Analysis of Sugar for Tetrachlorodibenzo-*p*-dioxin

Samples of sugar in four different stages of refinement were analyzed by packed column gas chromatography/high-resolution mass spectrometry (GC/HRMS) for the possible contamination by tetrachlorodibenzo-*p*-dioxin (TCDD). The samples were obtained from a sugar mill processing sugar cane from fields treated with the herbicide Silvex. No TCDD was detected at detection limits of 1 ppt or lower. In order to demonstrate the capability of detecting TCDD in sugar, at a subppt level, sugar samples fortified with native TCDD were also analyzed.

Silvex and (2,4,5-trichlorophenoxy)acetic acid (2,4,5-T) are used as herbicides in sugarcane fields to control unwanted grass and weeds. Both herbicides are known to be contaminated with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2,3,7,8-TCDD), which is present in 2,4,5-trichlorophenol, the synthetic precursor (Crummett and Stehl, 1973; Rappe and Marklund, 1978). 2,4,5-T is reported to contain 0.02 ppm of 2,3,7,8-TCDD, and in a typical application (2.2 kg/ha) approximately 44 μg of 2,3,7,8-TCDD/ha will be released. Although, plant accumulation of TCDD has been investigated under controlled conditions (Isensee and Jones, 1971) and in garden vegetables grown in TCDD-contaminated soil (Cocucci et al., 1979), leaf or root uptake of TCDD by sugarcane plants has not been studied.

This pilot study was undertaken to determine whether, owing to a possible uptake or carry-over on the surface of the plant, the TCDD will be carried through to various stages of refinement in the sugar manufacturing process. The samples utilized for this study were white sugar, raw sugar, molasses, and animal feed (which contained low-grade molasses), obtained from a sugar mill in Iberia parish in Louisiana. The mill received sugarcane grown in fields treated with the herbicide Silvex. To our knowledge, this is the first report of data from the analysis of sugar for TCDD.

EXPERIMENTAL SECTION

Safety Procedures. TCDD is highly toxic in animal tests and may be hazardous to human health. Techniques similar to those used for handling radioactive and infectious materials were used. Sample extraction and cleanup were carried out in an isolated laboratory open only to trained personnel. The laboratory is equipped with separately vented and filtered hoods. All mass spectrometry vacuum pumps are vented to hood lines or into traps containing charcoal. Periodically, wipe tests are performed on gas chromatographic and mass spectrometric equipment and on laboratory benches. To date, no TCDD has been found on the wipe samples at a detection limit of 0.02 pg/cm^2.

Reagents. Carbon tetrachloride, ethyl alcohol, hexane, and methylene chloride were glass-distilled OmniSolv, MCB, suitable for chromatography and residue analysis. Sulfuric acid and potassium hydroxide (analytical grade) were obtained from Mallinkrodt. Anhydrous sodium sulfate (analytical grade) was from Fisher Scientific Co. Aluminum oxide, neutral, activity grade I, was from Woelm Pharma. Dry nitrogen, boiled off from liquid nitrogen, and water, distilled in glass, were used. The native 2,3,7,8-TCDD and the internal standard 2,3,7,8-TCDD-$^{13}C_{12}$ used in quantification and in determining extraction and cleanup efficiency were supplied by KOR Isotopes.

Gas Chromatography/Mass Spectrometry. A Perkin-Elmer Sigma II gas chromatograph equipped with a 180 cm × 0.63 cm glass column was used. The column had a stationary phase of HNU permabond methyl silicone coated with 0.6% poly(S-179) (HNU Systems, Inc.). Chromatographic conditions were as follows: initial temperature at 250 °C; program rate at 10 °C/min; final temperature at 300 °C and held at the final temperature until the internal standard, 2,3,7,8-TCDD-$^{13}C_{12}$, had eluted; injection port temperature of 250 °C; helium carrier gas flow rate of 30 mL/min. This column is not suitable for isomer-specific 2,3,7,8-TCDD analysis.

A Kratos MS50 ultra-high-resolution (ultimate resolution 220 000) mass spectrometer was used for the analysis. The instrument was equipped with an electron impact ion source and a custom-built eight-channel multiple ion monitor. The source was operated at 70-eV ionizing energy, an accelerating voltage of 8 kV, and a temperature of 250 °C. The mass analysis was carried out at a resolution of 10 000 (10% valley definition), which is sufficient to separate $C_{12}H_4O_2{}^{35}Cl_3{}^{37}Cl$ (TCDD) from $C_{12}H_3{}^{35}Cl_5$ (PCB) and $C_{14}H_8{}^{35}Cl{}^{37}Cl_3$ (DDE). The latter two interferences are often found in biological and environmental samples.

The GC/HRMS interface was a simple glass-lined stainless steel capillary that was coupled to a glass jet separator and held at 250 °C.

A signal averager (Nicolet Model 1170) was used to acquire data during analysis. The acquired data were subsequently transferred to a Kratos DS55 data system for storage on magnetic tape or to an X-Y recorder (Houston Model 2000) for hard copy to be used in calculating the concentration and the percent recovery.

Sample Preparation and Cleanup. The animal feed sample was extracted in a Soxhlet extraction apparatus fitted with a fritted glass thimble. The animal feed sample (ca. 50 g) was placed in the thimble and fortified with the internal standard, 2,3,7,8-TCDD-$^{13}C_{12}$ (2.5 ng). Hexane was used as the solvent, and the extraction was carried out for 24 h.

