Health Consultation

Exposure Investigation

Biological Monitoring for Exposure to Herbicides

HIGHWAY 36 CORRIDOR

LANE COUNTY, OREGON

Cost Recovery Number: AA1200

MARCH 5, 2012

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service
Agency for Toxic Substances and Disease Registry
Division of Health Assessment and Consultation
Atlanta, Georgia 30333

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Executive Summary

The Agency for Toxic Substances and Disease Registry (ATSDR), the Pesticide Analytical Response Center (PARC) led by the Oregon Health Authority (OHA), and the Environmental Protection Agency (EPA) conducted an Exposure Investigation (EI). The purpose of this EI was to assess whether, and to what extent, residents of western Lane County, Oregon, who live near clear-cut areas, are being exposed to the herbicides, atrazine and 2,4-dichlorophenoxyacetic acid (2,4-D).

In this EI, we conducted urine biomonitoring in 64 volunteers from 38 households who live near forested areas where herbicides are applied to tracts of land that have been recently clear-cut. While forestry is the predominant land use in this area, pesticides are also used within or near the sample area for agricultural, road right-of-way, residential, and other uses.

After the land is clear-cut and tree seedlings are planted, landowners often apply herbicides one to three times to suppress the growth of competing plants. Urine samples for this EI were collected on August 30 and 31, 2011, prior to the forestry fall spraying season. At this time of year, herbicide use in forest areas is at or near its lowest level.

None of the urine samples contained a detectable concentration of atrazine or its metabolites, indicating there was no recent exposure at the time of testing.

The concentrations of 2,4-D in urine samples from the EI participants were compared to a national sample from the National Health and Nutrition Examination Survey (NHANES). Based on this comparison, the fraction of the EI participants above the NHANES 75th percentile was higher than expected. This suggests an increased exposure relative to the rest of the United States.

Despite an apparent greater exposure than the US population, these data indicate that, at the time of testing, the participants were not exposed to 2,4-D at levels that are expected to cause adverse health effects.

Further testing is needed to assess short-term exposures that could occur immediately following herbicide application to recently clear-cut forestlands.

Introduction

People living in the Triangle Lake area near Highway 36 in western Lane County, Oregon, near clear-cut spray areas have expressed concern for several years over possible exposures to herbicides from spraying on nearby forestland. Some local residents believe these exposures have resulted in symptoms and health effects such as rashes, dizziness, fertility issues, difficulty in breathing, excess miscarriages, birth defects, and rare cancers in young people.

Forest management practices vary with different landowners, but larger landowners typically clear-cut forest stands that they consider ready for harvest, and then replant the harvested areas. Control of vegetation that would compete with newly planted seedlings is needed to allow the seedlings to grow to meet landowner objectives and to comply with the reforestation requirements in the Oregon Forest Practices Act (OAR 629-610-0040(4))[1]. To control competing vegetation, larger landowners typically apply herbicides one to three times (sometimes, one or more applications are needed) within the first four years after harvest.

Herbicides are applied in three main ways. Aerial application involves the use of aircraft, primarily helicopters, to broadcast spray clear-cut areas. Hand spraying involves the use of ground-based workers with backpack sprayers. A third ground-based approach involves hack-and-squirt (cutting the competing vegetation and spraying herbicides on the exposed stem) or injecting herbicides into the stem of the competing vegetation.

Because some residents live in close proximity to the clear-cut areas, it is possible that herbicide drift from the spray areas could settle on residential properties, resulting in exposures. Other possible exposure pathways include surface water runoff from sprayed areas onto down-gradient residential properties, contamination of springs and ground water used for domestic purposes, and contamination of homegrown and wild foods, and home-raised animals used for food.

Typically, the forest applications occur in late summer and early spring. Different tracts of land are treated at different times at the discretion of the individual landowners. Atrazine and 2,4-dichlorophenoxyacetic acid (2,4-D) are two of the herbicides that are commonly applied to the replanted areas, although several other herbicides are also used[2]. The mixture and application rate of herbicides vary by season of application and type of competing vegetation.

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[1] http://www.oregon.gov/ODF/privateforests/fpaKeys.shtml

[2] Landowners have reported to the Oregon Department of Forestry that the following herbicides were applied to clear-cut areas in the Highway 36 corridor during the past several years: Atrazine, Hexazinone, Imazapyr, Sulfometuron Methyl, Metsulfuron Methyl, 2,4-D, Clopyralid, Glyphosate, Triclopyr, Aminopyralid, and Picloram.

In the spring of 2011, a researcher at Emory University analyzed urine samples from 21 residents of Lane County for herbicides. The researcher reported that the participating residents had elevated concentrations of 2,4-D and a metabolite of atrazine (diaminochlorotriazine [DACT]) in their urine (Barr 2011).

In response to these findings and the concerns of the community, Oregon's multi-agency coordinating body, the Pesticide Analytical Response Center (PARC), initiated an investigation. As the lead PARC member agency for this investigation, the Oregon Health Authority (OHA) requested assistance from ATSDR in conducting biomonitoring to evaluate the residents' exposures to herbicides used on clear-cut areas. To assess these possible exposures, ATSDR tested participants' urine for the herbicides 2,4-D and atrazine, including its principal metabolites.

Atrazine is a moderately persistent pesticide in the environment. In soil, atrazine has a half-life of 14-109 days; whereas in water, its half-life can be 200 days or more (ATSDR, 2003). 2,4-D is not persistent in terrestrial environments (half-life = 6.2 days), moderately persistent in aerobic aquatic environments (half-life = 45 days), and highly persistent in anaerobic terrestrial and aquatic environments (half-life = 231 days) (US EPA, undated).

While this report focuses on findings from the urine biomonitoring, these results are only one part of a larger PARC-led investigation that includes sampling of food, drinking water, and soil from the properties of participants in this investigation. These urine biomonitoring results will be reported again in the context of the broader investigation in a Public Health Assessment to be authored by the OHA as other sampling data become available.

Project Overview

Purpose

The purpose of this Exposure Investigation (EI) was to conduct urinary biomonitoring to measure exposure to the herbicides, atrazine and 2,4-D, in residents living along the Highway 36 corridor. These chemicals were selected as target compounds because: (1) the National Center for Environmental Health laboratory has existing analytical methods for these chemicals, (2) these chemicals (or their metabolite(s)) are included in the National Health and Nutrition Examination Survey (NHANES), (3) these chemicals are commonly sprayed on the clear-cut areas, and (4) to follow-up an earlier report that elevated concentrations of these chemicals were detected in some residents.

