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ORIGINAL ARTICLE

Evaluation of Pentachlorophenol Residues in Some Hygienic Papers Prepared from Virgin and Secondary Pulp by Electron Capture Gas Chromatographic Method

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KEYWORDS

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ABSTRACT: In this study, residual amount of pentachlorophenol (PCP) as the most important paper preservative, which is extremely hazardous pollutant, was determined in some tissue papers and napkins. Twenty-five samples of two producing hygienic paper factories prepared from virgin and secondary pulp were analyzed for the presence of trace amount of PCP. The analytical procedure involved direct extraction of PCP from hygienic paper and its determination by gas chromatography with electron capture detection. The statistical results for the analysis of all samples revealed that there were significant differences between mean of PCP in hygienic papers prepared from virgin and secondary pulp (P<0.05). This method gave recoveries of 86-98% for hygienic paper made from virgin pulp and 79-92% for hygienic paper made from secondary pulp. The limit of detection (LOD) and limit of quantification (LOQ) for PCP were 6.3 and 21.0 μg/kg, respectively. The analytical method has the requisite sensitivity, accuracy, precision and specificity to assay PCP in hygienic papers. This study demonstrates a concern with exposition to PCP considering that hygienic paper is largely consumed in the society.

INTRODUCTION

Chlorophenols have found predominant application in wood preservation and paper production. These compounds present a lipophilic character, which contributes to their bioaccumulation in the food chain [1].

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Pentachlorophenol (PCP) has been related as the most toxic compound among chlorophenols [2]. Due to its high volatility and solubility in water in its ionized form, there is a widespread contamination of PCP in the environment. Depending on the temperature and type of wood, up to 80% of PCP may evaporate from treated wood within 12 months [3]. Other impurities, found in PCP and their possible metabolic products, are also subject to investigation. Despite restrictions on the use of PCP since 2005-2006, this compound had wide spread application in preservation of wood and its derivatives. Residual amount of this pesticide could contaminate paper intended for hygienic paper constituting a public health risk.

PCP can not only give rise to acute toxicity but also cause immunological and endocrine disorders and infertility problems in human [4-6]. WHO has classified PCP as a possible carcinogenic agent to humans [3]. The use of this chemical has been banned or severity restricted by most countries. There is a compromising between European and German regulatory bodies about the limit of PCP value for waste wood to be recycled and it was set at 5 mg/Kg as dry substances [7]. The US and European Union legislations regulated the maximum levels of phenolic compounds in drinking water as 1.0 and 0.5 µg/L respectively [8]. PCP was also widely used in the form of its sodium salt (Na-PCP) as wood preservative to control mould and insects that causes sap stain and deterioration of lignocellulosic substrata [9]. The elevated environmental persistence of PCP and the increased demand for utilization of recyclable paper materials in food packaging give rise to concerns about the presence of this chemical as a potential contaminant from paperboard to food.

Due to necessity of water quality control, previously we described a preliminary investigation on environmental waters in our region [10]. A brief study on the published literatures revealed that among the various methods developed for PCP analysis in different matrices, the majority are focused on its determination in water resources [11-12] and food packaging materials [13]. Some methods for PCP determination in honey [14-15] gelatin samples [16] have been also described. There are also many reports on the release and control of chlorophenols in landfills [17], wastewaters [18] and drinking water [19]. Previous methods often involve acidification of the sample to convert PCP to its non-ionized form, extraction with an organic solvent, cleaning into an alkaline solution, and determination by gas chromatography or other chromatographic methods. These methods are usually costly, time-consuming, and have an expensive sample clean, besides a low sample throughput.

All of the pollutants in the environment have a potential of exposure to the human and causing health risk. These compounds must be monitored in the environment. Their routes should be followed up to the target products. We have previously reported some analytical methods for monitoring many pollutants and toxins in the environment and final products [20-24].

In this study, we aimed to evaluate the incidence of PCP in some hygienic paper samples prepared from virgin and secondary pulp commercially available in Iran by a validated gas chromatographic analytical method with a simple sample treatment.

MATERIALS AND METHODS

Reagents

Tissue paper and napkin samples were purchased from a range of commercial retail markets in Tehran City with domestic origins during 2011 and 2012 before expired and transferred to Reference Laboratories of Food and Drug Control for analysis. All solutions were prepared in double-distilled deionized water; using analytical grade reagents. Acetic anhydride, potassium hydroxide, sodium carbonate and sulfuric acid were supplied from Merck Int. Acetone, hexane and isopropanol were of pesticide residue grade from Merck Int. Analytical reference standard of pentachlorophenol was from Dr. Eh-

renstorfer GmbH (Augsburg, Germany).Working solutions were prepared in hexane, making appropriate dilutions to give standard solutions ranging from 0.02 to 12.5 mg/kg.

