# 7. ANALYTICAL METHODS

The purpose of this chapter is to describe the analytical methods that are available for detecting, measuring, and/or monitoring phosphate ester flame retardants, their metabolites, and other biomarkers of exposure and effect to phosphate ester flame retardants. The intent is not to provide an exhaustive list of analytical methods. Rather, the intention is to identify well-established methods that are used as the standard methods of analysis. Many of the analytical methods used for environmental samples are the methods approved by federal agencies and organizations such as EPA and the NIOSH. Other methods presented in this chapter are those that are approved by groups such as the Association of Official Analytical Chemists (AOAC) and the American Public Health Association (APHA). Additionally, analytical methods are included that modify previously used methods to obtain lower detection limits and/or to improve accuracy and precision.

## 7.1 BIOLOGICAL MATERIALS

Methods for the determination of phosphate ester flame retardants in biological materials are summarized in Table 7-1. There is virtually no information regarding the toxicokinetics of these chemicals in humans, including information on possible metabolites; consequently, there are no methods for determining metabolites. Thus far, detection of the parent compound is the only method available for detecting exposure to phosphate ester flame retardants.

There are very few published articles regarding bioanalytical methods for phosphate ester flame retardants, but interest in these compounds as emerging pollutants has stimulated development of a select few (Shah et al. 2006). The bioanalytical methods presented are preliminary and are not accepted methods of accurately determining phosphate ester flame retardants in biological materials.

Alkyl and aryl phosphate ester flame retardants were measured in blood using a method developed by Jonsson and Nilsson (2003). The sample preparation from blood plasma consisted of a hollow fibre-based XT-tube extractor to perform the liquid-liquid microextraction in hexane/methyl tert-butyl ether 2:1 (v/v). A top gas chromatograph equipped with a TS-2 nitrogen-phosphorus detector (NPD) was employed to analyze the phosphate esters. The detection limits were 0.3 ng/mL for TPP and 36 ng/mL for TBEP. Recoveries varied between 40 and 80% with a relative standard deviation (RSD) around 4%

## Table 7-1. Analytical Methods for Determining Phosphate Ester Flame Retardants in Biological Materials

| Sample matrix | Preparation method | Analytical method | Sample detection limit | Analyte | Percent recovery | Reference |
|---|---|---|---|---|---|---|
| Urine | MISPE >80% recovery | LC-ESI-MS | 0.025 ng/µL | TPP | 72–75<br>RSD: 11–12% | Moller et al. 2004 |
| | | | No data | TnBP | No data | |
| Blood plasma | Liquid-liquid microextraction | GC-MS-NPD | 0.3 ng/mL | TPP | 40–80<br>RSD: 4% | Jonsson and Nilsson 2003 |
| | | | 36 ng/mL | TnBP | 40–80<br>RSD: 4% | |
| | | | No data | TBEP, TCEP, TCPP | 40–80<br>RSD: 4% | |
| Blood Plasma | Methanol extraction, acetonitrile precipitation, then SPME | GC-ICP-MS | 17 ng/L | TnBP | 43<br>RSD 11% | Shah et al. 2006 |
| | | | 240 ng/L | TCEP | 49<br>RSD 7% | |
| | | | 24 ng/L | TPP | 66<br>RSD 14% | |
| Tissue | Extract with benzene or acetone/hexane; fractionation by GPC | GC-MS-NPD | No data | TPP, TnBP, TBEP, TDCP, TCEP *o*-TCP *m*-TCP | 2.5 ng: 78–96<br>10 ng: 52–100<br>25 ng: 88–105 | LeBel and Williams 1983 |
| Seminal fluid | Steam distillation; continuous liquid-liquid extraction | LC-MS-NCI | 0.01 µg | TDCP | No data | Hudec et al. 1981 |

ESI = electronspray; GC = gas chromatography; GPC = gel permeation chromatography; ICP = inductively coupled plasma; LC = liquid chromatography; MISPE = molecularly imprinted polymer solid-phase extraction; MS = mass spectrometry; *m*-TCP = *met*a-tricresyl phosphate; NCI = negative chemical ionization; NPD = nitrogen-phosphorus detection; *o*-TCP = *ortho*-tricresyl phosphate; RSD = relative standard deviation; SPME = solid phase microextraction; TBEP = tris(2-butoxyethyl) phosphate; TCEP = tris(2-chloroethyl) phosphate; TCPP = tri-(2-chloro-isopropyl) phosphate; TiBP = triisobutyl phosphate; TnBP = tributyl phosphate; TOF = time of flight; TPP = triphenyl phosphate

for most compounds. The method was also able to detect and quantify other phosphate ester flame retardants such as TnBP, TCEP, and TCPP, although the detection limit was not sufficiently low.

Another analytical method to determine phosphorus-specific compounds in human plasma was published by Shah et al. (2006). This technique uses solid-phase microextraction (SPME) followed by gas chromatography (GC) inductively coupled plasma (ICP) mass spectrometry (MS) and high resolution GC time of flight (TOF) MS. The detection limits from blood plasma were 17 ng/L for TnBP, 240 ng/L for TCEP, and 24 ng/L for TPP.

