FORMATION OF POLYCHLORINATED DIBENZO-*p*-DIOXINS DURING THE EXTRACTION OF PENTACHLOROPHENOL-CONTAMINATED GUAR GUM

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Abstract
During the extraction of pentachlorophenol-contaminated guar gum, significant biases are observed when acetone is used either as a solvent or part of a mixture of solvents. These biases may be up to five times the consensus TEQ values, or six times that of the major TEQ contributor, HpCDD. Even the small amount of acetone used during the spiking step can result in a significant bias; e.g., +50 percent for OCDD, and +10 percent for the WHO-1998 TEQ (five times the error). The postulated mechanism for the formation of PCDDs implicates precursor molecules such as polychlorinated hydroxydiphenylethers (a.k.a. predioxins). The role of acetone is through the acid-catalyzed formation of the enol form. The latter becomes a receptor for HCl, which is intramolecularly eliminated from the predioxin isomers/congeners with the hydroxyl and chlorine groups in *ortho* positions. The significance of this observation in PCP-contaminated environmental samples is assessed.

Introduction
Guar gum is a polysaccharide derived from the endosperm of the guar bean from the plant *Cyamopsis tetragonolobus.* It has a number of industrial uses, from lubrication to acting as a binder in pills; however, it is most widely used as a food additive because of its excellent thickening properties. The majority of guar gum being produced today comes from India. Several batches of guar gum imported to the EU from India were found in the summer of 2007 to have been contaminated with pentachlorophenol. This series of events led to a realization that the international community (and specifically the EU) would benefit from an inter-laboratory comparison (i.e., round-robin study) of methods used to analyze guar gum samples.

At the conclusion of an EU Community Reference Laboratory-initiated round-robin study[1]—involving over 50 laboratories and two PCP-contaminated guar gum samples—a number of perplexing observations were made by the organizers. A close examination of the entire set of data revealed that, with the exception of two groups, laboratories making use of acetone during the extraction had significantly higher results for PCDDs, but not for PCDFs and PCBs. All implicated laboratories verified that there were no errors (e.g., spiking, calculations), and that all measurement system performance indicators were normal. Our attention naturally turned towards finding other more subtle causes to explain the apparent irregularity. In particular, the ratios between OCDD and OCDF, which were predetermined in the submitted guar gum samples, increased three fold (with OCDF's concentration remaining constant) when acetone is used during the extraction step relative to when it is not. Since the ratio between the labeled extraction standards ($^{13}C_{12}$-OCDD and $^{13}C_{12}$-OCDF) is also a constant, and these recoveries were normal, we concluded that PCDDs were formed during the extraction whenever acetone is used, and not because of differences in extraction efficiencies or in physico-chemical properties. Before conducting additional experiments, a mechanism was postulated[2]. Following the supplementary tests, the mechanism was further refined and a more comprehensive version was presented in November 2008 (Figure 1)[3]. In essence, the role of precursors such as polychlorinated hydroxydiphenylethers—known impurities in PCP formulations[4]—and acetone as a receptor for intramolecularly eliminated HCl, was favored over a higher reaction order implicating two molecules of PCP and one of acetone. Closure to a six-member ring dioxin structure occurs concurrently with the elimination of HCl. The objective of this paper is to report on the authentication of the initial observations, and to follow through with the postulated mechanism by confirming the presence of predioxins in the PCP-contaminated guar gum, establishing their role as precursors to the formation of PCDDs, and to examine the potential ramifications of using acetone during the spiking step or the extraction of PCP-contaminated environmental samples.

Materials and Methods
The sample extractions, fractionations, analyses, and quality assurance/quality control procedures were carried out according to an enhanced version of USEPA Method 8290. Derivatization was performed using 1:1 (v:v) addition of N,O-bis-trimethylsilyl-trifluoroacetamide (BSTFA; Supelco, Bellefonte, PA), followed by vortexing for ~15 s. Derivatized extracts were analyzed on a Waters/Micromass GCT TOF mass spectrometer (Milford, MA) using electron ionization. The GCT acquired data over an m/z range spanning 60-600 Da, with an accumulation time of 0.5 s. Mass resolving power was approximately 6K, and mass accuracy was within 2 mDa on average. Extracts generated for GC/TOF analysis were the result of extraction using toluene. Round-robin samples "A" and "B" contain PCP at 235 ppb and 17 ppm, respectively.