Sample Preparation

Sample preparations were based on direct extraction and derivatization with acetyl group in a solution of sodium carbonate in the presence of acetic anhydride and hexane. Twenty-five samples from two producing hygienic paper factories were analyzed. For this purpose, exactly 10 gr of each sample used to determine the presence of residual amount of PCP. From this representative sample, pieces were cut (1×1 cm) and exactly 5 gr weighed into 250 ml test tubes. After extraction and derivatization with organic solvent, the hexane layer was analyzed without further purification. In practice, small strips (0.5 gr) of cardboard samples were added to 20 ml of 0.1 mol/L Na_2CO_3 and mixed for 1 min. Then, 5 ml hexane and 2 ml acetic anhydride were added to the tubes, which were sealed and shaken for 30 min. After appropriate incubation, these tubes were allowed for phase separation, and the hexane layer was transferred to a volumetric flask of 10 ml. A blank reagent was also prepared using the same procedure. For preparation of standards, PCP solutions in hexane up to 5.0 ml at concentrations of 0.02, 0.1, 0.5, 2.5, 5.0, 12.5 mg/kg were mixed with 20 ml of 0.1 mol/L Na_2CO_3 and 2 ml acetic anhydride. After 30 min agitation, the tubes were allowed to phase separation and the hexane layer was removed for calibration curve in GC analysis (Figure 1) [7].

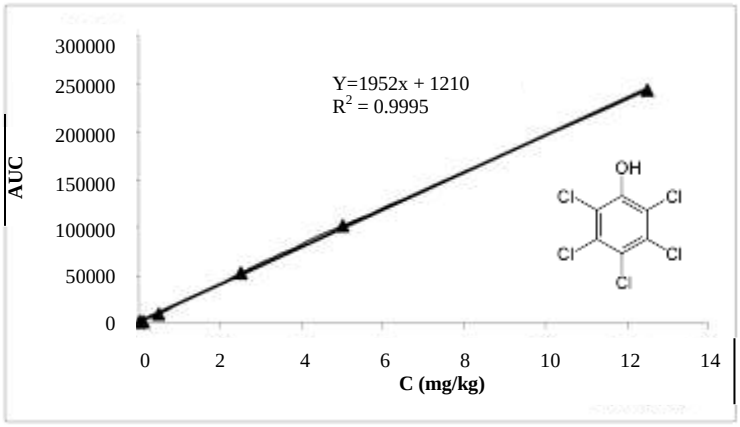

Figure 1. Calibration curve obtained for PCP

Gas Chromatography analysis

A gas chromatography system (Varian Analytical Instruments, Australia) equipped with a ^{63}Ni electron capture detector (Varian Star Model 3600) and STAR workstation software was used to determine PCP in hygienic paper samples. The separation was performed on a 12 m × 0.20 mm ID fused silica capillary SPB-5 column with a film thickness of 0.33 μm supplied by Supelco. Injections in splitless mode were made with an injection volume of 0.5 μL. The carrier was ECD grade hydrogen gas and the makeup was ECD grade nitrogen gas. The column oven temperature as well as the injector and detector temperatures and some other parameters can be seen in Table 1 [12].

Table 1. Gas chromatographic conditions for determination of PCP in hygienic papers

Column	SPB-5, 12 m × 0.20 mm i.d. (0.33 μm film thickness)
Carrier gas	Hydrogen, 1.0 ml/min
Makeup gas	Nitrogen, 1.0 ml/min
Injection mode	Splitless
Oven temperature program	100°C (2 min), with 10 °C/min to 250 °C (2 min)
Injector temperature	250 °C
Detector temperature	300 °C

RESULTS AND DISCUSSION

From all twenty-five hygienic papers (napkins and tissue papers) investigated for presence of PCP, only one sample was positive for PCP residues at concentration above the international permissible limit. The GC chromatogram of PCP for hygienic papers made from recycled paper and a typical GC chromatogram for its spiked PCP standard solution have been shown in Figure 2. The area under the curve (AUC) for PCP peak in this chromatogram was used for calculation of PCP concentration in the sample via calibration curve in Figure 1. Based on this calculation the assigned value for AUC was replaced as Y in calibration curve equation (Y=19526X + 1210). The obtained results revealed only a positive PCP test for recycled papers in concentration more than 0.5 mg/kg. Although all other samples were less than the PCP value compromised by European and German regulatory bodies for waste wood to be recycled but considering its potential for migration, it must be noted that any presence of PCP in food is forbidden and there is no maximum permissible limit for that.

