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1 Figure legends



Figure 1. PCA-LDA of ATR-FTIR spectral data extracted from the testicular cells 3 of rats exposed to 4-Nonylphenol (NP) at each concentration vs. control. Three-4 dimensional (3-D) PCA-LDA scores plots for ATR-FTIR spectra regions of 1800-900 5 cm⁻¹ (A) and of 3200-2800 cm⁻¹ (B). Linear discriminant 1 (LD1) scatter plots from 6 PCA-LDA for ATR-FTIR spectra regions of 1800-900 cm⁻¹ (C) and of 3200-2800 cm⁻¹ 7 ¹(**D**). Confidence ellipsoids (90%) were drawn in each 3D scores plot. The data of each 8 LD1 scatter plot is represented as mean \pm standard deviations. *n*=6 for each group. 9 Significance of category segregation was determined using one-way ANOVA with the 10 Fisher's LSD or Dunnett's T3 post hoc test, ***P < 0.001 versus the control group (0 11 mg/kg NP). 12



Figure 2. Cluster vector plots comparing the control (red line at origin) and 4-14 Nonylphenol (NP)-treated groups. (A) 21-day-old rats; (B) 35-day-old rats; (C) 50-15 day-old rats. The spectra cut at 1800-900 cm⁻¹ (left column), were baseline-corrected 16 and normalized to the Amide I peak prior to PCA-LDA, and the spectra cut between 17 3100 and 2800 cm⁻¹ (right column), were baseline-corrected and vector-normalized. 18 Plots were generated following PCA-LDA and show the top eight discriminating 19 wavenumbers (cm⁻¹) responsible for the separation between NP exposure and control 20 groups (0 mg/kg NP). Data represent the average of six rats per group. 21



Figure 3. Comparison of discriminating wavenumbers (cm⁻¹) with tentative 24 biochemical assignments between control and 4-Nonylphenol (NP)-treated groups. 25 ATR-FTIR spectra were from the testicular cells of mice exposed to different 26 concentrations of NP. (A) Lipid-to-protein ratio (1740 cm⁻¹/1400 cm⁻¹ ratio); (B) 27 Peptide aggregation (1630 cm⁻¹/1650 cm⁻¹ ratio); (C) Amide I-to-Amide II ratio (1655 28 $cm^{-1}/1545 cm^{-1}$ ratio); (D) Phosphate-to-carbohydrate ratio [(1055-1045) cm⁻¹/(1555-29 1535) cm⁻¹ ratio]. All the data are represented as mean \pm standard deviations. *n*=6 for 30 each group. **P*<0.05, ***P*<0.01, ****P*<0.001 *versus* control group (0 mg/kg NP), one-31 way ANOVA with the Fisher's LSD or Dunnett's T3 post hoc test. 32



Figure 4. PCA-LDA and resultant cluster vectors plots for Raman spectra 34 extracted from testicular interstitial tissue in rats treated and untreated with 4-35 Nonylphenol (NP). (A) Top row: 21-day-old rats. (B) Middle row: 35-day-old rats. (C) 36 Bottom row: 50-day-old rats. Three-dimensional (3-D) PCA-LDA scores plots (A, B 37 and C), Linear discriminant 1 (LD1) scatter plots (D, E and F), cluster vectors plots (G, 38 **H** and **I**), for Raman spectra region at 1800-900 cm⁻¹ (fingerprint region). Spectra were 39 baseline-corrected and normalized to the Amide I peak. Confidence ellipsoids (90%) 40 41 were drawn in each 3D scores plot. The data of each LD1 scatter plot is represented as 42 mean \pm standard deviations. Cluster vector plots were generated following PCA-LDA and show the top eight discriminating wavenumbers responsible for the separation 43 between NP exposure and control groups. Data represent the average of six mice per 44 group. Significance of category segregation was determined using one-way ANOVA 45 with the Fisher's LSD or Dunnett's T3 post hoc test, ***P <0.001 versus control group 46 (0 mg/kg NP).47



Figure 5. Comparison of discriminating wavenumbers (cm⁻¹) with tentative 49 biochemical assignments between control and 4-Nonylphenol (NP)-treated groups. 50 51 Raman spectra were from the testicular interstitial tissue of mice exposed to different concentrations of NP. (A) Protein-to-lipid ratio (1650 cm⁻¹/1440 cm⁻¹ ratio); (B) 52 Unsaturated lipids (1654 $\text{cm}^{-1}/1445 \text{ cm}^{-1}$ ratio); (C) Saturated lipids (1303 $\text{cm}^{-1}/1267$ 53 cm⁻¹ ratio). All the data are represented as mean \pm standard deviations. *n*=6 for each 54 group. *P < 0.05, **P < 0.01, ***P < 0.001 versus control group (0 mg/kg NP), one-way 55 56 ANOVA with the Fisher's LSD or Dunnett's T3 post hoc test.

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Figure 6. PCA-LDA scores plots and resultant cluster vectors plots for ATR-FTIR 61 spectra acquired from Sertoli cells exposed to 4-Nonylphenol (NP) at various doses 62 (2.5, 5, 10 and 20 µM) compared to the control (0 µM NP). Upper row: two-63 dimensional (2D) PCA-LDA scores plot of Linear discriminant 1 (LD1) versus Linear 64 discriminant 2 (LD2) (A), LD1 scatter plots (C) and cluster vectors plots (E) for ATR-65 FTIR spectra region at 1800-900 cm⁻¹ with baseline-correction and normalization to the 66 Amide I peak (1650 cm⁻¹). Lower row: an expanded view (**B**, **D** and **F**) of the CH 67 stretching region 3100-2800 cm⁻¹, baseline-corrected and vector-normalized. 68 Confidence ellipsoids (90%) were drawn in each 2-D scores plot. The data of each LD1 69 70 scatter plot is represented as mean \pm standard deviations of three experiments. Cluster vectors plots were generated following PCA-LDA and show discriminating 71 wavenumbers. *P < 0.05, ***P < 0.001 versus control group (0 μ M NP), one-way 72 73 ANOVA with the Fisher's LSD or Dunnett's T3 post hoc test.



Figure 7. Comparison of discriminating wavenumbers (cm⁻¹) with tentative 75 biochemical assignments between the control and 4-Nonylphenol (NP)-treated 76 Sertoli cells. ATR-FTIR spectra were from Sertoli cells treated with 0, 2.5, 5, 10 and 77 20 μ M NP for 12 h. (A) Lipid-to-protein ratio (1740 cm⁻¹/1400 cm⁻¹ ratio); (B) Peptide 78 aggregation (1630 cm⁻¹/1650 cm⁻¹ ratio); (C) Amide I-to-Amide II ratio (1655 cm⁻¹ 79 $^{1}/1545 \text{ cm}^{-1} \text{ ratio}$; (**D**) Phosphate-to-carbohydrate ratio [(1055-1045) cm⁻¹/(1555-1535)] 80 cm^{-1} ratio]. All the data are represented as mean \pm standard deviations of three 81 experiments. *P<0.05, **P<0.01, ***P<0.001 versus control group (0 mg/kg NP), one-82 way ANOVA with the Fisher's LSD or Dunnett's T3 post hoc test. 83