Bisphenol A Is Released from Used Polycarbonate Animal Cages into Water at Room Temperature

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Bisphenol A (BPA) is a monomer with estrogenic activity that is used in the production of food packaging, dental sealants, polycarbonate plastic, and many other products. The monomer has previously been reported to hydrolyze and leach from these products under high heat and alkaline conditions, and the amount of leaching increases as a function of use. We examined whether new and used polycarbonate animal cages passively release bioactive levels of BPA into water at room temperature and neutral pH. Purified water was incubated at room temperature in new polycarbonate and polysulfone cages and used (discolored) polycarbonate cages, as well as control (glass and used polypropylene) containers. The resulting water samples were characterized with gas chromatography/mass spectrometry (GC/MS) and tested for estrogenic activity using an MCF-7 human breast cancer cell proliferation assay. Significant estrogenic activity, identifiable as BPA by GC/MS (up to 310 μg/L), was released from used polycarbonate animal cages. Detectable levels of BPA were released from new polycarbonate cages (up to 0.3 μg/L) as well as new polysulfone cages (1.5 μg/L), whereas no BPA was detected in water incubated in glass and used polypropylene cages. Finally, BPA exposure as a result of being housed in used polycarbonate cages produced a 16% increase in uterine weight in prepubertal female mice relative to females housed in used polypropylene cages, although the difference was not statistically significant. Our findings suggest that laboratory animals maintained in polycarbonate and polysulfone cages are exposed to BPA via leaching, with exposure reaching the highest levels in old cages. ***Key words:*** **animal caging, bisphenol A, endocrine disruptor, estrogen, leaching, polycarbonate, polysulfone. *Environ Health Perspect* 111:1180–1187 (2003). doi:10.1289/ehp.5993 available via *http://dx.doi.org/* [Online 5 February 2003]**

Bisphenol A (BPA) is a monomer used in the manufacture of many types of products, including polycarbonate plastic food storage containers (i.e., baby bottles and water carboys), as well as the epoxy resin used as the lacquer lining of food or beverage cans and in some dental sealants. Exposure to the monomer has been reported to occur as a result of the migration of BPA out of these products when they are exposed to high heat or alkaline conditions (Brotons et al. 1995; Olea et al. 1996). Krishnan et al. (1993) reported that polycarbonate flasks leached BPA into media in the autoclaving process. Likewise, BPA is known to be released from reusable polycarbonate baby bottles as a result of washing (Consumers Union 1999). Recent research in Japan has shown that leaching of BPA from polycarbonate products increases as a function of the amount of wear on the product because of repeated use (Takao et al. 1999). There is considerable interest in whether use of BPA in food and beverage containers as well as in other products can result in significant release of BPA, because BPA has been shown to be estrogenic in both *in vitro* cell culture (Krishnan et al. 1993; Nagel et al. 1997) and *in vivo* rodent assays. This concern is increased by findings that levels of BPA detected in human fetal umbilical cord blood (Schönfelder et al. 2002b) are within the range of effects in studies with rats and mice.

Polycarbonate animal cages are commonly used to house laboratory animals, such as rodents and aquatic invertebrates and vertebrates, because of the assumption that these cages have superior heat-resistant properties and structural strength. This assumption is also typically made with regard to other polycarbonate products, such as baby bottles. Because of their presumed durability, the polycarbonate cages are generally used long after they show significant signs of wear. Continued use of discolored, clearly worn cages may occur because the plastic cages (and other plastic products) are commonly assumed to be manufactured from highly stable polymers. The objective of our study was to examine this assumption by observing whether used polycarbonate animal cages would release free BPA (unreacted BPA monomer or BPA from hydrolysis) under normal use conditions of room temperature (23 ± 2°C) and a neutral solvent, high-pressure liquid chromatography (HPLC)-grade water (pH 7). These experimental conditions are directly relevant because it is common for aquatic laboratory animals to be housed in polycarbonate cages. However, the potential for effects of BPA leaching from polycarbonate products on rodents cannot be discounted because effects at very low doses (micrograms per kilogram per day) of BPA have been reported (Elswick et al. 2000; Farabollini et al. 1999; Gupta 2000; Honma et al. 2002; Howdeshell et al. 1999; Markey et al. 2001a; Nagel et al. 1997; Palanza et al. 2002; Rubin et al. 2001; Sakaue et al. 2001; Schönfelder et al. 2002a; Steinmetz et al. 1998; vom Saal et al. 1998)

We also evaluated new polycarbonate cages as well as new polysulfone cages, another type of plastic manufactured from BPA. Polysulfone is marketed as having a higher temperature and chemical tolerance than polycarbonate cages and thus may be less likely to leach BPA. The bioactivity of the cage water samples was tested in an *in vitro* cell proliferation assay using estrogen-sensitive MCF-7 human breast cancer cells to determine whether the BPA measured by gas chromatography/mass spectrometry (GC/MS) was sufficient to elicit a biological response in human breast cancer cells. Finally, the *in vivo* estrogenic bioactivity of the used polycarbonate cages was tested by measuring the uterine wet weight of prepubertal female mice housed in the cages.

Materials and Methods

Animal caging. New and used polycarbonate rodent cages (Allentown Caging Equipment, Allentown, NJ) were obtained from two different animal care facilities at the University of Missouri. The new polycarbonate cages had not been used to house animals, whereas the used polycarbonate cages that were selected were visibly worn, with patches of opaque plastic and some areas of rough, pitted surface inside the cage. New polysulfone cages (Allentown Caging Equipment) were also tested. Glass casserole dishes were used as negative controls because they do not contain any plastic products. Used polypropylene rodent cages (Lab Products, Inc., Maywood, NJ) were selected as a negative control caging material because they are not manufactured with BPA. All plastic cages were standard size

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