If humans were to ingest these herbicides, they would be rapidly excreted in the urine. The urinary elimination half-life for atrazine is 24-28 hours; the urinary elimination half-life of 2,4-D is 18 hours (Gilman et al. 1998; Sauerhoff et al. 1977). Therefore, urinary biomonitoring for these chemicals would reflect recent exposures to these herbicides.

Urine samples were collected on August 30-31, 2011, prior to fall spraying operations. Thus, this EI did not measure acute exposures to herbicides from spraying operations. Rather, it measured the participants' exposures to these herbicides from potential sources such as residual environmental contamination of air, water, homegrown and wild plants and animals, and other dietary[3] sources of contamination.

The participants of this EI were self-selected (not randomly selected) residents who lived within 1.5 miles of a 2010-2011 clear-cut spray area. Therefore, the test results from this investigation are specific to these participants and are not generalizable to the community-at-large or to other populations

Investigators and Collaborators

The ATSDR Exposure Investigation and Site Assessment Branch (EISAB) was the lead for this Exposure Investigation. This EI was a collaborative effort of ATSDR and the OHA. The National Center for Environmental Health (NCEH) laboratory contributed to the investigation by analyzing the urine samples. The specific roles of the agencies that participated in this investigation were:

The ATSDR Exposure Investigation and Site Assessment Branch (EISAB):

(1) Developed the EI protocol

(2) Worked with OHA to conduct the field activities and obtain written informed consent/assent/parental permission for testing

(3) Collected urine samples from the participants and shipped them to the NCEH laboratory for analysis

(4) Evaluated the analytical test results

(5) Notified the participants of their individual test results

(6) Prepared this report that summarizes the collective findings of the EI

The OHA:

(1) Identified and recruited participants for the EI

(2) Made appointments for sample collection

(3) Worked with ATSDR to conduct the field activities and notify the community of the findings of the EI

[3] For example, in market basket surveys, the U. S. Food and Drug Administration detected low concentrations of 2,4-D (0.002 – 0.016 ppm) in bread and cereal products (US FDA, 2006).

(4) Co-authored the EI report

The National Center for Environmental Health (NCEH):

(1) Provided supplies for collecting urine samples

(2) Analyzed the urine samples for 2,4-D, atrazine, and six of atrazine's metabolites

METHODS

Criteria for participation

Residents who lived in Lane County, Oregon, within 1.5 miles of a clear-cut area that had been sprayed with herbicides during 2010-2011 were eligible for this study. Field studies have shown that following helicopter spraying of pesticides on forestland in mountainous terrain, pesticide drift and deposition can be detected as far as 6 kilometers (3.9 miles) from the spray area (Hitch et al. 1995).

People with occupational exposure to herbicides (e.g., sprayers) were not eligible for this investigation. The only age restriction was that participants had to be at least 6 years old. This age restriction was necessary because the test results were compared to data from the NHANES national surveys, which include people 6 years of age or older (CDC, 2009).

Recruiting participants

Based on the above criteria, OHA recruited participants for this EI. Recruitment efforts included:

(1) OHA staff and a representative of ATSDR attended a public meeting on July 14, 2011, to discuss the EI and notify the community of the upcoming opportunity to participate in the testing.

(2) The Oregon Department of Forestry identified areas that had been clear-cut in 2010-2011. Clear-cut areas are typically sprayed two to three times in the first 18 months post clear-cut. Therefore, people living near these clear-cut areas were in areas likely to be sprayed.

(3) OHA contacted people who were interested in participating, who lived within 1.5 miles of the harvest boundaries of the spray areas (using GIS), and recruited them for testing.

Eligible participants had to meet the following conditions: were at least six years old, lived within the recruitment area, had no occupational exposure to pesticides, and provided informed consent/assent/parental permission.

Field activities

OHA staff made appointments to meet with the participants in their homes. Two teams, consisting of one ATSDR staff person and one OHA staff person, conducted the home visits. During this visit, agency staff administered the appropriate consent/assent/parental permission forms. In addition, OHA sent the forms to the participants prior to the home visit to ensure an adequate time to review. These forms are included in the attached protocol (Appendix A).

ATSDR then gave each participant a urine collection cup with his/her identification number. We instructed the participant to collect a urine sample as described in the Urine Collection Instructions (Appendix B of the protocol). The participants collected a urine sample of at least 40 ml in the privacy of their bathroom. The participant then capped the cup and returned the freshly voided urine sample to us. We transferred aliquots of the urine sample to cryovials and froze the samples on dry ice. Once collected, the samples were kept frozen on dry ice and locked in the trunk of our car.

To protect anonymity, the samples were labeled with a coded identification number provided by the NCEH laboratory. Each team prepared one field blank with distilled water for each day that samples were collected.

Sample handling and shipping

The urine samples were shipped within 48 hours of collection. ATSDR staff packaged the urine samples on dry ice, enclosed a chain-of-custody form, and shipped them by priority overnight delivery to the NCEH laboratory in Atlanta, Georgia, for analysis.

Laboratory processing and analysis

The NCEH laboratory analyzed the urine samples.

The urine samples were analyzed for 2,4-D, and for atrazine and its principal metabolites using published methodology (Olsson et al. 2004; Kuklenyik et al. 2011). Each analytical batch included low- and high-concentration quality control materials, standards, blanks, and the study samples. The QC concentrations were evaluated using standard statistical probability rules (Caudill et al. 2008). The analysis for atrazine included the following chemicals: atrazine, atrazine mercapturate, desethyl atrazine mercapturate, diaminochloroatrazine, desethyl atrazine, desisopropyl atrazine, and desisopropyl atrazine mercapturate. Urinary creatinine was measured to correct for urinary dilution. Results were reported in units of micrograms of analyte per gram of creatinine (µg/g) and micrograms of analyte per liter of urine (µg/L) for comparison to NHANES data.

The concentrations of 2,4-D were measured in urine by high performance liquid chromatography-isotope dilution tandem mass spectrometry using a modification of the method described in Olsson et al. 2004. A 1 mL sample of urine was extracted with a

mix-mode solid-phase extraction sorbent using a semi-automated 96-well plate technology to achieve sample purification and a concentration factor of 25. The urine extracts were then analyzed using reversed phase high performance liquid chromatography, and the target analytes were quantified by isotope dilution tandem mass spectrometry. The limit of detection (LOD) for 2,4-D was 0.1 µg/L.