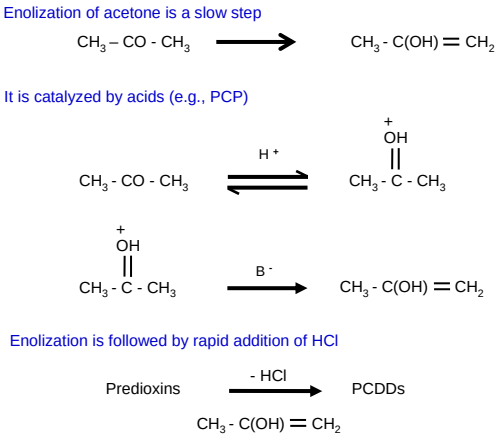

Figure 1: Proposed mechanism for the role of acetone during the formation of PCDDs from predioxins

Results and Discussion

As soon as the acetone was identified as a potential culprit, a series of additional confirmatory tests were conducted[3]. These studies examined the influence of acetone, pH, extraction technique, and extraction temperature; part of this is summarized in Figure 2. It is worth mentioning that multiple repeat analyses conducted on the same sample using a toluene extraction led to results that are consistently within two percent of the consensus values with an RSD of two percent (N=4). Moreover, the HpCDD congener represents the majority of the TEQ in the guar gum samples. An intramolecular elimination reaction of HCl (Figure 1) is more likely than a higher reaction order involving two PCP molecules (or any combinations with fewer chlorine atoms) and the enolic form of acetone. We therefore focused our efforts on confirming the presence of the precursors in the PCP-contaminated guar gum, and attempted to establish their role as precursors in the formation of PCDDs.

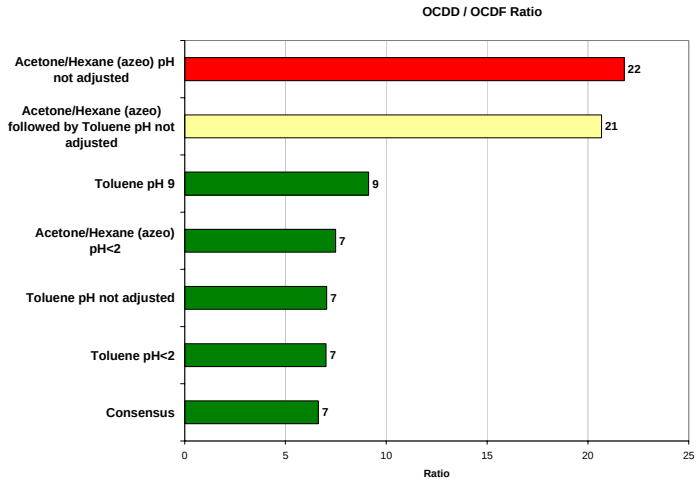

Figure 2: Ratio between OCDD and OCDF in the Round-Robin Sample "A" as a function of the extraction conditions

Confirmation of the Presence of Precursors. In an effort to confirm the presence of predioxin congeners in the PCP-contaminated guar gum sample, an aliquot of Sample "A" was extracted with toluene (sonication; pH unchanged and kept at ~6). Following a concentration step, the residue was derivatized and analyzed by GC/MS using a TOF instrument operating in the electron ionization mode. The derivatization step is necessary since it is known that the injector temperature (e.g., 250°C) is sufficient to convert predioxins into dioxins. The results (Fig. 3) show the three possible nonachlorinated hydroxydiphenylethers. Their mass spectra and elemental compositions are consistent with the derivatized forms. Only one of the three isomers has a structure favorable for the formation of OCDD. Similarly, up to six separate GC/MS peaks were detected for the octachlorinated species, some of which can be responsible for the formation of HpCDDs including the major contributor to the TEQ. No predioxins were detected in a control sample where approximately the same amount of PCP (standard) that would be extracted from one gram of guar gum was used.

