Supplemental Data Figure 1

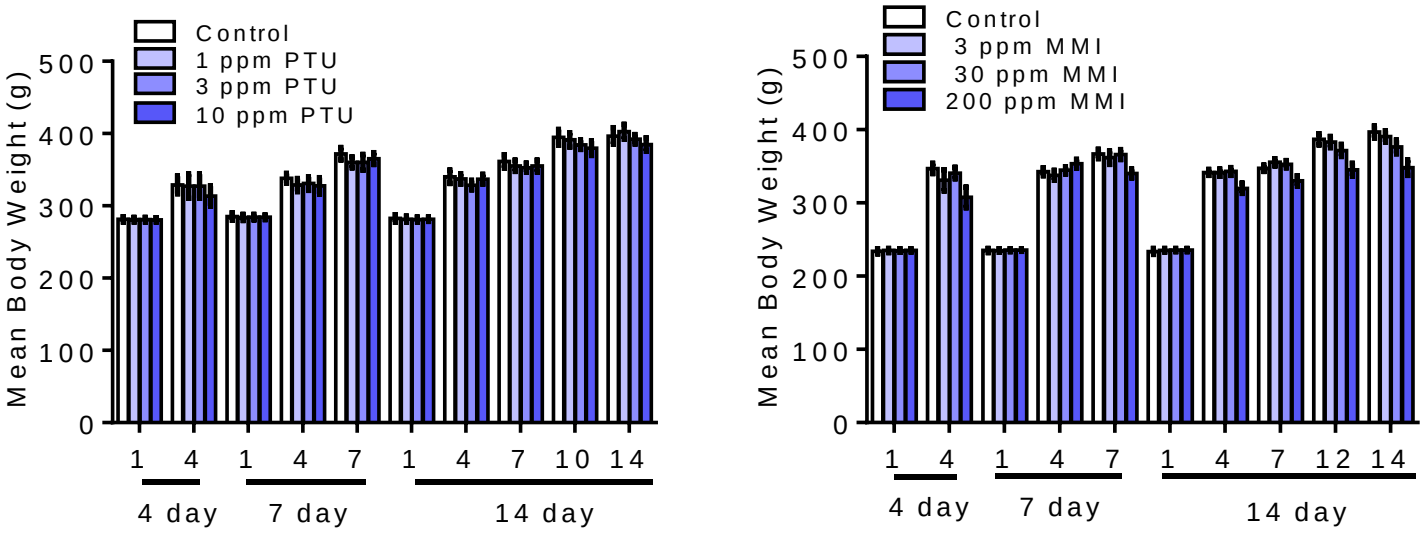


Figure S1: No significant body weight changes were observed at any exposure duration \ with PTU or MMI treatment as compared with control ($p<0.05$).

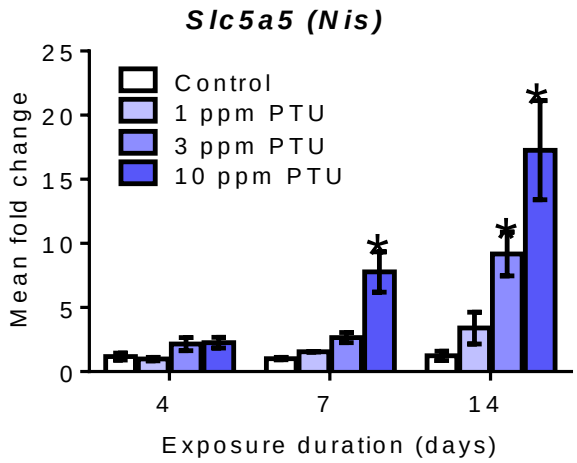
Supplemental Data Figure 2

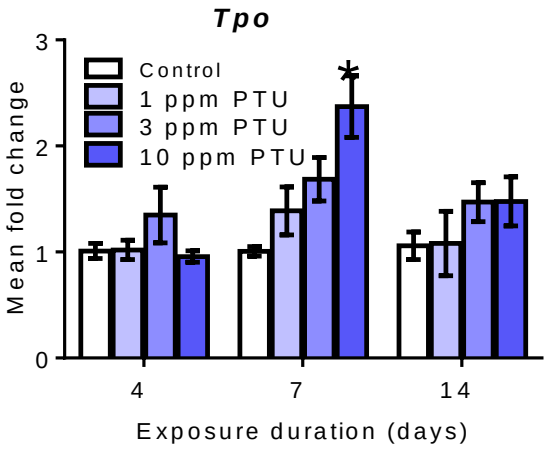
RNA extraction and Quantitative RT-PCR (qRT-PCR)