The urinary concentrations of atrazine and six of its metabolites and hydrolysis products, were determined using a two dimensional high performance liquid chromatography (2D-HPLC) coupled with tandem mass spectrometry approach similar to the one described in Kuklenyik et al. 2011. Atrazine and six atrazine metabolites in one milliliter of urine were extracted using automated off-line solid phase extraction before separation by 2D-HPLC and quantification by positive ion atmospheric pressure chemical ionization isotope dilution tandem mass spectrometry. The LODs for atrazine and its metabolites were: atrazine (0.0615 µg/L), atrazine mercapturate (0.0555 – 0.0561 µg/L), desethyl atrazine mercapturate (0.0713 – 0.1061µg/L), diaminochloroatrazine (0.1447 – 0.1633 µg/L), desethyl atrazine (0.0833 – 0.0858 µg/L), desisopropyl atrazine (0.2329 – 0.2500 µg/L), and desisopropyl atrazine mercapturate (0.0532 – 0.0537 µg/L).

Urine creatinine was measured by an enzymatic method (Roche's Plus Product) on a Hitachi 912 Chemistry Analyzer.

Results

Participants

ATSDR and OHA conducted the field activities for this EI on August 30-31, 2011. During this time, we collected urine samples from 66 people in 38 households.

Two of the urine samples we collected were from children who were below the age of 6 years old. The NHANES comparison data were obtained from people who were 6 years old and above. Therefore, the test results for the two children below the age of 6 were excluded from the data analysis for this EI. However, as a public health service, we analyzed the urine samples from these two young children and provided the test results to their parents. The concentrations of 2,4-D in urine samples from these two children were below the EI group mean, and the atrazine and metabolite concentrations were below the limit of detection.

The ages of the 64 participants in this EI ranged from 6 to 80 years old, and the average age of a participant was 52 years old. There were 32 males and 32 females in the EI population.

Test Results

2,4-Dichlorophenoxyacetic acid (2,4-D)

2,4-D is rapidly excreted into the urine by the organic acid active transport process in the kidney (Berndt and Koscher, 1973). This mechanism of elimination is generally independent of urine flow (Boeniger et al. 1993). Therefore, the concentration of 2,4-D in urine is influenced by the urinary flow rate and how dilute or concentrated the urine sample is. To correct for urinary dilution, the urinary 2,4-D concentrations were normalized by expressing the concentrations in units of µg/g creatinine. Results were also reported in mass/volume concentration units of µg/L (Table 1). Most studies in the scientific literature report urinary 2,4-D concentrations in units of µg/L, so these units will be used in most of the discussion below.

Table 1. Concentrations of 2,4-D (µg/L and µg/g creatinine) in urine samples from EI participants (n=64)

Concentration units	Mean	Median	Geometric mean	Range	No. > NHANES 95th percentile	95th percentile of EI (CI)	2001-2002 NHANES 95th percentile (CI)
µg/L	1.14	0.33	0.37	<LOD-29.98	4	1.39 (0.98-29.98)	1.27 (1.02-1.37)
µg/g creatinine	1.15	0.37	0.40	<LOD-37.33	6	1.46 (0.92-37.33)	1.08 (0.93-1.26)

CI = 95% confidence interval
LOD = Limit of Detection
NHANES = National Health and Nutrition Examination Survey

The concentrations of 2,4-D in the urine samples ranged from below the limit of detection (0.1 µg/L) to 30 µg/L (Table 1). 2,4-D was detected in the urine of 59 of the 64 participants over the age of 6 years. In order to calculate the mean concentrations in Table 1, samples below the limit of detection (LOD) were assumed to be the LOD divided by the square root of two.

The mean and geometric mean concentrations of 2,4-D in the NHANES sample were not calculated, since less than 60 percent of the samples had a 2,4-D concentration above the limit of detection (LOD). The urine samples from the EI participants were tested with a more sensitive analytical method that had a lower LOD (0.1 µg/L) than the NHANES LOD (0.2 µg/L).

The concentrations of 2,4-D in the urine samples were compared to national survey data from CDC's *Fourth National Report on Human Exposure to Environmental Chemicals* (CDC, 2009). This report contains data from the National Health and Nutrition Examination Surveys (NHANES). These data are the best available reference values for the United States population. The NHANES test population is considered representative of the civilian, non-institutionalized population of the United States in age, sex, and race/ethnicity. However, a limitation of the NHANES comparison ranges is that they may not be representative of the United States population for other factors that could

influence test results such as geographical variability, season of the year, and urban vs. rural residence.

ATSDR classifies individuals with a urine analyte concentration over the 95th percentile of the NHANES national population as having an unusual exposure. This is a statistical determination, not a health-based determination.

Table 1 compares the 95th percentile of the EI participants to the 95th percentile of the NHANES population. As indicated by the data in Table 1, the 95th percent confidence interval[4] for the 95th percentile of the EI participants overlaps the 95th percent confidence interval for the 95th percentile of the NHANES participants. These overlapping confidence intervals indicate that the 95th percentiles for the two populations are not different at a level of statistical significance.

We conducted one sample binomial tests[5] to answer the question of whether the number of EI participants above the NHANES 95th and 75th percentiles were more (or less) than expected. As indicated by the data in Table 2, the number of EI participants above the NHANES 95th percentile did not reach statistical significance. Therefore, it cannot be concluded that the number of EI participants above the NHANES 95th percentile was more than expected. However as indicated by the data in Table 3, the number of EI participants above the NHANES 75th percentile did reach statistical significance. Therefore, it can be concluded that the number of EI participants above the NHANES 75th percentile was more than expected.

Table 2. One sample binomial test for samples above the NHANES 95th percentile.

Concentration units	Values above NHANES 95th percentile		One sample binomial test	
	Number	Percent	95% Exact CI	Two-sided Exact p-value*
µg/L	4	6.25	1.73-15.24	0.7972
µg/g creatinine	6	9.38	3.52-19.30	0.2002

CI = 95% confidence interval
NHANES = National Health and Nutrition Examination Survey
*statistically significant if $p \leq 0.05$.

