7. ANALYTICAL METHODS

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

Diazinon is widely used for agricultural purposes, which may result in human exposure during application, and residues on or in foods can result in exposure of humans by ingestion. Although all indoor and outdoor residential use has been phased out and cancelled, additional exposure potentials exist as a result of home gardening activities and pet pest control. Consequently, methods for the determination of diazinon in biological samples can be used to verify that exposure and absorption has occurred. Since diazinon is rapidly metabolized, determination of the parent compound can only provide evidence of very recent exposures (see Chapter 3). Methods have been reported for metabolites, and these are briefly discussed below and under Biomarkers of Exposure. Table 7-1 lists the applicable analytical methods for determining diazinon and its metabolites in biological fluids and tissues.

The principal method used for the detection of diazinon or its metabolites in biological samples is gas chromatography (GC) using a flame photometric detector (FPD), a mass spectroscopy detector (MS), an electron capture detector (ECD), or a flame ionization detector (FID). The preparation of samples usually involves variations of solid-phase extraction (SPE), and/or liquid/liquid extraction with organic solvents.

Garcia-Repetto et al. (2001) reported a method for diazinon identification and quantification in human blood using SPE, GC-nitrogen phosphorus detection (NPD) analysis followed by GC-MS confirmation. The average recovery of diazinon in blood is 87.92%, which is in the acceptable range established by the EPA. The limit of detection (LOD) and the limit of quantitation (LOQ) reported in the study are 1.97 and 6.58 μg/L, respectively. This method has improved a previous method that involved liquid-liquid

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

Sample matrix[a]	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Human blood	Addition of 1 mg/L azobenzene, 0.2 g ammonium sulfate and 2 mL 0.1 M sulfuric acid to a 0.5 mL sample of blood. Mixture is sealed and heated in a vial. Samples are collected by HS-SPME.	GC/MS	0.01 µg/g	Absolute recovery compared to a methanolic solution: 2.9%	Musshoff et al. 2002
Human fatty tissue (from greater omentum)	Tissue pulverization and extraction with acetone. Concentration and purification by sweep co-distillation and Florisil/anhydrous sodium sulfate column chromatography. Elution with 20% ether in hexane followed by hexane. Addition of internal standard.	GC/NPD	No data	No data	Kirkbride 1987
Human adipose, bile, blood, brain, stomach contents, kidney, and liver	Maceration of 0.5 g sample in tissue grinder with acetonitrile. Addition of aqueous sodium sulfate and partitioning into hexane. Concentration and clean up using Florisil column.	GC/ECD; GC/FID	No data	No data	Poklis et al. 1980
Human urine (DEP, DETP)	Dilution of urine with acetonitrile, azeotropic distillation for water removal, evaporation of solvent, redissolution in acetone and derivatization using penta-fluorobenzyl bromide.	GC/FPD	DEP: 0.072 ppm; DETP: 0.041 ppm	DEP: 96 (4.7% RSD); DETP: 99 (2.4% RSD) at 0.8 ppm	Reid and Watts 1981
Human urine (2-isopropyl-6-methyl-4-pyrimidinol)	Solid phase extraction with or without liquid/liquid partitioning.	GC/MSD	0.01 ng	101.3% for SPE alone; 100.8% for partitioning and SPE	Yokley et al. 2000
Human urine (2-isopropyl-6-methyl-4-pyrimidinol)	Hydrolysis with ∃-glucuronidase, solid phase extraction, liquid/liquid extraction, and evaporation.	HPLC-MS/MS	0.2 ng/mL urine	116% low dose; 93% high dose	Olsson et al. 2003
Bovine liver, rumen content (partially digested grain and vegetation mixture)	Extraction of homogenized sample with methanol-dichloromethane (10–90, v/v) followed by gel permeation chromatography and silica gel solid phase extraction clean-up.	GC/FPD	0.01–0.05 µg/g using 5 g sample	Rumen content: 95 (3% RSD) at 0.1 µg/g; liver: 88 (5% RSD) at 0.05 µg/g	Holstege et al. 1991

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

Sample matrix[a]	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Animal fat	Sweep codistillation, Florisil clean up-elution with methylene chloride-light petroleum-acetonitrite (50:48.5:1.5).	GC/FPD	No data	90 (6% RSD) at 0.4 mg/L	Brown et al. 1987

[a]Diazinon is the target analytes unless otherwise specified.

