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Cytotoxicity of municipal solid waste incinerator ash wastes toward mammalian kidney cell lines

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Abstract

In this study, three municipal solid waste incinerator (MSWI) ash wastes—bottom ash, scrubber residue, and baghouse ash—were extracted using a toxicity characteristic leaching procedure (TCLP) extractant. These so-called final TCLP extracts were applied to African green monkey kidney cells (Vero), baby hamster kidney cells (BHK-21), and pig kidney cells (PK-15), multi-well absorption reader analysis was performed to test how the cytotoxicity of the incineration ashes would affect the digestive systems of animals. Ion-coupled plasma analyses indicated that the baghouse ash extract possessed the highest pH and heavy metal concentration, its cytotoxicity was also the highest. In contrast, the bottom ash and the scrubber residue exhibited very low cytotoxicities. The cytotoxicities of mixtures of baghouse ash and scrubber residue toward the three tested cell lines increased as the relative ratio of the baghouse ash increased, especially for the Vero cells. The slight cytotoxicity of the scrubber residue arose mainly from the presence of Cr species, whereas the high cytotoxicity of the baghouse ash resulted from its high content of heavy metals and alkali ions. In addition, it appears that the dissolved total organic carbon content of these ash wastes can reduce the cytotoxicity of ash wastes that collect in animal cells.

Keywords: Fly ash; Cytotoxicity; Vero cell; BHK-21; PK-15

1. Introduction

Incineration has become the most popular technique for treating municipal solid wastes (MSW) in Taiwan. MSW incinerators generate several types of ash waste, including boiler ash, scrubber residue, baghouse ash, and bottom ash (The International Ash Working Group, 1997; Brocca et al., 1997; Chang and Huang, 1999; Huang et al., 2003a). A widely employed method for analyzing the hazardous nature of a waste is the toxicity characteristic leaching procedure (TCLP). Previously, we studied the chemical composition and co-leaching behavior of pure scrubber residues and pure baghouse ashes from seven of Taiwan's MSW incineration plants (Huang and Chu, 2003b, 2005a; Huang et al., 2004, 2005b). Although all of the conclusions we drew took the physicochemical analysis data of the TCLP extracts of ash wastes into consideration, we did not perform integral assessment testing of the harmful effects of the whole TCLP extracts.

Cytotoxicity assessments of several environmental samples—such as particulate pollutants (Hsiao et al., 2000; McDonald et al., 2004; Seagrave and Nikula, 2000), MSWI ash wastes and their leachates (Kaneko, 1996; Diabate et al., 2002; Lapa et al., 2002; Ali et al., 2004; Chen and Lin, 2006; Lin and Chen, 2006; Ali et al., 2007; Chen and Lin, 2007), the flue gas of a heating MSWI baghouse ash (Huang and Shue, 2007), and coal ash leachates (Karuppiah and Gupta, 1997)—have attracted considerable attention recently. Most noteworthy, Lin and Chen (2006) and Chen et al. (2006) reported that the toxicity of bottom ash (BA), scrubber ash (SA), and cyclone ash (CA) increases in the sequence SA > BA > CA. Although Lin

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and Chen (2006) found that SA was more toxic than either Cr(III) or Cr(VI), the sample of SA that they used was actually a mixture of semi-dry scrubber residue and baghouse ash in a randomly mixed ratio because they had sampled the SA from the baghouse filter system of an MSWI plant. Because the ratio of these two ash wastes in an MSWI plant is under dynamic flux, the deduced series of toxicities might not be entirely accurate.

We have previously reported the co-leaching behavior of Pb ions from mixtures of semi-dry scrubber residue and baghouse ash (Huang and Chu, 2005a). More recently, from testing in lung cells we determined an empirical equation for predicting the cytotoxicity of the exhaust gas of a heated MSWI baghouse ash according to the physicochemical properties of its extracts (Huang and Shue, 2007).

