

**The National Toxicology Program Research on Synthetic Turf/Recycled Tire Crumb Rubber: Characterization of the Leachability and Cytotoxicity of Crumb Rubber In Vitro**

W. M. Gwinn, M. Bell, D. Crizer, G. K. Roberts, S. Masten, D. Dixon, M. DeVito, and E. Tokar.

*NIEHS, Research Triangle Park, NC.*

Public health concern for playing on synthetic turf fields with crumb rubber (CR) infill has increased in recent years. CR manufactured from recycled tires contains potential carcinogenic and toxic substances and there is potential for widespread exposure with over 12,000 synthetic turf fields in the US. The NTP is currently conducting research to improve the understanding of potential human exposure and health impacts following CR exposure. The objectives of this study were to determine the leachability and cytotoxicity of CR in vitro using human peripheral lung (HPL-1D) cells and keratinocytes (HaCaT cells) to reflect inhalation and dermal routes of exposure. CR (100 mg/ml) was mixed and incubated in cell type-specific culture media for 3 h, 1, 4 or 7 d at room temperature, 37°C or 60°C. CR-conditioned media was then sterile-filtered and serially-diluted to 50, 25, 12.5 and 6.25 mg/ml for cell exposures. HPL-1D or HaCaT cells were exposed to CR-conditioned media for 24 or 72 h and cell viability was measured via MTS cell proliferation assay. Cytotoxicity was observed for both cell lines in a concentration-dependent manner following incubation of CR at multiple temperatures and durations. For HPL-1D cells, 100, 50 and 25 mg/ml CR-conditioned media were cytotoxic at 24 and 72 h for all CR incubation times at 60°C. For HaCaT cells, 100 and 50 mg/ml CR-conditioned media were cytotoxic at 24 and 72 h for CR incubation times of 1, 4 or 7 d (100 mg/ml) and 4 or 7 d (50 mg/ml) at 60°C. For both cell types, cytotoxicity was also observed following CR incubation at 37°C but was most pronounced at 60°C. Untargeted LC-MS was used to characterize the chemical composition of CR-conditioned media. Compounds found to be elevated included 2-mercaptobenzothiazole, which is a known rodent carcinogen used in the vulcanization of rubber. Other compounds of interest included N,N'-diphenylguanidine, 1,2-benzisothiazoline-3-one and multiple phthalates. Studies are in-progress to address the in vitro cytotoxicity of CR using human small intestinal cells, to reflect oral exposure, and the effects of CR incubation in more physiologically-relevant biofluids. In conjunction with chemical characterization and in vivo testing of CR, this study will contribute to what is known about potential human health effects of playing on synthetic turf fields made from recycled tires.