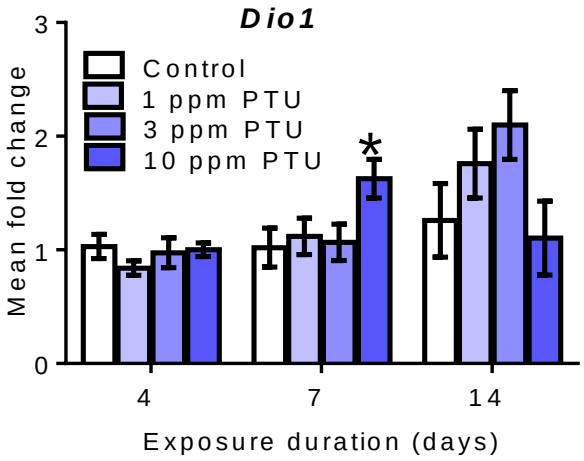
Gene expression analysis was performed on thyroid tissues from PTU and MMI treated rats. RNA was extracted and quantitative real-time polymerase chain reactions (qRT-PCR) was performed according to standard procedures as described by (Gilbert *et al.*, 2016). Thyroid hormone regulating genes were selected as the target genes and thyroperoxidase (*Tpo*), solute carrier family 5 member 5 (*Slc5a5*), iodothyronine deiodinase 1 (*Dio1*) and thyroglobulin (*Tg*), were evaluated. The reference gene was beta-2-microglobulin (*B2m*), mRNA levels were analyzed in triplicate technical replicates, and the $2^{-\Delta\Delta Ct}$ method applied for relative gene quantification.

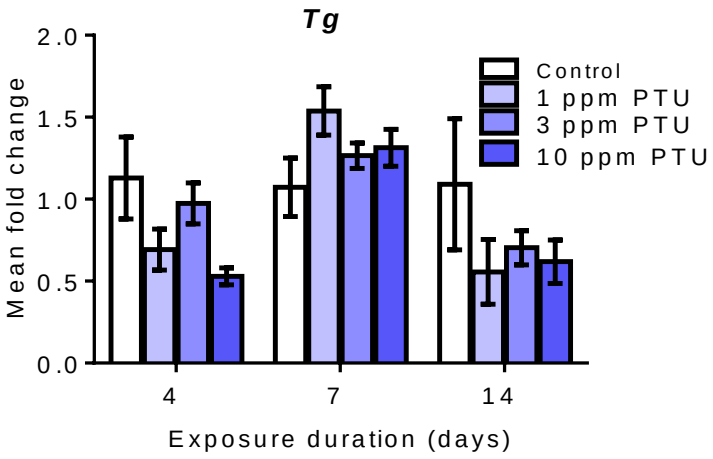
Gene expression profile of rat thyroid gland after exposure to PTU or MMI

