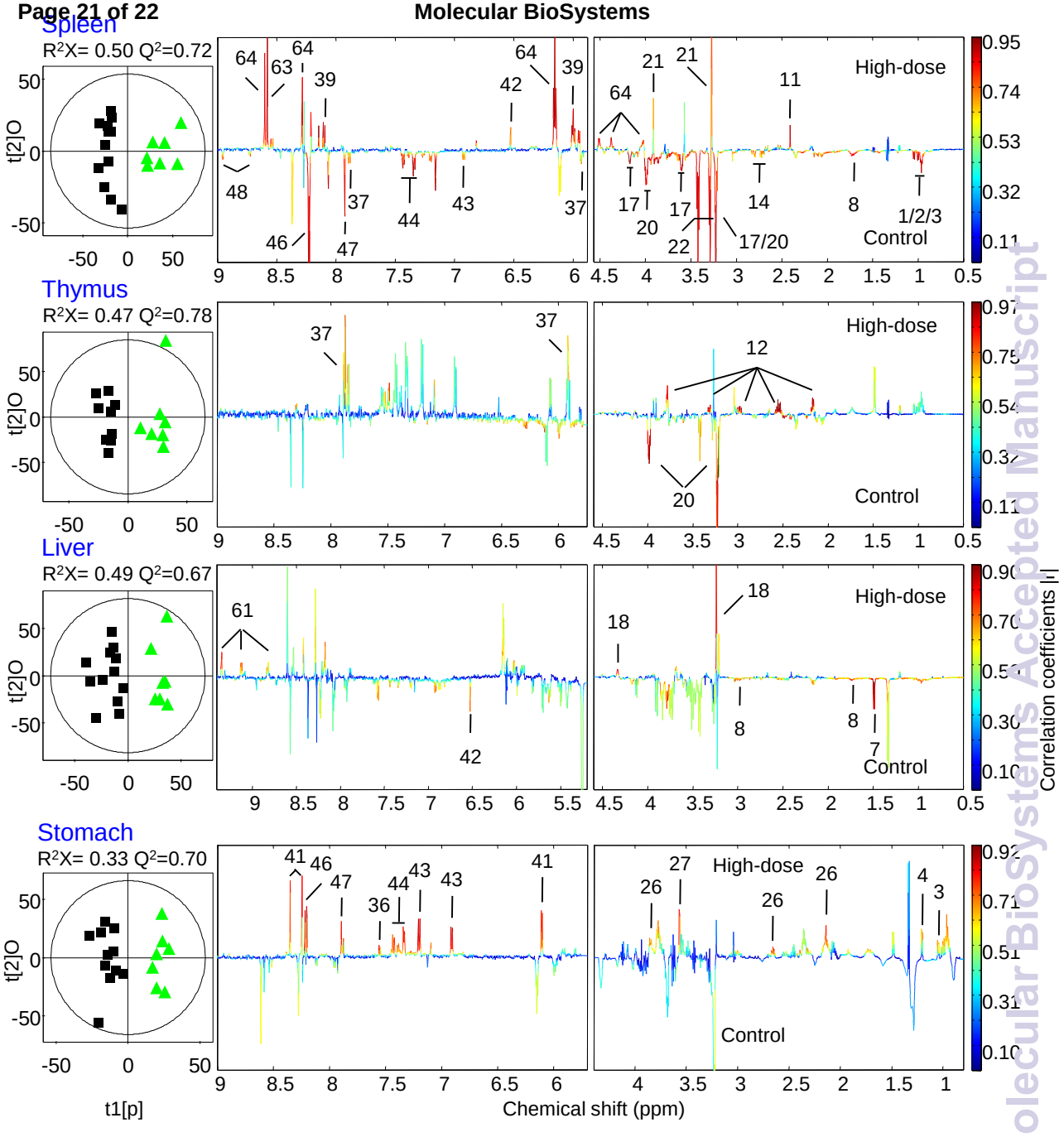

Figure 5