DEP = O,O-Diethyl phosphate; DETP = O,O-Diethyl phosphorothionate; ECD = electron capture detector; FID = flame ionization detector; FPD = flame photometric detector; GC = gas chromatography; HPLC = high-performance liquid chromatography, HS = head space, MS = mass spectrometry; MS/MS = tandem mass spectrometry, MSD = mass selective detection; NPD = nitrogen phosphorus detector, RSD = relative standard deviation; SPE = solid-phase extraction; SPME = solid-phase microextraction

extraction with *n*-hexane and benzene resulting in more complex chromatograms. Not only is the method more precise, it also eliminates hazardous waste emissions and exposure of technicians to toxic solvents.

A method for the determination of diazinon in human serum has been published by researchers at the Centers for Disease Control and Prevention (Liu et al. 1994) in which 2-dimensional chromatography was used to determine 15 pesticides in 4 minutes. Supercritical fluid extraction (SFE) was used to recover pesticides into methylene chloride and this extract was analyzed using two 2-meter columns connected by an on-column thermal desorption modulator. Sensitivity for diazinon was reported to be 1.8 pg on-column; no details about overall recoveries were provided (Liu et al. 1994).

Yokley et al. (2000) developed a method for valid, precise, and accurate determination of the metabolite, 6-methyl-2-(1-methylethyl)-4(1H)-pyrimidinone (G-27550), of diazinon in urine. The sample can be prepared by SPE with an LOQ of 1.0 μg/L, or by SPE in conjunction with liquid/liquid partitioning (LOQ of 0.50 μg/L). Average recoveries of G-27550 for each sample preparation method are 101.3 and 100.8%, respectively. The final analysis was done by GC/mass selective detection (MSD). The LOD for G-27550 was 0.01 ng. The report states that this is an accurate Good Laboratory Practice (GLP)-validated method that may be used as a biomonitoring tool to determine potential diazinon exposure in humans (Yokley et al. 2000).

A method for the rapid quantification of diazinon metabolite 2-isopropyl-6-methyl-pyrimidin-4-ol in human urine using liquid chromatography/electrospry ionization-tandem mass spectrometry has been published (Olsson et al. 2003).

Diazinon was determined in bovine liver and rumen content by GC/FPD by Holstege et al. (1991) using a method with an LOD reported to be 0.01–0.05 μg/g using a 5-g sample. Recoveries were reported to be 95% from rumen content and 88% from liver. Brown et al. (1987) used GC/FPD and sweep codistillation to determine diazinon in animal fat. The recovery was stated to be 90% (6% relative standard deviation [RSD]) at 0.4 ppm; no LOD information was given (Holstege et al. 1991).

7.2 ENVIRONMENTAL SAMPLES

Table 7-2 lists the methods used for determining diazinon and some of its degradation products in environmental samples. The principal separation and detection methods of diazinon and degradation products in environmental samples include GC or high performance liquid-chromatography (HPLC), in