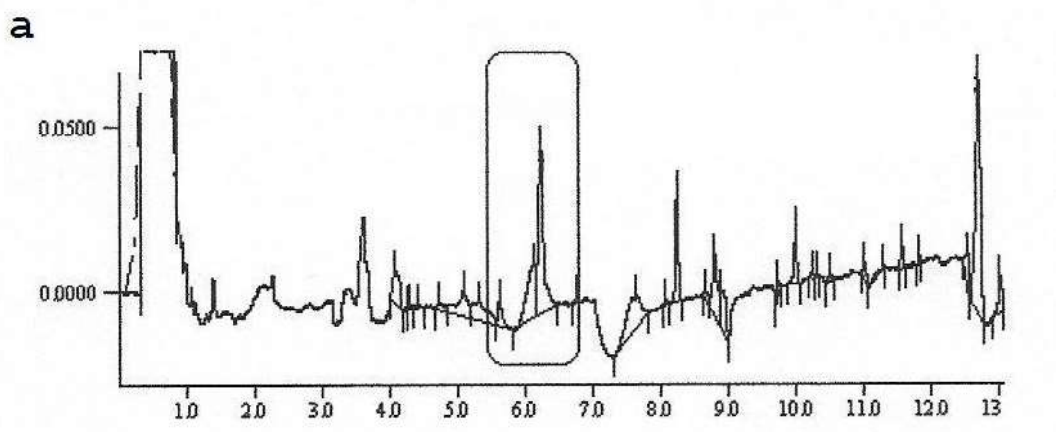

Figure 2. GC-ECD chromatograms of PCP for (a) A recycled hygienic paper spiked with 2 mg/kg PCP,

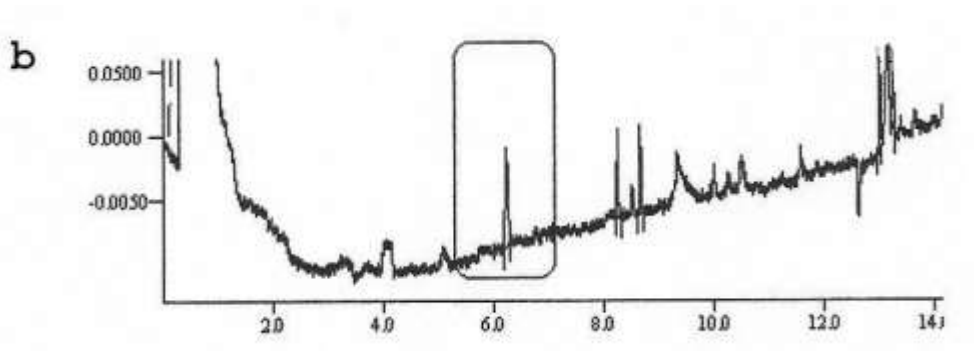

Figure 2. Continued. (b) A recycled hygienic paper which was positive for PCP

Although PCP residue was not positive in 96% in total of analyzed hygienic recycled paper samples in comparison with standard legislations, but for many years PCP had been a major selected fungicide agent of paper pulp, which is raw material of food packaging. While the mean concentration of PCP in virgin pulp was 0.089 mg/kg, this value was significantly higher in secondary pulp with a mean concentration of 0.401 mg/kg. The statistical results for the analysis of these samples revealed that there were significant differences between mean of PCP in hygienic papers prepared from virgin and secondary pulp (P<0.05). Since the prohibition of PCP use for preservation of wood and cellulose pulp, recently occurred in Brazil, from June 2006, still can have doubts on the presence of PCP in cardboard destined to foods. Based on the recent legislations, PCP does not have to be detected in levels higher than 0.15 mg/kg in the cardboard packaging material and has made special attention to ensure the necessary levels of control of any substances that might be transferred to the food in contact with the paperboard [25]. There is a potential health hazard for detection of PCP in packaging so the migration of PC from paperboard packaging into the drinking water and some foodstuffs has been demonstrated [26]. PCP is not detected in virgin paper products but it was detected in recycled paper packaging in the range of 0.05 to 0.08 mg/gr [27]. A general evaluation relative to extraction methods employed indicates that specific extraction step would be used for each sample group i.e. gelatin and packaging.

The analytical method used in this research was very simple and straightforward. It involves acidification of the sample to convert PCP to its non-ionized form, followed by extraction into an organic solvent. So this method can be easily employed in food packaging quality control laboratories. The other important note is that PCP is a hydrophobic ionizable organic compound and its distribution strongly depends on the pH of the aqueous phase as well as the ionic strength. Experimental results in this research were also revealed that in sample treatment pH dependence is only reflecting in the aqueous phase above 7.0. Under pH 3.0 the fraction of neutral species is almost 100%, while above 7.0 the anionic PCP is predominant [28]. Poor sample treatment leads to low resolution in separation of peaks in the chromatogram and this is a potential source of interference. In the literature analytical interferences was reported that might become a problem in PCP analysis for residues particularly at low measurement levels. As it was used in this research, in some other papers derivatization of PCP with appropriate compounds has been reported to be effective to reduce peak tailing and increase sensitivity. This was performed by derivatization with acetyl group and this allows the chromatographic separation of the acetylated chlorophenols with symmetric peak shapes [29-32].

