RISK EVALUATION FOR INDIANA BATS CONSUMING INSECTS COLLECTED ALONG LITTLE SULPHUR CREEK

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Background

In the summer of 2000, a study was conducted to determine concentrations of explosive chemicals, phosphorous, and metals in insects in the foraging area of the Indiana Bat at the Ammunition Burning Grounds (ABG) at NSWC Crane (NSWC, 2001). All study procedures were conducted in accordance with a QAPP approved by USEPA Region 5 (NSWC, 2000). A report describing the study procedures and results was prepared by the Navy and submitted to USEPA on August 27, 2001 (NSWC, 2001). The study consisted of collecting insects from three locations adjacent to Little Sulphur Creek immediately downstream of the ABG. Insects were collected from two locations using light traps, and insects were collected from a third location using a funnel trap known as a Malaise trap. The traps were operated for four nights each in the months of June, July, and August, 2000. The insects were separated into three groups prior to analysis: terrestrial insects (not including Lepidoptera), aquatic insects, and Lepidoptera insects.

The insect tissue samples were analyzed for explosive compounds, total phosphorous, and metals. None of the explosive compounds were detected in any of the insect samples. The concentrations of total phosphorous in the insects from the site were similar to the total phosphorous concentrations in the control insects (crickets). The metals concentrations in the insects from the site were greater than the concentrations in the control insects. Also, the concentrations of three metals (cadmium, copper, and lead) were greater than background levels from the literature for those three metals. No background levels were found in the literature for the other metals. The elevated levels of metals may have been caused by paint chips that peeled off the insect traps and became mixed with the insects. The metal concentrations in the insects were incorporated into a food chain model for the little brown bat to determine if there were any risks to bats from consuming the insects. This report describes the results of this risk evaluation.

Food Chain Model Procedure

The little brown bat model was used as a surrogate species for the Indiana bat, which has been known to forage in the riparian area south of the ABG at NSWC Crane, because exposure factors were available for the little brown bat.

Risks to the little brown bat from metals in the insects were determined by estimating the Chronic Daily Intake (CDI) and comparing the CDI to Toxicity Reference Values (TRVs) representing acceptable daily doses in mg/kg/day. The TRVs were developed from No-Observed-Adverse-Effect-Levels (NOAELs) and Lowest-Observed-Adverse-Effect-Levels (LOAELs) obtained from wildlife studies, if available. The majority of the TRVs are obtained from the ORNL Toxicological Benchmarks for Wildlife: 1996 Revision (Sample et al., 1996). Table 1 presents the TRVs that were used in this report and the derivation of the TRVs using the body-weight scaling equation presented below. Table 2 presents the sources and ecological endpoints for the TRVs. When a subchronic study was used to develop the TRV, the final value was multiplied by a factor of 0.1 to account for uncertainty between subchronic and chronic effects. Also, when a LOAEL study was used to develop the NOAEL TRV, the LOAEL was multiplied by a factor of 0.1 to obtain the NOAEL.

In accordance with U.S. Region 5 policy, the NOAEL (or LOAEL) from the test species (i.e., rat, mouse) was adjusted to a NOAEL (or LOAEL) for the surrogate species (the little brown bat) using the following body weight scaling equation from Sample et al., (1996):

$$NOAEL_w = NOAEL_t * (bw_t/bw_w)^{1/4}$$

Where: $NOAEL_w$ = No Observed Adverse Effect Level for the surrogate wildlife species
$NOAEL_t$ = No Observed Adverse Effect Level for the test species
bw_t = body weight of the test species
bw_w = body weight of the little brown bat

Exposure of the little brown bat to the metals in the insects was determined by estimating the daily doses in mg/kg-day using the following exposure equations.

$$\text{CDI Dose (mg/kg-day)} = \frac{(FI * FC)}{BW}$$

Where: CDI = Chronic Daily Intake (mg/kg-day)
FI = Food ingestion rate (mg/day)
FC = Food concentration (insect concentrations) (mg/kg)
BW = Body weight (kg)

The insect concentrations were reported in NSWC, (2001) on a dry weight basis. The ingestion rate of the little brown bat was reported on a wet weight basis, so the wet weight metal

concentrations were calculated by multiplying the dry weight concentration (mg/kg) by the percent solid for each insect group (see Table 3).

The lower bound of the threshold effects is based on consistently conservative assumptions and NOAEL toxicity values (U.S. EPA, 1997). This bound presents the highest potential risks. The upper bound is based on observed impacts or predictions that ecological effects could occur and is developed using consistent assumptions, site-specific data, LOAEL toxicity values, or an impact evaluation (U.S. EPA, 1997). This bound presents the average potential risk. Both the upper and lower bounds were evaluated to provide the overall range of potential risks as presented in the following table:

Conservative Scenario	**Average Scenario**
Highest receptor body weight for NOAEL calculation	Average receptor body weight for LOAEL calculation
Lowest receptor body weight for CDI equation	Average receptor body weight for CDI equation
Conservative receptor ingestion rate	Average receptor ingestion rate
Use NOAELS	Use LOAELs

The exposure assumptions (i.e., ingestion rate, body weight) for the little brown bat were obtained from Sample et al., (1997). Table 4 presents the exposure factors that were used for the food chain model and the values that were used to calculated the exposure factors. As indicated above, the little brown bat was used as a surrogate for the Indiana bat because more exposure factors were available for the little brown bat. However, because the Indiana bat is the ultimate receptor, the foraging area used by the Indiana bat is incorporated into this risk evaluation. An Indiana bat maternal colony has been reported to range from a linear strip of creek vegetation 0.8 km in length to a foraging area of 1.2 km in length (USFWS, 1999).

The risk characterization compares the exposure to the ecological effects. An Ecological Effects Quotient (EEQ) approach was used to characterize the risk to the bat. An EEQ of greater than "1.0" is considered to indicate a potential risk. The EEQ is not an expression of probability, and the meaning of values greater than 1.0 must be interpreted in light of attendant uncertainties in risk management. The EEQ for the bat wildlife model was calculated as follows:

$$EEQ = \frac{Dose}{TRV}$$

Where: EEQ = Ecological Effects Quotient, (unitless)
Dose = Daily Intake Dose, (mg/kg-day)
TRV = Toxicity Reference Value (NOAEL or LOAEL), (mg/kg-day)

Results

Tables 5 and 6 presents the results of the food chain models using the conservative and average scenarios for the three insect groups. The greatest EEQs were calculated for the model using the aquatic insects for both the conservative and average scenario. Five metals had EEQs that were greater than 1.0 based on the conservative inputs and using the NOAEL as the TRV for bats consuming the aquatic insects (Table 5). Two and three metals had EEQs that were greater than 1.0 for the bats consuming Lepidoptera and terrestrial insects, respectively, under the same conservative exposure scenario (Table 5).

Based on the average scenario, no metals had EEQs greater than 1.0 for bats that consumed Lepidoptera using either the NOAEL of LOAEL as the TRV (Table 6). Only two metals had EEQs greater than 1.0 for bats that consumed terrestrial insects using the NOAEL, but no EEQs were greater than 1.0 using the LOAEL. Finally, two metals had EEQs greater than 1.0 for bats that consumed terrestrial insects using the NOAEL, and one metal had an EEQs that was greater than 1.0 using the LOAEL (chromium).

Chromium was the only metal that had an EEQ greater than 1.0 using the average exposure factors and the LOAEL as the TRV. The TRV used for chromium is for chromium (VI) to be conservative, however, chromium in biological materials is usually in the chromium (III) form (Eisler, 1986). As listed on table 2, the NOAEL for chromium (III) is 2,737 mg/kg whereas the NOAEL for chromium (VI) is 3.28 mg/kg; no LOAEL was available for chromium (III) but if would be greater than the NOAEL. Therefore, had the NOAEL for chromium (III) been used in the food chain model, none of the chromium EEQs would have been greater than 1.0.

Summary/Conclusions/Recommendations

The two exposure scenarios (conservative and average) were presented to show the range of risks to bats. NOAELS are the highest concentrations in a particular study where no adverse effects were observed, whereas LOAELS are the lowest concentrations in a particular study where adverse effects were observed. Therefore, an EEQs that is greater than 1.0 using a NOAEL but less than 1.0 using the LOAEL is not likely to cause a significant risk to ecological receptors.

As presented in NSWC (2001), the total weight of the Lepidoptera (650 grams – wet weight) and terrestrial insects (439 grams – wet weight) that were collected were much greater than the weight of the aquatic insects (55 grams – wet weight). Although the bats consuming the aquatic insects had an EEQ slightly greater than 1.0 based on average exposure assumptions and the LOAEL for chromium [based on chromium (VI)](EEQ=1.2), aquatic insects should only comprise a small portion of the diet for the bats. Since none of the LOAEL EEQs for the bats that consume terrestrial insects and Lepidoptera exceeded 1.0, and these insects represent a larger percentage (by weight) of the insects collected, it is unlikely that the concentrations of metals in the insects will harm the bats. As indicated above, risks from chromium are expected to be low because most of the chromium in tissue is chromium (III), which is much less toxic than chromium (VI), which was used for the food chain model. Finally, these food chain models assume that the bats will consume all of their food from along Little Sulfur Creek but bats will obtain some of their food from other areas at the Base. Therefore, potential risks to bats from consuming insects along Little Sulphur Creek are low, and a study to obtain base-specific background concentrations of metals in insects does not appear to be necessary.

TABLE 1

TOXICITY REFERENCE VALUES FOR THE LITTLE BROWN BAT
AMMUNITION BURNING GROUNDS
NSWC CRANE, CRANE INDIANA

Parameter	Test Species				Little Brown Bat (conservative value)(1,2)		Little Brown Bat (average value)(1,3)	
	NOAEL	LOAEL	Species	Body Weight (kg)	NOAEL	LOAEL	NOAEL	LOAEL
Aluminum	1.93	19.3	mouse	0.03	2.5	25.2	2.8	27.6
Antimony	0.125	1.25	mouse	0.03	0.16	1.6	0.18	1.8
Arsenic	0.126	1.26	mouse	0.03	0.16	1.6	0.18	1.8
Barium	5.1	19.8	rat	0.435/0.35(1)	13.0	47.8	14.2	52.3
Beryllium	0.66	6.6	rat	0.35	1.6	15.9	1.7	17.4
Cadmium	1	10	rat	0.303	2.3	23.3	2.5	25.5
Chromium	3.28	13.14	rat	0.35	7.9	31.7	8.7	34.7
Hexavalent Chromium	3.28	13.14	rat	0.35	7.9	31.7	8.7	34.7
Cobalt	1.2	12	rat	0.35	2.9	29.0	3.2	31.7
Copper	11.71	15.14	mink	1	36.8	47.5	40.2	52.0
Cyanide	68.7	687	rat	0.273	156	1559	170	1705
Iron	50	500	rabbit	3.8	219	2191	240	2397
Lead	8	80	rat	0.35	19.3	193	21.1	211
Manganese	88	284	rat	0.35	212	686	232	750
Mercury	0.015	0.025	mink	1	0.047	0.078	0.05	0.09
Nickel	40	80	rat	0.35	96.6	193	105.6	211
Selenium	0.2	0.33	rat	0.35	0.48	0.80	0.53	0.87
Silver	1.81	18.1	mouse	0.03	2.4	23.6	2.6	25.9
Thallium	0.0074	0.074	rat	0.365	0.018	0.18	0.02	0.20
Vanadium	0.21	2.1	rat	0.26	0.47	4.7	0.5	5.1
Zinc	160	320	rat	0.35	386	773	422	845

Unite are mg/kg-day
NOAEL - No Observered Adverse Effect Level
LOAEL - Lowest Observered Adverse Effect Level
1 - The NOAEL and LOAEL for the bat was calculated using the following equation: $NOAEL_w$ (or $LOAEL_w$) =$NOAEL_t$ (or $LOAEL_t$)*$(bw_t/bw_w)^{0.25}$

Where: $NOAEL_w$ (or $LOAEL_w$) = NOAEL (or LOAEL) for the bat
$NOAEL_t$ (or $LOAEL_t$) = NOAEL (or LOAEL) for the test species
bw_w - body weight of the bat
bw_t - body weight of the test species

2 - The conservative values were calculated using the maximum body weight of 10.27 g for the bat (see Table 4).
3 - The average values were calculated using the average body weight of 7.2 g for the bat (see Table 4).

TABLE 2

ENDPOINTS AND SOURCES FOR TOXICITY REFERENCE VALUES FOR MAMMALS
AMMUNITION BURNING GROUNDS
NSWC CRANE, CRANE INDIANA

Parameters	Concentration (mg/kd-day)	Endpoint	Effect	Chronic/ Subchronic	Species	Primary Reference	Source of Reference
Aluminum	19.3	LOAEL	reproductive	chronic	mouse	Ondreicka et al., 1966	Sample et.al., 1996
Antimony	1.25	LOAEL	lifespan	chronic	mouse	Schroeder et al., 1968b	Sample et.al., 1996
Arsenic	1.261	LOAEL	reproductive	chronic	mouse	Schroeder and Mitchner, 1971	Sample et.al., 1996
Barium	5.1	NOAEL	growth	chronic	rat	Perry et al., 1983	Sample et.al., 1996
Barium	198	LOAEL	mortality	subchronic	rat	Borzelleca et al., 1988	Sample et.al., 1996
Cadmium	1	NOAEL	reproduction	chronic	rat	Sutou et al., 1980b	Sample et.al., 1996
Cadmium	10	LOAEL	reproduction	chronic	rat	Sutou et al., 1980b	Sample et.al., 1996
Chromium(III)	2737	NOAEL	reproduction	chronic	rat	Ivankovic and Preussmann, 1975	Sample et.al., 1996
Chromium(VI)	3.28	NOAEL	BW/food cons.	chronic	rat	Mackenzie, 1958	Sample et.al., 1996
Chromium(VI)	13.14	LOAEL	mortality	subchronic	rat	Steven et al., 1976	Sample et.al., 1996
Copper	11.71	NOAEL	reproduction	chronic	mink	Aulerich et al., 1982	Sample et.al., 1996
Copper	15.14	LOAEL	reproduction	chronic	mink	Aulerich et al., 1982	Sample et.al., 1996
Lead	8	NOAEL	reproductive	chronic	rat	Azar et al., 1973	Sample et.al., 1996
Lead	80	LOAEL	reproductive	chronic	rat	Azar et al., 1973	Sample et.al., 1996
Manganese	88	NOAEL	reproductive	chronic	rat	Laskey et al., 1982	Sample et.al., 1996
Manganese	284	LOAEL	reproductive	chronic	rat	Laskey et al., 1982	Sample et.al., 1996
Mercury	0.032	NOAEL	reproductive	chronic	rat	Verschuuren et al., 1976	Sample et.al., 1996
Mercury	0.16	LOAEL	reproductive	chronic	rat	Verschuuren et al., 1976	Sample et.al., 1996
Nickel	40	NOAEL	reproductive	chronic	rat	Ambrose et al., 1976	Sample et.al., 1996
Nickel	80	LOAEL	reproductive	chronic	rat	Ambrose et al., 1976	Sample et.al., 1996
Silver	18.1	NOAEL	systemic	subchronic	mouse	Rungby, 1984	ATSDR, 1989
Zinc	160	NOAEL	reproductive	chronic	rat	Schlicker and Cox, 1986	Sample et.al., 1996
Zinc	320	LOAEL	reproductive	chronic	rat	Schlicker and Cox, 1986	Sample et.al., 1996

TABLE 3

DRY WEIGHT TO WET WEIGHT CONVERSION FOR INSECTS
AMMUNITION BURNING GROUNDS
NSWC CRANE, INDIANA

	Terrestrial		Aquatic		Lepidoptera	
Inorganics	Dry Weight[1] mg/kg	Wet Weight[2] mg/kg	Dry Weight[1] mg/kg	Wet Weight[2] mg/kg	Dry Weight[1] mg/kg	Wet Weight[2] mg/kg
ALUMINUM	93.8	31.6	134	44.2	10.7	2.4
ANTIMONY	0.138	0.05	0.113	0.04	0.0205	0.005
ARSENIC	3.25	1.1	0.597	0.20	0.169	0.04
BARIUM	11.7	3.9	20.7	6.8	6.58	1.5
CADMIUM	0.871	0.29	0.83	0.27	0.698	0.16
CHROMIUM	38.6	13.0	209	69.0	28.6	6.4
COPPER	74.8	25.2	114	37.6	36.1	8.1
LEAD	16	5.4	16.5	5.4	1.75	0.39
MANGANESE	74.2	25.0	77.4	25.6	28.5	6.4
MERCURY	0.083	0.03	0.072	0.02	0.04	0.01
NICKEL	30.5	10.3	147	48.5	21.1	4.7
SILVER	0.0515	0.02	0.55	0.18	0.0942	0.02
ZINC	582	196	913	301	234	52.5
Percent Solid	0.3367		0.3302		0.2243	

1 - Source of data is NSWC, 2001
2 - The wet weight concentration was calculated by multpilying the dry weightconcentration by the percent solid.

TABLE 4

CALCULATION OF EXPOSURE PARAMETERS FOR THE LITTLE BROWN BAT
AMMUNITION BURNING GROUNDS
NSWC, CRANE, INDIANA

Exposure Parameters	Little Brown Bat
Body Weights (g)	6.15
	6.15
	6.03
	6.99
	10.27
	7.77
Minimum (Conservative)	***6.03***
Maximum	***10.27***
Average	***7.2***
Food Ingestion Rate (g/g-day) (1)	1.12
	0.23
	0.48
Minimum	***0.23***
Maximum	***1.12***
Average	***0.61***
Food Ingestion Rate (kg/day)	
Conservative	***0.0081***
Average	***0.0044***
Water Ingestion Rate (g/g-day)	0.177
	0.205
Minimum	***0.177***
Maximum	***0.205***
Average	***0.191***
Water Ingestion Rate (L/day)	
Conservative	***0.0015***
Average	***0.0014***
Home Range (km) (1)	0.80
	1.20
Minimum (km) (1)	***0.80***
Maximum (km) (1)	***1.20***
Average (km) (1)	***1.00***

Notes:

Source of the Little Brown Bat data is Sample et al., (1997) except for the home range, which is USFWS, (1999).

1 - Home range for the Indiana Bat is presented in km of shoreline.

Ingestion Rates (kg/day or L/day)

- Conservative value = Max Ingestion Rate (g/g-day)* Avg. Body Weight
- Average value = Avg. Ingestion Rate (g/g-day)*Avg. Body Weight

TABLE 5

TERRESTRIAL WILDLIFE MODEL ECOLOGICAL EFFECTS QUOTIENT CALCULATION
LITTLE BROWN BAT - CONSERVATIVE INPUTS
AMMUNITION BURNING GROUNDS
NSWC, CRANE, INDIANA

TERRESTRIAL INSECTS

Parameter	Insect Concentration[1] (mg/kg)	Dose[2] (mg/kg-day)	NOAEL (mg/kg-day)	LOAEL (mg/kg-day)	NOAEL EEQn	LOAEL EEQl
Inorganics						
ALUMINUM	32	43	2.5	25	17	1.7
ANTIMONY	0.046	0.063	0.16	1.63	0.38	0.038
ARSENIC	1.1	1.5	0.2	1.6	9.0	0.90
BARIUM	3.9	5.3	13	48	0.41	0.11
CADMIUM	0.3	0.40	2.3	23	0.17	0.017
CHROMIUM	13	18	7.9	32	2.2	0.55
COPPER	25	34	37	48	0.92	0.72
LEAD	5.4	7.3	19	193	0.38	0.038
MANGANESE	25	33.7	212	686	0.16	0.049
MERCURY	0.028	0.038	0.047	0.078	0.80	0.48
NICKEL	10	14	97	193	0.14	0.072
SILVER	0.017	0.023	2.4	23.6	0.010	0.0010
ZINC	196	265	386	773	0.68	0.34

AQUATIC INSECTS

Parameter	Insect Concentration (mg/kg)	Dose (mg/kg/day)	NOAEL (mg/kg/day)	LOAEL (mg/kg/day)	NOAEL EEQn	LOAEL EEQl
Inorganics						
ALUMINUM	44	60	2.5	25	24	2.4
ANTIMONY	0.037	0.050	0.16	1.6	0.31	0.031
ARSENIC	0.20	0.27	0.16	1.6	1.6	0.16
BARIUM	6.8	9.2	13	48	0.71	0.19
CADMIUM	0.27	0.37	2.3	23	0.16	0.016
CHROMIUM	69	93	7.9	32	12	2.9
COPPER	38	51	37	48	1.4	1.1
LEAD	5.45	7.4	19	193	0.38	0.038
MANGANESE	26	35	212	686	0.16	0.05
MERCURY	0.024	0.032	0.047	0.078	0.68	0.41
NICKEL	49	66	97	193	0.68	0.34
SILVER	0.18	0.25	2.4	24	0.10	0.010
ZINC	301	407	386	773	1.1	0.53

LEPIDOPTERA

Parameter	Insect Concentration (mg/kg)	Dose (mg/kg/day)	NOAEL (mg/kg/day)	LOAEL (mg/kg/day)	NOAEL EEQn	LOAEL EEQl
Inorganics						
ALUMINUM	2.4	3.2	2.5	25	1.3	0.13
ANTIMONY	0.0046	0.0062	0.16	1.6	0.038	0.0038
ARSENIC	0.038	0.051	0.16	1.6	0.31	0.031
BARIUM	1.5	2.0	13	48	0.15	0.042
CADMIUM	0.16	0.21	2.3	23	0.091	0.009
CHROMIUM	6.4	8.7	7.9	32	1.1	0.27
COPPER	8.1	11	37	48	0.30	0.23
LEAD	0.4	0.5	19	193	0.027	0.0027
MANGANESE	6.4	8.6	212	686	0.041	0.013
MERCURY	0.0090	0.012	0.047	0.1	0.26	0.15
NICKEL	4.7	6.4	97	193	0.066	0.033
SILVER	0.021	0.029	2.4	24	0.012	0.0012
ZINC	52	71	386	773	0.18	0.092

Cells are shaded if the EEQ is greater than 1.0.

Definitions:
NOAEL - No Observed Adverse Effects Level
LOAEL - Lowest Observed Adverse Effects Level
EEQn - Ecological Effects Quotient based on the NOAEL
EEQl - Ecological Effects Quotient based on the LOAEL

TABLE 6

TERRESTRIAL WILDLIFE MODEL ECOLOGICAL EFFECTS QUOTIENT CALCULATION
LITTLE BROWN BAT - AVERAGE INPUTS
AMMUNITION BURNING GROUNDS
NSWC, CRANE, INDIANA

TERRESTRIAL INSECTS

Parameter	Insect Concentration[1] (mg/kg)	Dose[2] (mg/kg-day)	NOAEL (mg/kg-day)	LOAEL (mg/kg-day)	NOAEL EEQn	LOAEL EEQl
Inorganics						
ALUMINUM	32	19	2.8	28	7.0	0.70
ANTIMONY	0.046	0.0	0.2	1.8	0.16	0.016
ARSENIC	1.1	0.7	0.2	1.8	3.7	0.37
BARIUM	3.9	2.4	14	52	0.17	0.046
CADMIUM	0.29	0.2	2.5	25	0.070	0.0070
CHROMIUM	13	7.9	8.7	35	0.92	0.23
COPPER	25	15	40	52	0.38	0.30
LEAD	5.4	3.3	21	211	0.16	0.016
MANGANESE	25	15	232	750	0.066	0.020
MERCURY	0.028	0.0	0.1	0.1	0.33	0.20
NICKEL	10	6.3	106	211	0.059	0.030
SILVER	0.017	0.0	2.6	26	0.0041	0.00041
ZINC	196	120	422	845	0.28	0.14

AQUATIC INSECTS

Parameter	Insect Concentration (mg/kg)	Dose (mg/kg/day)	NOAEL (mg/kg/day)	LOAEL (mg/kg/day)	NOAEL EEQn	LOAEL EEQl
Inorganics						
ALUMINUM	44	27	2.8	28	10	1.0
ANTIMONY	0.037	0.0	0.2	1.8	0.13	0.013
ARSENIC	0.20	0.1	0.2	1.8	0.67	0.067
BARIUM	6.8	4.2	14.2	52	0.29	0.08
CADMIUM	0.27	0.2	2.5	25	0.07	0.01
CHROMIUM	69	42	8.7	35	4.9	1.2
COPPER	38	23	40.2	52	0.57	0.44
LEAD	5.4	3.3	21.1	211	0.16	0.016
MANGANESE	26	16	232	750	0.07	0.021
MERCURY	0.024	0.0	0.1	0.1	0.28	0.17
NICKEL	49	30	106	211	0.28	0.14
SILVER	0.18	0.1	2.6	26	0.043	0.0043
ZINC	301	184	422	845	0.44	0.22

LEPIDOPTERA

Parameter	Insect Concentration (mg/kg)	Dose (mg/kg/day)	NOAEL (mg/kg/day)	LOAEL (mg/kg/day)	NOAEL EEQn	LOAEL EEQl
Inorganics						
ALUMINUM	2.4	1.5	2.8	28	0.5	0.1
ANTIMONY	0.0046	0.0	0.2	1.8	0.016	0.0016
ARSENIC	0.038	0.0	0.2	1.8	0.13	0.013
BARIUM	1.5	0.9	14	52	0.063	0.017
CADMIUM	0.16	0.1	2.5	25	0.04	0.004
CHROMIUM	6.4	3.9	8.7	35	0.5	0.11
COPPER	8.1	4.9	40	52	0.12	0.10
LEAD	0.39	0.2	21	211	0.011	0.0011
MANGANESE	6.4	3.9	232	750	0.017	0.0052
MERCURY	0.0090	0.0	0.1	0.1	0.11	0.064
NICKEL	4.7	2.9	106	211	0.027	0.014
SILVER	0.021	0.0	2.6	26	0.0050	0.00050
ZINC	52	32	422	845	0.076	0.038

Cells are shaded if the EEQ is greater than 1.0.

Definitions:
NOAEL - No Observed Adverse Effects Level
LOAEL - Lowest Observed Adverse Effects Level
EEQn - Ecological Effects Quotient based on the NOAEL
EEQl - Ecological Effects Quotient based on the LOAEL

References

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INSECT CAPTURE AND ANALYSIS
FOR
EVALUATION OF CONTAMINANTS
IN THE
FORAGE AREA OF THE INDIANA BAT
AT
AMMUNITION BURNING GROUNDS

NAVAL SURFACE WARFARE CENTER
CRANE DIVISION
CRANE, INDIANA

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Enclosure (1)

1. INTRODUCTION 1
2. BACKGROUND 1
2.1 FACILITY LOCATION 1
2.2 PROJECT BACKGROUND 2
2.3 PREVIOUS STUDIES 3
3. METHODOLOGY 4
3.1 FIELD 4
3.2 LABORATORY 6
3.2.1 Analytical Phases 6
3.2.2 Sample Preparation 6
4. ANALYTICAL RESULTS 7
4.1 INITIAL JUNE SPLIT 7
4.2 SAMPLES FOR ANALYSES 7
5. RESULTS 8
6. RECOMMENDATIONS 9
7. REFERENCES 11

Tables 12
Appendix A A-1
Appendix B B-1
Appendix C C-1

ACRONYMS

ABG = Ammunition Burning Grounds
CCRA = Current Conditions Risk Assessment
CoC = Chain of Custody
COPEC = Contaminant of Potential Ecological Concern
ECB = WES Environmental Chemistry Branch
LCS = Laboratory Control Spike
MDL = Method Detection Limit
MS = Matrix Spike
MSD = Matrix Spike Duplicate
NSWC = Naval Surface Warfare Center
QC = Quality Control
QAPP = Quality Assurance Project Plan
RCRA = Resource Conservation and Recovery Act
RFI = RCRA Facility Investigation
TtNUS = Tetra Tech NUS
U.S. EPA = United States Environmental Protection Agency
U.S. FWS = U.S. Fish and Wildlife Service
WES = Waterways Experiment Station, U.S. Army Corps of Engineers

1. INTRODUCTION

This report presents the results of the study to determine potential impacts to the food chain of the Indiana bat (*Myotis sodalis*) foraging in the riparian area south of the Ammunition Burning Grounds (ABG) at Crane Division, Naval Surface Warfare Center (NSWC Crane).

M. sodalis feeds strictly on flying insects, typically in riparian areas, consuming both terrestrial and aquatic insects. There is an on-going debate over opportunistic versus selective feeding in insectivorous bats. Diet apparently varies seasonally and within different ages, sexes, and reproductive-status groups. For example, the Indiana Bat Recovery Plan notes that moths (Lepidoptera) are major prey items identified in several studies, but caddisflies (Trichoptera) and flies (Diptera) are major prey items documented in another (UFWS 1999). Lee (1993) found that Lepidoptera was the most important prey item of *M. sodalis*, with some intraspecific variation (i.e., slight differences in feeding behavior between adult males versus juveniles and pregnant, lactating, and nonreproductive females). He also suggests that *M. sodalis* may switch between selective and opportunistic feeding depending on prey availability. Ultimately, the selection of prey depends on the environment in which they forage and hence, the insects that are available.

Contaminant concentrations in flying insects are needed to estimate the magnitude of contaminant exposure that *M. sodalis* may experience from consuming insects along Little Sulphur Creek below the ABG. Numerous studies have attempted to estimate the bioavailability of pollutants in the food chain to insectivorous animals through the use of sediment partitioning models. However, direct measurement of contaminants in flying insects is the preferred approach since it contributes the least uncertainty to exposure estimates. That is, direct sampling of the food chain allows better prediction of the actual contaminant loading to the bat.

The small size of most insects requires many individuals to be pooled to ensure that each sample has sufficient biomass to meet analytical detection limits for trace elements. This method sacrifices information on individual variation, but since *M. sodalis* is arguably an opportunistic feeder, differences between insect species is of little importance. What is important in this particular study is the differences between aquatic and terrestrial species in order to determine the potential contaminant migration route, if there is any. Lepidopterans were also separated out since, as stated previously there is indication that moths make up a majority of the bat's diet.

2. BACKGROUND

2.1 FACILITY LOCATION

NSWC Crane is located in southwestern Indiana, approximately 75 miles southwest of Indianapolis, and 71 miles northwest of Louisville, Kentucky. NSWC Crane occupies 62,463 acres (approximately 100 square miles) of the northern portion of Martin County and small portions of neighboring Greene, Daviess, and Lawrence Counties. The base is located in a rural agricultural and wooded area, and is situated on a topographic plateau known as the Crawford Upland, dissected by well-defined stream valleys, causing elevation differences of over 300 feet in some areas. Surficial geology consists of Pennsylvanian and Mississippian age sandstones, shales, and limestones.

Little Sulphur Creek is approximately 4.6 miles long from its northern most headwaters to its intersection of Sulphur Creek south of NSWC Crane. The creek consists of a north and a south fork emanating just west of the ABG operational area, which then joins approximately in the center of the ABG treatment area. From the ABG, a single, rocky and sandy channel

meanders south a distance of approximately 1.1 miles until reaching the southern installation boundary. The width of the channel varies in width from a few feet in the upper reaches to about 25 feet in the valley flats. The stream is intermittent in that its flow varies considerably with the seasons. Some flow is usually present in the north and south forks above the center of the ABG. Immediately downstream of the ABG operational area, surface flow ceases in the dry months as the surface water is captured by vertical infiltration into a pseudo-karst conduit leaving a dry stream bed. In dry weather, the stream bed remains dry downstream of the capture zone to about three-fourths of a mile below the ABG where Spring C issues from the Beech Creek limestone on the east valley wall and rejuvenates Little Sulphur Creek. Flow is further augmented downstream by ground water issuing from the Beech Creek limestone as springs A and B on the west valley wall. In the dry season, all of the water flowing in Little Sulphur Creek below the ABG originates from Springs A, B, and C. During times of heavy or extended periods of precipitation, the conduit fills and surface water then flows through the entire Little Sulphur Creek channel. Water from Spring A has been shown by dye trace tests to have connection to limestone openings beneath the east-central portion of the ABG (Murphy and Ciocco 1990, and Baedke 1998). Runoff from the ABG soils potentially contributes contamination to LSC. Prior operational practices no longer in use (e.g., open burning of explosives directly on the ground) have resulted in contamination of surface soils with metals and explosives. These contaminants may migrate to Little Sulphur Creek during large-scale rain events.

2.2 PROJECT BACKGROUND

Crane Division, Naval Surface Warfare Center (NSWC Crane) conducted an ecological risk assessment for Resource Conservation Recovery Act (RCRA) corrective action activities and to support a RCRA Subpart X (open burning-open detonation) permit. A bat survey along the streams near the areas of concern was included as part of the ecological risk assessment. On June 25, 1996, a single male Indiana bat was caught. The capture occurred south of the ABG on Little Sulphur Creek in the SE corner of Section 28, T5N, R3W. Figure (1) shows the approximate location where the Indiana Bat was captured.

Subsequent conversations with the United States Environmental Protection Agency (U.S. EPA) and the U.S. Fish and Wildlife Service (U.S. FWS) led to the recommendations for follow-on studies to determine if the operations at the ABG are impacting *M. sodalis*. NSWC Crane responded with a proposal to conduct a survey, such as a light trap survey, of the available prey in the area the bat was captured, in order to determine if contaminants are mobilizing through the bat's food chain.

The collection of insects is not an exact science. The mass collected is not controlled by the sampler, but rather influenced by such factors as climate (temperature, humidity, presence or absence of drought or rainfall, and cloud cover), number and size of insects available for capture, effectiveness of the traps at attracting available insects, time of year, etc. Therefore, it was agreed that three months of sampling would be needed in order to collect a sufficient mass of insects for analyses. The plan was to field sort the moths from the rest of the insects prior to freezing and shipping. Each month's collection would then be shipped to the laboratory for storage and sorting the rest of the insects into those that had aquatic vs. terrestrial juvenile life stages. Since the earliest shipment of insects would not be analyzed right away, the U.S. EPA recommended that NSWC Crane do an additional initial collection of insects. This initial set would be split at the laboratory and used to determine if any explosives degradation occurred during storage. As such, all parties agreed that this set would not be sorted in order to ensure sufficient mass for explosives analysis.

NSWC Crane contacted the U.S. Army Corps of Engineers, Waterways Experiment Station (WES) for assistance in the project. Dr. Al Cofrancesco, a research entomologist, provided support in field collection (i.e., collecting equipment and its usage) and insect identification. Ms. Karen Myers, a biologist for WES, assisted in preparation of the Quality Assurance Project Plan for this project and lead the analytical work.

Sampling took place in June, July, and August 2000. Experientially, June, July, and August are the peak times for insect availability in south central Indiana. Sampling across three months allowed for collecting insects that emerged at different times during the season, as well as providing for optimum sample volume. Additionally, this availability of the most insects, combined with the presence of the bats feeding, as well as full-scale operation of the ABG, allowed for a more representative sample of the bat's diet and greatest potential for identifying contamination in the bat's food chain.

The particulars of each field sampling event included:

- Insect collection;
- Sorting of Lepidopterans in the field; and
- Sample preservation, packaging, and shipping.

WES then took care of:

- Sorting fractions;
- Sample preparation;
- Analysis for inorganics and explosives; and
- Data reporting.

2.3 PREVIOUS STUDIES

Surface water and sediments of Little Sulphur Creek have been previously addressed in three studies: A RCRA Facility Investigation (RFI) Phase II Release Assessment conducted by WES (1998); an RFI Phase III Release Characterization conducted by TtNUS (2001); and a current contamination conditions risk assessment by Tetra Tech NUS (TtNUS 1999).

WES conducted a Phase II RFI for surface water and sediment in 1992 and published a report in 1998. This report concluded that contaminants were generally detected more frequently in sediments than in surface waters. This implies that contaminants accumulate and persist in sediments but are diluted and flushed seasonally in the surface waters. When compared to background levels, the report found that aluminum, antimony, arsenic barium, cadmium, chromium, copper, lead, nickel, manganese, magnesium, mercury, silver, thallium, vanadium, and zinc were potential site-related contaminants at various points along Little Sulphur Creek in surface water and/or sediments. Three explosives, RDX, HMX, and 2,4-DNT were detected only at three surface water sites below the ABG.

TtNUS conducted a Phase III RFI in 2001. Only the unvalidated data was available. Initial indication is that levels of antimony, arsenic, barium, beryllium, cadmium, chromium, copper, iron, lead, manganese, nickel, thallium, and zinc exceeded risk-based target levels in surface water and/or sediments. Detected explosives below the ABG in surface water and sediments include TNT, 2,4-DNT, 2-Amino-4,6-Dinitrotoluene, 4-Amino-4,6-Dinitrotoluene, RDX, and HMX.

The 1999 Current Conditions Risk Assessment (CCRA) by TtNUS included a ecological risk assessment. The report included an evaluation of the data from the 1998 WES report, but stated that the metals data for the surface water and sediment were rejected for use in the CCRA due to questionable QA/QC. The CCRA sampled surface water and sediment to fill data gaps created by the rejected data. For surface water, five inorganics exceeded ambient water quality criteria (AWQC): aluminum (included since there was no AWQC), cadmium, lead, mercury, and zinc. Sediment inorganic contaminants of potential ecological concern (COPECs) that exceeded screening criteria: aluminum, antimony, arsenic, barium, cadmium, copper, lead, and zinc (aluminum and barium were included since there was no sediment screening criteria). However, through population sampling, the CCRA did determine the presence of pollution intolerant macroinvertebrate species including mayfly nymphs, stonefly larvae, and caddisfly larvae. In summary, the U.S. EPA approved CCRA states, "The majority of ecological risks posed by COPECs at the ABG/OJT, appear to be limited to the aquatic habitats at this SWMU. Elevated levels of barium, lead, and zinc in the sediments at the site may have slight adverse effects to wildlife; however, population studies and tissue samples for fish and macroinvertebrates did not show any evidence of adverse effects. Elevated levels of various compounds in the surface water may have a potential adverse impact to wildlife at this site; however, impacts as a result of these COPECs would be very localized and unlikely to impact the viability of any one species at the site given the availability of similar habitat in close proximity to these locations. Population studies at this SWMU support this conclusion, as animal, fish, macroinvertebrate, and vegetation species are diverse and abundant, and are similar to what would be expected to occur in a non-impacted area". (TtNUS 1999).

3. METHODOLOGY

3.1 FIELD

On October 19, 1999, representatives from the USFWS, U.S. EPA, and NSWC Crane met to review and agree upon the proposed sampling locations at the ABG. Appendix A contains the letter from the USFWS dated October 20, 1999, wherein agreement was made by all parties on the locations for insect collection. One of the light traps was situated on the Jeep Trail below the ABG, near Bridge 3090. The second light trap was placed in the floodplain adjacent to Little Sulphur Creek immediately downstream of the ABG, as shown on Figure 1.

Two types of traps were used for field collection. The first collecting apparatus was a large light trap that utilized both black lights and a mercury vapor lamp. Using different light sources, this light trap produced a wide spectrum of light stimulus to attract phototropic insects from a considerable distance (see Figure 2). The trap contained three large drawers. The bottom drawer contained a metal pan, wherein, sheets of cotton were placed and then approximately 800 ml of ethyl acetate was poured over the cotton. The ethyl acetate would evaporate up through the two upper drawers and funnel area where the insects landed. After breathing the chemical most of the insects fell through the collecting funnel and into the top drawer. This drawer is composed of large mesh screen, allowing smaller insects to drop through to the second drawer containing a fine mesh screen.

Since not all insects are attracted to light, a second trap type was also used. This type of trap, known as a Malaise trap, consists of insect netting designed in a configuration to funnel flying insects into a collecting container (Figure 3).

The Malaise Trap was set up on the Old Jeep Trail area of the ABG so as not to be influenced by the two light traps. The Malaise collecting container was initially operated with a small amount of water in an attempt to drown any collected insects. However, relatively few insects

were found in the water. This, along with the fact that the water seemed only to complicate emptying the container, the decision was made, after a few sampling events, to operate the collecting head dry. As expected, the mass of insects from the Malaise trap was much less than the mass of insects collected by the two light traps. All traps were set up in late afternoon so as to reduce the potential for collecting insects that fly only during daylight hours.

All three traps were operated for four nights in each of the months of June, July, and August 2000, resulting in 36 trap nights. However, an initial collection of insects was made June 12, 2000 – June 19, 2000 for the purposes of determining if explosives degradation would occur in the insect tissue while the insects were stored over the summer. This first set of June collections were not sorted in the field or the laboratory, but rather combined in order to ensure that a sufficient mass of tissue would be available for comparison of analytical splits. The container was taken back to Building 3245 and placed in a freezer. Each morning's collection was placed in a separate container so as to prevent thawing of the previous night's sample. Initially, the plan was to operate the traps for four nights for the initial June collection. However, on the morning of June 14, 2000, both light traps were off with no insects collected. Therefore, an additional night of sampling was done the evening of June 18, 2000. Appendix B contains the field sampling logs. Sampling containers were packed into two coolers, each containing a chain-of-custody (CoC). The coolers were then sent to the WES Environmental Chemistry Branch (ECB). The CoCs and shipping documents are provided in Appendix C.

The upper drawer of the light traps typically contained only the largest of insects, such as the Regal moth (*Citheronia regalis*), Imperial Moth (*Eacles imperialis*), and Luna Moth (*Actias Luna*). Though other large insects were initially trapped in the upper drawer, presumably they were able to crawl through the screen to the lower drawer prior to being overcome by the ethyl acetate. Even then, numerous beetles appeared to be only mildly intoxicated by the ethyl acetate.

After the first June sampling event to examine contaminant degradation, the intent was to place insects from the upper drawers and lower drawers into separate containers for the subsequent sampling events. However, after removal of the Lepidopterans, there were so few insects in the upper drawers, the decision was made to place all insects in one container. Additionally, numerous smaller insects were present on the upper portion of the traps around the lights above the funnel, as well as on the exterior portions of the traps. An attempt was made to collect all insects on the traps, not just from the drawers. Some insects that had not been affected by the ethyl acetate were able to escape from the hands of the samplers and the fate that awaited the other insects at WES. All identifiable Lepidopterans (post-initial June sampling effort) were field sorted and containerized apart from the rest of the insects.

Paint chips occasionally would flake off and fall into the drawers. These were typically easily removed. However, occasional heavy rains resulted in numerous very small crumbled paint flakes in the samples. Attempts were made to remove these paint chips with tweezers, but it was difficult to differentiate between insect parts and the black paint pieces beyond a certain size of chip. Removal efforts were further hampered by the agglomeration of wet insects. Climatological data is also provided in Appendix B.

Sample shipments took place in two coolers accompanied by CoCs on June 19, June 26, July 24, and August 28, 2000. Samples were shipped via overnight courier to Dr. Cofrancesco at WES for storage and sorting. The CoCs and shipping documents are provided in Appendix C, while Appendix B contains the field sampling logs.

3.2 LABORATORY

3.2.1 Analytical Phases

From an analytical perspective, the project involved three primary phases. The initial, or preliminary phase, was to refine existing methods and document the procedures for analyzing explosives, metals, and phosphorus in an insect matrix. The second phase was to collect macroinvertebrate samples associated with the food chain of the federally endangered Indiana Bat in the area of the ABG associated with the location of the capture of a single male bat. In the third phase, the insect samples were sorted and analyzed to determine concentrations of metals and explosives. This information will then be used to determine the need for other studies, that may entail additional sampling of insects or use of a surrogate bat to determine possible contaminant effects to the bat.

The sample size ultimately controlled the extent that project objectives could be pursued, especially with respect to the total number of analyses and quality control (QC) efforts. In order to maximize the QC and total number of analyses, crickets were obtained and used as surrogates for the initial phase of the project. Work with the crickets assisted in determining changes to preparation and analytical procedures, interferences, lower reporting and method detection limits (LRL and MDL, respectively), required sample volumes, and the extent of QC. The initial surrogate cricket work, along with a pre-obtained insect sample volume, determined the actual detection limits achievable for this project. Table 3 of the approved Quality Assurance Project Plan (QAPP) (NSWC 2000) for this project lists the LRLs & MDLs derived from the cricket studies.

The first phase of refining existing methods and documenting the procedures for analyzing explosives and metals in an insect matrix was completed in December 1997. The second, or insect collection phase was completed August 2000. The third, or analytical phase was completed November 2000.

3.2.2 Sample Preparation

Two coolers were received by the WES ECB on 20 June 00. Upon receipt of the first June collection of insects from NSWC Crane, the WES ECB checked the temperature inside the coolers (-1° and -4°C). The samples were stored in their freezer prior to processing. The insects were not sorted. The individual samples were weighed. The total weight was 616.61 grams. Processing included the addition of 60mLs of Mili Q water to facilitate grinding. After the grinding, the ground insects were combined and weighed again. This wet weight was 654.49 grams, with the additional weight being due to the water that was added prior to grinding. This sample was then split into two aliquots. The first portion was submitted for further analyses. The wet weight of the first aliquot was 330.12 grams.

On 21 June 2000, the insect tissue was placed in a freeze drier until 26 June 2000. The dry weight was 101.52 grams equating to 31% solids.

The second half of the split was stored frozen (-20°C) until all the other insect samples arrived. The remaining samples would then be processed and analyzed simultaneously. All samples were stored in the same manner (same freezer, same type of holding container, etc.). Results from the two splits would then be compared to see if any degradation of explosives takes place in the insects while frozen. This was important since additional insect samples would arrive later in June and remain frozen until sampling was completed in August and the insects could later be analyzed.

4. ANALYTICAL RESULTS

All samples were analyzed for explosives, metals, and total phosphorus according to the methods described in the approved QAPP. As outlined in the QAPP, crickets were obtained for use as laboratory controls spikes (LCS) or method blanks. Summary results of explosives, total phosphorus, and metals analyses are presented in Table 1, Table 2 and, Table 3, respectively.

4.1 INITIAL JUNE SPLIT

Explosives
The split composited insect tissue was analyzed with a matrix duplicate, a matrix spike (MS), and a matrix spike duplicate (MSD). Recoveries ranged from 84.0% for the MSD for TNB to 125.9% for the MS for TNX. Table 5 of the approved QAPP contains the accuracy requirements for this project. The crickets were sacrificed with ethyl acetate and processed in the same manner as the rest of the insects. The LCS ranged from 88.5% for 3-NT to 111.5% for 2A-DNT. Tetryl was not reported because there was no recovery demonstrated in insect tissue during MDL studies.

4.2 SAMPLES FOR ANALYSES

Over the course of the summer, samples were sent from NSWC Crane to Dr. Cofrancesco. These samples were placed in the freezer with the ground composite sample (the second half of the split from the degradation sample). Once all of the samples were received, sorting began. Sorting was done tray by tray in a room kept at approximately 4°C. Subsequent insect samples (June, July, and August) were grossly sorted into aquatic and terrestrial juvenile life stages plus Lepidopterans (moths). These samples were delivered to the WES Chemistry Branch on 1 Nov 00 along with previously ground composite insects.

For processing, the sorted insects were removed from their containers, weighed, and ground in a blender as previously described. The composited insects were thawed, placed in freeze drier vessels and processed as well. Tissue was weighed, blended, and placed in the freeze drier on 15 Nov 00. Tissue was removed from the freeze drier on 20 Nov 00. Afterward the sample was ground and placed in jars in the freezer.

