Determination of Part-per-Trillion Concentrations of 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin in Fish

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An analytical procedure has been developed and utilized for the determination of approximately 10 to 100 parts per trillion (10^{-12} g/g) concentrations of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) in fish. The technique involves digestion and extraction of the matrix followed by a series of adsorbent, and chemically-modified adsorbent, liquid column chromatographic clean-up steps. A final "residue polishing" step via elevated temperature reversed-phase high performance liquid chromatography is applied prior to detection by multiple ion mode gas chromatography–mass spectrometry. Using ^{13}C-labeled TCDD as an internal standard and carrier, the procedure has been validated for rainbow trout from approximately 10 to 100 ppt TCDD. Relative to this range, TCDD recovery is 75% ± 25%, and the precision of a single determination at the 95% confidence level (2σ) is ±20% relative at 50 ppt TCDD concentration.

One compound which has been the subject of intense analytical and toxicological investigations in recent years is 2,3,7,8 tetrachlorodibenzo-*p*-dioxin (TCDD). As a result of the presence of TCDD as a contaminant in 2,4,5-trichlorophenoxy acetic acid (2,4,5-T) and related ester herbicides and the biological effects associated with exposure to TCDD, many reports have discussed both the determination of this compound and its biological activity.

Much of the work up to 1974 is contained in (*1*), while work since that time is more scattered through the literature. Co-workers have described methods and their application to the determination of part-per-trillion (ppt, 10^{-12} g/g) level in a variety of environmental matrices using gas chromatography–mass spectrometry (GC-MS) (*2–9*). O'Keefe, Meselson, and Baughman (*10*) have recently described a neutral clean-up procedure for this determination in milk. A similar method has been described by Fukuhara et al. for low amounts of TCDD in seafoods (*11*). Workers at the National Institute for Environmental Health Sciences (*12*) have also described a procedure which utilizes negative chemical ionization GC-MS which apparently yields high sensitivity. In all the above methods, however, the presence of other lipophilic substances represents a severe limitation. As Hummel (*3*) has pointed out, many commonly-observed environmental pollutants can be interferences. As part of an investigation into the presence of some halogenated aromatic compounds in fish and water, we have analyzed a variety of fish for the presence of polychlorinated biphenyls (PCB), polybrominated biphenyl (PBB), 4,4'-dichlorodiphenylethylene (DDE), and TCDD.

In order to avoid interference from million-fold excess concentrations of these materials, we have developed a multistep clean-up procedure which is very effective at removing these halogenated aromatic compounds as well as yielding high recovery and good precision for TCDD.

EXPERIMENTAL

Apparatus. As described by us and co-workers (*6, 9*), we have utilized low resolution GC-MS for the primary determination, with GC-high resolution MS for confirmation of identity. These low resolution GC-MS determinations were performed on a Hewlett Packard model 5992A (400 resolution) equipped with a single stage jet separator using helium at 20 cm^3/min for the carrier gas. The GC column was a 210 cm by 2-mm (i.d.) glass column packed with 0.6% OV-17/0.4% Poly S 179 on a specially deactivated Chromosorb W-AW (80/100) support. The final cleanup is achieved on a liquid chromatograph consisting of an Altex Model 110 pump, Rheodyne Model 7120 injector valve with 20-μL loop, a 250 mm × 4.6 mm DuPont Zorbax ODS column and a Perkin-Elmer LC-55 variable wavelength detector, using methanol as the mobile phase.

These analyses at ppt levels require careful attention to the purity of all reagents. The procedures described here include provision for cleaning adsorbents and reagents to remove traces of phthalate plasticizers and miscellaneous chlorinated organic compounds.

Reagents. *44% Sulfuric Acid on Silica.* Chromatographic grade silicic ac[redacted] It is then removed from the furnace and cooled to ambient temperature, and subsequently rinsed with consecutive ~75-mL portions of methanol and methylene chloride (Burdick and Jackson distilled-in glass quality). The methylene chloride saturated material is returned to the tube furnace which has been set to 50 °C and the dry nitrogen purge reestablished. Over a period of ~25 min the furnace temperature is increased in a step-wise manner to 180 °C. This technique can be described as a modified sweep co-distillation of the adsorbent using methylene chloride saturated nitrogen, and has been found to be an effective means for trace contaminant removal from silica. The effluent gases from this operation must be vented to a fume hood. The silica is then activated for an additional period of ~90 min at 180 °C. Dried adsorbent is cooled and placed in an appropriately sized glass bottle. Sufficient concentrated sulfuric acid is added directly to the silica to yield an acid concentration of 44% based upon total weight. The material is manually shaken until no clumping can be observed, and is then transferred to a glass bottle and stored in a desiccator over phosphorus pentoxide until used. Caution. This reagent retains all of the properties of concentrated sulfuric acid[redacted]

[redacted] % sulfuric acid on silica [redacted]

Basic Alumina. Chromatographic grade aluminum oxide as 100/200 mesh Bio-Rad Basic Alumina AG-10 is initially dried in a glass tube furnace for ~60 min at 300 °C under a continuous

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