

Interlaboratory validation of a new assay for DNA-protein crosslinks

Max Costa ^{a,*}, Anatoly Zhitkovich ^a, Michael Gargas ^b, Dennis Paustenbach ^b,
Brent Finley ^b, Jim Kuykendall ^c, Ruth Billings ^d, Timothy J. Carlson ^d,
Karen Wetterhahn ^c, Jian Xu ^f, Steven Patierno ^f, Matthew Bogdanffy ^g

Abstract

In 1992, a simple and sensitive assay for detecting DNA-protein crosslinks was developed [1]. In an effort to facilitate the greater use of the assay, a number of studies were conducted to evaluate its reliability and reproducibility. During this work, the assay was used to assess whether various metals and other compounds could induce crosslinks in cultured human lymphocytes (Epstein-Barr virus-transformed Burkitt's Lymphoma cell line). Potassium permanganate, mercury chloride, lead nitrate, magnesium perchlorate, aluminum chloride, and cadmium chloride did not induce DNA-protein crosslinks at either cytotoxic or non-cytotoxic levels. Copper sulfate, arsenic trioxide, and potassium chromate induced DNA-protein crosslinks only at cytotoxic concentrations. Acute lethality of the cells was assessed immediately after exposure to metals by trypan blue exclusion while long-term lethality was assessed by cell proliferation and trypan blue exclusion following an incubation period of 5 days after exposure to the metal compound. All metals exhibited more toxicity in the long-term lethality assay compared to the short-term assay. The cultured human lymphocytes treated with various doses of lead acetate, cadmium chloride, arsenic trioxide and copper sulfate, as well as *cis*-platinum and chromate, were sent to four different laboratories to compare the reliability and reproducibility of the DNA-protein crosslink assay. Depending on the chemical studied, there were quantitative differences in the results observed among the various laboratories using the assay. However, all laboratories generally showed that *cis*-platinum, chromate, arsenic trioxide and copper sulfate induced DNA-protein crosslinks at levels that produced acute cytotoxicity, whereas cadmium chloride and lead acetate did not.