[4] A confidence interval is a range of values used to estimate the true value of a population parameter.

[5] The binomial test is an exact test of the statistical significance of deviations from a theoretically expected distribution of observations into two categories.

Table 3. One sample binomial test for samples above the NHANES 75th percentile.

Concentration units	Values above NHANES 75th percentile		One sample binomial test	
	Number	Percent	95% Exact CI	Two-sided Exact p-value*
µg/L	40	62.5	49.51-74.30	< 0.0001
µg/g creatinine	32	50.0	37.23-62.77	< 0.0001

CI = 95% confidence interval
NHANES = National Health and Nutrition Examination Survey
*statistically significant if $p \leq 0.05$.

Atrazine

None of the urine samples from the EI participants contained atrazine or any of its metabolites at a concentration above the LOD. The 95th percentile concentration of atrazine mercapturate in the NHANES report (2001-2002 data) was below the LOD. There are no NHANES data for any of the other atrazine metabolites. The urine samples from the EI participants were tested for atrazine and its metabolites using an analytical methodology with a lower LOD than used in NHANES (see Methods section for detection limits).

Discussion

2,4-D

The concentration of 2,4-D in 58 of the 64 urine samples was below the 95th percentile of the NHANES comparison population (1.08 µg/g creatinine). Four of the urine concentrations (1.12, 1.16, 1.46, and 1.75 µg/g) slightly exceeded the NHANES 95th percentile concentration, and two of the urine concentrations (8.26 and 37.33 µg/g) substantially exceed the 95th percentile comparison value. None of the participants with an elevated urinary 2,4-D concentration were from the same household.

A recent study of urine concentrations of 2,4-D in non-occupationally exposed people reported higher background exposures than for NHANES. Morgan et al. (2008) measured 2,4-D in urine samples from randomly-selected, pre-school children and their adult caregivers in predominantly urban areas in Ohio and North Carolina. The 95th percentile concentrations of 2,4-D in urine samples from children and adults from Ohio were 4.3 and 3.3 µg/L, respectively, and in children and adults from North Carolina, 1.9 and 2.8 µg/L, respectively. The reason for these higher exposure levels compared to NHANES is not known, but it suggests there may be some variability in background 2,4-D exposures in different parts of the country. Also, the younger age of the children (2-5 years old) in this study may have been a factor.

Urine concentrations of 2,4-D in people who are occupationally exposed to 2,4-D are substantially higher (Table 2). Gary et al. (2001), measured concentrations of 2,4-D in urine samples from forest or roadside pesticide applicators in Minnesota . Based on a small number of workers (n = 4-8 per group), the average concentrations of 2,4-D in urine ranged from 0.5 µg/L in controls to 454 µg/L in backpack sprayers.

Thomas et al. (2010) measured 2,4-D concentrations in first morning void urine samples collected from farmer applicators before they handled any pesticides. In these pre-spray urine samples, the geometric mean concentration of 2,4-D was 7.8 µg/L and ranged from not detected (ND) to 210 µg/L. In urine samples collected the day after spraying, the geometric mean urine concentration increased to 25 µg/L, and the individual concentrations ranged from 1.6 to 970 µg/L.

In another study of farm applicators, the geometric mean concentration of 2,4-D in urine samples collected from farmers one day before spraying was 3.8 µg/L. In urine samples collected one-day after application, the geometric mean 2,4-D concentration increased to 64.2 µg/L. (Alexander et al. 2007).

Table 4. Urine concentrations of 2,4-D (µg/L) from occupational exposure studies.

Study	Population	2,4-D concentration (mean)	2,4-D (range)
Gary et al. 2001 (Forest/Roadside Applicators)	Control	0.5	ND – 1.8
	Skidder	18	0.85 - 58
	Aerial sprayer	43	ND - 97
	Boom sprayer	252	86 - 490
	Backpack sprayer	454	28 - 1700
Thomas et al. 2010 (farm applicators)	Pre-application	7.8 (GM)	ND - 210
	Post-application	25 (GM)	1.6 - 970
Alexander et al. 2007 (farm applicators)	Pre-application	3.8 (GM)	0.5 - 231
	Post-application	64.2 (GM)	1.5 - 1856

GM = geometric mean
ND = not detected

As compared to the data in Table 2, the highest urine concentrations of 2,4-D detected in the EI participants (14 and 30 µg/L) were within the low to middle range of concentrations detected in workers with occupational exposures.

The health significance of the urinary concentrations of 2,4-D in the EI participants was assessed by comparison to its Biomonitoring Equivalent (BE) of 200 µg/L (Alyward and Hayes, 2008; Alyward et al. 2010). A BE is derived by using pharmacokinetic principles to estimate 2,4-D concentrations in urine that would result from 2,4-D exposures. A BE represents the estimated concentration of 2,4-D that would be present in the urine of someone who was chronically exposed to 2,4-D at a dose level equal to EPA's reference dose (RfD). An RfD is an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily oral exposure to the human population (including sensitive

subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime. The maximum concentration of 2,4-D detected in an EI participant (30 µg/L) was about 7-fold less than the BE (200 µg/L), and the average concentration (1.14 µg/L) was 175-fold less than the BE. Therefore, the 2,4-D exposure in the highest exposed individual (even if chronic) is predicted to be below dose levels that have been reported to cause adverse health effects. For acute exposures to 2,4-D, BEs of 400 µg/L (women of reproductive age) and 1,000 µg/L (rest of population) were developed (Alyward and Hayes, 2008). These BEs were based on acute (1-day) RfDs developed by the EPA. The highest concentration of 2,4-D in a urine sample from an EI participant (30 µg/L) was 13 and 33-fold less than the acute BEs.

Atrazine

The urine samples were analyzed for atrazine and its metabolites using a sensitive, state-of-the-science analytical method (Kuklenyik et al. 2011). None of the urine samples from the EI participants contained a concentration of atrazine or any of its metabolites above the LOD. Thus, there was no evidence that any of the EI participants were recently exposed to atrazine at the time of the EI.

In a study of children in Minnesota, atrazine mercapturate was detected in urine samples from 6 of 262 (2.3 percent) children with a concentration range of not detected to 16 µg/L (Adgate et al. 2001).

