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Dioxins contamination in the feed additive (feed grade cupric sulfate) tied to chlorine industry

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The sources of polychlorinated dibenzo-p-dioxins/dibenzofurans (PCDD/Fs) found in animal feed additive (feed grade cupric sulfate, CuSO₄) were investigated and traced back to the formation of chlorinated organic compounds in the chlor-alkali industry. PCDD/Fs could be transported through the supply chain: hydrochloric acid (HCl) by-produced during formation of chlorinated organic compounds in chlor-alkali industry → spent acid etching solution (acid-SES) generated in printed circuit board production → industrial cupric salt → CuSO₄ in animal feed, and finally enter the food chain. The concentration ranges in HCl and acid-SES were similar, of which the level in acid-SES was also consistent with that in various cupric salt products including CuSO₄ based on Cu element content. PCDD/Fs also showed very similar congener profiles in all the sample types. This indicates a probable direct transport pathway of PCDD/Fs into the food chain, which may eventually be exposed to humans through consumption. To date this is the first study in China that systematically reports on the PCDD/Fs transport from industrial pollution sources to industrial processes and finally enters the human food chain.

Dioxins (polychlorinated dibenzo-p-dioxins/dibenzofurans, PCDD/Fs) are considered byproducts of numerous industrial activities and combustion processes¹, and classified as unintentionally produced persistent organic pollutants (UP-POPs). Dioxin emissions lead directly to the contamination of the environment and pose a threat to human health. Most dioxin exposure to humans derives from food (>90%)² and they can bioaccumulate and biomagnify through the food chain. Dioxin contamination episodes involving food and feed have raised great public concern around the world^{3–6}. Generally they could be traced back to contaminated raw materials, resulting in discontinued production and elimination of contaminated batches at great cost for relevant industries. However, in-depth investigation on the potential sources of dioxins in some contaminated products is still limited.

As one of the most important mineral supplements, feed grade cupric sulfate (CuSO₄) is widely employed for animal nutrition after removing hydrate molecules from industrial products and by purification. It is estimated that approximately 27000 tonnes of feed grade CuSO₄ were produced as feed additive in China in 2013⁷, and most of them were added into pig feeds (unpublished data). Thereby the quality of feed grade CuSO₄ could be closely associated with animal and human health. Industrial CuSO₄ is traditionally produced from copper slag or ore resources. However, in the last two decades, the technology using recovered copper to produce cupric salts including CuSO₄ has been widely adopted by many manufacturers. For example, environmental protection enterprises use spent etching solutions (SES) generated from printed circuit board production for CuSO₄ production^{8,9}, which is considered to be more cost-effective and environmentally friendly compared with the traditionally methods. Although the annual amount of CuSO₄ produced by this method is not available, it could be conjectured that a large amount could be produced along with the rapid development of the recycling market in China.

In general, impurities, such as lead, iron, arsenic and tin, are of great concern in these cupric salts including CuSO₄. Few reports are available concerning the presence of organic pollutants in this product, especially regarding PCDD/Fs. A recent report¹⁰ showed that high levels of PCDD/Fs were observed in spent acid etching solutions (acid-SES) and their downstream products (e.g., industrial, plating grade and feed grade CuSO₄), which indicated dioxins contamination in these industrial products and could further lead to high increased exposure to

