Bioactive Contaminants Leach from Disposable Laboratory Plasticware

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Recent reports of leaching of bisphenol A and antimony into foods and beverages from polycarbonate and polyethylene terephthalate containers, respectively, have drawn attention to plastics as potential sources of bioactive environmental contaminants (*1*, *2*). However, numerous other processing additives coat, or intercalate within, polymeric structures, and these also migrate into foods stored in plastic containers (*3*).

Disposable plasticware is used in life science laboratories worldwide. Although labeling of plastics as "sterile" appears to offer researchers some assurance that products are free of bioactive contaminants, the presence of processing additives is unavoidable. Herein, we report identification of two additives leaching from disposable plasticware and demonstrate potent effects on enzyme and receptor proteins.

Observations of anomalous kinetics with human monoamine oxidase-B (hMAO-B), which recognizes numerous xenobiotic substrates, led us to examine disposable tubes used in our assays as a potential source of interferences. Water rinsed through several brands and sizes of plastic tubes adopted inhibitory potency versus hMAO-B; when dimethyl sulfoxide (DMSO) (10%, v/v) was used instead, inhibition was more pronounced, and marked enzyme activation was observed in one case (Fig. 1A). Samples of two tubes (indicated) were rinsed with water (W) or methanol (M), and leachates were dried and subjected to mass spectrometry. Fragmentation spectra for major species present (Fig. 1B and fig. S1A) identified a biocide, di(2-hydroxyethyl)methyldodecylammonium (DiHEMDA), and a slip agent, 9-octadecenamide (oleamide), in W and M leachates, respectively (Fig. 1C, inset). Pure samples of DiHEMDA (*4*) and oleamide were confirmed as hMAO inhibitors, showing selectivity for hMAO-B (Fig. 1C). Inhibition by leachates from oleamide-positive tubes increased markedly over a 10-day plastic exposure period at 20°C (fig. S1B).

Repeated (10 times) pipetting of 10% DMSO with pipette tips from several suppliers resulted in extraction of species that had significant effects on hMAO-B (fig. S1C). One or more unidentified compounds causing hMAO-B activation also leached into 10% DMSO from wells of polystyrene or acrylic 96-well microplates (fig. S1D).

Oleamide is an endogenous signaling molecule that binds to numerous receptor and channel proteins (*5*), including the γ-aminobutyric acid type A ($GABA_A$) receptor. Specific binding of the $GABA_A$ radioligand [^{3}H]Ro15-4513 to rat brain membranes was inhibited significantly by DMSO leachate from Eppendorf (Eppendorf AG, Hamburg, Germany) microfuge tubes (Fig. 1D).

Related slip agents such as erucamide and stearamide are endogenous molecules used routinely in plastic manufacturing (*3*), whereas quaternary ammonium compounds are included as biocides or antistatic agents. Many such biocides bind substantially to proteins and DNA and have recently been linked with fertility problems in mice (*6*). Our findings that processing agents leach from laboratory plasticware into biological media and solvents, particularly when liquids are stored in plastic vessels, identify a likely source of error in many assay systems.

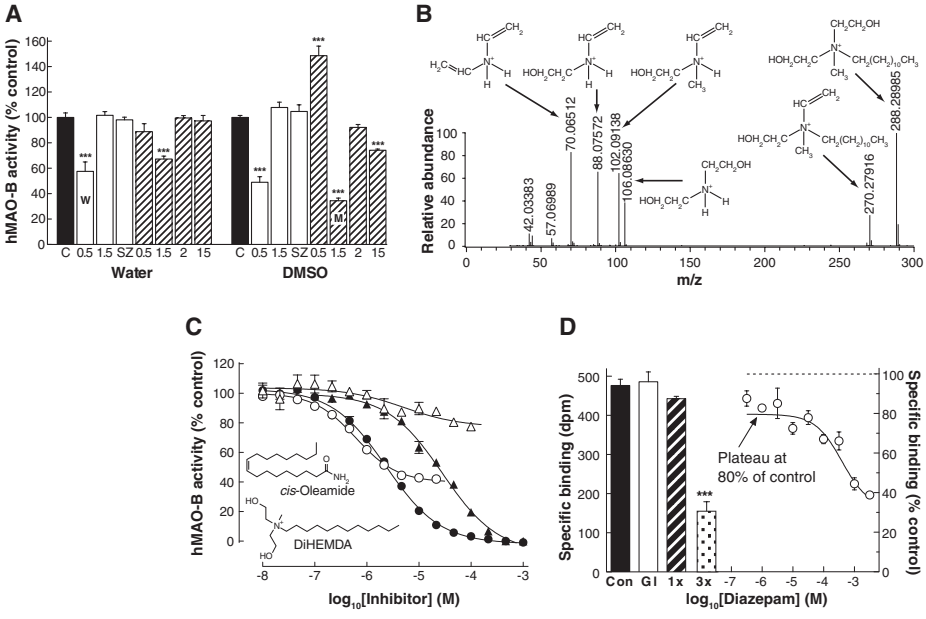

Fig. 1. (**A**) Effects on hMAO-B of water ($N = 3$) or 10% DMSO ($N = 7$) (40% of tube volume, 1 hour, 20°C) from Fisherbrand (Fisher Scientific, Ottawa, Canada) (clear bars) or Sarstedt (Sarstedt AG, Nümbrecht, Germany) (hatched bars) plastic tubes or glass vials (C). *x* axis labels indicate tube volumes (ml). C indicates control; SZ, siliconized 0.5-ml tube; W and M, water and methanol rinses analyzed by tandem mass spectrometry (MS/MS). Data are mean ± SEM. ***$P < 0.001$ compared with control (one-way analysis of variance with Dunnett's post hoc test). (**B**) MS/MS data for the major peak obtained from a water rinse of a Fisherbrand 0.5-ml tube reveal the presence of DiHEMDA. (**C**) Inhibition of human MAO-A (triangles) or MAO-B (circles) by oleamide (open symbols) or DiHEMDA (solid symbols) (structures inset). (**D**) (Left) Effects on binding of [^{3}H]Ro15-4513 to rat brain $GABA_A$ channels of DMSO (0.2% in assay) rinsed (at 100%) through a glass (Gl) tube or through one (1×) or three consecutive (3×) Eppendorf brand 1.5-ml tubes, compared with a DMSO-free control (Con). (Right) Competition binding curve to diazepam, prepared in DMSO in microfuge tubes, reveals a lowered plateau relative to a DMSO (in glass) control.

References and Notes

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7. Supported by the Canadian Institutes of Health Research (A.H., S.M.D., and G.B.B.), the Canada Foundation for Innovation (G.B.B.), the Natural Sciences and Engineering Research Council of Canada (A.J.), and the National Institute of Health (D.E.E.). We thank O. Degenhardt, I. Paulsen, and G. Rauw for technical assistance.

Supporting Online Material

www.sciencemag.org/cgi/content/full/322/5903/917/DC1
Materials and Methods
Fig. S1
References

26 June 2008; accepted 2 September 2008
10.1126/science.1162395

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