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Supplemental Material

Metabolic Effects of a Chronic Dietary Exposure to a Low-Dose Pesticide Cocktail in Mice: Sexual Dimorphism and Role of the Constitutive Androstane Receptor

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Figure S1. Diagram of the murine CAR gene. (A) Boxes represent exons 1 and 2 and arrows the forward and reverse primers CAR 2H3S and CAR R2 in WT animal, CAR 2H3S and CAR R3 in $CAR^{-/-}$ mice. Homologous recombination resulted in replacement of exon 1 and 2 with the β -gal and *neo* resistance genes. (B) Primer Sequences for the 5' strand mCAR 2H3S, and for the 3' strand mCAR R2 in WT and mCAR-R3 in $CAR^{-/-}$ animals. (C) The sizes of the generated DNA sequences using PCR were 315 and 500 bp in WT and $CAR^{-/-}$ mice respectively.

Figure S2. Pesticide metabolites identified in urine samples from male and female mice exposed for 48 weeks. (A) An example MS/MS spectrum of m/z 230 corresponding to [M-H] of THPI conjugated to sulphate (Captan SO₃). (B) Chemical structures of pesticide metabolites identified in urine samples. Position of glucuronide. Boscalid Glc ac (a and b) and mercapturic conjugation of boscalid are undetermined.

Figure S3. Quantity of ingested pesticides per gram of WT mouse. (A) Level of exposure based on pesticide levels in pellets as determined by GC/MS LC/MS/MS and measured food consumption as a function of exposure duration; (B) Mean \pm standard error of the mean pesticide exposure in male (M) and female (F) mice over the 52-week exposure period. *** $P < 0.001$ compared to respective TDI as determined using the Student's t-test. $n = 18$ mice per group. TDI, Tolerable Daily Intake; BW body weight.

Figure S4. Food and water intake in WT male (M) and female (F) mice who were fed pesticide chow (P) or control chow (C). Data are presented as mean \pm standard error of the mean. * $P < 0.05$ compared to mice fed control chow as determined using a two-way ANOVA. *** $P < 0.001$ between male and female mice fed control chow as determined using a two-way ANOVA.

Figure S5. Body weight of WT males (**A**) fed control and pesticide chow and of females (**B**) fed control and pesticide chow after 16, 36 and 48 weeks of exposure. Results are the mean \pm standard error of the mean with n=5 (cages 1 and 2) and n=4 (cages 3 and 4).

Figure S6. Blood glucose and blood insulin measured in WT male (**A**) and female (**B**) mice after 52 weeks of control (**C**) or pesticide (**P**) chow. Data are presented as mean \pm standard error of the mean. n= 18 mice per group.

Figure S7. Analysis of plasma from WT male (**A, B**) and female (**C, D**) mice fed control (**C**) or pesticide (**P**) for 52 weeks. Data are presented as the mean \pm standard error of the mean. *P<0.05 **P<0.01 ***P<0.001. P-values represent the difference between mice fed control chow and those fed pesticide chow as determined using a Student's t-test; n=18 mice per group. FFA, free fatty acid; TG, triglyceride; LDL, low density lipoprotein; HDL, high density lipoprotein.

Figure S8. O-PLS-DA score plots derived from the plasma spectra of WT males (**A**), WT females (**B**), CAR^{-/-} males (**C**) and CAR^{-/-} females (**D**) after 48 weeks of either control (**C**) or pesticide (**P**) chow. Plasmatic biomarkers of pesticide exposure were investigated using ¹H-NMR based metabolomics. Q²Y represents the goodness of fit for the PLS-DA models and p-values were derived using 1000 permutations of the Y matrix.

Figure S9. Hepatic lipid analysis of WT male (M) and female (F) mice after 52 weeks of eating either control (**C**) or pesticide (**P**) chow. (**A**) Heat map with hierarchical clustering, allowing the definition of 6 lipid clusters, as labeled on the left side of the map. (**B**) The relative PS32:0, PS34:0, PS36:1 abundances for cluster 5 (lipid species specifically down-regulated in males exposed to pesticides). (**C**) The relative SM18:1/16:0, PC30:0, PC 32:0 abundances for cluster 4 (lipid species up-regulated in males and females exposed to pesticides). (**D**) The relative C18:2n-6, Cerd18:1/C26:1, SM18:1/24:1 abundances for cluster 2 (lipid species down-regulated in males and females exposed to pesticides). (**E**) The relative TG51, TG53, PI36:0 abundances for cluster 1 (lipid species specifically up-regulated either in males or in females exposed to pesticides). Data are presented as the mean of relative abundance in each lipid species \pm standard error of the mean. * P<0.05, ** P<0.01, *** P<0.001. P-values represent the difference between mice fed control chow and those fed pesticide chow as determined using a Student's t-test; n=18 mice per group. PS, Phosphatidylserin; SM Sphingomyelin; PC, Phosphatidylcholine; FAME, Fatty acid methyl ester, TG triglycerides; PI, phosphatidylinositols, PE, phosphatidylethanolamine.