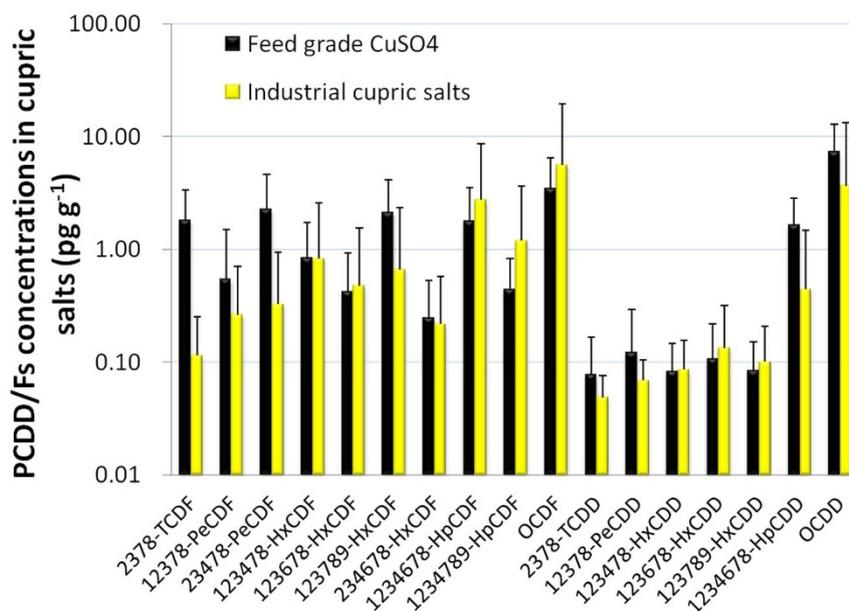


Figure 1 | The concentrations (mean + S.D.) and congener profiles of PCDD/Fs in the feed grade CuSO_4 and industrial cupric salt samples ($n=26$).

livestock and humans due to their use in agriculture. These suggested that CuSO_4 could be of significant concern in the transport of dioxins contamination to the food chain.

Here the sources of PCDD/Fs found in this animal feed additive (feed grade CuSO_4) were systematically investigated and traced back to the formation of chlorinated organic compounds in the chlor-alkali industry. To date this is the first study in China reporting the PCDD/Fs transport from industrial pollution sources to the industrial processes and finally were delivered to the human food chain, which could lead to increased health risk through consumption.

Results

PCDD/Fs investigation was performed for seventeen 2,3,7,8-substituted congeners. The feed grade CuSO_4 samples were collected from different manufacturers ($n=14$) and the results showed that PCDD/Fs concentrations were in the range of 8.58–41.2 pg g^{-1} , corresponding to 0.35–3.92 $\text{pg WHO-TEQ}_{2005} \text{g}^{-1}$ (Table S1). More than a half of the values exceeded the maximum levels established by European Commission (1 pg WHO-TEQ g^{-1})¹¹ if these CuSO_4 were to be served as feed additive. The isomer profiles showed that

octachlorodibenzo-p-dioxin (OCDD) and octachlorodibenzofuran (OCDF) were the predominant congeners, and PCDFs were commonly more abundant in PCDD/Fs (Fig. 1).

These feed grade products were generally obtained from industrial products via hot-air drying at 200–300°C. Therefore, industrial CuSO_4 were sampled from some plants as well as the other two cupric salt products including cupric chloride (CuCl_2) and tribasic copper chloride ($\text{Cu}_2(\text{OH})_3\text{Cl}$). The results showed that comparable levels and isomer profiles of PCDD/Fs were observed in various cupric salts (Fig. 1, Table S1), which were also consistent with those reported by Qing et al.¹⁰, indicating the ubiquity and similar origin of PCDD/Fs in the cupric salts.

These cupric salts including CuSO_4 were produced from SES, which is a waste solution from the chemical etching of printed circuit boards in the electronic industry. SES is grouped into three different groups: acid, alkaline and micro-etch solutions. Samples from these different groups of SES were therefore collected for PCDD/Fs analysis. The results showed that the pollutants could only be detected in the acid-SES samples and the concentrations were in the range of 0.06–687 pg mL^{-1} (Table S2), where approximately a quarter of the values were higher than 10 pg mL^{-1} . The congener profiles exhibited