The total weights shown in Table 4 are the weights of the insects as received by the WES laboratory from NSWC Crane prior to addition of the Mili Q water. For the sample that was composited in June and then split, the initial total weight is listed since the total weight of the splits is unknown. The Wet Weight lists the weights of the selected aliquots from the sorted fractions, after processing. For the composited June sample, out of the initial wet weight of 654.49 grams, 330.12 grams were analyzed during the first round, leaving a total of 324.37 grams from which the 295.44 gram sample was pulled (note that the large sample size is due WES analyzing inorganic fractions as well as explosives. Since these were not required nor lend themselves to useful interpretation, they are not discussed in this report). The listed Dry Weights are the weights of the aliquots after drying.

Explosives
The second half of the split from the composited insects was analyzed with MS/MSD. Spike recoveries ranged from 112.5% for 2A-DNT to 83% for 2,2'-Dinito-4,4'-Azoxytoluene. As before, the cricket control tissue was used as the matrix blank and LCS. LCS values ranged from 112% for DNX and 4,4'-Dinitro-2,2'-Azoxytoluene to 86% for 2,2'-Dinitro-4,4'-Azoxytoluene. Similar to the initial split, no data was reported for Tetryl.

Inorganics
Total Phosphorus: Due to changes in laboratory structure, management and procedures, samples for total phosphorus were sent to the part of the WES laboratory located in Omaha, Nebraska. The SOP for their analytical method was previously submitted to the Navy.

Metals: The Terrestrial insect tissue was analyzed for an MS/MSD. Matrix spike recoveries ranged from 119.2% for Manganese to 93.6% for silver and zinc. LCS recoveries ranged from 81.6% for zinc to 102.5% for manganese. No LCS data were reported for aluminum and magnesium.

5. RESULTS

As seen from Table 4, the traps produced sufficient mass for analyses. The process of operating three traps for four nights in each of the months of June, July, and August resulted in a wide spectrum of insect species collected. Lepidoptera constituted the greatest mass collected, followed by terrestrials, then aquatic.

Explosives
As shown in Table 1, explosives were not detected. Since no explosives were found in either the initial June split or any subsequent samples, the degradation of explosive compounds in an insect matrix could not be quantified. Here is possibly an example of where direct measurement of contamination in the food chain is preferable to a sediment partitioning model. Previous studies have found explosives in sediments (WES 1999, TtNUS 2001). If one used a partitioning model, explosives contamination of the food chain may have been indicated.

Inorganics
Total Phosphorus: The total phosphorus results are presented in Table 2. Though phosphorus compounds have been treated at the ABG (e.g., red and white phosphorus), phosphorus is also found in nearly every living organism. As can be seen in Table 2, none of the phosphorus results for the NSWC Crane insects exceeded the values of the cricket control.

Metals: An attempt was made to compare the results of the NSWC Crane metals analyses to values reported in journal articles. The only literature found was for metals in pre-emergent aquatic macroinvertebrates. Obviously, this study analyzed the adults. However, most adult forms typically do not feed – at least not on contaminated sediments. The primary role of the adult aquatic insect is to reproduce. A few live less than one day. So some extrapolations can be made. However, little is known about the levels of various metals left behind in the transformation from the nymph to the adult form.

Another difficulty encountered is in finding whole body data from a control setting. Since the bat ingests the entire insect, the NSWC Crane study examined whole-insect body burdens. In reality, bats will often consume only parts of insects – which is why wings of moths are often found below outdoor lights – and portions of the insects will also pass through the bat's gut. The purpose of this study however, is to quantify contaminant concentrations in whole-insect bodies in order to conservatively qualify the potential for harmful effects to the bat through bioaccumulation. Almost all of the literature sources examined depurated (i.e., gut content removed) collections from a contaminated setting. Some researchers have attempted to compare gut content with respect to whole body contaminant burdens. Unfortunately, only one published report was found that provided a few trace element concentrations for non-depurated insects from a non-contaminated source.

Cain (1995) evaluated the influence of gut content on immature caddisflies (Trichoptera) and stoneflies (Plecoptera). The insects evaluated were collected from a reportedly uncontaminated stream in western Montana and looked only at four metals: cadmium, copper, iron, and lead.

Since iron was not an element evaluated in the NSWC Crane study, only cadmium, copper, and lead results are presented (see Table 5).

Cadmium: Cain reported a potential maximum cadmium value of 0.3 mg/kg (Table 5), which is less than the reported value of 0.83 mg/kg for the NSWC Crane aquatics (Table 3). The highest value for cadmium (0.871 mg/kg) was found in the terrestrial insects. The lowest was for lepidoptera at 0.698 mg/kg and exceeded the respective cricket control by 3262%.

Copper: Cain reported copper values of a potential maximum value of 28 mg/kg (Table 5). The NSWC Crane aquatics reported a value greater than this. Table 3 shows that the aquatic copper value was 114 mg/kg, the highest value for copper of the NSWC Crane insects. Note that the lowest NSWC Crane value was for Lepidoptera at 36.1 mg/kg. Though this value is more than twice the cricket control, it is within an order of magnitude.

Lead: Cain reported a potential maximum value of 1.4 mg/kg. The NSWC Crane aquatics had the highest lead value of the three groups at 16.5 mg/kg. Again, the lepidoptera had the lowest lead value at 1.75 mg/kg. This value however, exceeded the respective cricket control by 3813%.

Adequate outside data was not available for comparison of the other eleven metals. The following is simply a synopsis of the data.

Table 6 shows the maximum and minimum percentage differences for the metals in the insects over the controls. The maximum values are highlighted, while the minimum values are in bold. Since Antimony, mercury, and silver, were not detected in the cricket controls, one-half of the detection limit was used for the percentage calculation.

The lowest metal concentrations for 13 of the 14 analytes were found in Lepidoptera. The exception being for silver wherein the lowest value was found in the terrestrial insects. Interestingly, the insects with the lowest recorded concentrations for 13 out of the 14 metals, also constituted the largest mass collected (Table 4). Unless this is some artifact of collection bias, this possibly benefits the bat, if indeed Lepidoptera constitute the majority of the prey for *M. sodalis*.

Paragraph 8 of Section 3.1 briefly discussed the presence of paint flakes in the samples. Though this might account for some elevated levels of metals, Dr. Cofrancesco provided assurance that he did not use metal based paints, and certainly not lead containing paint.

6. RECOMMENDATIONS

Since no explosive compounds were detected in any of the samples there is no need to evaluate explosives any farther. Furthermore, since the total phosphorus results were less than or equal to the crickets used as controls, there does not appear to be a problem with total phosphorus. Metals are clearly a concern identified in this project. The levels of metals seem to indicate contaminated conditions. However, it may be premature at this point to act upon this conclusion. First of all, most of the data comparison assumes that crickets serve as a suitable control. Clearly, all Orders of insects inherently contain naturally different levels of metals, possibly even down to the Species level.

Secondarily, little outside data was found for comparative purposes. Only one article was identified that was sufficiently similar so as to be useful for comparison (Cain 1995). Cain looked at four metals. Only three of which were the same looked at in this project. Then in only four Species of two Orders of immature aquatic insects. The other eleven metals for the aquatics, plus all 14 for the terrestrial and Lepidoptera did not have outside data to evaluate

against. Possibly another difference is the location. Cain studied insects from the Clark Fork River in western Montana. It is unclear, yet quite likely that insects will have different levels of naturally occurring metals based upon location. Additionally, some reported concentration differences may be attributable to differences in preparation and analytical techniques between the Cain and NSWC Crane studies.

So why was the Cain study even used for evaluation? One reason was to show that efforts had been made to find articles for comparison. Section 1.4.2 of the approved QAPP stated that the data would be compared to published literature values for metals. The result was that there seems to be suprisingly little data reported on the background levels of metals in insects. Another reason was to get an indication as to whether or not there may be a problem with contamination in the NSWC Crane insects. Since the Cain article was found to be reasonably similar, it was presented to show the need for additional studies.

Remember, as stated in section 2.3, the ecological portion of the approved CCRA concluded that though contamination was present, it did not appear to impact macroinvertebrate populations. Population studies identified animal, fish, macroinvertebrate (including pollution intolerant organisms), and vegetation species similar to what would be expected to occur in a non-impacted area.

In conclusion, to adequately quantify whether or not the metals found in the NSWC Crane insects are abnormally high, more data is needed. Unfortunately, we only have one data point. Therefore, the recommendation is to collect a background sample of insects using the same materials and methods, or similar if equipment is unavailable. Since the question of the paint from the traps is a possible source of contamination, the same traps should be used for the background collection. If the same traps are not available, then one of the set of traps should be used to collect an additional round of insects from the ABG area. For simplicity, since the Malaise trap produced very few insects, the Malaise would not be used in the background study. If there is indeed a contaminant problem, collecting a background sample would allow NSWC Crane to more closely determine the true magnitude of the problem.

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Tables

Table 1 - Explosive Results

Description		Units	MDL	Terrestrial	Aquatic	Lepidoptera	1st June Split	2nd June Split	1st Cricket Control	2nd Cricket Control
HMX	HMX	mg/kg dry	3.2	<3.2	<3.2	<3.2	<3.2	<3.2	< 3.2	<3.2
RDX	RDX	mg/kg dry	0.80	<0.80	<0.80	<0.80	<0.80	<0.80	< 0.8	<0.80
TNB	1,3,5-Trinitrobenzene	mg/kg dry	1.2	<1.2	<1.2	<1.2	<1.2	<1.2	< 1.2	<1.2
DNB	1,3-Dinitrobenzene	mg/kg dry	1.0	<1.0	<1.0	<1.0	<1.0	<1.0	< 1	<1.0
TETRYL	Tetryl	mg/kg dry	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
TNT	2,4-6-Trinitrotoluene	mg/kg dry	1.3	<1.3	<1.3	<1.3	<1.3	<1.3	< 1.3	<1.3
4A-DNT	4-Amino-2,6-Dinitrotoluene	mg/kg dry	2.50	<2.50	<2.50	<2.50	<2.50	<2.50	< 2.5	<2.50
2A-DNT	2-Amino-4,6-Dinitrotoluene	mg/kg dry	1.20	<1.20	<1.20	<1.20	<1.20	<1.20	< 1.2	<1.20
2,6-DNT	2,6-Dinitrotoluene	mg/kg dry	1.30	<1.30	<1.30	<1.30	<1.30	<1.30	< 1.3	<1.30
2,4-DNT	2,4-Dinitrotoluene	mg/kg dry	0.70	<0.70	<0.70	<0.70	<0.70	<0.70	< 0.7	<0.70
NB	Nitrobenzene	mg/kg dry	1.70	<1.70	<1.70	<1.70	<1.70	<1.70	< 1.7	<1.70
2-NT	2-Nitrotoluene	mg/kg dry	1.90	<1.90	<1.90	<1.90	<1.90	<1.90	< 1.9	<1.90
3-NT	3-Nitrotoluene	mg/kg dry	1.60	<1.60	<1.60	<1.60	<1.60	<1.60	< 1.6	<1.60
4-NT	4-Nitrotoluene	mg/kg dry	2.10	<2.10	<2.10	<2.10	<2.10	<2.10	< 2.1	<2.10
MNX	MNX	mg/kg dry	1.80	<1.80	<1.80	<1.80	<1.80	<1.80	< 1.8	<1.80
TNX	TNX	mg/kg dry	1.80	<1.80	<1.80	<1.80	<1.80	<1.80	< 1.8	<1.80
DNX	DNX	mg/kg dry	1.80	<1.80	<1.80	<1.80	<1.80	<1.80	< 1.8	<1.80
4,4-AZOX	2,2'-Dinitro-4,4'-Azoxytoluene	mg/kg dry	2.10	<2.10	<2.10	<2.10	<2.10	<2.10	< 2.1	<2.10
2,2-AZOX	4,4'-Dinitro-2,2'-Azoxytoluene	mg/kg dry	2.10	<2.10	<2.10	<2.10	<2.10	<2.10	< 2.1	<2.10
3,5-DNA	3,5-Dinitroaniline			92.80%	86%	100%	100%	102%	100.2	84.20%

Table 2 - Total Phosphorus Results	
Description	Ttl. Phosphorus
Units	mg/kg dry
MDL	1
Terrestrial	6600
Aquatic	5900
Lepidoptera	5400
Cricket control	6600

Table 3 - Metals Results							
Description		Units	MDL	Terrestrial	Aquatic	Lepidoptera	Cricket Control
Sb	Antimony	mg/kg dry	0.014	0.138	0.113	0.0205	<0.014
As	Arsenic	mg/kg dry	0.073	3.25	0.597	0.169	0.0745
Cd	Cadmium	mg/kg dry	0.005	0.871	0.83	0.698	0.0214
Cr	Chromium	mg/kg dry	0.065	38.6	209	28.6	0.23
Cu	Copper	mg/kg dry	0.05	74.8	114	36.1	17.1
Pb	Lead	mg/kg dry	0.007	16	16.5	1.75	0.0459
Hg	Mercury	mg/kg dry	0.2	0.083	0.072	<0.040	<0.040
Ni	Nickel	mg/kg dry	0.032	30.5	147	21.1	0.249
Ag	Silver	mg/kg dry	0.026	0.0515	0.55	0.0942	<0.026
Zn	Zinc	mg/kg dry	0.19	582	913	234	143
Al	Aluminum	mg/kg dry	0.078	93.8	134	10.7	7.77
Ba	Barium	mg/kg dry	0.009	11.7	20.7	6.58	0.476
Mg	Magnesium	mg/kg dry	0.13	1510	1430	1300	1190
Mn	Manganese	mg/kg dry	0.01	74.2	77.4	28.5	27.3

Table 4– Insect Weight Summaries (grams)				
Group	Total Weight (grams)[1]	Wet Weight (grams)[2]	Dry Weight (grams)[3]	Avg. % Solids
1st June Split	616.61[4]	330.12	101.52	0.3092
Aquatic	54.56	82.78	27.33	0.3302
Terrestrial	438.74	213.27	71.48	0.3367
Lepidoptera	649.51	440.19	101.08	0.2243
2nd June Split	616.61[4]	295.44	96.57	0.3268

[1]Actual total weights received by the laboratory
[2]Weight of aliquots selected for sampling post-processing (e.g., water added, blended, etc.)
[3]Aliquots after removal from freeze drier
[4]Total weight prior to split (½ were analyzed during the first phase)

Table 5 - Whole Body Metal Concentration (mg/kg dry weight)[1]			
	Cd	Cu	Pb
Stonefly	0.2±0.02	22±2	0.4±0.1
Caddisfly	0.2±0.1	20±8	0.8±0.6

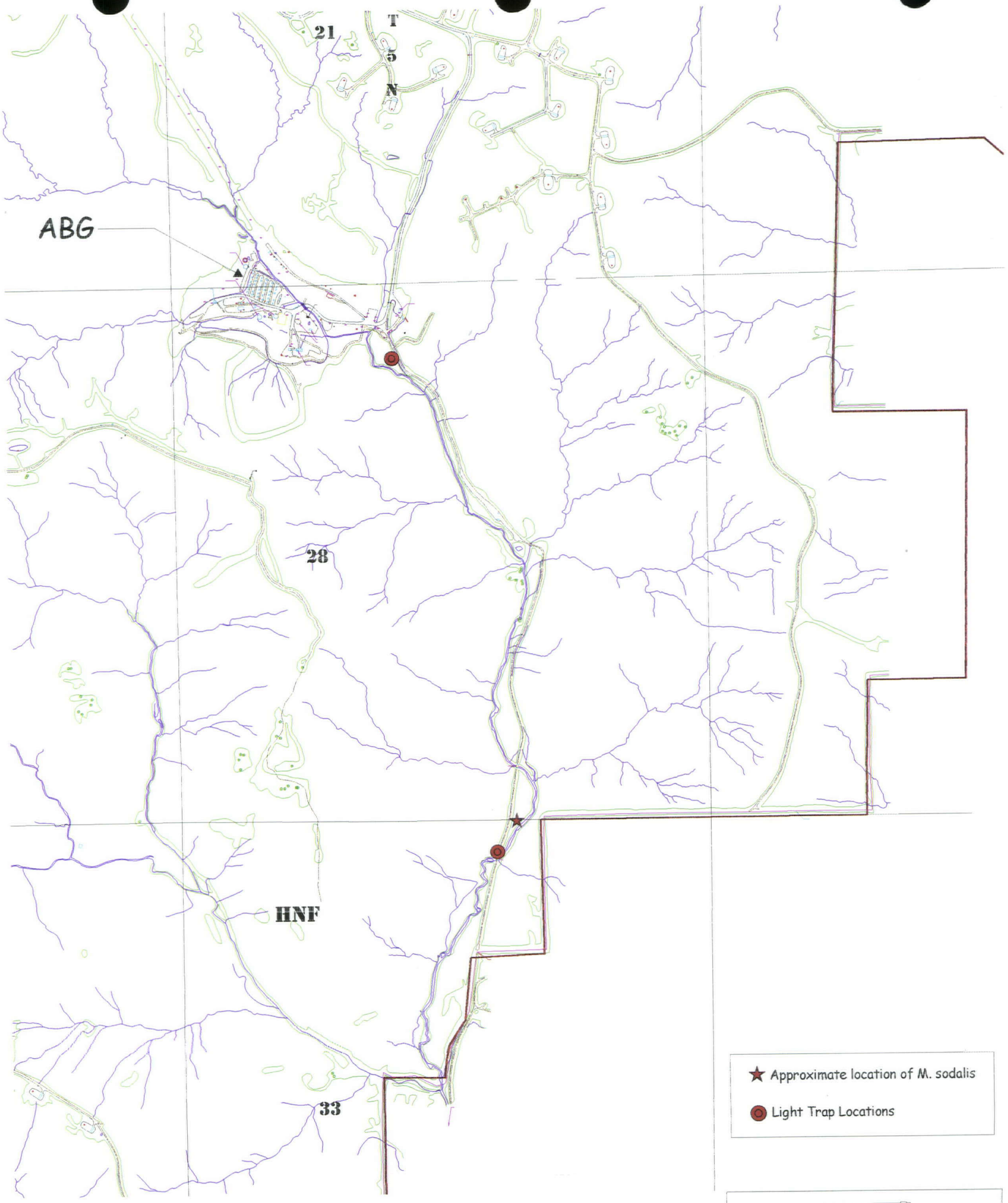
[1]After Cain (1995)

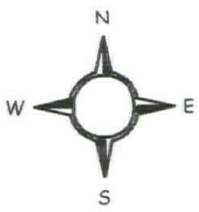
Table 6 - Max. & Min. %s Compared to Cricket Control

Description		Units	MDL	Terrestrial	Aquatic	Lepidoptera	Cricket Control	% Exceed. by Min.	% Exceed. by Max.
Sb	Antimony	mg/kg dry	0.014	0.138	0.113	**0.0205**	<0.014	293	1971
As	Arsenic	mg/kg dry	0.073	3.25	0.597	**0.169**	0.0745	227	4362
Cd	Cadmium	mg/kg dry	0.005	0.871	0.83	**0.698**	0.0214	3262	4070
Cr	Chromium	mg/kg dry	0.065	38.6	209	**28.6**	0.23	12435	90870
Cu	Copper	mg/kg dry	0.05	74.8	114	**36.1**	17.1	211	667
Pb	Lead	mg/kg dry	0.007	16	16.5	**1.75**	0.0459	3813	35948
Hg	Mercury	mg/kg dry	0.2	0.083	0.072	**<0.040**	<0.040	1	415
Ni	Nickel	mg/kg dry	0.032	30.5	147	**21.1**	0.249	8474	59036
Ag	Silver	mg/kg dry	0.026	**0.0515**	0.55	0.0942	<0.026	396	4231
Zn	Zinc	mg/kg dry	0.19	582	913	**234**	143	164	638
Al	Aluminum	mg/kg dry	0.078	93.8	134	**10.7**	7.77	138	1725
Ba	Barium	mg/kg dry	0.009	11.7	20.7	**6.58**	0.476	1382	4349
Mg	Magnesium	mg/kg dry	0.13	1510	1430	**1300**	1190	109	127
Mn	Manganese	mg/kg dry	0.01	74.2	77.4	**28.5**	27.3	104	284

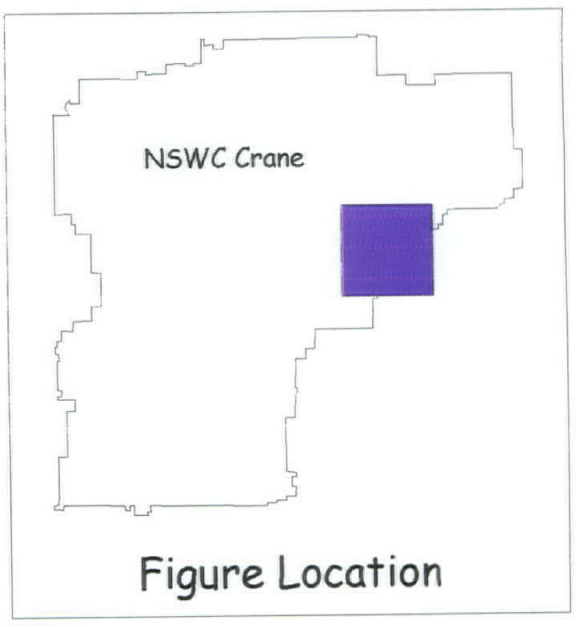
Figure 1

Sampling Location





800 0 800 Feet



Location of ABG wrt Bat Capture & Trap Locations

Figure (1)

Figure 2

Light Trap

Figure 3

Malaise Trap

Appendix A

Letter from USFWS

United States Department of the Interior

FISH AND WILDLIFE SERVICE

BLOOMINGTON FIELD OFFICE (ES)
620 South Walker Street
Bloomington, IN 47403-2121
(812) 334-4261 FAX (812) 334-4273

IN REPLY REFER TO:

October 20, 1999

Mr. Thomas J. Brent
Department of the Navy
Crane Division - Code 09510
Naval Surface Warfare Center
300 Highway 361
Crane, Indiana 47522-5000

Dear Mr. Brent:

As you requested, this letter is intended to follow up on the site inspection that was conducted on Crane yesterday. Specifically, potential insect collection sites along Little Sulphur Creek were inspected by you, Daniel Mazur (EPA), Scott Pruitt (USFWS, Bloomington Field Office), and me. After inspecting these sites, we concurred that the 2 sampling sites should include: 1) 1 site in floodplain vegetation adjacent to Little Sulphur Creek immediately downstream of the Ammunition Burning Grounds (ABG); and 2) a second site further downstream (near the previous sampling site).

If you have any questions please contact me at (812) 334-4261, extension 211.

Sincerely,

Lori Pruitt

Lori Pruitt

cc: Peter Ramanauskas, EPA

Appendix B

Field Sample Logs

INDIANA BAT FOOD CHAIN STUDY
SAMPLE LOG

Department of the Navy
Naval Surface Warfare Center
Crane, IN 47522

sheet # 1a

TRAP TYPE	TRAP SITE
Light	B3090
SAMPLE DATE	**TIME OF TRAPPING**
6/12 - 6/13/00	1730 - 0735

OBSERVATIONS

p.m. - Cloudy/humid 1st June Sample (degradation study)
a.m - Raining (hard).
Trap was running. All lights on. Bottom drawer covered/full w/ of small moths.

SAMPLE DATE	TIME OF TRAPPING
6/13 - 6/14/00	1710 - ?

OBSERVATIONS

Removed screen from "water collector". p.m. Clear humid
a.m. Trap off - no bugs. Will have to do an addl. collection this weekend.
Possibly due to position of gas can & there being less gas than last night.
Skies were clear

SAMPLE DATE	TIME OF TRAPPING
6/14 - 6/15	1655 - 0720

OBSERVATIONS

p.m. ptly. Cloudy.
a.m. Cloudy. 1 Flourescs. lt. burned out. Fewer insects (esp moths) than 6/13 a.m. collection. Larger moths than 6/13 a.m.

SAMPLE DATE	TIME OF TRAPPING
6/15 - 6/16/00	1710 - 0713

OBSERVATIONS

p.m. mostly clear. Warm. Not as humid
a.m. Warm, mostly cloudy. Not as humid.

SAMPLE NUMBER	NUMBER OF CONTAINERS

SAMPLER'S SIGNATURE	CODE

DATE SAMPLES SENT	ANALYTICAL LAB

INDIANA BAT FOOD CHAIN STUDY
SAMPLE LOG

Department of the Navy
Naval Surface Warfare Center
Crane, IN 47522

sheet # 1

TRAP TYPE	TRAP SITE
Light	B3090
SAMPLE DATE	**TIME OF TRAPPING**
6/18 - 6/19/00	1745-0730

OBSERVATIONS

p.m. Cloudy - ~72°F

a.m. partly cloudy. cool & hazy. Top light off (H_g - Vapor).

Changed transformer.

SAMPLE DATE	TIME OF TRAPPING

OBSERVATIONS

SAMPLE DATE	TIME OF TRAPPING

OBSERVATIONS

SAMPLE DATE	TIME OF TRAPPING

OBSERVATIONS

SAMPLE NUMBER	NUMBER OF CONTAINERS

SAMPLER'S SIGNATURE	CODE

DATE SAMPLES SENT	ANALYTICAL LAB

INDIANA BAT FOOD CHAIN STUDY
SAMPLE LOG

Department of the Navy
Naval Surface Warfare Center
Crane, IN 47522

sheet # 16

TRAP TYPE	TRAP SITE
Light	LSC Floodplain
SAMPLE DATE	**TIME OF TRAPPING** (took ~30 min. to setup Malaise)
6/12 - 6/13/00	1800 - ?

OBSERVATIONS

p.m. Cloudy
a.m. Raining (hard). Trap was off upon arrival. May be due to failure to pull gas from external tank. Will try to set up higher tonight. Had ~75% vol. of B3050 trap. Lots of moths, some large beetles.

SAMPLE DATE	TIME OF TRAPPING
6/13 - 6/14/00	1730 - ~~07~~ ?

OBSERVATIONS

Removed screen from water collector. p.m. Clear + humid
a.m. Trap off. Will have to do an add'l. collection this wknd. Possibly due to connections being shut (unable to vent/drain) &/or gas may have been shut off. Skies were clear.

SAMPLE DATE	TIME OF TRAPPING
6/14/00 - 6/15/00	1720 - 0714

OBSERVATIONS

PM partly cloudy
a.m. Cloudy. Fewer insects than B3050 L.T. No large moths.

SAMPLE DATE	TIME OF TRAPPING
6/15 - 16/00	1730 - ~~0710~~ CDF 0705

OBSERVATIONS

p.m. mostly clear, warm, not as humid
a.m. Warm. Mostly cloudy. Generator ran out of gas as I was walking toward it, ∴ didn't get to check lights.

SAMPLE NUMBER	NUMBER OF CONTAINERS

SAMPLER'S SIGNATURE	CODE

DATE SAMPLES SENT	ANALYTICAL LAB

INDIANA BAT FOOD CHAIN STUDY
SAMPLE LOG

Department of the Navy
Naval Surface Warfare Center
Crane, IN 47522

sheet #/b

TRAP TYPE	TRAP SITE
Light	Flood plain LSC
SAMPLE DATE	**TIME OF TRAPPING**
6/18 - 6/19/00	1805 - ?

OBSERVATIONS

p.m. cloudy ~72°F

Trap off in a.m. Partly cloudy, cool, & hazy

SAMPLE DATE	TIME OF TRAPPING

OBSERVATIONS

SAMPLE DATE	TIME OF TRAPPING

OBSERVATIONS

SAMPLE DATE	TIME OF TRAPPING

OBSERVATIONS

SAMPLE NUMBER	NUMBER OF CONTAINERS

SAMPLER'S SIGNATURE	CODE

DATE SAMPLES SENT	ANALYTICAL LAB

INDIANA BAT FOOD CHAIN STUDY
SAMPLE LOG

Department of the Navy
Naval Surface Warfare Center
Crane, IN 47522

sheet # 1c

TRAP TYPE	TRAP SITE
Malaise	Near 03-07 well
SAMPLE DATE	**TIME OF TRAPPING**
6/12 - 6/13/00	1750 - 0800

OBSERVATIONS

p.m. Cloudy
a.m. raining (hard).
fair collection of insects. Still probably <0.1% of Light traps.
More than I recall from last year.

SAMPLE DATE	TIME OF TRAPPING
6/13 - 6/14/00	1650 - 0735

OBSERVATIONS

p.m. Clear / humid
a.m. Clear - not as humid. Due to other traps having ~~Ø~~ bugs, placed this Collection in w/ yesterday's.

SAMPLE DATE	TIME OF TRAPPING
6/14 - 6/15/00	1700 - 0716

OBSERVATIONS

p.m. ptly cloudy
a.m. Cloudy

SAMPLE DATE	TIME OF TRAPPING
6/15 - 16/00	1655 - ~~0705~~ CDF 0710

OBSERVATIONS

p.m. mostly clear - warm, not ss humid.
a.m. Mostly cloudy, Not as humid.

SAMPLE NUMBER	NUMBER OF CONTAINERS

SAMPLER'S SIGNATURE	CODE

DATE SAMPLES SENT	ANALYTICAL LAB

INDIANA BAT FOOD CHAIN STUDY
SAMPLE LOG

Department of the Navy
Naval Surface Warfare Center
Crane, IN 47522

sheet # 10

TRAP TYPE	TRAP SITE
Malaise	Near well 0307
SAMPLE DATE	**TIME OF TRAPPING**
6/18 - 6/19/00	1750 - 0725

OBSERVATIONS

p.m. Cloudy ~72°F

a.m. Partly cloudy, cool, & hazy. Least amt. of insects so far.

SAMPLE DATE	TIME OF TRAPPING

OBSERVATIONS

SAMPLE DATE	TIME OF TRAPPING

OBSERVATIONS

SAMPLE DATE	TIME OF TRAPPING

OBSERVATIONS

SAMPLE NUMBER	NUMBER OF CONTAINERS

SAMPLER'S SIGNATURE	CODE

DATE SAMPLES SENT	ANALYTICAL LAB

INDIANA BAT FOOD CHAIN STUDY
SAMPLE LOG

Department of the Navy
Naval Surface Warfare Center
Crane, IN 47522

sheet # 2a

TRAP TYPE	TRAP SITE
Light	B 3090
SAMPLE DATE	**TIME OF TRAPPING**
6/19 - 6/20/00	1700 - ??

OBSERVATIONS

p.m. clear ~70°F Removed flaking paint

a.m. Cool & sprinkling. Trap off in a.m. Practically no insects other than moths in upper drawer. ∴ elected to just use 2 containers instead of 3. Emptied trap: 0720

SAMPLE DATE	TIME OF TRAPPING
6/20/00 - 6/21/00	~~1645~~ 1715 - 0735

OBSERVATIONS

p.m. Δ start time due to generator problems (low oil). Cloudy

a.m. Tree down on way to this trap. Had to get moved. Heavy rains overnight → lots of small insects & paint chips from L.T. Few bugs in upper drawer ∴ placed all in lower drawer container (only 1 lg. orange moth).

SAMPLE DATE	TIME OF TRAPPING
6/21/00 - 6/22/00	1705 - 0710

OBSERVATIONS

p.m. Clear. Had to fill this gen. w/oil again.

a.m. mostly cloudy

SAMPLE DATE	TIME OF TRAPPING
6/22/00 - 6/23/00	1645 - 0730

OBSERVATIONS

p.m. Mostly clear — lower bridge Crane-INS-62300

a.m. clear sunny — Crane-LEP-62300 bridge

SAMPLE NUMBER	NUMBER OF CONTAINERS

SAMPLER'S SIGNATURE	CODE

DATE SAMPLES SENT	ANALYTICAL LAB

INDIANA BAT FOOD CHAIN STUDY
SAMPLE LOG

Department of the Navy
Naval Surface Warfare Center
Crane, IN 47522

sheet # 2

TRAP TYPE	TRAP SITE
Light	LSC Flood Plain
SAMPLE DATE	**TIME OF TRAPPING**
6/19 - 6/20/00	1635 - 0710

OBSERVATIONS

p.m. Clear - ~70°F Removed flaking paint.

a.m. Sprinkling. Used 2 containers instead of 3 due to lack of insects in upper drawer.

SAMPLE DATE	TIME OF TRAPPING
6/20/00 - 6/21/00	1735 - 0705

OBSERVATIONS

p.m. Cloudy.

a.m. Heavy rain o-night.

Began emptying @ 0830. Used separate container for lower drawer. No large moths

Malaise insects included in lower drawer container.

SAMPLE DATE	TIME OF TRAPPING
6/21/00 - 6/22/00	1720 - 0700

OBSERVATIONS

p.m. clear. oil in gen. OK.

a.m. mostly cloudy. Finished emptying @ 0815. By then skies were clear. No med. size "Megaloptera" (or (g.).

SAMPLE DATE	TIME OF TRAPPING
6/22/00 - 6/23/00	1700 - 0720

OBSERVATIONS

p.m. mostly clear emptied: 0825 lower Floodplain Crane-INS-62300

a.m. sunny clear Crane-LEP-62300 Floodplain ~~Floodplain~~

malaise in floodplain sample

SAMPLE NUMBER	NUMBER OF CONTAINERS

SAMPLER'S SIGNATURE	CODE

DATE SAMPLES SENT	ANALYTICAL LAB

INDIANA BAT FOOD CHAIN STUDY
SAMPLE LOG

Department of the Navy
Naval Surface Warfare Center
Crane, IN 47522

sheet #2c

TRAP TYPE	TRAP SITE
Malaise	Well 0307
SAMPLE DATE	**TIME OF TRAPPING**
6/19 - 6/20/00	1730 - 0715

OBSERVATIONS

p.m. clear ~70°F
a.m. sprinkling.

SAMPLE DATE	TIME OF TRAPPING
6/20/00 - 6/21/00	1720 - 0730

OBSERVATIONS

p.m. Cloudy.
a.m. Heavy rains o-night.
Insects included in lower drawer Floodplain sample

SAMPLE DATE	TIME OF TRAPPING
6/21/00 - 6/22/00	1710 - 0705

OBSERVATIONS

p.m. clear
a.m. mostly cloudy

SAMPLE DATE	TIME OF TRAPPING
6/22/00 - 6/23/00	1650 - 0725

OBSERVATIONS

p.m. mostly clear a.m. sunny clear
malaise in floodplain sample

SAMPLE NUMBER	NUMBER OF CONTAINERS

SAMPLER'S SIGNATURE	CODE

DATE SAMPLES SENT	ANALYTICAL LAB

INDIANA BAT FOOD CHAIN STUDY
SAMPLE LOG

Department of the Navy
Naval Surface Warfare Center
Crane, IN 47522

sheet # 3

TRAP TYPE	TRAP SITE
Light	B-3090
SAMPLE DATE	**TIME OF TRAPPING**
7/17/00 - 7/18/00	1745 - 0722

OBSERVATIONS

p.m. Cloudy warm
1 flo. light out p.m. overcast
Finished collection @ 0812 Top drawer placed in bottom drawer (only 6 beetles)

SAMPLE DATE	TIME OF TRAPPING
7/18/00 - 7/19/00	1653 - 0725

OBSERVATIONS

p.m. partly cloudy
a.m. cloudy/rainy (heavy rain overnight) Finished @ 0815
Due to quantity of wet bugs, Bridge collection (upper & lower drawers are in a seperate container from Floodplain. Few bugs in upper dr[illegible]).

SAMPLE DATE	TIME OF TRAPPING
7/19/00 - 7/20/00	1643 - 0712

OBSERVATIONS

P.M. Cloudy
a.m. Sunny & Cool. Fewer insects than previous mornings, but not as active due to cool temps. Stopped (finished) emptying @ 0744.
No "top drawer" collection - too few bugs.

SAMPLE DATE	TIME OF TRAPPING
7/20-7/21/00	1656 - 0715

OBSERVATIONS

p.m. Cool & mostly sunny
a.m. Cool & clear (almost cold). Finished emptying @ 0742
1 large megalopteran

SAMPLE NUMBER	NUMBER OF CONTAINERS

SAMPLER'S SIGNATURE	CODE

DATE SAMPLES SENT	ANALYTICAL LAB

INDIANA BAT FOOD CHAIN STUDY
SAMPLE LOG

Department of the Navy
Naval Surface Warfare Center
Crane, IN 47522

sheet # 3a

TRAP TYPE	TRAP SITE
Light	LSC Floodplain
SAMPLE DATE	**TIME OF TRAPPING**
7/17/00 - 7/18/00	1800 - 0713.

OBSERVATIONS

p.m. Cloudy warm

a.m. overcast. Emptied @ 0825 finish collection @ 0900

Top drawer placed w/ Bottom drawer (only 6 beetles).

SAMPLE DATE	TIME OF TRAPPING
7/18/00 - 7/19/00	1716 - 0714

OBSERVATIONS

p.m. partly cloudy / a.m. Dragon Fly + 2 locusts. Obvious signs of bat feeding @ this trap (wings) - practically every a.m.

a.m. Heavy rain overnight. Morning - Cloudy Started Collection @ 0840 finished @ 0922

Due to lg. qty. of wet bugs, we collected the Bridge samples separate from the Flood plain sample. Too few bugs in top drawer to collect separately (mostly Leps.).

SAMPLE DATE	TIME OF TRAPPING
7/19/00 - 7/20/00	1702 - 0705

OBSERVATIONS

p.m. cloudy.

a.m. sunny & cool. Started emptying @ 0751. Finished @ 0834. (now - partly cloudy).

Bugs were more active than those in the B3090 light trap.

No "top drawer" collection - too few insects.

SAMPLE DATE	TIME OF TRAPPING
7/20/00 - 7/21/00	1708 - 0708

OBSERVATIONS

p.m. cool + mostly sunny

a.m. Cool & clear (almost cold). Started emptying @ 0752

finished @ 0820

SAMPLE NUMBER	NUMBER OF CONTAINERS

SAMPLER'S SIGNATURE	CODE

DATE SAMPLES SENT	ANALYTICAL LAB

INDIANA BAT FOOD CHAIN STUDY
SAMPLE LOG

Department of the Navy
Naval Surface Warfare Center
Crane, IN 47522

sheet # 3

TRAP TYPE	TRAP SITE
Malaise	Well 0307

SAMPLE DATE	TIME OF TRAPPING
7/17/00 - 7/18/00	1755 - 0719

OBSERVATIONS

p.m. Cloudy warm
a.m. overcast. Contained 1 hornet - probably from a.m., but left w/in.

SAMPLE DATE	TIME OF TRAPPING
7/18/00 - 7/19/00	1703 - 0718

OBSERVATIONS

p.m. partly Cloudy.
a.m. Heavy rain overnight (Cloudy in a.m.).

SAMPLE DATE	TIME OF TRAPPING
07/19/00 - 07/20/00	1650 - 0708

OBSERVATIONS

p.m. cloudy

SAMPLE DATE	TIME OF TRAPPING
07/20/00 - 07/21/00	1700 - 0712

OBSERVATIONS

p.m. cool + mostly sunny
a.m. Cool & Clear (almost cold).

SAMPLE NUMBER	NUMBER OF CONTAINERS

SAMPLER'S SIGNATURE	CODE

DATE SAMPLES SENT	ANALYTICAL LAB

INDIANA BAT FOOD CHAIN STUDY
SAMPLE LOG

Department of the Navy
Naval Surface Warfare Center
Crane, IN 47522

sheet # 4a

TRAP TYPE	TRAP SITE
Light	B-3090
SAMPLE DATE	**TIME OF TRAPPING**
8/21 - 22/00	1725 - 0704

OBSERVATIONS

p.m. Clear
a.m mostly Cloudy Did some sorting in field, but ret'd to office for most (placed in a 3rd box). Only used 2 containers when done.
No large moths (Regal or Imperial). ~~Kept~~ in cooler until sorted. Finished @ 0843

SAMPLE DATE	TIME OF TRAPPING
8/22 - 23/00	1650 - 0706

OBSERVATIONS

p.m. partly cloudy
a.m. Heavy rain. No Regal or Imperial Moths. Most Placed insects in a separate box to be sorted back @ B3245

SAMPLE DATE	TIME OF TRAPPING
8/23 - 24/00	1715 - ??

OBSERVATIONS

p.m. Clear
a.m. Cloudy - Rain overnight. ~~Trap~~ Generator had quit running sometime last night. Cool to touch. However, there were several bugs, although not as many as usual. Did some sorting in field, but brought most back for sorting @ B3245

SAMPLE DATE	TIME OF TRAPPING
8/24 - 25/00	1712 - ??

OBSERVATIONS

p.m. Clear. Had to use alternate generator as Honda gen. had apparently seized up. a.m. Alt. gen. apparently quit early in the eve. Very few insects. ∴ sorted in field.

SAMPLE NUMBER	NUMBER OF CONTAINERS

SAMPLER'S SIGNATURE	CODE

DATE SAMPLES SENT	ANALYTICAL LAB

INDIANA BAT FOOD CHAIN STUDY
SAMPLE LOG

Department of the Navy
Naval Surface Warfare Center
Crane, IN 47522

sheet # 4b

TRAP TYPE	TRAP SITE
Light	LSC Floodplain
SAMPLE DATE	**TIME OF TRAPPING**
8/21-22/00	1738 - 0658

OBSERVATIONS

p.m. Clear

~~p.m.~~ a.m. Mostly cloudy. Thousands of small winged insects. No (Regal or Imperial) large moths. Placed insects & leps. from lower drawer into a 3rd container for sorting later. Placed in cooler & kept there. Finished @ 0543

SAMPLE DATE	TIME OF TRAPPING
8/22-23/00	1707 - 0659

OBSERVATIONS

p.m. partly cloudy

a.m. Heavy rain. ~4 Hornets (European?). No Regal or Imperial ~~moths~~

Most Insects placed in a separate container to be sorted back @ B3245.

SAMPLE DATE	TIME OF TRAPPING
8/23-24/00	1732 - 0704

OBSERVATIONS

p.m. Clear

a.m. Cloudy. Rain overnight. 2 June moths & 1 Megaloptera. Most of (in lower drawer) tiny black bugs were determined inseparable containing paint chips & discarded. Did some sorting in field. Ret'd rest via cooler to B3245 to sort there.

SAMPLE DATE	TIME OF TRAPPING
8/24-25/00	1726 - 0710

OBSERVATIONS

p.m. Clear

SAMPLE NUMBER	NUMBER OF CONTAINERS

SAMPLER'S SIGNATURE	CODE

DATE SAMPLES SENT	ANALYTICAL LAB

INDIANA BAT FOOD CHAIN STUDY
SAMPLE LOG

Department of the Navy
Naval Surface Warfare Center
Crane, IN 47522

sheet # 4c

TRAP TYPE	TRAP SITE
Malaise	Near 03-07 Well
SAMPLE DATE	**TIME OF TRAPPING**
8/21-22/00	1732-0702

OBSERVATIONS

p.m. Clear
a.m. Mostly Cloudy

SAMPLE DATE	TIME OF TRAPPING
8/22-23/00	1657-0702

OBSERVATIONS

p.m. partly cloudy
a.m. Heavy rain-

SAMPLE DATE	TIME OF TRAPPING
8/23-24/00	1722-0708

OBSERVATIONS

p.m. Clear
a.m. Cloudy - Rain Overnight

SAMPLE DATE	TIME OF TRAPPING
8/24-25/00	1719-0714

OBSERVATIONS

p.m. Clear
a.m. Light fog - otherwise clear

SAMPLE NUMBER	NUMBER OF CONTAINERS

SAMPLER'S SIGNATURE	CODE

DATE SAMPLES SENT	ANALYTICAL LAB

TODAY'S DATE: 18-SEP-00

JUN-00 FOR BLOOMINGT, IN (824')

LAT=39.2N LON= 86.5W

	TEMPERATURE							PRECIPITATION			
	ACTUAL			NORMAL							
	HI	LO	AVG	HI	LO	AVG	DEPT	AMNT	SNOW	SNCVR	HDD
1	87	62	75	79	57	68	+7	0.00	0.0	0	0
2	83	60	72	79	58	68	+4	0.00	0.0	0	0
3	73	50	62	79	58	69	-7	0.00	0.0	0	3
4	79	54	67	79	58	69	-2	0.06	0.0	0	0
5	70	52	61	80	59	69	-8	0.03	0.0	0	4
6	71	47	59	80	59	70	-11	0.00	0.0	0	6
7	78	43	61	80	59	70	-9	trace	0.0	0	4
8	84	57	71	81	60	70	+1	0.00	0.0	0	0
9	87	56	72	81	60	70	+2	0.00	0.0	0	0
10	89	66	78	81	60	71	+7	0.01	0.0	0	0
11	87	72	80	81	60	71	+9	0.25	0.0	0	0
12	90	70	80	82	61	71	+9	0.01	0.0	0	0
13	88	71	80	82	61	71	+9	0.33	0.0	0	0
14	87	69	78	82	61	72	+6	0.84	0.0	0	0
15	80	67	74	82	62	72	+2	0.00	0.0	0	0
16	80	68	74	82	62	72	+2	1.05	0.0	0	0
17	75	63	69	83	62	72	-3	0.71	0.0	0	0
18	70	61	66	83	62	73	-7	0.08	0.0	0	0
19	80	58	69	83	62	73	-4	0.00	0.0	0	0
20	86	62	74	83	63	73	+1	trace	0.0	0	0
21	79	66	73	83	63	73	+0	0.41	0.0	0	0
22	84	64	74	84	63	73	+1	0.00	0.0	0	0
23	87	63	75	84	63	74	+1	0.00	0.0	0	0
24	84	67	76	84	64	74	+2	0.84	0.0	0	0
25	84	67	76	84	64	74	+2	0.01	0.0	0	0
26	80	64	72	84	64	74	-2	0.01	0.0	0	0
27	81	62	72	85	64	74	-2	0.21	0.0	0	0
28	78	57	68	85	64	75	-7	trace	0.0	0	0
29	78	57	68	85	64	75	-7	0.00	0.0	0	0
30	78	53	66	85	65	75	-9	0.00	0.0	0	0

TOTALS FOR BMG

HIGHEST TEMPERATURE	90	TOTAL PRECIP	4.85
LOWEST TEMPERATURE	43	TOTAL SNOWFALL	0.0
AVERAGE TEMPERATURE	71.1	NORMAL PRECIP	3.60
DEPARTURE FROM NORM	-0.7	% OF NORMAL PRECIP	135
HEATING DEGREE DAYS	17		
NORMAL DEGREE DAYS	8		

TODAY'S DATE: 18-SEP-00

JUL-00 FOR BLOOMINGT, IN (824')

LAT=39.2N LON= 86.5W

	TEMPERATURE							PRECIPITATION			
	ACTUAL			NORMAL							
	HI	LO	AVG	HI	LO	AVG	DEPT	AMNT	SNOW	SNCVR	HDD
1	82	53	68	85	65	75	-7	trace	0.0	0	0
2	85	66	76	85	65	75	+1	trace	0.0	0	0
3	86	73	80	85	65	75	+5	0.54	0.0	0	0
4	86	70	78	85	65	75	+3	0.48	0.0	0	0
5	82	71	77	86	65	76	+1	0.06	0.0	0	0
6	88	71	80	86	65	76	+4	0.00	0.0	0	0
7	82	61	72	86	66	76	-4	0.00	0.0	0	0
8	83	57	70	86	66	76	-6	trace	0.0	0	0
9	85	58	72	86	66	76	-4	trace	0.0	0	0
10	88	71	80	86	66	76	+4	0.18	0.0	0	0
11	81	70	76	86	66	76	+0	0.96	0.0	0	0
12	82	70	76	86	66	76	+0	0.00	0.0	0	0
13	86	67	77	86	66	76	+1	trace	0.0	0	0
14	85	64	75	86	66	76	-1	0.00	0.0	0	0
15	82	63	73	86	66	76	-3	0.00	0.0	0	0
16	80	58	69	86	66	76	-7	0.00	0.0	0	0
17	82	57	70	86	66	76	-6	0.00	0.0	0	0
18	83	67	75	86	66	76	-1	0.02	0.0	0	0
19	75	63	69	86	66	76	-7	0.42	0.0	0	0
20	74	58	66	86	66	76	-10	0.00	0.0	0	0
21	77	56	67	86	66	76	-9	0.00	0.0	0	0
22	77	51	64	86	66	76	-12	0.00	0.0	0	1
23	77	53	65	86	66	76	-11	0.00	0.0	0	0
24	80	54	67	86	66	76	-9	0.00	0.0	0	0
25	80	51	66	86	66	76	-10	trace	0.0	0	0
26	82	56	69	86	66	76	-7	0.00	0.0	0	0
27	85	57	71	86	66	76	-5	0.00	0.0	0	0
28	82	63	73	86	66	76	-3	0.13	0.0	0	0
29	72	64	68	86	66	76	-8	0.77	0.0	0	0
30	79	67	73	86	66	76	-3	0.10	0.0	0	0
31	77	63	70	86	65	76	-6	0.03	0.0	0	0

TOTALS FOR BMG

HIGHEST TEMPERATURE	88	TOTAL PRECIP	3.69
LOWEST TEMPERATURE	51	TOTAL SNOWFALL	0.0
AVERAGE TEMPERATURE	71.7	NORMAL PRECIP	4.72
DEPARTURE FROM NORM	-4.2	% OF NORMAL PRECIP	78
HEATING DEGREE DAYS	1		
NORMAL DEGREE DAYS	0		

TODAY'S DATE: 18-SEP-00

AUG-00 FOR BLOOMINGT, IN (824')

LAT=39.2N LON= 86.5W

	TEMPERATURE							PRECIPITATION			
	ACTUAL			NORMAL							
	HI	LO	AVG	HI	LO	AVG	DEPT	AMNT	SNOW	SNCVR	HDD
1	82	61	72	86	65	76	-4	0.03	0.0	0	0
2	84	64	74	86	65	76	-2	0.27	0.0	0	0
3	80	63	72	86	65	75	-3	0.00	0.0	0	0
4	80	61	71	86	65	75	-4	0.00	0.0	0	0
5	77	57	67	86	65	75	-8	0.11	0.0	0	0
6	86	73	80	85	65	75	+5	0.67	0.0	0	0
7	82	67	75	85	65	75	+0	1.14	0.0	0	0
8	79	67	73	85	65	75	-2	0.22	0.0	0	0
9	88	73	81	85	64	75	+6	0.08	0.0	0	0
10	83	62	73	85	64	75	-2	trace	0.0	0	0
11	82	59	71	85	64	74	-3	trace	0.0	0	0
12	80	55	68	85	64	74	-6	0.00	0.0	0	0
13	79	52	66	85	64	74	-8	0.00	0.0	0	0
14	83	58	71	84	64	74	-3	0.00	0.0	0	0
15	87	65	76	84	63	74	+2	0.00	0.0	0	0
16	85	60	73	84	63	74	-1	0.00	0.0	0	0
17	89	60	75	84	63	74	+1	0.68	0.0	0	0
18	79	63	71	84	63	73	-2	0.12	0.0	0	0
19	77	55	66	84	63	73	-7	0.00	0.0	0	0
20	76	54	65	84	63	73	-8	0.00	0.0	0	0
21	80	52	66	83	62	73	-7	0.00	0.0	0	0
22	79	62	71	83	62	73	-2	trace	0.0	0	0
23	83	68	76	83	62	73	+3	0.53	0.0	0	0
24	80	61	71	83	62	72	-1	0.02	0.0	0	0
25	81	55	68	83	62	72	-4	0.00	0.0	0	0
26	78	58	68	83	62	72	-4	0.10	0.0	0	0
27	83	61	72	82	61	72	+0	trace	0.0	0	0
28	81	60	71	82	61	72	-1	0.02	0.0	0	0
29	85	66	76	82	61	71	+5	0.00	0.0	0	0
30	88	67	78	82	61	71	+7	trace	0.0	0	0
31	88	62	75	82	60	71	+4	trace	0.0	0	0

TOTALS FOR BMG

HIGHEST TEMPERATURE	89	TOTAL PRECIP	3.99
LOWEST TEMPERATURE	52	TOTAL SNOWFALL	0.0
AVERAGE TEMPERATURE	71.7	NORMAL PRECIP	3.99
DEPARTURE FROM NORM	-1.9	% OF NORMAL PRECIP	100
HEATING DEGREE DAYS	0		
NORMAL DEGREE DAYS	0		

Appendix C

Shipping Documents

SHIPPING CONTAINER TALLY → 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50

REQUISITION AND INVOICE / SHIPPING DOCUMENT

Form Approved
OMB No. 0704-0246

Public reporting burden for this collection of information is estimated to average 15 minutes per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden; to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0246), Washington, DC 20503.

