7. ANALYTICAL METHODS

The purpose of this chapter is to describe the analytical methods that are available for detecting, measuring, and/or monitoring DEET, its metabolites, and other biomarkers of exposure and effect to DEET. 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.

7.1 BIOLOGICAL MATERIALS

DEET is used globally as a commercial insect repellent, which results in the direct exposure of humans. Studies have shown that DEET is absorbed and most is metabolized before excretion. Analytical methods for the determination of DEET and its metabolites in biological materials and environmental media (e.g., waste water samples) may be used to verify that exposure and absorption has occurred.

Dermal absorption of DEET following applications of various DEET products has been reported between 5.6 and 16.7% of the amount applied (Blomquist and Thorsell 1977; Feldman and Maibach 1970; Selim et al. 1995). The majority of dermally absorbed DEET is metabolized and excreted in human urine (Selim et al. 1995). The main human urinary metabolites of DEET are DCBA and EACB. Additional metabolites may include ET, DHMB, *m*-toluic acid, and ACB. Additional information and standards relating to metabolites of DEET would prove useful for better analytical analysis of both biological and environmental samples.

Several methods have been validated for the analysis of DEET in biological samples. The principal method used for the detection of DEET and/or its metabolites in biological samples is high performance liquid chromatography (HPLC) and GC coupled with MS. Sample preparation is typically performed using solid-phase extraction (SPE) and/or LLE with organic solvents such as methanol, methylene chloride, and acetonitrile. Those methods are generally suitable for the analysis of DEET by itself or simultaneously with other similar substances (e.g., repellents and pesticides).

Identification of DEET and DEET metabolites in human urine has been performed using GC glass capillary columns and MS elucidations with both electron impact (EI) and chemical ionization-methane (MCI) mass spectra. Failure to control food and beverage intake, including caffeine, and the presence of plasticizers complicated the evaluation (Wu et al. 1979). A method for the rapid quantification of DEET in human urine using HPLC and a triple-quadrupole tandem MS using an atmospheric pressure chemical ionization application has been published (Olsson et al. 2004). Sample preparation involves enzyme hydrolysis, SPE, and concentration, and throughput is ~50 samples/day.

Qiu and Jun (1996) have used SPE and reverse-phase liquid chromatography (LC) with UV detection at 220 nm for the quantification of DEET in both dog and human plasma. Extraction was achieved with reverse-phase C_8 (yielding faster throughput) or C_{18} SPE cartridges using acetonitrile-ammonium acetate solutions as wash and elution solvent systems as well as for the mobile phase for the chromatography. This method had an overall absolute recovery of 97.7%, with a range of recovery, dependent on DEET concentration, of 96.9–100.2%, accuracy range of 1.5–5.1%, precision of 2.6–11.1%, and an LOQ of 15 ng/mL. Abu-Qare and Abou-Donia (2001c) have developed an analytical method using HPLC with reverse-phase C_{18} columns and UV detection, with a reported LOD of 50 ng/mL and LOQ of 50–100 ng/mL, for the simultaneous quantitative and qualitative detection of DEET and its metabolites, in rat plasma and urine that could be used in the monitoring of human plasma and urine. Barr et al. (2002) have developed a sensitive analytical method for quantifying serum and plasma concentrations of DEET in human blood, reporting an LOD of 10 pg/g. The method employs SPE using an OASIS cartridge with a mixed polarity phase followed by isotopic dilution GC-high resolution (HR)-MS for analysis.

Smallwood et al. (1992) demonstrated that DEET can be detected in both serum and urine after dermal exposure to DEET by HPLC. DEET quickly metabolizes in the body; therefore, urine concentrations of DEET specifically are not the most accurate reflection of dermal exposure concentrations. Kuklenyik et al. (2013) have successfully developed a rapid HPLC-MS/MS method to measure concentrations of DEET in addition to two of its oxidative metabolites, DHMB and DCBA, in human urine. Because DEET, DHMB, and DCBA undergo metabolism to form conjugates, they must be hydrolyzed in order to evaluate total concentrations. Enzymatic hydrolysis of the urine sample is achieved via previously described methods (Olsson et al. 2004) using β-glucuronidase/sulfatase. Separation is done on a reverse-phase analytical column and detection is achieved via atmospheric pressure chemical ionization in positive ion mode. Detection limits for these three chemicals are reported to be between 0.1 and 1.0 ng/mL.