In urine, Moller et al. (2004) detected and quantified TnBP and TPP hydrolysis products. The method is capable of extracting the corresponding diesters of TnBP and TPP via molecularly imprinted polymer solid-phase extraction (MISPE) and liquid chromatography (LC) electrospray (ESI) MS method using a Hypercarb LC column with a graphitized carbon stationary phase. The detection limit was 0.025 ng/μL for diphenyl phosphate, the hydrolysis product of TPP.

In Canada, TDCP was detected in human adipose tissue by LeBel and Williams (1983, 1986) in concentrations that ranged from not detectable (<0.001 μg/kg) to 257 μg/kg. In later studies, samples from four out of six cities showed no detectable TDCP; however, two concentrations ranged up to 32 μg/kg (LeBel and Williams 1983, 1986; LeBel et al. 1989). Using LC negative chemical ionization (NCI) MS with a limit of detection of 0.01 μg, Hudec et al. (1981) found TDCP in the seminal fluid of 34 out of 123 student donors. The TDCP concentrations ranged from 5 to 50 μg/L.

## 7.2 ENVIRONMENTAL SAMPLES

Methods for the determination of phosphate ester flame retardants in environmental samples are summarized in Table 7-2.

Standard environmental analysis methods are available for several of the selected phosphate ester flame retardants from the U.S. Geological Survey (USGS) and NIOSH (NIOSH 1994a, 1994b; USGS 2001). All standardized methods, as well as literature methods available, utilize either liquid- or gas-based chromatography with predominantly GC flame photometric detection (FPD) being employed. Methods for analyzing phosphate ester flame retardants in air, water, soil, and other environmental media are prevalent throughout the literature.

## Table 7-2. Analytical Methods for Determining Phosphate Ester Flame Retardants in Environmental Samples

| Sample matrix | Preparation method | Analytical method | Sample detection limit | Percent recovery | Analyte(s) | Reference |
|---|---|---|---|---|---|---|
| Air (indoor) | Collection on filter, extract with ethyl ether, filter | NIOSH 5034 GC-FPD | 2 µg/sample | No data | TnBP | NIOSH 1994a |
| Air (indoor) | Collection on filter, extract with ethyl ether, filter | NIOSH 5034 GC-FPD | 0.05 µg/sample | 101.3% | *o*-TCP | NIOSH 1994c |
| Air (indoor) | Absorbed on a filter, extracted with dichloromethane | GC-MS | 3 ng/mL or 13 ng/$m^3$ | 92–109 | TnBP, TiBP, TCP, TPP | Solbu et al. 2007 |
| Air (outdoor) | Trap with glycerol-Florisil column, elute with MeOH/water, extract with hexane | GC-FPD | 1 ng/$m^3$ | No data | Trialkyl/aryl phosphates | IPCS 1991b (EHC-111) |
| Water, sediments, solid wastes, sludges | Collection on filter, extract with ethyl ether, filter | NIOSH 5038 GC-FPD | 10 µg/sample | No data | TPP | NIOSH 1994b |
| Water, sediments, solid wastes, sludges | Extracted with $CH_2Cl_2$ or MeCN, gel permeatation, SPE | GC-FPD EPA-8141a; EPA-1618 | Not specified | No data | TCP | EPA 1989, 1994 |
| Filtered waste water and water | Filtered, extracted with an SPE cartridge containing a polystyrene divinylbenzene phase, dried, rinsed with DCM/ether, and concentrated | USGS-NWQL O-1433-01 GC-MS | TnBP 0.1 µg/L | TnBP: 110[a], 5.97% RSD | TnBP, TBEP, TPP, TDCP, TCEP | USGS 2001 |
| | | | TBEP 0.2 µg/L | TBEP: 103.4[a], 12.52% RSD | | |
| | | | TPP 0.06 µg/L | TPP: 90[a], 4.5% RSD | | |
| | | | TDCP: 0.08 µg/L | TDCP: 96.4[a], 5.29% RSD | | |
| | | | TCEP: 0.08 µg/L | TCEP: 100[a], 5.04% RSD | | |
| Water (drinking) | Adsorb resin, elute | GC-NPD | 1 ng/L | No data | Trialkyl/aryl phosphates | LeBel et al. 1979 |

## Table 7-2. Analytical Methods for Determining Phosphate Ester Flame Retardants in Environmental Samples

| Sample matrix | Preparation method | Analytical method | Sample detection limit | Percent recovery | Analyte(s) | Reference |
|---|---|---|---|---|---|---|
| Sediments (sea or river) | Extract with MeCN or acetone, Florisil column | GC-FPD | TnBP: 2 ng/g<br>TCPP: 5 ng/g<br>TCEP: 5 ng/g<br>TPP: 5 ng/g<br>TCP: 20 ng/g | 83–98 | TnBP, TCEP, TPP, TCPP, TCP | Ishikawa et al. 1985 |

[a]Percent recovery and relative standard deviation were reported by NEMI (2009).