1. FROM: (Include ZIP Code)
NAVSURFWARCENDIV
CODE 1121 BLDG 41SE
300 HIGHWAY 361
CRANE, IN 47522-5001

2. TO: (Include ZIP Code)
USAE Waterways Experiment Station
3909 Halls Ferry Road
CEWES ERD-A
Vicksburg, MS 39180

3. SHIP TO - MARK FOR
Al Cofrancesco (PH 601-634-3182)
OR Mike Grodowitz (PH 601-634-2972)

SHEET NO.	NO. OF SHEETS	5. REQUISITION DATE	6. REQUISITION NUMBER
1		2000-06-19	

7. DATE MATERIAL REQUIRED (YYMMDD): 00/06/20
8. PRIORITY: 03
9. AUTHORITY OR PURPOSE:
10. SIGNATURE: CODE 095
11a. VOUCHER NUMBER & DATE (YYMMDD): N00164-0176-A934
12. DATE SHIPPED (YYMMDD):
b.
13. MODE OF SHIPMENT:
14. BILL OF LADING NUMBER:
15. AIR MOVEMENT DESIGNATOR OR PORT REFERENCE NO.

4. APPROPRIATIONS SYMBOL AND SUBHEAD	OBJ. CL.	BUR. CONT. NO.	SUBAL-LOT.	AUTHORIZATION ACCT'G ACTIVITY	TRANS. TYPE	PROPERTY ACCT'G ACTIVITY	COUN-TRY	COST CODE	AMOUNT
	97X4930	NH1J	000	77777	0	000164	2F	000000 096958373454	

ITEM NO. (a)	FEDERAL STOCK NUMBER, DESCRIPTION, AND CODING OF MATERIAL AND / OR SERVICES (b)	UNIT OF ISSUE (c)	QUANTITY REQUESTED (d)	SUPPLY ACTION (e)	TYPE CON-TAINER (f)	CON-TAINER NOS. (g)	UNIT PRICE (h)	TOTAL COST (i)
01	SHIP TWO (2) COOLERS CONTAINING SAMPLES FOR ANALYSIS PO	EA	2					

16. TRANSPORTATION VIA MATS OR MSTS CHARGEABLE TO
17. SPECIAL HANDLING

18. RECAPITULATION OF SHIPMENT	TOTAL CON-TAINERS	TYPE CON-TAINER	DESCRIPTION	TOTAL WEIGHT	TOTAL CUBE
ISSUED BY					
CHECKED BY					
PACKED BY					
			← TOTAL →		

19. RECEIPT				
CONTAINERS RECEIVED EXCEPT AS NOTED	DATE (YYMMDD)	BY	SHEET TOTAL	
QUANTITIES RECEIVED EXCEPT AS NOTED	DATE (YYMMDD)	BY	GRAND TOTAL	
POSTED	DATE (YYMMDD)	BY	20. RECEIVER'S VOUCHER NO.	

DD Form 1149, DEC 93
306/080

51 52 53 54 55 56 57 58 59 60 61 62 63 64 65 66 67 68 69 70 71 72 73 74 75 76 77 78 79 80 81 82 83 84 85 86 87 88 89 90 91 92 93 94 95 96 97 98 99 100

Previous editions are obsolete.

S/N 0102-LF-017-7900

(Navy Overprint 1994)

Cooler 2/2

CHAIN OF CUSTODY

CRANE DIVISION, NAVAL SURFACE WARFARE CENTER
ENVIRONMENTAL PROTECTION DEPT.
NAVSURFWARCENDIV 5090/6 (REV. 5/93)

SAMPLING DATE	SAMPLE I.D.	CONTAINER (PARAMETERS)
13,15,16,18/00	CRAN-DEG-600	EXPLOSIVES

SAMPLER PREPARER (SIGNATURE)	TRANSFERRED TO (SIGNATURE)	DATE	TIME	ORGANIZATION
Thomas J. Beat	ALCO FRANCESCO	6/19/00		NSWC → WES

Cooler 1/2

CHAIN OF CUSTODY

CRANE DIVISION, NAVAL SURFACE WARFARE CENTER
ENVIRONMENTAL PROTECTION DEPT.
NAVSURFWARCENDIV 5090/6 (REV. 5/93)

SAMPLING DATE	SAMPLE I.D.	CONTAINER (PARAMETERS)
6/13,15,16,19/00	CRANE-DEG-600	EXPLOSIVES

SAMPLER PREPARER (SIGNATURE)	TRANSFERRED TO (SIGNATURE)	DATE	TIME	ORGANIZATION
Thomas J. Brent	AL COFRANCESCO	6/19/00		NSWC → WES

SHIPPING CONTAINER TALLY → 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50

REQUISITION AND INVOICE/SHIPPING DOCUMENT

Form Approved
OMB No. 0704-0[illegible]
Expires Dec 31, [illegible]

The public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden, to Department of Defense, Washington Headquarters Services, Directorate for Information Operations and Reports (0704-0246), 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302. Respondants should be aware that not withstanding any other provision of law, no person shall be subject to any penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number.

PLEASE DO NOT RETURN YOUR FORM TO THIS ADDRESS. RETURN COMPLETED FORM TO THE ADDRESS IN ITEM 2.

1. FROM: (Include ZIP Code)
NAVSURFWARCENDIV
CODE 1121 BLDG 41SE
300 HIGHWAY 361
CRANE, IN 47522-5001

SHEET NO.	NO. OF SHEETS	5. REQUISITION DATE	6. REQUISITION NUMBER
1	1	2000-06-26	

7. DATE MATERIAL REQUIRED (YYYYMMDD)	8. PRIORITY
2000-06-27	03

2. TO: (Include ZIP Code)
USAE WATERWAYS EXPERIMENT STATION
3909 HALLS FERRY ROAD (CEWES ERD-A)
VICKSBURG, MS 39180

9. AUTHORITY OR PURPOSE
SAMPLES FOR ANALYSIS

10. SIGNATURE	11a. VOUCHER NUMBER & DATE (YYYYMMDD)
DANA WILLIAMS	N00164-0178-8105

3. SHIP TO - MARK FOR
AL COFRANCESCO (601)634-3182 OR
MIKE GRODOWITZ (601)634-2972

12. DATE SHIPPED (YYYYMMDD)	b.
13. MODE OF SHIPMENT	14. BILL OF LADING NUMBER

15. AIR MOVEMENT DESIGNATOR OR PORT REFERENCE NO.

4. APPROPRIATIONS DATA

			AMOUNT
TRANSPORTATION:	97X4930.	NH1J 000 77777 0 000164 2F 000000 837345409695	
LABOR:	97X4930.	NH1J 000 77777 0 000164 2F 000000 711240009695	
MATERIAL:	97X4930.	NH1J 000 77777 0 000164 2F 000000 711240009695	

ITEM NO. (a)	FEDERAL STOCK NUMBER, DESCRIPTION, AND CODING OF MATERIAL AND/OR SERVICES (b)	UNIT OF ISSUE (c)	QUANTITY REQUESTED (d)	SUPPLY ACTION (e)	TYPE CONTAINER (f)	CONTAINER NOS. (g)	UNIT PRICE (h)	TOTAL COST (i)
	POC: DANA WILLIAMS PHONE NO: 812-854-3590 RETURN COPY TO: TOM BRENT SHOP CD: 09695							
01	TWO (2) COOLERS OF SAMPLES	EA	2					

16. TRANSPORTATION VIA MATS OR MSTS CHARGEABLE TO

17. SPECIAL HANDLING

18. RECAPITULATION OF SHIPMENT

ISSUED BY	TOTAL CONTAINERS	TYPE CONTAINER	DESCRIPTION	TOTAL WEIGHT	TOTAL CUBE
DW3948					
CHECKED BY					
PACKED BY					
			← TOTAL →		

19. RECEIPT

CONTAINERS RECEIVED EXCEPT AS NOTED	DATE (YYYYMMDD)	BY	SHEET TOTAL
QUANTITIES RECEIVED EXCEPT AS NOTED	DATE (YYYYMMDD)	BY	GRAND TOTAL
POSTED	DATE (YYYYMMDD)	BY	20. RECEIVER'S VOUCHER NO.

DD FORM 1149, JAN 1997

51 52 53 54 55 56 57 58 59 60 61 62 63 64 65 66 67 68 69 70 71 72 73 74 75 76 77 78 79 80 81 82 83 84 85 86 87 88 89 90 91 92 93 94 95 96 97 98 99 100

PREVIOUS EDITION MAY BE USED.

CHAIN OF CUSTODY

CRANE DIVISION, NAVAL SURFACE WARFARE CENTER
ENVIRONMENTAL PROTECTION DEPT.
NAVSURFWARCENDIV 5090/6 (REV. 5/93)

SAMPLING DATE	SAMPLE I.D.	CONTAINER (PARAMETERS)
6/22/00	CRANE-LEP-62200	Inserts (metals + Expl.).
6/20/00	CRANE-LEP-62000	" "
6/20/00	CRANE-INS-62000	" "
6/23/00	CRANE-INS-62300	" "
6/23/00	CRANE-LEP-62300	" "

SAMPLER PREPARER (SIGNATURE)	TRANSFERRED TO (SIGNATURE)	DATE	TIME	ORGANIZATION
Thomas J. Brent	AL COFRANCESCO	6/26/00	1200	NSWC → WES

CHAIN OF CUSTODY

CRANE DIVISION, NAVAL SURFACE WARFARE CENTER
ENVIRONMENTAL PROTECTION DEPT.
NAVSURFWARCENDIV 5090/6 (REV. 5/93)

SAMPLING DATE	SAMPLE I.D.	CONTAINER (PARAMETERS)
6/21/00	CRANE-INS-62100	INSECTS (Metals + EXPL).
6/21/00	CRANE-INS-62100	INSECTS (Metals + EXPL).
6/21/00	CRANE-LEP-62100	" "
6/22/00	CRANE-INS-62200	" "
6/23/00	CRANE-LEP-62300	" "
6/23/00	CRANE-INS-62300	" "

SAMPLER PREPARER (SIGNATURE)	TRANSFERRED TO (SIGNATURE)	DATE	TIME	ORGANIZATION
Thomas J. Brant	AL COFRANCESCO	6/26/00	1200	NSWC → WES

SHIPPING CONTAINER TALLY → 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50

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CRANE, IN 47522-5001

SHEET NO. 1 | NO. OF SHEETS 1 | 5. REQUISITION DATE 2000-07-24 | 6. REQUISITION NUMBER

7. DATE MATERIAL REQUIRED (YYYYMMDD) 2000-07-25 | 8. PRIORITY 03

2. TO: (Include ZIP Code)
USAE WATERWAYS EXPERIMENT STATION
3909 HALLS FERRY ROAD (CEWES ERD-A)
VICKSBURG, MS 39180

9. AUTHORITY OR PURPOSE
SAMPLES FOR ANALYSIS

10. SIGNATURE
DANA WILLIAMS

11a. VOUCHER NUMBER & DATE (YYYYMMDD)
N00164-0206-9103

3. SHIP TO - MARK FOR
AL COFRANCESCO (601) 634-3182 OR
MIKE GRODOWITZ (601) 634-2972

12. DATE SHIPPED (YYYYMMDD)

b.

13. MODE OF SHIPMENT

14. BILL OF LADING NUMBER

15. AIR MOVEMENT DESIGNATOR OR PORT REFERENCE NO.

4. APPROPRIATIONS DATA

			AMOUNT
TRANSPORTATION:	97X4930.	NH1J 000 77777 0 000164 2F 000000 837345409695	
LABOR:	97X4930.	NH1J 000 77777 0 000164 2F 000000 711240009695	
MATERIAL:	97X4930.	NH1J 000 77777 0 000164 2F 000000 711240009695	

ITEM NO. (a)	FEDERAL STOCK NUMBER, DESCRIPTION, AND CODING OF MATERIAL AND/OR SERVICES (b)	UNIT OF ISSUE (c)	QUANTITY REQUESTED (d)	SUPPLY ACTION (e)	TYPE CON-TAINER (f)	CON-TAINER NOS. (g)	UNIT PRICE (h)	TOTAL COST (i)
	POC: DANA WILLIAMS PHONE NO: 812-854-3590 RETURN COPY TO: TOM BRENT SHOP CD: 09695							
01	TWO (2) COOLERS OF SAMPLES	EA	2					

16. TRANSPORTATION VIA MATS OR MSTS CHARGEABLE TO

17. SPECIAL HANDLING

18. RECAPITULATION OF SHIPMENT

ISSUED BY	TOTAL CON-TAINERS	TYPE CON-TAINER	DESCRIPTION	TOTAL WEIGHT	TOTAL CUBE
DW3948					
CHECKED BY					
PACKED BY					
			← TOTAL →		

19. RECEIPT

CONTAINERS RECEIVED EXCEPT AS NOTED	DATE (YYYYMMDD)	BY	SHEET TOTAL
QUANTITIES RECEIVED EXCEPT AS NOTED	DATE (YYYYMMDD)	BY	GRAND TOTAL
POSTED	DATE (YYYYMMDD)	BY	20. RECEIVER'S VOUCHER NO.

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51 52 53 54 55 56 57 58 59 60 61 62 63 64 65 66 67 68 69 70 71 72 73 74 75 76 77 78 79 80 81 82 83 84 85 86 87 88 89 90 91 92 93 94 95 96 97 98 99 100

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CHAIN OF CUSTODY

CRANE DIVISION, NAVAL SURFACE WARFARE CENTER
ENVIRONMENTAL PROTECTION DEPT.
NAVSURFWARCENDIV 5090/6 (REV. 5/93)

SAMPLING DATE	SAMPLE I.D.	CONTAINER (PARAMETERS)
7/18/00	CRANE-LEP-71800	Insects (Metals + Explosives)
7/18/00	CRANE-INS-71800	" (" + ")
7/19/00	CRANE-LEP-71900	" (" + ")
7/19/00	CRANE-INS-71900	" Bridge (" + ")
7/19/00	CRANE-INS-71900	" Floodplain (" + ")

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Christine D. Freeman	Al Cofrancesco	7/24/00	1200	NSWC → WES

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CRANE DIVISION, NAVAL SURFACE WARFARE CENTER
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NAVSURFWARCENDIV 5090/6 (REV. 5/93)

SAMPLING DATE	SAMPLE I.D.	CONTAINER (PARAMETERS)
7/20/00	CRANE-LEP-72000	Insects (Metals + Explosives)
7/20/00	CRANE-INS-72000	" (" + ")
7/21/00	CRANE-LEP-72100	" (" + ")
7/21/00	CRANE-INS-72100	" (" + ")

SAMPLER PREPARER (SIGNATURE)	TRANSFERRED TO (SIGNATURE)	DATE	TIME	ORGANIZATION
Christine D. Freeman	Al Cofrancesco	7/24/00	1200	NSWC → WES

SHIPPING CONTAINER TALLY → 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50

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SHEET NO. 1 | NO. OF SHEETS 1 | 5. REQUISITION DATE 2000-08-28 | 6. REQUISITION NUMBER

7. DATE MATERIAL REQUIRED (YYYYMMDD) 2000-08-28 | 8. PRIORITY 03

2. TO: (Include ZIP Code)
USAE WATERWAYS EXPERIMENT STATION
3909 HALLS FERRY ROAD (CEWES ERD-A)
VICKSBURG, MS 39180

9. AUTHORITY OR PURPOSE
SAMPLES FOR ANALYSIS

10. SIGNATURE
DANA WILLIAMS

11a. VOUCHER NUMBER & DATE (YYYYMMDD)
N00164-0241-0267

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12. DATE SHIPPED (YYYYMMDD)

b.

13. MODE OF SHIPMENT

14. BILL OF LADING NUMBER

15. AIR MOVEMENT DESIGNATOR OR PORT REFERENCE NO.

4. APPROPRIATIONS DATA

			AMOUNT
TRANSPORTATION:	97X4930.	NH1J 000 77777 0 000164 2F 000000 837345409695	
LABOR:	97X4930.	NH1J 000 77777 0 000164 2F 000000 837345409695	
MATERIAL:	97X4930.	NH1J 000 77777 0 000164 2F 000000 837345409695	

ITEM NO. (a)	FEDERAL STOCK NUMBER, DESCRIPTION, AND CODING MATERIAL AND/OR SERVICES (b)	UNIT OF ISSUE (c)	QUANTITY REQUESTED (d)	SUPPLY ACTION (e)	TYPE CONTAINER (f)	CONTAINER NOS. (g)	UNIT PRICE (h)	TOTAL COST (i)
	POC: DANA WILLIAMS PHONE NO: 812-854-3590 RETURN COPY TO: TOM BRENT SHOP CD: 09695							
01	TWO (2) COOLERS OF SAMPLES	EA	2					

16. TRANSPORTATION VIA MATS OR MSTS CHARGEABLE TO

17. SPECIAL HANDLING

18. RECAPITULATION OF SHIPMENT

ISSUED BY	TOTAL CONTAINERS	TYPE CONTAINER	DESCRIPTION	TOTAL WEIGHT	TOTAL CUBE
DW3948					
CHECKED BY					
PACKED BY					
			← TOTAL →		

19. RECEIPT

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QUANTITIES RECEIVED EXCEPT AS NOTED	DATE (YYYYMMDD)	BY	GRAND TOTAL
POSTED	DATE (YYYYMMDD)	BY	20. RECEIVER'S VOUCHER NO.

DD FORM 1149, JAN 1997

51 52 53 54 55 56 57 58 59 60 61 62 63 64 65 66 67 68 69 70 71 72 73 74 75 76 77 78 79 80 81 82 83 84 85 86 87 88 89 90 91 92 93 94 95 96 97 98 99 100

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CHAIN OF CUSTODY

CRANE DIVISION, NAVAL SURFACE WARFARE CENTER
ENVIRONMENTAL PROTECTION DEPT.
NAVSURFWARCENDIV 5090/6 (REV. 5/93)

SAMPLING DATE	SAMPLE I.D.	CONTAINER (PARAMETERS)
8/24/00	CRANE-INS-82400	Insects (Metals + Explosives)
8/24/00	CRANE-LEP-82400	" (" + ")
8/25/00	CRANE-INS-82500	" (" + ")
8/25/00	CRANE-LEP-82500	" (" + ")

SAMPLER PREPARER (SIGNATURE)	TRANSFERRED TO (SIGNATURE)	DATE	TIME	ORGANIZATION
Christine D. Freeman	Al Cofrancesco	8/28/00	1200	NSWC → WES

CHAIN OF CUSTODY

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ENVIRONMENTAL PROTECTION DEPT.
NAVSURFWARCENDIV 5090/6 (REV. 5/93)

SAMPLING DATE	SAMPLE I.D.	CONTAINER (PARAMETERS)
8/22/00	CRANE-INS-82200	Insects (Metals + Explosives)
8/22/00	CRANE-LEP-82200	" (" + ")
8/23/00	CRANE-INS-82300	" (" + ")
8/23/00	CRANE-LEP-82300	" (" + ")

SAMPLER PREPARER (SIGNATURE)	TRANSFERRED TO (SIGNATURE)	DATE	TIME	ORGANIZATION
Christine D. Freeman	Al Cofrancesco	8/28/00	1200	NSWC → WES

QUALITY ASSURANCE PROJECT PLAN
FOR
INSECT COLLECTION AND ANALYSIS AT THE AMMUNITION BURNING GROUNDS
CRANE DIVISION, NAVAL SURFACE WARFARE CENTER

REVISION 3
DATE
MARCH 2000

NSWC Crane Project Manager

Thomas J. Brent (3/21/00)

Laboratory Manager

16 Mar 2000

U.S. EPA, Region V, RCRA Permit Writer

5/30/2000

U.S. EPA, Region V, Regional Quality Assurance Manager

Allen A. Dobson 5-30-00

TABLE OF CONTENTS

LIST OF ACRONYMS ..... *ii*
ABG Ammunition Burning Grounds ..... *ii*

1. **PROJECT DESCRIPTION** **1**

1. 1. INTRODUCTION 1
1. 1. 1. OVERALL PROJECT OBJECTIVES *1*
1. 1. 2. PROJECT STATUS/PHASE *2*
1. 2. LOCATION 2
1. 2. 1. FACILITY LOCATION *2*
1. 2. 2. PROJECT LOCATION *3*
1. 3. SITE HISTORY 3
1. 3. 1. PROJECT SITE HISTORY *3*
1. 3. 2. PAST DATA COLLECTION ACTIVITIES *4*
1. 3. 3. CURRENT STATUS *5*
1. 4. PROJECT OBJECTIVES 6
1. 4. 1. SPECIFIC OBJECTIVES AND ASSOCIATED TASKS *6*
1. 4. 2. PROJECT TARGET PARAMETERS AND INTENDED DATA USAGES *7*
1. 4. 3. DATA QUALITY OBJECTIVES *8*
1. 5. SAMPLING LOCATION 9
1. 6. PROJECT SCHEDULE 9
1. 6. 1. SAMPLE COLLECTION DATES *9*
1. 6. 2. ANTICIPATED ANALYTICAL TIMEFRAMES *10*
1. 7. SORTING 10

2. **PROJECT ORGANIZATION AND RESPONSIBILITY** **11**

3. **QUALITY ASSURANCE OBJECTIVES FOR MEASUREMENT DATA** **12**

3. 1. PRECISION 12
3. 2. ACCURACY 13
3. 3. COMPLETENESS 14
3. 4. REPRESENTATIVENESS 14
3. 5. METHOD DETECTION LIMITS 14
3. 6. LEVEL OF QUALITY CONTROL EFFORT 15

4. **SAMPLING PROCEDURES** **16**

5. **CUSTODY PROCEDURES** **17**

5. 1. FIELD SAMPLE COLLECTION DOCUMENTATION 17
5. 2. CHAIN-OF-CUSTODY DOCUMENTS 17
5. 3. LABORATORY SAMPLE CUSTODY 18

6. **CALIBRATION PROCEDURES AND FREQUENCY** **19**

6. 1. FIELD INSTRUMENT CALIBRATION 19
6. 2. LABORATORY INSTRUMENT CALIBRATION 19

7. **ANALYTICAL PROCEDURES** **20**

7. 1. FIELD ANALYTICAL PROCEDURES 20
7. 2. LABORATORY ANALYTICAL PROCEDURES 20

8. **INTERNAL QUALITY CONTROL CHECKS** **21**

8. 1. FIELD QUALITY CONTROL CHECKS 21
8. 2. LABORATORY QUALITY CONTROL CHECKS 21

9. **DATA REDUCTION, VALIDATION, AND REPORTING** **22**
9. 1. DATA REDUCTION 22
9. 1. 1. Field data reduction procedures *22*
9. 1. 2. Laboratory data reduction procedures *22*
9. 2. DATA REVIEW/VALIDATION 23
9. 2. 1. Procedures Used to Evaluate Field Data *23*
9. 2. 2. Procedures to Review Laboratory Data *23*
9. 2. 3. Procedures to Validate Laboratory Data *24*
9. 3. DATA REPORTING 24
9. 3. 1. Field Data Reporting *24*
9. 3. 2. Laboratory Sorting Data Reporting *24*
9. 3. 3. Laboratory Analytical Data Reporting *24*
9. 4. DATA ASSESSMENT 25
10. **REFERENCES** **26**

List of Acronyms

ABG	Ammunition Burning Grounds
CAAA	Crane Army Ammunition Activity
COPEC	Contaminants of Potential Ecological Concern
FSP	Field Sampling Plan
ICP-MS	Inductively-coupled plasma - mass spectrometry
IRP	Installation Restoration Program
LCS	Laboratory Control Sample
LCSD	Laboratory Control Sample Duplicate
LD50	Lethal Dose (which causes 50% mortality)
LOAEL	Lowest Observable Adverse Effect Level
LRL	Lower Reporting Limit
MDL	Method Detection Limit
MS/MSD	Matrix Spike/Matrix Spike Duplicate
NA	Not Applicable
NAD	Naval Ammunition Depot
NIST	National Institute of Standards and Technology
NOAEL	No Observable Adverse Effect Level
NSWC Crane	Crane Division, Naval Surface Warfare Center
PEP	Pyrotechnics, Explosives, and Propellants
QAPP	Quality Assurance Project Plan
QA/QC	Quality Assurance/Quality Control
QC	Quality Control
RCRA	Resource Conservation Recovery Act
RDX	Hexahydro-1,3,5-trinitro-1,3,5,-triazine
RFI	RCRA Facility Investigation
RPD	Relative Percent Difference
RPHPLC	Reversed Phase High Performance Liquid Chromatography
SOPs	Standard Operating Procedures
SWMU	Solid Waste Management Unit
TCE	Trichloroethylene
U.S. EPA	U.S. Environmental Protection Agency, Region V
USFWS	U.S. Fish and Wildlife Service
WES	Waterways Experiment Station

1. PROJECT DESCRIPTION

This project is for collecting and analyzing insects from the Ammunition Burning Grounds (ABG) at Crane Division, Naval Surface Warfare Center (NSWC Crane). This Quality Assurance Project Plan (QAPP) presents the organization, objectives, planned activities, and specific Quality Assurance/Quality Control (QA/QC) procedures for handling and storage, chain-of-custody, and laboratory and field analyses.

1. 1. INTRODUCTION

This QAPP presents the objectives, tasks, and QA/QC procedures associated with conducting sampling, preparation, and analyses of insects for metals and explosives compounds.

1. 1. 1. OVERALL PROJECT OBJECTIVES

The project has three primary phases. The initial, or preliminary phase, was to refine existing methods and document the procedures for analyzing explosives and metals in an insect matrix. The second objective is to collect macroinvertebrate samples associated with the food chain of the federally endangered Indiana Bat (*Myotis sodalis*) in the area of the ABG associated with the location of the capture of a single male bat. The third phase is to prepare and analyze the insect samples for the purpose of determining the levels, if present, of metals and explosives in the insect tissue. This information will then be used to determine the need for other studies, that may entail additional sampling of insects or use of a surrogate bat to determine possible contaminant effects to the bat.

The collection of insects is not an exact science. The mass collected is not controlled by the sampler, but rather influenced by such factors as climate (temperature, humidity, presence or absence of drought, and cloud cover), number of insects available for capture, effectiveness of the traps at attracting available insects, time of year, etc. The sample size ultimately controls the extent that the objectives can be pursued, especially with respect to the total number of analyses and quality control (QC) efforts. Therefore, crickets were obtained and used as surrogates for the initial phase of the project. Work with crickets assisted in determining changes to preparation and analytical procedures, interferences, detection limits, required sample volumes, and the extent of QC. The initial surrogate cricket work, along with a pre-obtained insect sample volume, determined the actual detection limits achievable for this project. Table 1 is the sample network/design.

Table 1 - Sampling Network/Design			
Type of Sampling	**Target Constituent**	**Objective**	**Limit of Detection**
Cricket Surrogate	Metals Explosives	To determine changes to preparation and analytical procedures, interferences, detection limits, required sample volumes, and the extent of QC.	NA
Light Traps	Metals Explosives	❑ **Primary Objective:** To determine whether contaminants at the site are present in food sources for the Indiana Bat. ❑ **Secondary Objective:** To obtain representative samples of the Bat's food	Reporting limits low enough to determine potential harm to the Bat posed by the contaminants.
Malaise Trap	Metals Explosives		

1. 1. 2. PROJECT STATUS/PHASE

The project includes three periods of insect collection in order to capture insects that emerge at different times during the season. Samples will be collected in June, July, and August 2000. Experientially, this is the peak time for availability of insects. This availability of the most insects, combined with the presence of bats feeding as well as full-scale operation of the ABG, allows for a more representative sample of the bat's diet and potential for contamination. The particulars of each sampling round will include:

- Insect collection;
- Sample preservation, packaging, and shipping;
- Sample preparation;
- Analysis for metals and explosives; and
- Data reporting.

The first phase of refining existing methods and documenting the procedures for analyzing explosives and metals in an insect matrix was completed in December 1997. The second phase will be completed August 2000. The third phase will be completed October or November 2000. At the conclusion of the three phases of this project, data gaps may be identified. Data gaps may lead to additional work during the summer and fall of 2001. If additional work is required beyond the phases of this project, a new Field Sampling Plan (FSP) with established objectives may be submitted for that work.

1. 2. **LOCATION**

A brief description of the facility and project locations are presented in the following sections.

1. 2. 1. FACILITY LOCATION

NSWC Crane is located in southwestern Indiana, approximately 75 miles southwest of Indianapolis, and 71 miles northwest of Louisville, Kentucky. NSWC Crane occupies 62,463 acres (approximately 100 square miles) of the northern portion of Martin County and small

portions of neighboring Greene, Daviess, and Lawrence Counties. The base is located in a rural agricultural and wooded area, and is situated on a topographic plateau known as the Crawford Upland, dissected by well-defined stream valleys, causing elevation differences of over 300 feet in some areas. Surficial geology consists of Pennsylvanian and Mississippian age sandstones, shales, and limestones.

1. 2. 2. PROJECT LOCATION

Insect collection occurs in the vicinity of the capture of a single male Indiana Bat, south of the ABG. The ABG is located in the east-central portion of the facility in the northwest corner of Section 28 and the southwest corner of Section 21, Township 5N, Range 3W, as shown on Figure (1). The ABG was identified as a Solid Waste Management Unit (SWMU) in the facility's operating permit. Covering approximately 50 acres, the ABG is located in a valley at the headwaters of Little Sulphur Creek (Figure (1)). The eastern boundary of the operating unit is the beginning of a pseudo-karst zone characterized by springs and a sinking stream.

1. 3. **SITE HISTORY**

NSWC Crane provides material, technical, and logistical support to the Navy for equipment, weapons systems, and expendable and nonexpendable ordnance items. The facility was opened in 1941 as the Naval Ammunition Depot (NAD), Burns City to serve as an inland munitions production and storage center. In 1943, the name was changed to NAD Crane in honor of Commodore William Montgomery Crane, the first chief of the Navy's Bureaus of Ordnance. The name changed again in 1975, to Naval Weapons Support Center, to reflect the facility's growing involvement in high-technology weapons systems. In 1977, it was decided by the Secretary of Defense to combine all conventional ammunition acquisition under the responsibility of a single service. The ammunition production and storage function was passed to the Army and the Crane Army Ammunition Activity (CAAA) was established as a Crane tenant to accomplish this task for Naval ammunition. CAAA has assumed ordnance production, storage, and related responsibilities under the single service management directive. All environmental activities on the installation, including permitting activities, remain the responsibility of the Navy. In 1992, the name was changed again to the Crane Division, Naval Surface Warfare Center. Although ordnance production and storage still resides at NSWC Crane, today, NSWC Crane serves a modern and sophisticated Navy as a recognized leader in diverse and highly technical product lines, such as microwave devices, acoustic sensors, small arms, microelectronic technology, and more.

1. 3. 1. PROJECT SITE HISTORY

The ABG has been used extensively since the 1940's for the thermal treatment of military pyrotechnics, explosives, and propellants (PEP), and materials potentially PEP contaminated. CAAA has operated the ABG since 1978. Thermal treatment is via open burning, conducted under a U.S. Environmental Protection Agency (U.S. EPA), Region V, Resource Conservation Recovery Act (RCRA) Subpart X permit issued January 13, 2000.

1. 3. 2. PAST DATA COLLECTION ACTIVITIES

A hydrogeological investigation of the ABG and surrounding area was conducted by Army Corps of Engineers, Waterways Experiment Station (WES) in 1986-87. The Hunt (1988) report identified factors influencing or controlling the flow of potentially contaminated groundwater into and out of the ABG. Work included emplacement of 66 exploration borings and monitoring wells in three aquifers, geologic and hydrologic field mapping, literature survey and consultation with geologists at the Indiana Geological Survey, Bloomington. The study area included the area surrounding the ABG, especially that to the south.

The report by Hunt (1988) concluded that flow through solution passages in the Beech Creek limestone is the primary conduit for groundwater leaving the ABG and that flow through the conduits can be "rapid." The report recommended that increased emphasis be given to monitoring of springs and that injection of tracers (i.e., dye tracer test) be considered to confirm direction and rate of movement away from the ABG through solution conduits. In 1989, WES installed five additional monitoring wells (July and August 1989) in the vicinity of the ABG.

The Federal portion of the RCRA Permit, dated December 10, 1989, established the HSWA Corrective Action Requirements and Compliance Schedules (RCRA Section 3004). The compliance schedules obligated NSWC Crane to perform RCRA Facility Investigations (RFIs) at 30 SWMUs, and, if contamination was found, to conduct Corrective Measures Studies and implement Corrective Measures, if needed. The Permit's RFI compliance schedule for the ABG established work plan submittals for the following: (a) Modified RFI Phase III Release Characterization for Groundwater; (b) RFI Phase III Release Characterization for Soil; and (c) RFI Phase II Release Assessment for Surface Water Bodies. In April 1990, NSWC Crane submitted the Modified RFI Phase III Release Characterization Work Plan for Groundwater. The Work Plan scheduled the submittal of the Dye Tracer Report, the proposal to conduct a second Dye Tracer Test, progress reports, and the RFI Phase III Final Report for Groundwater. The RCRA Section 3004 Corrective Action Requirements of the Storage Permit have incorporated the Installation Restoration Program (IRP). RCRA will be the primary vehicle to further investigate and remediate the IRP sites.

As a part of the water sampling and chemical analysis program initiated by NSWC Crane in 1987, laboratory analysis and laboratory QA and QC data have been reported to NSWC Crane in a series of reports. The information from these reports formed the basis of a summary report, dated April 1, 1992, and prepared under contract by COMARCO, Environmental Services Division of Bloomfield, Indiana. The groundwater was tested for a wide range of chemical parameters at various limits during the program. These parameters are discussed in 40 CFR 265.92, 265.93, and 265.94 Appendix III.

Murphy (1994) provided additional analyses of the groundwater data pertaining to hexahydro-1,3,5-trinitro-1,3,5,-triazine (RDX), Trichloroethylene (TCE), and barium in the final RFI Phase III Groundwater Report for the ABG. Murphy concluded that four monitoring well sites within the ABG were notably higher in RDX and/or TCE than other wells.

To investigate background conditions and to characterize the source(s) of contamination in the soils, an RFI Phase III Part 1 soils investigation was conducted by WES in 1990. Twelve auger borings were placed and soil samples were collected. Chemical analysis of the soil samples indicated contamination by explosives and metals.

Murphy and Wade (1998) conducted a Phase II Surface Water Assessment at the ABG and submitted a Final report. The report concluded that explosives, metals, and other inorganics, and certain volatile and semivolatile compounds have been released to the water and bottom sediments of streams at and below the ABG.

In 1993, Albertson (1998) conducted an RFI Phase III Part 2 soils investigation. Thirty-three surface (grab) soil samples were collected and 32 soil borings were made to determine the extent of soil contaminants identified in the RFI Phase III Part 1 soils investigation. The soils analyses indicated that ABG waste disposal activities have contributed residues of explosives compound and metal contaminants to the soils. PAHs and VOAs were also released, but in concentrations that were generally below 1 mg/kg. The explosives HMX, RDX, TNB, TNT, 2,4-DNT, 2,6-DNT, 2A-DNT, and 4A-DNT were detected. Explosives with the highest concentrations were HMX, RDX, and TNT. Several metals and inorganic constituents in ABG soils had consistently higher concentrations than nearby background soils. In some cases the concentrations of metals and inorganics were 100 times the background which suggested the release of these constituents to ABG soils. Constituents that were greater than 100 times background included cadmium, calcium, copper, lead, zinc, and tin.

In 1997, light traps and a malaise trap was used to collect insects at the ABG. Although not used for analyses, the information was useful in determining the mass of insects that potentially could be collected and what level of sorting could be accomplished and still provide meaningful data. The 1997 physical collection results are provided as Appendix A. A conference call was then held on September 30, 1999 to discuss the sampling plan and sorting. These items are discussed in the FSP, provided in Appendix B.

1. 3. 3. CURRENT STATUS

NSWC Crane conducted an ecological risk assessment for RCRA corrective action activities and to support the RCRA Subpart X permit. A bat survey along the streams near the areas of concern was included as part of the ecological risk assessment and compliance with the Endangered Species Act. On June 25, 1996, a single male Indiana Bat (*M. sodalis*) was caught. The capture occurred south of the ABG on Little Sulphur Creek approximately in the SE corner of Section 28, T5N, R3W, as shown on Figure (1). Following the capture, NSWC Crane proposed to the U.S. Fish and Wildlife Service (USFWS), during informal consultation, to conduct a survey, such as a blacklight survey, of the available prey in the area the bat was captured, in order to determine if contaminants are mobilizing through the bat's food chain. On October 19, 1999, representatives from the USFWS and U.S. EPA met to review and agree upon the proposed sampling locations at the ABG. Per the letter from the USFWS dated October 20, 1999, one of these light traps will be situated on the Jeep Trail below the ABG, near Bridge 3090. The second light trap will be placed in the floodplain adjacent to Little Sulphur Creek immediately downstream of the ABG, as shown on Figure (1).

1. 4. PROJECT OBJECTIVES

1. 4. 1. SPECIFIC OBJECTIVES AND ASSOCIATED TASKS

The ultimate goal of this project is to gather sufficient information to evaluate the potential for contamination to be present in the food chain of *M. sodalis* and to determine whether ecological risks are associated with the levels of contaminants, if found. It may not be possible to accomplish this objective fully during this project, however, because of the slight possibility of an insufficient amount of insect tissue.

1.4.1.a. Preliminary tasks associated with this project are:

1.4.1.a.1. Collect a limited sample volume (reflecting a possible food source of the endangered bat) to allow some specific objectives to be accomplished in this phase as outlined in (1.4.1.b) below.

1.4.1.a.2. Determine from literature reviews, toxicity levels for each analyte listed in Table 2.

1.4.1.a.3. Establish analytical procedures for insect tissue capable of providing acceptable recoveries for the analytes listed in Table 2.

1.4.1.a.3.a. Refine existing explosives methods to allow compound identification in the absence of major intereferences.

1.4.1.a.3.b. Refine existing metals digestion procedures to reduce or eliminate the effects of interferences from the insect matrix during metals analysis.

1.4.1.a.3.c. For explosives and metals, demonstrate method sensitivities [method detection limits (MDLs)] for each analyte in Table 2 for the cricket matrix.

1.4.1.a.3.d. Determine the sample size needed to achieve MDLs sensitive enough to meet the analytical requirements of this phase and future project phases.

1.4.1.b. Specific objectives for the analysis of potentially impacted insects are highly dependent on the outcome of §1.4.1.a above. The preliminary tasks listed in §1.4.1.a were completed in December 1997. Results of that study are provided in Appendix C. The modified SOPs are provided in Appendix D. The following objectives will be accomplished in phase II beginning in June 2000.

1.4.1.b.1. Insect samples will be preliminarily sorted into fractions of the collected sample that reflect three exposures prior to preparation for analysis: moths, general aquatic, and general terrestrial food sources.

1.4.1.b.2. Each of the Table 2 analytes will be reported with a sensitivity ranging down to levels regarded as toxicologically significant as determined in §1.4.1.a.2 above, if available or practicable.

1.4.1.b.3. The data package resulting from the analyses in this phase shall be sufficiently comprehensive in its documentation to demonstrate the reliability of the data. The items to be included in the data package originating from the analytical laboratory are listed in §9.3.2 below.

1.4.1.b.4. The accuracy (percent recovery) data for the Table 2 analytes must be of an accurate nature. Precision measures must be regarded as acceptable. If adequate sample is available duplicate analyses (field duplicates and MSDs) will be performed. If the amount of sample is limited, the laboratory control samples (LCS) will be duplicated instead. The targeted analytical program for explosives will include Matrix Spike/Matrix Spike Duplicate (MS/MSD) and field duplicate analysis. At a minimum it will include MS recoveries and LCS recoveries. The analytical program for metals will include MS/MSD if sample size permits.

Table 2 - **Target Compounds**

EXPLOSIVES				INORGANICS		
	ORL-Rat LD50	NOAEL[1]	LOAEL[1]		NOAEL[1]	LOAEL[1]
1.RDX	Low toxicity when digested or inhaled	No data	1.5	1. cadmium	No data	No data
2. TNT	High toxicity when digested or inhaled	0.4	No data	2. lead	No data	No data
3. HMX	1500 mg/kg (mouse)	No data	150	3. zinc	No data	No data
4. 1,3-DNB	83 mg/kg	0.4	1.2[2]	4. mercury	No data	No data
5. Tetryl	Moderately toxic when digested or inhaled	No data	No data	5. copper	No data	No data
6. NB	640 mg/kg	No data	No data	6. barium	45	75
7. TNB	450 mg/kg	2.68	13.31	7. silver	No data	No data
8. 4-ADNT	Not available	No data	No data	8. aluminum	No data	No data
9. 2-ADNT	Not available	No data	No data	9. antimony	No data	0.35
10. 2,6-DNT	177 mg/kg	No data	No data	10. arsenic	No data	No data
11. 2,4-DNT	268 mg/kg	No data	34	11. chromium	1468	No data
12. 2-NT	891 mg/kg	No data	No data	12. manganese	No data	No data
13. 3-NT	1072 mg/kg	No data	No data	13. magnesium	No data	No data
14. 4-NT	1960 mg/kg	No data	No data	14. nickel	5	50
15. Azoxytoluene	Not available	No data	No data			
16. MNX	Not available	No data	No data			
17. TNX	Not available	No data	No data			

[1]mg/kg/day based or oral rat studies.
[2]estimated based on NOAEL data

1. 4. 2. PROJECT TARGET PARAMETERS AND INTENDED DATA USAGES

The list of target parameters for this project is included in Table 2. Intended data usages are to ultimately determine whether or not the bat is affected by Table 2 analytes. However, data usages may be limited by factors determined above. The data shall be compared to literature values for metals as developed above or to ecological based levels for explosives, and to ecological risk based levels established as part of the overall risk assessment for the ABG. Results of this study will be used as described in §1.1.1.

1. 4. 2. 1. Field Parameters

No field measurements will be collected for this project.

1. 4. 2. 2. Laboratory Parameters

The target compounds for this project are presented in Table 2 along with LD50, no observable adverse effect level (NOAEL), and lowest observable adverse effect level (LOAEL) data for oral dosages in rats (ORL-Rat) for those compounds for which it is available.