In this present study, we wished to determine how the toxicity of incineration ashes affects the digestive systems of animals. Thus, we measured the cytotoxicity of the TCLP extracts of MSWI ash wastes and mixtures of pure semi-dry scrubber residue and baghouse ash toward African green monkey kidney cells (Vero), baby hamster kidney cells (BHK-21), and pig kidney cells (PK-15). In addition, we developed three empirical equations to predict the cytotoxicity of the TCLP extracts of these ash wastes and mixtures according to their physicochemical properties, we discuss their accuracy herein.

2. Materials and methods

2.1. Sampling of ash wastes

An MSW incineration plant having a treating capacity of 1350 tons/day was selected as the site from which to collect combustion ash wastes. The air pollution control device (APCD) of the incinerator was a semi-dry lime scrubbing system equipped with a fabric filter. The APCD residues, including the scrubber residue, baghouse ash, and incinerator bottom ash, were sampled separately and stored in high-density polyethylene (HDPE) bottles at temperatures below 4 °C prior to analysis. When sampled, the bottom ash had not been mixed with either the boiler ash or the grid ash.

2.2. Toxicity leaching extraction procedure (TCLP) test

The TCLP, following USA EPA Method #1311, is a widely employed essential test for assessing the leaching properties of hazardous wastes. The sampled ashes were graded by passing them through a 10-mm standard sieve. Because the values of ashes' pH were greater than 5.0, therefore the pH of used extractant was 2.88. Samples were mixed with an acidic extractant in a liquid-to-solid ratio of 20:1 and agitated at a rotation rate of 30 rpm for 18 h. A Perkin–Elmer Optima 3000XL ion-coupled plasma (ICP) detector was used to monitor the concentrations of the species in the final extract (following US EPA Method #7420). A Fisher Scientific Accruement pH meter was used to probe the pH of the solution. The acetic acid-based extractant was used as a negative control for the cytotoxicity test (see Section 2.4 for details).

2.3. Determination of total organic carbon in final extracts

The total organic carbon (TOC) contents of the final extracts were measured using a Shimadzu TOC-5000 instrument. Potassium hydrogen phthalate, sodium carbonate, and sodium hydrogen carbonate were used as standards, the maximum heating temperature was 680 °C.

2.4. Cytotoxicity test

2.4.1. Principle of testing method

The cytotoxicity of each extract was measured toward African green monkey kidney cells (Vero), baby hamster kidney cells (BHK-21), and pig kidney cells (PK-15). Theses cell lines were purchased from the Bioresource Collection and Research Center (BCRC), Taiwan, the code numbers were BCRC-60013 (Vero), BCRC-60057 (PK-15), and BCRC-60006 (CHO). The mode of toxicity of the incineration ashes toward the digestion systems of animals was tested in this study. The measured biological endpoint was the release of lactate dehydrogenase (LDH) from cells when they died. The quantitative data were measured using a Dynex-MRX reader (Dynex Technologies, USA). Fetal bovine serum (FBS), Dulbecco's modified Eagle medium (DMEM), and trypsin versene solution (TVS) were obtained from HyClone Co. (USA). The cytotoxicity detection kit (LDH) was obtained from Roche Applied Science. The dilution of all extracted samples was performed using deionized water.

2.4.2. Cell line conditioning

Vero cells were cultured for several days in DMEM containing 1% FBS. The cultured cells were then transferred to a 96-well plate at a cell density of 10 000 cells per well and continually incubated at 37 °C for 24 h under an atmosphere of 5% CO_2. A 0.25% TVS was used as the digest solution to separate the single cell layer from the bottom of the incubation bottle to form a suspended solution. The PK-15 cells were cultured for several days in DMEM containing 10% FBS. The cultured cells were transferred to a 96-well plate at a concentration of 10 000–20 000 cells per well and continually incubated at 37 °C overnight under an atmosphere of 5% CO_2. Again, 0.25% TVS was used as the digest solution to separate the single cell layer from the bottom of the incubation bottle to form a suspended solution. The BHK-21 cells were cultured for several days in DMEM containing 10% FBS. The cultured cells were transferred to a 96-well plate at a concentration of 10 000–20 000 cells per well and continually incubated at 37 °C overnight under an atmosphere of 5% CO_2. Once again, 0.25% TVS was used as the digest solution to separate the single cell layer from the bottom of the incubation bottle to form a suspended solution.