Abstract 1. The liver metabolizes thyroxine (T4) through two major pathways: deiodination and conjugation. Following exposure to xenobiotics, T4 conjugation increases through the induction of hepatic uridine diphosphate glucuronosyltransferase (UGT) in rodents; however, it is uncertain to what degree different species employ deiodination and conjugation in the metabolism of T4. The objective of this study was to compare the metabolism of T4 in untreated and 2,2',4,4',5,5'-hexachlorobiphenyl (PCB 153)-treated primary sandwich-cultured hepatocytes from rat (SCRH) and human (SCHH). 2. Basal metabolite concentrations were 13 times higher in the medium of SCRH compared to SCHH. Metabolite distribution in the medium of SCRH versus SCHH was as follows: T4G (91.6 versus 5.3%); T4S (3.6 versus 4.4%) and T3 + rT3 (4.9 versus 90.3%). PCB 153 induced T4G in the medium of SCRH and SCHH; however, T4S and T3 + rT3 were changed but to a much lesser degree. 3. The results indicate that baseline T4 glucuronidation is greater in SCRH compared to SCHH. These data also suggest that glucuronidation may be a more important pathway for T4 metabolism in rats and deiodination may be a favored pathway in humans; however, with PCB 153 treatment these data support glucuronidation as a primary route of T4 metabolism in both rat and humans.