Analyte selection is based on previous investigations, as discussed in §1.3.2. The RFI ground water, surface water, sediment, and soils reports were reviewed for contaminants of significance. This information was compared to the contaminants of potential ecological concern (COPEC) listed in the Current Contamination Conditions Risk Assessment (Zeal, 1999). The order of listing of the inorganic constituents in Table 2 is based upon RFI multimedia prevalence, listing as a COPEC, and COPEC media prevalence. For example, cadmium was identified as a contaminant in all RFIs for all media, and was listed as a COPEC for all media. Whereas, nickel was identified as a contaminant in the two soil RFIs and the sediment RFI but was not listed as a COPEC. The second phase of this project may not contain enough sample volume to analyze for all of the inorganic constituents. As such, analysis will proceed in the order the compounds are listed as sample size allows. In addition to the above constituents, phosphorus will be analyzed in the first phase cricket study as well as for one sample of insects collected during the second phase.

1. 4. 3. DATA QUALITY OBJECTIVES

Single operator precision and accuracy for the analytical methods selected will be demonstrated in the first phase of this project. Sensitivity was also determined during the first phase.

The primary objective of this project is to obtain sufficient data of known quality to determine whether contaminants at the ABG are present (and if so, at what concentration) in food sources for the Indiana Bat. Table 3 compares the lower reporting limits (LRL) of explosives in an insect matrix to the known oral LD50 determined in rats. It also compares the MDL of metals from the inductively coupled plasma - mass spectrometry (ICP-MS) to the target detection limits obtained from the USFWS.

Table 3 – Estimated reporting limits compared to known ORL-Rat LD50s or target MDLs[1]					
EXPLOSIVES			INORGANICS		
Analyte	LRL(mg/kg)	LD50(mg/kg)	Analyte	MDL (mg/kg)	Target MDL (mg/kg)
HMX	3.2	1500	Silver	0.026	
RDX	0.8		Aluminum	0.078	5
TNB	1.2	450	Arsenic	0.073	0.5
1,3-DNB	1.0	83	Barium	0.009	1
Tetryl			Cadmium	0.005	0.1
NB	1.7	640	Chromium	0.065	0.5
TNT	1.3		Copper	0.05	0.5
4-ADNT	2.5		Magnesium	0.13	5
2-ADNT	1.2		Manganese	0.01	1
2,4-DNT	0.7	268	Nickel	0.032	0.5
2,6-DNT	1.3	177	Lead	0.007	0.5
2-NT	1.9	891	Antimony	0.014	
3-NT	1.6	1072	Zinc	0.19	1
4-NT	2.1	1960	Mercury	0.20	0.20
MNX					
TNX					
Azoxytoluene					

[1]The LRL and MDL were determined in the laboratory using cricket tissue. Note that these reporting limits were determined in Fall 1997 and are only estimates of method and instrument sensitivity that will exist at the time of actual analysis.

1. 5. SAMPLING LOCATION

The principle area for insect collection is shown on Figure (1). This is the same area as the previous capture of the Indiana Bat taking into account foraging range and proximity to the potential source of contaminants. It is assumed that the bat was foraging along Little Sulphur Creek at the time of the capture. Therefore, the intent of the sampling was to collect insects within the bats forage locale.

1. 6. PROJECT SCHEDULE

1. 6. 1. SAMPLE COLLECTION DATES

See the FSP provided in Appendix B.

1. 6. 2. ANTICIPATED ANALYTICAL TIMEFRAMES

The phase I study was completed December 1997. Samples will be collected from June through August 2000. The sorting and analytical matrix table is in the FSP (Appendix B). Samples will be sorted and weighed as they arrive at WES. Following sorting, the samples will be turned over to the analytical laboratory for analyses. Analyses will begin in late August or early September in accordance with the approved QAPP. An analytical data package will follow in October or November 2000.

1. 7. **SORTING**

For a discussion of sorting issues, refer to the FSP (Appendix B).

2. PROJECT ORGANIZATION AND RESPONSIBILITY

NSWC Crane has overall responsibility for all phases of this study. Personnel from WES provide field and analytical assistance. A WES entomologist supervised initial setup of the insect traps at the ABG, and WES is providing sampling and analysis support. Responsible personnel and their functions are shown in Table 4.

Table 4 - **Project Responsibilities**

Project Member	Function
Thomas J. Brent NSWC Crane Installation Restoration Project Manager	Overall project coordination, including scheduling, assistance in sample collection, and report writing. Assists in the development of the QA plans.
Karen Myers WES Chemist	Receives, stores, analyzes, and disposes of samples. Assists in the development of the QA plans. Also documents analytical methods used and reports analytical results.
Dr. Al Cofrancesco WES Entomologist	Established field collection, sorting, preservation, and shipping procedures, as well as report writing.
Allen Debus U.S. EPA Quality Assurance Expert	Review and approve the QAPP and overall support throughout the project. May also conduct audits of the WES laboratory as well as review analytical procedures. Will assist in data validation.
Peter Ramanauskas U.S. EPA RCRA Corrective Action Expert	Overview of all site activities to ensure regulatory compliance. Reviews and approves all phases of the project.
Lori Pruitt USFWS Biologist	Overview of site activities to ensure compliance with the Endangered Species Act.

The laboratory chemist will have overall responsibility for ensuring analytical quality assurance. This responsibility includes, but is not limited to, receipt and inspection of the incoming sample containers, controlling and monitoring access and storage of samples and extracts, coordinate laboratory analyses, monitoring analytical and project QA requirements, conduct detailed review and verification of analytical data in reports prior to submission to NSWC Crane.

3. QUALITY ASSURANCE OBJECTIVES FOR MEASUREMENT DATA

The primary objective for this project is to gather sufficient defensible information to evaluate the potential for contamination to be present in the food chain of *M. sodalis*. This will be accomplished by implementing field sampling, chain-of-custody, laboratory analysis, and reporting procedures that include adequate quality assurance/quality control to assure that the data are of known and verifiable quality. Specific procedures for sampling, chain-of-custody, laboratory instrument calibration, reporting of data, internal quality control, audits, preventive maintenance of field equipment, and corrective action are described in Appendix D of this QAPP. Because of the unique nature of the biological matrix, laboratory preparatory and determinative Standard Operating Procedures (SOPs) have been evaluated and modified for this project. Specific procedures for laboratory analysis have been determined from the cricket study and are included in Appendix D of this QAPP.

3. 1. Precision

Precision examines the distribution of the reported values about their mean, and is determined through duplicate measurements. Precision may be affected by the natural variation of the matrix or contamination within the matrix, as well as by errors made in field and/or laboratory handling or homogenization procedures. To minimize precision errors, preparatory methods will include adequate homogenization procedures.

Precision in the field is assessed through the collection and measurement of field duplicates. The preferred rate of field duplication is 1 duplicate in 10 samples. However, based upon the rather unknown and finite size of the sample collection, true field duplication may not be possible.

Precision in the laboratory is assessed through the calculation of relative percent differences (RPD). For this project, due to finite sample amounts, MSDs will be substituted for matrix duplicates. If sample volume is insufficient for spike duplication, an LCS duplicate will be substituted.

Precision of duplicate samples is calculated using the equation below:

Relative percent difference:

$$RPD = \frac{|S1 - S2|}{(S1 + S2)/2} \times 100$$

where: S_1 and S_2 represent sample and duplicate sample results or MS/MSD results.

Precision measurements for this study must be regarded as acceptable. The default control limits given in Table 5 , will be utilized for this study.

Table 5 - **Analytical methods and data quality objectives.**				
Analyte Group[1]	Laboratory SOP Number	Equivalent U.S. EPA Method Number[2]	Precision (RPD)	Accuracy (%R)
Explosives	M-8330-00-ECC-OP/A	8330	35	75 - 125
Mercury	M-7471A-ECC-IA	7471A	35	75 - 125
Metals digestion-microwave	M-3051-00-ECC-IP	3051		
ICP/MS metals analysis (option 1)	M-6020-00-ECC-IA	6020	35	75 - 125
Total Phosphorous	M-365.2-MRL-GC	365.2	35	75 - 125
ICP metals analysis (option 2)	M-6010B-ECC-IA	6010B	35	75 - 125

[1] See Table 2 for specific analytes within each group.
[2] SW-846

3. 2. Accuracy

Accuracy measures the bias in a measurement system. Accuracy may be difficult to measure for the entire data collection activity. Sources of error are the sampling process, field contamination, preservation, handling, sample matrix, sample preparation, and analysis techniques.

Laboratory analytical accuracy is assessed through the analysis of known QC samples such as LCS and external reference materials and through the analysis of spiked samples such as MS and surrogate spikes. Accuracy is determined by calculating percent recoveries using the equations given below.

For LCS and surrogate spikes:

$$\% \text{ Recovery} = \frac{A}{B} \times 100$$

where: A = concentration of analyte measured
B = known true value

For MS/MSDs:

$$\% \text{ Recovery} = \frac{\{(\text{Sample} + \text{Spike Result}) - \text{Sample Result}\}}{\text{Spike Added}}$$

Accuracy control limits for LCS and surrogate spikes are provided in the SOPs found in Appendix D. Although accuracy control limits are unavailable for insect matrices, analysis of MSs will serve as an indicator for any unexpected matrix effects. The default control limits given in Table 5, will be utilized for this study.

See Crane Hr Ser 095/190 of 9/1/00

Table 5 - **Analytical methods and data quality objectives.**

Analyte Group[1]	Laboratory SOP Number	Equivalent U.S. EPA Method Number[2]	Precision (RPD)	Accuracy (%R)
Explosives	M-8330-00-ECC-XX	8330	35	75 - 125
Mercury	M-7471-00-WES-XX	7471	35	75 - 125
Metals digestion-microwave	M-3051-00-WES-XX	3051		
ICP/MS metals analysis (option 1)	M-6020-00-WES-XX	6020	35	75 - 125
ICP metals analysis (option 2)	M-6010-00-WES-XX	6010A	35	75 - 125

[1] See Table 2 for specific analytes within each group.
[2] SW-846

3. 2. Accuracy

Accuracy measures the bias in a measurement system. Accuracy may be difficult to measure for the entire data collection activity. Sources of error are the sampling process, field contamination, preservation, handling, sample matrix, sample preparation, and analysis techniques.

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$$\% \text{ Recovery} = \frac{A}{B} \times 100$$

where: A = concentration of analyte measured
B = known true value

For MS/MSDs:

$$\% \text{ Recovery} = \frac{\{(\text{Sample} + \text{Spike Result}) - \text{Sample Result}\}}{\text{Spike Added}}$$

Accuracy control limits for LCS and surrogate spikes are provided in the SOPs found in Appendix D. Although accuracy control limits are unavailable for insect matrices, analysis of MSs will serve as an indicator for any unexpected matrix effects. The default control limits given in Table 5, will be utilized for this study.

3. 3. Completeness

Laboratory completeness is a measure of the amount of valid measurements obtained from all the measurements taken in the project. For this project, the number of measurements to be made will depend upon the actual sample mass obtained after sorting and upon the sample size required for each suite of analyses (determined from the cricket study). Laboratory completeness for this project will be greater than 95 percent. Following completion of analytical testing, the percent completeness will be calculated by the following equation:

$$\% \text{ Completeness} = \frac{\text{(number of valid measurements)}}{\text{(number of measurements planned)}} \times 100$$

3. 4. Representativeness

Representativeness expresses the degree to which sample data accurately and precisely represent the characteristics of a population of samples. Representativeness is a qualitative parameter that is most concerned with the proper design of the sampling program or subsampling of a given sample. Employing appropriate sampling strategies and techniques best satisfies the representativeness criterion.

Following the criteria set forth in the FSP ensures representativeness for this project. The sampling network was designed to provide data representative of facility conditions. During development of this network, consideration was given to habitat, foraging habits, and existing analytical data. The rationale of the sampling network is discussed in the detail in the FSP (Appendix B).

Representativeness in the laboratory is ensured by using the proper analytical procedures and meeting sample holding times. Representativeness is enhanced by making certain that all subsamples taken from a given sample are representative of the entire sample. To this end, special emphasis will be placed on initial homogenization of the sample before it is split for further processing.

3. 5. Method Detection Limits

Analytical reporting limits for the parameters targeted in this project must be lower than the target detection limits supplied by the USFWS for metals and lower than the ORL-Rat LD50 for explosives (Table 3). Estimated reporting limits for the target analytes are included in Table 3, LRL for explosives and MDL for inorganics. Method detection limits are determined according to 40CFR, Appendix B to Part 136-Definition and Procedure for the Determination of the Method Detection Limit-Revision 1.11. The laboratory SOP for MDL determinations is found in Appendix C. The cricket, *Acheta domestica*, was used as a surrogate for the MDL study for explosives. As stated in the laboratory SOP, all metal MDLs are calculated from water spikes. Conversion into mass units is based upon the digestion weights and volumes. Reporting limits are calculated from the MDL values and are at or near the low standard.

3. 6. Level of Quality Control Effort

Method blank, duplicate, and spiked samples will be analyzed to assess the quality of the data resulting from the field sampling and analytical programs. Method blank samples are generated within the laboratory and used to assess contamination resulting from laboratory procedures. Duplicate samples are analyzed to check for sampling and analytical reproducibility. Duplicate samples may include field duplicates, MS/MSD, or LCS/LCSD. Matrix spikes will be performed in duplicate where possible with one MS/MSD set collected for every 20 or fewer field samples. Field duplicates will be collected and analyzed where sample size permits (see §3.1).

4. SAMPLING PROCEDURES

The insect trapping procedures are described in Appendix B. Trapping will occur three times during the summer season (June, July, and August) in order to capture insects with different emergent periods. Samples will be frozen for preservation, packaged in coolers, and shipped to WES. The FSP (Appendix B) outlines all the sampling procedure information. No further sample collection is currently planned. Additional sampling would be preceded by a new sampling plan.

5. CUSTODY PROCEDURES

Records generated as a result of analytical sampling activities are quality records and will be processed in accordance with the requirements of this QAPP. Sampling and analytical documents are essential for ensuring the integrity and defensibility of data used to make decisions in determining impact upon the endangered species. Sample custody is addressed in three parts: field sample collection, laboratory analysis, and final evidence files. Evidence files include all originals of field notes, sorting logs, laboratory reports, notebooks, custody records and narratives. These original documents will be maintained under document control in a secure area or in the possession of the project member responsible for that phase of the project. Copies of the evidence files will be included in the final data submittals to the Navy.

5. 1. Field Sample Collection Documentation

The following information will be recorded on the sampling record at the time of sampling:

- Trap type;
- Trap site;
- Sample date;
- Time of trapping; and
- General observations (e.g., weather, status of lights in a.m., anesthetic used, etc.).

A copy of the sampling record is provided in Appendix B.

5. 2. Chain-of-Custody Documents

Samples are considered to be under a person's custody if:

- the item is in actual possession of a person; or
- the item is in the view of the person after being in actual possession of the person; or
- the item is in actual physical possession but is locked up to prevent tampering; or
- the item is in a designated and identified secure area.

Each sample will be assigned a unique identification number and that number entered on the chain-of-custody form. The chain-of-custody form includes the following information.

- Sample identification number;
- Sample date;
- Analysis required;
- Sampler's name; and
- Release and acceptance information including date, location, and the technician's signature.

Custody will be relinquished from the site by using the signature blocks at the bottom of the custody form. The original chain-of-custody record accompanied the shipment to WES by a commercial carrier. The original chain-of-custody record and the commercial carrier waybill will be kept as part of the project record files.

Upon receipt at WES, all samples proceed through an orderly processing sequence specifically designed to ensure continuous integrity of both the sample and other information pertinent to the analysis. All samples will be checked and verified for proper chain-of-custody records, preservation, leaking sample containers, proper label identification, and any associated discrepancies.

If no discrepancies are identified, the sample chain-of-custody record will be signed. If any discrepancies are found, WES will contact and discuss with NSWC Crane. After sorting, the samples will be assigned unique identification numbers to reflect the sorting scheme, new chain-of-custody documents will be initiated if needed to reflect any changes in the numbering system before the samples are delivered to the analytical laboratory for preparation and analysis.

5. 3. Laboratory Sample Custody

When the samples are received in the laboratory, custody will be transferred to the chain-of-custody officer and a unique laboratory identification number will be assigned for tracking and filing purposes. Receipt of samples will be noted in a bound chain-of-custody log. The samples will be placed in a secured freezer or refrigerator until required for sorting or analysis, respectively. Custody will be transferred to the analysts as needed. Each transfer will be recorded sequentially in a bound logbook. The laboratory QA system and the use of an internal chain-of-custody procedure ensure that the samples are appropriately tracked from receipt through completion of the analytical process.

6. CALIBRATION PROCEDURES AND FREQUENCY

6. 1. Field Instrument Calibration

Not applicable

6. 2. Laboratory Instrument Calibration

Calibration of instruments is required to ensure that the analytical system is operating correctly and functioning at the proper sensitivity to meet the project-specific quantitation limits that will be determined upon the completion of the cricket study under way at WES. Instruments utilized for this study will be calibrated daily or prior to analysis. A detailed description of the calibration process is presented in the analytical SOPs found in Appendix D.

Explosives analysis will be performed by reversed phase high performance liquid chromatography (RPHPLC) following SOP number M-8330-00-ECC-XX dated June 17, 1999. This SOP is based upon U.S. EPA SW- 486 Method 8330, 1994. Primary source standards are prepared from neat crystalline stock explosives standards obtained from the Army Environmental Center at Aberdeen Proving Ground, Maryland. These standards are used to prepare the initial 5-point calibration curve, continuing calibration verification standard, surrogates, LCS, and MSs. Secondary source stock standards are purchased as certified solutions for use as the initial calibration verification standard. Standards preparation is discussed in Section 7 of the SOP. Modifications to this SOP for this project are included with the SOP in Appendix D.

Metals analysis will be performed by either ICP/MS following U.S. EPA SW- 846 Method 6020, 1994 or by inductively coupled plasma - atomic emission spectroscopy (ICP) following SOP number M-6020-00-WES-XX dated June 1, 1998. Primary source standards are prepared from both single and multi-element standards purchased from Alfa Aesar and SPEX Chemical. Initial calibration verification standards are prepared from second source stocks purchased specifically for that purpose. Certificates of analysis accompanying the purchased solutions state that concentrations are checked against the National Institute of Standards and Technology (NIST) standard reference materials. Standards preparation is discussed in Section 7 of the SOP. Modifications to this SOP for this project are included with the SOP in Appendix D.

Mercury analysis will be performed by manual cold vapor with fluorescence detection following SOP number M-7471A-00-WES-XX. Standards preparation is discussed in Section 7 of the SOP. Primary and second source standards are purchased from SPEX Chemical. Certificates of analysis accompanying the purchased solutions state that concentrations are checked against NIST standard reference materials. Modifications to this SOP for this project are included with the SOP in Appendix D.

In all cases, analysts maintain logbooks identifying the calibration standards and date of calibration associated with each sample set analyzed on individual instruments.

7. ANALYTICAL PROCEDURES

7. 1. Field Analytical Procedures

Not applicable

7. 2. Laboratory Analytical Procedures

All analyses will be conducted by the USACE Waterways Experiment Station Environmental Chemistry Branch, 3909 Halls Ferry Road, Vicksburg, Mississippi, 39180. The laboratory director is Ann B. Strong, telephone - 601-634-2726. The sample custodian and chain-of-custody officer is Linda K. Stevenson, telephone - 601-634-3625.

A study was undertaken utilizing crickets (*Acheta domestica*) to determine the correct methods for sample preparation and extraction/digestion of both explosives and metals and to identify any modifications necessary to eliminate or minimize the effects of interferences extracted from the biological matrix. Because the sample size is finite and the amount of sample needed to obtain low detection limits is expected to be large, a method validation study cannot be performed on the actual sample matrix. The cricket, *Acheta domestica*, has been used as a test matrix in the course of method development. Results are in Appendix C. A single operator precision and accuracy study will be performed before the actual analysis is performed.

Table 5 lists the laboratory SOP numbers of the methods selected, their corresponding U.S. EPA reference method and the default target control limits for precision and accuracy for each of the analyte groups targeted for this project. As stated in Section 6, modifications to the SOPs are included in Appendix D.

8. INTERNAL QUALITY CONTROL CHECKS

8. 1. Field Quality Control Checks

Since sample sizes are not predictable, there may not be enough insect mass to provide a field duplicate. If sufficient sample is available, a sample will be split and analyzed as a field duplicate.

8. 2. Laboratory Quality Control Checks

WES has a QC program in place to ensure the reliability and validity of the analysis performed in the laboratory. Analytical procedures are documented in the SOPs, each of which includes a QC section that addresses the minimum QC requirements for the procedure. The internal quality control checks to be used in this investigation are listed in Table 6 below.

Table 6 - **Quality Control Checks**

QC Check	Explosives	Metals
Field Duplicate	?	?
Method Blank	X	X
Matrix Duplicate		X[1]
MS[3]	X[1]	X[1]
MSD[3]	X[1]	X[1]
LCS[3]	X	X
LSD[3]	X[2]	X[2]
Surrogate Spike	X	
Standard Reference Material		X

? Addition of this sample will depend upon the amount of sample available for analysis.
[1] Ability to prepare this control check depends upon amount of sample available and sample size. QC samples will be prepared and analyzed at a rate of 5%.
[2] The laboratory control sample will be duplicated only if there is insufficient sample to prepare a MSD.
[3] Spiking solutions shall contain all target analytes.

WES will report the results of the QC samples with the analytical data for field samples. The data package will include a full deliverable package (without forms) capable of allowing the recipient to reconstruct QC information and compare it to QC criteria.

9. DATA REDUCTION, VALIDATION, AND REPORTING

9. 1. Data Reduction

9. 1. 1. Field data reduction procedures

Notebooks and chain-of-custody forms will be kept for all field activities.

9. 1. 2. Laboratory data reduction procedures

9. 1. 2. 1. Insect Sorting Procedures

Notebooks and chain-of-custody forms will be kept for the sorting process.

9. 1. 2. 2. Laboratory Analytical Procedures

All raw analytical data pertaining to sample and batch preparation will be recorded in numerically identified laboratory notebooks. Pertinent information recorded in these notebooks includes the laboratory sample identification number, the analytical method used, the data of preparation/analysis, the matrix sampled, solvents and lot numbers used, QC samples included in the batch, concentrations of spikes, and the name of the analyst.

For this project, the equations that will be employed in reducing the raw data into concentrations of mass of analyte per unit mass of sample are presented in Section 9 of the appropriate SOP.

9. 1. 2. 2. 1. For metals and explosives, the concentration of elements in solid matrices is reported in mg/kg (dry basis) of analyte and is calculated as follows:

$$\text{mg/kg analyte in sample} = \frac{A \times V}{W}$$

where: A = final concentration read from calibration curve in mg/L
V = final volume of processed sample (mL)
W = weight of sample digested (g)

where: W = (wet weight of sample) x (% solids / 100)

9. 1. 2. 2. 2. For mercury, the concentration is reported in mg/kg on a dry weight basis and is calculated as follows:

$$\text{mg/kg analyte in sample} = \frac{A}{W}$$

where: A = μg mercury read from standard curve
W = weight of sample digested (g)

where: W = (wet weight of sample) x (% solids / 100)

9. 1. 2. 2. 3. Percent recovery:

For LCS, recovery is calculated using:

$$\% \text{ Recovery} = \frac{A}{B} \times 100$$

where: A = concentration of analyte measured
B = known true value

For MS/MSDs, recovery is calculated using:

$$\% \text{ Recovery} = \frac{\{(\text{Sample} + \text{Spike Result}) - \text{Sample Result}\}}{\text{Spike Added}}$$

9. 1. 2. 2. 4. Relative percent difference

$$RPD = \frac{|S1 - S2|}{(S1 + S2)/2} \times 100$$

where: S_1 and S_2 represent sample and duplicate sample results, or MS/MSD results.

9. 2. Data Review/Validation

9. 2. 1. Procedures Used to Evaluate Field Data

The project manager will keep field notebooks, data sheets and chain-of-custody forms for each sampling event. All field notes will be checked for accuracy, legibility and completeness.

9. 2. 2. Procedures to Review Laboratory Data

9. 2. 2. 1. Insect Sorting Procedures

Notebooks and chain-of-custody forms will be kept for the sorting process. All notes will be checked for accuracy, legibility and completeness.

9. 2. 2. 2. Laboratory Analytical Procedures

All analytical data generated by WES will be reviewed prior to report generation to assure the validity of the reported data. This data review process consists of a technical data review by the analyst to ensure: sample preparation information is correct and complete; analysis information is correct and complete; the appropriate SOPs have been followed; the analytical results are correct and complete; QC samples are within established limits; and the documentation is complete. This data review will be documented by using a checklist form with a signature and date entered by the reviewer. After the data package is complete, it undergoes an administrative review performed by either the quality assurance officer or the program administrator.

9. 2. 3. Procedures to Validate Laboratory Data

An independent validation of the data will be required of the final data package. The U.S. EPA and NSWC Crane will perform an independent validation on the final data package. Where applicable, procedures will follow appropriate U.S. EPA Functional Guidelines for Data Validation based upon project specific objectives. Data validation shall include a review of holding times, of calibration criteria, method blanks, spike recoveries, control samples, target compound identification, and quantitation.

9. 3. **Data Reporting**

9. 3. 1. Field Data Reporting

Copies of all field records will be included in the files maintained by the Navy.

9. 3. 2. Laboratory Sorting Data Reporting

Results of sorting will produce the following data:

- Date of issuance;
- Project name;
- Condition of samples as received;
- Mass of June splits for explosives degradation determination;
- Mass/month of each of the following: terrestrial and aquatic insects, and lepidoptera; and
- Signature of the entomologist.

9. 3. 3. Laboratory Analytical Data Reporting

The final data package will be delivered to the Navy 60 days after analysis begins. The data package will include a full deliverable package (excluding forms) capable of allowing the validation process to be accomplished. The report package will consist of the record of chain-of-custody, a case narrative, and the chemical data package.

The narrative will contain the following information:

- Date of issuance;
- Project name;
- Condition of samples as received;
- Laboratory analysis performed;
- Any deviation from intended analytical strategy;
- Number of samples and respective matrices;
- Quality control procedures utilized and also references to the acceptance criteria (note that standard laboratory criteria may have to be suspended based upon interferences caused by the matrix);
- Discussion of technical problems or other observations which may have created analytical difficulties;
- Discussion of any laboratory quality control checks which failed to meet project criteria (note that standard laboratory criteria may have to be suspended based upon interferences caused by the matrix); and
- Signature of the laboratory director.

The chemical data package will consist of:

- Case narrative for each sample delivery group;
- Summary page indicating dates of analyses for samples and laboratory quality control checks;
- Cross-referencing of laboratory sample to project sample identification numbers;
- Data qualifiers to be used should be adequately described;
- Copies of sample preparation and analyses logbooks and bench sheets;
- Sample results;
- Raw data for sample results and laboratory quality control samples;
- Matrix spike and MSD recoveries (or LCS and LCSD if insufficient sample for MSD), method blank results, calibration check compounds, and system performance check compounds; and
- Labeled and dated chromatograms/spectra of sample results and laboratory quality control checks;

9. 4. Data Assessment

Results of analytical data will be compared to the LD50, NOAEL, and LOAEL for explosives and the NOAEL and LOAEL for inorganics. Data will be assessed by the USFWS for potential deleterious effects to the Indiana Bat.

10. REFERENCES

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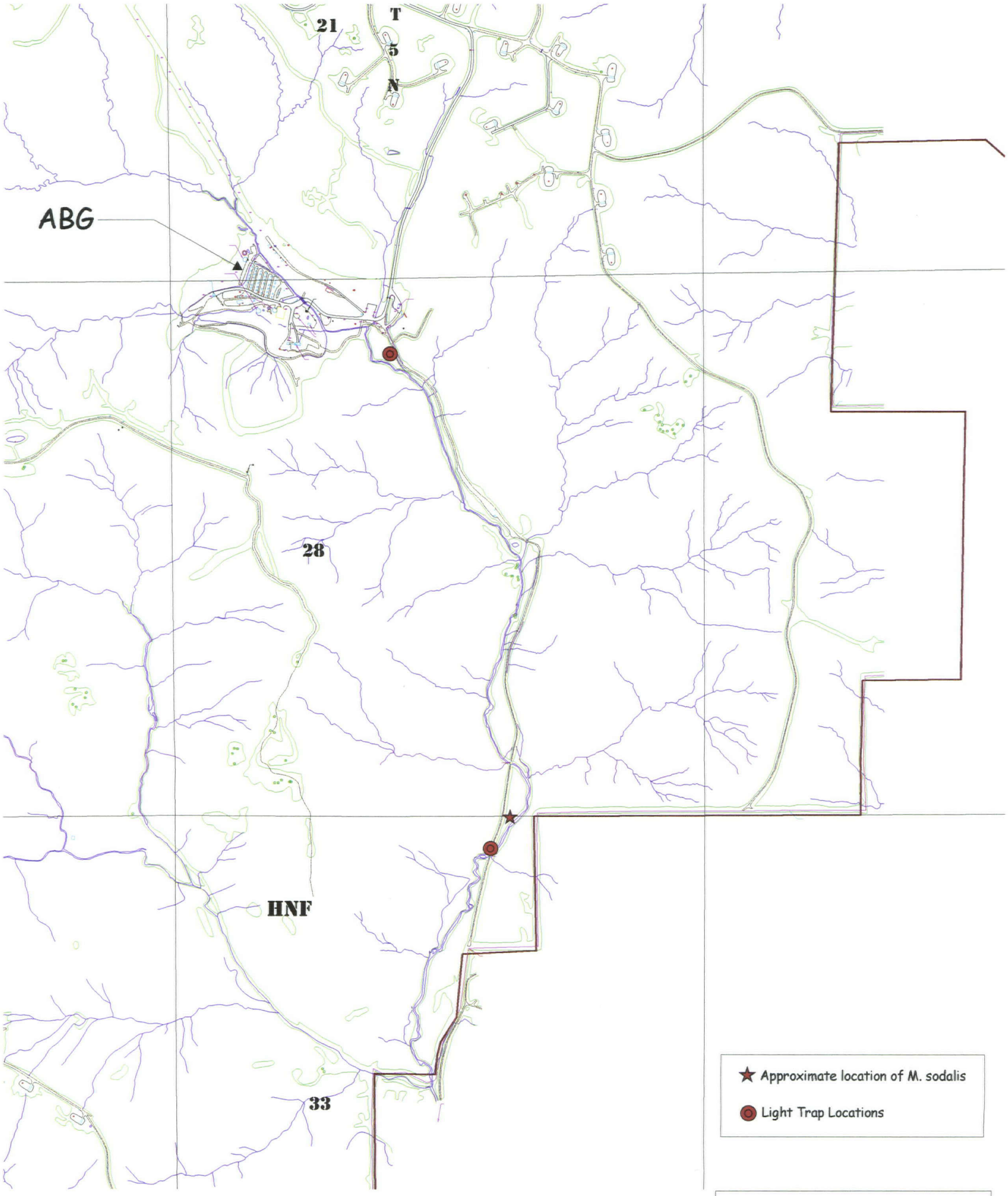

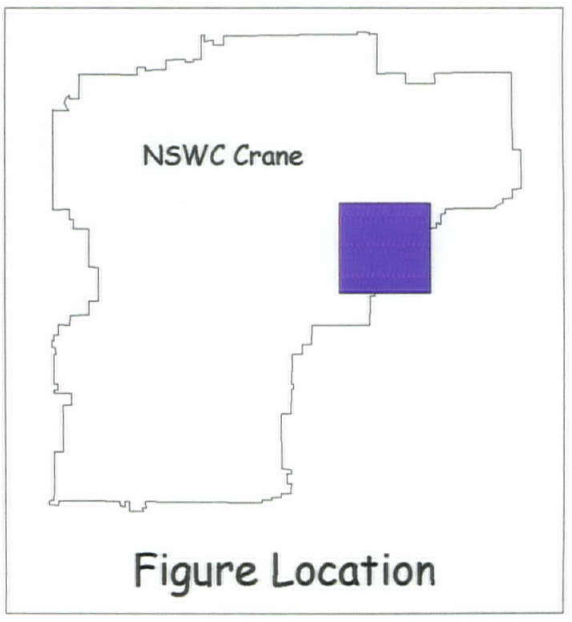

Location of ABG wrt Bat Capture & Trap Locations

Figure (1)

APPENDIX A
1997 INSECT COLLECTION RESULTS
(WEIGHT/ORDERS/SAMPLE)

AL Cofrunceso - B182

Received 12/98

1

Sample No. B3090-807-0897

INSECTS	Wt. of Insects Only gm
Lepidoptera - Terrestrial	13.13
Coleoptera - T	0.04
Trichoptera - Aquatic	0.21
Diptera - mixed	0.05
Homoptera - T	0.06
Neuroptera - A	0.07
Hymenoptera - T	0.07
Sub Total	**13.63**
1 dish not included	0.60
TOTAL	**14.23**

2

Sample No. B3089-807-0897

INSECTS	Wt. of Insects Only gm
Lepidoptera T	8.06
Coleoptera T - Small A	0.16
Trichoptera A	0.23
Diptera - Mixed	0.23
Homoptera - T	0.10
Neuroptera - A	0.07
Plecoptera - A	no wt.
Hymenoptera - T	0.07
Sub Total	**8.85**
1 dish not included	0.36
TOTAL	**9.21**

3

Sample No. B3089-619-2097

INSECTS	Wt. of Insects Only gm
Lepidoptera - T	29.85
Coleoptera - T	11.85
Trichoptera - A	1.78
Diptera - A	0.12
Ephemeropter - A	0.55
Homoptera - T	0.22
Plecoptera A	4.23
Hymenoptera - T	0.17
Sub Total	**48.77**
2 dishes not included	9.59
TOTAL	**58.36**

4

Sample No. B3089-806-0797

INSECTS	Wt. of Insects Only gm
Lepidoptera	6.32
Coleoptera	0.17
Trichoptera	0.12
Diptera	0.07
Homoptera	0.02
Sub Total	**6.70**
1 dish not included	0.28
TOTAL	**6.98**

5

Sample No. Crane Malaise 797

INSECTS	Wt. of Insects Only gm
Lepidoptera	0.07
Diptera	0.04
Homoptera	0.02
Sub Total	**0.13**
Misc./Trash not included	0.02
TOTAL	**0.15**

6

Sample No. B3090-806-0797

INSECTS	Wt. of Insects Only gm
Lepidoptera	5.95
Coleoptera	0.63
Trichoptera	0.12
Diptera	0.10
Homoptera	0.03
Neuroptera	0.00 no wt.
Hymenoptera	0.07
Sub Total	**6.90**
1 dish not included	0.34
TOTAL	**7.24**

7 Sample No. B3090-805-0697

INSECTS	Wt. of Insects Only gm	
Lepidoptera	7.84	
Coleoptera	0.11	
Trichoptera	1.09	
Diptera	0.13	
Homoptera	0.24	
Neuroptera	0.02	
Hymenoptera	0.00	no wt.
Sub Total	**9.43**	
1 dish not included	0.09	
TOTAL	**9.52**	

8 Sample No. B3097-717-1897

INSECTS	Wt. of Insects Only gm
Lepidoptera	9.72
Coleoptera	9.24
Trichoptera	3.81
Diptera	0.09
Hemiptera	0.02
Neuroptera	0.33
Hymenoptera	0.05
Plecoptera	0.40
Homoptera	0.20
Sub Total	**23.86**
1 dish not included	2.32
TOTAL	**26.18**

9 Sample No. B3089-716-1797

INSECTS	Wt. of Insects Only gm
Lepidoptera	15.32
Coleoptera	14.29
Trichoptera	2.76
Diptera	0.23
Hemiptera	0.21
Neuroptera	0.20
Orthoptera	0.05
Plecoptera	0.20
Hymenoptera	0.02
Sub Total	33.28
1 dish not included	3.36
TOTAL	36.64

10 Sample No. B3089-717-1897

INSECTS	Wt. of Insects Only gm
Lepidoptera	16.25
Coleoptera	14.56
Trichoptera	2.46
Diptera	0.11
Hemiptera	0.30
Neuroptera	0.43
Plecoptera	0.48
Hymenoptera	0.11
Sub Total	34.70
1 dish not included	4.28
TOTAL	38.98

11

Sample No. B3089-716-1797

INSECTS	Wt. of Insects Only gm
Lepidoptera	5.99
Coleoptera	7.18
Trichoptera	2.63
Diptera	0.14
Neuroptera	0.20
Plecoptera	1.55
Hymenoptera	0.05
Homoptera	0.17
Sub Total	**17.91**
1 dish not included	2.37
TOTAL	**20.28**

12

Sample No. B3090-718-1997

INSECTS	Wt. of Insects Only gm
Lepidoptera	25.27
Coleoptera	6.15
Trichoptera	6.07
Diptera	0.07
Neuroptera	0.12
Plecoptera	0.48
Hymenoptera	0.02
Homoptera	0.09
Orthoptera	0.01
Sub Total	**38.28**
2 dishes not included	1.17
TOTAL	**39.45**

13 Sample No. B3090-619-2097

INSECTS	Wt. of Insects Only gm
Lepidoptera	64.14
Coleoptera	8.74
Trichoptera	8.55
Diptera	0.25
Neuroptera	0.22
Plecoptera	0.95
Hymenoptera	0.16
Homoptera	0.09
Ephemeroptera	0.18
Mecoptera	0.08
Sub Total	**81.27**
2 dishes not included	5.07
TOTAL	**86.34**

14 Sample No. B3089-618-1997

INSECTS	Wt. of Insects Only gm
Lepidoptera	34.07
Coleoptera	10.39
Trichoptera	5.30
Diptera	0.51
Plecoptera	1.16
Hymenoptera	0.08
Homoptera	0.38
Sub Total	**51.89**
1 dish not included	0.80
TOTAL	**52.69**

15

Sample No. B3090-715-1697

INSECTS	Wt. of Insects Only gm
Lepidoptera	14.24
Coleoptera	10.22
Trichoptera	5.74
Diptera	0.36
Plecoptera	0.32
Homoptera	0.05
Neuroptera	0.08
Sub Total	31.01
1 dish not included	1.28
TOTAL	32.29

16

Sample No. B3090-715-1697

INSECTS	Wt. of Insects Only gm
Lepidoptera	0.16
Parts of insects	0.02
Total	0.18

17

Sample No. B3089-715-1697

INSECTS	Wt. of Insects Only gm
Lepidoptera	13.66
Coleoptera	12.92
Trichoptera	5.27
Diptera	0.53
Plecoptera	0.13
Homoptera	0.09
Hymenoptera	0.05
Sub Total	32.65
3 dishes not included	3.48
TOTAL	36.13

Sample No. B3089-805-0697

INSECTS	Wt. of Insects Only gm
Lepidoptera	9.77
Coleoptera	0.33
Trichoptera	1.01
Diptera	0.33
Homoptera	0.16
Hymenoptera	0.07
Sub Total	**11.67**
3 dishes not included	0.34
TOTAL	**12.01**

APPENDIX B
FIELD SAMPLING PLAN

FIELD SAMPLING PLAN
FOR
INSECT COLLECTION AT THE
AMMUNITION BURNING GROUNDS
CRANE DIVISION, NAVAL SURFACE WARFARE CENTER

BACKGROUND

Crane Division, Naval Surface Warfare Center (NSWC Crane) conducted an ecological risk assessment for Resource Conservation Recovery Act (RCRA) corrective action activities and to support a RCRA Subpart X (open burning-open detonation) permit. A bat survey along the streams near the areas of concern was included as part of the ecological risk assessment. On June 25, 1996, a single male Indiana bat (*Myotis sodalis*) was caught. The capture occurred south of the Ammunition Burning Grounds (ABG) on Little Sulphur Creek approximately in the SE corner of Section 28, T5N, R3W.

On June 28, 1996, Thomas J. Brent from NSWC Crane and Carol Witt-Smith of the United States Environmental Protection Agency, Region V (U.S. EPA) met with representatives from the U.S. Fish and Wildlife Service (USFWS) to discuss follow-up actions. NSWC Crane is in the process of determining if operations at the ABG are impacting *M. sodalis*. Thus, NSWC Crane proposed to conduct a survey, such as a blacklight survey, of the available prey in the area the bat was captured, in order to determine if contaminants are mobilizing through the bat's food chain.

FIELD COLLECTION

NSWC Crane has contacted the U.S. Army Corps of Engineers, Waterways Experiment Station (WES) to assist in the project. Dr. Al Cofrancesco is a research entomologist who will provide support in field collection and identification. Ms. Karen Myers will lead the analytical work.

Two types of traps are used for field collection. The first collecting apparatus is a large light trap that utilizes both black lights and a mercury vapor lamp. This trap attracts nighttime flying insects from a long distance. The different light sources give a wide spectrum of light stimulus that attracts the insects. The trap contains three large drawers. The bottom drawer contains a metal pan. Inside the pan, sheets of cotton will be placed and then approximately 800 ml of ethyl acetate is poured over the cotton. The ethyl acetate evaporates up through the two upper drawers and funnel area where the insects land. After breathing the chemical the insects fall through the collecting funnel and into the top drawer. This drawer is composed of large mesh screen and the smaller insects dropped through to the second drawer.

On October 19, 1999, representatives from the USFWS and U.S. EPA met to review and agree upon the proposed sampling locations at the ABG. Per the letter from the USFWS dated

October 20, 1999, one of these light traps will be situated on the Jeep Trail below the ABG, near Bridge 3090. The second light trap will be placed in the floodplain adjacent to Little Sulphur Creek immediately downstream of the ABG, as shown on Figure 1 of the Quality Assurance Project Plan.

Due to the remote location of the sampling sites, the lights receive power from gas-powered generators. The generators are fitted with an external gas supply to provide continuous fuel through the night. The generators will be placed on gravel or plywood sheets to ensure that hot exhausts will not create a fire hazard for dry vegetation. Typically, the traps will be setup at dusk and taken down and emptied around 8:00 a.m. Both generators and all lights will be confirmed operable in the evening when started and morning when stopped. Any discrepancies such as non-functioning lights or inoperable generators will be noted on the sample record and corrected prior to the next sampling event. An example copy of the sample record is provided in Attachment (1). If inoperable generators or non-functioning lights are discovered, NSWC Crane may elect to conduct an additional night of sampling - depending on the volume insects collected in the trap already.

Since light traps bias collection for light-attracted insects, a Malaise trap will also be used. This type of trap consists of insect netting designed in a configuration to funnel flying insects into the collecting container. The Malaise trap will be set up at dusk so as not to collect insects that fly only during daylight hours. The collecting container will be operated with a small amount of water or other attractant. The Malaise Trap will be set up on the Old Jeep Trail area of the ABG so as not to be influenced by the two light traps. The mass of insects from the Malaise is expected to be much less than the mass of insects from the two light traps.

All three traps will operate for four nights in each of the months of June, July, and August resulting in 36 trap nights. However, an additional 4 nights of sampling will occur in June to allow for the split sample as discussed below. This sampling strategy allows for collecting insects with differing emergences, as well as providing for optimum sample volume.

For the first June sampling event, all insects from all three traps will be placed in a single container. The container will be taken back to Building 3245 and placed in a freezer. Each morning's collection will be placed in a separate container so as to prevent thawing of the previous night's sample. Depending on the size of the containers and the volume of insects in the traps, multiple containers may have to be used for a single morning's collection. The resulting four-night's containers will then be shipped to WES under a chain-of-custody for grinding and splitting, as discussed below. A sample chain-of-custody is provided in Attachment (2).

Each morning following the next twelve sampling nights, NSWC Crane personnel will separate out the moths (Lepidopterans) and place those in a container. For ease of later sorting, insects in the upper drawer from the two light traps will be placed in a single container. Insects from the lower drawer from the two light traps, plus the Malaise trap, will then be placed in another single container. Containers will then be taken back to Building 3245 and stored in a freezer. Separate containers will be used for each night's collection so as

to avoid thawing of previously collected insects. Depending on the size of the containers and the volume of insects in the traps, multiple containers may have to be used for a single morning's collection. After one month's collection, the containers will be packaged and shipped to WES under a chain-of-custody. Therefore, four shipments of four sampling nights will be made to Dr. Cofrancesco at WES. The sorting and analytical matrix is provided in Table 1.

Table 1 - Sorting and Analysis Table

	June	June	July	August
Terrestrial	1	2	2	2
Aquatic	1	3	3	3
Lepidoptera	1	4	4	4

1. Collect. No sorting. Grind, split, and weigh splits. Analyze ½ now & ½ late Aug. early Sept.
2. Separate from Aquatic. Obtain weights/month. Store frozen until all 3 months arrive. Analyze as 1 sample.
3. Separate from Terrestrial. Obtain wts./month. Store frozen until all 3 months arrive. Analyze as 1 sample.
4. Collect. Obtain weights/month. Store frozen until all 3 months arrive. Analyze as 1 sample.

SAMPLE IDENTIFICATION

Three sample number formats will be used. The sample number formats will be:

CRANE-DEG-NNN
CRANE-LT-AAA-NNNNN
CRANE-MALAISE-NNN

Where:

CRANE — Sampling project.

DEG — The first June sample for evaluating explosives degradation in insect tissue. Collections from all 3 traps for all 4 nights will receive 1 sample number.

LT — Designates the two light traps. One light trap is located just south of the ABG, while the other is approximately 0.75 miles south of the first near the location of the original Indiana Bat capture. Since samples from the two traps will be combined, designating the difference between the 2 traps is unnecessary.

AAA — Alphabetic field. Will either be: LEP for Lepidopterans that are sorted in the field, or INS for all other insects to be sorted at WES.

NNNNN — Numeric field. The traps are run overnight thus, sampling crosses 2 dates. This is the date (morning) of sample collection, after the light trap has run all night. An example would be 70900, which would be a sample collected the morning July 09, 2000. The trap would have been started the night of July 08, 2000.

MALAISE Designates collections from the Malaise trap.

NNN Numeric field used for both the first June collection and the Malaise displaying month and year. The first June collection will be "600" for June 2000. Historically, the Malaise trap produces little insect volume. Therefore, fresh (morning) collections from the Malaise trap will be combined with those collections stored in the freezer for 4 nights.

Examples: CRANE-DEG-600 = sample taken early June 2000 for evaluating explosive degradation.

CRANE-LT-LEP-62200 = Lepidopterans collected from the light traps and sorted in the field the morning of June 22, 2000.

CRANE-MALAISE-800 = August collection from the Malaise trap.

SORTING

Dr. Cofrancesco will sort the insects at WES for identification purposes. As agreed to during the conference call of September 30, 1999, no attempt will be made to determine the differences between the collection devices or locations during this sampling effort.

Upon receipt of the first June collection of insects from NSWC Crane, WES will immediately grind the insects, then split the sample in roughly equal halves (by mass). No attempt at identification will be made prior to grinding. WES will analyze one half of the split for chemical contamination within the normal 7 day holding time. The second half of the split will remain frozen for the approximately four months that all the other insect samples will be frozen (same freezer, same type of holding container, etc.). Once all three months' samples have arrived, when all the other insect samples will be chemically analyzed (after having been separated and identified), the second half of the original split will also be analyzed. The two results (June and September) from the split sample can be compared to see if any chemical degradation takes place in the insects while frozen.

Subsequent insect samples (June, July, and August) will be grossly sorted into aquatic and terrestrial juvenile life stages plus Lepidopterans. Upon removal from the freezer, a timer will be set for 10 minutes and at the end of that time, the insects will be returned to the freezer and another container will be pulled (if available) for sorting. The insects will stay frozen for the entire holding time, except for the minimal time when they are being separated and identified.

All of the sorted fractions will be weighed and turned over to the chemist. Although an objective of the sorting is to determine the differences in contamination between insects that have aquatic and terrestrial juvenile stages, the potential exists that some of the sorted fractions may not have sufficient mass for analysis.

