Does Dietary Copper Supplementation enhance or diminish PCB126 Toxicity in Rodent Liver?

Running Head (limit 50 characters, currently 43) Copper Supplementation and PCB126 Toxicity

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Supplemental Data

Supplemental Table 1: Composition of AIN-93G modified copper diets

Diet	Low Cu (2 ppm)	Adequate Cu (6 ppm)	Supplemental Cu (10 ppm)
Constituent	**g/kg**	**g/kg**	**g/kg**
Casein, low Cu & Fe	200	200	200
L-Cystine	3.0	3.0	3.0
Corn Starch	397	397	397
Maltodextrin	132	132	132
Sucrose	100	100	100
Soybean Oil	70.0	70.0	70.0
Cellulose	50.0	50.0	50.0
Cu Deficient Mineral Mix	35.0	35.0	35.0
Cupric Carbonate	0.0036	0.0108	0.0180
Vitamin Mix, AIN-93-VX	10.0	10.0	10.0
Choline Bitartrate	2.5	2.5	2.5
THBQ, antioxidant	0.014	0.014	0.014

Supplemental Table 2: Primer sequences used in hepatic metalloprotein gene expression determination

	Forward	Reverse
Ceruloplasmin	5’-tcttggaatccttggtcctg-3’	5’-tctttggggacagtccattc-3’
Tyrosinase	5’-aacccagggccttgtgcttcct-3’	5’-ggcatgagggctggccatttg-3’
CytOx (Subunit I)	5’-ggagctggaacaggatgaac-3’	5’-gagaggtgtctgatattggg-3’
CytOx (Subunit IV)	5’-acttcggtgtgccttcggg-3’	5’-aaaggctgctccagtcggc-3’
Metallothionein Isoform 1	5’-caccgttgctccagattcac-3’	5’-gcagcagcactgttcgtcac-3’
Metallothionein Isoform 2	5’-atctccaactgccgcctcc-3’	5’-tgcacttgtccgaagcctct-3’
B-actin	5’-tagagccaccaatccacacag-3’	5’-cagccttccttcctgggtatg-3’

Supplemental Table 3a: Liver (µg/g), kidney (µg/g), and blood selenium (µg/L) under each experiment condition and significance of various comparisons (adjusted using Dunnett's test)

	Liver Selenium (µg/g tissue wet weight)				Kidney Selenium (µg/g tissue wet weight)				Blood Selenium (µg/L)			
Treatment	**Dietary Copper Level**				**Dietary Copper Level**				**Dietary Copper Level**			
	Low (2 ppm)	**Adequate (6 ppm)**	**High (10 ppm)**	**Overall**	**Low (2 ppm)**	**Adequate (6 ppm)**	**High (10 ppm)**	**Overall**	**Low (2 ppm)**	**Adequate (6 ppm)**	**High (10 ppm)**	**Overall**
Corn Oil	0.617 ± 0.026 (-,*)	0.784 ± 0.031 (-,-)	0.663 ± 0.026 (-,*)	-	0.89 ± 0.36 (-,-)	0.97 ± 0.03 (-,-)	1.09 ± 0.02 (-,*)	-	418 ± 14 (-,-)	412 ± 20 (-,-)	418 ± 23 (-,-)	-
1 µmol/kg PCB126	0.460 ± 0.017 (†,-)	0.488 ± 0.027 (†,-)	0.502 ± 0.019 (†,-)	†	0.90 ± 0.02 (-,-)	0.97 ± 0.03 (-,-)	1.05 ± 0.05 (-,-)	-	443 ± 8 (-,*)	377 ± 13 (-,-)	392 ± 15 (-,-)	-
5 µmol/kg PCB126	0.421 ± 0.014 (†,-)	0.443 ± 0.021 (†,-)	0.502 ± 0.020 (†,*)	†	0.99 ± 0.02 (†, *)	1.15 ± 0.03 (†,-)	1.14 ± 0.04 (-,-)	†	455 ± 13 (-,*)	402 ± 13 (-,-)	407 ± 10 (-,-)	-
Overall	*	-	-		*	-	*		*	-	-	

Results are expressed as mean ± SEM. Each group contained 4-6 animals. One-way ANOVA was used to examine the difference between each PCB126 level and the corn oil treatment, "†" in the parentheses indicates a significant difference due to PCB treatment. Similarly, a "*" in the parentheses indicates significant differences between low or supplemental and the adequate dietary copper level. Significance for each factor based on two-way ANOVA is indicated in the bottom margin for copper diet (*) and in the right margin for PCB treatment (†). The level for significance is 0.05.

