The Preparation and Validation of Isotope Labeled and Unlabeled Reference Standards for Endocrine Disruptor Compounds (EDCs)

Daniel L. Bolt, Terry Grim, Cambridge Isotope Laboratories, 50 Frontage Road, Andover MA, 01810 USA
Michael Re, Lawrence H. Keith, Radian International LLC, 8501 Mopac Blvd, Austin TX, 78759 USA

Abstract

Recently, concern has been raised over the ubiquitous use of chemicals and the potential for underlying disruption of critical human endocrine systems. The compounds initially identified as targets by the World Wildlife Federation, USEPA and CDC include a large number of disparate chemicals found in a multitude of matrices and acting at extremely low levels. The combination of these factors points to a need for precise and accurate measurement processes for diverse analytes in multiple matrices at very low detection. The combination of 1) gas or liquid chromatography, 2) mass spectrometric detection and 3) stable isotope standards has proven to be an effective one for other problems of this type such as halogenated dioxins and furans and PCBs. Cambridge Isotope Laboratories, Inc. (CIL) and Radian International LLC (Radian) propose a list of validated reference standards, both unlabeled and isotope labeled, to facilitate the development of reliable measurement systems for this important new class of compounds.

Introduction

Endocrine Disruptor Compounds (EDCs) are chemicals that exhibit estrogen mimicry. EDCs "convince" certain receptors that they are estrogen. When this happens, fetal development can be impaired in many ways. There is evidence to suggest that humans (as well as other species) have suffered adverse health effects from exposure to these chemicals which interact with the endocrine system[1]. However, unlike many compounds with related toxicity characteristics, EDCs have been shown to be structurally diverse, making it difficult to identify potential EDCs. They share common properties, among the most important of which is an affinity for the estrogen receptor protein. Some screening approaches such as radioligand binding assays and the DNA-band shift assays show promise for identification of EDCs, but many questions remain.

Many of the synthetic chemicals suspected of being EDCs are ubiquitous in the modern world. Among classes of compounds believed to be EDCs are Pesticides (including herbicides,

fungicides etc.,), phthalate esters (extensively used as plasticizers), alkylphenol ethoxylates (metabolic products of common surfactants), PCBs and halogenated dibenzo-p-dioxins and dibenzofurans. Many of these chemicals are being made at the rate of thousands of tons each year in the US alone, while plasticizers are prepared on the order of hundreds of thousands of tons each year. In order to assess these problems and quantify the extent of the problem in the environment, reliable measurement systems mist be created. The work described is a first attempt to prepare a comprehensive set of validated reliable analytical standards, both unlabeled and stable isotope labeled, to serves as a basis for such a program.

Experimental Methods

Analytical methods rely on reference materials for accurate reporting of data. Especially when faced with the daunting task of measuring so many different types of compounds in multiple matrices, the analyst should be armed with a full array of validated reference materials. Further, the addition of stable isotope analogs of the analytes of interest and the use of mass spectrometric detection has become the definitive method for reliable quantitation. This powerful combination also yields high precision and virtually eliminated false positives allowing for very low detection limits. The isotope dilution method of quantitation has been widely used in the analysis of halogenated dioxins and furans and the same established protocols used with the dioxin and PCB program were established for the preparation of unlabeled and isotope labeled EDC standards[2]. The general procedure prior to actual gravimetry is:

1. Compounds are synthesized according to the most unambiguous and well-documented literature routes.

2. Materials are characterized by a variety of analytical methods including HPLC, GC/FID, GC/MS, ^{1}H and/or ^{13}C NMR, melting point determinations to establish absolute identity, chemical purity and isotopic enrichment.

3. Solvents (used in the preparation of solution standards) are redistilled and checked for purity by GC/FID and GC/MS.

4. Balances are maintained by a qualified balance technician, are standardized by comparison to NIST class "s" weights and made ready for gravimetry.

Having prepared for the weighings, the materials are gravimetrically prepared and diluted to a nominal concentration in the previously redistilled solvent. Next, the solution is compared by multiple injections on GC/MS to NIST standards (where possible) or other commercially available certified standards. Statistical controls are applied. The tolerance level versus the certified standard is 5% RSD. Finally, the materials are ampouled and sealed in a manner that maintains the integrity and stability of the standard solutions. Before entering inventory, useful technical data sheets (TDS) and material safety data sheets (MSDS) are prepared. On-going

shelf life and stability testing allows CIL and Radian to monitor the quality of materials over tine and to test for lot-to-lot variability.