INDIANA BAT FOOD CHAIN STUDY
SAMPLE LOG

Department of the Navy
Naval Surface Warfare Center
Crane, IN 47522

TRAP TYPE	TRAP SITE
SAMPLE DATE	TIME OF TRAPPING

OBSERVATIONS

SAMPLE DATE	TIME OF TRAPPING

OBSERVATIONS

SAMPLE DATE	TIME OF TRAPPING

OBSERVATIONS

SAMPLE NUMBER	NUMBER OF CONTAINERS
SAMPLER'S SIGNATURE	CODE
DATE SAMPLES SENT	ANALYTICAL LAB

Attachment (1)

CHAIN OF CUSTODY

CRANE DIVISION, NAVAL SURFACE WARFARE CENTER
ENVIRONMENTAL PROTECTION DEPT.
NAVSURFWARCENDIV 5090/6 (REV. 5/93)

SAMPLING DATE	SAMPLE I.D.	CONTAINER (PARAMETERS)

SAMPLER PREPARER (SIGNATURE)	TRANSFERRED TO (SIGNATURE)	DATE	TIME	ORGANIZATION

Attachment (2)

CHAIN OF CUSTODY

CRANE DIVISION, NAVAL SURFACE WARFARE CENTER
ENVIRONMENTAL PROTECTION DEPT.
NAVSURFWARCENDIV 5090/6 (REV. 5/93)

SAMPLING DATE	SAMPLE I.D.	CONTAINER (PARAMETERS)

SAMPLER PREPARER (SIGNATURE)	TRANSFERRED TO (SIGNATURE)	DATE	TIME	ORGANIZATION

CHAIN OF CUSTODY

CRANE DIVISION, NAVAL SURFACE WARFARE CENTER
ENVIRONMENTAL PROTECTION DEPT.
NAVSURFWARCENDIV 5090/6 (REV. 5/93)

SAMPLING DATE	SAMPLE I.D.	CONTAINER (PARAMETERS)

SAMPLER PREPARER (SIGNATURE)	TRANSFERRED TO (SIGNATURE)	DATE	TIME	ORGANIZATION

CHAIN OF CUSTODY

CRANE DIVISION, NAVAL SURFACE WARFARE CENTER
ENVIRONMENTAL PROTECTION DEPT.
NAVSURFWARCENDIV 5090/6 (REV. 5/93)

SAMPLING DATE	SAMPLE I.D.	CONTAINER (PARAMETERS)

SAMPLER PREPARER (SIGNATURE)	TRANSFERRED TO (SIGNATURE)	DATE	TIME	ORGANIZATION

APPENDIX C
MDL DETERMINATION SOP
(CRICKET STUDY RESULTS)

Cricket Study

In order to prepare for analysis of insects from Crane Naval Surface Warfare Center, the ECB at WES performed a preliminary study utilizing crickets (*Acheta domestica*) to determine the most appropriate methods for sample preparation and extraction/digestion of explosives and metals. Crickets obtained from Armstrong Cricket Farm in Monroe, LA, were placed in a closed container and sacrificed with ethyl acetate after which they were stored in the freezer until needed. Crickets used in the study ranged between 0.25 and 0.60 grams in weight and measured between 1.5 and 2.5 cm in length. Percent solids for the crickets were 31%.

Initially, the cricket tissue was prepared for additional processing in three ways: 1) the thawed tissue was ground with a ceramic mortar and pestle and analyzed "wet"; 2) the frozen tissue was freeze-dried, then ground with a mortar and pestle; and 3) the thawed tissue was refrozen with liquid nitrogen, ground with a mortar and pestle, and separated into a portion to be freeze-dried for explosives and a second portion to be analyzed "wet" for metals.

Freeze-drying procedure:

Like sized aliquots of whole crickets or diced tissue are placed in freeze drying flasks, covered with parafilm and placed into a freezer. When completely frozen the flasks are placed on a Labconco 4.5 liter freeze drying system. Samples are dried overnight or until the flask reaches room temperature. The dried tissue is emptied into a ceramic mortar and ground to a powder with a ceramic pestle. The tissue is stored in the freezer until needed for extraction/digestion in preparation for analysis.

Explosives:

For explosives (Method 8330), the freeze-dried sample was found to have fewer chromatographic interferences than did the sample obtained from the "wet" extraction. As a result, freeze-drying was selected as the pre-extraction method for explosives.

Explosives extraction will follow SOP M-8330-00-WES-XX . 0.25 g of freeze-dried tissue will be extracted with 10 mL of acetonitrile, sonicated for 18 hours, cut with calcium chloride solution and filtered. The cricket tissue did not require further clean-up prior to analysis.

Using the above procedure, the following detection and reporting limits were obtained:

Explosives	MDL (mg/kg)	LRL (mg/kg)
HMX	1.0	3.2
RDX	0.3	0.8
TNB	0.4	1.2
DNB	0.3	1.0
Tetryl *		
NB	0.8	1.7
TNT	0.4	1.3
4-A-DNT	0.8	2.5
2-A-DNT	0.4	1.2
2,4-DNT	0.2	0.7
2,6-DNT	0.4	1.3
2-NT	0.6	1.9
3-NT	0.5	1.6
4-NT	0.6	2.1

- Tetryl recoveries were low. The analysis will be repeated before Crane tissue is analyzed.

Mercury was selected as the indicator metal because of its volatility. Portions of NIST reference material as well as fish tissue previously analyzed for mercury in our lab were subjected to freeze drying prior to analysis for mercury. Additional sample was also prepared by grinding frozen tissue made brittle by the addition of liquid nitrogen to the mortar during the grinding process. This process took less than 30 minutes. After the liquid nitrogen evaporated, the tissue quickly thawed. This thawed tissue was prepared for mercury analysis along side the freeze-dried material. A comparison of recoveries indicated that the freeze-drying did not affect mercury recovery. Freeze-drying was, therefore selected as the best pre-preparative method for the insect tissue. Analysis will follow SOP M-7471-00-WES-XX using 0.5 grams of freeze-dried material.

EPA method 3051, microwave digestion, was chosen to prepare the cricket tissue for analysis by ICP/MS. The microwave program is listed below:

Program Variables
File Name = Insects
Inorganic Sample Digestion

Stage	1	2	3	4	5
Power	60 %	60 %	60 %	60 %	60 %
Pressure	0020	0040	0085	0135	0175
Run Time	05:00	05:00	05:00	05:00	05:00
Time @ P	05:00	05:00	05:00	05:00	05:00
Temperature	120 C	140 C	160 C	170 C	180 C
Fan Speed	100 %	100 %	100 %	100 %	100 %

Number of Vessels: 12
Volume per Vessel: 10 mL
Sample Wt.: 0.5 g
Acid: HNO3

Cricket tissue prepared by method 3051 was scanned using an ICP/MS. The digest will need to be diluted 1 to 10 prior to analysis. This dilution is also performed on soil and salt water matrices analyzed by ICP/MS in our lab.

Using the above methods for metals, the following detection limits should be achievable:

Analyte	Target MDL (mg/L)	Est MDL (mg/L) *
Silver	-	0.026
Aluminum	5	0.078
Arsenic	0.5	0.073
Barium	1	0.009
Cadmium	0.1	0.005
Chromium	0.5	0.065
Copper	0.5	0.05
Magnesium	5	0.13
Manganese	1	0.01
Nickel	0.5	0.032
Lead	0.5	0.007
Antimony	-	0.014
Zinc	1	0.19
Mercury	0.20	0.20

* The MDL s were estimated from MDL s developed for water samples.

The method detection limits (MDLs) listed above are clearly below the target detection limits. We believe, barring unexpected interferences not observed in the crickets, we will be able to achieve the reporting limits necessary to fulfill the objectives of this project. We did not evaluate P by ICP/MS, but have obtained a standard. We do not know if we will have interferences for the P analysis using this analytical method.

Sample Size:

Initial results from the sorting indicate that the total insect mass per box will be around 16 to 18 grams maximum. This should produce about 5 to 6 grams of dry mass per box. To obtain the required QC analyses (field dup, MS & MSD), we will need to use a different sample as a QC sample for each analytical procedure (Hg, metals, and explosives). I have estimated the minimum sample size using the required weights.

Procudure	Sample (g)	Field Dup (g)	MS (g)	MSD (g)	Total/Procedure
Metals	1	1	1	1	4
Mercury	1	1	1	1	4
Explosives	.5	.5	.5	.5	2
T Phos **	5	5	5	5	20

** analysis by nutrient method

Procedure	Min sample required for all (g)	Min sample without T Phos (g)
Metals	10.5	5.5
Mercury	10.5	5.5
Explosives	9	4
T Phos	22.5	-

Please note that some of these weights do not allow for any re-extractions.

APPENDIX D
STANDARD OPERATING PROCEDURES

Missouri River Laboratory
USACE

CEERD EE-Q
420 SOUTH 18TH STREET
OMAHA, NE 68102-2586

TITLE: Total Phosphorus
(EPA Method 365.2)

SOP NUMBER: M-365.2-MRL-GC, Ver. 1.0

PREPARED BY: Primo Arora 9.17.96

REVIEWED BY: Vicki L. Rich 9-17-96
Technical Specialist Date

Denise C MacNulgan 9-17-96
QA Officer Date

Douglas B. Taggart 9-17-96
Laboratory Director Date

SOP MANUAL CONTROL NO.: _____

1.0 Scope and Application

1.1 This method is applicable to the determination of "total Phosphorus" in drinking, surface and saline water, domestic, industrial waste and solid waste. This method is applicable to water samples containing more than 0.01 mg/L total phosphorus. Total phosphorus is defined as all of the phosphorus present in the sample, regardless of form, as measured by the digestion procedure.

2.0 Method Summary

2.1 Samples are digested to hyrolyze phorphorus to ortho phosphate. The acidic digestate is neutralized and analyzed for ortho phosphate. Ortho phosphate reacts with molybdenum (VI) in an acid medium to form a phosphoanti-molybdenum complex which is subsequently reduced with ascorbic acid to form a mixed valence complex with an absorbance maximum at 880 nm.

3.0 Health and Safety

3.1 The toxicity or carcinogenicity of each compound, reagent or sample used or tested in this procedure has not been precisely determined. Therefore, all chemicals/samples should be treated as if they pose a health hazard and be handled in a fume hood. The analyst is required to wear the appropriate protective clothing while preforming these procedures such as a lab coat, gloves and safety glasses.

3.2 Prior to performing this procedure, the analyst should be familiar with the proper use of liquid spill kits and containment procedures. Spill clean-up kits are located next to the hood. These kits shall be used as directed by the manufacturer for small or medium spills. The kit mixture, which is a dry powder, will be poured around the spill perimeter and then sprinkled on the main body of the spill. The safety offer is to be contacted for removal of the spill. In the event of a large spill, the area of the spill will be evacuated immediately and the Safety Officer contacted.

3.3 For specific information regarding the toxicity of the reagents used in this procedure and other related health and safety issues including the proper storage and handling of reagents and chemicals, the analyst should consult the appropriate Materials Safety Data Sheets (MSDSs). The reagent MSDSs are located in room 421. Consult the Lab Safety Officer, Prem Arora for more information.

4.0 Sample Preservation, Containers, Handling, and Storage

4.1 General requirements for sample preservation, sample containers, and sample storage are detailed in SOP No. Q-005-MRL-SH, Sample Receipt, Login, and Storage.

4.2 Samples should be collected in plastic or glass bottles and kept tightly closed and refrigerated at 4°C until determination.

4.3 Samples are preserved by the addition of 2 mL of concentrated sulfuric acid per liter of sample and cooled immediately. The holding time for acid preserved samples is 28 days.

5.0 Interferences and Potential Problems

5.1 Ferric iron up to 40 mg/L, copper up to 10 mg/L and silica up to 10 mg/L do not interfere.

5.2 Samples with background absorbance at the analytical wavelength may interfere.

5.3 Commercial detergents containing phorphorus should never be used to clean glassware used in phosphorus determination. Acid washed glassware should be used for all reagents and calibrants. Wash glassware with 1:1 hydrochloric acid and rinse throughly with reagent water. Store the glassware filled with reagent water. If the glassware is reserved for the use only in phosphorus determination, treatment with hydrochloric acid is necessary only occasionally.

6.0 Equipment/Apparatus

6.1 Rapid Flow Alpkem Instrument using Method Number A303-S050-03, REV. A.

6.1.1 Flowcell: Part # A303-0103-01

6.1.2 Lamp: Part # A381-0047-01

6.1.3 Pump: Part # A-302

6.1.4 Polyethylene transmission tubing: Part #R3603

6.1.5 Filters: Part # A305-1460-00

6.2 Analytical balance, capable of weighing to 0.0001 g.

6.3 Volumetric Flasks - Class A, various sizes: Fisher Cat.# 10-210-5C to 10-210-5G

6.4 Volumetric Pipets - Class A, 1-30 mL: Fisher Cat.# 13-650-2B to 13-650-2T

6.5 Stir Plate and magnetic stir bars: Corning Stirrer/Hot Plate Cat.# 11-495-40A

6.5 Drying oven: Thelco Laboratory Oven Model 130

6.6 Ultrasonic bath: Fisher Cat.# 15-336-1

6.7 Membrane filter, 0.45 μm: Whatman Cat.# 09-904-4

6.8 pH paper - full range: pHydrin Insta-Check 0-13

6.9 Medicine dropper, glass or plastic

7.0 Reagents

The traceability of all reagents, standards, and solutions used in this analysis shall be documented. All standards and solutions shall be assigned unique identifiers which will be noted in the appropriate log books.

7.1 Stock Antimony Potassium Tartrate: Dissolve 3.0 ±0.1 g of antimony potassium tartrate ($K(SbO)C_4H_4O_6.1/2H_2$) in approximately 900 mL of reagent contained in a 1-L volumetric flask. Dilute the solution to the mark with reagent water and mix it well.

7.2 Stock Molybdate/Antimony: Slowly add 70.0 ±0.1 mL of sulfuric acid to approximately 600 mL of reagent water contained in a 1-L volumetric flask and mix it well. Add 6.0 ±0.1 g of ammonium molybdate ($(NH_4)_6MO_7O_{24}\cdot 4H_2O$) to the sulfuric acid solution and stir until the ammonium molybdate dissolves. Add 50.0 ±0.1 mL of stock antimony potassium tartrate and mix the solution well. Dilute the solution to the mark with reagent water and mix it well. Do not refrigerate this reagent. Discard the solution if it becomes blue. Caution: The mixing of sulfuric acid with water releases a great amount of heat.

7.2.1 Working Molybdate/Antimony: Mix together 100.0 ±0.1 mL of the stock molybdate/antimony and 4 drops of Dowfax. Prepare daily the quantity sufficient for the day's run.

7.3 Stock Ascorbic Acid: Dissolve 6.0 ±0.1 g of ascorbic acid ($C_6H_8O_6$) in a mixture of 200.0 ±0.1 mL of acetone (CH_3COCH_3) and 200.0 ±0.1 mL of reagent water. Store the solution at 2 - 6°C. Prepare this reagent weekly.

7.3.1 Working Ascorbic Acid: Mix together 10.0 ±0.1 mL of stock ascorbic acid and 50.0 ±0.1 mL of reagent water. Prepare this reagent daily.

7.4 Stock 10 N Sodium Hydroxide: Add cautiously and with continuous stirring, 400.0 ±0.1 g sodium hydroxide (NaOH) to approximately 700 mL of reagent water contained in a 1-L volumetric flask. Cool the solution in an ice bath when adding the sodium hydroxide. When the solution is cool, dilute it to the mark with reagent water and mix it well.

7.4.1 Working Sodium Hydroxide: Add 12.5 ±0.1 mL of the 10 N sodium hydroxide to 50.0 ±0.1 mL of reagent water contained in a 100-mL volumetric flask. Dilute the solution to the mark with reagent water.

7.5 Stock Sodium Chloride, 0.5%: Dissolve 5.0 ±0.1 g of sodium chloride (NaCl) in approximately 950 mL of reagent water contained in a 1-L volumetric flask. Dilute the solution to the mark and mix it well.

7.5.1 Working Sodium Chloride: Mix together 100.0 ±0.1 mL of stock sodium chloride and 4 drops of Dowfax. Prepare daily and quantity sufficient for the day's run.

7.6 Stock Mercuric Sulfate: To a 100-mL volumetric flask, add 40 mL reagent water, 10.0 ±0.1 mL concentrated sulfuric acid, and 8.0 ±0.1 g mercuric oxide (red). Stir until dissolved, dilute to the mark with reagent water and mix well.

7.7 Digestion Solution: To a 1-L volumetric flask, dissolve 133.0 ±0.1 g potassium sulfate in about 700 mL reagent water and 200.0 ±0.1 mL concentrated sulfuric acid. Add 25.0 ±0.1 mL of the stock mercuric sulfate solution and dilute to the mark with reagent water. Mix well.

7.8 Sampler Wash Solution: Since the digested samples, after being brought up to 25 mL, have an acid concentration of 5%, use a solution of the same composition for the sampler wash. Add 50.0 ±0.1 mL of concentrated sulfuric acid to approximately 800 mL of reagent water. Dilute to the mark with reagent water and mix well.

7.9 To prepare the stock standard, (1000 mg/L) dissolve 4.3937 ±0.0001 g of potassium dihydrogen phosphate (KH_2PO_4, dried at 110°C) in approximately 900 mL of reagent water contained in a 1-L volumetric flask. Dilute the solution to the mark with reagent water. Preserve the stock calibrant with 2 drops of chloroform and refrigerate at 2 - 6°C. Mix the solution well.

7.10 Prepare an intermediate calibrant with (5 mg/L) by pipetting 5.0±0.1 mL of stock calibrant into approximately 800 mL of acidified reagent water (2 mL of sulfuric acid per liter of solution) contained in a 1-L volumetric flask. Dilute the solution to the mark with the acidified reagent water and mix it well. (A combined intermediate calibrant for the Total Kjeldahl Nitrogen and Total Phosphorus analyses can be made by pipetting 20 mL of stock ammonium chloride and 5 mL of stock potassium dihydrogen phosphate in approximately 800 mL of the acidified reagent water contained in a 1-L volumetric flask. Dilute the solution to the mark with reagent water and mix it well.)

7.11 Prepare the working standards by pipetting the designated volumes of intermediate calibrant, tabulated below, to the required number of 100 mL volumetric flasks that each contain 80 mL of the acidified reagent water. Dilute each solution to the mark with the acidified reagent water and mix it well. Prepare working calibrants daily. Digest the standards.

Intermediate Solution (mL)	Concentration (mg/L)
20	1
10	.5
5	.2
2	.1
1	.05

8.0 Procedure

8.1 Set up the total phosphorus manifold as illustrated on the Alpkem chart provided by Perstorp (instrument manufacturer). Place the manifold lines on reagent water with Dowfax (2 mL/L) followed by cleaning with 1 N sodium hydroxide.

8.2 Now place the manifold lines on reagent water with Dowfax (2 mL/L) to allow the instrument to warm up for approximately 20 minutes and produce a steady baseline (fluctuations of absorbance less than 0.04). Then transfer the lines to the reagents and obtain a steady baseline.

8.3 Place working standards in order of decreasing concentration in sampler. Complete filling the sample tray with samples and begin analysis as per manufacturer's instructions.

8.4 Digestion Procedure: Place 25.0 ± 0.1 mL of the sample in a digestion tube and add 5.0 ± 0.1 mL of the digestion solution and mix throughly (vortex) for a minimum of 30 seconds. Add 4 to 8 boiling stones. Place tubes in the block digester for one hour at 160°C. Continue to digest for 1.5 hours at 380°C. Cool sample and add 20 mL ammonia-free water and mix throughly (vortex) for a minimum of 30 seconds. Bring the volume of the digested sample to 25 mL. Mix well. The digested sample will now have an acid concentration of 5%.

9.0 Calculations

None

10.0 Quality Assurance/Quality Control

10.1 A preparation batch of samples would be defined as a group of up to 15 field samples of similar matrix type that are extracted on the same day using the same reagents for the same analysis. In addition to the 15 samples, each batch would also contain at a minimum a method blank (MB), a matrix duplicate (MD), a matrix spike/matrix spike duplicate (MS/MSD), and a laboratory control sample (LCS).

10.2 All sample identification numbers, batch numbers, and/or project numbers must be properly recorded using the extraction log data sheet. Record the date the extraction was started and completed, weight of the sample extracted, volume and type of extraction fluid used, and the results of the extraction.

10.3 For each batch of samples analyzed a method blank must be prepared with each batch of samples. The method blank should be prepared using the same extraction fluid used to extract the samples. The extraction blank is carried through the entire extraction process and is used to monitor potential reagent/laboratory contamination.

10.4 For each batch of samples analyzed a laboratory control sample must be prepared and analyzed. The LCS would be prepared using the primary source standard and would contain all method target analytes unless project-specific requirements are identified.

10.5 The use of the matrix spike and matrix spike duplicate will depend in part on the role of the laboratory. When the laboratory serves the role as the primary laboratory, then the site-specific documents should be consulted. The sample to be used for the MS/MSD may be specified in the field. This previously designated sample would then be spiked with the site-specific target analytes at a concentration equivalent to the site action level. The MS/MSD would be prepared using the primary source standards. If this information was not specified or unknown, then the laboratory would choose a representative sample from each batch of samples analyzed. If samples from multiple sites were to be analyzed in the same batch, then multiple sets of MS/MSDs may be required.

When the laboratory serves as the QA laboratory, the above scenario may not be practical to implement. If the site-specific requirements are unknown and samples from multiple sites are analyzed in the same batch, then the laboratory should select a single sample for spiking. The order of preference for spiking levels is as follows:

- If the target analyte concentrations are known, spike to increase the background concentration by a factor of approximately two
- If an action level exists, spike at this level
- If neither of the first two conditions apply, spike at a level between the low and the mid-point of the calibration curve.

10.6 The use of the matrix duplicate may also depend in part on the role of the laboratory. The selection of a matrix duplicate will be performed as described for the MS/MSD in Section 10.4.

10.7 The analyst must demonstrate proficiency in performing the analysis as outlined in SOP No. Q-016-MRL-QC, Technical Training. Method proficiency must be redemonstrated anytime a major method modification is made.

10.8 Corrective action:

10.8.1 If there is baseline noise, do one or more of the following: filter or remake the reagents, flush flowcell to clean, replace pump tubing, replace flowcell, replace lamp.

10.8.2 If there is loss of sensitivity, do one or more of the following: prepare fresh reagents, replace pump tubing, flush flowcell to clean, use correct filter or flowcell, use correct pump tubing, use correct calibration setting, replace filter, replace lamp, check to see if appropriate gas is being used as the segmentation gas, check to see if the pump tubes are in the correct containers, check the system pH.

10.8.3 If there is a drift or baseline shift, do one or more of the following: flush flow cell, prepare fresh reagents, replace pump tubing, secure flowcell, replace lamp, flush flowcell with cleaning solution.

10.8.4 If there is non-linearity or the coefficient of correlation is not at least 0.999, do one or more of the following: prepare fresh standards or reagents, replace pump tubing, verify 5.00 V is set using the lowest coarse gain setting.

10.8.5 If there is carry over or poor washout, do one or more of the following: verify the intersample bubbles maintain integrity into the cartridge, verify or increase wash time, turn on the pecking action of the sampler if it is called for, check to see if appropriate gas is being used as the segmentation gas.

10.8.6 If there is no reaction, do one or more of the following: remake standards or reagents, use correct filters and flowcells, verify 5.00 V is set using the lowest coarse gain setting, place lines in correct containers.

10.8.7 If the matrix spike and matrix spike duplicate recoveries are not within laboratory acceptable limits, do one or more of the following: prepare a new spike sample, use another sample for spiking, reanalyzed the sample and the spiking.

10.8.8 If the LCS recovery are not within laboratory acceptable limits, do one or more of the following: Prepare a new check sample, reanalyze the original check sample.

10.8.9 Some problems can arise that are too numerous to list in this SOP. When these problems do occur, the analyst and if necessary, the supervisor determine what coarse of action should be followed.

11.0 Data Validation

11.1 The analyst is responsible for verifying the information recorded on the log sheet (see Figure 1) is complete and accurate.

11.2 Additional levels of review are performed as described in SOP No. Q-024-MRL-DR, Data Reduction and Review/Validation, using the predesigned checklist as shown in Figure 2.

12.0 Waste Disposal

This procedure generates wastes that must be disposed of in accordance to all Federal and local regulations. Refer to the Missouri River Laboratory's Waste Management Plan.

13.0 References

13.1 Methods for Chemical Analysis of Water and Wastes, March 1984, EPA-600/4-79-020, "Sample Preservation", Page xvii, Environmental Monitoring and Support Laboratory, Office of Research and Development, U.S. Environmental Protection Agency, Cincinnati, Ohio 45286.

13.2 Methods for Chemical Analysis of Water and Wastes, March 1984, EPA-600/4-79-020, "Phosphorus All Forms", Method 365.3 (Colorimetric, Ascorbic Acid, Two Reagent).

13.3 Murphy, J. and J.P. Riley, "A Modified Single Solution Method for the Determination of Phosphate in Natural Waters", Anal. Chem. Acta., Volume 27, Page 31-36.

13.4 Whitledge, T.E., S.C. Malloy, C.J. Patton and C.D. Wirick, Automated Nutrient Analyses in Seawater, Technical Report, Brookhaven National Laboratory, Upton, New York, 1981, Page 48.

13.5 SOP No.: Q-005-MRL-SH, Sample Receipt, Login, and Storage.

13.6 SOP No.: Q-016-MRL-QC, Technical Training and Personnel Qualifications.

13.7 SOP No.: Q-024-MRL-DR, Data Reduction and Review/Validation (In-House/Contractor Data).

13.8 SOP No.: Q-008-MRL-GL, Reagent Water Generation and Quality Monitoring.

13.9 Missouri River Laboratory Waste Management Plan

Total Phorphorus Analysis
Missouri River Laboratory

Batch No.:	Method: ☐ SOP # M-365.2-MRL-GC
Start Date:	

Date	Lab ID	Matrix	Peak Height	Dilution Factor	Concentration (mg/L)

Analyst: _____ Date: _____

2nd Reviewer: _____ Date: _____

Figure 1. Total Phorphorus Analysis Log Sheet

Total Phorphorus Analysis
Missouri River Laboratory

Batch No.:	Method: ☐ SOP # M-365.2-MRL-GC
Start Date:	

Date	Lab ID	Matrix	Peak Height	Dilution Factor	Concentration (mg/L)

Analyst: _____ Date: _____

2nd Reviewer: _____ Date: _____

Figure 1. Example of Completed Total Phorphorus Analysis Log Sheet

Lab Data Review Check List
Total Phorphorus Analysis
Missouri River Laboratory

Project Number(s)	Method : SOP No. M-365.2-MRL-GC
Batch Number(s)	

Review Item	Yes (x)	No (x)	N/A (x)	2nd Level Review (x)
Were the project number, batch number, and sample identification number(s) properly recorded?				
Was the weight of soil samples extracted and the final extraction volume properly recorded?				
Was the volume of water sample analyzed and the final volume properly recorded?				
Were the sample dilution or concentration factors properly recorded?				
Was a method blank prepared at the required frequency using a blank solid/liquid matrix?				
Was a Laboratory Control Sample (LCS) prepared at the required frequency?				
Were a matrix spike (MS) and a matrix spike duplicate (MSD) prepared at the required frequency?				
Were the matrix duplicates (MD) prepared at the proper frequency?				
Were samples analyzed within holding times?				
Are there any Corrective Action Reports associated with this sample batch?				
Are copies of the Corrective Action Reports attached?				

NOTES:

Analyst: _____ Date: _____

2nd Level Reviewer: _____ Date: _____

Figure 2. Lab Data Review Check List Total Phorphorus Analysis

Lab Data Review Check List
Total Phorphorus Analysis
Missouri River Laboratory

Project Number(s)	Method : SOP No. M-365.2-MRL-GC
Batch Number(s)	

Review Item	Yes (x)	No (x)	N/A (x)	2nd Level Review (x)
Were the project number, batch number, and sample identification number(s) properly recorded?				
Was the weight of soil samples extracted and the final extraction volume properly recorded?				
Was the volume of water sample analyzed and the final volume properly recorded?				
Were the sample dilution or concentration factors properly recorded?				
Was a method blank prepared at the required frequency using a blank solid/liquid matrix?				
Was a Laboratory Control Sample (LCS) prepared at the required frequency?				
Were a matrix spike (MS) and a matrix spike duplicate (MSD) prepared at the required frequency?				
Were the matrix duplicates (MD) prepared at the proper frequency?				
Were samples analyzed within holding times?				
Are there any Corrective Action Reports associated with this sample batch?				
Are copies of the Corrective Action Reports attached?				

NOTES:

Analyst: _____ Date: _____

2nd Level Reviewer: _____ Date: _____

Figure 2. Example of Completed Lab Data Review Check List Total Phorphorus Analysis

CEERD-EE-C
3909 HALLS FERRY ROAD
VICKSBURG, MS 39180

TITLE: Acid Digestion of Sediments, Sludges, and Soils (SW-846 Method 3050A)

SOP NUMBER: M-3050A-ECC-IP, Ver. 1.1

PREPARED BY: Anne Halpenny-Weatherby 10/1/1999
Date

REVIEWED BY: Karen F Myers 10/01/99
Technical Specialist Date

Karen F Myers 10/01/99
QA Officer Date

Ann B Strong 10/01/99
Laboratory Director Date

SOP MANUAL CONTROL NO.: _____

1.0 Scope and Application

1.1 This method is an acid digestion procedure used to prepare sediments, sludges, and soil samples for analysis by flame or furnace atomic absorption spectroscopy (FLAA and GFAA, respectively) or by inductively coupled plasma emission spectrometry (ICP) or inductively coupled plasma mass spectrometry (ICP-MS).

1.2 Samples prepared by this method may be analyzed by ICP for all the listed metals, or by FLAA or GFAA as indicated below in Table 1.

Table 1. Techniques for Metals Analysis

Analyte	Technique	Symbol	CAS #
Aluminum	ICP/FLAA	Al	7429-90-5
Arsenic	ICP-MS/ICP/GFAA	As	7440-38-2
Barium	ICP/FLAA	Ba	7440-39-3
Beryllium	ICP-MS/ICP/FLAA/GFAA	Be	7440-41-7
Cadmium	ICP-MS/ICP/FLAA/GFAA	Cd	7440-43-9
Calcium	ICP/FLAA	Ca	7440-70-2
Chromium	ICP-MS/ICP/FLAA/GFAA	Cr	7440-47-3
Cobalt	ICP-MS/ICP/FLAA/GFAA	Co	7440-48-4
Copper	ICP/FLAA	Cu	7440-50-8
Iron	ICP-MS/ICP/FLAA/GFAA	Fe	7439-89-6
Lead	ICP-MS/ICP/FLAA/GFAA	Pb	7439-92-1
Magnesium	ICP/FLAA	Mg	7439-95-4
Manganese	ICP/FLAA	Mn	7439-96-5
Molybdenum	ICP-MS/ICP/FLAA/GFAA	Mo	7439-98-7
Nickel	ICP/FLAA	Ni	7440-02-0
Osmium	ICP/FLAA	Os	
Potassium	ICP/FLAA	K	7440-09-7
Selenium	ICP/GFAA	Se	7782-49-2
Silver	ICP/FLAA	Ag	7440-22-4
Sodium	ICP/FLAA	Na	7440-23-5
Thallium	ICP-MS/ICP/FLAA/GFAA	Tl	7440-28-0
Vanadium	ICP/FLAA/GFAA	V	7440-62-2
Zinc	ICP/FLAA	Zn	7440-66-6

2.0 Method Summary

A representative 1-g (dry weight) sample is digested in nitric acid and hydrogen peroxide. The digestate is then refluxed with either nitric acid or hydrochloric acid. Hydrochloric acid is used for flame AA and ICP analyses and nitric acid is used for furnace AA work.

2.1 Dilute hydrochloric acid is used as the final reflux acid for the ICP analysis of As and Se, and the flame AA or ICP analysis of Ag, Al, Ba, Be, Ca, Cd, Co, Cr, Cu, Fe, K, Mg, Mn, Mo, Na, Ni, Os, Pb, Tl, V, and Zn.

2.2 Dilute nitric acid is employed as the final dilution acid for the furnace AA analysis of As, Be, Cd, Cr, Co, Fe, Pb, Mo, Se, Tl, and V.

2.3 The diluted samples have an approximate acid concentration of 5.0% (v/v).

3.0 Health and Safety

3.1 Use of this procedure requires the handling of reagents and corrosive acids, therefore, protective equipment must be utilized. Minimum personal protection includes the use of laboratory safety glasses, a lab coat or apron, and protective gloves. All digestions must be performed in a fume hood.

3.2 For specific information regarding the toxicity of the acids used in this procedure and other related health and safety issues including the proper storage and handling of reagents and chemicals, the analyst should consult the appropriate Material Safety Data Sheets (MSDS) located in room 111.

3.3 Use of this procedure requires the handling of concentrated acids, nitric and hydrochloric acid. Prior to performing this procedure, the analyst should be familiar with the proper use of corrosive liquid spill kits and containment procedures. Spill kits are located in room 126.

4.0 Sample Preservation, Containers, Handling, and Storage

4.1 Requirements for sample preservation, sample containers, and sample storage are detailed in SOP No. Q-005-ECC-SH, Sample Receipt, Login, and Storage Procedures.

4.2 This method requires the digestion of 1 g of dry gound sample. It is recommended that a minimum of 50 g be available to allow for QC analyses and possible reanalyses.

4.3 All containers used to store digestates must be pre-washed with detergents, acids, and reagent water. Refer to SOP No. Q-012-ECC-GL, Glassware Cleaning.

4.4 Samples for metals analysis must be digested and analyzed within 6 months of sample collection. Digestates are stored in high-density, polyethylene (HDPE) bottles at room temperature.

5.0 Interferences and Potential Problems

5.1 Sludge samples can contain diverse matrix types, each of which may present its own analytical challenge. Spiked samples and any relevant standard reference material should be processed to aid in determining whether Method 3050A is applicable to a given waste.

5.2 Refer to the appropriate analytical method SOPs for a discussion of the possible interferences that may be encountered during the analysis of the digestates.

5.3 During the digestion, the samples must be monitored closely so as to prevent the sample from boiling or going to dryness. The temperature of the heating device should be adjusted so that a gentle sample reflux develops and the sample slowly evaporates, but does not boil or bump. If the samples begin to boil, immediately lower the temperature of the heating device.

5.4 Samples must be covered between digestion steps to avoid picking up dust particles.

5.5 The sample digestion area must be kept as clean as possible. This involves regular cleaning of the area and hoods.

6.0 Equipment/Apparatus

6.1 Technicon BD-20 digestion tubes.

6.2 Qualitative filter paper, S & S, White ribbon, # 589.

6.3 Graduated cylinders — Class A, 100-mL, 500-mL.

6.4 Volumetric flasks — Class A, 100-mL, 1000-mL.

6.5 Automatic pipets or acid-dispensing bottles, accurate to within ± 0.1 mL. Brinkman Dispensette, 2ml to 10mL.

6.6 Nalgene polyethylene or equivalent material, squeeze bottles, for dispensing reagent water and for general rinsing purposes.

6.7 Filter funnels,Urbanti,high speed filter funnels, molded TPX, 70mm.

6.8 Hotplate or equivalent heating device, Tecator Digestion Block 2020 or 1015 with autostep 1012 controller.

6.9 Analytical Balance — Capable of accurately weighing to the nearest 0.01 g.

6.10 Polyethylene bottles for storing digestates, Eagle Picher, 125mL, HDPE, wide mouth nalgene, Level 1, wash C

7.0 Reagents

7.1 Reagent water — As defined in SOP No. Q-008-ECC-GL, Reagent Water Generation and Quality Monitoring.

7.2 Acids must be of sufficient purity for the analytical methods to be employed. Acids must be analyzed to determine levels of impurities. If a method blank with the acid is < MDL, the acid can be used. This should be verified for each new lot of acid.

7.2.1 Concentrated nitric acid, (HNO_3) —, Fisher TraceMetal grade, Cat.# A-509 or equivalent.

7.2.2 Concentrated hydrochloric acid, (HCl) —, Fisher TraceMetal grade Cat.# A-508 or equivalent.

7.2.3 Nitric acid, (1:1). Using a 500-mL graduated cylinder, measure 250 ± 50 mL of reagent water into a 1-L volumetric flask. Using the same 500-mL graduated cylinder, measure 500 ± 10 mL of concentrated nitric acid and slowly add it to the volumetric flask containing the reagent water. Bring to volume with reagent water, mix. This reagent can be stored in a 1-L plastic bottle at room temperature.

7.3 Hydrogen peroxide (30%), H_2O_2 — ACS reagent grade. This solution is commercially available. H_2O_2 must be stored in a refrigerator away from flammable and combustible materials. Once opened, 30% H_2O_2 should be disposed of after 6 months.

7.4 Metal stock calibration solutions shall be purchased as certified solutions from commercial suppliers (SPEX plasma grade or equivalent). Certificates must be kept on file. Calibration standards are from Alfa Aesar, STK# 35422. All stock standards must be replaced after one year.

7.4.1 Primary source standards shall be used to prepare the initial calibration curve, continuing calibration verification (CCV) standard, laboratory control sample (LCS), and matrix spikes (MS).

7.4.2 Secondary source standards shall be used to verify the initial calibration (ICV) curve only. These standards shall be purchased

from a different vendor, unless the primary vendor can supply different lot numbers.

7.5 Matrix spike (MS) standard

7.5.1 The matrix spike standard shall be prepared from the calibration stock standard (primary) in Section 7.4.1. by adding 1mL of stock to the matrix spike sample.

7.5.2 The spike should be at a level that would approximately double the concentration of the target analytes present. In the absence of target analytes, the spike would be made at the site action level, assuming that this level did not also correspond to the value of the low standard used. If the action level is the same as the low standard used, then the spiking would occur at a slightly higher level, i.e., at the value of the next calibration standard used. If the action level is not known, the spiking level would then default to a concentration between the low and mid-level of the initial calibration standards.

7.5.3 All method analytes should be contained in the MS spike. Subsets may be used based on project specific requirements.

7.6 Laboratory Control Sample (LCS)

7.6.1 The LCS shall be prepared from the calibration stock standard (primary) in Section 7.3.1. by adding 1mL of stock standard to a blank.

7.6.2 The LCS is prepared from reagent water and contains all of the method target analytes. A subset of the method target analytes could be used based on the project-specific requirements. The spiking level used would be at the same level as the site-specific action limit. If site-specific action limits are not available, the spiking level shall be at a concentration between the low and mid-level of the initial calibration standards.

8.0 Procedure

All glassware and digestion vessels used to contain a sample must be labeled The digestion log (see Figure 1) is to be filled out with each step of the procedure.

8.1 Mix the sample thoroughly to achieve homogeneity, (see SOP No. Q-021-ECC-GL, Subsampling of Containers). Dry a representative amount of wet sample at 60 C overnight. When cool using a mortar and pestle, grind sample until it passes through a .40 mesh sieve. For each digestion procedure, weigh 1-g of dry gound sample to the nearest 0.01 g and transfer to a tube. Record weight on digestion log (see Figure 1). Spike QC samples as appropriate.

8.2 A separate determination of percent solids must be performed on a homogeneous aliquot of the sample if required by the project. Refer to SOP No. M-2216-ECC-GL, Determination of Water (Moisture) Content of Soil (ASTM Method 2216).

8.3 Using a dispensing bottle, add 10 mL of 1:1 HNO_3, mix the slurry. Program the autostep controller to a temperature of 130°C with a ramp time of 20 minutes and a total run time of 8 hours. When samples are high in organics it may be necessary to start at a lower temperature. A block temp of 130 will give a sample temp of 90 to 95°C in the sample Heat the sample to 95 ± 5°C and reflux for 10 to 15 minutes without boiling. It is suggested that the temperature of the heating device or representative sample be monitored near the center of the heating device. Record the temperature on the digestion log in Figure 1 at the beginning and end of the digestion process.

8.4 Allow the sample to cool, add 5 mL of concentrated HNO_3, and reflux for 30 minutes. Repeat this last step to ensure complete oxidation. Allow the solution to evaporate to 5 mL without boiling.

8.5 After the sample has cooled, add 2 mL of reagent water and 3 mL of 30% H_2O_2 using dispensing bottles. and return the sample to the heating device for warming and to start the peroxide reaction. Care must be taken to ensure that losses do not occur due to excessively vigorous effervescence. Heat until effervescence subsides and cool the vessel .

8.6 Continue to add 30% H_2O_2 in 1-mL aliquots with warming until the effervescence is minimal or until the general sample appearance is unchanged.

NOTE: Do not add more than a total of 10 mL 30% H_2O_2.

8.7 If the sample is being prepared for the ICP analysis of As and Se, or the flame AA or ICP analysis of Ag, Al, Ba, Be, Ca, Cd, Co, Cr, Cu, Fe, K, Mg, Mn, Mo, Na, Ni, Os, Pb, Tl, V, and Zn, then add 5 mL of concentrated HCl and 10 mL of reagent water with dispensing bottles. Return the vessel to the heating device, and reflux for an additional 15 minutes without boiling. After cooling, filter the sample through a filter (section 6.3) into a volumetric flask, or centrifuge. Rinse the filter and solids with ~~an~~ additional 10 to 15 mL reagent water and dilute to a 100 mL volume with reagent water using a volumetric flask. Thoroughly mix the sample to ensure the digestate is completely homogenous. The diluted sample has an approximate acid concentration of 5.0% (v/v) HCl and 5.0% (v/v) HNO_3. The sample should be transferred to a clean, acid-rinsed, labeled HDPE bottle.

8.8 If the sample is being prepared for the furnace analysis of As, Be, Cd, Co, Cr, Fe, Mo, Pb, Se, Tl, and V, continue heating the acid-peroxide digestate until the volume has been reduced to approximately 5 mL. After cooling,filter the sample through a filter (section 6.3) into a volumetric flask or centrifuge. Rinse the filter and solids with additional reagent water and dilute to 100 mL

with reagent water using a volumetric flask. Thoroughly mix the sample to ensure the digestate is completely homogenous. The diluted digestate solution contains approximately 5% (v/v) HNO_3. The sample should be transferred to a clean, acid-rinsed, labeled HDPE bottle.

8.9 Prior to analysis, digestates are stored at room temperature.

9.0 Calculations

The final metal concentration in the sample should be reported in mg/L or μg/L as appropriate following analysis by ICP, ICP-MS, FLAA or GFAA. All dilution factors or concentration factors used in the digestion must be factored into the final sample concentration. Refer to the appropriate determinative method(s) for sample calculations.

10.0 Quality Assurance/Quality Control

10.1 A preparation batch of samples would be defined as a group of up to field twenty samples of similar matrix type that are extracted at the same time using the same reagents for the same analysis. In addition to the twenty samples, each batch would also contain at a minimum a method blank, a laboratory control sample (LCS), a matrix spike (MS), and a matrix duplicate (MD).

10.2 A method blank (MB) consisting of consisting of 100 ± 1 mL of reagent water shall be prepared with each batch of samples. Acceptance criteria are presented in the appropriate determinative method.

10.3 A Laboratory Control Sample (LCS) consisting of 100 ± 1 mL of reagent water spiked with all the target metals prior to digestion, is digested and analyzed with each batch of samples. Control charts will be maintained for the LCS for all method target analytes. Acceptance criteria are specified in the appropriate determinative method. The order of preference for spiking levels is as follows:

- If an action level exists, spike at this level. If the action level is at the lowest calibration standard, the concentration of the LCS should be slightly higher.
- If the above condition does not apply, spike at a level between the low and mid-level of the calibration standards.

10.4 The use of the matrix spike and will depend in part on what role the laboratory is playing. When the laboratory serves the role as the primary laboratory, then site-specific documents should be consulted. The sample to be used for the MS may be specified in the field. This previously designated sample would then be spiked with the site-specific target analytes at a

concentration equivalent to the site action level. If this information was not specified or unknown, then the laboratory would choose a representative sample from each batch of samples analyzed. If samples from multiple sites were to be analyzed in the same batch, then multiple MSs may be required.

When the laboratory serves the role as the QA laboratory, the above scenario may not be practical to implement. If the site-specific requirements are unknown and samples from multiple sites are analyzed in the same batch, then the laboratory should select a single sample for spiking. The order of preference for spiking levels is as follows:

- If the target analyte concentrations are known, spike to increase the background concentration by a factor of approximately two
- If an action level exists, spike at this level
- If neither of the first two conditions apply, spike at a level between the low and mid-level of the calibration standards.

10.5 The use of the matrix duplicate may also depend in part on the role the laboratory is playing. The selection of a matrix duplicate will be performed as described for the MS in Section 10.4.

10.6 The analyst must demonstrate proficiency in performing this method as outlined in SOP No. Q-016-ECC-QC, Technical Training. Method proficiency must be redemonstrated anytime a major method modification is made.

11.0 Data Validation

11.1 The digestion analyst is responsible for verifying the information recorded on the digestion log (Figure 1) is complete and accurate.

11.2 Additional levels of review are performed as described in SOP No. Q-024-ECC-DR, Data Reduction and Review/Validation (In-House/Contractor Data), using a predesigned form as shown in Figure 2.

12.0 Waste Disposal

This procedure generates corrosive and metallic wastes that must be disposed of in accordance to all federal and local regulations. Refer to the laboratory's Waste Management Plan. Metal digestion extracts are stored in the triple-door refrigerator in the bay area. Chain of custody extracts are stored in the walk-in cooler in the bay area. metal digestion extracts are stored for six months, afterwards, they are returned to the principal investigator of origin for waste disposal.

13.0 References

13.1 Test Method for Evaluating Solid Waste, SW-846, Third Edition, Update I, July 1992, Method 3050A.

13.2 SOP No. Q-008-ECC-GL, Reagent Water Generation and Quality Monitoring.

13.3 SOP No. Q-021-ECC-GL, Subsampling of Containers.

13.4 SOP No. Q-005-ECC-SH, Sample Receipt, Login, and Storage.

13.5 SOP No. M-2216-ECC-GL, Determination of Water (Moisture) Content of Soil (ASTM Method 2216).

13.6 SOP No. Q-024-ECC-DR, Data Reduction and Review/Validation (In-House/Contractor Data).

13.7 SOP No. Q-012-ECC-GL, Glassware Cleaning.

13.8 SOP No. Q-003-ECC-QC, Standards Preparation, Traceability, and Storage.

13.9 SOP No. Q-004-ECC-QC, Nonconformances and Corrective Action.

13.10 SOP No. Q-016-ECC-QC, Technical Training.

13.11 ERDC WES site Waste Management Plan.

Digestion Log For Metals Analysis
ERDC EL ECC

Batch No.:	SOP # _____	Matrix: ☐ Solid ☐ Liquid
Start Date:	Project No.:	Analysis Method: ICP, FLAA, GFAA

Type	Sample ID No.	Sample Amount (mL) (g)	Filtration necessary?	Final Volume (mL)	Description/Sample Comments
MB					
LCS					
1					
2					
3					
4					
5					
6					
7					
8					
9					
10					
11					
12					
13					
14					
15					
16					
17					
18					
19					
20					
MD					
MS					
MSD					

LCS Spiking Standard ID: _____ Amount Added _____ mL

MS/MSD Spiking Standard ID: _____ Amount Added _____ mL

Witness: _____ Date: _____

Reagent Lot No(s): _____ Temp. of Heating Device: Start: _____ End: _____

_____

_____

Analyst: _____ Reviewed By: _____ Date: _____

Figure 1.