DCM = dichloromethane; FPD = flame photometric detection; GC = gas chromatography; MS = mass spectrometry; *m*-TCP = *meta*-tricresyl phosphate; NIOSH = National Institute for Occupational Safety and Health; NPD = nitrogen-phosphorus detection; *o*-TCP = *ortho*-tricresyl phosphate; RSD = relative standard deviation; SPE = solid phase extraction; TBEP = tris(2-butoxyethyl) phosphate; TCEP = tris(2-chloroethyl) phosphate; TCPP = tri-(2-chloroisopropyl) phosphate; TiBP = triisobutyl phosphate; TnBP = tributyl phosphate; TPP = triphenyl phosphate; USGS = U.S. Geological Survey

Drinking water has been analyzed for TPP using a modified version of EPA method 525.5 using C-18 bonded solid phase extraction columns (Stiles et al. 2008) and also using GC-MS with NPD (LeBel et al. 1979). TPP adsorbed to sediments and soils can also be analyzed using a method developed by Degeus et al. (1994) using thermionic detection (TID) with supercritical fluid chromatography (SFC).

An alternative method for testing for phosphate ester flame retardants was developed by Lombardo and Egry (1979) based on an AOAC method developed for analysis of pesticide residues in fatty foods. The method extracted the hydraulic fluid from contaminated fish samples and analyzed them by gas-liquid chromatography (GLC) with phosphorus selective detection. The analysis yielded concentrations of TPP concentrations of 0.06 and 0.12 ppm in carp and 0.15 ppm in goldfish collected from Waukegan Harbor, Illinois.

Several other methods were developed for detection of phosphate esters in various types of media. Lamouroux et al. (2000) report an LC-MS method for determining degradation products of TnBP used in nuclear fuel processing. The TnBP content affects the performance of the extracting solvent; therefore, determining the diester and monoester content is desired.

Nagase et al. (2003) used GC-FPD to detect phosphate ester flame retardants in polyurethane foam cushions. The detection limits were 0.3–0.9 μg/g. The recoveries from a 0.05 g sample of soft polyurethane foam were 80–90%, when the spiked amounts were 0.25–1 μg. The compounds were detected from soft polyurethane foam at a level of 0.4–23.3 μg/g.

## 7.3 ADEQUACY OF THE DATABASE

Section 104(i)(5) of CERCLA, as amended, directs the Administrator of ATSDR (in consultation with the Administrator of EPA and agencies and programs of the Public Health Service) to assess whether adequate information on the health effects of phosphate ester flame retardants is available. Where adequate information is not available, ATSDR, in conjunction with NTP, is required to assure the initiation of a program of research designed to determine the health effects (and techniques for developing methods to determine such health effects) of phosphate ester flame retardants.

The following categories of possible data needs have been identified by a joint team of scientists from ATSDR, NTP, and EPA. They are defined as substance-specific informational needs that if met would reduce the uncertainties of human health assessment. This definition should not be interpreted to mean

that all data needs discussed in this section must be filled. In the future, the identified data needs will be evaluated and prioritized, and a substance-specific research agenda will be proposed.

### 7.3.1 Identification of Data Needs

#### Methods for Determining Biomarkers of Exposure and Effect.

***Exposure.*** *In vitro* analytical methods have shown to detect TnBP, TPP, TCEP, TCPP, and TBEP in bodily fluids using GC-MS (Moller et al. 2004; Jonsson and Nilsson 2003). It is not documented whether these methods could be applied to analysis of TiBP or TDCP in bodily fluids. TDCP is the only analyte of the selected phosphate esters reported to be analyzed in human tissue and seminal fluid (Hudec et al. 1981; Lebel and Williams 1983, 1986). There are no specific biomarkers of exposure other than the phosphate esters themselves. A data need exists for additional bioanalytical methods for TiBP and TDCP. As information becomes available regarding the metabolism of these chemicals in humans, appropriate methods need to be developed for the detection and quantification of metabolites in tissues and biological fluids.

***Effect.*** No significant health effects have been reported in humans exposed to the phosphate ester flame retardants discussed in this profile in the limited studies available of workers exposed to TDCP or TPP (Stauffer Chem Co. 1983; Sutton et al. 1960). Consequently, no associations have been established between body burdens of phosphate esters and health effects. More information is needed regarding the toxicity and toxicokinetics of these substances to determine whether the existing analytical methods reported in Table 7-1 are adequate in selectivity and sensitivity to measure phosphate esters in biological materials at levels associated with adverse health effects.

**Methods for Determining Parent Compounds and Degradation Products in Environmental Media.** GC coupled with various detection methods (MS, FPD, or NPD) provide sufficiently accurate, precise, and repeatable methods for determining phosphate ester concentrations in the environment (Ishikawa et al. 1985; LeBel et al. 1979; NIOSH 1994a, 1994b; Solbu et al. 2007; USGS 2001; IPCS 1991a, 1991b). These analytical methods can adequately measure phosphate esters in air, water, soil, and sediments at concentrations in the ng/L or $ng/m^3$ range.

### 7.3.2 Ongoing Studies

No ongoing studies pertaining to analytical methods for phosphate ester flame retardants were identified in a search of the Federal Research Progress database (FEDRIP 2009).