Supplemental Table 3b: Liver (µg/g), kidney (µg/g), and blood iron (mg/L) under each experiment condition and significance of various comparisons (adjusted using Dunnett's test)

Treatment	Liver Iron (µg/g tissue wet weight)				Kidney Iron (µg/g tissue wet weight)				Blood Iron (mg/L)			
	Dietary Copper Level				Dietary Copper Level				Dietary Copper Level			
	Low (2 ppm)	Adequate (6 ppm)	High (10 ppm)	Overall	Low (2 ppm)	Adequate (6 ppm)	High (10 ppm)	Overall	Low (2 ppm)	Adequate (6 ppm)	High (10 ppm)	Overall
Corn Oil	115 ± 2 (-,*)	137 ± 8 (-,-)	133 ± 5 (-,-)	-	78.2 ± 3.4 (-,-)	74.7 ± 2.4 (-,-)	83.4 ± 4.6 (-,-)	-	510 ± 18 (-,-)	517 ± 30 (-,-)	552 ± 22 (-,-)	-
1 µmol/kg PCB126	112 ± 12 (-,-)	107 ± 6 (†,-)	106 ± 5 (†,-)	†	71.7 ± 3.9 (-,-)	78.1 ± 4.8 (-,-)	82.5 ± 8.8 (-,-)	-	452 ± 30 (-,-)	513 ± 35 (-,-)	547 ± 24 (-,-)	-
5 µmol/kg PCB126	106 ± 10 (-,-)	107 ± 3 (†,-)	100 ± 7 (†,-)	†	71.5 ± 5.3 (-,*)	87.7 ± 5.2 (-,-)	78.1 ± 2.5 (-,-)	-	425 ± 11 (†,*)	522 ± 26 (-,-)	470 ± 32 (-,-)	†
Overall	-	-	-		-	-	-		*	-	-	

Results are expressed as mean ± SEM. Each group contained 4-6 animals. One-way ANOVA was used to examine the difference between each PCB126 level and the corn oil treatment, "†" in the parentheses indicates a significant difference due to PCB treatment. Similarly, a "*" in the parentheses indicates significant differences between low or supplemental and the adequate dietary copper level. Significance for each factor based on two-way ANOVA is indicated in the bottom margin for copper diet (*) and in the right margin for PCB treatment (†). The level for significance is 0.05.

Supplemental Table 3c: Liver (µg/g), kidney (µg/g), and blood zinc (mg/L) under each experiment condition and significance of various comparisons (adjusted using Dunnett's test)

	Liver Zinc (µg/g tissue wet weight)				Kidney Zinc (µg/g tissue wet weight)				Blood Zinc (mg/L)			
	Dietary Copper Level				Dietary Copper Level				Dietary Copper Level			
Treatment	**Low (2 ppm)**	**Adequate (6 ppm)**	**High (10 ppm)**	**Overall**	**Low (2 ppm)**	**Adequate (6 ppm)**	**High (10 ppm)**	**Overall**	**Low (2 ppm)**	**Adequate (6 ppm)**	**High (10 ppm)**	**Overall**
Corn Oil	27.0 ± 0.8 (-,*)	30.9 ± 0.7 (-,-)	30.4 ± 1.0 (-,-)	-	19.6 ± 0.5 (-,-)	19.8 ± 0.4 (-,-)	21.8 ± 0.6 (-,*)	-	6.58 ± 0.13 (-,-)	6.40 ± 0.15 (-,-)	6.67 ± 0.31 (-,-)	-
1 µmol/kg PCB126	24.5 ± 0.5 (-,-)	25.8 ± 0.4 (†,-)	29.2 ± 1.6 (-,*)	†	19.5 ± 0.4 (-,-)	20.8 ± 0.4 (-,-)	21.2 ± 1.2 (-,-)	-	5.98 ± 0.17 (-,-)	5.68 ± 0.26 (-,-)	5.95 ± 0.20 (-,-)	†
5 µmol/kg PCB126	22.6 ± 1.1 (†,-)	25.1 ± 1.1 (†,-)	26.9 ± 1.0 (-,-)	†	20.2 ± 0.3 (-,*)	22.6 ± 0.5 (†,-)	21.5 ± 0.5 (-,-)	-	5.82 ± 0.28 (†,-)	6.08 ± 0.25 (-,-)	6.07 ± 0.28 (-,-)	†
Overall	-	-	*		*	-	-		-	-	-	

