

## 6. ANALYTICAL METHODS

The purpose of this chapter is to describe the analytical methods that are available for detecting, and/or measuring, and/or monitoring titanium tetrachloride, its metabolites, and other biomarkers of exposure and effect to titanium tetrachloride. 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 National Institute for Occupational Safety and Health (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.

### 6.1 BIOLOGICAL SAMPLES

Titanium tetrachloride hydrolyzes into titanium dioxide and hydrochloric acid upon contact with water or moisture in the air. Titanium tetrachloride cannot be detected in biological materials; however, titanium dioxide and titanium metal can be detected and may be used as indicators of exposure to titanium tetrachloride, although the presence of these materials in biological tissue does not necessarily mean the exposure occurred. See Table 6-1 for a summary of the analytical methods most commonly used to detect titanium tetrachloride and titanium dioxide in biological materials. The primary method used to detect titanium dioxide in lung tissue is scanning and/or transmission electron microscopy (STEM). Electron probe X-ray microanalysis (EMX-SM) and energy dispersive X-ray analysis (EDXA) have been used in conjunction with STEM (Ferin et al. 1976; Redline et al. 1986). Sample preparation consists of fixation of the tissue sample in osmium tetroxide and/or glutaraldehyde, dehydration in ethanol, embedding in epoxy, followed by sectioning and staining with uranyl acetate and lead citrate (Ferin et al. 1976; Ophus et al. 1979). These methods can detect particles as small as 0.2  $\mu\text{m}$ .

No analytical methods were found for determining titanium dioxide in urine. However, titanium metal can be determined in urine by inductively coupled argon plasma, atomic emission spectroscopy

**Table 6-1. Analytical Methods for Determining Titanium and Titanium Dioxide in Biological Samples**

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Urine (Ti metal)	Creatine determination, pH adjustment, addition of polydithiocarbamate resin, ashing of filter and resin, redissolution.	ICP-AES (NIOSH Method 8310)	0.02 ppm (wt/vol)	86 at 2 ppm (8% RSD).	NIOSH 1994a
Lung tissue (TiO <sub>2</sub> as particles)	Fixation in osmium tetroxide, dehydration in ethanol, embedding in epoxy, sectioning and staining with uranyl acetate and lead citrate.	TEM, EMX-SM; STEM	<0.2 µm	Not reported	Ferin et al. 1976
Lung tissue (TiO <sub>2</sub> )	Drying of sample to constant weight followed by grinding.	OES	<100 ppm (wt/wt)	Not reported	Elo et al. 1972
Lung tissue (TiO <sub>2</sub> and Ti pigments)	Fixation of sample in glutaraldehyde and osmium tetroxide, dehydration in ethanol, embedding in epoxy resin, sectioning and staining with uranyl acetate and lead citrate.	TEM/EDS	Not reported	Not reported	Ophus et al. 1979
Lung tissue (TiO <sub>2</sub> and Ti pigments)	Dehydration of sample, mounting on carbon stub with carbon cement and coating with gold	SEM/EDS	Not reported	Not reported	Ophus et al. 1979
Lung tissue (TiO <sub>2</sub> and Ti pigments)	Ashing of sample followed by filtration of lung-tissue/ash dispersions through silver membrane	XD	Not reported	Not reported	Ophus et al. 1979

EMX-SM = electron probe X-ray microanalyzer; EDS = energy dispersive X-ray microanalysis; EDXA = energy dispersive X-ray analysis; ICP-AES = inductively coupled argon plasma, atomic emission spectroscopy; NIOSH = National Institute for Occupational Safety and Health; OES = optic emission spectrograph; RDS = relative standard deviation; SEM = scanning electron microscopy; STEM = scanning transmission electron microscopy; TEM = transmission electron microscopy; Ti = titanium; TiO<sub>2</sub> = titanium dioxide; wt/vol = weight/volume; wt/wt = weight/weight; XD = X-ray diffraction

## 6. ANALYTICAL METHODS

(ICP-AES). This method is very sensitive, with a detection level of 20 ppb and a good recovery of 86% (NIOSH 1994a).

No information was located on detecting titanium dioxide in blood, adipose tissue, feces, or human milk.

