Bioactive Leachates from Lab Plastics

Use of Plastic Disposables May Compromise Bioassay Results

Published and anecdotal evidence suggests that bioassay results may be compromised by pharmacologically active compounds leaching from plastic ware into buffers and solvents. False positives, false negatives, lost productivity and unnecessary expense can be minimized if researchers follow some simple guidelines.

Introduction

Pipette tips, microfuge tubes, multiwell plates, syringes, conical centrifuge tubes; disposable plastic items used routinely in tens of thousands of laboratories worldwide, by academic and industrial scientists. That the use of such plastic items may interfere with bioassays has been considered by manufacturers of laboratory ware; siliconized or otherwise coated plastics to which proteins will not bind, and plastics certified as DNAase-, RNAase- and pyrogen-free, are testament to such concerns. However, are these precautions sufficient to ensure the integrity of bioassay results? The identification of bisphenol-A in liquids stored in polycarbonate bottles [1] suggests that a clean, sterile or inert plastic surface does not guarantee that liquids will not be contaminated by species migrating from the plastic into solution. Indeed, recent data from our laboratories and from others indicate that this is likely to be a widespread problem impacting results from all areas of life science research.

Most laboratory disposables are manufactured from polypropylene, polyethylene or polystyrene. Softer plastics such as polypropylene are typically used in the manufacture of pipette tips and microfuge tubes, while more rigid plastics such as polystyrene are commonly used in multiwell plates, test tubes and centrifuge tubes. A variety of reagents may be added to these plastics during the manufacturing process, to achieve several desired outcomes. For example, addition of slip agents and plasticizers may lead to reduced viscosity of molten plastics at lower temperatures and reduced tackiness of hardened plastics, and will also facilitate easier removal of hardened plastics from moulds.
Some heavy metals act as catalysts in the plastic polymerization process while antibacterial detergents prevent bacterial colonization of plastic surfaces and reduce the build-up of static charge. Some detergents are also used to solubilize dyes that impart color to pipette tips and microfuge tubes. Since these additives intercalate within the plastic polymer structure, there is the potential for any of these classes of compounds, or for unpolymerized plastic monomers, to leach into liquids that come into contact with plastic surfaces. Indeed, concerns over the potential detrimental health effects of bisphenol-A in beverages, along with data confirming the presence of slip agents in foods or food-mimetics stored in plastic containers [2], indicate that such leaching is a common occurrence.

Bioactive Leachates

In addition to the obvious difficulties that may be caused to analytical chemists through introduction of plastic-derived contaminants to sample solutions, the bioactive nature of many of these leachates indicates that life scientists doing bioassays should also be concerned. Recently, we observed significant unpredictability in the activity of human monoamine oxidase-B, with the cause eventually attributed to the presence in our assays of a quaternary ammonium biocide, diHEMDA, that had leached from microfuge tubes used to prepare substrate dilutions [3]. The degree of inhibition achieved with diHEMDA was surpassed by oleamide, a fatty acid amide slip agent that leached from a different brand of microfuge tubes into our assay buffers. Enzyme inhibition also occurred on exposure of substrate solutions to pipette tips (fig. 1).

Results from mass spectral analyses also suggested the presence of other related slip agents, including stearamide and erucamide, in washes from microfuge tubes and pipette tips. While these compounds were found to be devoid of effects upon monoamine oxidase, a recent study [4] has confirmed that erucamide, leached from plastic pipette tips, stimulated cAMP production through effects upon G protein-coupled fatty acid receptors. These researchers became suspicious when determinations of pharmaceutical solubility in a validated microplate assay system generated inconsistent results, anomalies eventually attributed to erucamide from pipette tips.

The use of dyes to create yellow and blue tips, or a range of possible colors of microfuge tubes, requires the presence of surfactants to solubilize the dye compounds. Markedly reduced activity of mitochondrial respiration in patient biopsy samples screened at a metabolic diseases clinic was attributed to inhibition of complex I by nonylphenol ethoxylate (NP-10), a surfactant used to solubilize the blue dye present in pipette tips purchased by the clinic [5]. A similar relationship between plastic coloring and biological effect was observed in our laboratory during studies of binding of radiolabeled benzodiazepines to GABA-A receptors. Dilute DMSO washed through amber microfuge tubes caused significant inhibition of drug binding, an effect.
not observed when colorless microfuge tubes were used (fig. 2).

Following publication of our observations with monoamine oxidase-B, we received extensive feedback from other researchers who had encountered similar problems. For example, Tinuvin 770 (2,2,6,6-tetramethyl-4-piperidinyl) sebacate, an additive in plastics used to make medical syringes, is a potent inhibitor both of neuronal nicotinic acetylcholine receptors and of some calcium channels [6]. Currents induced by acetylcholine, or by the glutamate receptor agonist NMDA, were potently blocked by UV-absorbing species that have been identified. The prevalence of quaternary ammonium compounds as solubilizers and anti-static agents may predispose cell surface cation channels to inhibition, through binding of these cationic surfactants to the negatively-charged pore turret regions of these channels. Protein crystallographers should also be aware of the possibility that plastic leachates may co-crystallize with pure proteins, perhaps altering protein stability or conformation. For example, 1,4-diphenyl-2-butene leached from polystyrene and could be observed occupying the active site of crystaline human monoamine oxidase-B [9].

Avoiding Problems

There are several steps researchers can take to minimize the likelihood of their data being compromised by leachates. Some manufacturers provide information on the additives content of their plastics; for example, Eppendorf brand microfuge tubes of different colors may be found in the literature. Many similar tales of woe and of some calcium channels [6]. Currents induced by acetylcholine, or by the glutamate receptor agonist NMDA, were potently blocked by UV-absorbing species that have been identified. The prevalence of quaternary ammonium compounds as solubilizers and anti-static agents may predispose cell surface cation channels to inhibition, through binding of these cationic surfactants to the negatively-charged pore turret regions of these channels. Protein crystallographers should also be aware of the possibility that plastic leachates may co-crystallize with pure proteins, perhaps altering protein stability or conformation. For example, 1,4-diphenyl-2-butene leached from polystyrene and could be observed occupying the active site of crystaline human monoamine oxidase-B [9].

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