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

Sample matrix[a]	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Air, gloves (surrogate for dermal exposure)	Preconcentration from air sample using PUF. Soxhlet extraction of PUF or gloves with 5% ethyl ether/hexane. Addition of deuterated internal standards and concentration using K-D and nitrogen blowdown.	Capillary GC/MS (can use multiple ion detection)	55 ng/m^3 (5.5 m^3 sample)	73% (14% RSD)	Hsu et al. 1988
Air (diazinon, diazoxon)	Preconcentration using ORBO-42 pesticide adsorbent tubes (Supelco). Extraction with acetone, evaporation just to dryness and redissolution in 100 µL acetone containing internal standard.	Capillary GC/NPD	No data	90% at 0.1% and 1 µg/m^3 (diazinon)	Williams et al. 1987
Air	Preconcentration of pesticide onto OVS-2 tube (13 mm quartz filter, XAD-2, 270 mg/140 mg. Elution with 90% toluene/10% acetone.	GC/FPD (NIOSH Method 5600)	0.0004 mg/m^3 (400 ng/m^3) for 120 L sample.	94% (2.7% RSD at 2.4 µg (0.01 µg/m^3, 240 L sample)	NIOSH 1994
Air	Air is drawn through a glass tube with a glass fiber filter and XAD-2 adsorbent. The samples are desorbed with toluene.	GC/FPD	3.0 µg/m^3	91.2%	OSHA 1986
Drinking water	Preconcentration onto 5 µm C_{18}-silica or 7 µm polystyrene-divinyl benzene co-polymer with subsequent backflush onto analytical HPLC column.	RP-HPLC/UV (254 mn)	0.03–0.06 µg/L (ppb)	91% (±10% RSD) at sample volumes up to 300 mL	Driss et al. 1993
Ground-water and finished drinking water	Extraction with methylene chloride. Drying and concentration of extract then resolution in MTBE.	GC/NPD	0.13 µg/L	94% (18% RSD)	EPA 1995b
Drinking water, river water	Preconcentration of 2.5 mL water onto C_{18} extraction disks, rinsing with additional 1 mL and purging disk with gas to remove residual water. Elution with ethyl acetate directly onto GC pre-column with solvent venting.	GC/NPD	Tap water: 20 pg/mL; river water: 20–50 pg/mL	>95% (<4% RSD at 200 ppt)	Kwakman et al. 1992

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

Sample matrix[a]	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Pond water	Micro liquid-liquid extraction of 1.5 mL water with 1.5 mL methyl t-butyl ether; 500 µL of extract slowly introduced into GC pre-column with solvent venting.	cap. GC/FPD	0.02 µg/L	102% (5% RSD) at 0.50 µg/L level	van der Hoff et al. 1993
Surface water	Adsorption of pesticides from 2 L of water onto XAD-2 and XAD-7 resins. Elution with methylene chloride, water removal and use of K-D to reduce volume.	GC/chemical ionization ion trap MS	0.0005 µg/L	103.8% (14% CV) at 1 ppb level	Mattern et al. 1991
Raw water and finished drinking water	SPE then elution under vacuum with ethyl acetate and dichloromethane. The extract is dried and concentrated.	GC/MS	0.015 µg/L	101% (3% RSD)	EPA 2000b
Finished drinking water, source water, or drinking water in any treatment stage	LSE followed by extraction with ethyl acetate and methylene chloride then evaporation of solvent.	GC/MS	0.11 µg/L	83% (9% RSD)	EPA 1995a
Filtered waste water and natural-water samples	Filtration of field samples using glass-filter fibers and SPE. Elution of dry SPE cartridges with dichloromethane and diethyl ether (4:1) followed by gentle evaporation of extract.	GC/MS	0.07 µg/L	93% (4% RSD)	USGS 2002b
Water	Filtration using glass-fiber filters followed by SPE. Elution of dry SPE columns with ethyl acetate then evaporation.	GC/FPD	0.01 µg/L	88% (16% RSD)	USGS 2002c
Water	Filtration of 1 L of water followed by extraction 3 times with 100 mL methylene chloride after addition of 20 g sodium sulfate. Concentration using K-D and solvent exchange to benzene. Concentrations done under nitrogen. Fractionation by HPLC.	GC/FPD (P-mode)	0.025 µg/L	92% (2% RSD)	Seiber et al. 1990