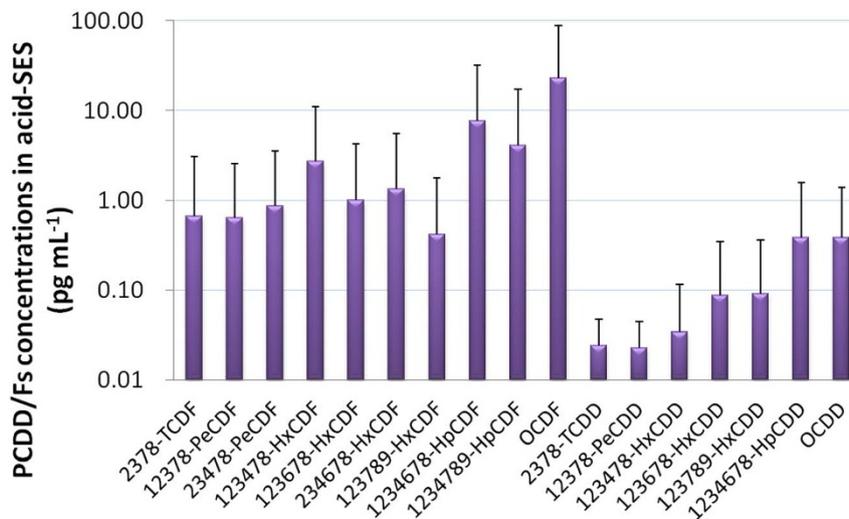


Figure 2 | The concentrations (mean + S.D.) and congener profiles of PCDD/Fs in the acid-SES samples ($n=34$).

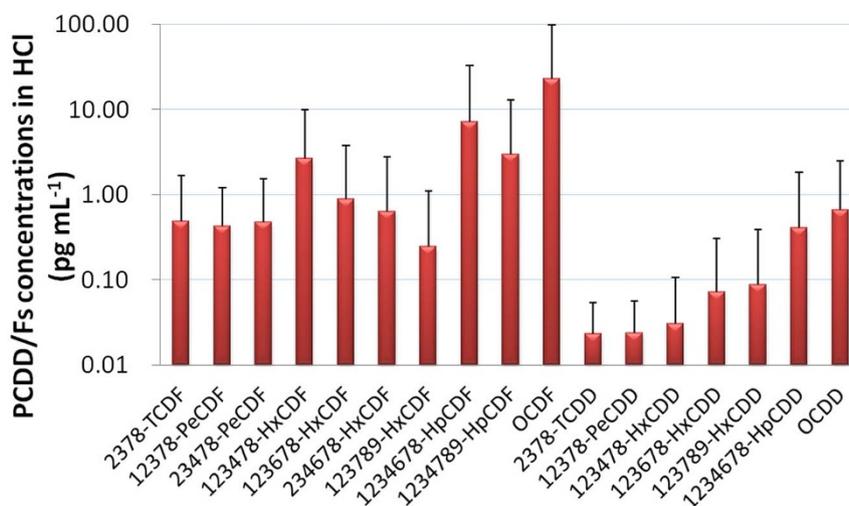


Figure 3 | The concentrations (mean + S.D.) and congener profiles of PCDD/Fs in the HCl samples (n=17).

comparable patterns with those in the downstream products of cupric salts (Fig. 2) and significant correlations (performed by SPSS 13.0) were observed between the mean concentrations in the acid-SES and cupric salts (Table S3). Considering that industrial sulfuric acid (H_2SO_4) was used in $CuSO_4$ production after neutralization of acid-SES and alkaline-SES, H_2SO_4 samples were also collected, but PCDD/Fs were only detected at a very low level (Table S2). These results suggested that PCDD/Fs in $CuSO_4$ are closely associated with the acid-SES.