Evaluation of fetal exposure is a key concern that was addressed by Bradman et al. (2003). Amniotic fluid was evaluated using an MS analytical method previously intended for detection of DEET in urine. The LOD for urine was reported as 0.1 µg/L, with 98% recovery, and the LOD for amniotic fluid was reported as 0.40 µg/L, with 100% recovery. Although DEET was not detected in the amniotic samples evaluated in the study, it was noted that the analytical method for measuring DEET in urine is transferable to amniotic fluids with little modification.

It has been reported that high-level exposure to DEET in combination with other chemicals may increase adverse effects (Abu-Qare and Abou-Donia 2001b, 2001c; Abou-Donia et al. 2001a; Kuklenyik et al. 2013). Cherstniakova et al. (2006) developed rapid and sensitive methods for simultaneous determination of DEET and permethrin, and DEET and pyridostigmine bromide, in human plasma using GC-MS and HPLC, respectively. Abu-Qare et al. (2001) found that urinary excretion of 3-nitrotyrosine (a biomarker of oxidative stress) in rats increased when an oral dose of pyridostigmine bromide and a dermal dose of DEET were administered alone and in combination. Due to the possibility of the combined exposure scenarios, the method developed by Abu-Qare and Abou-Donia (2001b) using reverse-phase HPLC and UV detection, mentioned above, was developed for the simultaneous determination of diazinon, permethrin, DEET, and their metabolites in rat plasma and urine using solid-phase extraction of DEET and its metabolites. HPLC methods for the separation and quantification of chlorpyrifos, pyridostigmine bromide, N,N-diethyl-*m*-toluamide, and their metabolites in rat plasma and urine had detection limits ranging from 20 and 150 ng/mL (Abu-Qare and Abou-Donia 2001c). HPLC methods for the simultaneous determination of malathion, permethrin, DEET, and their metabolites in rat plasma and urine have been developed (Abu-Qare and Abou-Donia 2001d).

Analytical methods for the determination of DEET in biological materials and fluids are summarized in Table 7-1.

7.2 ENVIRONMENTAL SAMPLES

Analysis of environmental samples is similar to or the same as that of biological samples. The primary methods of analyzing for DEET in water samples involve SPE or LLE followed by GC, HPLC, and MS. Those methods are generally suitable for the analysis of DEET by itself or simultaneously with other similar substances (e.g., repellents and pesticides).

Table 7-1. Analytical Methods for Determining DEET and Transformation Products in Biological Samples

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Human blood	Blood containing heparin centrifuged and plasma collected; serum samples denatured with ammonium sulfate and centrifuged; SPE with OASIS cartridges.	GC-HR-MS	10 pg/g	43	Barr et al. 2002
Human serum	LLE with MTBE or SPE	GC-MS or HPLC-UV	Not reported	>90	Cherstniakova et al. 2006
Human urine and serum	Urine: extraction with diethyl ether; evaporation and dilution with methanol Serum: centrifuge and mix with 20% saline; SPE with C_{18} Sep-Pak Vac cartridges; wash with water; elute with methanol; evaporate	HPLC-UV	0.09 µg/mL (urine; serum)	90–91 (urine) 92–100 (serum)	Smallwood et al. 1992
Human urine	Hydrolysis with β-glucuronidase, SPE OASIS cartridge; LLE; evaporate; 50 samples/day throughput	HPLC-APCI-MS/MS/MS	0.1 ng/mL	At 5 ppb: 96 ppb (4.4 SD) At 50 ppb: 93 (2.7 SD)	Olsson et al. 2004
Human urine (DEET and selected metabolites)	Urine samples in sodium carbonate extracted with DCM/ ethyl alcohol and centrifuged; aqueous-phase pH adjusted and re-extracted; organic phase dried, evaporated, and reconstituted in methanol	GC-MS	Not reported	Not reported	Wu et al. 1979
Human urine (DEET and selected metabolites)	Hydrolysis with β-glucuronidase/ sulfatase in 0.1 M sodium acetate buffer; mixed, incubated at 37°C for 17 hours, then vortex mixed	HPLC MS/MS	0.1 ng/mL (DEET); 0.1 ng/mL (DHMB); 1 ng/mL (DCBA)	95	Kuklenyik et al. 2013
Human tissue	Minced tissue homogenized with water; HCl added and filtered; filtrate pH adjusted; hexane added and centrifuged; direct injection of hexane aliquot	GC	Not reported	45–60	Crowley et al. 1986