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

Sample matrix[a]	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Water	SPME of filtered water sample; thermal desorption of diazinon from SPME fiber.	GC/AED	1 µg/L with carbon line (193 nm); 3 µg/L with S line (181 nm)	No data (precision 8–12 relative standard deviation)	Eisert et al. 1994
Water	Extraction of analytes from water using SPE; elution with ethyl acetate (108 µL) directly onto retention gap with solvent venting.	GC/AED	1 ng/L (100 mL sample) with P channel	105% (4% RSD) at 5 µg/L	Hankemeier et al. 1995
Industrial and municipal waste water	Extraction of 1 L of sample with 60 mL methylene chloride 3 times. Water removal from extract and solvent exchange to hexane during K-D concentration.	GC/FPD or thermionic detection (P-mode); GC/MS for qualitative identifications recommended. (Method 1657)	0.6 µg/L	67% (6% RSD)	EPA 1993a
Waste water	Extraction of 1 L of water with 15% methylene chloride in hexane using a separatory funnel. Concentration using K-D. Cleanup (if needed) by Florisil fractionation or acetonitrile partition.	GC/FPD (P-mode) or GC/thermionic detection. GC/MS for qualitative compound identification recommended. (Method 614)	0.012 µg/L	94% (5.2% RSD)	EPA 1993b
Water	Direct injection or liquid/liquid extraction and concentration.	HPLC/UV	0.5 mg/L (direct injection); 0.5 µg/L (liquid/liquid extraction)	No data	Mallet et al. 1990
Bed sediment (lake and stream), aqueous suspended sediment and soil	Extraction with Soxhlet apparatus of minimum 25-g equivalent dry-weight samples using 350 mL dichloromethane and 25 mL methanol (93:7). Concentration and filtration of extract. Elution with dichloro-methane through chromato-graphic column. Concentration and resolution in ethyl acetate.	GC/FPD	1.24 ppb	71% (7% RSD)	USGS 2002d

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

Sample matrix[a]	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Soil (diazinon, diazoxon, 2-isopropyl-4-methyl-6-hydroxy-pyrimidine)	Extraction of soil with hexane:acetone (1:1), centrifugation, separation of hexane from acetone/water layer. Extraction of acetone/ water phase with chloro-form:diethyl ether (1:1), solvent exchanged to methanol. Hexane layer contained diazinon, chloroform/diethyl ether fraction contained 2-isopropyl-4-methyl-6-hydroxy-pyrimidine.	TLC	No data	No data	Sethunathan and Yoshida 1969
Soil (diazinon, 2-isopropyl-4-methyl-6-hydroxy-pyrimidine)	Sequential Soxhlet using acetone then methanol.	GC, TLC, GC/MS	No data	No data	Burkhard and Guth 1979
Water, soil	*Water:* Addition of deuterated standards to 1 L water and extraction 3 times with 200 mL methylene chloride. Water removal with anhydrous sodium sulfate then concentration using K-D and nitrogen blowdown. *Soil:* Addition of 10 mL water and deuterated standards to 50 g of soil followed by equilibration for 1 hour. Sonication 3 times with acetone/hexane. Phase separation followed by water removal using sodium sulfate, concentration using K-D, and nitrogen blow-down. Spiking with phenanthrene-d_{10} before analysis.	GC/MS (SIM)	100–200 ppt for water, 2–4 ppb for soil	Water: 89.4% (4.4% RSD) at 1 ppb Soil: 103% (15% RSD) at 20 ppb	Lopez-Avila et al. 1985
Cucumber, lettuce, grapes	Chopping of produce and extraction with acetone/ methylene chloride/petroleum ether (1:1:1). Evaporation to dryness and redissolution in acetone and concentration.	SFC/NPD	No data	No data	Zegers et al. 1994a

