

Mechanism of Action of 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin Antagonists: Characterization of 6-[¹²⁵I]Methyl-8-iodo-1,3-dichlorodibenzofuran–Ah Receptor Complexes¹

J. Piskorska-Pliszczynska, B. Astroff, T. Zacharewski, M. Harris, R. Rosengren, V. Morrison, L. Safe, and S. Safe²

Department of Veterinary Physiology and Pharmacology, Texas A&M University, College Station, Texas 77843-4466

6-Methyl-8-iodo-1,3,-dichlorodibenzofuran (I-MCDF) and its radiolabeled analog [¹²⁵I]MCDF have been synthesized and used to investigate the mechanism of action of 1,3,6,8-substituted dibenzofurans as 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) antagonists. Like 6-methyl-1,3,8-trichlorodibenzofuran (MCDF), I-MCDF partially antagonized the induction by TCDD of microsomal aryl hydrocarbon hydroxylase (AHH) and ethoxyresorufin *O*-deethylase (EROD) activities in rat hepatoma H-4-II E cells and male Long-Evans rat liver. Incubation of rat liver cytosol with [¹²⁵I]MCDF followed by velocity sedimentation analysis on sucrose gradients gave a specifically bound peak which sedimented at 9.6 S. This radioactive peak was displaced by coinubation with a 200-fold excess of unlabeled I-MCDF, 6-methyl-1,3,8-trichlorodibenzofuran (MCDF), 2,3,7,8-tetrachlorodibenzofuran (TCDF), and benzo [*a*]pyrene. Based on the velocity sedimentation results and the elution profile from a Sephacryl S-300 gel permeation column, the Stokes radius and apparent molecular weights of the cytosolic [¹²⁵I]MCDF–Ah receptor complex were 6.5 nm and 259,200, respectively. In addition, the nuclear [¹²⁵I]MCDF–receptor complex eluted at a salt concentration of 0.29 M KCl from a DNA–Sephrose column. Velocity sediment analysis of the nuclear [¹²⁵I]MCDF–Ah receptor complex from rat hepatoma H-4-II E cells gave a specifically bound peak at 5.6 ± 0.8 S. All of these properties were similar to those observed using [³H]-TCDD as the radioligand. In addition, there were several ligand-dependent differences observed in the properties of the I-MCDF and TCDD receptor complexes; for example, the [¹²⁵I]MCDF rat cytosolic receptor complex was unstable in high salt buffer and was poorly transformed into a form with increased binding affinity on DNA–Sephrose columns; Scatchard plot analysis of the saturation binding of [³H]TCDD and [¹²⁵I]MCDF with rat hepatic cytosol gave K_D values of 1.07 and 0.13 nM and B_{max} values of 137 and 2.05 fmol/mg protein, respectively. The nuclear extract from rat hepatoma H-4-II E cells treated with I-MCDF or TCDD interacted with a dioxin-responsive element in a gel retardation assay. These results suggest that the mechanism of antagonism may be associated with competition of the antagonist receptor complex for nuclear binding sites.