Lab Data Review Check List
Metals Digestion Log
ERDC EL EEC

Project Number(s):
Batch Number(s):
Method SOP No.:

Review Item	Yes (x)	No (x)	N/A (x)	2nd Level Review (x)
Were the following recorded properly? - batch number - sample identification number(s) - dates				
Were the weight/volume of sample digested and the final digested volume properly recorded?				
Were sample dilutions or concentration factors properly recorded?				
Was a method blank prepared at the required frequency using a blank matrix?				
Was a Laboratory Control Sample (LCS) prepared at the required frequency?				
Were a matrix spike (MS) and matrix spike duplicate (MSD) prepared at the proper frequency?				
Were the matrix duplicates (MD) prepared at the proper frequency?				
Are there any Corrective Action Reports associated with this sample batch?				
Are copies of Corrective Action Reports attached?				
Were the spiking volume, stock source, and spike concentration properly recorded for the MS/MSD and LCS?				

NOTES: _____

Analyst: _____ Date: _____

2nd Level Reviewer: _____ Date: _____

Figure 2.

CEERD-EE-C
3909 HALLS FERRY ROAD
VICKSBURG, MS 39180

ENVIRONMENTAL CHEMISTRY CENTER
ENVIRONMENTAL LABORATORY
ERDC

TITLE: Microwave Assisted Acid Digestion Of Sediments, Sludges, Soils, And Oils (SW-846 Method 3051)

SOP NUMBER: M-3051-ECC-IP, Ver. 1.0

PREPARED BY: Donald E. Parker 9/14/99
Date

REVIEWED BY: Karen F Myers 10/11/99
Technical Specialist Date

Karen F Myers 10/11/99
QA Officer Date

10/15/99
Laboratory Director Date

SOP MANUAL CONTROL NO.: _____

1.0 Scope and Application

1.1 This method is applicable to the microwave assisted acid digestion of sludges, sediments, soils, and oils for the elements listed in Table 1.

Table 1. Techniques for Analyte Analysis

Analyte	Technique	Symbol	CAS #
Aluminum	ICP/ICP-MS/GFAA	Al	7429-90-5
Antimony	ICP/ICP-MS/GFAA	Sb	7440-36-0
Arsenic	ICP/ICP-MS/GFAA	As	7440-38-2
Boron	ICP/ICP-MS	B	
Barium	ICP/ICP-MS/GFAA	Ba	7440-39-3
Beryllium	ICP/ICP-MS/GFAA	Be	7440-41-7
Cadmium	ICP/ICP-MS/GFAA	Cd	7440-43-9
Calcium	ICP/ICP-MS/GFAA	Ca	7440-70-2
Chromium	ICP/ICP-MS/GFAA	Cr	7440-47-3
Cobalt	ICP/ICP-MS/GFAA	Co	7440-48-4
Copper	ICP/ICP-MS/GFAA	Cu	7440-50-8
Iron	ICP/ICP-MS/GFAA	Fe	7439-89-6
Lead	ICP/ICP-MS/GFAA	Pb	7439-92-1
Magnesium	ICP/ICP-MS/GFAA	Mg	7439-95-4
Manganese	ICP/ICP-MS/GFAA	Mn	7439-96-5
Molybdenum	ICP/ICP-MS/GFAA	Mo	7439-98-7
Nickel	ICP/ICP-MS/GFAA/	Ni	7440-02-0
Potassium	ICP/ICP-MS/GFAA	K	7440-09-7
Selenium	ICP/ICP-MS/GFAA	Se	7782-49-2
Silver	ICP/ICP-MS/GFAA	Ag	7440-22-4
Sodium	ICP/ICP-MS/GFAA	Na	7440-23-5
Strontium	ICP/ICP-MS	Sr	
Thallium	ICP/ICP-MS/GFAA	Tl	7440-28-0
Vanadium	ICP/ICP-MS/GFAA	V	7440-62-2
Zinc	ICP/ICP-MS/GFAA	Zn	7440-66-6

1.2 This method provides a rapid multi-element acid leach digestion. If a decomposition including hydrochloric acid is required for certain elements, it is recommended that Method 3050A be used. Digestates produced by the method are suitable for analysis by flame atomic absorption (FLAA), graphite furnace atomic absorption (GFAA), inductively coupled plasma emission spectroscopy (ICP-AES) and inductively coupled plasma mass spectrometry (ICP-MS).

2.0 Method Summary

A representative sample of approximately 0.5 g is digested in 10 mL of concentrated nitric acid for 10 minutes in a Teflon digestion vessel. The vessel is capped and heated in the microwave unit. After cooling, the vessel contents are filtered and diluted, or diluted and then centrifuged, or allowed to settle.

3.0 Health and Safety

3.1 Kitchen-type, or modified kitchen-type microwave ovens do not contain sufficient safety devices, and are not acceptable for use with this method. Laboratory microwave ovens have been specifically designed for this procedure, and must be used.

3.2 Acceptable digestion vessels include unlined fluorocarbon (PFA or TFM) containers with pressure relief mechanisms or containers with fluorocarbon liners and pressure relief mechanisms.

3.3 Use of this procedure requires the handling of reagents and corrosive acids, therefore protective equipment must be utilized. Minimum personal protection includes the use of laboratory safety glasses, a lab coat or apron, and protective gloves. During the manual operation of the microwave oven, and particularly in case of vessel failure, acid and nitric oxide fumes will vent. Therefore, the microwave oven must be located next to the fume hood, and vented into it according to manufacturer instructions. Do not leave the microwave unattended during operation. Keep away from the close proximity of the oven as much as possible.

Wait a sufficient amount of time, a minimum of 20 minutes, after the heating cycle for the digestion vessels to cool. **Never open vessels abruptly, and only open vessels in the fume hood.** Try to keep distance between the operator and the vessels they are opening.

3.4 For specific information regarding the toxicity of the acids used in this procedure and other related health and safety issues including the proper storage and handling of reagents and chemicals, the analyst should consult the appropriate Material Safety Data Sheets (MSDSs) located in room 111, Building 3299.

3.5 Use of this procedure requires the handling of concentrated acid, (nitric acid). Prior to performing this procedure, the analyst should be familiar with the proper use of corrosive liquid spill kits and containment procedures. Spill kits are located in room 129 of Building 3299.

TREAT ACID SPILLS AS FOLLOWS:

a. Wear personal protective equipment (PPE) such as gloves and eye protection.
b. For large spills, notify the supervisor.
c. Isolate the spill by encircling the spill with neutralizer (sodium carbonate).
d. Cover the spill by slowly pouring neutralizer over it. Avoid splattering.
e. Mix neutralizer into the spill and check the pH. If necessary, add more neutralizer.
f. Sweep up waste and dispose.

3.6 Caution must be used when using Nitric Acid because of its strong oxidizing powers and its toxic by products. All digestions must be performed in a laboratory fume hood.

3.6.1 Nitric acid decomposes at its boiling point of 78 °C. Digestions are carried out at 95 °C. Decomposition products of nitric acid yield two highly toxic gases Nitric oxide (NO) - 25 ppb tolerance, and Nitrogen dioxide (NO2) - 3 ppm tolerance.

3.6.2 Nitric acid is a strong oxidizer and is incompatible with Acetic acid, and Acetone and must be stored separately. Contact with cellulose (finely divided wood, i.e., sawdust) combusts spontaneously.

3.7 The following safety guide lines for laboratory microwave systems must be followed:

3.7.1 Never heat liquids in a closed container that is not equipped with a pressure relief device.

3.7.2 Never insert metallic objects such as wire into the inlet/outlet ports nor modify the ports. Serious microwave leakage and /or electric shock may result. The inlet/outlet ports of the MDS-2100 are electrically grounded to the microwave cavity and are designed to prevent leakage of microwave energy.

3.7.3 Never insulate the exterior of a CEM digestion vessel. Air circulation is critical to cooling and proper operation of the digestion vessel.

3.7.4 Never install a MDS-2100 inside a laboratory fume hood where corrosive chemicals are stored. The fumes from stored chemicals may attack external surfaces and electrical components.

3.7.5 Exercise extreme caution when microwave heating any liquid that can decompose at elevated temperatures. Decomposition gases may evolve

too rapidly for the pressure relief devices to dissipate, causing vessel failure.

3.7.6 Use caution when microwave heating digestion containers other than those supplied by CEM. Some polymers such as nylon, polyester, or acrylic absorb microwave radiation directly and can become so hot they will melt and even ignite.

3.7.7 Microwave heating of alkaline or salt solutions in open or closed vessels will concentrate these solutions, causing precipitation of salts and formation of crystal deposits on vessel walls. In a microwave field these crystal deposits may cause electrical arcing and/or localized not spots on vessel walls, leading to vessel failure.

3.7.8 All vessel surfaces other than the sample-containing portion of the vessel must be dry and free of particulate matter to avoid localized microwave heating and possible vessel damage. Always DI water rinse vessels before use.

3.7.9 Samples such as carbonates, sulfides, and other materials that react to form gaseous reaction products should be weighed, added to vessels, and allowed to stand until the reaction subsides. Then the vessels may be sealed and heated in the MDS-2100.

3.7.10 When performing acid digestion of samples containing volatile or easily oxidized compounds, initially weigh no more than 0.10 grams (for solids) into the vessel and observe the reaction before capping the vessel. For liquids, if an organic vapor from the sample is detected by smell, use hotplate digestion in lieu of microwave digestion.

3.7.11 Do not attempt to digest organic samples large than 0.5 grams if the organic content and composition of the sample are unknown. For sample unknowns be alert for any organic odors. If an organic vapor from the sample is detected by smell, use hotplate digestion in lieu of microwave digestion.

3.7.12 TCLP extracts will concentrate lower molecular weight and water soluble organic compounds (alcohols, ketones, and glycols) if they are present in the waste sample.

3.7.13 US EPA TCLP microwave methods call for using a 45 mL sample per vessel. At this volume the concentration of highly reactive organic compounds such as acetone, methy ethyl ketone, ethylene glycol antifreeze, etc can easily exceed the 0.5 gram limitation for organic samples in sealed microwave digestion vessels. BTU analysis is an acceptable method for determining organic content of samples. Be alert for any organic vapor odors the sample might have. In such cases use hotplate digestion.

4.0 Sample Preservation, Containers, Handling, and Storage

4.1 Requirements for sample preservation, sample containers, and sample storage are detailed in SOP No. Q-005-ECC-SH, Sample Receipt, Login, and Storage Procedures.

4.2 All glassware, as well as containers used to store digestates must be prewashed with detergents, acid, and reagent water. Refer to SOP No. Q-012-ECC-GL, Glassware Cleaning.

4.3 Metals digestion and analysis must be completed within 180 days of sample collection. Digestates are stored in low-density, polyethylene (LDPE) bottles at room temperature.

5.0 Interferences and Potential Problems

5.1 Very reactive or volatile materials that may create high pressures when heated may cause venting of the vessels with potential loss of sample and analytes.

5.2 Samples that contain carbonates or other carbon dioxide generating compounds may cause enough pressure to vent the vessel. Refer to Section 3.0. If this situation is anticipated, the analyst should use a smaller sample volume. If a smaller volume must be used, document on a Corrective Action Report, as described in SOP No. Q-004-ECC-QC, Nonconformances and Corrective Actions.

6.0 Equipment/Apparatus

6.1 Microwave apparatus requirements CEM MDS-2100.

6.1.1 The microwave unit must provide programmable power with a minimum of 574 W and can be programmed to within ± 10 W of the required power.

6.1.2 The microwave unit cavity must be corrosion resistant and well ventilated.

6.1.3 All electronics must be protected against corrosion for safe operation.

6.1.4 The system requires Teflon PFA digestion vessels (120-mL capacity) capable of withstanding pressures up to 190 ± 10 psi and capable of controlled pressure relief at pressures exceeding 190 ± 10 psi.

6.1.5 A rotating turntable is employed to insure homogeneous distribution of microwave radiation within the unit. The speed of

the turntable should be a minimum of 3 rpm.

6.2 Graduated cylinder, Class A ÿÿÀ ß 50-or 100-mL capacity.

6.3 Quantitative filter paper S&S White label, or equivalent.

6.4 Analytical balance 300-g capacity, capable of weighing to nearest 0.001g.

6.5 Filter funnels, Urbanti,high speed filter funnels, molded TPX, 70mm.

6.6 Polyethylene bottles for storing digestates, Eagle Picher, 125mL, HDPE, wide mouth nalgene, Level 1, wash C. or equivalent

6.7 Automatic pipets or acid-dispensing bottle, accurate to within ± 0.1 mL Brinkman Dispensette, 2ml to 10mL

6.8 Volumetric flasks, Class A, 50-mL capacity.

7.0 Reagents

7.1 Reagent Water As defined in SOP No. Q-008-ECC-GL, Reagent Water Generation and Quality Monitoring.

7.2 Acids must of sufficient purity for the analytical methods to be employed. Acids must be analyzed to determine levels of impurities. If a method blank made with the acid is < MDL, the acid can be used. This should be verified for each new lot of acid.

7.2.1 Nitric acid, concentrated, HNO_3 - Fisher TraceMetal grade, Cat# A-509 or equivalent.

7.2.2 Hydrochloric acid, concentrated, HCl - Fisher TraceMetal grade, Cat# A-508 or equivalent.

7.3 Metal stock calibration solutions shall be purchased as certified solutions from commercial suppliers (SPEX plasma grade or equivalent) Certificates must be kept on file. All stock standards must be replaced after one year.

Primary source standards shall be used to prepare the initial calibration curve, continuing calibration verification (CCV) standard, laboratory control sample (LCS), and matrix spikes and their duplicates (MS/MSDs).

7.3.1 Stock standard solutions for ICP digestions:

VHG Labs. Cat. # W1-100
Ag, Be, Cd (5 μg/ml)
Cu (25 μg/ml)

Co, Mn, Ni, Pb, Zn, (50 μg/ml)
Fe (100 μg/ml)
Tl (200 μg/ml)

VHG Labs Cat. # W2-100:
Cr (20 μg/ml)
Sb, V (50 μg/ml)
Al, As, Ba, Se (200 μg/ml)

7.3.2 Stock standard solution for GFAA digestions

Claritas PPT Cat # Cl -Spike -1:
Fe (500 μg/ml)
Ba, Zn, (250 μg/ml)
Co, Cr, Cu, Mn, Ni, Sb, V (100 μg/ml)
As, Pb, (50 μg/ml)
Ag, Be, Cd, Se, Tl (25 μg/ml)

7.3.3 Stock standard solutions for ICP-MS digestions.

Claritas PPT Cat. # CL - Spike - 1 (10 ml to 100 ml in 5% HNO_3)
Fe (50 μg/ml)
Ba, Zn (25 μg/ml)
Co, Cr, Cu, Mn, Ni, Sb, V (10 μg/ml)
As, Pb, (5 μg/ml)
Ag, Be, Cd, Se, Tl (2.5 μg/ml)

7.4 Matrix spike (MS) standard

7.4.1 The matrix spike standard shall be prepared from the calibration stock standard (primary) in Section 7.3.1. by adding 0.5 mL of the appropriate stock (ICP, ICP-MS or GFAA) to the matrix spike sample.

7.4.2 The spike should be at a level that would approximately double the concentration of the target analytes present. In the absence of target analytes, the spike would be made at the site action level, assuming that this level did not also correspond to the value of the low standard used. If the action level is the same as the low standard used, then the spiking would occur at a slightly higher level, i.e., at the value of the next calibration standard used. If the action level is not known, the spiking level would then default to a concentration between the low and mid-level of the initial calibration standards.

7.4.3 All method analytes should be contained in the MS spike. Subsets may be used based on project specific requirements.

7.5 Laboratory Control Sample (LCS)

7.5.1 The LCS shall be prepared from the stock standard (primary) in Section 7.3.1. by adding 0.5 mL of the appropriate stock (ICP, ICP-MS or GFAA) to the blank sample. Because of the difficulties associated with obtaining a representative blank solid matrix gree of metals contamination, no solid matrix is used. Instead the spike is added to an empty sample vessel as used in the preparation of other samples.

7.5.2 The LCS is prepared from reagent water and shall contain all of the method target analytes. A subset of the method target analytes could be used based on the project-specific requirements. The spiking level used would be at the same level as the site-specific action limit. If site-specific action limits are not available, the spiking level shall be at a concentration between the low and mid-level of the initial calibration standards.

Digestate Concentrations For Matrix Spike (MS) and Laboratory Control Samples (LCS)

Analyte	ICP (µg/mL)	GFAA (µg/mL)	ICP-MS (µg/mL)
Ag	0.05	0.25	0.025
Al	2.0	--	--
As	2.0	0.5	0.05
Ba	2.0	2.5	0.25
Be	0.05	0.25	0.025
Cd	0.05	0.25	0.025
Co	0.5	1.0	0.1
Cr	0.2	1.0	0.1
Cu	0.25	1.0	0.1
Fe	1.0	5.0	0.5
Mn	0.5	1.0	0.1
Ni	0.5	1.0	0.1
Pb	0.5	0.5	0.05
Sb	0.5	1.0	0.1
Se	2.0	0.25	0.025
Tl	2.0	0.25	0.025
V	0.5	1.0	0.1
Zn	0.5	2.5	0.25

8.0 Procedure

8.1 Calibration of Microwave Equipment

8.1.1 Measurement of the available power for heating is evaluated so that absolute power in watts may be transferred from one microwave unit to another. For cavity type microwave equipment, this is accomplished by measuring the temperature rise in 1 kg of water exposed to microwave radiation for a fixed period of time. The analyst can relate power in watts to the partial power setting of the unit. The calibration format required for laboratory microwave units depends on the type of electronic system used by the manufacturer to provide partial microwave power. Few units have an accurate and precise linear relationship between percent power settings and absorbed power. Where linear circuits have been utilized, the calibration curve can be determined by a three-point calibration method (8.1.3), otherwise, the analyst must use the multiple point calibration method (8.1.2). (Prior to the use of the three-point calibration curve, linearity must be initially verified using the multiple-point calibration procedure.) The MDS-2100 has a microwave power output of 950 watts at 100% power.

8.1.2 The multiple point calibration involves the measurement of absorbed power (p) over a large range of power settings. For a 600 W unit, the following power settings are measured; 100, 99, 98, 97, 95, 90, 80, 70, 60, 50, and 40% using the procedure described in Section 8.1.4. These data are clustered about the customary working power ranges. Nonlinearity has been commonly encountered at the upper end of the calibration. If the unit's electronics are known to have nonlinear deviations in any region of proportional power control, it will be necessary to make a set of measurements that bracket the power to be used. The final calibration point must be at the partial power setting that will be used in the test. The power setting (x-axis) is plotted versus the absorbed power (y-axis). This setting should be checked periodically to evaluate the integrity of the calibration. The multiple point calibration must be performed if a significant change in the calculated power setting for this test point is detected (± 10 W). This calibration must also be performed on new units brought into service.

8.1.3 The three-point calibration involves the measurement of absorbed power (p) at three different power settings. Measure the absorbed power at 100% and 50% using the procedure described in Section 8.1.4, and calculate the power setting corresponding to the required test power in watts as determined using the equation in Section 9.1 from the (2-point) line. Measure the absorbed power (p) at that partial power

setting. If the measured absorbed power (p) does not correspond to the specified power within ± 10 W, use the multiple point calibration in Section 8.1.2.

8.1.4 Equilibrate a large volume of water to room temperature (23 ± 2 ÿÿv C). One kg of reagent water is weighed (1000.0 g ± 0.1 g into a Teflon beaker or a beaker made of some other material that does not significantly absorb microwave energy (glass absorbs microwave energy and is not recommended). The initial temperature of the water should be 23 ± 2 C measured to ±0.05 C. The covered beaker is circulated continuously (in the normal sample path) through the microwave field for 2 minutes at the desired partial power setting with the unit's exhaust fan on maximum (as it will be during normal operation). The beaker is removed and the water vigorously stirred. Use a magnetic stirring bar inserted immediately after microwave irradiation and record the maximum temperature within the first 30 seconds to ±0.05 C. Use a new sample for each additional measurement. If the water is reused both the water and the beaker must have returned to 23 ±2 C. Three measurements at each power setting should be made. The mean of the three power measurements should be calculated.

The absorbed power (p) is determined as described in Section 9.

NOTE: Stable line voltage is necessary for accurate and reproducible calibration and operation. The line voltage should be within manufacturer's specification, and during measurement and operation not vary by more than ± 2V. A constant power supply may be necessary for microwave use if the source of the line voltage is unstable.

Electronic components in most microwave units are matched to the unit's function and output. When any part of the high voltage circuit, power source, or control components in the unit have been serviced or replaced, it will be necessary to recheck the units' calibration power. If the power output has changed significantly (±10 W), then the entire calibration should be reevaluated.

8.2 Sample Digestion

All digestion vessels and volumetric ware must be carefully acid washed and rinsed with reagent water. When switching between high concentration samples and low concentration samples, all digestion vessels should be cleaned by leaching with hot (1:1) hydrochloric acid (greater than 80 C, but less than boiling) for a minimum of two hours followed with hot

(1:1) nitric acid (greater than 80 C, but less than boiling) for a minimum of two hours and rinsed with reagent water and dried in a clean environment. This cleaning procedure should also be used whenever the prior use of the digestion vessels is unknown or cross contamination from vessels is suspected. Polymeric or glass volumetric ware and storage containers should be cleaned by leaching with more dilute acids (approximately 10% V/V) appropriate for the specific plastics used and then rinsed with reagent water and dried in a clean environment. To avoid precipitation of silver, ensure that all HCl has been rinsed from the vessels. All glassware and polymeric funnels used in metals digestion are rinsed with reagent grade water, placed into a 20 % HCl solution for 30 minutes, rinsed again with reagent grade water, then dried. The digestion log (see Figure 1) is to be filled out with each step of the procedure.

8.2.1 Dry samples are used in the digestion procedure. A 10 g portion of the sample is placed in a beaker and dried overnight at 60 °C. The sample is ground in a mortar and sieved though a 40 mesh screen.

8.2.2 Weigh the Teflon PFA digestion vessel, valve and cap assembly to 0.01 g prior to use and record on digestion log (see Figure 1).

[CAUTION: If the sample smells highly organic, additional gasses may develop during digestion. This could result in a dangerous situation. Notify the QA Officer, and determine if a smaller sample volume is possible. Alternatively, a different digestion method can be used.]

8.2.3 Weigh 0.5 g of sample to the nearest 0.01 g into the Teflon PFA sample vessel with the number of the vessel recorded on the digestion log following the sample identification. For soils, sediments, and sludges, use no more than 0.50 g. For oils, use no more than 0.25 g.

8.2.4 In a fume hood, using an acid-dispensing bottle, add 10.0 ± 0.1 mL of concentrated (70%) nitric acid to each vessel. Allow any reactions to stop before sealing. Seal each vessel according to manufacturer instructions. Measure the mass of each vessel and record in the digestion log.

8.2.5 Evenly distribute the vessels in the carousel. The carousel can hold 12 samples. Blanks are treated as samples for the purpose of balancing the power unit. When fewer than 12 vessels are used in the digestion the power setting must be adjusted in accordance with the manufacturers recommendations. This provides an energy balance since the microwave power absorbed is proportional to the total mass in the cavity. Alternative power settings for different

manufacturers are acceptable as long as they bring the samples to 175 C in less than 5.5 minutes and keeps the temperature between 170 - 180 C during the next 4.5 minutes. This may also be accomplished by the use of temperature feedback, using a monitor vessel.

8.2.6 Place the carousel in the unit; be sure to seat it carefully on the turntable. Program the microwave unit for the first stage of the power program to give the required power calculated as described in Section 9.1. Set power to 100, PSI to 70, time to 10.0, TAP to 4.3 and temp to 175. This sequence brings the temperature of samples to 175 C in less than 5.5 minutes and remain between 170 - 180 C for the balance of the 10 minute irradiation period. The pressure should peak at less than 6 atm for most soil, sludge, and sediment samples. To prevent catastrophic venting, one may need to start with a lower power setting before applying full power.

NOTE: The pressure will exceed these limits in the case of high concentrations of carbonate or organic compounds. In these cases the pressure will be limited by the relief pressure of the vessel 190 ± 10 psi.

Start the turntable motor and be sure the vent fan is running on high and the turntable is turning. Start the microwave generator.

8.2.7 At the end of the microwave program, allow the vessels to cool for a least 5 minutes in the unit before removal to avoid possible injury if a vessel vents immediately after microwave heating. The samples may be cooled outside the unit by removing the carousel and allowing the samples to cool on the bench or in a water bath. When the vessels have cooled to room temperature, weigh and record the weight of each vessel assembly on the digestion log to the nearest 0.01 g. If the weight of the sample plus acid has decreased by more than 10%, discard the sample.

8.2.8 Complete the preparation of the sample by carefully uncapping and venting each vessel in a fume hood. Transfer the sample to an acid-cleaned 50-mL volumetric flask and dilute to volume with reagent water. If the digested sample contains particulates which may clog nebulizers or interfere with injection of the sample into the instrument, the sample shall be filtered through acid rinsed qualitative filter paper prior to being 50 mL transferred to a volumetric flask. The sample is now ready for analyses and can be stored in a 125 mL acid-cleaned polyethylene bottle. The digestate contains approximately

10% V/V nitric acid.

8.3 Digestates are stored at 4 °C in a triple door refrigerator in room 131A. Prior to analysis, digestates are allowed to come to room temperature.

9.0 Calculations

The absorbed power (Sec. 8.1.4) is determined as:

$$\ddot{y} = \frac{\text{[illegible]}}{\text{[illegible]}}$$

where:

P	=	Apparent power absorbed by the sample in watts (W)
K	=	Conversion factor for thermochemical calories sec^{-1} to watts (=4.184 J/cal)
C_p	=	Heat capacity, thermal capacity, or specific heat of water (cal g^{-1} C^{-1})
m	=	Mass of the solid sample in grams (g)
T	=	Final temperature minus the initial temperature (C)
t	=	Time in seconds (s).

Using the experimental conditions of 2 minutes and 1 kg of distilled water (heat capacity at 25 C is 0.9997 cal g^{-1} C^{-1}) the calibration equation simplifies to:

[illegible]

10.0 Quality Assurance/Quality Control

10.1 A preparation batch of samples would be defined as a group of up to field twenty samples of similar matrix type that are extracted at the same time using the same reagents for the same analysis. In addition to the twenty samples, each batch would also contain at a minimum a method blank, a laboratory control sample (LCS), a matrix spike (MS), and a matrix duplicate (MD). A matrix spike duplicate (MSD) is digested if required by principle investigator to meet project data quality objectives.

10.2 A method blank (MB) consisting of reagent water and shall be prepared with each batch of samples. Acceptance criteria are presented in the appropriate determinative method.

10.3 A Laboratory Control Sample (LCS) consisting of reagent water spiked with all the target metals prior to digestion, is digested and analyzed with each batch of samples. Control charts will be maintained for the LCS for all method target analytes. Acceptance criteria are specified in the appropriate

determinative method. The order of preference for spiking levels is as follows:

If an action level exists, spike at this level. If the action level is at the lowest calibration standard, the concentration of the LCS should be slightly higher.

If the above condition does not apply, spike at a level between the low and mid-level of the calibration standards.

10.4 The use of the matrix spike (and matrix spike duplicate) will depend in part on what role the laboratory is playing. When the laboratory serves the role as the primary laboratory, then site-specific documents should be consulted. The sample to be used for the MS/MSD may be specified in the field. This previously designated sample would then be spiked with the site-specific target analytes at a concentration equivalent to the site action level. If this information was not specified or unknown, then the laboratory would choose a representative sample from each batch of samples analyzed. If samples from multiple sites were to be analyzed in the same batch, then multiple sets of MS/MSDs may be required.

When the laboratory serves the role as the QA laboratory, the above scenario may not be practical to implement. If the site-specific requirements are unknown and samples from multiple sites are analyzed in the same batch, then the laboratory should select a single sample for spiking. The order of preference for spiking levels is as follows:

If the target analyte concentrations are known, spike to increase the background concentration by a factor of approximately two

If an action level exists, spike at this level

If neither of the first two conditions apply, spike at a level between the low and mid-level of the calibration standards.

10.5 The use of the matrix duplicate may also depend in part on the role the laboratory is playing. The selection of a matrix duplicate will be performed as described for the MS/MSD in Section 10.4.

10.6 The analyst must demonstrate proficiency in performing this method as outlined in SOP No. Q-016-ECC-QC, Technical Training. Method proficiency must be redemonstrated anytime a major method modification is made.

11.0 Data Validation

11.1 The digestion analyst is responsible for verifying the information recorded on the digestion log (Figure 1) is complete and accurate.

11.2 Additional levels of review are performed as described in SOP No. Q-024-EEC-DR, Data Reduction and Review/Validation (In-House/Contractor Data),

using a predesigned form as shown in Figure 2.

12.0 Waste Disposal

This procedure generates corrosive and metallic wastes. Metal digestion extracts are stored in the triple door refrigerator in room 131A. Metal digestion extracts are stored for six months. Afterwards they are either returned to the principle investigator for disposal or are disposed of by ECC. All waste disposal must comply with all Federal and local regulations. Refer to the ERDC-WES site Waste Management Plan.

13.0 References

13.1 Test Method for Evaluating Solid Waste, SW-846, Third Edition, Update II, September 1994, Method 3051.

13.2 SOP No. Q-005-ECC-SH, Sample Receipt, Login, and Storage Procedures.

13.3. SOP No. Q-012-ECC-GL, Glassware Cleaning.

13.4 SOP No. Q-008-ECC-GL, Reagent Water Generation and Quality Monitoring.

13.5 Introduction to Microwave Sample Preparation: Theory and Practice. Kingston, H.M., Jassie, L.B., Eds., ACS Professional Reference Book Series, American Chemical Society, Washington, DC, 1988. Chapters 6 and 11.

13.6 SOP No. Q-003-ECC-QC, Standards Preparation, Traceability, and Storage.

13.7 SOP No. Q-021-ECC-GL, Subsampling of Containers.

13.8 SOP No. Q-016-ECC-QC, Technical Training.

13.9 SOP No. Q-004-ECC-QC, Nonconformances and Corrective Action.

13.10 SOP No. Q-024-ECC-DR, Data Reduction and Review/Validation (In-House/Contractor Data).

13.11 SOP No. M-2216-ECC-GL, Determination of Water (Moisture) Content of Soil (ASTM Method 2216).

13.12 ERDC WES site Waste Management Plan.

Microwave Digestion Log For Metals Analysis
ERDC EL EEC

Batch No.:	SOP # _____	Matrix: ÿÿÀ ß Solid Liquid
Start Date:	Project No.:	Analysis Method: ICP, FLAA, GFAA

Type	Sample ID No.	Digestion vessel ID	Sample Amount (mL) (g)	mL Acid Added	Mass of Vessel		Filtration necessary?	Description/ Sample Comments
					before heating	after heating		
MB								
LCS								
1								
2								
3								
4								
5								
6								
7								
8								
9								
10								
11								
12								
13								
14								
15								
16								
17								
18								
19								
20								
Dup								
MS								
MSD								

LCS Spiking Standard ID: _____ Amount Added _____ mL

MS/MSD Spiking Standard ID: _____ Amount Added _____ mL

Witness: _____ Date: _____

Reagent Lot No(s): _____

_____

_____

Analyst: _____ Reviewed By: _____ Date: _____

Figure 1.

Lab Data Review Check List
Metals Digestion Log
ERDC EL EEC

Batch Number(s):
Method SOP No.:

Review Item	Yes (x)	No (x)	N/A (x)	2nd Level Review (x)
Were the following recorded properly? - batch number - sample identification number(s) - dates				
Were the weight/volume of sample digested and the final digested volume properly recorded?				
Were sample dilutions or concentration factors properly recorded?				
Was a method blank prepared at the required frequency using a blank matrix?				
Was a Laboratory Control Sample (LCS) prepared at the required frequency?				
Were a matrix spike (MS) and matrix spike duplicate (MSD) prepared at the proper frequency?				
Were the matrix duplicates (MD) prepared at the proper frequency?				
Are there any Corrective Action Reports associated with this sample batch?				
Are copies of Corrective Action Reports attached?				
Were the spiking volume, stock source, and spike concentration properly recorded for the MS/MSD and LCS?				

NOTES: _____

_____

_____

Analyst: _____ Date: _____

2nd Level Reviewer: _____ Date: _____

Figure 2.

ENVIRONMENTAL CHEMISTRY CENTER
ENVIRONMENTAL LABORATORY
ERDC

CEERD-EE-C
3909 HALLS FERRY ROAD
VICKSBURG, MS 39180

TITLE: Inductively Coupled Plasma-Atomic Emission Spectroscopy (SW-846 Method 6010B)

SOP NUMBER: M-6010B-ECC-IA, Ver. 1.0

PREPARED BY: Anne Halpenny-Weatherby 4/17/1999
Date

REVIEWED BY: Karen T Myers 3/29/99
Technical Specialist Date

Karen T Myers 3/29/99
QA Officer Date

Ann B Stroy 4/21/99
Laboratory Director Date

SOP MANUAL CONTROL NO.: _____

1.0 Scope and Application

1.1 Inductively coupled plasma-optical emission spectroscopy (ICP-OES) determines trace elements, including metals, in solution. All matrices, including groundwater, aqueous samples, TCLP and EP extracts, industrial and organic wastes, soils, sludges, sediments, and other solid wastes, require digestion prior to analysis, following SW-846 methods 3005A-3051. Groundwater samples that have been prefiltered and acidified need not be digested. Samples which are not digested must either be matrix matched with the standards or use an internal standard.

1.2 Elements for which this SOP is applicable are listed in Table 1. This list is a default list to be used in the absence of a project-specific list, which would take preference. Detection limits, sensitivity, and optimum ranges of the metals will vary with the matrices and model of spectrometer. The data shown in Table 1 provide detection wavelengths and estimated instrument detection limits for analytes in clean aqueous samples. See Table 1.

1.3 Use of this method is restricted to spectroscopists who are knowledgeable in the correction of spectral, chemical, and physical interferences.

2.0 Method Summary

2.1 Prior to analysis, samples must be digested using appropriate sample preparation methods (e.g., SW-846 Methods 3005A-3051). When analyzing for dissolved constituents, acid digestion is not necessary if the samples are filtered and acid preserved prior to analysis.

2.2 This SOP describes the simultaneous, multi-elemental determination of elements by ICP. The method measures element-emitted light by optical spectrometry.

2.2.1 Samples are nebulized and the resulting aerosol is transported to the plasma torch. Element-specific emission lines are produced in the r-f inductively coupled plasma. The emission lines are dispersed by a grating spectrometer, and the line intensities are measured by a charge-coupled device. All lines are to be monitored or interferences may be overlooked.

2.2.2 Background correction is required for trace element determination. Background signals must be measured adjacent to analyte lines on samples during analysis. The position selected for the background-intensity measurement, on either or both sides of the analytical line, will be determined by the complexity of the spectrum adjacent to the analyte line. The position used must be free of spectral interference and reflect the same change in background intensity as occurs at the analyte wavelength measured. Background photo-multiplier correction is not required in cases of line broadening where a background correction measurement would actually degrade the analytical result. The

possibility of additional interferences named in Section 5.0 should also be recognized and appropriate corrections made; tests for their presence are described in Step 11.10.

3.0 Health and Safety

3.1 Use of this procedure requires the handling of samples and standards containing corrosive acids and protective equipment must be utilized. Minimum personal protection includes the use of laboratory safety glasses, a lab coat or apron, protective gloves.

CAUTION: Be careful when diluting and mixing acids. ALWAYS pour acid into water when mixing. Gently heat acid mixtures (NEVER HEAT RAPIDLY), to prevent splatter from extremely exothermic reactions typical of acid-water mixtures, etc.

3.2 All digestions must be performed in a laboratory fume hood.

3.3 For specific information regarding the toxicity of the acids used in this procedure and other related health and safety issues including the proper storage and handling of reagents and chemicals, the analyst should consult the appropriate Material Safety Data Sheets (MSDSs). The location of the MSDSs is in Room 111, Building 3299.

3.4 Use of this procedure requires the handling of samples containing corrosive acid. Prior to performing this procedure, the analyst should be familiar with the proper use of corrosive liquid spill kits and containment procedures. The spill kits are located inside the door of Room 129, Building 3299 The use and disposal of the spill kits are by the instructions included with the kits.

4.0 Sample Preservation, Containers, Handling, and Storage

4.1 Requirements for sample preservation, sample containers and sample storage are detailed in SOP No. Q-005-ECC-SH, Sample Receipt, Login, and Storage.

4.2 All glassware must be prewashed with detergents, acid, and reagent water. Refer to SOP No. Q-012-ECC-GL, Glassware Cleaning.

4.3 Metals digestion and analysis must be completed within 180 days of sample collection.

4.4 Digestates are stored in low-density, metal-free polyethylene (LDPE) bottles or equivalent containers (e.g., centrifuge tubes) at room temperature.

5.0 Interferences and Potential Problems

5.1 Spectral interferences are caused by

- Overlap of a spectral line from another element at the analytical or background measurement wavelengths.

- Unresolved overlap of molecular band spectra.

- Background contribution from continuous or recombination phenomena.

- Stray light from the line emission of high-concentration elements.

Spectral overlap can be compensated for by computer-correcting the raw data after monitoring and measuring the interfering element. Interferent elements must be analysed at the same time as the element of interest. The appropriate Interferent element correction (IEC) table must be activated in the method. Unresolved overlap requires selection of an alternative wavelength. Stray light can usually be compensated for by a background correction adjacent to the analyte line.

Users of all ICP instruments must verify the absence of spectral interference from an element in a sample for which there is no instrument detection channel. Recommended wavelengths are listed in Table 1 and potential spectral interferences for the recommended wavelengths are given in Table 2. The data in Table 2 are intended as rudimentary guides for indicating potential interferences; for this purpose, linear relations between concentration and intensity for the analytes and the interferences can be assumed.

5.1.1 The interference is expressed as analyte concentration equivalents arising from 100 mg/L of the interference element. The interference effects must be evaluated for each individual instrument since the intensities will vary with operating conditions, power, viewing height, argon flow rate, etc. The user should be aware of the possibility of interferences other than those specified. Both positive and negative interferences may occur.

5.1.2 The dashes in Table 2 indicate that no measurable interferences were observed even at higher interferant concentrations. Generally, interferences were discernible if they produced peaks, or background shifts, corresponding to 2 to 5% of the peaks generated by the analyte concentrations.

5.1.3 At present, information on the listed silver and potassium wavelengths is not available, but it has been reported that second-order energy from the magnesium 383.231 nm wavelength interferes with the listed potassium line at 766.491 nm.

5.2 Physical interferences are effects associated with the sample nebulization and transport processes. Changes in viscosity and surface tension can cause significant inaccuracies, especially in samples containing high dissolved solids or high acid concentrations. Physical interferences are reduced by using a peristaltic pump and/or an internal standard such as Yttrium (Y). Another problem that can occur with high dissolved solids is salt buildup at the tip of the nebulizer, which affects aerosol flow rate and causes instrumental drift. The

problem can be controlled by wetting the argon prior to nebulization, using a tip washer, diluting the sample, or using a high solids tolerant nebulizer. Also, it has been reported that better control of the argon flow rate improves instrument performance; this is accomplished with the use of mass flow controllers.

5.3 Chemical interferences include molecular compound formation, ionization effects, and solute vaporization effects. Normally, these effects are not significant with the ICP technique until sodium level reaches between 4 and 500 mg/L in solutions on the standard ICP. The axial ICP can be affected by ionization at lower levels. If observed, they can be minimized by careful selection of operating conditions (incident power, observation position, and so forth), and by procedures such as using ionization modifiers such as yttrium for ICP analysis. This is important when running the axial ICP. Chemical interferences are highly dependent on matrix type and the specific analyte element.

6.0 Equipment/Apparatus

6.1 Inductively coupled plasma emission spectrometer

6.1.1 Perkin Elmer Optima 3000DV. The Optima 3000dv is a fully computer controlled, bench mounted ICP optical emission spectrometer system capable of providing high quality multielement analysis rapidly. It is a dual detector system with dual viewing capability. A data system allows interelement correction calculations, application of internal standard and simultaneous measurement of spectral background. Nebulizer argon flow rate is automatically controlled using a mass flow controler in 0.01 L/min increments. A peristaltic pump is used for sample solution.

6.1.2 40 MHz, free running RF generator with true power control, compliant with FCC regulations. Power levels can be adjusted in 5-watt increments.

6.1.3 Data System- The optima is interfaced to a Digital Celebris XL 5100 pentium 100 computer. The system allows the continuous acquistion and storage on machine readable media of all emission spectra obtained during the run of samples.

6.1.3.1 Hardware - A 1 gigabyte hard drive with data backup by a tape drive. A Digital VCR17-HA monitor (17") is used for the viewing of the data. A Hewlett Packard deskjet 1600C printer is used for the hard copy printout of the data results.

6.1.3.2 Software - Microsoft Windows 95 operating system with ICP Winlab software, version 1.47.

6.1.4 Perkin-Elmer AS-90 Auto sampler.

6.1.5 Argon gas supply — Boil-off from liquid argon. Liquid argon of 99.9996% purity is used to produce the argon gas that is needed.

NOTE: Operating conditions for the ICP and related equipment are fully outlined in the instructions provided by the instrument manufacturer. Daily operation procedures include: (1) Check tubing, (2) Visually inspect torch. (3) Ensure there is sufficient wash (3% HNO3). Plasma is ignited automatically by selecting the plasma icon and turning plasma on. For operation with organic solvents, use of an auxiliary argon inlet is recommended, as are solvent-resistant tubing, increased plasma (coolant) argon flow, decreased nebulizer flow, and increased RF power to obtain stable operation and precise measurements. Sensitivity, instrumental detection limit, precision, linear dynamic range, and interference effects must be established for each individual analyte line on that particular instrument. All measurements must be within the instrument linear range where spectral interference correction factors are valid. The analyst must (1) verify that the instrument configuration and operating conditions satisfy the analytical requirements and (2) maintain quality control data confirming instrument performance and analytical results.

6.2 Pipets — Microliter with disposable tips. Sizes can range from 5 to 1,000 µL, as required. Pipet tips used should be suitable for trace metal analysis (e.g., pyrogen-free, trace metal certified, Fisher Cat # 21-197-8K or equivalent). Rainin EDP-Plus Electronic Digital pipets with the appropriate liquid ends, ie, 25-250 µL, 100-1000 µL, 250-2500 µL, and 0.5-10.0 mL.

6.3 Volumetric Flasks — Class A, assorted sizes including 100, 200, 500, and 1000 mL.

6.4 Volumetric pipets — Class A, assorted sizes from 0.5 to 50 mL.

6.5 LDPE storage bottles, 125 and 250 mL.

7.0 Reagents

7.1 Reagent Water — Refer to SOP No. Q-008-ECC-GL, Reagent Water Generation and Quality Monitoring.

7.2 Concentrated nitric acid (HNO_3), Fisher TraceMetal grade, Cat# A-509 or equivalent. Acid must be analyzed to determine levels of impurities. If a method blank made with the acid is < MDL, the acid can be used. This should be verified with each new lot of acid.

7.3 Concentrated hydrochloric acid (HCl), Fisher TraceMetal grade, Cat# 508 or equivalent. Acid must be analyzed to determine levels of impurities. If a method blank made with the acid is < MDL, the acid can be used. This should be verified with each new lot of acid.

7.4 Individual stock solutions or mixed calibration solutions are purchased as prepared certified solutions from commercial suppliers (Alfa Aesar). Refer to

SOP No. Q-003-ECC-QC, Standards Preparation, Traceability, and Storage for requirements. Purchased stock solutions are stable for one year.

7.4.1 Primary source standards from Alfa Aesar shall be used to prepare the initial calibration curve, continuing calibration verification (CCV) standard, laboratory control sample (LCS), and matrix spikes (MS) and their duplicates (MSDs) when applicable. See Primary Source Table below.

Primary Source Table

Solution	Elements	Conc (ug/ml)	Catalog #	Acid	Ical A*	Ical B*	10 ppm Ag
Alfa Aesar solution A	Ag	200		HCl			5mls in 100mls
	Ba, Co, Cu, Fe, Mn, Ni, Pb, Tl, Zn	1000	Alfa Aesar 35417		10ml in 100 mls		
	Be	400					
	Cd	500					
Alfa Aesar solution B	Al, As, Cr, Sb,Se,V	1000		HCl		10 mls in 100 mls	
	Ca, Mg, K, Na	10,000					

* Intermediate calibration standard (ICAL)

7.4.2 Secondary source standards shall be used to verify the initial calibration (ICV) curve only. These standards shall be purchased from a different vendor, unless the primary vendor can supply different lot numbers. See ICV Table below

ICV Table

Solution	Elements	Conc (μg/ml)	Catalog #	ICV mls of soln
Quality Control Std 21	As,Be,Ca,Cd Co,Cr,Cu,Fe, Mg,Mn,Mo, Ni,Pb,Sb,Se Sr,Tl,V,Zn	100	Spex Certiprep QC-21	1ml/100ml
Quality Control 7A	Al,Ba,Na	100	Spex Certiprep QC-7A	1ml/100mls
	Ag	50		
	K	1000		

7.5 If purchasing a certified prepared standard solution is not an option, then the standard stock solutions shall be made from ultrahigh purity grade chemicals/metals material. Prepared stock solutions are stable for one year. Each stock solution shall be analyzed separately to determine possible spectral interference or the presence of impurities.

7.6 Calibration Standards — Calibration standards are prepared by combining appropriate volumes of the individual stock solutions with reagent water (mixed standards). Intermediate calibration standards are good for 6 months. Working calibration standards are to be prepared every two weeks. Silver calibration standards must be prepared daily. Care should be taken when preparing mixed standards to ensure that the elements are compatible and stable together. Standards should be monitored for stability and replaced if change is found. Some typical mixed standards are:

Mixed Standard Solutions

	Elements
I	Be, Cd, Mn, Pb, Se and Zn
II	Ba, Co, Cu, Fe, and V
III	As and Mo
IV	Al, Ca, Cr, K, Na, Ni, Li and Sr
V	Ag, Mg, Sb and Tl
VI	P

See Standards preparation table below

Standard Preparation Table

Solution	Elements	Conc. PPM	Std 1	std 2	std 3	CCV	low chk
			# mls in 100ml volumetric				
Ag 10	Ag						
Ical a	Be	20	0.1	1.00	10.00	5.00	0.025
	Cd	50					
	Cu,Ba,Co, Fe,Mn,Ni, Pb,Tl,Zn,	100					
Ical B	Ca,Mg,Na, K	1000	0.1	1.00	10.00	5.00	0.025
	As,Al,Cr, Sb,Se,V	100					

7.7 The continuing calibration verification (CCV) standard is prepared from the primary calibration stock standards by the analyst by combining compatible elements at concentrations equivalent to the midpoint of their respective calibration curves. See Standards preparation table section 7.6

7.8 The interference check standard (ICS) is prepared to contain known concentrations of interfering elements that will provide an adequate test of the correction factors. The interference check standards shall be purchased from a commercial source (e.g., Alfa Aesar Plasma Interference Check Standards, Set of 3, or equivalent). Spike the sample with the elements of interest at approximate concentrations of 10 times the instrumental detection. In the absence of measurable analyte, over-correction could go undetected because a negative value could be reported as zero. If the particular instrument will display over correction as a negative number, this spiking procedure will not be necessary.