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

Sample matrix[a]	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Green beans, lettuce, carrot, bell pepper	Homogenization of produce with acetonitrile. Addition of NaCl to affect phase separation, removal of acetonitrile, water removal volume reduction, addition of deuterated internal standards.	GC/MS	50 ppb	88% (17% RSD)	Liao et al. 1991
Kale, endive, carrots, lettuce, apples, potatoes, strawberries	Extraction of crops with ethyl acetate and granular sodium sulfate, filtration, concentration with K-D. Sweep co-distillation cleanup for GC.	GC thermionic detector	No data for GC	No data	AOAC 1990a
Numerous non-fatty crops	Extraction with acetonitrile and partition into petroleum ether. Concentration using K-D and purification using Florisil column chromatography.	GC/KCl thermionic detector; identifications by combin-ations of gas, thin layer, and paper chroma-tography; polarographic confirmatory method	Polarographic method: 0.2 ppm based on 1 g crop in 1 mL cell	80%	AOAC 1990a, 1990b, 1990c
Soybeans and rice	Grinding of 25 g samples and extraction with 150 mL of 2:1 acetone: methanol; filtration and reduction of volume to 100 mL. Addition of water, NaCl followed by extraction with methylene chloride (2x); solvent evaporation and redissolution in methylene chloride:cyclohexane (1:1) and fractionation on Bio-Bead S-X3. Evaporation under N_2 stream and redissolution in 2 mL hexane.	GC/NPD or GC/MS (SIM)	Rice: 0.01 ppm soybeans: 0.05 ppm	Rice: 83.4% (1.5% RSD) at 1 ppm soybeans: 62.7% (8.6% RSD) at 1 ppm	Hong et al. 1993
Sweet cherries for baby food	Extraction with methylene chloride and cleaning with quaternary aminesilane-silica-dichloromethane.	GC/electron capture detection/ FPD and GC/NPD/ FPD	30 ppt	≥70% at 0.01 ppm	Bicchi et al. 1997

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

Sample matrix[a]	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Strawberries and cherries	Spike samples were sliced and homogenized.	HS-SPME	8.4 ppb in strawberries; 10.2 ppb in cherries	75–88%	Lambropoulou and Albanis 2003
Various fruits and vegetables	Homogenization of sample (adding water if needed) and adsorption on activated Florisil to produce a free-flowing powder. Elution with ethyl acetate or methylene chloride.	GC/NPD	4 ppb	91–103% at 0.05 mg/kg	Kadenczki et al. 1992
Various produce	Homogenization of sample and extraction with acetonitrile, filtration, addition of salt and solvent evaporation. Redissolution of residue in acetone for analysis.	GC/FPD or alkali FID	100 ppb	96% (17% RSD)	Hsu et al. 1991
Various prepared foods	Blending of sample with acetone, filtration and transfer to Hydromatrix column. Elution with methylene chloride and concentration.	GC/FPD	No data	91% at 100 ppb	Hopper 1988
Pasta, eggs	Blending of samples with acetone and extraction with dichloromethane and acetone, water removal and volume reduction. Cleanup using carbon-celite (pasta) or C_{18} SPE (eggs).	GC/FPD	~1 ppb	Pasta: 80% at 30 ppb; eggs: 93% at 13 ppb	Leoni et al. 1992
Cow's milk	Extraction of milk 3 times with 70% acetonitrile in water, filtration, removal of fat by zinc acetate addition, and partitioning with methylene chloride. Reduction of volume after drying.	GC/FPD (P-mode)	10 ppb	89% (3.8% RSD) at 100 ppb	Toyoda et al. 1990
Cow's milk	Homogenization of milk, acetonitrile and ethanol followed by equilibration with a mixture of light petroleum-acetonitrile-ethanol and separation of the upper phase and elution through a solid matrix cartridge. Concentration and drying of the eluates to a residue that is dissolved.	GC/FPD	No data (0.003 MDL)	84% at 0.42 µg/mL	Di Muccio et al. 1996

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

Sample matrix[a]	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Lanolin	Dissolution in hexane and extraction with acetonitrile. Addition of 5% NaCl in water to acetonitrile and back-extraction with hexane. Washing of hexane extract with water, volume reduction and fractionation using Florisil.	GC/FPD (526 nm); GC/AED; GC/MS	GC/FPD 0.03 ppm; GC/AED 0.6 ppm (phosphorus monitor); 0.3 ppm (sulfur monitor); GC/MS 0.6 ppm	90% (6.4% RSD) at 1 ppm; 95% (5.6% RSD) at 2 ppm	Miyahara et al. 1992

[a]Unless otherwise stated, diazinon was determined.