Further investigation was conducted on the chemical etching process of printed circuit boards. Acid etchants containing copper chloride ($CuCl_2$) are extensively used, e.g., a cupric chloride-based hydrochloric acid solution comprising of water, hydrochloric acid (HCl), $CuCl_2$ and oxidizers⁹. HCl and oxidizers (chlorate) were then sampled and the analytical results showed extremely low PCDD/Fs concentrations in the oxidizers (Table S4). However, high levels of the pollutants were found in the HCl samples (range 0.52–540 $pg\ mL^{-1}$) (Tables S4), and distribution patterns were consistent with those observed in the acid-SES (Fig. 3). This implied that PCDD/Fs might be transferred from HCl to the downstream products. Previous study¹⁰ argued that PCDD/Fs could be generated when the mixture of HCl and oxidizer (chlorate) is in contact with printing ink and laminate during acid etching processes, while no direct proof was given. In order to test our hypothesis that PCDD/Fs are transported from HCl to acid-SES, we supplied one tonne of HCl with no detectable levels of PCDD/Fs to a printed circuit board manufacturer and the acid-SES samples were collected in the following 8 days. As expected, the PCDD/Fs level was significantly reduced by 90% in 8 days (Table S2, Fig. 4 and S1), while the congener distribution showed consistent profiles in the acid-SES after HCl replacement (Fig. S1), which indicated that HCl solution was the main factor affecting the dioxin levels in acid-SES rather than the acid etching processes.

HCl is mostly made by absorbing hydrogen chloride in the chlor-alkali industry, which includes two synthesis approaches: 1) direct combination with chlorine (Cl_2) and hydrogen (H_2); 2) by-produced during formation of chlorinated and fluorinated organic compounds, e.g., Teflon, chloroacetic acid and polyvinyl chloride (PVC). Investigation indicated that the HCl used for acid etching processes were mainly from by-production during synthesis of chlorinated organic compounds, which contains more impurities and has lower purity. In this study, both types of HCl were collected from different manufacturing plants for PCDD/Fs determination. The results showed that they were not detected in the HCl produced by direct synthesis (Table S4). However, some amounts could be

measured in those by-produced HCl with an average concentration of $8.95\ pg\ mL^{-1}$, and the range of levels are consistent with those in the corresponding HCl used for chemical etches, as well as the congener profiles (Fig. 3, Table S4).

Discussion

Dioxin emissions from the chlor-alkali industry has been reported in the last two decades^{12,13}. The electrode sludge from chlorine production contains high levels of PCDFs (“chlorine pattern”), which was assumed to be related to the chlor-alkali process utilizing graphite electrodes^{12–14}. Moreover, studies on Chinese chlor-alkali sites also indicated high levels of PCDD/Fs where graphite anode was used for electrolysis^{15,16}. Chlorination of cyclic aromatic hydrocarbons (such as dibenzofuran) present in the coal tar used as a binding agent in the graphite electrodes has been proposed as the primary PCDD/Fs source¹⁷. However, the diaphragm and mercury cell processes with graphite anodes have been superseded by newer membrane cell process using activated titanium anodes since 2004 in China. Furthermore, results from the HCl produced by direct synthesis also suggested that PCDD/Fs were neither generated during chemical reaction between H_2 and Cl_2 , nor carried over by Cl_2 from the chlor-alkali process. However, it has been recognized that PCDD/Fs could be formed during the manufacture of PVC and PVC intermediates such as ethylene dichloride (EDC) and vinyl chloride monomer (VCM)¹⁸. Various cyclic aromatic hydrocarbons contained in pet-

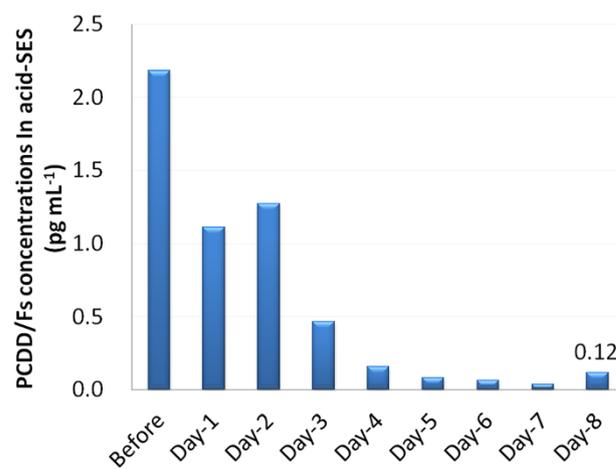


Figure 4 | The PCDD/Fs concentrations in the acid-SES samples collected for verification experiment with HCl replacement.