Bakke et al. (2009) measured pesticide exposures in corn farmers in Iowa. During the off-season, atrazine mercapturate was detected in 13 percent of farmers at an average concentration of 0.12 µg/L. During the growing season, atrazine mercapturate was detected in urine samples from 85 percent of the farmers at an average concentration of 3.5 µg/L.

Even higher concentrations of other atrazine metabolites have been detected in turf pesticide applicators (Barr et al. 2007) and pesticide factory workers (Catenacci et al. 1993).

A BE for atrazine has not been developed because of incomplete knowledge of the toxicokinetics of atrazine and its metabolites and their relative contribution to the toxic effects of atrazine.

Limitations of the Exposure investigation

Because of the short biological half-lives of 2,4-D and atrazine, the test results reflected the participants' recent exposure to these herbicides. Test results for samples collected at another time of the year or after a spray event could be different.

Urinary concentrations of 2,4-D and atrazine in an individual can vary over the course of a day (Morgan et al. 2008; Catenacci et al. 1993). In this EI, we collected spot urine samples. Urine samples collected at a different time of day could yield different results.

The participants of this EI were self-selected volunteers. The test results are specific to the participants and are not generalizable to the community-at-large.

Notifying the Community of Test Results

ATSDR sent a letter to each EI participant with his or her test results and an interpretation. The letter contained information for contacting an ATSDR or OHA staff person if they wanted to discuss their test results.

Conclusions

(1) None of the urine samples contained atrazine or its metabolites at a concentration above the analytical limit of detection. Therefore, there was no evidence of recent exposure to atrazine at the time of testing.

(2) The concentrations of 2,4-D in urine samples from the EI participants were compared to a national sample from the National Health and Nutrition Examination Survey (NHANES) for people 6 to 59 years old. Based on this comparison, the fraction of the EI participants above the NHANES 75th percentile was higher than expected. This suggests an increased exposure relative to the rest of the United States.

(3) Despite an apparent greater exposure than the US population, these data indicate that, at the time of testing, the participants were not exposed to 2,4-D at levels that are expected to cause adverse health effects.

Recommendations

(1) Further testing is needed to assess short-term exposures to herbicides that could occur immediately following the spraying of clear-cut areas.

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Appendix A

Exposure Investigation Protocol

Biological Monitoring for Exposure to Herbicides

Highway 36 Corridor

Lane County, Oregon

Cost Recovery Number AA1200

August 18, 2011

Prepared by:

Kenneth Orloff, PhD

ATSDR/DHAC/EISAB

Introduction

Since 2005, people living in the Triangle Lake area near Highway 36 in Lane County, Oregon, have expressed concern over possible exposures to herbicides from aerial spraying on nearby forestland. After the trees in an area are clear-cut, the area is replanted with tree seedlings, and the area is sprayed with herbicides to reduce the growth of competing weeds and underbrush.

The steep incline of some of the clear-cut areas makes it difficult to access these areas. Therefore, helicopters are often used to aerially spray herbicides over the clear-cut areas. Residents of the area have reported that herbicides drift from the spray areas and settle on their property, resulting in residential exposures. Surface water runoff from sprayed areas may also result in exposures to people living down gradient from the spray areas.

After tree seedlings are planted in a clear-cut area, the area is typically sprayed twice a year with herbicides[6]. These sprayings occur in late summer and early spring. Different tracts of land are sprayed at different times at the discretion of the individual landowners. State law requires that landowners notify the Oregon Department of Forestry (ODF) two weeks before a spraying event occurs. However, the time interval between notification and spraying can vary at the discretion of the landowner. After spraying has occurred, the ODF can request the landowners to report which herbicides were used. Atrazine and 2,4-dichlorophenoxyacetic acid (2,4-D) are two of the herbicides commonly used.

Atrazine is a moderately persistent pesticide in the environment. In soil, atrazine has a half-life of 14-109 days; whereas in water, its half-life can be 200 days or more (ATSDR, 2003). 2,4-D is non-persistent (half-life = 6.2 days) in terrestrial environments, moderately persistent (half-life = 45 days) in aerobic aquatic environments, and highly persistent (half-life = 231 days) in anaerobic terrestrial and aquatic environments (US EPA, undated).

If humans were to ingest these herbicides, they would be rapidly excreted in the urine with a urinary elimination half-life of 18 hours for 2,4-D and 24-28 hours for atrazine (Sauerhoff et al. 1977, Gilman et al. 1998). Therefore, urinary biomonitoring for these contaminants would reflect relatively recent exposures to these herbicides.

In the spring of 2011, a researcher at Emory University collected urine samples from 21 area residents and tested them for herbicides. The researcher reported that the participating residents had elevated concentrations of 2,4-D and a metabolite of atrazine (diaminochlorotriazine [DACT]) in their urine.

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[6] Landowners have reported to the Oregon Department of Forestry that the following herbicides were applied to clear-cut areas in the Highway 36 corridor during the past two years: Atrazine, Hexazinone, Imazapyr, Sulfometuron Methyl, Metsulfuron Methyl, 2,4-D, Clopyralid, Glyphosate, Triclopyr, Aminopyralid, and Picloram.

In response to these preliminary findings and the concerns of the community, the Oregon Health Authority (OHA) has proposed additional investigations to evaluate the residents' potential exposure to environmental herbicides. Other agencies, including the U.S. Environmental Protection Agency, Oregon Department of Environmental Quality, and Oregon Department of Agriculture, will evaluate potential herbicide and pesticide contamination of water, air, and home-grown/raised food.

As part of this overall effort, OHA requested assistance from ATSDR in conducting urinary biomonitoring to evaluate the residents' exposures to herbicides used on clear-cut areas. Although testing by other agencies may include other herbicides and pesticides, ATSDR's urinary biomonitoring will be limited to 2,4-D, atrazine, and its principal metabolites. These chemicals were selected as target compounds because the National Center for Environmental Health laboratory has existing analytical methods for these chemicals. They are also chemicals frequently sprayed over the clear-cut areas.

The urinary biomonitoring results and evaluation are specific to the community tested. The results are not generalizable to other populations who live near areas where aerial spraying occurs. This protocol and subsequent report will be limited to the urinary biomonitoring investigation and will not include other investigations being conducted by other agencies. Upon the completion of these multiple investigations, the OHA will issue a report that integrates the findings of these investigations.

