

CHLORINATED DIOXINS AND FURANS FROM KELP AND COPPER SULFATE: INITIAL INVESTIGATIONS OF DIOXIN FORMATION IN MINERAL FEED SUPPLEMENTS

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Introduction

In 2002, dioxins were discovered in animal feed ingredients during a random sampling by Irish officials and subsequently traced to particular mineral supplements produced at a Minnesota plant in the United States. These products sold under the names of SQM Mineral Products (Sea-Questra-Mineral) and Carbosan Mineral Products provide trace minerals complexed to polysaccharides for delivery of trace minerals.¹ The products were voluntarily recalled by the company until the source of the dioxins could be identified and the dioxins eliminated from the supplements. Preliminary investigations by the company and federal agencies indicated that the dioxins were apparently produced during the manufacturing process of supplements containing copper, zinc, manganese, magnesium and iron. An initial examination of the ingredients and the process for the production of the SQM copper product failed to identify any specific ingredient or provide an apparent explanation as to the source or particular process responsible for their presence. In an attempt to identify the particular ingredient or process responsible, the USEPA obtained the ingredients and the details of the production process from the manufacturer for the copper product. The ingredients were analyzed and found to be free of dioxins/furans (D/Fs) contamination at sub parts-per trillion levels while a sample of the product itself contained D/Fs at approximately 1300 ppt TEQ. The production process was simulated in the laboratory and successfully produced the D/Fs at similar concentrations with virtually an identical isomer distribution as that found in the manufactured product. Additional studies were initiated to identify the specific ingredients required for dioxin formation and to provide further insight into the conditions necessary for their production.

Materials and Methods

The ingredients used in the production of the SQM Copper were: feed grade kelp (the giant brown kelp, *Macrocystis pyrifera*), copper sulfate, dextrose monohydrate, Zeosyl (silicone dioxide), white mineral oil and water. The water used in all experiments described herein was Fisher Optima. The individual ingredients and a sample of the manufacturers' product were processed and analyzed for the presence of dioxins and furans. Initially 25-50 grams (g) samples of the individual ingredients were processed and analyzed. All the samples that were free flowing were pulverized with a mortar and pestle, mixed with sodium sulfate, fortified with 16, C13 labeled 2,3,7,8-pcdd/f recovery surrogates and extracted with benzene for 24 hours using soxhlets. Six g of the mineral oil was fortified with recovery surrogates and all the samples were cleaned up with acid/base silica, alumina and carbon column chromatography. All sample sets included a method blank and a fortified control consisting of the lab prepared product dried at room temperature.

Samples were analyzed by high resolution gas chromatography/ high resolution mass spectrometry on a Kratos Concept employing a 60 meter by 0.32 μ m fused silica DB-5ms column from J&W Scientific. Details of the analytical methods, acquisition parameters and QA/QC guidelines are similar to those described in EPA's method 1613 and Ferrario et.al.²

Specific details about the manufacturers procedure and the exact formulations are not described due to their proprietary nature. In order to simulate the production process, the ingredients were combined according to the procedure and then dried at 50° C until all visible water had evaporated. Individual sub-samples of the mixture were then heated to various temperatures while being supplied with a stream of purified compressed air until completely dry and "crumbly" or, for samples that were heated above 275° C, until the first visible signs of smoke appeared. The samples were mixed approximately every two minutes. The temperature was measured using a Thermolyne Pyrometer with both an immersion and surface probe. One probe was inserted directly into the mixture and the other was applied to the surface of the hotplate. The original experiments were conducted in a small Blue M oven but due to residual contamination the oven was replaced with a hot plate. Moreover, the soxhlets were also abandoned in favor of an extraction employing stirring with benzene and using all disposable glassware. The results were comparable to those employing the more conventional procedures.

Results and Discussion

Before experiments were initiated to simulate the process, all the ingredients listed in the Methods section were analyzed and found to be free of dioxin and furan contamination at detection limits of 0.08 ppt, tetras: 0.4 ppt, penta- through heptafurans: 1.0 ppt heptadioxin and OCDF and 1.6 ppt OCDD. The sample of SQM copper obtained from the manufacturer contained D/Fs at 1308 ppt WHO-TEQ. (Table 1.). Initial experiments to determine the effectiveness of the system in producing D/Fs from the mixture resulted in the formation of D/Fs at TEQs ranging from approximately 150 to over 8000 ppt TEQ and D/F ratios of 0.39 to 0.59. The isomer distribution is quite similar to those from other combustion sources, with all the isomers from tetra- through octa represented. This variability in TEQ reflects the lack of control of essential reaction conditions in the experimental system and our inability to precisely control the temperature and define reaction endpoints. Nonetheless, the similarity in the congener profile and isomer distribution confirms the systems utility in simulating the production process (Table 1.) and provides the means to explore the process and conditions responsible for D/F formation.

Experiments to determine the temperature required for D/F formation were conducted by heating the kelp/mineral mixture as described above at various temperatures (50, 100, 130, 175, 200, 260, and 425° C). It was our original intention to have samples heated to 300 and 350° C but were unable to control the temperature once the water had completely evaporated and the mixture was dry. Small amounts of smoke immediately preceded and/or accompanied a spike in temperature from about 275 to 425° C during the time it took to remove the flask from the hotplate. All samples that reached these temperatures and emitted any smoke were found to contain significant levels of D/Fs.