7.9 Matrix Spike (MS) Standard -

7.10.1 The matrix spike standard shall be prepared from the calibration stock standard (primary) in Section 7.5. Post digestion spikes and filtered water matrix spikes are prepared by adding 0.05mls each of Ical A and B to 10mls of sample. Predigestion Matrix spiking procedures are dependent on the digestion procedure used or required by the project manager. For specific digestion method numbers and application see section 8.1

7.10.2 The spike should be at a level that would approximately double the concentration of the target analytes present. In the absence of target analytes, the spike would be made at the site action level, assuming that this level did not also correspond to the value of the low standard used. If the action level is the same as the low standard used, then the spiking would occur at a slightly higher level, i.e., at the value of the next calibration standard used. If the action level is not known, the spiking level would then default to a concentration between the low and mid-level calibration standards.

7.10.3 Ideally, all method analytes should be contained in the MS spike. Subsets may be used based on project specific requirements.

7.11 Laboratory Control Sample (LCS)

7.11.1 The LCS shall be prepared from the calibration stock standard primary(in Section 7.4.) 0.05 mls each of Ical A and B is added to 10 mls of reagent water. Predigestion LCS requirements are covered in the appropriate digestion

procedures. See section 7.10.1

7.11.2 The LCS shall be prepared in the appropriate matrix (reagent water); and contains all of the method target analytes. A subset of the method target analytes could be used based on the project specific requirements. The spiking level used would be at the same level as the site-specific action limit. If site-specific action limits are not available or where these levels are very high, the spiking level shall be at a concentration between the low and mid-level calibration standards.

8.0 Procedure

8.1 Sample Preparation — Refer to the appropriate SW-846 digestion method below for soil or water.

Method # 3005a, Acid digestion of Waters for Total Recoverable or Dissolved metals for analysis by FLAA ir ICP spectroscopy.

Method # 3015 Microwave Assisted Acid digestion of Aqueous Samples and Extracts.

Method # 3050b Acid digestion of Sediments Sludges and Soils.

Method # 3051 Microwave Assisted Acid digestion of Sediments, Sludges, Soils and Oils.

8.2 Set up the instrument with proper operating parameters. The instrument must be allowed to become thermally stable before beginning (usually requiring 1 hour of operation prior to calibration). Perform a mercury realignment by selecting spectrometer control under tools icon. Select mercury realign.

8.3 Initial Calibration — The calibration curve shall consist of a blank and at least three mixed standard concentrations (low, mid, high) for each element. Alternatively, a single standard and a blank may be used for initial calibration as long as verification is performed using both mid and low-level standards. Flush the system with rinsing solution (3% nitric acid solution) between each standard. (Use the average intensity of three integrations [minimum of 5 seconds each] for both calibration and sample analysis to reduce random error. The relative standard deviation of these replicate integrations should be less than 10% for the low standards and 5% for the higher standards. (Correlation coefficient $r \geq 0.995$) If not, corrective action must be performed such as remaking the standards, check pump tubing and recalibrate.

8.4 Reanalyze the highest mixed calibration standard as if it were a sample. Concentration values obtained should not deviate from the actual values by more than 5%. If they do, troubleshoot the system to correct for this condition. (1) Check solution. (2) Check calibration curve. (3) Check tubing. Continue until an acceptable run is made for this highest mixed calibration

standard.

8.5 Rinse the system with rinsing solution for at least 1 minute. Analyze the ICV — concentration values must be within 10% of known values. (If single point calibration was performed, both a mid and low-level verification shall be performed.)

8.6 Rinse the system with rinsing solution for at least 50 seconds. Analyze the ICB. ICB must be less than 2 times the MDL. If not the ICB must be reanalysed.

8.7 Rinse the system with rinsing solution for at least 1 minute. Analyze the ICS(s). Concentrations must be within ± 20% of the known values (refer to Section 11.3). If not, check background correction points and Interelement correction factors.

8.8 Sample analysis can now begin. Rinse the system with rinsing solution between samples and QC checks. If data system automates analytical run, program samples into software. If automation is not available, all runs must be recorded using a run log as shown in Figure 2. The software generates the run log similiar to example below.

Analytical Sequence

Sample	Comment
Reagent Water	Check instrument zero
Calibration Blank	Blank working standard
Standard 1 (S1)	Working standard #1 (low)
Standard 2 (S2)	Working standard #2 (mid)
Standard 3 (S3)	Working standard #3 (high)
Standard 3 (S3)	QC--must be within 5% of known value
ICV	QC check--must be within 10% of known value
ICS	Interference Check Standard(s)
Rinse	To remove any ICS memory
Samples 1-10	
CCB	Acceptance criteria in Section 11
CCV	QC check must be within 10% of known value

Repeat box until samples exhausted; samples include MD, MS/MSDs, LCS, and method blanks. Then end run with:

CCB

CCV

ICS

8.8 Continue with sample analysis, checking the calibration blank and midpoint standard (CCV) after every 10 samples. For acceptance criteria see section 11.4 and 11.5

8.9 The analytical sequence must be ended with an acceptable calibration blank, mid-point standard (CCV) analysis and interference check standard. For criteria see section 8.6 and 8.7

8.10 Each sample shall be integrated three times. The RSD between these integrations needs to be evaluated. If RSD of triplicate integrations is consistently >10% and highly variable, this indicates a possible problem with the ICP sample introduction system. Replace tubing or clean nebulizer if necessary.

8.11 If the concentration found is greater than the highest standard, the sample must be diluted with the same concentration of acids used in the standard and in the original sample digestion and reanalyzed.

9.0 Calculations

9.1 The concentration of elements (µg/L) in the samples is read from the calibration curve or directly from the instrument if operating in the direct concentration mode. Samples that required dilution are adjusted according to the following equation:

$$\mu g/L \text{ metal in sample} = \frac{A\ (C + B)}{C}$$

where:

A = sample concentration from calibration curve
B = volume (mL) of calibration blank used to dilute sample
C = volume (mL) of sample used

9.2 The concentration of elements in solid matrices is reported in mg/Kg (dry basis) of waste as follows:

$$mg/Kg \text{ metal in sample} = \frac{A \times V}{W}$$

where:

A = final concentration read from calibration curve (see Section 9.1) in µg/L
V = Final volume of processed sample (L)
W = Weight of sample digested (g) (dry basis)

9.3 RSD is calculated by the instrument and is reported as part of the run data.

9.4 The relative percent difference (RPD) between duplicate determinations must be calculated as follows:

$$RPD = \frac{|D(l) - D(2)|}{(D(1) + D(2))/2} \times 100$$

where:

RPD = relative percent difference.
D(1) = first sample value.
D(2) = second sample value (duplicate)

A control limit of 20% RPD should not be exceeded for analyte values greater than 100 times the instrumental detection limit. If this limit is exceeded, the reason for the out-of-control situation must be found and corrected, and any samples analyzed during the out-of-control condition must be reanalyzed.

10.0 Quality Assurance/Quality Control

10.1 A preparation batch of samples is defined as a group of up to twenty field samples of similar matrix type that have been prepared at the same time or time sequence with the same lots of reagents for the same analysis. In addition to the twenty samples, each preparatory batch will contain at a minimum, a method blank, a laboratory control sample, a matrix spike, (a matrix spike duplicate, when applicable) and a matrix duplicate. An analytical, or instrumental batch is defined as samples that are analyzed together within the same time period or in continuous sequential time periods. Within the analytical are included individual QC requirements as defined by the analytical (determinative) method. Preparation batches of samples may be continuously strung together in these run sequences, as long as the analytical batch QC requirements meet the acceptance criteria established within the appropriate SOP. Each analytical sequence must be documented using the run log similar to that given in Figure 2. The run log is generated by the instrument software.

10.2 Run a calibration curve on a daily basis that employs a minimum of a calibration blank and three standard concentrations. Alternatively, a single standard and a blank may be used for initial calibration as long as verification is performed using both mid and low-level standards. Before beginning the sample run, the highest mixed calibration standard shall be analyzed as if it were a sample. Acceptance criteria are presented in Section 11.1.

10.3 After the highest standard, analyze the initial calibration verification (ICV) standard(s) containing all target analytes. Acceptance criteria listed in Section 11.2.

10.4 Check the instrument calibration by analyzing interference check standards (ICS) as follows. Verify the interelement and background correction factors at the beginning and end of an analytical run or twice during every 8-hour work shift, whichever is more frequent. Acceptance criteria are presented in Section 11.3.

10.5 Check the calibration by analyzing the continuing calibration blank (CCB) and continuing calibration verification (CCV) standard after every 10 samples and at the end of and analytical run. Acceptance criteria listed are in Section 11.4.

10.6 A method blank shall accompany each sample batch to determine if contamination or any memory effects are occurring. A method blank is a volume of reagent water acidified with the same amounts of acids as were used for preparation of the standards and samples. This sample is carried through the entire digestion and analysis procedure. Acceptance criteria are presented in Section 11.5.

10.7 A laboratory control sample must be prepared and analyzed with each batch of samples. The LCS would be prepared using the primary source standard and would contain all method target analytes. Control charts will be maintained for the LCS for all target analytes. Acceptance criteria are presented in Section 11.6.

10.8 The use of the matrix spike (matrix spike duplicate, when applicable) and matrix duplicate will depend in part on what role the laboratory is playing. When the laboratory serves the role as the primary laboratory, then site-specific documents should be consulted. The sample to be used for the MS/MD or MSD may be specified in the field. This previously designated sample would then be spiked with the site-specific target analytes at a concentration equivalent to the site action level. The MS /(MSD) would be prepared using the primary source standards. If this information was not specified or unknown, then the laboratory would choose a representative sample from each batch of samples analyzed. If samples from multiple sites were to be analyzed in the same batch, then multiple sets of MS/(MSD)s may be required. When the laboratory serves the role as the QA laboratory, the above scenario may not be practical to implement. If the site-specific requirements are unknown and samples from multiple sites are analyzed in the same batch, then the laboratory should select a single sample for spiking. Each batch of samples would then contain at least one MS/MD pair. Refer to the appropriate metals preparation SOP. Acceptance criteria are presented in Section 11.7 for percent recovery and RPD (when applicable).

10.9 The use of the matrix duplicate may also depend in part on the role the laboratory is playing. The selection of a matrix duplicate will be performed as described for the MS/(MSD) in Section 10.8. Acceptance criteria are presented in Section 11.8 for RPD.

10.10 All sample analytical results used for final data reporting must be above the MDL and below the high standard of the calibration curve. Corrective actions are described in Section 11.9.

10.11 Whenever a new or unusual sample matrix is encountered, a series of tests shall be performed prior to reporting concentration data for analyte elements. These tests, as outlined in 11.10.1 and 11.10.2, will ensure the analyst that neither positive nor negative interferences are operating on any of the analyte elements to distort the accuracy of the reported values.

10.12 Data shall be checked to ascertain if it conforms to accepted practices for reporting of results at or near the MDL. The reporting limits which are required are (listed in order of preference); 1) project-specific requirements, or 2) the lowest standard of the standard curve. Results between the MDL and the low standard shall be reported as estimates.

10.13 MDLs are determined in reagent water and verified on a project-specific schedule or annually if no project-specific requirements are in place. Refer to SOP No. Q-019-ECC-QC, Method Detection Limits (MDLs), Method Quantitation Limits (MQLs), and Laboratory Reporting Limits (LRLs).

10.14 The analyst must demonstrate proficiency in performing the analysis as outlined in SOP No. Q-016-ECC-QC, Technical Training. Method proficiency must be redemonstrated anytime a major method modification is made, a major software revision is added, or a major instrument modification is made. Demonstration of method proficiency may also be required after major instrument maintenance. This is decided on a case by case basis through discussions with the Section Chief, Laboratory Director, and Laboratory QA Officer.

11.0 Data Validation

11.1 After running the calibration standards, the highest standard is to be analyzed as a sample, prior to actual sample analysis. Concentration values obtained should not deviate from the actual values by more than 5%. If they do, troubleshoot the system to correct for this condition. The following procedures are suggested:

- Check calibration standards and recalibrate

- Perform routine maintenance such as checking the tubing, nebulizer or torch.

-Recalibrate and reanalyze.

Continue until an acceptable run is made for this highest mixed calibration standard.

11.2 The results of the ICV for all analytes are to be within 10% of known values. If this criteria is not met, recalibrate and reanalyze, using the same standards. If acceptance criteria are still not met. Check ICV preparation. See section 11.1 for other protocol. If still not acceptable, refer to

manufacturers's instructions or call service representative.

11.3 The analyst shall verify that the ICS has been analyzed at the required frequency. The prepared ICS is to be run at the beginning and end of each analytical run, or at the beginning and end of an 8 hour shift, whichever occurs more often. Results should be within ± 20% of the true value for all target analytes within the prepared ICS sample. If this criteria is not met, check the background correction protocols (IEC factors) currently in place for appropriateness and recalculate if needed. If this is the initial ICS run after daily calibration, recalibrate and reanalyze. If the ICS did not check at the end of an 8 hour shift, readjust background correction factors, and if needed, reanalyze any samples in the previous run that may have been affected.

11.4 Continuing Calibration

11.4.1 The results of the continuing calibration verification (CCV) check standard shall agree to within 10% of the expected value. If not, correct the problem, and reanalyze the previous ten samples since the last acceptable CCV.

11.4.2 The results of the calibration blank are to be less than the MDL. If the calibration blank is not less than the MDL, terminate the analyses, correct the problem, recalibrate, and reanalyze the samples analyzed since the last acceptable calibration blank. If the blank is less than 10% of the action level of interest and no sample is within 10% of the action limit, analyses need not be rerun and recalibration need not be performed before continuation of the run.

11.5 Assess the method blanks. The analyst shall confirm that this blank was analyzed at the required frequency.

The method blank should not exhibit any contamination of any analyte above the MDL for any of the method target analytes. Corrective action should be performed any time method target analytes are detected above the MDL to reduce and control contamination. Corrective action will be required if site-specific target analytes are detected at greater than 5% of the regulatory limit for that analyte or if the concentration in the blank is greater than 5% of that in the sample. The first step of corrective action is to assess the effect on the samples. Corrective action would include reanalysis of field and QC samples in the batch if some or all of the samples also contained levels of target analytes that exceeded the above criteria. If none of the field samples had values above the stated criteria, then reanalysis may not be necessary. The source of contamination should still be investigated and reduced/eliminated. Any time contamination is noted in the method blank, the situation and impact on the data should be discussed in the case narrative.

11.6 Verify that the LCSs were prepared at the required frequency. Plot the target analytes on appropriate control charts. (Refer to SOP No. Q-009-ECC-QC, Control Chart Generation, Maintenance, and Usage.) If all target analytes are

not within control limits, reanalyze the LCS digestate. If still unacceptable, redigest and reanalyze a new LCS and all associated samples. If the LCS is still unacceptable, then the entire procedure must be systematically investigated to locate the source of error.

Note: Even though control charts must be maintained for the LCSs, the acceptance criteria derived from these charts may not be appropriate if the charted ranges are too wide. A maximum default range should be used. It is recommended that the maximum default range be set to 80-120%. Lab control ranges are listed in Appendix A.

The RPD of interbatch LCSs should fall within the control limits determined from the precision control charts (Lab control ranges are being developed. Default ranges are used at present.) However, if the RPD is outside these control limits, the batch will not be rejected, as long as the LCS recovery is acceptable. This precision information should be evaluated to see if systematic problems can be identified. If problems are suspected, the method should be fully evaluated.

11.7 Verify that matrix spike/(matrix spike duplicates if appropriate) were analyzed at required frequency. The analyst shall also verify that the samples were spiked at the appropriate level. The order of preference for spiking levels is as follows; 1) If the target analyte concentrations are known, spike to increase the background concentration by a factor of approximately two, 2) if an action level exists, spike at this level, or 3) if neither of the first two conditions apply, spike at a level that corresponds between the low and mid-level calibration standards. Acceptance criteria are that all % Recovery and/or RPD results meet project established goals. If no project goals are specified, then results must be within the indicated control limits on the appropriate LCS control charts. See Appendix A for lab LCS %recovery ranges. RPD ranges are the default ± 25 % until lab ranges completed. If these conditions are not met, perform the following corrective actions as appropriate.

- If both LCS and MS recoveries and/or precision are unacceptable (+/_ 25%), then the entire batch of field and QC samples must be redigested and reanalyzed.

- If the MS recovery is unacceptable recovery, but the LCS is acceptable, then a potential matrix effect has been identified. Reanalyze the MS digestates to verify a matrix effect. If a matrix effect is still suspected, then the project manager must be contacted to discuss further alternatives and the potential impact on the project. These further alternatives may include redigestion/reanalysis.

11.8 Verify matrix duplicates were analyzed at required frequency (one per digestion run). Acceptance criteria are that all RPD results meet project established goals. If no project goals are specified, then results must be within the indicated control limits on the appropriate LCS precision control charts. The acceptance criteria derived from these charts may not be

appropriate if the charted ranges are too wide. A maximum default range of 25% RPD should be used. (Lab RPD ranges are being reviewed If these conditions are not met, perform the following corrective actions as appropriate.

- Reanalyze the sample and duplicate digestates.

- If the duplicate precision is unacceptable, then a potential matrix effect has been identified. The project manager must be contacted to discuss further alternatives and the potential impact on the project. These further alternatives may include redigestion/reanalysis.

11.9 The analyst must verify all reported results are derived from analytical results that are above the MDL or below the highest calibration standard. Verify the results are reported as follows:

- Sample concentrations that have been analyzed using the digestate in its most concentrated form, and are below the laboratory reporting limit (LRL), should report the result as less than the LRL.

- For sample results (again using the digestate in its most concentrated form) that are above the MDL but below the LRL, results need to be flagged as estimates (J values).

- For samples that exceed the calibration curve, dilute and analyze an appropriate sample aliquot.

11.10 For each new matrix, ensure that the following series of tests were performed and meet the listed acceptance criteria. If criteria are not met, the method of standard additions (MSA) should be considered. The project manager should be consulted.

11.10.1 Serial dilution

If the analyte concentration is sufficiently high (minimally, a factor of 10 above the instrumental detection limit after dilution), an analysis of a 1:4 dilution should agree within ± 10% of the original determination. If not, a chemical or physical interference effect should be suspected.

11.10.2 Post-digestion spike addition

An analyte spike added to a portion of a prepared sample, or its dilution, should be recovered to within 75% to 125% of the known value. The spike addition should produce a minimum level of 10 times and a maximum of 100 times the instrumental detection limit. If the spike is not recovered within the specified limits, a matrix effect should be suspected.

CAUTION: If spectral overlap is suspected,use of computerized compensation (Interelement Correction), an alternate

wavelength, or comparison with an alternate method (Data is compared with ICP/MS and HGA data) is recommended.

11.11 Besides the items listed in Sections 11.1 through 11.10, the analyst should also verify the additional items as noted in Figure 1.

11.12 Additional levels of review are performed as described in SOP No. Q-024-ECC-DR, Data Reduction and Review/Validation (In-House/Contractor Data), using a predesigned form as shown in Figure 1.

12.0 Waste Disposal

This procedure generates acidic wastes. All wastes should be disposed of according to local regulatory as well as laboratory established guidelines. Refer to the ERDC WES site Waste Management Plan.

13.0 References

13.1 Method 6010A, Test Methods for the Analysis of Solid Waste, Physical/Chemical Methods, Update II, Third Edition, July 1992.

13.2 Methods 3005A - 3051, Test Methods for the Analysis of Solid Waste, Physical/Chemical Methods, Update II, Third Edition, July 1992.

13.3 SOP No. Q-005-ECC-SH, Sample Receipt, Login, and Storage.

13.4 SOP No. Q-012-ECC-GL, Glassware Cleaning.

13.5 SOP No. Q-008-ECC-GL, Reagent Water Generation and Quality Monitoring.

13.6 SOP No. Q-009-ECC-QC, Control Chart Generation, Maintenance, and Usage

13.7 SOP No. Q-016-ECC-QC, Technical Training.

13.8 SOP No. Q-019-ECC-QC, Method Detection Limits (MDLs), Method Quantitation Limits (MQLs), and Laboratory Reporting Limits (LRLs).

13.9 SOP No. Q-024-ECC-DR, Data Reduction and Review/Validation (In-House/Contractor Data).

13.10 ERDC WES site Waste Management Plan.

Table 1
Recommended Wavelengths and Estimated Method Detection Limits.

Analyte	CAS #	Wavelength (nm)[a]	Estimated Element Detection Limit (µg/L)[b]	Method detection Limits. (ug/L)
Aluminum	7429-90-5	308.215	5	25
Antimony	7440-36-0	206.833	10	50
Arsenic	7440-38-2	188.979	3	20
Barium	7440-39-3	317.933	.5	1
Beryllium	7440-41-7	313.042	0.2	1
Cadmium	7440-43-9	226.052	0.2	5
Calcium	7440-70-2	317.933	20	200
Chromium	7440-47-3	267.716	0.3	2
Cobalt	7440-48-4	228.616	0.5	1
Copper	7440-50-8	324.754	0.8	5
Iron	7439-89-6	238.204	0.1	20
Lead	7439-92-1	220.353	2	10
Lithium	7439-93-2	670.784	5	5
Magnesium	7439-95-4	279.079	10	200
Manganese	7439-96-5	257.610	0.5	1
Molybdenum	7439-98-7	202.030	0.6	2
Nickel	7440-02-0	231.604	0.6	5
Phosphorous	7723-14-0	213.618	51	51
Potassium	7440-09-7	766.491	200(c)	200(c)
Selenium	7782-49-2	196.026	2	50
Silver	7440-22-4	328.068	1	5
Sodium	7440-23-5	588.995	20	200
Strontium	7440-24-6	407.771	0.03	0.3
Thallium	7440-28-0	190.864	7	50
Vanadium	7440-62-2	292.402	0.3	1
Zinc	7440-66-6	206.200	1	10

(a) The wavelengths listed are recommended because of their sensitivity and overall

acceptance.
These wavelengths are also based on Perkin Elmers reccommendations.

(b) The estimated instrumental detection limits shown are taken from Reference 1 in Section 13. They are given as a guide for an instrumental limit. The actual method detection limits are sample dependent and may vary as the sample matrix varies. Detection limits are based on axial detection.

(c) Highly dependent on operating conditions and plasma position.

Table 2
Analyte Concentration Equivalents Arising From Interference at the 100 mg/L Level.

Analyte	Wavelength (nm)	Interferant (a,b,c)									
		Al	Ca	Cr	Cu	Fe	Mg	Mn	Ni	Tl	V
Al	308.215	--	--	--	--	--	--	0.21	--	--	1.4
Sb	206.833	0.47	--	2.9	--	0.08	--	--	--	0.25	0.45
As	193.696	1.3	--	0.44	--	--	--	--	--	--	1.1
Ba	455.403	--	--	--	--	--	--	--	--	--	--
Be	313.042	--	--	--	--	--	--	--	--	0.04	0.05
Cd	226.052	--	--	--	--	0.03	--	--	0.02	--	--
Ca	317.933	--	--	0.08	--	0.01	0.01	0.04	--	0.03	0.03
Cr	267.716	--	--	--	--	0.003	--	0.04	--	--	0.04
Co	228.616	--	--	0.03	--	0.005	--	--	0.03	0.15	--
Cu	324.754	--	--	--	--	0.003	--	--	--	0.05	0.02
Fe	259.940	--	--	--	--	--	--	0.12	--	--	--
Pb	220.353	0.17	--	--	--	--	--	--	--	--	--
Mg	279.079	--	0.02	0.11	--	0.13	--	0.25	--	0.07	0.12
Mn	257.610	0.005	--	0.01	--	0.002	0.002	--	--	--	--
Md	202.030	0.05	--	--	--	0.03	--	--	--	--	--
Ni	231.604	--	--	--	--	--	--	--	--	--	--
Se	196.026	0.23	--	--	--	0.09	--	--	--	--	--
Na	588.995	--	--	--	--	--	--	--	--	0.08	--
Tl	190.864	0.30	--	--	--	--	--	--	--	--	--
V	292.402	--	--	0.05	--	0.005	--	--	--	0.02	--
Zn	213.856	--	--	--	0.14	--	--	--	0.29	--	--

(a) Dashes indicate that no interference was observed in the presence of the following interferants:

Al, Ca, Fe, and Mg at 1000 mg/L, and Cr, Cu, Mn, Tl, and V at 200 mg/L.

(b) The figures recorded as analyte concentrations are not the actual observed concentrations; to obtain those figures, add the listed concentration to the interferant figure.

(c) Other lines can be used if they can provide the needed sensitivity and are treated with the same corrective techniques for spectral interferences.

NOTE: Negative concentration equivalents can arise from some interferants.

Data Review Check List
ICP Metals
ERDC EL ECC

Project Number(s)	
Batch Number(s)	
SOP No.	

Review Item	Yes (x)	No (x)	N/A (x)	2nd Level Review (x)
1. Does the daily standard curve consist of a Calibration Blank and the required minimum number of calibration standards?				
2. Is the low standard near, but above, the MDL?				
3. Is the highest initial calibration standard reanalyzed immediately after calibration and results within QC limits?				
4. Are the CCV standards analyzed at required frequency and at the end of the analytical sequence and all parameters within QC limits?				
5. Are the CCB standards analyzed at required frequency and at the end of the analytical sequence and all parameters within QC limits?				
6. Are all sample holding times met?				
7. Are all samples with concentrations > the highest standard used for initial calibration diluted and reanalyzed?				
8. Is the method blank run at the desired frequency and is its concentration for target analytes less than the LRLs?				
9. Is the ICV from a second source and is its percent recovery within QC limits?				
10. Is the Matrix Spike/Matrix Spike Duplicate run at the desired frequency and is the percent recovery/RPD within QC limits?				
11. Is the Matrix Duplicate run at the desired frequency and is the RPD within QC limits?				
12. Is a Serial Dilution analysis performed at the desired frequency and within QC limits?				
13. Are post-digestion spikes analyzed at the desired frequency and within QC limits?				
14. Are Interference check standards analyzed at the beginning and end of analytical run or at minimum frequencies and within QC limits?				

Figure 1.

Data Review Check List
ICP Metals
ERDC EL ECC

Review Item	Yes (x)	No (x)	N/A (x)	2nd Level Review (x)
15. Are all nonconformances include and noted?				
16. Is the correct methodology used for sample prep and analysis?				
17. Are all calculations checked at the minimum frequency?				
18. Did analyst sign/date the appropriate printouts and report sheets?				
19. Are all sample ID and units checked for transcription errors?				

Comments on any "No" response:

_____

_____

_____

_____

_____

_____

_____

Analyst: _____ Date: _____

2nd Level Reviewer: _____ Date: _____

Figure 1.(cont.).

ICP Run Log
ERDC EL EEC

Instrument ID:		
Date:		SOP No.

No.	Lab ID	Dil. Factor	Conc. Factor	Conc. from Instrument	Final Conc.
1					
2					
3					
4					
5					
6					
7					
8					
9					
10					
11					
12					
13					
14					
15					
16					
17					
18					
19					
20					
21					
22					

Notes: _____

Figure 2.

ENVIRONMENTAL CHEMISTRY CENTER
ENVIRONMENTAL LABORATORY
ERDC

CEERD-EE-C
3909 HALLS FERRY ROAD
VICKSBURG, MS 39180

TITLE: Inductively Coupled Plasma - Mass Spectrometry (SW-846 Method 6020)

SOP NUMBER: M-6020-ECC-IA, Ver. 1.0

PREPARED BY: _____ 6/30/99
Date

REVIEWED BY: _____ 6/30/99
Technical Specialist Date

_____ 7/8/99
QA Officer Date

_____ 7/16/99
Laboratory Director Date

SOP MANUAL CONTROL NO.: _____

1.0 Scope and Application

1.1 Inductively coupled plasma-mass spectrometry (ICP-MS) is applicable to the determination of sub-ug/L concentrations of a large number of elements in water samples and in waste extracts or digests [1.2]. When dissolved constituents are required, samples must be filtered and acid-preserved prior to analysis. No digestion is required prior to analysis for dissolved elements in water samples. Acid-digestion prior to filtration and analysis is required for groundwater, aqueous samples, industrial wastes, soils, sludges, sediments, and other solid wastes for which total (acid-leachable) elements are required. All matrices, including groundwater, aqueous samples, industrial and organic wastes, soils, sludges, sediments, and other solid wastes, require digestion prior to analysis, following SW-846 methods 3005A-3051.

1.2 ICP-MS has been applied to the determination of over 60 elements in various matrices. Analytes for which EPA has demonstrated the acceptability of Method 6020 in a multi-laboratory study on solid wastes are listed in Table 1. Acceptability of the method for an element was based upon the multi-laboratory performance compared with that of either furnace atomic absorption spectroscopy or inductively coupled plasma - atomic emission spectroscopy. It should be noted that multi-laboratory study was conducted in 1986. Instrument detection limits, sensitivities, and linear ranges will vary with the matrices, instrumentation, and operating conditions. In relatively simple matrices, detection limits will generally be below 0.02 ug/L. [This list is a default list to be used in the absence of a project-specific list, which would take preference. Detection limits, sensitivity, and optimum ranges of the metals will vary with the matrices and model of ICPMS. The data shown in Table 1 provide instrument detection limits for analytes in clean aqueous samples.]

1.3 If Method 6020 is used to determine any analyte not listed in Table 1, it is the responsibility of the analyst to demonstrate the accuracy and precision of the Method in the matrix to be analyzed. The analyst is always required to monitor potential sources of interferences and take appropriate action to ensure data of known quality (see Section 10.4).

1.4 Use of this method is restricted to spectroscopists who are knowledgeable in the recognition and the correction of mass, chemical, and physical interferences in ICP-MS.

1.4.1 ICP-MS Analyst. Qualifications for these individuals should be at a minimum of a bachelor's degree in chemistry or any related scientific/engineering discipline with specialized training in ICP Mass Spectrometry. The experience should be a minimum of two years of applied experience with ICP-MS analysis of environmental samples.

1.4.2 ICP-MS Operator Qualifications for these individuals should be at a minimum of a bachelor's degree in chemistry or any related scientific/engineering discipline with 1 year of experience in operating and maintaining ICP instrumentation.

1.5 An appropriate internal standard is required for each analyte determined by ICP-MS. Recommended internal standards are 6Li, 45Sc, 72Ge, 89Y, 103Rh, 115In, 159Tb, 165Ho, and 209Bi. The lithium internal standard should have an enriched abundance of 6Li, so that interference from lithium native to the sample is minimized. Other elements may need to be used as internal standards when samples contain significant native amounts of the recommended internal standard elements.

2.0 Method Summary

2.1 Prior to analysis, samples must be digested using appropriate sample preparation methods (e.g., SW-846 Methods 3015, 3050B, 3051, 3052). When analyzing for dissolved constituents, acid digestion is not necessary if the samples are filtered and acid preserved prior to analysis.

2.2 Method 6020 describes the multi-elemental determination of analytes by ICP-MS. The method measures ions produced by a radio-frequency inductively coupled plasma. Analyte species originating in a liquid are nebulized and the resulting aerosol transported by argon gas into the plasma torch. The ions produced are entrained in the plasma gas and introduced, by means of an interface, into a mass spectrometer. The ions produced in the plasma are sorted according to their mass-to-charge ratios and quantified with a channel electron multiplier. Interferences must be assessed and valid corrections applied or the data flagged to indicate problems. Interference correction must include compensation for background ions contributed by the plasma gas, reagents, and constituents of the sample matrix.

3.0 Health and Safety

3.1 Use of this procedure requires the handling of samples and standards containing corrosive acids and protective equipment must be utilized. Minimum personal protection includes the use of laboratory safety glasses, a lab coat or apron, protective gloves.

CAUTION: Be careful when diluting and mixing acids. ALWAYS pour acid into water when mixing. Gently heat acid mixtures (NEVER HEAT RAPIDLY), to prevent splatter from extremely exothermic reactions typical of acid-water mixtures, etc.

3.2 All digestions must be performed in a laboratory fume hood.

3.3 For specific information regarding the toxicity of the acids used in this procedure and other related health and safety issues including the proper storage and handling of reagents and chemicals, the analyst should consult the appropriate Material Safety Data Sheets (MSDS). The location of the MSDS is in Room 111, Building 3299.

3.4 Use of this procedure requires the handling of samples containing corrosive acid. Prior to performing this procedure, the analyst should be familiar with the proper use of corrosive liquid spill kits and containment procedures. The spill kits are located in Room 126, Building 3299. The use and disposal of the spill kits are by the instructions included with the kits.

4.0 Sample Preservation, Containers, Handling, and Storage

4.1 Requirements for sample preservation, sample containers and sample storage are detailed in SOP No. Q-005- ECC-SH, Sample Receipt, Login, and Storage.

4.2 All glassware must be prewashed with detergents, acid, and reagent water. Refer to SOP

No. Q-012- ECC-GL, Glassware Cleaning.

4.3 Metals digestion and analysis must be completed within 180 days of sample collection.

4.4 Digests are stored in low-density, metal-free polyethylene (LDPE) bottles or equivalent containers (e.g., centrifuge tubes) at room temperature.

4.5 Only polyethylene or fluorocarbon (TFE, PFA, or FEP) containers are recommended for use in Method 6020.

5.0 Interferences and Potential Problems

5.1 Isobaric elemental interferences in ICP-MS are caused by isotopes of different elements forming atomic ions with the same nominal mass-to-charge ratio (m/z). A data system must be used to correct for these interferences. This involves determining the signal for another isotope of the interfering element and subtracting the appropriate signal from the analyte isotope signal. Since commercial ICP-MS instruments nominally provide unit resolution at 10% of the peak height, very high ion currents at adjacent masses can also contribute to ion signals at the mass of interest. Although this type of interference is uncommon, it is not easily corrected, and samples exhibiting a significant problem of this type could require resolution improvement, matrix separation, analysis using another verified and documented isotope or use of another method.

5.2 Isobaric molecular and doubly-charged ion interferences in ICP-MS are caused by ions consisting of more than one atom or charge, respectively. Most isobaric interferences that could affect ICP-MS determinations have been identified in the literature [13.13,13.14]. Examples including ArCl(+) ions on the 75As signal and MoO(+) on the cadmium isotopes. While the approach used to correct for molecular isobaric interferences is demonstrated below using the natural isotope abundances from the literature [13.15], the most precise coefficients for an instrument can be determined from the ratio of the net isotope signals observed for a standard solution at a concentration providing suitable (<1 percent) counting statistics. Because the 35Cl natural abundance of 75.77 percent is 3.13 times the 37Cl abundance of 31.23 percent, the chloride correction for arsenic can be calculated (approximately) as follows (where the 38Ar37Cl(+) contribution at m/z 75 is a negligible 0.06 percent of the 40Ar35Cl(+) signal):

corrected arsenic signal (using natural isotopes abundances for coefficient approximations) =

(m/z 75 signal) - (3.13) (m/z 77 signal) + (2.73) (m/z 82 signal), (where the final term adjusts for any selenium contribution at 77 m/z),

NOTE: Arsenic values can be biased high by this type of equation when the net signal at m/z 82 is caused by ions other than 82Se (+)), (e.g., 81BrH(+) from bromine wastes [13.16]).

Similarly,

corrected cadmium signal (using natural isotopes abundances for coefficient approximations) = (m/z 114 signal) - (0.027)(m/z 118 signal) - (1.63)(m/z 108 signal), (where last 2 terms adjust for any tin or MoO(+) contributions at m/z 114).

NOTE: Cadmium values will be biased low by this type of equation when 92Zr0(+) ions contribute at m/z 108, but use of m/z 111 for Cd is even subject to direct (94ZrOH(+) and indirect (90ZrO(+)) additive interferences when Zr is present.

NOTE: As for the arsenic equation above, the coefficients in the Cd equation are ONLY illustrative. The most appropriate coefficients for an instrument can be determined from the ratio of the net isotope signals observed for a standard solution at a concentration providing suitable (<1 percent) counting precision.

The accuracy of these types of equations is based upon the constancy of the OBSERVED isotopic ratios for the interfering species. Corrections that presume a constant fraction of a molecular ion relative to the "parent" ion have not been found [13.17] to be reliable, e.g., oxide levels can vary. If a correction for an oxide ion is based upon the ratio of parent-to-oxide ion intensities, the correction must be adjusted for the degree of oxide formation by the use of an appropriate oxide internal standard previously demonstrated to form a similar level of oxide as the interferents. This type of correction has been reported [13.17] for oxide-ion corrections using ThO(+)/Th(+) for the determination of rare earth elements. The use of aerosol desolvation and/or mixed plasmas have been shown to greatly reduce molecular interferences [13.18]. These techniques can be used provided that method detection limits, accuracy, and precision requirements for analysis of the samples can be met.

5.3 Physical interferences are associated with the sample nebulization and transport processes as well as with ion-transmission efficiencies. Nebulization and transport processes can be affected if a matrix component causes a change in surface tension or viscosity. Changes in matrix composition can cause significant signal suppression or enhancement [13.19]. Dissolved solids can deposit on the nebulizer tip of a pneumatic nebulizer and on the interface skimmers (reducing the orifice size and the instrument performance). Total solid levels below 0.2% (2,000 mg/L) have been recommended [13.20] to minimize solid deposition. An internal standard can be used to correct for physical interferences, if it is carefully matched to the analyte so that the two elements are similarly affected by matrix changes [13.21]. When the intensity level of an internal standard is less than 70 percent or greater than 120 percent of the intensity of the first standard used during calibration, the sample must be reanalyzed after a fivefold (1+4) dilution has been performed.

5.4 Memory interferences can occur when there are large concentration differences between samples or standards which are analyzed sequentially. Sample deposition on the sampler and skimmer cones, spray chamber design, and the type of nebulizer affect the extent of the memory interferences which are observed. The rinse period between samples must be long enough to eliminate significant memory interference.

6.0 Equipment/Apparatus

6.1 Inductively coupled plasma-mass spectrometer:

6.1.1 Perkin Elmer SCIEX ELAN 6000 - The ELAN 6000 is a fully computer controlled, bench mounted ICP Mass Spectrometer system capable of providing resolution, better than or equal to 0.9 amu at 10% peak height with a mass range from 6 to 240 amu and a data system that allows corrections for isobaric interferences and the application of the internal standard technique. It has use of a mass-flow controller for the nebulizer argon and a peristaltic pump for the sample solution.

6.1.2 40-MHZ free-running Radio frequency generator compliant with FCC regulations.

6.1.3 Data System - The ELAN 6000 is interfaced to a Digital Celebris XL5100 Pentium 100 computer. The system allows the continuous acquisition and storage on machine-readable media of all mass spectra obtained throughout the duration of a run of samples.

6.1.3.1 Hardware - Both a 1 gigabyte and a 2 gigabyte hard drive with data backup by a tape drive. A Digital VRC21-HA monitor (21") is used for the viewing of the data. An HP LaserJet 4 Plus printer is used for the hard copy printout of the data results.

6.1.3.2 Software - Microsoft Windows NT Workstation Version 4.0 with Perkin-Elmer ELAN operating software 018051 Version 2.2. PE NELSON Turbochrom Workstation 79WS-0005 Version 6.1.0 is used to calculate the data obtained when using the DIONX IC for speciation.

6.1.4 Perkin-Elmer AS-90 Autosampler

6.1.5 Flow Injection Atomic Spectroscopy - The Perkin-Elmer FIAS 400MS provides sample preconcentration and matrix removal using on-line column techniques. hydride generation and amalgamation , all integrated with an automatic sampling system.

6.1.6 Electrothermal Vaporization (ETV) - Both Perkin-Elmer HGA-600MS and its dedicated autosampler, the AS-60, are fully controlled by the ELAN 6000 software.

6.1.7 Liquid Chomatograph - The DIONX DX300 IC is used for the speciation of metals species.

6.1.8 Argon gas supply — Boil-off from liquid argon. Liquid argon of 99.9996% purity is used to produce the argon gas that is needed.

NOTE: Operating conditions for the ICPMS and related equipment are fully outlined in the instructions provided by the instrument manufacturer. Sensitivity, instrumental detection limit, precision, and interference-effects must be established for each individual analyte mass on that particular instrument. The analyst must (1) verify that the instrument configuration and operating conditions satisfy the analytical requirements and (2) maintain quality control data confirming instrument performance and analytical results.

6.2 Pipets — Micro liter with disposable tips. Sizes can range from 5 to 10,000 µL, as required.

Pipet tips used should be suitable for trace metal analysis (e.g., pyrogen-free, trace metal certified, Fisher Cat # 21-197-8K or equivalent). Rainin EDP-Plus Electronic Digital pipets with the appropriate liquid ends, i.e., 25-250 µL, 100-1000 µL, 250-2500 µL, and 0.5-10.0 mL.

6.3 Volumetric Flasks — Class A, glass or polymethylpentane (PMP), 100 mL.

6.4 Volumetric pipets — Class A, assorted sizes from 0.5 to 50 mL.

6.5 FEP. fluorocarbon storage bottles, 125, 250, 500, and 1000 mL.

7.0 Reagents

7.1 Reagent Water — Refer to SOP No. Q-008-ECC-GL, Reagent Water Generation and Quality Monitoring.

7.2 Acids used in the preparation of standards and for sample processing must be of high purity. Redistilled acids are recommended because of the high sensitivity of ICP-MS. Nitric acid at less than 2 per cent (v/v) is required for ICP-MS to minimize damage to the interface and to minimize isobaric molecular-ion interferences with the analytes. Many more molecular-ion interferences are observed on the analytes when hydrochloric and sulfuric acids are used [13.13,13.14]. Concentrations of antimony and silver above 50-500 ug/L require 1% (v/v) HCl for stability; for concentrations above 500 ug/L Ag, additional HCl will be needed. Concentrated nitric acid (HNO_3), Fisher TraceMetal grade, Cat# A-509 or equivalent. Acid must be analyzed to determine levels of impurities. If a method blank made with the acid is < MDL (Section 10.21), the acid can be used. This should be verified with each new lot of acid. Nitric Acid used for preparation of standards is GFS Chemicals Item 621 Double Distilled HNO3 in Teflon bottles.

7.3 Concentrated hydrochloric acid (HCl), Fisher TraceMetal grade, Cat# 508 or equivalent. Acid must be analyzed to determine levels of impurities. If a method blank made with the acid is < MDL (Section 10.21), the acid can be used. This should be verified with each new lot of acid.

7.4 Individual stock solutions or mixed calibration solutions are to be purchased as prepared certified solutions from commercial suppliers (SPEX Claritas PPT grade or equivalent). Refer to SOP No. Q-003-ECC-QC, Standards Preparation, Traceability, and Storage for requirements. Purchased stock solutions are stable for one year.

7.4.1 Primary source standards shall be used to prepare the initial calibration curve, continuing calibration verification (CCV, QC3) standard, laboratory control sample (LCS), and matrix spikes and their duplicates (MS/MSD).

7.4.2 Secondary source standards shall be used to verify the initial calibration (ICV, QC1) curve only. These standards shall be purchased from a different vendor, unless the primary vendor can supply different lot numbers.

7.5 If purchasing a certified prepared standard solution is not an option, then the standard stock solutions shall be made from ultrahigh purity grade chemicals/metals material (99.99 or

greater purity). Prepared stock solutions are stable for one year. See Method SW846-6010A, Section 5.3, and 6020, Section 5.3, for instructions on preparing standard solutions from solids. Each stock solution shall be analyzed separately to determine the presence of impurities.

7.6 Calibration Standards —Mixed calibration standard solutions are prepared by diluting the stock-standard solutions to levels in the linear range for the instrument in a solvent consisting of 1 percent (v/v) HNO3 in reagent water. The calibration standard solutions must contain a suitable concentration of an appropriate internal standard for each analyte. Internal standards may be added on-line at the time of analysis using a second channel of the peristaltic pump and an appropriate mixing manifold. Generally, an internal standard should be no more than 50 amu removed from the analyte. Recommended internal standards include 6Li, 45Sc. 72Ge, 89Y, 103Rh, 115In, 159Tb, 169Ho, and 209Bi. Prior to preparing the mixed standards, each stock solution must be analyzed separately to determine possible spectral interferences or the presence of impurities. Care must be taken when preparing the mixed standards that the elements are compatible and stable. Transfer the mixed standard solutions to freshly acid-cleaned FEP fluorocarbon bottles for storage. Fresh mixed standards must be prepared as needed with the realization that concentrations can change on aging. Calibration standards must be initially verified using a quality control sample (see Section 7.9) and monitored weekly for stability. All working stock solutions and calibration standards are entered the ICPMS Standards Log Book.

7.6.1 The Stock solutions used for the calibration standards are: (Stock Standard 1) SPEX Instrument Calibration Standard 2, CL-CAL-2 (100 mg/L of Ag, Al, As, Ba, Be, Ca, Cd, Co, Cr, Cu, Fe, K, Mg, Mn, Mo, Na, Ni, Pb, Sb, Se, Sn, Sr, Ti, Tl, V, Zn) and (Stock Standard 2) SPEX Instrument Check Standard 3, CL-ICS-3 (200 mg/L of Ca, Fe, K, Mg, Na).

7.6.1.1 A working stock standard (Working Stock 1) of 10 mg/L is made from SPEX CL-CAL-2 (Stock Standard 1). Add 10 mL of CL-CAL-2 to 100 mL PMP Volumetric flask containing 70 mL of reagent water and 1 mL of HNO3. Dilute to 100 mL with reagent water.

7.6.1.2 An intermediate stock standard (Intermediate Stock 1) of 0.1 mg/L is made from the 10 mg/L SPEX CL-CAL-2 working stock standard. Add 0.1 mL of Working Stock Standard 1 to 10 mL PMP Volumetric flask. Dilute to 10 mL with reagent water.

7.6.1.3 Calibration Standards are prepared from stock solutions according to the following schedule. Add the appropriate volume of stock to 100 mL PMP Volumetric flask containing 70 mL of reagent water and 1 mL of HNO3. Dilute to 100 mL with reagent water. After the solution is made to volume, add 1 mL of Internal Standard working stock (7.6.2.1) to the volumetric and mix well.

Calibration Standard	Metals	Final Concentration	Volume of Stock Used	Specific Stock Use
		To get ___ µg/L	Use ____ mL	Of _____ in 100 mL
S1	All	1	1.0	Intermediate Stock 1
S2	All	10	0.1	Working Stock 1
S3	All	100	1.0	Working Stock 1
S4	Fe,Ca,Mg,K, Na	1,000	0.5	Stock Standard 2
S5	Fe,Ca,Mg,K, Na	10,000	5.0	Stock Standard 2

7.6.2 The stock internal standards used are: SPEC Lithium 6, CLLI62-1Y (100 mg/L); SPEX Bismuth, ZACMS-1 (100 mg/L) or CLBI2-1AY (10 mg/L); SPEX Holmium, ZACMS-2 (100 mg/L) or CLGE9-1AY (10 mg/L); SPEX Indium, ZACMS-3 (100 mg/L) or CLIN2-1AY (10 mg/L); SPEX Scandium, ZACMS-4 (100 mg/L) or CLSC2-1AY (10 mg/L); SPEX Terbium ZACMS-5 (100 mg/L) or CLTB2-1AY (10 mg/L); SPEX Yttrium, ZACMS-6 (100 mg/L) or CLY2-1AY (10 mg/L); SPEX Germanium, ZACMS-7 (100 mg/L) or CLGE9-1AY (10 mg/L); and SPEX Rhodium, CLRH1-1AY (10 mg/L).