A neutral extraction procedure with acetonitrile partitioning was utilized for the remaining samples. The sample (ca. 100 g) was dissolved in water (100 mL) and fortified with the internal standard, 2,3,7,8-TCDD-$^{13}C_{12}$ (2.5 ng). Acetonitrile (15 mL) was added and the resulting aqueous solution was extracted with three 30-mL portions of hexane. The hexane extracts were combined. For the method validation study, raw sugar samples were fortified with native 2,3,7,8-TCDD and 2,3,7,8-TCDD-$^{13}C_{12}$ and extracted in a similar manner.

The hexane extracts were washed with 10-mL portions of concentrated sulfuric acid until both acid and organic layers were colorless, followed by distilled water and then 1 M potssium hydroxide (30 mL). Finally, the hexane was washed twice with distilled water and dried with anhydrous sodium sulfate. The extract was subsequently concentrated to a final volume of 0.5–1.0 mL under a stream of dry nitrogen.

The extracts were cleaned up by using alumina chromatography as previously described (Gross et al., 1981) except the TCDD was eluted from the column with 25% methylene chloride in hexane instead of methylene chlo-

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Table I. Analytical Results for TCDD in Sugar

sample matrix	weight, g	TCDD found,[a] ppt	detection limit[b]	TCDD added, ppt	% recovery[c]
molasses	103	ND[e]	0.7	0	55
brown sugar	100	ND	0.5	0	75
white sugar	101	ND	1.0	0	40
animal feed	51	ND	0.9	0	60
brown sugar[d] (control)	100	ND	1.0	0	85
brown sugar[d]	101	0.5	0.2	0.30	70
brown sugar[d]	101	1.0	0.4	0.60	55

[a] Corrected for percent recovery losses. [b] The average detection limit was 0.7 ± 0.3 ppt. [c] Each sample had been fortified with 2.5 ng of 2,3,7,8-TCDD-$^{13}C_{12}$. The average recovery efficiency was 63 ± 13%. [d] TCDD mean accuracy was ±67% for fortified samples. [e] ND = none detected.

ride. In addition, the column was washed with 10% methylene chloride in hexane prior to the elution of TCDD.

Mass Analysis. The analysis was carried out by monitoring M^+ and $(M + 2)^+$ ions of TCDD at the masses 319.8965 ($C_{12}O_2H_4{}^{35}Cl_4$) and 321.8936 ($C_{12}O_2H_4{}^{35}Cl_3{}^{37}Cl$), respectively, and 333.9339, the mass of the internal standard ($^{13}C_{12}O_2H_4{}^{35}Cl_3{}^{37}Cl$). The PFK (perfluorokerosene) fragment of mass 331.97923 was used as the reference. Complete peak profiles were acquired at an amplifier bandwidth of 1000 Hz by scanning at a frequency of 2 Hz, corresponding in each case to a mass range of 300 ppm (0.096 amu). The signal averager was set up to accumulate the signal from the mass spectrometer over 70–100 sweeps per single GC/HRMS run starting with the elution of the internal standard at approximately 3.1 min. The retention time occurs later (ca. 3.5 min), giving a peak width at 10% height of approximately 40 s. The resulting signals were smoothed and printed out on an X-Y recorder. Examples of the actual data output have been presented elsewhere (Gross et al., 1981; Gross, 1982).

Calculation of Results. The concentration of TCDD was calculated by comparing the peak heights at m/z 333.9339 and m/z 321.8936 (internal standard ratio method). An average ratio of these peak intensities for calibration was obtained by analyzing six standard solutions of native 2,3,7,8-TCDD and the internal standard, 2,3,7,8-TCDD-$^{13}C_{12}$. These standard solutions were analyzed along with the actual unknowns. Residue levels of TCDD in actual samples were determined by comparing the ratio of signal intensities of m/z 321.8936 and m/z 333.9339 of the samples with that of the standards. If no signal was detected at m/z 321.8936, the detection limit was calculated by using a ratio of 2.5 times the noise amplitude and the signal response for m/z 333.9339 in a similar manner (2.5:1 signal to noise criterion).

The signal height of m/z 333.9339 was also utilized to calculate the recovery efficiency. In doing so, the absolute intensity of m/z 333.9339 for the sample was compared with that of an average response obtained for standard solutions of the internal standard.

Confirmation. The isotope ratio of the masses 319.8965 and 321.8936 was used as a criterion for the confirmation of the results. The observed signal for TCDD was considered to be valid if the isotope ratio was 0.77 ± 0.1, the 0.77 being the theoretical isotope ratio. Furthermore, the signal for the internal standard serves as a mass standard. A shift of 5 ppm (0.0016 amu) from the center of the acquired profile could be detected.

RESULTS AND DISCUSSION

No TCDD was detected in any of the sugar samples analyzed. The detection limits were in the range of 0.5–1 ppt with an average detection limit of 0.7 ± 0.03 ppt (see Table I). However, TCDD could be readily detected in method validation samples (see Table I). The average efficiency of recovery of the samples was 63 ± 13%, which is reasonably good. The mean percent accuracy for the two quality assurance samples fortified with native 2,3,7,8-TCDD was ±67%. The accuracy is reasonable considering the low level of fortification (0.3–0.6 ppt). The error could arise in the actual step of fortification, in sample handling during workup, or in the analysis. In a similar study (Harless et al., 1980), the mean percent accuracy obtained was ±53% for 10 samples fortified at levels of 0.2–5 ppt.

Conclusion. We do not consider this study to be conclusive in terms of the possible occurrence of 2,3,7,8-TCDD residues in sugar, because the number of real samples analyzed was limited. Future studies should be conducted with a larger number of samples. Nevertheless, we do conclude that the analytical methods appropriate for analysis of TCDD at the low-ppt level in tissue and soil can be used for analysis of this material in various sugar samples to give detection limits of 1 ppt or lower.

Registry No. 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin, 1746-01-6; sugar, 57-50-1.

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