Table 7-1. Analytical Methods for Determining DEET and Transformation Products in Biological Samples

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Rat plasma and urine (DEET and selected metabolites)	Acidify with 1 N acetic acid; vortex and centrifuge samples; SPE with C_{18} Sep-Pak Vac cartridges; wash with water; elute with methanol and acetonitrile	HPLC	50 ng/mL	78.4–89.1 (DEET, urine) 72.8–84.2 (DEET, plasma)	Abu-Qare and Abou-Donia 2001c
Rat plasma and urine (DEET and selected metabolites)	Acidify with 1 N acetic acid; vortex and centrifuge samples; SPE with C_{18} Sep-Pak Vac cartridges wash with water; elute with methanol and acetonitrile	HPLC	20 ng/mL	83	Abu-Qare and Abou-Donia 2001b
Dog and human plasma	Vortex sample; SPE with C_{18} cartridges; wash and elute with acetonitrile and ammonium acetate	HPLC-UV	15 ng/mL (LOQ)	96.9–100.2 based on DEET concentration	Qiu and Jun 1996

APCI = atmospheric pressure chemical ionization; DHMB = N,N-diethyl-3-(hydroxymethyl)benzamide; DCBA = *m*-diethylcarbonyl) benzoic acid; DCM = methylene chloride; GC = gas chromatography; HCl = hydrogen chloride; HPLC = high-performance liquid chromatography; LLE = liquid-liquid extraction; LOQ = limit of quantification; MS = mass spectrometry; MS/MS = isotope dilution tandem mass spectrometry; MTBE = methyl-tert-butyl ether; SD = standard deviation; SPE = solid-phase extraction; UV = ultraviolet

Cheng et al. (2006) used an accelerated solvent extractor with dichloromethane and methanol followed by separation using a silica gel chromatography column, followed by GC/MS to analyze aerosol samples.

He and Lee (1997) developed a method for combining capillary electrophoresis (CE) with field-amplified concentration (FAC) and SPE for rapid concentration, separation, and quantification of DEET and five organonitrogen pesticides in water samples. However, the method recovery for DEET was less than half that for the pesticides (5–50 ppb was recovered 40.5–37.8%) and the reason was not discovered. Knepper (2004) employed solid-phase enrichment of DEET in surface waters and WWTP effluents on a capillary column followed by quantification using GC/MS in single ion monitoring mode. LOQs for surface water and WWTP effluent were 0.03 and 0.1 µg/L, respectively. Sandstrom et al. (2005) analyzed whole surface water for DEET and a range of other substances using methylene chloride LLE followed by GC/MS operated in selected-ion monitoring mode. They achieved a detection level of 0.02 µg/L for DEET, if retention time and ionic abundance criteria were met; otherwise, the reporting limit was 0.08 µg/L. Surface water samples may be analyzed using SPE followed by ultrahigh pressure LC-MS. Loos et al. (2013b) developed a method employing these analytical techniques using a hybrid triple-quadrupole linear ion trap instrument. Wang and Gardinali (2013) reported the successful use of an SPE-HPLC-atmospheric pressure photoionization (APPI)-MS/MS method for the detection and quantification of DEET in filtered water.

Methods for analyzing DEET in soils were not readily available.

An analytical method for determination of DEET in soda water was reported by Chandramouli et al. (2004); however, analytical procedures for food were not located.

Details of commonly used analytical methods for several types of environmental samples are presented in Table 7-2.

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 DEET is available. Where adequate information is not available, ATSDR, in conjunction with NTP, is required to assure the initiation of a program of research

Table 7-2. Analytical Methods for Determining DEET and Transformation Products in Environmental Samples