Results are expressed as mean ± SEM. Each group contained 4-6 animals. One-way ANOVA was used to examine the difference between each PCB126 level and the corn oil treatment, "†" in the parentheses indicates a significant difference due to PCB treatment. Similarly, a "*" in the parentheses indicates significant differences between low or supplemental and the adequate dietary copper level. Significance for each factor based on two-way ANOVA is indicated in the bottom margin for copper diet (*) and in the right margin for PCB treatment (†). The level for significance is 0.05.

Supplemental Table 3d: Liver (µg/g), kidney (µg/g), and blood manganese (µg/L) under each experiment condition and significance of various comparisons (adjusted using Dunnett's test)

	Liver Manganese (µg/g tissue wet weight)				Kidney Manganese (µg/g tissue wet weight)				Blood Manganese (µg/L)			
	Dietary Copper Level				Dietary Copper Level				Dietary Copper Level			
Treatment	**Low (2 ppm)**	**Adequate (6 ppm)**	**High (10 ppm)**	**Overall**	**Low (2 ppm)**	**Adequate (6 ppm)**	**High (10 ppm)**	**Overall**	**Low (2 ppm)**	**Adequate (6 ppm)**	**High (10 ppm)**	**Overall**
Corn Oil	2.15 ± 0.07 (-,*)	2.52 ± 0.03 (-,-)	2.39 ± 0.10 (-,-)	-	0.91 ± 0.03 (-,-)	0.93 ± 0.03 (-,-)	1.05 ± 0.04 (-,-)	-	10.16 ± 0.35 (-,*)	8.38 ± 0.55 (-,-)	8.76 ± 0.50 (-,-)	-
1 µmol/kg PCB126	1.67 ± 0.03 (†,-)	1.69 ± 0.07 (†,-)	1.90 ± 0.10 (†,-)	†	0.90 ± 0.01 (-,-)	0.88 ± 0.02 (-,-)	0.96 ± 0.05 (-,-)	-	9.39 ± 0.54 (-,*)	6.75 ± 0.46 (-,-)	8.16 ± 0.62 (-,-)	†
5 µmol/kg PCB126	1.55 ± 0.10 (†,-)	1.79 ± 0.06 (†,-)	1.81 ± 0.09 (†,-)	†	0.95 ± 0.02 (-,-)	1.03 ± 0.02 (-,*)	1.04 ± 0.06 (-,-)	-	9.53 ± 0.54 (-,-)	8.18 ± 0.69 (-,-)	7.34 ± 0.49 (-,-)	-
Overall	*	-	-		-	-	-		*	-	-	

Results are expressed as mean ± SEM. Each group contained 4-6 animals. One-way ANOVA was used to examine the difference between each PCB126 level and the corn oil treatment, "†" in the parentheses indicates a significant difference due to PCB treatment. Similarly, a "*" in the parentheses indicates significant differences between low or supplemental and the adequate dietary copper level. Significance for each factor based on two-way ANOVA is indicated in the bottom margin for copper diet (*) and in the right margin for PCB treatment (†). The level for significance is 0.05.

Legends to the Supplemental Figures:

Supplemental Figure 1: Growth (a) and feed consumption (b) of vehicle- (Corn Oil) and PCB126- (1 μmol/kg and 5 μmol/kg) treated animals. Growth is defined as the weight gained relative to initial weight. High (5 μmol/kg) dose PCB126 significantly slowed growth relative to vehicle-treated control indicating acute toxicity. Feed consumption is defined as total feed consumed in grams following injection. PCB126 diminished feed consumption in a dose-dependent manner. Error bars represent SEM. † $p < 0.05$ as compared to Corn Oil vehicle control.