The linearity of the test method was evaluated by linear regression analysis, which calculated by the least square regression method [33]. The calibration curve constructed for PCP was linear over the concentration range of 21-12500 µg/kg. The areas under the curve (AUC) for PCP were plotted versus their concentration and linear regression analysis performed on the obtained curve. A correlation coefficient of 0.9995 with %R.S.D. Values ranging from 0.77 – 3.93% across the concentration range studied were obtained. Typically, the regression equation for the calibration curve was y = (19526 ± 7X) + (1210 ± 11). The calibration graph that obtained for the determination of PCP has been shown in Figure 1.

The limits of detection (LOD) and quantification (LOQ) were defined as 3 and 10 times the value of noise, respectively [30]. The standard deviation of the estimated concentration values of the lowest calibration point was used as a measure of the noise. The LOQ was 21.0 µg/kg with a resultant %R.S.D. of 3.73 (n=3). The LOD was 6.3 µg/kg.

To assess the recoveries, a known amount of PCP standard solution was added to samples of virgin and secondary pulp, which was then extracted, diluted and analyzed. Recovery of PCP during method development was evaluated by fortifying separate control hygienic paper made from virgin and secondary pulp samples in triplicate at 0.021 mg/kg (1* LOQ), 0.105 mg/kg (5*LOQ) and 0.210 mg/kg (10*LOQ). Recoveries of PCP from hygienic paper made from virgin and secondary pulp spiked at various concentrations has been shown in Table 2. This assay was repeated for three times over 3 consecutive days to obtain intermediate precision data. The resultant %R.S.D. for this study was 3.3% with corresponding percentage mean recovery value of 94.3 and 89.1 for virgin and secondary pulp respectively.

The precision and accuracy of the analytical method was also evaluated based on the ICH guideline [33]. The precision of each method indicates the degree of dispersion within a series on the determination of the same sample. Precision of this assay was investigated with respect to both repeatability and reproducibility. Repeatability was investigated by analyzing nine replicate samples of each 0.021, 0.105 and 0.210 mg/kg standards where the mean concentration from calibration curve were 0.024, 0.109 and 0.221 mg/kg with associated %R.S.D.'s of 4.1, 1.9 and 0.9 % respectively. Inter-day precision was assessed by analyzing the same three-concentrations over 3 consecutive days, resulting in mean concentrations of PCP of 0.026, 0.111 and 0.223 mg/kg and associated %R.S.D. of 3.9, 1.7 and 0.7% respectively. The degree of closeness of PCP measurements to their true actual values was determined through interpolation of replicate (n=6) AUCs for three levels of accuracy standards used in precision test from calibration curve in Figure 1. In each case, the percent of relative error and accuracy was calculated (Table 3).

Table 2. Recoveries of PCP from hygienic paper made from virgin and secondary pulp spiked at various concentrations

Spiking condition		Source of paper			
		Virgin pulp		Secondary pulp	
Spike level ($mg.kg^{-1}$)	Replicate	Found ($mg.kg^{-1}$)	Mean recovery (%)	Found ($mg.kg^{-1}$)	Mean recovery (%)
0.0210 (1* LOQ)	3	0.0185	88±2	0.0170	81±2
0.1050 (5* LOQ)	3	0.0955	91±3	0.0871	83±3
0.2100 (10* LOQ)	3	0.2016	96±2	0.1890	90±2

Table 3. Accuracy data for replicate analysis of PCP at various concentrations

True value mg.Kg^{-1}	Number of replicate	Mean of Con. from calib. Curve mg.Kg^{-1}	S.D. mg.Kg^{-1}	R.E. (%)
0.021	6	0.022	0.004	4.76
0.105	6	0.108	0.006	2.85
0.210	6	0.217	0.012	3.33

CONCLUSIONS

Chlorophenols have a relatively high volatility and water solubility and this has led to widespread contamination of the environment. Chlorophenols especially PCP has found worldwide application in wood preservation and paper production. Control of pentachlorophenol in tissue and napkins prepared from recycled pulp has a vital importance. Producing hygienic paper using virgin pulp or recycled first grade paper could have an important role in general health in society. Considering 4% for positive result in analysis of papers and napkins prepared from recycled pulp, it can be concluded that we should have an increasing the enforcement activities of the authorities, especially in relation to the investigation of illegal usage of certain pesticides in the tissue papers product. In addition, the future prospects are so that such an investigation will be continued in this mode. In quality control of hygienic papers, validated analytical method must be used for presence of PCP residues. This is more important in quality control of recycled hygienic papers. In official laboratories that investigated routine paper controls, it is necessary to use more sensitive and reliable analytical technique like mass spectrometry to confirm the identity of PCP peaks, and to guarantee the public health.

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