Sample matrix[a]	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Air	Collection with quartz filters followed by extraction with DCM/methanol (3:1 v/v)	GC with mass selective detector	No data	58	Cheng et al. 2006
Waste water influent and effluent	SPE; elution with 10/90 (v/v) methanol/MTBE followed by DCM	GC-MS/MS	0.1 ng/L	70–111	Trenholm et al. 2008
Waste water	Sample extraction with 15% DCM in hexane followed by concentration	GC-FID	No data	No data	EPA 1983
Filtered waste water and natural water samples	Field sample filtration using glass-filter fibers and SPE; elution of dry SPE cartridges with dichloromethane and diethyl ether followed by evaporation	GC/MS	0.14 µg/L	100 (9% RSD)	Zaugg et al. 2002
Whole water	CLLE with DCM	Capillary-column GC/MS	0.12 µg/L	Ground-water 98.57; surface water 71.31	USGS-06.pdf
Surface water, groundwater, drinking water	SPE; elution with methanol	UHPLC-MS/MS	1.0 ng/L	Not reported	Weeks et al. 2012
Drinking water	Grab samples from tap; SPE (Oasis HLB or Empore SDVB sorbent); elution with organic solvent, dried over column of sodium sulfate followed by evaporation	GC-MS full scan, SIM or SIS mode	0.019 µg/L (Oasis); 0.0042 µg/L (Empore)	97.9–106 (finished drinking water from ground sources) 92.9–97.5 (finished drinking water from surface sources)	EPA 2012b
Surface water	LLE with DCM	GC/MS SIM mode	20 ng/L	74±10	Sandstrom et al. 2005
Surface water, marine water	SPE; elution with methanol and evaporation	UHPLC-MS/MS	0.213 ng/L (LOQ)	Not reported	Loos et al. 2013b

Table 7-2. Analytical Methods for Determining DEET and Transformation Products in Environmental Samples

Sample matrix[a]	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Air	Collection with quartz filters followed by extraction with DCM/methanol (3:1 v/v)	GC with mass selective detector	No data	58	Cheng et al. 2006
Water, cola, and soft drinks	Extracted with DCM (water) or heptane (soda); dried with sodium sulfate; nonane added as keeper solvent; samples evaporated down to nonane amount	GC-HRMS	Not reported	Not reported	Chandramouli et al. 2004
Seawater	Extraction using a polymeric sorbent; elution with ethyl acetate followed by n-hexane/ ethyl acetate; rotary evaporation; iso-octane added as a keeper	GC/MS	26 pg/L	68±12	Weigel et al. 2002
Raw materials and cosmetic products	Samples prepared in ethyl acetate	HPTLC-UV	25 ng	Not reported	Markovic et al. 1999

CLLE = continuous liquid-liquid extraction; DCM = methylene chloride; GC = gas chromatography; FID = flame ionization detector; HLB = hydrophilic-lipophilic-balanced; HRMS = high-resolution mass spectrometry; LOQ = limit of quantification; MS = mass spectrometry; MS/MS = tandem mass spectrometry; MTBE = methyl-tert butyl ether; RSD = relative standard deviation; SDBV = styrene divinylbenzene; SIM = selected ion monitoring; SIS = selected ion storage; SPE = solid-phase extraction; UHPLC = ultra-high performance liquid chromatography; UV = ultraviolet absorbance detection

designed to determine the health effects (and techniques for developing methods to determine such health effects) of DEET.

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. Methods for the detection of DEET in human urine (Olsson et al. 2004; Smallwood et al. 1992; Wu et al. 1979) and serum (Cherstniakova et al. 2006; Smallwood et al. 1992) are available. These methods are sensitive and detect levels of DEET at background levels in the population, levels at which biological effects may occur. No data needs are identified for DEET-specific analytical methods. DEET rapidly metabolizes after absorption, however, suggesting that DEET concentrations in urine may not be the best biomarker. The Fourth National Report on Human Exposure to Environmental Chemicals (CDC 2017) includes results from the assessment of DEET levels and its metabolites, DHMB and DCBA, in NHANES for urine samples. An analytical method for detecting the main DEET metabolites, DHMB and DCBA, in urine has been validated by Kuklenyik et al. (2013). This area may be a potential focus for further investigation.

Methods for Determining Parent Compounds and Degradation Products in Environmental Media. Analytical methods are available to measure levels of DEET in air (Cheng et al. 2006) and water media (including waste water) (Trenholm et al. 2008; Weeks et al. 2012; Weigel et al. 2002; Zaugg et al. 2002). Studies describing methods for identifying DEET in soil or sediment samples would be useful; however, it is likely that liquid extraction of DEET from solid media followed by standard analytical methods described above for biological or environmental samples would be effective.

7.3.2 Ongoing Studies

No ongoing analytical studies for DEET were identified using the NIH RePORTER version 6.1.0 or the DTIC online database.