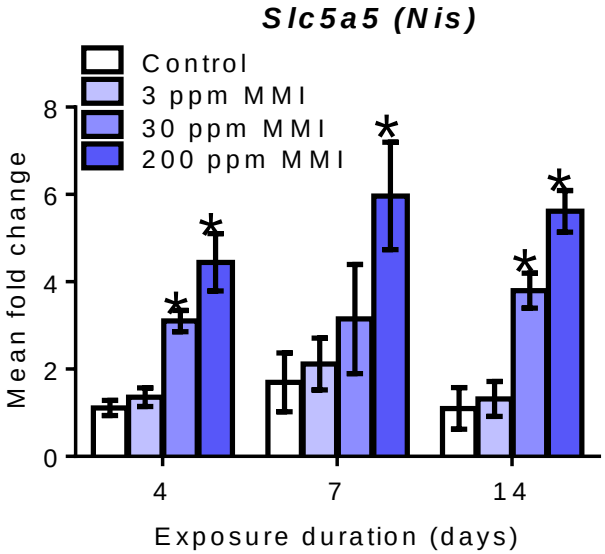
To further characterize the impact of MMI and PTU on thyroid hormone synthesis, glandular mRNA levels of thyroid hormone regulating genes were determined. Expression of *Slc5a5* (transcript encoding for sodium-iodine symporter, NIS) was up-regulated in a dose-dependent manner and was significantly increased with exposure to 10 ppm PTU at 7 [$F(3, 29) = 6.91$, $p<0.0001$] and 14 days; and by 3 ppm PTU after 14 days of exposure only [$F(3, 24) = 8.79$, $p<0.0001$). Expression of *Slc5a5* also showed dose and temporal concordance with MMI, progressively increasing at the two highest MMI doses as exposure duration increased from at 4, [$F(3, 29) =17.37$, $p<0.0001$)], to 7, [$F(3, 25) =3.40$, $p<0.03$)], to 14 days, [$F(3, 29) =22.56$, $p<0.0001$)]. Increased expression of *Slc5a5* suggests that the gland is responding to the activation of the feedback loop where serum levels of TSH are elevated. Expression of *Tpo* was significantly increased with 7-day exposure to 10 ppm PTU [$F(3,29) = 6.91$, $p<0.001$]. No change was observed in the expression of *Tg*, and *Dio1* with exposure to MMI while 4-day exposure to 10 ppm PTU significantly decreased *Tg* expression [$F(3, 29) =3.25$, $p=0.03$]. Increased, but not significant, expression of *Tpo* was also observed with14-day exposure to all MMI doses compared with controls.

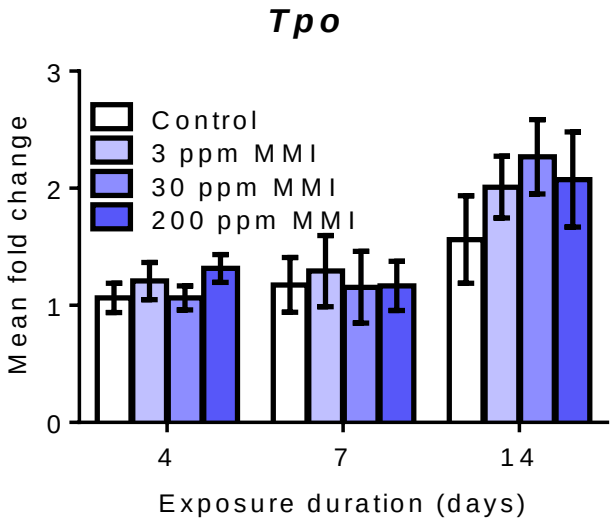

Slc5a5 (Nis)
Control
1 ppm PTU
3 ppm PTU
10 ppm PTU
Mean fold change
0
5
10
15
20
25
4
7
14
Exposure duration (days)
*
*
*


Tpo
Control
1 ppm PTU
3 ppm PTU
10 ppm PTU
Mean fold change
0
1
2
3
4
7
14
Exposure duration (days)
*


Dio1
Control
1 ppm PTU
3 ppm PTU
10 ppm PTU
Mean fold change
0
1
2
3
4
7
14
Exposure duration (days)
*


Tg
Control
1 ppm PTU
3 ppm PTU
10 ppm PTU
Mean fold change
0.0
0.5
1.0
1.5
2.0
4
7
14
Exposure duration (days)


Slc5a5 (Nis)
Control
3 ppm MMI
30 ppm MMI
200 ppm MMI
Mean fold change
0
2
4
6
8
4
7
14
Exposure duration (days)
*
*
*
*


Tpo
Control
3 ppm MMI
30 ppm MMI
200 ppm MMI
Mean fold change
0
1
2
3
4
7
14
Exposure duration (days)

