Bisphenol A is released from used polycarbonate rodent cages

KL Howdeshell, PH Peterman, RL Ruhlen, BM Judy, WV Welshons and FS vom Saal,
University of Missouri and Columbia Environmental Research Center (USGS), Columbia, MO 65211

Polycarbonate cages are commonly used to house rodents, and they are generally used long after they show significant signs of wear. Bisphenol A is a chemical monomer used in the production of polycarbonate animal caging, food packaging, and many other products. This monomer, which has been shown to be estrogenic in both *in vitr*o and *in vivo* assays, leaches from these products when they are exposed to high heat or alkaline conditions. Recent research in Japan has shown that leaching of bisphenol A from polycarbonate products increases as a function of age (Takao *et al*., J. Health Sci. 45:39, 1999). We report here that well-used, discolored polycarbonate rodent cages release a significant amount of bisphenol A into water at room temperature, while new polycarbonate cages and polypropylene cages do not release detectable levels of bisphenol A (detection limit = 10 ng).

Purified water (HPLC-grade, 250 ml) was added to two used and to two new polycarbonate plastic rodent cages (the water covered a surface area of 446 cm^2) and remained at room temperature for one week to determine the amount of bisphenol A that would migrate out of the cages into the water. Leaching from the polycarbonate cages was compared to leaching from glass pyrex casserole dishes and from used polypropylene cages (2 each), which are not made from bisphenol A. A 220 ml volume of water was extracted and analyzed by gas chromatography and quadrupole mass spectrometry (GC/QMS). The two well-used polycarbonate cages leached 51 and 110 ng of bisphenol A /ml water. Water from the glass dishes and polypropylene cages did not contain detectable bisphenol A. However, both polypropylene cages released a detectable amount of nonylphenol.

We conducted the same experiment a second time, and extracted leachate from the water for use in an estrogen-sensitive MCF-7 breast cancer cell proliferation assay. Water from each cage was collected after one week (total volume, 250 ml) and a 10 ml aliquot was dried down under nitrogen. The leachate was reconstituted in cell culture medium and added to wells containing MCF-7 cells for 4 days. A range of estradiol standards was included in the assay as a positive control. To determine that cell proliferation was due to estrogenic activity, we added a 0.1 μM concentration of the antiestrogen LY156758 (Raloxifene) to the medium in a duplicate set of samples. In the cell proliferation assay, we found the estrogenic activity in the water from the two well-used polycarbonate cages to be equivalent to 1 pM and 2.5 pM estradiol, or equivalent to 22.8 and 57.0 ng bisphenol A/ml. New polycarbonate cages showed no increase in proliferation similar to control cells not treated with estradiol. In each case, the addition of the antiestrogen blocked the increase in cell proliferation. The glass and polypropylene leachates also did not stimulate cell proliferation, revealing the absence of estrogenic activity in these samples. Our findings show that significant estrogenic activity, identifiable as bisphenol A by GC/QMS, is released from polycarbonate animal cages after they become visibly discolored, but not when they are new. Supported by NIH grant ES08293, University of Missouri grant VMFC0018, and the USGS.