AED = atomic emission detection; FPD = flame photometric detector; FPD = flame photometric detector; GC = gas chromatography; HPLC = high performance liquid chromatography; HS = head space, KCl = potassium chloride; K-D = Kuderna-Danish; LSE = liquid-solid extraction, MDL = method detection limit; MS = mass spectrometry; MTBE = methyl tert-butyl ether; NaCl = sodium chloride; NPD = nitrogen phosphorus detector; PUF = polyurethane foam; RSD = relative standard deviation; SFC = supercritical fluid chromatography; SIM = selected ion monitoring; SPE = solid phase extraction; SPME = solid-phase microextraction, TLC = thin layer chromatography; UV = ultraviolet absorbance detection

conjunction with a MS detector, an NPD, or an FPD. Sample preparation methods vary depending on the sample matrix (EPA 1995a, 1995b; Mallet et al. 1990; OSHA 1986). The method of Williams et al. (1987) is applicable to both diazinon and diazoxon. The NIOSH method (NIOSH 1994) has been fully validated for use in occupational settings where regulatory exposure limits are of concern.

Many methods were reported for the determination of diazinon in water. Sample preparation methods include either some form of liquid/liquid extraction or the use of SPE, usually C_{18}-silica, for isolation of diazinon residues. Mallet et al. (1990) reported a method for environmental water based on HPLC/ultra violet (UV) absorbance detection with either direct injection of the water or of an aliquot of an extract. The LODs were as low as 0.5 μg/L with the extraction approach. Mattern et al. (1991) reported a LOD for diazinon in surface water of 0.0005 ppb using GC in conjunction with chemical ionization ion trap MS. Lopez-Avila et al. (1985) reported an isotope dilution GC/MS selected ion monitoring (SIM) method that is applicable to water or soil after solvent extraction. Recoveries were stated to be 89% at 1 ppb in water and 103% at 20 ppb in soil. An LOD of 0.025 μg/kg was reported for diazinon in water with a recovery of 92% (2% RSD) by Seiber et al. (1990). SPE provides an easy method to isolate residues and can greatly reduce the amounts of solvent used in sample preparation. Driss et al. (1993) preconcentrated diazinon from drinking water onto C18-silica or polystyrene-divinylbenzene co-polymer with a subsequent backflush onto an HPLC column (UV detection). LODs as low as 30 μg/L were reported. Kwakman et al. (1992) preconcentrated diazinon from drinking and river water onto C_{18}-SPE disks and eluted the adsorbed compounds directly into a GC pre-column. Detection was by NPD and excellent LODs (20 pg/L) and recoveries (>95% with <4% RSD at 200 pg/L) were reported. Although most of the SPE methods boasted good recoveries and LODs, one reference noted that the pesticide can associate with dissolved organic matter (primarily humic materials) resulting in poor retention by the SPE material (Johnson et al. 1991). This can reduce method recoveries.

Diazinon can be measured in air after pre-concentration from air onto some adsorbent material with subsequent extraction. A method for diazinon in air has been reported that is based on the use of polyurethane foam (PUF) to adsorb the pesticide from the air as the air is pulled through the PUF (Hsu et al. 1988). The PUF is then Soxhlet-extracted and the extract volume reduced prior to capillary GC/MS analysis. An LOD of 55 ng/m^3 (5.5 m^3 sample) and recovery of 73% were reported. Another study was described in which the diazinon levels in indoor air were monitored following periodic application of the pesticide for insect control (Williams et al. 1987). In this method, air is pulled through a commercially available adsorbent tube to concentrate diazinon. The tube is then extracted with acetone prior to GC/NPD analysis. No data were provided for the LOD, but recoveries in excess of 90% were reported at

the 0.1 and 1 μg/m^3 levels. This paper also indicated that diazinon can be converted to diazoxon by ozone and NO_x in the air during the sampling process.