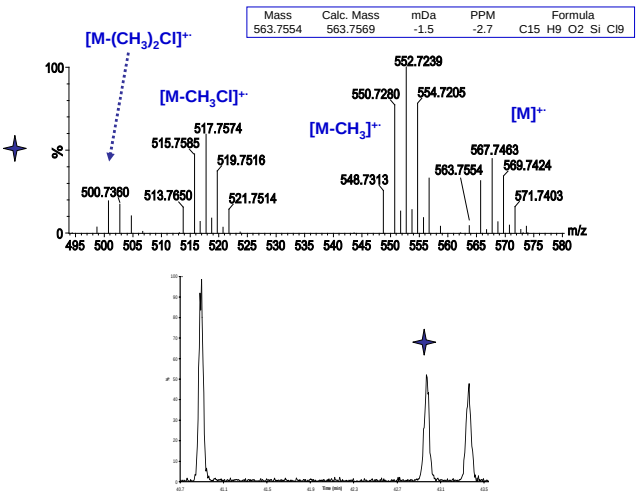

Figure 3: Extracted ion chromatogram of the $[M+4]^{+\cdot}$ obtained from a derivatized extract of PCP-contaminated guar gum. The associated mass spectra and accurate mass measurement (-2.7 ppm) for the three (TMS-derivatized) possible nonachlorinated hydroxydiphenylether isomers confirm the presence of predioxins.

Establishing the Role of the Precursors. Pure predioxin standards are not commercially available. Consequently, a separate aliquot of the PCP-contaminated guar gum Sample "A" was extracted with toluene using sonication. The pH was not adjusted prior to extraction. Using the pKa differences between PCP (*pKa: ~4*) and predioxins (*pKa: 7.7-8*), a series of selective partitioning steps led to an extract enriched in predioxins, and freed of OCDD. The extract was then split into two equal portions. A control sample using a PCP standard was prepared as well. The split extracts were analyzed for OCDD before and after a 16-H reflux with acetone. OCDD was not detected in the extracts not subjected to the acetone reflux. However, when the guar gum extract is refluxed in acetone, OCDD was found approximately 10 times the level in the corresponding fraction from the PCP standard.

Assessing the effect of acetone during the spiking step. Duplicate extractions of the guar gum samples "A" and "B" were carried out using hexane or acetone (1 to 3 mL) during the addition of the labeled extraction standards. The spiked samples were then subjected to toluene extractions. The pH of the samples was not adjusted prior the extraction. Following the regular sample fractionation steps, the extracts were analyzed for PCDD/Fs as usual. The results indicate that when the Soxhlet Dean-Stark set up is used, no increase in PCDD concentrations was observed most likely due to the fact that the contact time of the acetone was shortened when the small amount of acetone used during the spiking step ended up inside the Dean-Stark arm. When the arm is removed, the Soxhlet extraction of the 3-mL acetone spiked PCP-contaminated guar gum led to a bias for OCDD of over 50 percent (i.e., 776 ppt *vs.* 505 ppt consensus value; the TEQ increases eight to 10 percent for an assay where the TEQ's RSD is two percent). In the absence of acetone, we verified that a hexane spike does not result in the formation of PCDDs. Note that the artifact formation of PCDDs and the effect on the TEQ will depend on the amount of precursors as well as the kinetics of the reactions involved.

Other considerations. The data from extractions using reflux conditions, a Soxhlet Dean-Stark apparatus, or a Soxhlet with 3-mL acetone used during the spiking step suggests that the reaction takes place inside the boiling flask rather than inside the thimble where the sample matrix is located. Heat, and contact time may be influential factors as well as the concentrations of the reagents (predioxins and acetone). For instance, in the absence of a large excess of acetone, the weight of the sample undergoing extraction is expected to influence the extent of the artifact reaction due to the kinetics involved (reaction order), and the amount of precursors. With regard to environmental samples, it is necessary to consider that the levels of precursor impurities will vary between past PCP production batches, as well as from weathering effects; e.g., formation of the predioxins from polychlorinated diphenylethers undergoing oxidation in the environment. The ITEQ from duplicate analyses of a PCP-contaminated soil (pH~6; PCP~300 ppb) extracted with acetone-hexane was found 23 percent higher relative to not using acetone; i.e., from 454 ppt (RPD 1%) to 557 ppt (RPD 4%). On average, PCDDs increase 30 percent whereas PCDFs increase by 4 percent in this particular sample. Furthermore, a 10-fold increase in concentration is observed for 1,2,7,8-TCDD. The concentration for 1,2,3,7,8-PeCDD increases 66 percent [24 ppt (RPD 8%) to 41 ppt (RPD 4%)] while the 1,2,3,7,9-PeCDD isomer increases 300 percent. OCDD concentration increases by 39 percent (RPD 1-2%). Because of the artifact nature, the results for PCDDs are more erratic than for PCDFs. Finally, the data suggests that adjusting the pH (<2) with no acetone involved in the extraction results in the formation of 1,2,3,4-TCDD and/or 1,2,6,9-TCDD (coeluting pair).