Experiments were then conducted to determine if a single ingredient or all the ingredients used by the manufacturer to produce these SQM products were required for the formation of the D/Fs. The results conclusively demonstrated that the kelp alone was insufficient to generate the D/Fs but the kelp in combination with copper sulfate produced D/Fs at similar concentrations with a similar

isomer distribution as that seen in the commercial product and the products produced in the lab using all the ingredients (Table 1). All further experiments were then conducted with only a mixture of approximately equal weights of kelp, copper sulfate and water. Additional experiments to determine the affect of varying amounts of copper sulfate (0.5, 2.0, 5.0 x usual amounts) were inconclusive due to the lack of precision in the production of the D/Fs in replicate control samples using the usual proportions of kelp and copper sulfate. D/F concentrations in the experimental groups fell within the range of concentrations defined by the control samples.

In an attempt to isolate and identify possible starting materials, 50 g of kelp was then extracted three times with 100 ml water and the aqueous extracts combined and saved. The kelp was then air dried, mixed with copper sulfate and heated as described previously. A sample of unextracted kelp and copper sulfate was also heated to serve as a positive control. The sample containing the kelp that had been extracted with water produced only trace amounts of D/Fs (less than 1.0 ppt TEQ) while the positive control produced D/Fs at the expected elevated levels. The aqueous extract was then split and an aliquot representing 25 g of kelp was concentrated to less than 5 ml and the residue (3.6 g after drying) was added to a quartz fiber filter, mixed with equal amounts of copper sulfate and heated. The results (Table 1) show that the D/Fs were produced in similar amounts and with a congener profile consistent with those seen both in the commercial SQM product and the lab processed materials. Moreover, this result strongly indicates that both the chlorine source, most probably sodium chloride, and the carbon source are both water soluble components of the dried kelp.

An important commercial product derived from the giant brown kelp, *Macrocystis pyrifera*, is the phycocolloid algin and its derivatives. This hydrophilic colloid is used as emulsifying, stabilizing and suspending agents in a variety of products like ice cream, syrups, salad dressings, tooth paste, lotions, paints, polishes, etc. and is commercially available as alginic acid.³ To explore the possibility that this component of the kelp was acting as a carbon source, a sample of alginic acid was purchased from Aldrich, 10 g mixed with an equal amount of copper sulfate and water, and the mixture air dried. In addition, 1 g of sodium chloride was dissolved in water and added to another 5 g subsample of the alginic acid/ copper sulfate mixture. Both mixtures were then heated as described above. The results showed that only trace amounts of D/Fs were formed in the alginic acid/ copper sulfate mixture but that substantial quantities (330 ppt TEQ) were formed in the mixture that contained the sodium chloride. These results suggest that alginic acid, a known component of kelp, is probably one of the carbon sources for the formation of D/Fs in the SQM products and that the sodium chloride associated with the dried kelp is most likely the chlorine source.

These findings are consistent with those reported by Yasuhara, et. al.⁴ who investigated the role of inorganic chloride and lignin content in dioxin formation and found that the amounts of D/Fs produced were correlated to the amount of sodium chloride and lignin present in the starting materials by using combusted NaCl impregnated newspapers.

Besides providing a relatively simple model system to explore the identity and chemical nature of starting materials, the role of inorganic chlorides, and the reaction conditions responsible for dioxin formation, these experiments also provide an interesting example of the potential to reduce and/or eliminate dioxins in finished commercial products through the effective control of reaction

conditions or the use of alternate starting materials. Discussions with researchers at the production facility have confirmed that measures taken to control the temperature and maintain sufficient water content in the kelp/copper sulfate mixture eliminates the formation of dioxins. The substitution of purified carbohydrates for the kelp, lowering drying temperatures, appropriate equipment cleanout procedures and an ongoing QA program have all been implemented by the affected company to ensure that their products are free from dioxin contamination.

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Table 1. Concentration of D/Fs in Commercial and Experimental Products (pg/g:wet weight)

Description of Sample	SQM Copper	Exp Prdt	Kelp+CuS04	Kelp Extract	Alginic Acid/NaCl
PCDDs					
2,3,7,8-TCDD	15.3	96.0	2.52	10.9	82.3
1,2,3,7,8-PeD	176	557	57.1	208	207
1,2,3,4,7,8-HxD	214	600	132	1080	100
1,2,3,6,7,8-HxD	313	1160	219	1630	122
1,2,3,7,8,9-HxD	211	906	151	1050	121
1,2,3,4,6,7,8-HpD	3140	16700	4810	24500	632
OCDD	9060	52300	28300	72200	798
PCDFs					
2,3,7,8-TCDF	53.8	500	36.7	63.1	484
1,2,3,7,8-PeF	434	3440	283	701	666
2,3,4,7,8-PeF	591	4130	491	900	626
1,2,3,4,7,8-HxF	1750	12200	3500	11200	659
1,2,3,6,7,8-HxF	1470	11900	2850	8360	642
1,2,3,7,8,9-HxF	776	6270	2030	4520	150
2,3,4,6,7,8-HxF	1560	11900	3640	7180	449
1,2,3,4,6,7,8-HpF	11200	39100	3100	75600	1340
1,2,3,4,7,8,9-HpF	1610	12700	5520	9650	175
OCDF	13300	97500	69800	69400	523
TEQ					
PCDD	297	1090	161	846	330
PCDF	1010	7040	1840	4470	600
TOTAL	1300	8130	2000	5320	930