SFE is also used in sample preparation methods. Supercritical trifluoromethane has been shown to extract diazinon from glass beads with a recovery of 86% (Hillmann and Bächmann 1995). Organophosphorus pesticides have also been recovered from Tenax-GC, an adsorbent used to collect diazinon during air sampling, and analyzed directly by GC (Raymer and Velez 1991). Supercritical fluid chromatography (SFC) has also been used for the determination of diazinon in water where 75 μL were injected (Zegers et al. 1994b). Using thermionic detection, the LOD was about 1 μg/L (1 ppb) with a reproducibility of better than 7% at the 5–15 μg/L level. The same authors also published an SFC-based method for cucumber, lettuce, and grapes (Zegers et al. 1994a) but did not specify the LOD and recovery.

Three standardized methods were found in the *Official Methods of Analysis of the Association of Official Analytical Chemists* (AOAC 1990a, 1990b, 1990c). The first of these methods is based on the extraction of crops (kale, endive, carrots, lettuce, apples, potatoes, and strawberries) with ethyl acetate and isolation of the residue followed by a sweep codistillation cleanup prior to GC/thermionic detection (Method 968.24). In the second method (Method 970.52), the sample is extracted with acetonitrile, and the residue is partitioned into petroleum ether followed by Florisil clean-up and GC/potassium chloride (KCl) thermionic detection. Chemical identifications are based on combinations of gas, thin-layer, and paper chromatography. The recovery for diazinon in this method is stated to be greater than 80%; no data on limits of detection were given. The third method utilizes the same extraction and clean-up techniques as the second and then GC/FPD for detection (Method 970.53).

Several methods employ the homogenization of the plant material with aqueous acetonitrile (Hsu et al. 1991; Liao et al. 1991) or other polar organic solvents such as acetone/methanol mixtures (Hong et al. 1993). Phase separation is brought about with the addition of a salt. The acetonitrile approach is preferred by the California Department of Food and Agriculture because of the higher recoveries possible (see Table 7-2) (Lee et al. 1991). The advantage of acetonitrile is found in its ability to more readily solvate residues and in the ease with which the phase separation can be accomplished through the addition of salt (Lee et al. 1991). Reported LODs for diazinon were typically 10–50 ppb. One of the methods eliminated any clean-up steps after the initial extraction (Hsu et al. 1991) to provide a method with a faster turnaround time with some loss in sensitivity (LOD approximately 100 ppb) relative to the purified samples.

Methods found for the determination of diazinon in animal products also used homogenization with a polar organic solvent as the first step in residue recovery. Toyoda et al. (1990) isolated diazinon from cow's milk via partition into methylene chloride after extraction of the milk with 70% acetonitrile in water. Based on GC/FPD, an LOD of 10 ppb and a recovery of 89% (3.8% RSD) at 100 ppb were reported. Diazinon residues in eggs were studied (Leoni et al. 1992) after blending the eggs with acetone and partitioning into dichloromethane and acetone followed by C_{18}-silica SPE. Based on GC/FPD analysis, an LOD of 1 ppb and a recovery of 93% at 13 ppb were reported.

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 diazinon 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 diazinon.

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. Section 3.8.1 reported on biomarkers used to identify or quantify exposure to diazinon. Some methods for the detection of the parent compound in biological samples were described above. The parent chemical is quickly metabolized so the determination of metabolites can also serve as biomarkers of exposure. The use of liquid chromatography (LC) coupled to hybrid quadruple time-of-flight (QTOF) MS has recently been reported for the elucidation and confirmation of diazinon metabolites in biological samples (Ibanez et al. 2006). The most specific biomarkers will be those metabolites related to 2-isopropyl-6-methyl-4-pyrimidine (IMHP). Methods for the detection of this compound in human urine have been reported (Olsson et al. 2003; Yokley et al. 2000). Also a method for IMHP and its oxidized