The Peter Paradox. Two laboratories[5] followed the same acetone-toluene pH not-adjusted extraction procedures, made use of the same—but different from other participants—supplier of acetone, and did not experience the formation of PCDDs. Questions about the possible presence of preservatives (i.e., substances that compete for the enol carbon-carbon double bond or act as inhibitors) in the acetone from one supplier and/or other differences in the types of materials used to condition the sample before the extraction (e.g., acidic Celite), or a different mechanism than the one postulated need to be considered in order to resolve this paradox. In contrast, traces of basic substances (e.g., amines) possibly present in the solvent can facilitate the elimination of HCl. In some ways, this is reminiscent of the suitability of HPLC grade solvent for pesticide analyses. HPLC-grade solvent may contain impurities transparent to UV detection and are detectable by GC/MS. If the quality of the solvent plays a role, then when we define the suitability of our supplies, including specifying that our solvents are pesticide- or distilled-in-glass grade, we tend to focus on the absence of substances that contribute to our background. Stating that the solvent used is freed of dioxins and dioxin-like compounds is obviously a necessary but not a sufficient condition. An analytical protocol can be very detailed, and can still miss issues such as the ones discussed herein. What if the SRM/PE sample certified or verified concentrations were derived following a flawed methodology? It demonstrates how vigilant we must remain at all times if our goal is to achieve data reliability. Round-robin studies provide valuable feedback especially when they challenge our ways of doing and thinking. Our methods will greatly benefit from these studies if we recognize past mistakes, adapt and move on.

Conclusions

Predioxin impurities in PCP-contaminated guar gum are by and large responsible for the formation of polychlorinated dibenzo-*p*-dioxins during the laboratory extraction step when acetone is used as a solvent or as part of a mixture of solvents. Even the use of a small amount of acetone during the spiking step can lead to a significant bias. The artifact is prevented when the pH of the sample is adjusted (pH<2) before the extraction step; a precaution that is consistent with the postulated mechanism (competition by the excess acid for the addition across the carbon-carbon double bond in the enol form of acetone). Thus, adjusting the pH before the extraction[6] of samples where PCP contamination is suspected seems reasonable. However, more work is required to validate this action. At this moment, it is preferable to refrain from using acetone. Freeman and Srinivasa[7] reported on the role of acetone during the irradiation of the 3,4,5,6-tetrachloro-2-(pentachlorophenoxy)phenol isomer at 300 nm. When the irradiation takes place in cyclohexane, no dioxins are formed whereas, in the presence of acetone, the irradiation leads to the formation of dioxins and one furan. The authors observed that higher yields of OCDD are achieved when a tertiary amine is added to the acetone. This latter observation is consistent with the mechanism postulated in this paper (i.e., the amine is an excellent receptor for HCl). Finally, and unless we are more interested in being precisely wrong than accurate[8], it is essential that laboratories participating in the round-robin studies be allowed to follow their own methodology. Otherwise, we could easily end up with the participating laboratories following the same wrong procedure leading to a significant bias of the analytical results for years to come. The value of round-robin studies in perfecting our analytical methodologies is immense. The guar gum illustration is a classic story showing how method's performance and our understanding of intricate ultratrace analyses can be shaped if we just take the time to listen to the feedback offered by the study's whole data set, and our willingness and wisdom to maintain some flexibility.

Acknowledgments

The authors wish to thank the Community Reference Laboratory for Dioxins and PCBs in Feed and Food organizers.

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