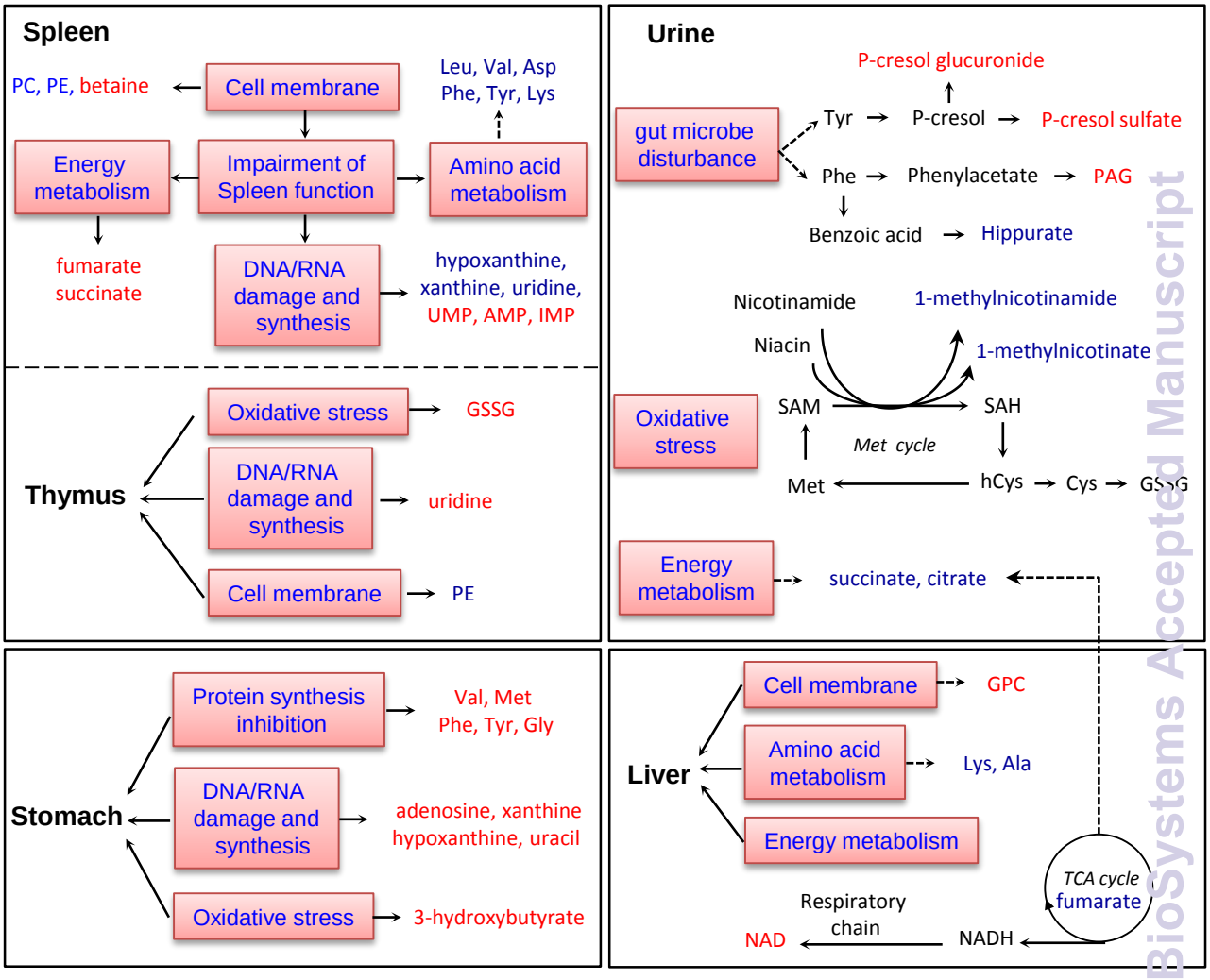

Figure 6