### 6.2 ENVIRONMENTAL SAMPLES

No methods for detecting titanium tetrachloride in environmental samples were located. However, titanium dioxide may be used as an indicator of titanium tetrachloride's presence in air and some food samples, but its presence does not necessarily mean that titanium tetrachloride is or was present in the environmental sample. See Table 6-2 for a summary of the analytical methods used to determine titanium dioxide in environmental samples.

The primary method for detecting titanium dioxide in air is by gravimetric filter weight (G/FW) (NIOSH 1980, 1994b, 1994c) although gravimetric methods alone are not specific for titanium. Air sampling may be performed by collection of a sample on a polyvinyl chloride membrane or DM800 filter, drying or heating, followed by equilibration of the sample in an environmental chamber prior to measurement of the mass of particles collected. Detection limits are in the ppm range (NIOSH 1980, 1994b, 1994c). Spectroscopic methods can detect titanium metal in air at a detection level of 2 µg, and atomic absorption spectrophotometry (AAS) can detect titanium metal at 1.9 µg/mL for 1% absorption (Anonymous 1975). The corresponding limits of detection in air are dependent upon the volumes of both the air sample and the resulting extract. Samples for both methods are collected on an electrostatic precipitator (ESP), filter paper, standard impinger, or a membrane filter. The sample is reacted with hydrogen peroxide for the general spectrophotometric method, or acidified with hydrochloric acid solution for the AAS method (Anonymous 1975). Caution must be used for the general calorimetric method because iron, nickel, chromium, vanadium, molybdenum fluoride, and large amounts of phosphates and alkali metal sulfates can interfere (Anonymous 1975).-If these substances are present, additional fractionation steps will be needed. High levels of iron, aluminum, and fluoride enhance the response from titanium in the AAS method and this could result in an overestimation of the titanium concentration (Anonymous 1975). ICP-AES is an alternative method to determine titanium dioxide in air, with detection limits in the ppb range and an excellent recovery of 96% (NIOSH 1994d).

**Table 6-2. Analytical Methods for Determining Titanium and Titanium Dioxide in Environmental Samples**

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Air (total particulates)	Collection of sample onto filter followed by drying and equilibration	G/FW (NIOSH Method 0500)	1.8 ppm (wt/vol) or 0.23 mg/m <sup>3</sup> for 133 L sample	100 (11% RSD) at 8 mg/m <sup>3</sup> .	NIOSH 1994b
Air (respirable particulates)	Collection of sample onto filter followed by drying and equilibration.	G/FW (NIOSH Method 0600)	0.58 ppm (wt/vol) or 0.075 mg/m <sup>3</sup> for 400 L sample	Not reported	NIOSH 1994c
Air (Ti, TiO <sub>2</sub> )	Collection of sample onto 0.8 µm filter, heating with ashing acid, dissolution of residue in dilution acid	ICP-AES (NIOSH Method 7300)	80 ppb for 100 L sample	96 at 2.5 µg/filter	NIOSH 1994d
Air (Ti metal)	Collection of sample with ESP, reaction with H <sub>2</sub> O <sub>2</sub>	Spectrophotometry	2 µg	Not reported	Anonymous 1975
Air (Ti metal)	Collection of sample with ESP, acidification	AAS	Instrument limit of detection = 1.9 µg/mL for 1% absorption; actual limit depends on sample and extract volumes	Not reported	Anonymous 1975
Cheese	Charring under IR lamp, ashing, addition of Na <sub>2</sub> SO <sub>4</sub> and H <sub>2</sub> SO <sub>4</sub>	Spectrophotometry	Not reported	Not reported	Leone 1973

AAS = atomic absorption spectrophotometry; ESP = electrostatic precipitator; G/FW = gravimetric filter weight; H<sub>2</sub>O<sub>2</sub> = hydrogen peroxide; H<sub>2</sub>SO<sub>4</sub> = sulfuric acid; ICP-AES = inductively coupled argon plasma, atomic emission spectroscopy; IR = infrared; Na<sub>2</sub>SO<sub>4</sub> = sodium sulfate; NIOSH = National Institute for Occupational Safety and Health; %RSD = percent relative standard deviation; Ti = titanium; TiO<sub>2</sub> = titanium dioxide; wt/vol = weight/volume

## 6. ANALYTICAL METHODS

Hydrochloric acid is a hydrolysis product of titanium tetrachloride and can be detected in air. Gaseous hydrochloric acid must first be separated from aerosols that contain chloride ions. Filter packs, diffusion denuders, and diffusion samplers are the most common methods used to determine hydrochloric acid in air (Kamrin 1992). However, if hydrochloric acid is found in air, it is not necessarily indicative of exposure to titanium tetrachloride.