Project Overview

A. Purpose

The purpose of this Exposure Investigation (EI) is to conduct urinary biomonitoring for exposure to the herbicides, atrazine and 2,4-dichlorophenoxyacetic acid, in residents living along the Highway 36 corridor. The biological monitoring and analyses will be conducted using validated, state-of-the-science analytical methodologies. The results of this investigation will tell the residents if they have been recently exposed to these herbicides and/or their metabolites. This biomonitoring will be conducted prior to fall 2011 spraying operations. Thus, this EI will measure the participants' chronic exposures to herbicides that could result from exposures to environmental contamination of air and water, and the foods that they eat.

The participants of this EI are self-selected residents who live near spray areas. As such, the test results from this investigation will be specific to these participants and are not generalizable to the community-at-large or to other populations

B. Investigators and Collaborators

The ATSDR Exposure Investigation and Site Assessment Branch (EISAB) will be the lead for this Exposure Investigation. This EI will be a collaborative effort of ATSDR and

the OHA. The National Center for Environmental Health (NCEH) laboratory will analyze the urine samples.

EISAB will:

(1) Develop the EI protocol and consent/assent/permission forms

(2) Work with OHA to get consent/assent/parental permission, collect urine samples from the participants, and ship them to the NCEH laboratory for analysis

(3) Evaluate the analytical test results

(4) Notify the participants of their individual test results

(5) Write a report that summarizes the collective findings of the EI

The National Center for Environmental Health (NCEH) will:

(1) Provide supplies for collecting urine samples

(2) Analyze the urine samples for the 2,4-D, atrazine, and six of its metabolites (see Section E for details).

The OHA:

(1) Identify and recruit participants for the EI

(2) Make appointments for sample collection

(3) Work with ATSDR to conduct the field activities

(4) Provide health education to the community on the findings of the EI

METHODS

A. Criteria for participation

Residents who live in Lane County, Oregon, near an herbicide spray area are eligible for this study. Preference will be given to people who have the highest potential for exposure, that is, those who live within 1.5 miles of a spray area. Field studies have documented that following aerial spraying of pesticides in mountainous terrain, pesticide residues can be detected up to 6 kilometers (3.7 miles) from the spray area (Allwine et al.

2002). However, if more participants volunteer than can be tested, preference will be given to those living closest to the spray area.

People with occupational exposure to pesticides (e.g., sprayers) will not be eligible for this investigation. The only age restriction is that participants must be 6 years of age or older. This age restriction is necessary because the test results will be compared to NHANES national survey data, which is limited to people 6 years of age or older (CDC, 2009).

B. Recruiting participants

Based on the above criteria, OHA will recruit participants for this EI. The target goal is to conduct urinary biomonitoring for about 80 residents of the area. Recruitment efforts will include:

(1) OHA staff and a representative of ATSDR attended a public meeting on July 14 to discuss the EI and notify the community of the upcoming testing.

(2) The Oregon Department of Forestry will identify areas that have been clear-cut in 2010-2011. Based on GIS information, OHA will contact people who live within 1.5 miles of the property boundaries of the spray area and invite them to participate in the testing.

Eligible participants must be at least six years of age, live within the recruitment area, have no occupational exposure, and provide consent/assent/parental permission.

C. Field activities

OHA staff will make appointments to meet with the participants in their homes. During this appointment, ATSDR/OHA staff will administer the appropriate consent/assent/parental permission form to the participants. These forms are included in Appendix A.

During the home appointment, we will give each participant a urine collection cup with his/her identification number. We will instruct the participant to collect a urine sample as described in the Urine Collection Instructions (Appendix B). The participant will collect a urine sample of at least 40 ml in the privacy of their bathroom. The participant will then cap the cup and return the freshly voided urine sample to us. We will transfer aliquots of the urine sample to cryovials and freeze the samples on dry ice. Once collected the samples will be kept frozen on dry ice and locked in the trunk of our car.

ATSDR will prepare one field blank of distilled, deionized water for each day that samples are collected. To protect anonymity, the samples will be labeled with a coded identification number provided by the NCEH laboratory.

D. Sample handling and shipping

The urine samples will be shipped within 48 hours after collection. ATSDR staff will package the urine samples on dry ice, enclose a chain-of-custody form, and ship them by overnight delivery to the NCEH laboratory in Atlanta, Georgia, for analysis.

E. Lab processing and analysis

The NCEH laboratory will analyze the urine samples.

Urine samples will be analyzed for 2,4-D and for atrazine and its principal metabolites (Barr et al. 2007). The analysis for atrazine will include the following chemicals: Atrazine (AZN), Diaminochloroatrazine (DACT), Desisopropilatrazine (DIA), Desethylatrazine (DEA), AZN-mercapturate, DIA-mercapturate and DEA-mercapturate. Urinary creatinine will also be measured to correct for urinary dilution. Results will be reported in units of µg/g of creatinine and µg/L of urine for comparison to the NHANES data sets.

The urinary concentrations of atrazine [2-chloro-4-(ethylamino)-6-(isopropylamino)-s-triazine] and six of its metabolites and hydrolysis products, will be determined using two dimensional high performance liquid chromatography (2D-HPLC) coupled with tandem mass spectrometry. Atrazine and six atrazine metabolites in one milliliter of urine are extracted using automated off-line solid phase extraction before separation by 2D-HPLC and quantification by positive ion atmospheric pressure chemical ionization isotope dilution tandem mass spectrometry. The limit of detection (LOD) in 1-mL of sample for atrazine and diaminochloroatrazine (DACT) is 0.5 ng/mL.

The concentrations of the herbicide 2,4-dichlorophenoxyacetic acid (2,4-D), is measured in urine by high performance liquid chromatography-isotope dilution tandem mass spectrometry using a modification of the method described in Olsson et al. 2004. A 1 mL sample of urine is extracted with a mix-mode solid-phase extraction sorbent using a semi-automated 96-well plate technology to achieve sample purification and a concentration factor of 25. The urine extracts are then analyzed using reversed phase high performance liquid chromatography and the target analytes are quantified by isotope dilution tandem mass spectrometry. The limit of detection of 2,4-D is about 0.1 ng/mL in 1 mL urine.