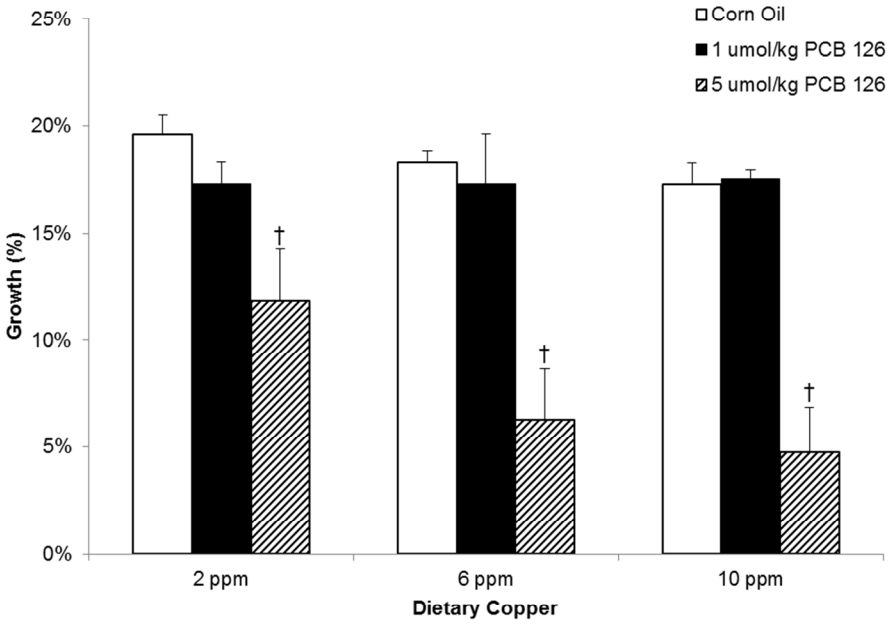
Supplemental Figure 2: Liver 4-HNE levels of vehicle- (Corn Oil) and PCB126- (1 μmol/kg and 5 μmol/kg) treated animals. Error bars represent SEM. * $p < 0.05$ as compared to adequate (6 ppm) dietary copper. † $p < 0.05$ as compared to Corn vehicle control.

Supplemental Figure 3a: Electron micrograph from hepatocytes of a rat fed adequate (6 ppm) copper and treated with corn oil (vehicle). N, nucleus; M, mitochondria; R, rough endoplasmic reticulum; S, smooth endoplasmic reticulum.

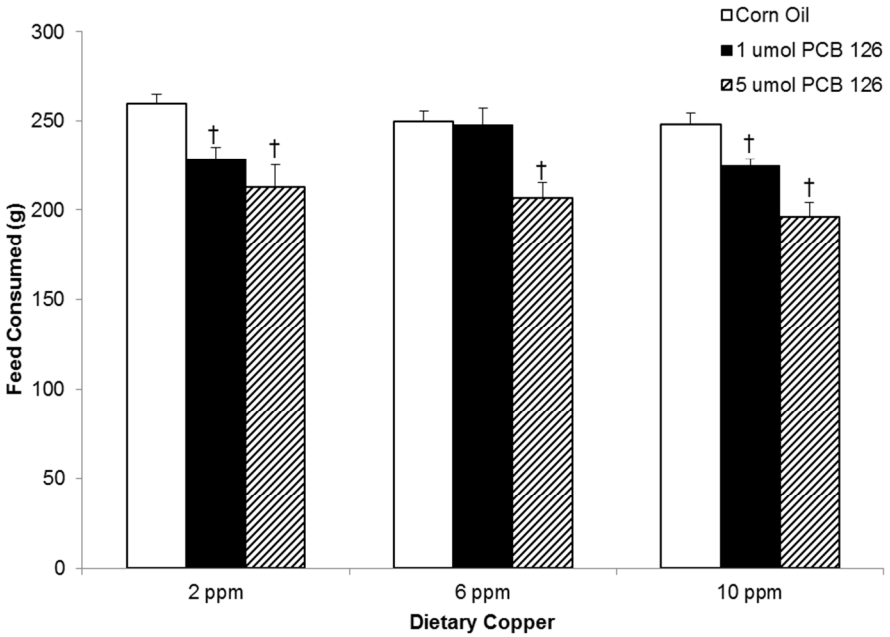
Supplemental Figure 3b: Electron micrograph from hepatocytes of a rat fed adequate (6 ppm) copper and treated with low (1 μmol/kg) dose PCB126. N, nucleus; M, mitochondria; R, rough endoplasmic reticulum; S, smooth endoplasmic reticulum; L, lipid.

