Dichloroanilines (DCAs) and dichloronitrobenzenes (DCNBs) are important chemical intermediates in the manufacture of a wide variety of industrial, agricultural, and pharmaceutical agents and have been found to induce nephrotoxicity in vivo and/or in vitro. CAs may also be metabolized to CNBs and vice versa. Among the DCNBs, 3,5-dichloronitrobenzene (3,5-DCNB) was the most potent nephrotoxicant in renal cortical slices. The current study was designed to explore the nephrotoxicity of 3,5-DCNB and its possible renal biotransformation using isolated renal cortical cells (IRCC) as the in vitro model. Briefly, IRCC (~4.0 million cells/ml, 3 ml) from male Fisher 344 rats were treated with DMSO (vehicle control) or 3,5-DCNB (0.5 or 1.0 mM), and cytotoxicity determined over 90 min by measuring lactate dehydrogenase release. In some experiments, a pretreatment was added before 3,5-DCNB (1.0 mM) followed by 60 min incubations. The pretreatments were antioxidants, cytochrome P450 (CYP) inhibitors, a cyclooxygenase inhibitor, a peroxidase inhibitor or flavin monooxygenase (FMO) inhibitors. All antioxidants and metabolizing enzyme inhibitor pretreatments completely or partially attenuated 3,5-DCNB cytotoxicity. These results suggest that the bioactivation of 3,5-DCNB is complex, as inhibition of enzyme systems as different as CYPs, FMOs, cyclooxygenase and peroxidases can provide some cytoprotection. It can also be concluded that free radicals play a role in 3,5-DCNB nephrotoxicity either via participating in the in vitro renal biotransformation of 3,5-DCNB or via being produced as a consequence of 3,5-DCNB biotransformation. Additional studies are needed to ascertain which pathways are critical for 3,5-DCNB nephrotoxicity. Supported by NIH Grant P20GM103434.