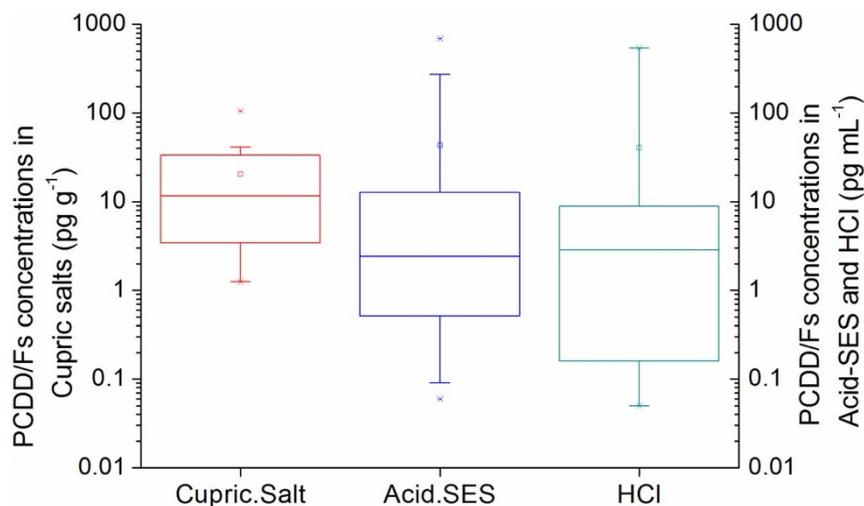


Figure 5 | The overall concentrations of PCDD/Fs in the cupric salt (n=26), acid-SES (n=34) and by-produced HCl (n=17) samples.

roleum could be preserved in petroleum products (e.g., fuel oil, paraffins, asphaltum, etc.) and then be chlorinated during the manufacture of petrochemicals in the chlor-alkali industry. This could be supported by our investigation on PCDD/Fs in the HCl by-produced during chlorinated paraffins (CPs) production and formation of other chlorinated organic compounds (Table S4). Therefore, PCDD/Fs in HCl were closely correlated with the chlorination of petroleum products containing cyclic aromatic hydrocarbons. Further study is necessary to confirm the generation of PCDD/Fs during this chemical process.

Regarding the PCDD/Fs levels along this production chain, it could be seen that the PCDD/F concentrations in the acid-SES were comparable to or even lower than those in the HCl (Fig. 5). This is probably because some other solutions (e.g., oxidizers) will also be introduced into the chemical etches process, which might dilute the contaminant levels in the waste (acid-SES). While in the cupric salts, it seems that the contamination level was approximately one order of magnitude higher than in the other two matrices. This could be mainly attributed to the fact that elemental Cu content was approximately 150 g L^{-1} in acid-SES, while it is 40% in CuSO_4 , 47% in CuCl_2 and 60% in $\text{Cu}_2(\text{OH})_3\text{Cl}$. The PCDD/Fs levels in the cupric salts were therefore comparable to those in the acid-SES based on elemental Cu, which suggested that PCDD/Fs might be transported without loss to the downstream products.

In conclusion, we have shown that PCDD/Fs most likely generated during the chlorination of petroleum products could be transported through the production chain: HCl by-produced during synthesis of chlorinated organic compounds \rightarrow acid-SES generated in printed circuit board production \rightarrow industrial cupric salt \rightarrow CuSO_4 in animal feed, and finally entering the human food chain. The dioxin distribution showed a very similar congener profile along the chain, e.g., PCDFs were commonly more abundant, and octa-chlorinated congeners (OCDD and OCDF) predominated among the PCDD/Fs. To date, this is the first study in China to systematically report that PCDD/Fs could be transported from industrial pollution source to downstream products. This sheds light on a potential transport pathway of industrial UP-POPs to the human food chain, which could increase the health risk to humans through consumption of livestock that are given the contaminated feed.