metabolite, 2-(1'-hydroxy-1'-methyl)-ethyl-6-methyl-4-hydroxypyrimidine, in dog urine has been described by Lawrence and Iverson (1975) with reported sensitivities in the sub-ppm range. Other metabolites most commonly detected are diethylphosphate (DEP) and diethylthiophosphate (DETP), although these compounds are not specific for diazinon as they also arise from other diethylphosphates and phosphorothioates (Drevenkar et al. 1993; Kudzin et al. 1991; Mount 1984; Reid and Watts 1981; Vasilic et al. 1993). Further studies designed to refine the identification of metabolites specific to diazinon and provide dosimetric data will be useful in the search for a more dependable biomarker of diazinon exposure.

Effect. Biomarkers of effect include plasma cholinesterase (ChE) and erythrocyte (RBC) and brain acetylcholinesterase (AChE), enzymes inhibited by insecticidal organophosphorus compounds (see Chapter 3). Rapid, simple, and specific methods should be sought to make assays readily available to the clinician. Currently, no effect specific to diazinon exposure has been identified by any study. Future studies designed to provide such information would be useful in identifying exposure to diazinon.

Methods for Determining Parent Compounds and Degradation Products in Environmental Media. Human exposure to diazinon occurs via inhalation of ambient air; ingestion of contaminated food and water; and dermal uptake through occupational and non-occupational contact with contaminated soils, surface water, and commercial preparations. Methods have been reported for the measurement of diazinon in various foods, soils, sludges, sediment, solid wastes, waste water, drinking water, and air. The methods of Hsu et al. (1988) (LOD of 55 ng/m^3) Burright (OSHA 1986) (LOD 3.0 μg/m^3) are adequate for the determination of diazinon in air. If a 70-kg individual is assumed, method LODs of 0.007 mg/L (7 ppb) and 0.007 mg/kg (7 ppb) in water and foods, respectively, are required for the method to be adequate at the oral intermediate MRL. All of the methods for detection of diazinon in water shown in Table 7-2 are adequate. With regard to foods, the methods of Kadenczki et al. (1992) and Leoni et al. (1992) for detection of diazinon are adequate. Methods for other non-fatty crops would need to be validated or developed if routine use were desired. Di Muccio et al. (1996) describe a quick and simple method for the determination of diazinon in cow's milk; however, no data were provided given on LODs. Additional methods for detection of diazinon in fatty foods are needed to permit the evaluation of the residues in those fatty media.

There are also methods for the analysis of diazinon degradation products in air, water, and soil. Ibanez et al. (2006) have reported a method for the elucidation and confirmation of degradation products in environmental samples. Williams et al. (1987) published a method for diazinon and its oxon (diazoxon)

in air. Other methods have been reported for diazinon, its oxon, and hydrolysis products in water (Suffet et al. 1967), soils and water (Lichtenstein et al. 1968), and soil (Burkhard and Guth 1979). The hydrolysis product IMHP was studied along with diazoxon in submerged soil (Sethunathan and Yoshida 1969). Suffet et al. (1967) demonstrated the ability of GC to separate diazinon, diazoxon, and IMHP. However, no validated methods for the determination of diazoxon or IMHP were found. Thus, additional methods are needed for the quantitative analysis of diazinon transformation products in environmental matrices. It will also be important to establish MRLs for the transformation products to put the analytical requirements into perspective.

7.3.2 Ongoing Studies

The following information was found as a result of a search of the Federal Research in Progress database (FEDRIP 2006).

Researchers at the University of Puerto Rico, Clemson University, and the University of Tennessee are collaborating in a Multi-State Regional project funded by the U.S. Department of Agriculture to develop environmentally friendly procedures and methods for water sampling of crop management chemicals, including diazinon, which can be used in field situations. Procedures for solid-phase field extraction techniques of water, and stability during storage and shipment of the field water samples to analytical laboratories for testing is being investigated. The study began on October 1, 2003 and is projected to end September 30, 2008. Recent results are to be published in the Journal of Agriculture and Food Science.