Figure S10. Partially assigned 600 MHz 1D NMR spectra of (**A**) aqueous liver extract and (**B**) urine from mice. Numerical keys are described in Table S4.

Figure S11. Identification of 2-ketoadipate in urine from WT female fed pesticide chow. (**A**) ¹H-¹H 800MHz TOCSY spectra of a representative female urine sample showing the cross-peaks between the different signals of 2-oxoadipate (multiplet at 1.84 ppm, triplet at 2.22 ppm, and triplet at 2.79 ppm). (**B**) A spike-in experiment was performed in a representative urine sample using the standard of 2-oxoadipate and confirmed an increase of the three above-described signals (as pointed by black arrows) in the spiked-in sample.

Figure S12. Area under the curve integrated for the 1.81-1.84 ppm (triplet) signal of 2-ketoadipate obtained from ¹H NMR urine samples analysis of WT female mice fed control (C) or pesticide (P) chow for 6, 36, or 48 weeks.

Figure S13. Venn diagram representing the number of hepatic genes specifically down-regulated after 52 weeks of pesticide exposure in male and female mice (n=6 mice per group). A total of 511 genes were specifically down-regulated in female mice (p<0.05), whereas 853 genes were significantly down-regulated in male mice. Using the David Bioinformatic resource, different GO terms were identified in female and male mice. Histograms show the enrichment score for each pathway. Gene number and the corresponding p-value are indicated to the right of the histograms.

Figure S14. Comparison of genes identified as upregulated in response to pesticide exposure in female mice with PPAR α -sensitive genes as identified from Montagner et al. (2016) and Regnier et al. (2017). **(A)** Venn diagram showing the numbers of hepatic genes specifically up-regulated in response to pesticides in female mice (p<0.05) in this study, the number PPAR α -sensitive genes, and the number of genes shared between them. Genes were considered PPAR α -sensitive when up-regulated (p<0.05) in response to pharmacological agonist (fenofibrate) in the liver of PPAR α hep^{+/+} but not in PPAR α hep^{-/-} mice (Montagner et al., 2016) and down-regulated (p<0.05) in the liver of fed PPAR α hep^{-/-} mice when compared to fed PPAR α hep^{+/+} mice (Régnier et al, 2017). **(B)** Expression profile for the 41 hepatic genes identified in our study and reported as related to PPAR α – dependent pathways by Montagner et al. (2016) and Regnier et al. (2017) in females and males fed pesticide chow for 52 weeks.

Figure S15. Glucose tolerance was assessed in **(A)** male and **(B)** female CAR^{-/-} mice at 16 weeks via i.p. glucose administration, at 36 and 48 weeks via oral glucose administration in the pesticide exposed (P) and control (C) groups. n=9 mice per group. Data are presented as mean \pm s.e.m.

Figure S16. Kaplan-Meier survival curve for male and female WT mice fed control or pesticide chow for 52 weeks.

Table S1. Oligonucleotide sequences used in real-time PCR.

Table S2. List of the metabolites of pesticides screened by UHPL-HRMS.

Table S3. Fasting blood glucose (mg/dL) in male and female mice fed either pesticide or control chow after 16, 36, and 48 weeks.

Table S4. ¹H and partial ¹³C assignments for identified metabolites. U, urine; L, liver.

Table S5. OPLS correlation coefficients determined for discriminate treatment models constructed from ¹H-NMR spectra for urine obtained from untreated mice and mice treated with pesticides at 6 weeks, 24 weeks, and 48 weeks. Metabolites with correlation coefficients greater than Rcrit at the 5% (p=0.05) level were selected as the discriminant biomarkers. A positive correlation coefficient corresponds to a relative increase in the concentration of metabolite in the pesticide-treated group and a corresponding decrease in the control group.