F. Evaluation of data

The concentrations of 2,4-D and atrazine mercapturate in the urine samples will be compared to national survey data from CDC's *Fourth National Report on Human Exposure to Environmental Chemicals* (CDC, 2009). This report contains data from the National Health and Nutrition Examination Surveys (NHANES). The NHANES test population is selected to be representative of the civilian, non-institutionalized population of the United States. We will not attempt to quantitatively interpret analytical results for chemicals for which we have no NHANES comparison values. Nevertheless, the

presence of metabolites of atrazine in a urine sample indicates that exposure has occurred, and this qualitative information could be useful.

ATSDR classifies individuals with a urine concentration in excess of the 95th percentile of the NHANES national population as having an unusual exposure. This is a statistical determination, not a health based determination. Information is not available to assess the health impact of urinary herbicide concentrations.

Because of the short biological half-lives of 2,4-D and atrazine, the test results will reflect the participants' recent exposure to these herbicides. Test results for samples collected at another time of the year or after a spray event could be different.

Risks to the Participants

Providing a urine sample poses no risk to the participants of this investigation.

Benefits to the Participants

The potential benefit to the participants of this investigation is that they will learn if they were exposed to the herbicides tested for and how their exposures compare to the U.S. population.

Notifying the Community of Test Results

ATSDR will send a letter to the EI participants to notify them of their test results. The letter will also contain information for contacting an ATSDR staff person if they want to further discuss their test results; however, this will not be a personal medical consultation regarding his/her health care.

At the conclusion of this investigation, ATSDR will prepare a written report that presents the findings of the EI. This report will contain no personal identifiers on order to protect the anonymity of the participants. The report will be available to federal, state, and local environmental and public health agencies, as well as to the general public.

The consent form will request permission from the participants for ATSDR to share their test results with other federal and state health and environmental agencies.

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Appendix A

U.S. Department of Health and Human Services

Agency for Toxic Substances and Disease Registry

Exposure Investigation

Lane County, Oregon

Adult Consent Form for Urine Testing

WHO ARE WE AND WHY ARE WE DOING THIS EXPOSURE INVESTIGATION (EI)?

We are from the Agency for Toxic Substances and Disease Registry (ATSDR), a sister agency to the Centers for Disease Control and Prevention (CDC). The purpose of the EI is to determine whether people who live near a Highway 36 herbicide spray area are being exposed to herbicides. The purpose of the EI is to determine whether people who live near a Highway 36 herbicide spray area are currently being exposed to the herbicides, 2,4-D and atrazine.

We are inviting you to have your urine tested for 2,4-D and atrazine and six of its break-down products.

We will only test your urine for these chemicals.

WHAT IS INVOLVED IN THIS TESTING?

We will give you a plastic cup to collect a urine sample. We will tell you how to collect your sample. It should take 5 minutes or less for you to collect your urine sample.

WHAT ARE THE BENEFITS FROM BEING IN THIS EI?

By being part of this EI, you will find out if you may have been recently exposed to these herbicides and how your exposure compares to others in the U.S.

This test will not tell you if your health may be harmed by these exposures.

There is no cost to you for this testing.

WHAT ARE THE RISKS OF THIS EI?

There is no risk from donating a urine sample

Some people may feel uncomfortable about having their urine tested for chemicals.

Some people may be concerned over their test results.

WILL I BE PAID?

You will not be paid for being in this EI.

WHAT ABOUT MY PRIVACY?

We will protect your privacy as much as the law allows. We will give you an identification (ID) number. This number, not your name, will go on your urine sample. We will not use your name in any report we write. We will keep a record of your name, address, and ID number so that we can send you the test results. Your name and address will be kept in a password-protected computer. Copies of your consent form will be kept in a locked file cabinet.

After we complete the EI, your urine sample will be destroyed.

HOW WILL I GET MY TEST RESULTS

We will mail your test results to you 3-4 months after your sample is collected. We will also give you a telephone number that you can call to discuss your test results or request a copy for your family doctor. ATSDR does not provide any follow-up medical care or evaluation.

MAY WE SHARE YOUR TEST RESULTS?

Sharing the test results with other agencies may help us to understand how people might be exposed to these herbicides. May we share these test results with other Federal and State health and environmental agencies?

YES _____ NO _____

WHAT IF I DON'T WANT TO DO THIS?

You are free to choose whether or not you want to be part of this testing. If you agree to be tested, you may change your mind at any time and drop out without penalty. You must sign this consent form to be tested.

WHOM DO I CONTACT IF I HAVE QUESTIONS?

If you have any questions about this testing, you can ask us now. If you have questions later, contact the Project Officer, Dr. Kenneth Orloff, at ATSDR at 770-488-0735 or 888-232-4636 or send him an e-mail at KEO1@CDC.GOV.

If you have questions about your rights as part of this EI, please call the CDC Human Research Protection Office at 1-800-584-8814. Leave a message with your name and

telephone number and say that you are calling about the Highway 36 EI. Someone will return your call.

VOLUNTARY CONSENT

I have read this form or it has been read to me. I have had a chance to ask questions about this testing and my questions have been answered. I know I can change my mind at any time. I will be given copy of this form to keep. I agree to be part of this testing.

_____ _____

Participant's Signature Date

_____

Participant's Printed Name

Age _____ Gender _____

Address _____

_____

_____

Telephone number _____

Lab ID Number _____

I have read the consent form to the person named above. He/she has asked questions about the investigation and had the questions answered.

_____

Signature of person administering consent form

_____

Printed name of person administering consent form

_____

Date

U.S. Department of Health and Human Services

Agency for Toxic Substances and Disease Registry

Exposure Investigation

Lane County, Oregon

Parental Permission Form for Urine Testing for Children Less than 7 Years of Age

WHO ARE WE AND WHY ARE WE DOING THIS EXPOSURE INVESTIGATION (EI)?

We are from the Agency for Toxic Substances and Disease Registry (ATSDR), a sister agency to the Centers for Disease Control and Prevention (CDC). The purpose of the EI is to determine whether people who live near a Highway 36 herbicide spray area are currently being exposed to the herbicides, 2,4-D and atrazine.

We are inviting your child to have his/her urine tested for 2,4-D and atrazine and six of its break- down products.

We will only test your child's urine for these chemicals.

WHAT IS INVOLVED IN THIS TESTING?

We will give you a plastic cup to collect your child's urine sample. We will tell you how to collect the sample. It should take 5 minutes or less for you to collect the urine sample.

WHAT ARE THE BENEFITS FROM BEING IN THIS EI?

By being part of this EI, you will find out if your child may have been recently exposed to these pesticides and how those exposures compare to others in the U.S.

