The objective of this study was to establish a technique for biological monitoring of occupational exposure to methyl tertiary-butyl ether (MTBE). We conducted both basic animal experiments and a field application study. In the first basic experiments, we focused on the biological measure of an MTBE metabolite, tertiary butyl alcohol (TBA) in urine. The experiments employed a hydrolysis technique using hydrochloric acid to break down the conjugation of TBA. With this technique, the TBA was clearly detected in the urine of rats exposed to MTBE, and the concentrations increased proportionally with an increase in the MTBE dose level. In the second field application study, we collected air samples and urine specimens from 35 road-toll station workers in Bangkok, Thailand. The MTBE in air samples ranged from 7.5 to 24.4 μg/m³, and the geometric mean was 13.9 μg/m³. The urine TBA concentration ranged from 124.1 to 756.0 μg/l, and the geometric mean was 232.7 μg/l. The workers who were exposed to higher levels of MTBE during their shifts showed higher urine TBA concentrations (r=0.498, p=0.002). The results suggest the usefulness of the established technique of biological monitoring of MTBE exposure by using urine TBA as the exposure index. The technique has enabled us to monitor occupational exposure of MTBE and will contribute to the health management of workers.