Supplemental Figure 3c: Electron micrograph from hepatocytes of a rat fed adequate (6 ppm) copper and treated with high (5 μmol/kg) dose PCB126. N, nucleus; M, mitochondria; R, rough endoplasmic reticulum; S, smooth endoplasmic reticulum; L, lipid.

(a: growth)



(b: feed consumption)



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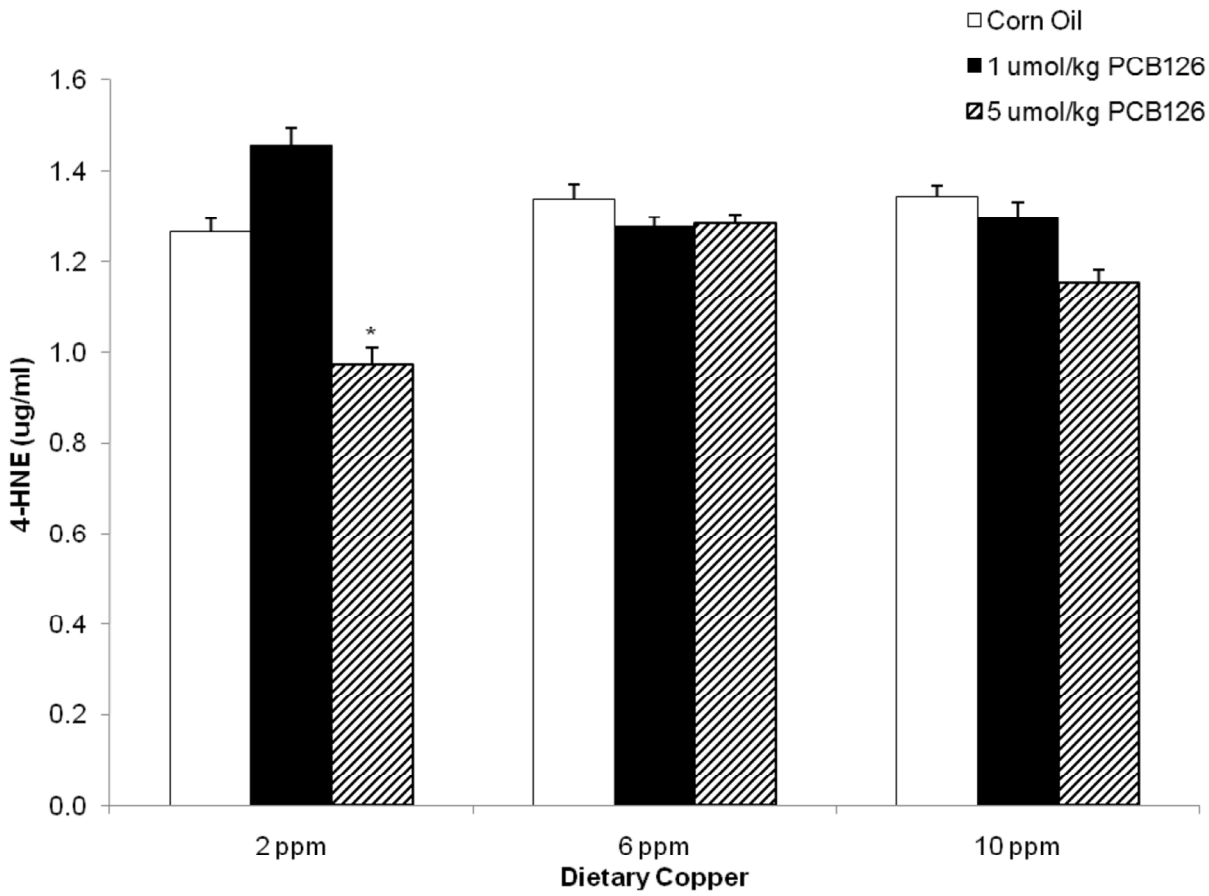
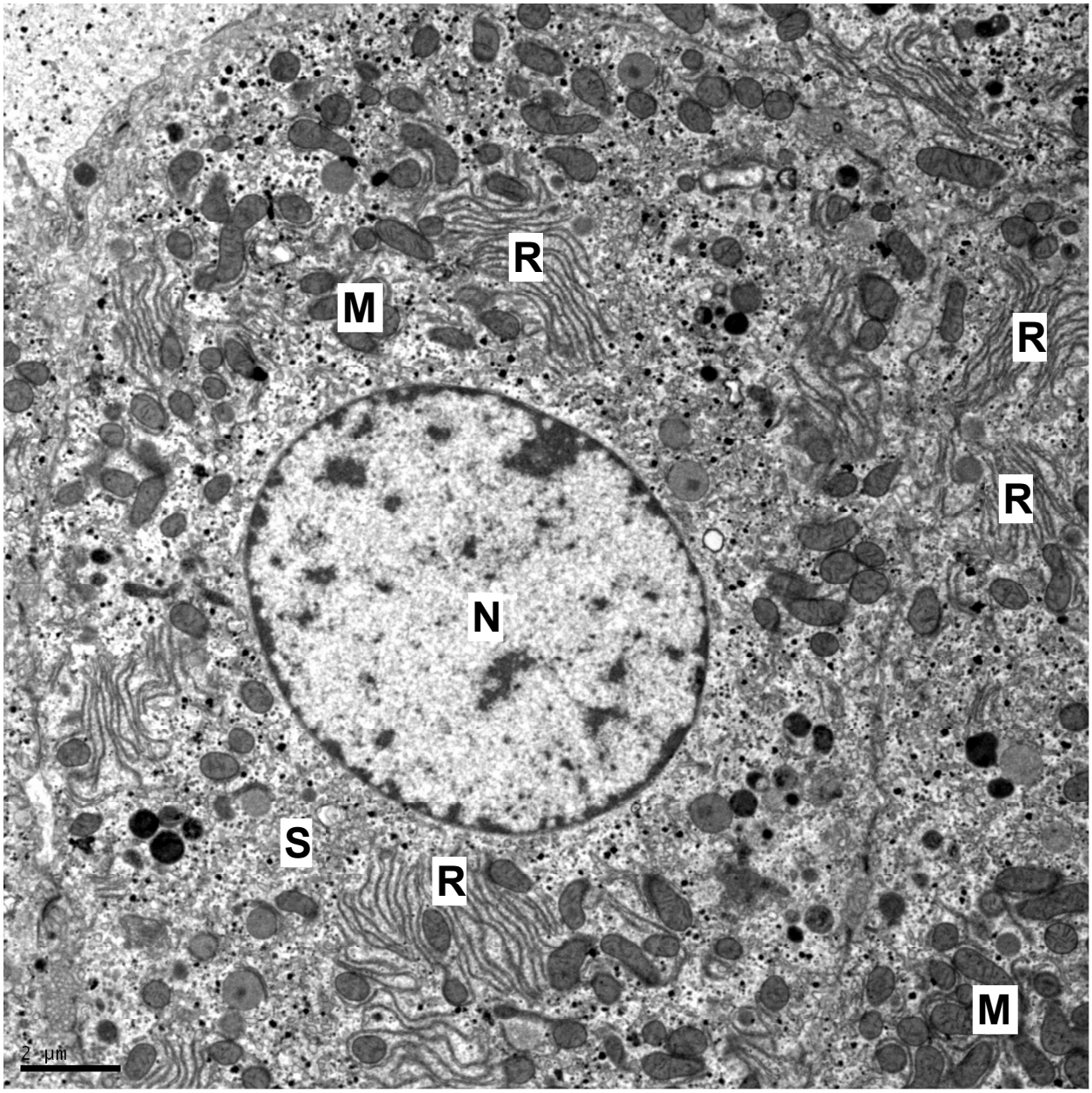
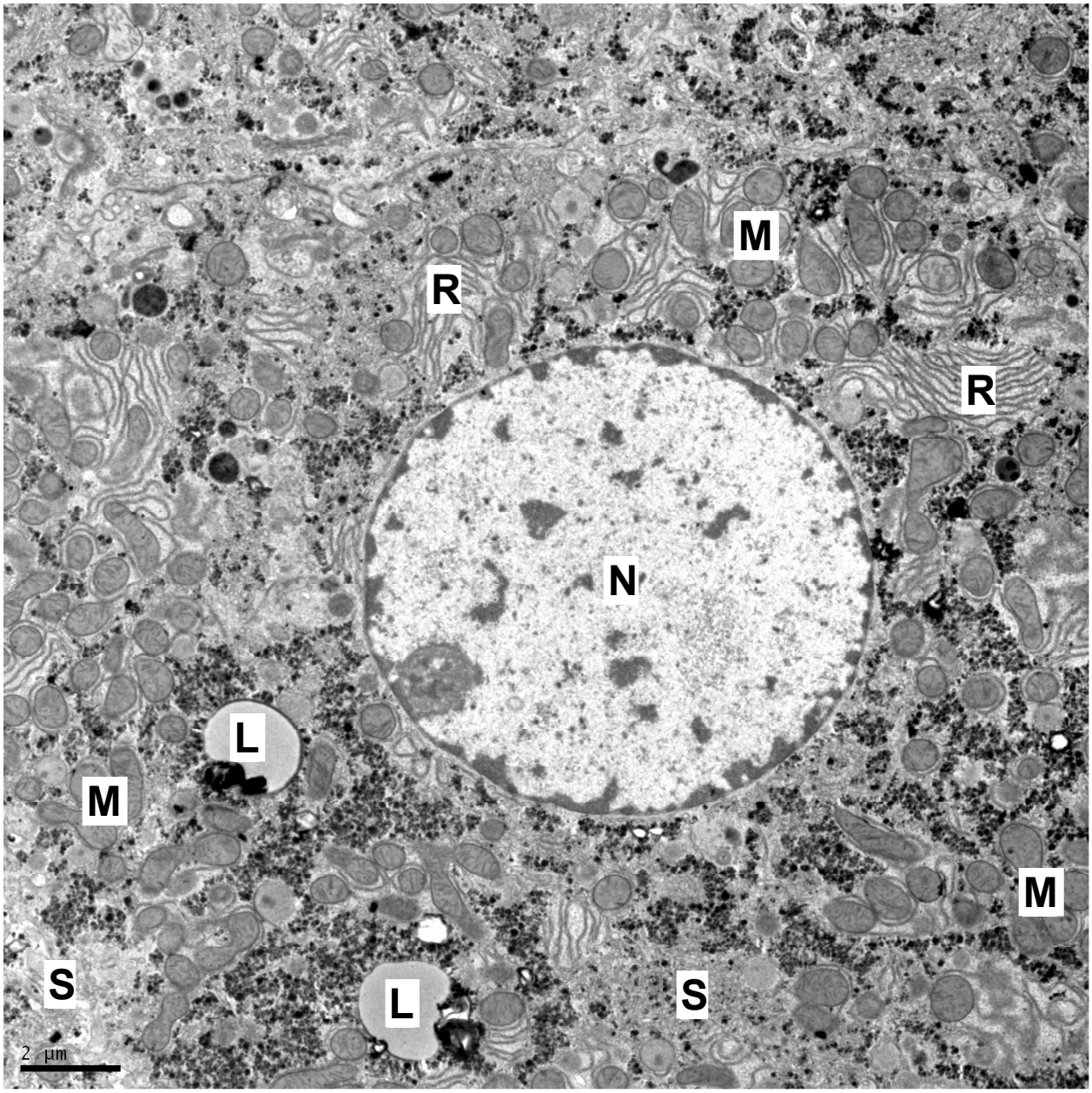