Results and Discussion

Driven by the analytical chemistry community's need for accurate low-level measurement of halogenated dioxins and furans, Cambridge Isotope Laboratories, Inc. (CIL) in conjunction with its long-term collaborator Radian International LLC (Radian), has spent over 15 years developing protocols for the preparation and validation of reference standards, both unlabeled and stable isotope labeled, for use with GC/MS and stable isotope dilution quantitation. These protocols are now applied to a new problem in analytical chemistry. That is, the detection and quantitation of a diverse group of chemicals known as endocrine disrupter compounds (EDCs). Organizations such as USEPA, World Wildlife Federation and the Centers for Disease Control and Prevention (USA) have put forth lists of potential EDCs for evaluation. CIL and Radian have responded by preparing over 40 potential EDCs as isotope labeled and unlabeled standards using the protocols described above. (See Table 1)

This list will continue to grow and we would hope that the entire list of over 80 potential EDCs will be available as both unlabeled and stable isotope labeled standard solutions by the end of 1997. All such materials will be prepared according the established protocols and with the associated reliability and quantitative features that the analytical community has come to expect from CIL/Radian standards.

Literature Cited

(1). Colborn T; Clement C, eds. *Advances in Modern Environmental Toxicology Vol XXI*, Princeton Scientific Publishing, Princeton NJ, 1992; p.401.

(2) Bolt, D.L.; Bradley, J.C. *Reference Materials for Environmental Analysis*; CRC Press Lewis Publishers; Boca Raton FL, 1997; p. 15.

Table 1: Available EDCs from CIL and Radian

Acenaphthene ($^{13}C_6$)	Di-n-hexyl Phthalate ($^{13}C_4$)
Alachlor ($^{13}C_6$)	Di-n-propyl Phthalate ($^{13}C_4$)
Aldrin ($^{13}C_4$)	Dieldrin ($^{13}C_4$)
Anthracene ($^{13}C_6$)	Diethyl Phthalate (D_4)
Atrazine ($^{13}C_3$)	Endosulfan I (D_4)
Benz[a]anthracene ($^{13}C_6$)	Heptachlor ($^{13}C_4$)
Benzo[b]fluoranthene ($^{13}C_6$)	Heptachlor epoxide ($^{13}C_1$)
Benzo[k]fluoranthene ($^{13}C_6$)	Hexachlorobenzene ($^{13}C_6$)
Benzo[a]pyrene ($^{13}C_4$)	PCB-169 ($^{13}C_{12}$)
Benzophenone (D_{10})	Indeno[1,2,3-cd]pyrene ($^{13}C_6$)
α-BHC ($^{13}C_6$)	Kepone ($^{13}C_8$)
β-BHC ($^{13}C_6$)	Malathion (diethyl-D_{10})
γ-BHC (Lindane) ($^{13}C_6$)	Metolachlor ($^{13}C_6$)
Bisphenol-A ($^{13}C_{12}$)	Methoxychlor ($^{13}C_{12}$)
Butylated Hydroxytoluene (BHT) (D_{12})	Mirex ($^{13}C_8$)
n-Butyl Benzene ($^{13}C_6$)	4-Nitrotoluene ($^{13}C_6$)
Butyl Benzyl Phthalate (D_4)	p-Nonylphenol ($^{13}C_6$)
Carbaryl ($^{13}C_6$)	Parathion (diethyl-D_{10})
Chlordane ($^{13}C_1$)	PCB-126 ($^{13}C_{12}$)
Chrysene ($^{13}C_6$)	Pentachloronitrobenzene ($^{13}C_6$)
4,4'-DDD (D_8)	Pentachlorophenol ($^{13}C_6$)
4,4'-DDE ($^{13}C_{12}$)	Phenanthrene ($^{13}C_6$)
4,4'-DDT ($^{13}C_{12}$)	Pyrene ($^{13}C_3$)
2,4-Dichlorophenoxyacetic Acid ($^{13}C_6$)	Simazine ($^{13}C_3$)
2,4-Dichlorophenol ($^{13}C_6$)	Styrene (D_8)
Di-ethylhexyl Adipate (3,3',4,4'-D_4)	PCB-77 ($^{13}C_{12}$)
Di-ethylhexyl Phthalate [Bis(2-ethylhexyl)] (D_4)	2,4,5-T ($^{13}C_6$)
Di-n-butyl Phthalate (D_4)	Trifluralin (D_6)

The native (unlabeled) analogs for all of these materials are available from CIL, as well as the native analogs for other labeled EDCs which are under development.

Quantities suitable for metabolic and decomposition studies of many of these compounds