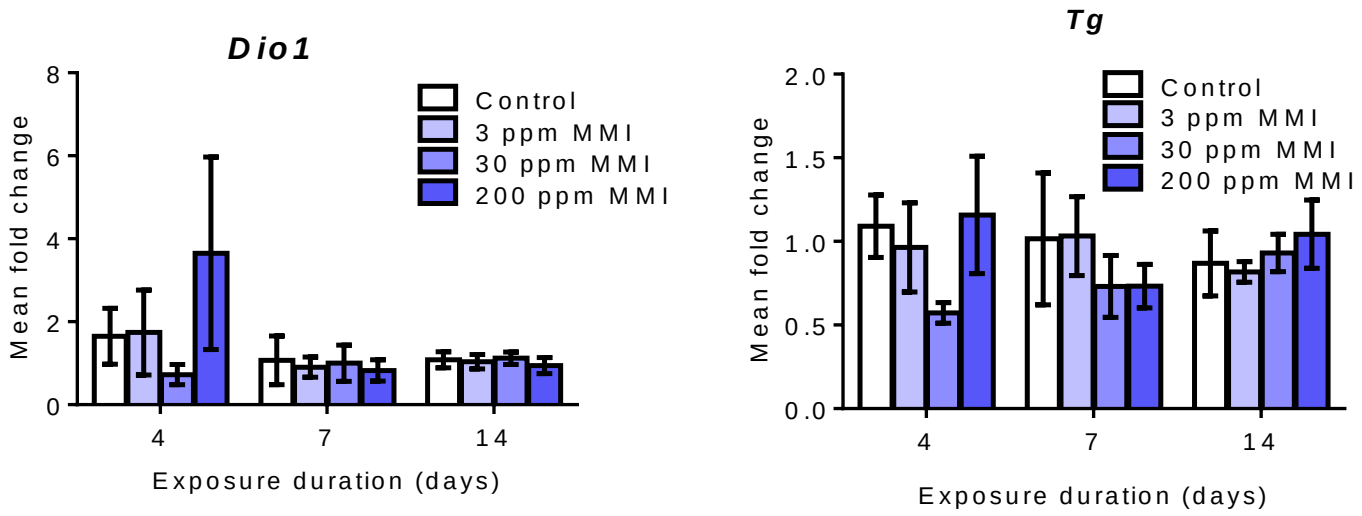
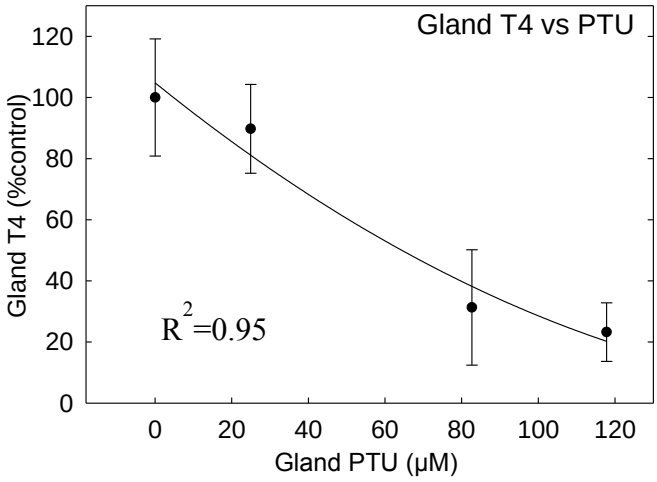
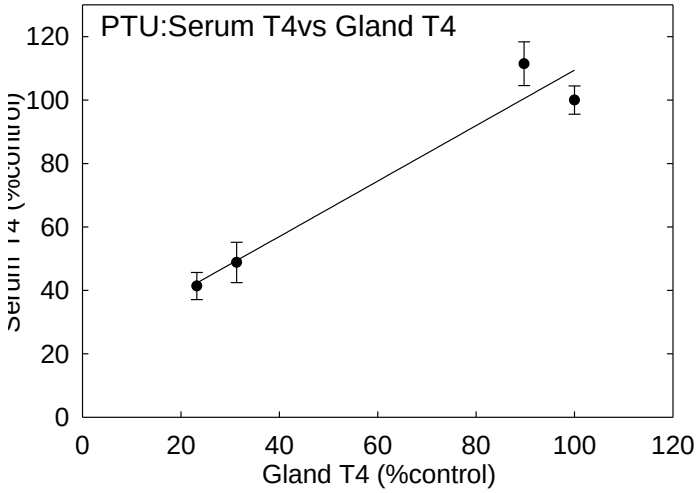
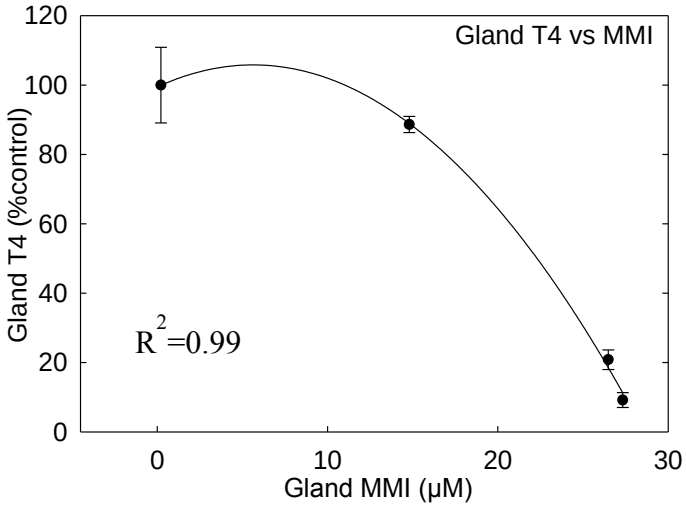
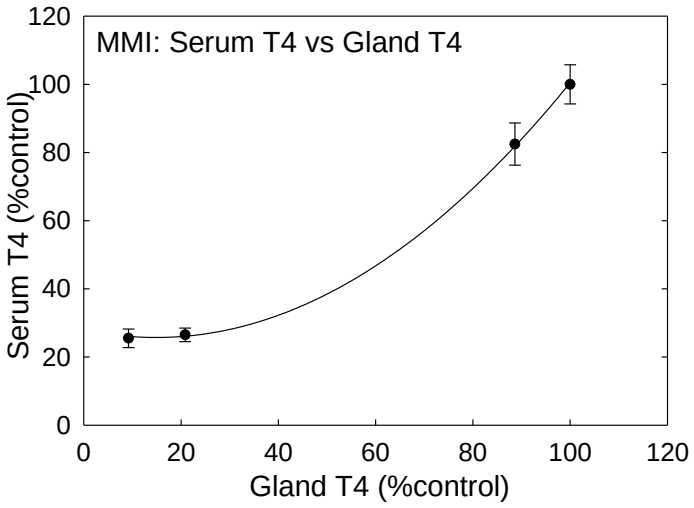


Figure S2: The gene *Slc5a5* was up-regulated with exposure to 10 ppm PTU for 7 days and 3 and 10 ppm for 14 days. Similarly, *Slc5a5* was also significantly upregulated with exposure to 30 and 200 ppm MMI for 4 and 14 days as well as 200 ppm for 7 days. While exposure to 10 ppm PTU for 7 day resulted in significant upregulation of *Tpo* and *Dio1*. N = 8 were analyzed per treatment per timepoint; *represent $p<0.01$ and bar graph shows mean± SEM.









Supplemental Data Figure 3

R^2=0.94

Figure S3: Levels of glandular and serum T4, PTU, and MMI were measured by LC/MS/MS then converted to percent control and mathematical relationships plotted A) Correlation between glandular T4 versus levels of PTU in the gland was fitted well by a polynomial equation. B) Serum and glandular T4 are also modeled well as linear equation. C) Gland T4 and MMI were modeled well by an exponential relationship. D) Serum and glandular T4 levels are well represented by quadratic relationship. All R^2 values for each relationship are shown. In all graphs the error bars represent ± SEM.