Methods

Sample Collection. A total of 112 samples including cupric salts (n=26), SESs (n=59), industrial H_2SO_4 (n=2), etchants including oxidizer (n=5) and HCl (n=20) were obtained from the plants in this study. Traceability of PCDD/Fs was processed along the production chain. Specifically, after determination of the pollutants in feed grade CuSO_4 , various cupric salts were collected from the plants (n=5) with higher

contamination levels for further investigation. Then one plant was chosen where the products (cupric salts) and raw materials (SES and H_2SO_4) were sampled for PCDD/Fs measurement. Thereafter, the plants which supplied these raw materials to produce cupric salt were investigated and the SES was collected including acid, alkaline and microetch solutions. Due to high levels of the pollutants in the acid-SES, a survey was carried out in seven typical printed circuit boards manufacturers, where the wastes (acid-SES) and raw materials (etchants including oxidizer and HCl) were sampled. Based on the analytical results, field samples (HCl) were collected from two HCl manufacturers. Meanwhile, HCl of different origins were obtained for further identification.

In the experiment to test the hypothesis that PCDD/Fs was transferred from HCl to acid-SES, the HCl (approximately 1 tonne) with no detectable PCDD/Fs was supplied to one printed circuit boards manufacturer, where high contaminations were observed in both HCl and acid-SES samples. Then the acid-SES samples were collected in the following 8 days for PCDD/Fs determination.

All the samples were sealed into polypropylene bottles and then transported back to the laboratory for analysis.

Analytical Method. For cupric salt samples including CuSO_4 , CuCl_2 and $\text{Cu}_2(\text{OH})_3\text{Cl}$, the extractions were performed on a Soxhlet system. Approximately 10.0 g cupric salt was weighed and extracted with 150 mL *n*-hexane/dichloromethane (DCM) (1 : 1, v/v) for 24 h. The surrogate standards (EPA 1613-LCS) were spiked prior to the extraction. After rotary evaporation, the extract was loaded to a multilayer silica column packed with 1 g silica gel, 4 g basic silica gel, 1 g silica gel, 8 g acid silica gel, 2 g silica gel and 2 cm anhydrous sodium sulfate from the bottom up, and eluted with 100 mL *n*-hexane. After concentration, the eluate was transferred to a Kuderna-Danish (K-D) concentrator for further concentration under a stream of nitrogen flow. Finally, it was transferred into GC vial with a liner and then concentrated into 20 μL nonane. Prior to instrumental analysis, the concentrate was spiked with the injection standards (EPA 1613-IS) for the recovery calculation of the surrogate standards.

For the liquid samples including SES, oxidizer and HCl, 100 mL sample was spiked with the surrogate standards and then extracted with DCM using liquid-liquid extraction (LLE). While for industrial H_2SO_4 , 50 mL sample was diluted with deionized water (100 mL) and then extracted with *n*-hexane using LLE. After removing moisture with anhydrous sodium sulfate, the extract was followed the same treatment process as mentioned previously and finally spiked with the injection standards.

PCDD/Fs were analyzed using high-resolution gas chromatography coupled with high-resolution mass spectrometry (HRGC/HRMS). The HRGC is an Agilent 6890N (Wilmington, USA) with CTC PAL autosampler, while the HRMS is an AutoSpec Ultima (Waters Micromass, UK) with electron impact (EI) ion source. The instrumental conditions were described in our previous publication¹⁹. Specification and quantification were performed for seventeen 2,3,7,8-substituted PCDD/Fs.

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Author contributions

P.W. jointly conceived the study with Q.H.Z., and prepared the manuscript; P.W., Y.H.L., S.A.X., R.F.G., G.L., H.D.Z., H.T.S. and X.M.L. collected the samples; P.W., D.W.R., C.F.Z. and Y.M.L. performed experiments and analyzed data; Q.H.Z. and G.B.J. gave technical support and conceptual advice.

Additional information

Supplementary information accompanies this paper at <http://www.nature.com/scientificreports>

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