The presence of titanium dioxide in cheese has been studied (Leone 1973). Sample preparation included charring and ashing, followed by dissolution of the sample in sulfuric acid. A yellow-orange color is produced. The addition of hydrogen peroxide to a cheese or air sample to calorimetrically determine titanium dioxide may cause interference from nickel, copper, cobalt, molybdenum, vanadium, and chromium, if present. It is unlikely, however, that these elements are present in cheese. Interferences in air samples can be overcome by the use of treated and untreated standards, or by precipitating the titanium dioxide (Anonymous 1975; Leone 1973).

No information was located on detecting titanium tetrachloride or titanium dioxide in water, soil, or sediment.

### 6.3 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 titanium tetrachloride is available. Where adequate information is not available, ATSDR, in conjunction with the 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 titanium tetrachloride.

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.

## 6. ANALYTICAL METHODS

**6.3.1 Identification of Data Needs**

**Methods for Determining Biomarkers of Exposure and Effect.** Methods exist for measuring titanium dioxide and titanium dioxide pigment, that is, different crystalline forms of titanium dioxide, in lung tissue (Elo et al. 1972; Ferin et al. 1976; Ophus et al. 1979) and for measuring titanium metal in urine (NIOSH 1994a). However, no methods were identified for detecting titanium tetrachloride in any biological materials. Most methods can only detect the presence and not the concentrations of titanium dioxide or titanium dioxide pigments in lung tissue (Elo et al. 1972; Ophus et al. 1979). STEM and EDXA methods cannot differentiate among different crystalline forms of titanium dioxide. However, X-ray diffraction (XD) methods can differentiate among the different crystalline forms and this information can be useful in the assessment of the bioavailability of the titanium. However, no method exists for determining background levels of titanium tetrachloride or titanium dioxide in the general population, or levels at which biological effects occur.

More sensitive methods for detecting long-term exposure to titanium dioxide or titanium metal in biological tissue are desirable in order to monitor levels of titanium dioxide, titanium dioxide pigment, or metal in titanium industry workers. In addition, methods should be developed that could easily differentiate among the different crystalline forms of titanium dioxide in lung tissue so that possible differences in toxic effect resulting from differing bioavailabilities can be studied.

No biomarkers of effect of titanium tetrachloride exist. However, after a worker's accidental exposure to titanium tetrachloride, a dark pigmentation formed around the scars left by the burns, suggesting that titanium metal or titanium dioxide may be a biomarker of exposure (Lawson 1961). Additional study is required to determine the cause of the dark pigmentation. Further development of methods for determining biomarkers of effect for titanium tetrachloride would be beneficial to determine whether or not an individual has been exposed to the compound.

**Methods for Determining Parent Compounds and Degradation Products in Environmental Media.** Human exposure to titanium tetrachloride is most likely to result from being splashed with the liquid. Titanium dioxide, a hydrolysis product of titanium tetrachloride, or titanium metal in workplace air may be indicative of exposure to titanium tetrachloride, titanium metal, or titanium dioxide. G/FW is the most common method for determining titanium and titanium dioxide in air as particulate matter (NIOSH 1980, 1994b, 1994c), and spectroscopic methods are most common

## 6. ANALYTICAL METHODS

for detecting titanium metal associated with particulates in air (Anonymous 1975; NIOSH 1994d). The sensitivity of the gravimetric methods is in the ppm range, and the sensitivity of the spectroscopic methods is in the ppb range with good recovery. Both methods can measure background levels in the environment and levels at which health effects may occur. A calorimetric method to determine the presence of titanium dioxide in cheese has also been developed (Leone 1973). The reliability and specificity of many of these methods have not been determined; therefore, methods to improve the reliability and specificity of titanium dioxide and titanium metal in air would be useful.

No methods for determining titanium tetrachloride or titanium dioxide in water, soil, or sediment were found.

### **6.3.2 Ongoing Studies**

No ongoing studies regarding analytical methods were located for titanium tetrachloride.

