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.

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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).

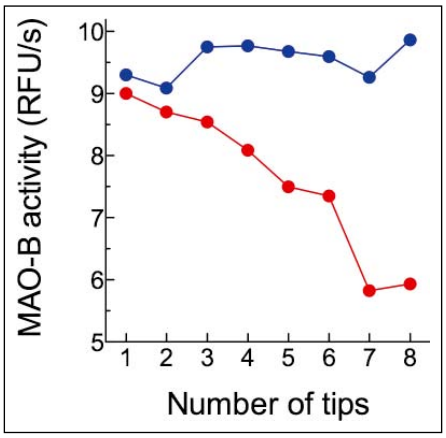

Fig. 1: Human monoamine oxidase-B activity measured by an in vitro fluorescence method following exposure of substrate solution to an increasing number of prewashed (DMSO then water; blue) or unwashed (red) Fisher brand pipette tips.

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

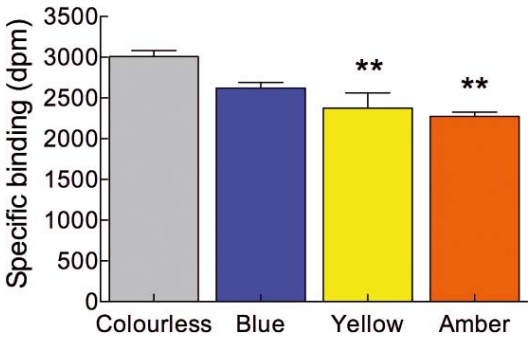

Fig. 2: Effect of DMSO (0.2 % v/v) washed through six consecutive Eppendorf brand microfuge tubes of different colors on benzodiazepine binding to GABAA receptors in rat brain membranes. ** P<0.01 compared with results from colorless tubes.

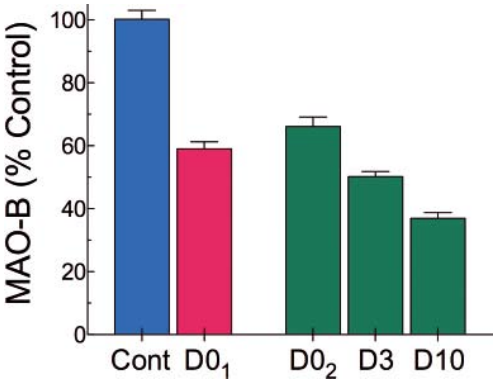

Fig. 3: Human monoamine oxidase-B activity in the presence of DMSO (2.5 % v/v) rinsed briefly through glass (Cont) or through Sarstedt brand 1.5 ml microfuge tubes (D01). Following an initial rinse, a second aliquant of DMSO was added to the same microfuge tubes and aliquots were taken immediately (D02) and after three (D3) and ten (D10) days and assessed for effects on monoamine oxidase-B.

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 a UV-absorbing species that leached from Falcon-brand plastic centrifuge tubes into water [7], while the presence of citric acid, used as a mould-releasing agent, on pipette tips has compromised protein assays and several enzyme assays (Promega, personal communication). It is probable that the presence of plastic-derived bioactive contaminants also explains anomalous effects at infinitely high dilutions attributed previously to water (e.g. [8]). The use of plastic multiwell plates may also compromise data; for example, some kinase enzymes are inhibited potently by a species leaching from polystyrene plates into wells close to plastic mould injection points, but not in wells elsewhere on the plate (P. Franks, personal communication). Many similar tales of woe may be found in the literature.

Cell culture seems particularly susceptible to the presence of bioactive leachates, given the extensive use of disposable plastics and prolonged contact of warm solutions with plastic surfaces. Many researchers report problems with one or more brands or batches of culture plates or tubes, although the cause of problems is rarely 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 crystalline 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 use virgin polypropylene for their colorless pipette tips and microfuge tubes, and no slip agents or other additives are present. Although the associated costs may be slightly higher, researchers should purchase plastic ware from a manufacturer that does not use additives and avoid buying from suppliers that refuse to confirm the absence of additives. Researchers should then confirm that the chosen brand of plastic ware is devoid of effects in their assay system, bearing in mind that reaction components may also leach into plastics, and also that leaching of some metals used as polymerization catalysts may be unavoidable. The use of colored plastics should be avoided whenever possible, since both dyes and surfactants may contaminate liquids. Finally, while pre-washing of plastics may remove surface contamination, benefits are transient as additives can migrate over time to the plastic surface from within the crystalline structure (fig. 3).

Ultimately, improved transparency from manufacturers regarding plastic additives coupled with a heightened awareness within the industry of these issues, and vigilance among life scientists in screening for effects of disposables on their own assays, should minimize generation and publication of compromised data in the future. Increased availability and affordability of additive-free plastics such as cyclic olefin polymer will also help in this regard.

Acknowledgements

Work in the author's laboratory is supported by the Canadian Institutes of Health Research (MOP77529 to A.H.).

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