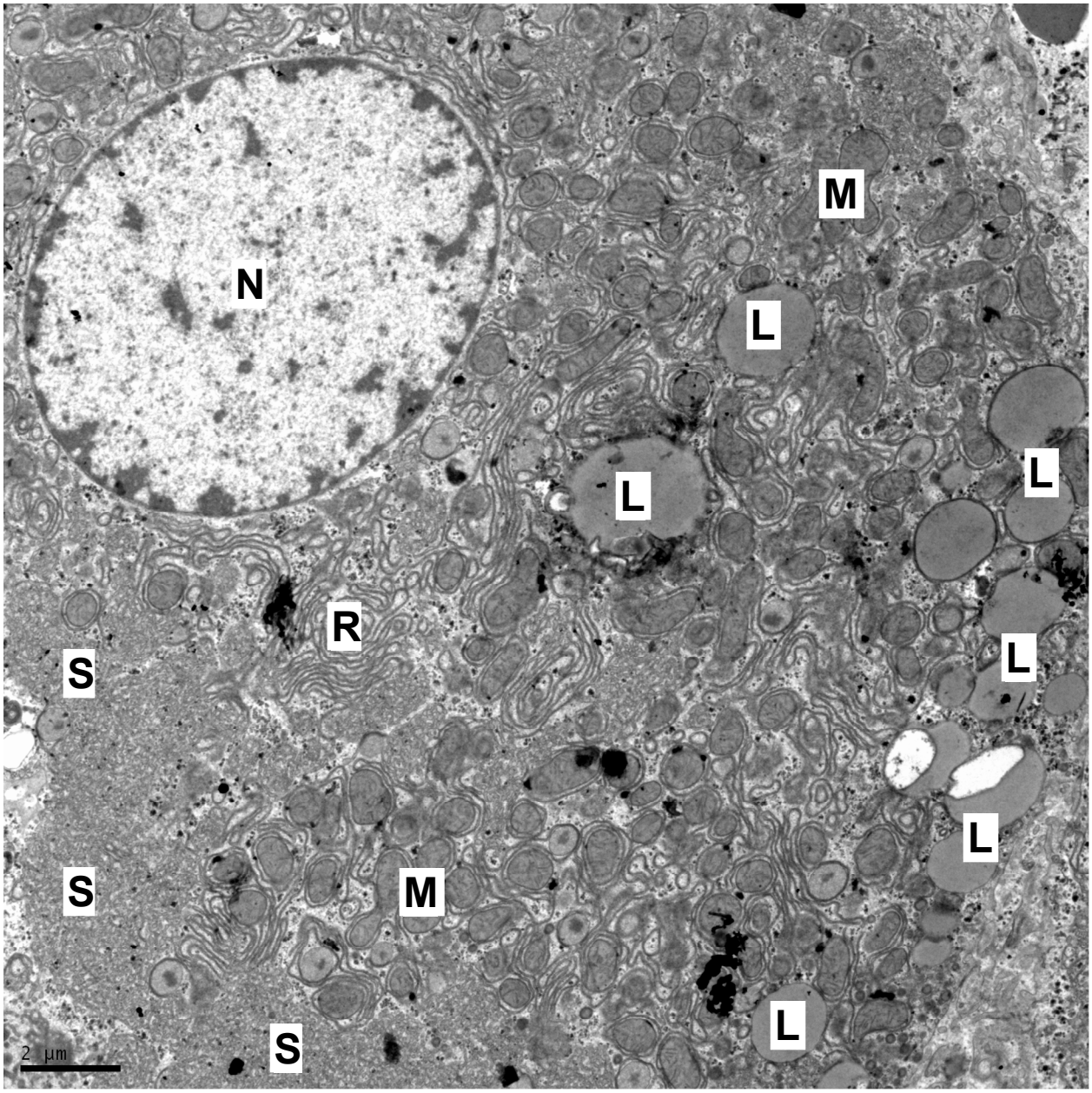
Supplemental Figure 2: Liver 4-HNE levels of vehicle- (Corn Oil) and PCB126- (1 µmol/kg and 5 µmol/kg) treated animals. Error bars represent SEM. * $p < 0.05$ as compared to adequate (6 ppm) dietary copper. † $p < 0.05$ as compared to Corn vehicle control.



Supplemental Figure 3a: Electron micrograph from hepatocytes of a rat fed adequate (6 ppm) copper and treated with corn oil (vehicle). N, nucleus; M, mitochondria; R, rough endoplasmic reticulum; S, smooth endoplasmic reticulum.



Supplemental Figure 3b: Electron micrograph from hepatocytes of a rat fed adequate (6 ppm) copper and treated with low (1 µmol/kg) dose PCB126. N, nucleus; M, mitochondria; R, rough endoplasmic reticulum; S, smooth endoplasmic reticulum; L, lipid.



Supplemental Figure 3c: Electron micrograph from hepatocytes of a rat fed adequate (6 ppm) copper and treated with high (5 µmol/kg) dose PCB126. N, nucleus; M, mitochondria; R, rough endoplasmic reticulum; S, smooth endoplasmic reticulum; L, lipid.