This test will not tell you if your child's health may be harmed by these exposures.

There is no cost to you for testing your child.

WHAT ARE THE RISKS OF THIS EI?

There is no risk from donating a urine sample. However, your child may feel uncomfortable about having their urine tested for chemicals. Some people may be concerned over their test results.

WILL I BE PAID?

Neither you nor your child will be paid or receive any type of compensation for being in this EI.

WHAT ABOUT MY CHILD'S PRIVACY?

We will protect your child's privacy as much as the law allows. We will give your child an identification (ID) number. This number, not your child's name, will go on the urine sample. We will not use your child's name in any report we write. We will keep a record of your child's name, address, and ID number so that we can send you the test result. Your child's name and address will be kept in a password-protected computer. Copies of your child's consent form will be kept in a locked file cabinet.

After we complete the EI, your child's urine sample will be destroyed.

HOW WILL I GET MY CHILD'S TEST RESULTS

We will mail your child's test results to you 3-4 months after the sample is collected. We will also give you a telephone number that you can call to discuss the test results or request a copy for your child's doctor. ATSDR does not provide any follow-up medical care or evaluation.

MAY WE SHARE YOUR CHILD'S RESULTS?

Sharing the test results with other agencies may help us to understand how people might be exposed to these herbicides. May we share these test results with other Federal and State health and environmental agencies?

YES _____ NO _____

WHOM DO I CONTACT IF I HAVE QUESTIONS?

If you have any questions about this testing, you can ask us now. If you have questions later, contact the Project Officer, Dr. Kenneth Orloff, at ATSDR at 770-488-0735/ 888-232-4636 or send an e-mail to KEO1@CDC.GOV.

If you have questions about your rights as part of this EI, please call the CDC Human Research Protection Office at 1-800-584-8814. Leave a message with your name and telephone number and say that you are calling about the Highway 36 EI. Someone will return your call.

PARENTAL PERMISSION

I have read this form or it has been read to me. I have had a chance to ask questions about this testing and my questions have been answered. I agree that my child can be

part of this testing. I know I, or my child, can change our minds at any time. I will be given a copy of this form to keep.

SIGNATURE

I give permission for my child to be tested.

_____
Printed Name of Child

_____ _____
Signature of Parent Date

_____
Printed Name of Parent

Age of child _____ Gender of child_____

Address of child:

_____

_____

_____

Telephone number _____

Lab ID Number _____

I have read the consent form to the person named above. He/she has asked questions about the investigation and had the questions answered.

_____
Signature of person administering consent form

_____

Printed name of person administering consent form

_____

Date

U.S. Department of Health and Human Services

Agency for Toxic Substances and Disease Registry

Exposure Investigation

Lane County, Oregon

Assent Form for Urine Testing for Children 7 to less than 18 Years of Age

WHO ARE WE AND WHY ARE WE DOING THIS EXPOSURE INVESTIGATION (EI)?

We are from the Agency for Toxic Substances and Disease Registry (ATSDR), a sister agency to the Centers for Disease Control and Prevention (CDC). The purpose of the EI is to determine whether people who live near a Highway 36 herbicide spray area are currently being exposed to the herbicides, 2,4-D and atrazine.

We are inviting you to have your urine tested for 2,4-D and atrazine and six of its break-down products.

We will only test your urine for these chemicals.

WHAT IS INVOLVED IN THIS TESTING?

We will give you a plastic cup to collect a urine sample. We will tell you how to collect your sample. It should take 5 minutes or less for you to collect your urine sample.

WHAT ARE THE BENEFITS FROM BEING IN THIS EI?

By being part of this EI, you will find out if you may have been recently exposed to these pesticides and how your exposure compares to others in the U.S.

This test will not tell you if your health may be harmed by these exposures.

There is no cost to you for this testing.

WHAT ARE THE RISKS OF THIS EI?

There is no risk from donating a urine sample. Some people may feel uncomfortable about having their urine tested for chemicals. Some people may be concerned over their test results.

WILL I BE PAID?

You will not be paid for being in this EI.

WHAT ABOUT MY PRIVACY?

We will protect your privacy as much as the law allows. We will give you an identification (ID) number. This number, not your name, will go on your urine sample. We will not use your name in any report we write. We will keep a record of your name, address, and ID number so that we can send you the test result. Your name and address will be kept in a password-protected computer. Copies of your consent form will be kept in a locked file cabinet.

After we complete the EI, your sample will be destroyed.

HOW WILL I GET TEST RESULTS

We will mail your test results to you 3-4 months after the sample is collected. We will also give you a telephone number that you can call to discuss the test results or request a copy for your family doctor. ATSDR does not provide any follow-up medical care or evaluation.

ASSENT

Your parents said it is all right for you to have this test. You don't have to if you don't want to.

MAY WE SHARE YOUR TEST RESULTS?

Sharing the test results with other agencies may help us to understand how people might be exposed to these herbicides. May we share these test results with other Federal and State health and environmental agencies?

YES _____ NO _____

WHAT IF I HAVE QUESTIONS?

If you have questions, you can ask us now. You can talk with your parents if you want. If you have questions later, ask your parent. They can call us for answers.

SIGNATURE

I have read this form or it has been read to me. I have had a chance to ask questions about this testing and my questions have been answered. I agree to be part of this testing. I know I can change my mind at any time. I will be given a copy of this form to keep.

_____ _____

Signature of Minor Date

_____

Printed Name of Minor

_____

Signature of Parent

Age of Participant _____ Gender of Participant_____

Address: _____

_____

_____

Telephone number _____

Lab ID Number _____

I have read the consent form to the person named above. He/she has asked questions about the investigation and had the questions answered.

_____

Signature of person administering consent form

_____

Printed name of person administering consent form

_____

Date

Appendix B

Urine Collection Instructions

Urine collection cups (which hold at least 120 ml) will be provided for each participant. Label each cup with a bar-coded specimen ID label. Instruct each study participant to do the following for a clean-catch urine collection.

- Wash hands and air dry.
- Do not remove the cap from the specimen cup until ready to void.
- Place the cap turned inside-upwards on a clean and stable surface while collecting urine.
- Collect at least 30-40 ml of urine in the cup; do not touch the inside of the cup or cap at any time.
- Recap the specimen cup.
- Return the cup to the ATSDR/OPEH staff person.