7.6.2.1 A working multi-element internal standard containing 10 mg/L of 6LI, Sc, Ge, and 5 mg/L of In, Ho, Bi, and Tb is made from the purchased 100 mg/L SPEX single-element internal stocks (see 7.6.2).

7.7 Blanks: Three types of blanks are required for the analysis. The calibration blank is used in establishing the calibration curve. The continuing calibration blank is used to monitor that it system stays free from contamination during the sample run. The preparation blank is used to monitor for possible contamination resulting from the sample preparation procedure. The rinse blank is used to flush the system between all samples and standards.

7.7.1 The calibration blank (ICB, QC2) and continuing calibration blank (CCB, QC4) consists of the same concentration(s) of the same acid(s) used to prepare the final dilution of the calibrating solutions of the analytes [often 1 percent HNO3, (v/v) in reagent water] along with the selected concentrations of internal standards such that there is an appropriate internal standard element for each of the analytes. Use of HCl for antimony and silver is cited in Section 7.2.

7.7.2 The method (or reagent) blank (PBW or PBS) must be carried through the complete preparations procedure and contain the same volumes of reagents as the sample solutions.

7.7.3 The rinse blank consists of 1 to 2 percent HNO3 (v/v) in reagent water. Prepare a sufficient quantity to flush the system between standards and samples.

NOTE: The ICS solutions in Table 2 are intended to evaluate corrections for known interferences on only the analytes in Table 1. If Method 6020 is used to determine an element not listed in Table 1, it is the responsibility of the analyst to modify the ICS solutions, or prepare an alternative ICS solution, to allow adequate verification of correction of interferences on the unlisted element (see section 10.4)

7.8 The interference check solution (ICS) is prepared to contain known concentrations of interfering elements that will demonstrate the magnitude of interferences and provide an adequate test of any corrections. Chloride in the ICS provides a means to evaluate software

corrections for chloride-related interferences such as 35Cl16O(+) on 51V(+) and 40Ar35Cl(+) on 75As(+). Iron is used to demonstrate adequate resolution of the spectrometer for the determination of manganese. Molybdenum serves to indicate oxide effects on cadmium isotopes. The other components are present to evaluate the ability of the measurement system to correct for various molecular-ion isobaric interferences. The ICS is used to verify that the interference levels are corrected by the data system within quality control limits.

7.8.1 These solutions must be prepared from ultra-pure reagents. They can be obtained commercially or prepared by the following procedure.

7.8.1.1 Mixed ICS solution I may be prepared by adding 13.903 g Al(NO3)3*9H2O, 2.498 g CaCO3 (dried at 180 C for 1 h before weighing), 1.000 g Fe, 1.658 g MgO, 2.305 g Na2CO3, and 1.767 g K2CO3 to 25 mL of reagent water. Slowly add 40 mL of (1+1) HNO3. After dissolution is complete, warm the solution to degas. Cool and dilute to 1,000 mL with reagent water.

7.8.1.2 Mixed ICS solution II may be prepared by slowly adding 7.444 g 85% H3PO4, 6.373 g 96% H2SO4, 40.024 g 37% HCl, and 10.664 g citric acid C6O7H8 to 100 mL of reagent water. Dilute to 1,000 mL with reagent water.

7.8.1.3 Mixed ICS solution III may be prepared by adding 1.00 mL each of 100-ug/mL arsenic, cadmium, chromium, cobalt, copper, manganese, nickel, silver, and zinc stock solutions to about 50 mL reagent water. Add 2.0 mL concentrated HNO3, and dilute to 100.0 mL with reagent water.

7.8.1.4 Working ICS Solutions

7.8.1.4.1 ICS-A may be prepared by adding 10 mL of mixed ICS solution I (7.8.1.1), 2.0 mL each of 100-ug/mL titanium stock solution (5.3.9) and molybdenum stock solution (5.3.10), and 5.0 mL of mixed ICS solution II (7.8.1.2). Dilute to 100 mL with reagent water. ICS solution A must be prepared fresh weekly.

7.8.1.4.2 ICS-AB may be prepared by adding 10 mL of mixed ICS solution I (7.8.1.1), 2.0 mL each of 100-ug/mL titanium stock solution (5.3.9) and molybdenum stock solution (5.3.10), 5.0 mL of mixed ICS solution II (7.8.1.2), and 2.0 mL of Mixed ICS solution III (7.8.1.3). Dilute to 100 mL with reagent water. Although the ICS solution AB must be prepared fresh weekly, the analyst should be aware that the solution may precipitate silver more quickly.

The interference check standards can be purchased from a commercial source (e.g., SPEX Claritas Interference Check Standards, or equivalent). Spike the sample with the elements of interest at concentrations comparable to those expected in the sample.

7.8.2 The interference check samples used at ECC are SPEX Interference Check Solution 1, CL-INT-A2 and SPEX Interference Check Solution 2, CL-INT-B2.

7.9 The quality control sample is the initial calibration verification solution (ICV), which must be prepared in the same acid matrix as the calibration standards. This solution must be an independent standard near the midpoint of the linear range at a concentration other than that used for instrument calibration. An independent standard is defined as a standard composed of the analytes from a source different from those used in the standards for instrument calibration. (When these standards are purchased, flexibility from this criteria may be exercised.) The continuing calibration verification (CCV) standard is prepared from the primary calibration stock standards by the analyst by combining compatible elements at concentrations equivalent to the midpoint of their respective calibration curves. The ICV and CCB must contain all calibrated analyte masses.

7.10 Mass spectrometer tuning solution. A solution containing elements representing all of the mass regions of interest (for example, 10 ug/L of Ba, Be, Ce, Co, In, Li, Mg, Rh, Tl, U, and Y) must be prepared to verify that the resolution and mass calibration of the instrument are within the required specifications (see Section 8.4.1). This solution is also used to verify that the instrument has reached thermal stability (See Section 8.4). The stock is SPEX Claritas CL-TUNE-1.

7.11 Matrix Spike (MS) Standard

7.11.1 The matrix spike standard shall be prepared from the calibration stock standard (primary) in Section 7.5. The matrix spike is done during the sample preparation procedure. Also Spike Sample Standard 1 (Water) from SPEX CertiPrep can be used. It is SPEX ClaritasPPT Spike Sample Standard 1 (Water) CL-SPIKE-1. This contains the following elements: 500 ug/ml of Fe; 250 ug/ml of Ba, Zn; 100 ug/ml of Co, Cr, Cu, Mn, Ni Sb, V; 50 ug/ml of As, Pb; 25 ug/ml of Ag, Be, Cd, Se, Tl. A working stock must be made from the original stock from SPEX by taking 5 ml of the SPEX stock and diluting to 100 ml of 5% HNO3. This should by made up fresh every 3 months.

7.11.2 The spike should be at a level that would approximately double the concentration of the target analytes present. In the absence of target analytes, the spike would be made at the site action level, assuming that this level did not also correspond to the value of the low standard used. If the action level is the same as the low standard used, then the spiking would occur at a slightly higher level, i.e., at the value of the next calibration standard used. If the action level is not known, the spiking level would then default to a concentration between the low and mid-level calibration standards.

7.11.3 Ideally, all method analytes should be contained in the MS spike. Subsets may be used based on project specific requirements.

7.12 Laboratory Control Sample (LCS)

7.12.1 The LCS shall be prepared from the calibration stock standard (primary) in Section 7.5 and is prepared by spiking a blank at a concentration between the low and mid-

level calibration standards.

7.12.2 The LCS shall be prepared in the appropriate matrix (reagent water); and contains all of the method target analytes. A subset of the method target analytes could be used based on the project specific requirements. The spiking level used would be at the same level as the site-specific action limit. If site-specific action limits are not available or where these levels are very high, the spiking level shall be at a concentration between the low and mid-level calibration standards.

7.12.3 One LCS should be prepared and analyzed for each sample batch at a frequency of one LCS for each 20 samples or less.

8.0 Procedure

8.1 Sample Preparation — Refer to the appropriate SW-846 digestion method (e.g. Methods 3005 - 3051).

8.2 Initiate appropriate operating configuration of the instruments computer according to the instrument manufacturer's instructions.

8.3 Set up the instrument with the proper operating parameters according to the instrument manufacturer's instructions.

8.4 Daily Operating Procedure: The analyst should follow the instructions provided by the instrument manufacturer. Allow at least 30 minutes for the instrument to equilibrate before analyzing any samples. This must be verified by analyzing a tuning solution (Section 7.10) at least four times with relative standard deviations of </= 5% for the analytes contained in the tuning solution.

NOTE: Precautions must be taken to protect the channel electron multiplier from high ion currents. The channel electron multiplier suffers from fatigue after being exposed to high ion currents. This fatigue can last from several seconds to hours depending on the extent of exposure. During this time period, response factors are constantly changing, which invalidates the calibration curve, causes instability, and invalidates sample analysis.

8.4.1 Conduct mass calibration and resolution checks in the mass regions of interest. The mass calibration and resolution parameters are required criteria which must be met prior to any samples being analyzed. If the mass calibration differs more than 0.1 amu from the true value, then the mass calibration must be adjusted to the correct value. The resolution must also be verified to be less than 0.9 amu full width at 10 percent peak height.

8.5 The following sequence of events should be followed to optimize the instrument on a daily basis (see Elan 6000 Operating Manual for actual instructions):

8.5.1 XY adjustment - This is done only when the cones or the torch is removed or adjusted.

8.5.2 Nebulizer Flow - This is done daily because this is highly dependent on the RF voltage.

8.5.3 Oxides - This procedure adjusts the gas flow to give a minimum value for oxides and double charged ions.

8.5.4 Ion Lens Voltage - This is done to monitor how clean the ion lens is. If the voltage for the lens reaches 10 Volts for Rhodium, then the lens needs to be cleaned.

8.5.5 Autolens Voltage - This is done when samples to be run will use both the pulse and analog detectors.

8.5.6 Daily Performance Check - This gives you values for certain analytes, the background, the oxides, double charged ions and the corresponding RSD's.

8.6 Calibrate the instrument for the analytes of interest (recommended isotopes for the analytes in Table 1 are provided in Table 3) using the calibration blank and at least a single initial calibration standard according to the instrument manufacturer's procedure. Flush the system with the rinse blank (7.7.3) between each standard solution. Use the average of at least three integrations for both calibration and sample analysis.

8.6.1 Initial Calibration — The calibration curve shall consist of a blank and at least three mixed standard concentrations (low, mid, high) for each element. Alternatively, a single standard and a blank may be used for initial calibration as long as verification is performed using both mid and low-level standards. The minimum default standard concentrations shall be: 1.0 ng/mL, 10.0 ng/mL, and 100.0 ng/mL for all metals except for Fe, Ca, Mg, K, and Na which is 100 ng/mL, 1000 ng/mL and 10000 ng/mL. Flush the system with rinsing solution between each standard. (Use the average intensity of three integrations for both calibration and sample analysis to reduce random error. The relative standard deviation of these replicate integrations should be less than 5 percent. If not, corrective action must be performed.) The calibration curve that is used is linear thru zero. This is done by the ELAN software by using linear regression. The correlation coefficient of the linear calibration curve should be minimum of 0.995. Typical correlation coefficient of the calibration curve will normally be greater that 0.9995.

8.7 All masses which could affect data quality should be monitored to determine potential effects from matrix components on the analyte peaks. The recommended isotopes to be monitored are listed in Table 3.

8.8 Initial Calibration Verification - Immediately after the calibration has been established, the calibration must be verified and documented for every analyte by the analysis of the initial calibration verification solution (Section 7.9). When measurements exceed +\- 10% of the expected value the analysis must be terminated, the problem corrected, the instrument recalibrated, and the new calibration verified. Any samples analyzed under an out-of-control calibration must be reanalyzed. During the course of an analytical run, the instrument may be "resloped" or recalibrated to correct for instrument drift. A recalibration must then be followed immediately by a new analysis of a CCV and CCB before any further samples may be analyzed.

8.9 Rinse Blank - Flush the system with the rinse blank solution (7.7.3) until the signal levels return to the method's levels of quantitation (usually about 30 seconds) before the analysis

of each sample. Nebulize each sample until a steady-state signal is achieved (usually about 30 seconds) prior to collecting data. Analyze the calibration verification solution (Section 7.9) and the calibration blank (Section 7.7.1) at a frequency of at least once every 10 analytical samples. Flow-injection systems may be used as long as they can meet the performance criteria of this method.

8.9.1 Sample analysis can now begin. Rinse the system with rinsing solution between samples and QC checks. If data system automates analytical run, program samples into software. If automation is not available, all runs must be recorded using a run log as shown in Figure 2. The software generates the run log. Set up the samples on the autosampler using the autosampler log as shown in Figure 3.

Analytical Sequence

Sample	Comment
Reagent Water	Check instrument zero
Calibration Blank	Blank working standard
Standard 1 (S1)	Calibration standard #1 (low)
Standard 2 (S2)	Calibration standard #2 (mid)
Standard 3 (S3)	Calibration standard #3 (high); Fe, Ca, Mg, K, Na (Low)
Standard 4 (S4)	Calibration Standard #4 (Mid) Fe, Ca, Mg, K, Na
Standard 5 (S5)	Calibration Standard #5 (High) Fe, Ca, Mg, K, Na
ICV (QC1)	QC check--must be within 10% of known value
ICB (QC2)	QC check--must be less than MDL
ICS	Interference Check Standard(s)
Rinse	To remove any ICS memory
Samples 1-10	Includes MD, MS/MSD, LCS and Method Blank
CCV (QC3)	QC check must be within 10% of known value
CCB (QC4)	Acceptance criteria in Section 11

Repeat above box until samples exhausted; samples include MD, MS/MSD, LCS, and method blanks. Then end run with:

ICS

CCV (QC3)

CCB (QC4)

8.9.2 Continue with sample analysis, checking the calibration blank and midpoint standard (CCV) after every 10 samples.

8.9.3 The analytical sequence must be ended with an acceptable calibration blank, mid-point standard (CCV) analysis and interference check standard.

8.9.4 Each sample shall be integrated three times. The RSD between these integrations needs to be evaluated. If RSD of triplicate integrations is consistently >10% and highly variable, this indicates a possible problem with the ICP sample introduction system. Replace tubing or clean nebulizer if necessary. This could also indicate that the sample is less than the MDL (Section 10.21).

8.10 Dilute and reanalyze samples that are more concentrated than the linear range for an analyte (or species needed for a correction) or measure an alternate less-abundant isotope. The linearity at the alternate mass must be confirmed by appropriate calibration (see Sec. 8.6 and

8.8).

9.0 Calculations

9.1 The quantitative values shall be reported in appropriate units, such as micrograms per liter (ug/L) for aqueous samples and milligrams per kilogram (mg/kg) for solid samples. If dilutions were performed, the appropriate corrections must be applied to the sample values.

9.1.1 If appropriate, or required, calculate results for solids on a dry-weight basis as follows:

(1) A separate determination of percent solids must be performed.
(2) The concentrations determined in the digest are to be reported on the basis of the dry weight of the sample.

$$\text{Concentration (dry weight)(mg/kg)} = \frac{C \times V}{W \times S}$$

Where:

C = Digest Concentration (mg/L)
V = Final volume in liters after sample preparation
W = Weight in kg of wet sample

$$S = \frac{\%\ \text{Solids}}{100}$$

Calculations should include appropriate interference corrections (see Section 5.2 for examples), internal-standard normalization, and the summation of signals at 206, 207, and 208 m/z for lead (to compensate for any differences in the abundances of these isotopes between samples and standards).

9.2 The concentration of elements (µg/L) in the samples is read from the calibration curve or directly from the instrument if operating in the direct concentration mode. Samples that required dilution are adjusted according to the following equation:

$$\mu\text{g/L metal in sample} = \frac{A(C+B)}{C}$$

where:

A = sample concentration from calibration curve
B = volume (mL) of calibration blank used to dilute sample
C = volume (mL) of sample used

9.3 The concentration of elements in solid matrices is reported in mg/Kg (dry basis) of waste as follows:

$$\text{mg/kg metal in sample} = \frac{AxV}{W}$$

where:

A = final concentration read from calibration curve (see Section 9.1) in µg/L
V = Final volume of processed sample (L)
W = Weight of sample digested (g) (dry basis)

10.0 Quality Assurance/Quality Control

10.1 All quality control data should be maintained and be available for easy reference or inspection.

10.2 Instrument Detection Limits (IDL) in ug/L can be estimated by calculating the average of the standard deviations of the three runs on three non-consecutive days from the analysis of a reagent blank solution with seven consecutive measurements per day. Each measurement must be performed as though it were a separate analytical sample (i.e., each measurement must be followed by a rinse and/or any other procedure normally performed between the analysis of separate samples). IDL's must be determined at least every three months and kept with the instrument logbook. Refer to SW-846, Chapter One for additional guidance.

10.3 The intensities of all internal standards must be monitored for every analysis. When the intensity of any internal standard fails to fall between 70 and 120 percent of the intensity of that internal standard in the initial calibration standard, the following procedure is followed. The sample must be diluted fivefold (1+4) and reanalyzed with the addition of appropriate amounts of internal standards. This procedure must be repeated until the internal-standard intensities fall within the prescribed window. The intensity levels of the internal standards for the calibration blank (Section 7.7.1) and instrument check standard (Section 7.8) must agree within +/- 20 percent of the intensity level of the internal standard of the original calibration blank solution. If they do not agree, terminate the analysis, correct the problem, recalibrate, verify the new calibration, and reanalyze the affected samples.

10.4 To obtain analyte data of known quality, it is necessary to measure more than the analytes of interest in order to apply corrections or to determine whether interference corrections are necessary. If the concentrations of interference sources (such as C, Cl, Mo, Zr, W) are such that, at the correction factor, the analyte is less than the limit of quantification and the concentration of interferents are insignificant, then the data may go uncorrected. Note that monitoring the interference sources does not necessarily require monitoring the interferant itself, but that a molecular species may be monitored to indicate the presence of the INTERFERANT. When correction equations are used, all QC criteria must also be met. Extensive QC for interference corrections are required at all times. The monitored masses must include those elements whose hydrogen, oxygen, hydroxyl, chlorine, nitrogen, carbon and sulfur molecular ions could impact the analytes of interest. Unsuspected interferences may be detected by adding pure major matrix components to a sample to observe any impact

on the analyte signals. When an interference source is present, the sample elements impacted must be flagged to indicate (a) the percentage interference correction applied to the data or (b) an uncorrected interference by virtue of the elemental equation used for quantitation. The isotope proportions for an element or molecular-ion cluster provide information useful for quality assurance.

NOTE: Only isobaric elemental, molecular, and doubly charged interference corrections which use the observed isotopic-response ratios or parent-to-oxide ratios (provided an oxide internal standard is used as described in Section 5.2) for each instrument system are acceptable corrections for use in Method 6020.

10.5 Dilution Test: If the analyte concentration is within the linear dynamic range of the instrument and sufficiently high (minimally, a factor of at least 100 times greater than the concentration in the reagent blank, refer to Section 7.7.2), an analysis of a fivefold (1+4) dilution must agree within +/- 10% of the original determination. If not, an interference effect must be suspected. One dilution test must be included for each twenty samples (or less) of each matrix in a batch.

10.6 Post-Digestion Spike Addition: An analyte spike added to a portion of a prepared sample, or its dilution, should be recovered to within 75 to 125 percent of the known value or within the laboratory derived acceptance criteria. The spike addition should be based on the indigenous concentration of each element of interest in the sample. If the spike is not recovered within the specified limits, the sample must be diluted and reanalyzed to compensate for the matrix effect. Results must agree to within 10% of the original determination. The use of a standard-addition analysis procedure may also be used to compensate for this effect (Refer to Method 7000).

10.7 A Laboratory Control Sample (LCS) should be analyzed for each analyte using the same sample preparations, analytical methods and QA/QC procedures employed for the test samples. One LCS should be prepared and analyzed for each sample batch at a frequency of one LCS for each 20 samples or less. The LCS would be prepared using the primary source standard and would contain all method target analytes. Control charts will be maintained for the LCS for all target analytes. (Data is being collected.) Acceptance criteria are presented in Section 11.6.

10.8 Check the instrument standardization by analyzing appropriate quality control solutions as follows:

10.8.1 Check instrument calibration using a calibration blank (Section 7.7.1) and the initial calibration verification solution (Section 7.9).

10.8.2 Verify calibration at a frequency of every 10 analytical samples with the instrument check standard (Section 7.11) and the calibration blank (Section 7.7.1). These solutions must also be analyzed for each analyte at the beginning of the analysis and after the last sample. Acceptance criteria listed are in Section 11.4.

10.8.3 The results of the initial calibration verification solution and the instrument check standard must agree within +/- 10% of the expected value. If not, terminate the analysis, correct the problem, and recalibrate the instrument. Any sample analyzed

under an out-of-control calibration must be reanalyzed.

10.8.4 The results of the calibration blank must be less than 3 times the current IDL for each element. If this is not the case, the reason for the out-of-control condition must be found and corrected, and affected samples must be reanalyzed. If the laboratory consistently has concentrations greater than 3 times the IDL, the IDL may be indicative of an estimated IDL and should be re-evaluated.

10.9 Verify the magnitude of elemental and molecular-ion isobaric interferences and the adequacy of any corrections at the beginning of an analytical run or once every 12 hours, whichever is more frequent. Do this by analyzing the interference check solutions A and AB. The analyst should be aware that precipitation from solution AB may occur with some elements, specifically silver. Refer to Section 3.0 for a discussion on interferences and potential solutions to those interferences if additional guidance is needed.

10.10 Analyze one duplicate sample for every matrix in a batch at a frequency of one matrix duplicate for every 20 samples.

10.10.1 The relative percent difference (RPD) between duplicate determinations must be calculated as follows:

$$RPD = \frac{|D(1) - D(2)|}{(D(1) + D(2))/2} \times 100$$

where:

RPD = relative percent difference.
D(1) = first sample value.
D(2) = second sample value (duplicate)

A control limit of 20% RPD should not be exceeded for analyte values greater than 100 times the instrumental detection limit. If this limit is exceeded, the reason for the out-of-control situation must be found and corrected, and any samples analyzed during the out-of-control condition must be reanalyzed.

10.11 A preparation batch of samples is defined as a group of up to twenty field samples of similar matrix type that have been prepared at the same time or time sequence with the same lots of reagents for the same analysis. In addition to the twenty samples, each preparatory batch will contain at a minimum, a method blank, a laboratory control sample, a matrix spike, a matrix spike duplicate and a matrix duplicate. An analytical, or instrumental batch is defined as samples that are analyzed together within the same time period or in continuous sequential time periods. Within the analytical are included individual QC requirements as defined by the analytical (determinative) method. Preparation batches of samples may be continuously strung together in these run sequences, as long as the analytical batch QC requirements meet the acceptance criteria established within the appropriate SOP. Each analytical sequence must be documented using the run log in Figure 2.

10.12 Run a calibration curve on a daily basis that employs a minimum of a calibration blank and three standard concentrations. Alternatively, a single standard and a blank may be used for

initial calibration as long as verification is performed using both mid and low-level standards. Acceptance criteria are presented in Section 11.1.

10.13 Analyze the initial calibration verification (ICV) standard(s) containing all target analytes. Acceptance criteria listed in Section 11.2.

10.14 Check the instrument calibration by analyzing interference check standards (ICS) as follows. Verify the interelement factors at the beginning and end of an analytical run or twice during every 8-hour work shift, whichever is more frequent. Acceptance criteria are presented in Section 11.3.

10.15 A method blank shall accompany each sample batch to determine if contamination or any memory effects are occurring. A method blank is a volume of reagent water acidified with the same amounts of acids as were used for preparation of the standards and samples. This sample is carried through the entire digestion and analysis procedure. Acceptance criteria are presented in Section 11.5.

10.16 The use of the matrix spike and matrix spike duplicate will depend in part on what role the laboratory is playing. When the laboratory serves the role as the primary laboratory, then site-specific documents should be consulted. The sample to be used for the MS/MSD may be specified in the field. This previously designated sample would then be spiked with the site-specific target analytes at a concentration equivalent to the site action level. The MS/MSD would be prepared using the primary source standards. If this information was not specified or unknown, then the laboratory would choose a representative sample from each batch of samples analyzed. If samples from multiple sites were to be analyzed in the same batch, then multiple sets of MS/MSD may be required. When the laboratory serves the role as the QA laboratory, the above scenario may not be practical to implement. If the site-specific requirements are unknown and samples from multiple sites are analyzed in the same batch, then the laboratory should select a single sample for spiking. Each batch of samples would then contain at least one MS/MSD pair. Refer to the appropriate metals preparation SOP. Acceptance criteria are presented in Section 11.7 for percent recovery and RPD.

10.17 The use of the matrix duplicate may also depend in part on the role the laboratory is playing. The selection of a matrix duplicate will be performed as described for the MS/MSD in Section 10.7. Acceptance criteria are presented in Section 11.8 for RPD.

10.18 All sample analytical results used for final data reporting must be above the MDL and below the high standard of the calibration curve. Corrective actions are described in Section 11.9.

10.19 Whenever a new or unusual sample matrix is encountered, a series of tests shall be performed prior to reporting concentration data for analyte elements. These tests, as outlined in 11.10.1 and 11.10.2, will ensure the analyst that neither positive nor negative interferences are operating on any of the analyte elements to distort the accuracy of the reported values.

10.20 Data shall be checked to ascertain if it conforms to accepted practices for reporting of results at or near the MDL. The reporting limits which are required are (listed in order of preference); 1) project-specific requirements, or 2) the lowest standard of the standard curve. Results between the MDL and the low standard shall be reported as estimates.

10.21 At a minimum, MDL's are determined in reagent water annually. However MDL's are performed at more frequent intervals when dictated by project-specific requirements. Additionally, MDL check samples are analyzed quarterly to verify MDL's. Refer to SOP No. Q-019-ECC-QC, Method Detection Limits (MDL), Method Quantitation Limits (MQL), and Laboratory Reporting Limits (LRL).

10.22 The analyst must demonstrate proficiency in performing the analysis as outlined in SOP No. Q-016-ECC-QC, Technical Training. Method proficiency must be demonstrated anytime a major method modification is made, a major software revision is added, or a major instrument modification is made. Demonstration of method proficiency may also be required after major instrument maintenance. This is decided on a case by case basis through discussions with the Section Chief, Laboratory Director, and Laboratory QA Officer.

10.23 Internal Standards (IS) - The internal standards are evaluated in the following manner: In standards, the IS are in control when they are between 80% - 120%, and in samples they are in control when they are between 70% - 120%.

11.0 Data Validation

11.1 After running the calibration standards, the highest standard is to be analyzed as a sample, prior to actual sample analysis. Concentration values obtained should not deviate from the actual values by more than 5%. If they do, troubleshoot the system to correct for this condition. Continue until an acceptable run is made for this highest mixed calibration standard.

11.2 The results of the ICV for all analytes are to be within 10% of known values. If this criteria is not met, recalibrate and reanalyze, using the same standards. If acceptance criteria are still not met, recheck standard curve and ICV preparation and/or perform routine instrument maintenance (e.g., replace tubing, clean nebulizer/torch), recalibrate and reanalyze. If still not acceptable, refer to manufacturers's instructions or call service representative.

11.3 The analyst shall verify that the ICS has been analyzed at the required frequency. The prepared ICS is to be run at the beginning and end of each analytical run, or at the beginning and end of an 8 hour shift, whichever occurs more often. Results should be within ± 20% of the true value for all target analytes within the prepared ICS sample. If this criteria is not met, check the background correction protocols currently in place for appropriateness and recalculate if needed. If this is the initial ICS run after daily calibration, recalibrate and reanalyze. If the ICS did not check at the end of an 8 hour shift, reanalyze any samples in the previous run that may have been affected.

11.4 Continuing Calibration

11.4.1 The results of the continuing calibration verification (CCV) check standard shall agree to within 10% of the expected value. If not, correct the problem, and reanalyze the previous ten samples since the last acceptable CCV.

11.4.2 The results of the calibration blank are to agree to within three standard deviations of the mean blank value. If not, repeat the analysis two more times and average the

results. If the average is not within three standard deviations of the background mean, correct the problem, recalibrate, and reanalyze the samples analyzed since the last acceptable calibration blank.

11.5 Assess the method blanks. The analyst shall confirm that this blank was analyzed at the required frequency.

The method blank should not exhibit any contamination of any analyte above the MDL for any of the method target analytes. Corrective action to reduce and control contamination should be performed any time method target analytes are detected above the MDL. Corrective action will be required if site-specific target analytes are detected at greater than 5% of the regulatory limit for that analyte or if the concentration in the blank is greater than 5% of that in the sample. The first step of corrective action is to assess the effect on the samples. Corrective action would include reanalysis of field and QC samples in the batch if some or all of the samples also contained levels of target analytes that exceeded the above criteria. If none of the field samples had values above the stated criteria, then reanalysis may not be necessary. The source of contamination should still be investigated and reduced/eliminated. Any time contamination is noted in the method blank, the situation and impact on the data should be discussed in the case narrative.

11.6 Verify that the LCS were prepared at the required frequency. Plot the target analytes on appropriate control charts. (Refer to SOP No. Q-009-ECC-QC, Control Chart Generation, Maintenance, and Usage.) If all target analytes are not within control limits, reanalyze the LCS. If still unacceptable, redigest and reanalyze a new LCS and all associated samples. If the LCS is still unacceptable, then the entire procedure must be systematically investigated to locate the source of error.

Note: Even though control charts must be maintained for the LCS, the acceptance criteria derived from these charts may not be appropriate if the charted ranges are too wide. A maximum default range should be used. It is recommended that the maximum default range be set to 80-120%. (Data is being collected.)

The RPD of interbatch LCS should fall within the control limits determined from the precision control charts. However, if the RPD is outside these control limits, the batch will not be rejected, as long as the LCS recovery is acceptable. This precision information should be evaluated to see if systematic problems can be identified. If problems are suspected, the method should be fully evaluated.

11.7 Verify that matrix spike/matrix spike duplicates were analyzed at required frequency. The analyst shall also verify that the samples were spiked at the appropriate level. The order of preference for spiking levels is as follows; 1) If the target analyte concentrations are known, spike to increase the background concentration by a factor of approximately two, 2) if an action level exists, spike at this level, or 3) if neither of the first two conditions apply, spike at a level that corresponds between the low and mid-level calibration standards. Acceptance criteria are that all % Recovery and/or RPD results meet project established goals. If no project goals are specified, then results must be within the indicated control limits on the appropriate LCS control charts. If these conditions are not met, perform the following corrective actions as appropriate.

- If both LCS and MS/MSD recoveries and/or precision are unacceptable, then the entire batch of field and QC samples must be redigested and reanalyzed.

- If the MS/MSD recovery is unacceptable, but the LCS is acceptable, then a potential matrix effect has been identified. Reanalyze the MS/MSD digestates to verify a matrix effect. If a matrix effect is still suspected, then the project manager must be contacted to discuss further alternatives and the potential impact on the project. These further alternatives may include redigestion/reanalyzis.

11.8 Verify matrix duplicates were analyzed at required frequency (one per digestion run). Acceptance criteria are that all RPD results meet project established goals. If no project goals are specified, then results must be within the indicated control limits on the appropriate LCS precision control charts. The acceptance criteria derived from these charts may not be appropriate if the charted ranges are too wide. (Data is being collected.) A maximum default range of 25% RPD should be used. If these conditions are not met, perform the following corrective actions as appropriate.

- Reanalyze the sample and duplicate digestates.

- If the duplicate precision is unacceptable, then a potential matrix effect has been identified. The project manager must be contacted to discuss further alternatives and the potential impact on the project. These further alternatives may include redigestion/reanalysis.

11.9 The analyst must verify all reported results are derived from analytical results that are above the MDL or below the highest calibration standard. Verify the results are reported as follows:

- Sample concentrations that have been analyzed using the digestate in its most concentrated form, and are below the laboratory reporting limit (LRL), should report the result as less than the LRL.

- For sample results (again using the digestate in its most concentrated form) that are above the MDL but below the LRL, results need to be flagged as estimates (J values).

- For samples that exceed the calibration curve, dilute and analyze an appropriate sample aliquot.

11.10 For each new matrix, ensure that the following series of tests were performed and meet the listed acceptance criteria. If criteria are not met, the method of standard additions (MSA) should be considered. The project manager should be consulted.

11.10.1 Serial dilution

If the analyte concentration is sufficiently high (minimally, a factor of 10 above the instrumental detection limit after dilution), an analysis of a 1:4 dilution should agree within ± 10% of the original determination. If not, a chemical or physical interference effect should be suspected.

11.10.2 Post-digestion spike addition

An analyte spike added to a portion of a prepared sample, or its dilution, should be recovered to within 75% to 125% of the known value. The spike addition should produce a minimum level of 10 times and a maximum of 100 times the instrumental detection limit. If the spike is not recovered within the specified limits, a matrix effect should be suspected.

11.11 Besides the items listed in Sections 11.1 through 11.10, the analyst should also verify the additional items as noted in Figure 1.

11.12 Additional levels of review are performed as described in SOP No. Q-024-ECC-DR, Data Reduction and Review/Validation (In-House/Contractor Data), using a predesigned form as shown in Figure 1.

12.0 Waste Disposal

This procedure generates acidic wastes. All wastes should be disposed of according to local regulatory as well as laboratory established guidelines. Refer to the ERDC-WES site Waste Management Plan.

13.0 References

13.1 Method 6020, Test Methods for the Analysis of Solid Waste, Physical/Chemical Methods, Update II, Third Edition, July 1992.

13.2 Methods 3005A - 3051, Test Methods for the Analysis of Solid Waste, Physical/Chemical Methods, Update II, Third Edition, July 1992.

13.3 SOP No. Q-005-ECC-SH, Sample Receipt, Login, and Storage.

13.4 SOP No. Q-012-ECC-GL, Glassware Cleaning.

13.5 SOP No. Q-008-ECC-GL, Reagent Water Generation and Quality Monitoring.

13.6 SOP No. Q-009-ECC-QC, Control Chart Generation, Maintenance, and Usage

13.7 SOP No. Q-016-ECC-QC, Technical Training.

13.8 SOP No. Q-019-ECC-QC, Method Detection Limits (MDL), Method Quantitation Limits (MQL), and Laboratory Reporting Limits (LRL).

13.9 SOP No. Q-024-ECC-DR, Data Reduction and Review/Validation (In-House/Contractor Data).

13.10 ERDC -WES Waste Management Plan.

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13.13 Tan, S.H., and Horlick, G., Appl. Spectrosc. 40, 445 (1986).

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13.22 Wolf, Ruth, "ELAN 6000 Method 6020 Quick Start Guide", Perkin Elmer Application Brief ENVB-307, (1996).

13.23 Wolf, Ruth, et. al., "RCRA SW-846 Method 6020 for the ICP-MS, Analysis of Soils and Sediments", Perkin Elmer Application Note ENVA-301, (1996).

Table 1
Elements Approved For ICP-MS Determination and Estimated Instrumental Detection Limits.

Analyte	CAS #	Estimated Element Detection Limit (µg/L)(a)	Method Reporting Limits. (ug/L)
Aluminum	7429-90-5	0.026	0.100
Antimony	7440-36-0	0.005	0.020
Arsenic	7440-38-2	0.024	0.100
Barium	7440-39-3	0.003	0.010
Beryllium	7440-41-7	0.004	0.020
Cadmium	7440-43-9	0.005	0.020
Calcium	7440-70-2	20.0	50.0
Chromium	7440-47-3	0.022	0.100
Cobalt	7440-48-4	0.002	0.010
Copper	7440-50-8	0.017	0.050
Iron	7439-89-6	1.00	5.00
Lead	7439-92-1	0.002	0.010
Magnesium	7439-95-4	0.05	0.200
Manganese	7439-96-5	0.003	0.010
Nickel	7440-02-0	0.011	0.040
Potassium	7440-09-7	5.0	10.0
Selenium	7782-49-2	0.100	0.200
Silver	7440-22-4	0.010	0.030
Sodium	7440-23-5	0.50	1.00
Thallium	7440-28-0	0.001	0.010
Vanadium	7440-62-2	0.025	0.100
Zinc	7440-66-6	0.063	0.200

(a) The estimated instrumental detection limits shown are taken from an actual IDL analysis on the Elan 6000 ICPMS. They are given as a guide for an instrumental limit. The actual method detection limits are sample dependent and may vary as the sample matrix varies.

Table 2
Recommended Interference Check Sample Components And Concentrations.

Solution component	Solution A Concentration (mg/l)	Solution AB Concentration (mg/l)
Al	100.0	100.0
Ca	100.0	100.0
Fe	100.0	100.0
Mg	100.0	100.0
Na	100.0	100.0
P	100.0	100.0
K	100.0	100.0
S	100.0	100.0
C	200.0	200.0
Cl	1000.0	1000.0
Mo	2.0	2.0
Ti	2.0	2.0
As	0.0	0.0200
Cd	0.0	0.0200
Cr	0.0	0.0200
Co	0.0	0.0200
Cu	0.0	0.0200
Mn	0.0	0.0200
Ni	0.0	0.0200
Se	0.0	0.100
Ag	0.0	0.0200
V	0.0	0.100
Zn	0.0	0.0200

TABLE 3.
RECOMMENDED ISOTOPES FOR SELECTED ELEMENTS

Element of Interest	Element Symbol	Mass
Aluminum	Al	27(*)
Antimony	Sb	121, 123(*)
Arsenic	As	75(*)
Barium	Ba	138, 137, 136, 135(*), 134
Beryllium	Be	9(*)
Bismuth (IS)	Bi	209
Cadmium	Cd	114(*), 112, 111(*), 110, 113, 116, 106
Calcium (I)	Ca	42, 43, 44(*), 46, 48
Chlorine (I)	Cl	35, 37, (77,82)(a)
Chromium	Cr	52(*), 53(*), 50(*), 54
Cobalt	Co	59(*)
Copper	Cu	63(*), 65(*)
Germanium (IS)	Ge	70, 72(*), 73, 74, 76
Holmium	Ho	165
Indium (IS)	In	115(*), 113
Iron (I)	Fe	56(*), 54(*), 57(*), 58
Lanthanum (I)	La	139
Lead	Pb	208(*), 207(*), 206(*), 204
Lithium (IS)	Li	6(b), 7
Magnesium (I)	Mg	24, 25(*), 26(*)
Manganese	Mn	55 (*)
Molybdenum (I)	Mo	98, 96, 92 97(*), 94, (108)(a)
Nickel	Ni	58, 60(*), 62, 61(*), 64
Potassium (I)	K	39(*)
Rhodium (IS)	Rh	103
Scandium (IS)	Sc	45

Element of Interest	Element Symbol	Mass
Silver	Ag	107(*), 109(*)
Sodium (I)	Na	23(*)
Terbium (IS)	Tb	159
Thallium	Tl	205(*), 203
Tin (I)	Sn	120, 118(*)
Yttrium (IS) (I)	Y	89 (c)
Zinc	Zn	64, 66(*), 68(*), 67(*), 70
Carbon (I)	C	12(*), 13

NOTE: Method 6020 is recommended for only those analytes listed in Table 1. Other elements are included in this table because they are potential interferents (labeled I) in the determination of recommended analytes, or because they are commonly used internal standards (labeled IS). Isotopes are listed in descending order of natural abundance. The most generally useful isotopes, have an (*) next to them, although certain matrices may require the use of alternative isotopes.

(a) These masses are also useful for interference correction (Section 3.2).
(b) Internal standard must be enriched in the 6Li isotope. This minimizes interference from indigenous lithium.
(c) Yttrium causes an interference indirectly with 111Cd.

Data Review Check List
ICPMS Metals
ERDC EL EEC

Project Number(s)	
Batch Number(s)	
SOP No.	

Review Item	Yes (x)	No (x)	N/A (x)	2nd Level Review (x)
1. Does the daily standard curve consist of a Calibration Blank and the required minimum number of calibration standards?				
2. Is the low standard near, but above, the MDL?				
3. Are the CCV standards analyzed at required frequency and at the end of the analytical sequence and all parameters within QC limits?				
4. Are the CCB standards analyzed at required frequency and at the end of the analytical sequence and all parameters within QC limits?				
5. Are all sample holding times met?				
6. Are all samples with concentrations > the highest standard used for initial calibration diluted and reanalyzed?				
7. Is the method blank run at the desired frequency and is its concentration for target analytes less than the LRLs?				
8. Is the ICV from a second source and is its percent recovery within QC limits?				
9. Is the Matrix Spike/Matrix Spike Duplicate run at the desired frequency and is the percent recovery/RPD within QC limits?				
10. Is the Matrix Duplicate run at the desired frequency and is the RPD within QC limits?				
11. Is a Serial Dilution analysis performed at the desired frequency and within QC limits?				
12. Are post-digestion spikes analyzed at the desired frequency and within QC limits?				
13. Are Interference check standards analyzed at the beginning and end of analytical run or at minimum frequencies and within QC limits?				

Figure 1.

Data Review Check List
ICP Metals
ERDC EL ECC

Review Item	Yes (x)	No (x)	N/A (x)	2nd Level Review (x)
14. Are all nonconformances include and noted?				
15. Is the correct methodology used for sample prep and analysis?				
16. Are all calculations checked at the minimum frequency?				
17. Did analyst sign/date the appropriate printouts and report sheets?				
18. Are all sample ID and units checked for transcription errors?				

Comments on any "No" response:

_____

_____

_____

_____

_____

_____

_____

Analyst: _____ Date: _____

2nd Level Reviewer: _____ Date: _____

Figure 1.(cont.).

Autosampler Log - ICPMS
USAERDC-WES Environmental Laboratory

Protocol:	Batch ID:
Method:	Description:
OID No.:	File Name:
	Calibration File Name:
Parameters:	Sample Flush (sec/+-rpm):
	Read Delay (sec/+-rpm):
	Wash (sec/+-rpm):

A/S Loc	Sample ID	Dil.	Wash	A/S Loc	Sample ID	Dil.	Wash
1				31			
2				32			
3				33			
4				34			
5				35			
6				36			
7				37			
8				38			
9				39			
10				40			
11				41			
12				42			
13				43			
14				44			
15				45			
16				46			
17				47			
18				48			
19				49			
20				50			
21				51			
22				52			
23				53			
24				54			
25				55			
26				56			
27				57			
28				58			
29				59			
30				60			

Notes:

Figure 2.

ENVIRONMENTAL CHEMISTRY CENTER
ENVIRONMENTAL LABORATORY
ERDC

CEERD-EE-C
3909 HALLS FERRY ROAD
VICKSBURG, MS 39180

TITLE: Mercury In Solid Or Semisolid Waste (Manual Cold-Vapor Technique) (SW-846 Method 7471A)

SOP NUMBER: M-7471A-ECC-IA, Ver. 1.0

PREPARED BY: Charles L. White 3/17/99
Date

REVIEWED BY: Charles L. White 3/17/99
Technical Specialist Date

Karen F Myers 3/30/99
QA Officer Date

Wm B Shroy 4/13/99
Laboratory Director Date

SOP MANUAL CONTROL NO.: _____

1.0 Scope and Application

1.1 This SOP details a cold-vapor atomic fluorescence procedure (CVAF) for determining the concentration of mercury in soils, sediments, wastes, bottom deposits, and sludge-type materials.

1.2 This procedure is used for the determination of total mercury.

2.0 Method Summary

2.1 Prior to analysis by the mercury cold-vapor technique, all samples must be prepared according to the procedure discussed in Section 8.0 of this SOP.

2.2 This is a cold-vapor atomic fluorescence technique and is based on the emittance of radiation at 253.7 nm by mercury vapor. During the digestion step, inorganic forms of mercury and organo-mercury compounds are oxidized by potassium permanganate and potassium persulfate and converted to mercuric ions. This method has been shown to be effective for measuring a number of organic mercurials including phenyl mercuric acetate and methyl mercuric chloride. The effectiveness of this procedure for other organic mercurials should be evaluated with duplicates and matrix spikes. (Refer to Section 10.0 of this SOP for quality assurance information.)

2.3 After digestion, the mercuric ion is reduced to the elemental state and aerated from the solution in a closed system.

2.4 The mercury vapor passes through a cell positioned in the light path of an atomic fluorescence spectrophotometer.

2.5 The sample fluorescence (as measured by peak height) is measured and quantified on a calibration curve constructed from known mercury standards.

2.6 The typical detection limit using 0.5 grams of sample is 0.04 mg/Kg.

3.0 Health and Safety

3.1 Use of this procedure requires the handling of corrosive acids and protective equipment must be utilized.

3.2 All sample handling must be performed in a fume hood.

3.3 For specific information regarding the toxicity of the reagents used in this procedure and other related health and safety issues including the proper storage and handling of reagents and chemicals, the analyst should consult the appropriate Material Safety Data Sheets (MSDSs) which are located in Room 111 of Building 3299.

3.4 Use of this procedure requires the handling of concentrated acid (sulfuric acid).

Prior to performing this procedure, the analyst should be familiar with the proper use of corrosive liquid spill kits and containment procedures. Minimum personal protection includes the use of laboratory safety glasses, a lab coat or apron, and protective gloves. All digestions must be performed in a laboratory fume hood.

4.0 Sample Preservation, Containers, Handling, and Storage

4.1 Requirements for sample preservation, sample containers, and sample storage are detailed in SOP No. Q-005-ECC-SH, Sample Receipt, Login and Storage.

4.2 All samples must be preserved and stored at 4 ± 2 °C until analyzed. The holding time for mercury in solid matrices is 28 days from the date of collection.

5.0 Interferences and Potential Problems

5.1 Potassium permanganate is added to eliminate possible interference from sulfide. Sulfide concentrations as high as 20 mg/Kg have been shown not to interfere with the recovery of added inorganic mercury from reagent water.

5.2 Copper has also been reported to interfere with the analysis of mercury, however, copper concentrations as high as 10 mg/Kg had no effect on recovery of mercury from spiked samples.

5.3 Samples high in chlorides require additional permanganate (as much as 25 mL) because, during the oxidation step, chlorides are converted to free chlorine, which also absorbs radiation of 253.7 nm. Care must therefore be taken to ensure that free chlorine is absent before the mercury is reduced and swept into the cell. This may be accomplished by using an excess of hydroxylamine hydrochloride solution (25 mL). Both inorganic and organic mercury spikes have been quantitatively recovered from seawater by using this technique. Excessive amounts of chlorine have not been found to cause interferences with a fluorescence detector.

6.0 Equipment/Apparatus

6.1 P.S. Analytical Merlin Plus with a Merlin Fluorescence Detector - A closed sample presentation area where the sample enters the detector as a gas, which is channeled through a chimney past a light source and a photomultiplier tube which are at right angles to each other. The mercury vapour absorbs the light and then fluoresces at 253.7.

6.2 High intensity Mercury lamp developed by Cathodeon which works by isolation of the required excitation and emission wavelenths using a fixed 254 nm filter and a Photomultiplier tube which detects fluorescence from the sample.

6.3 P.S. Analytical TouchStone Software version 3.4 with a printer - The output

from the Merlin Detector is fed to the computer using the Merlin's BCD signals. The software controls the operation of the individual components and collects, calculates, and reprints the results of blanks, samples, and standards.

6.4 Liquid Argon Tank plumbed into a regulator set at 35 psi is used to deliver flow at 1.0 liters/min.

6.5 Flowmeter — Capable of measuring an air flow of 1 L/min.

6.6 Gas/liquid separator - strips mercury from the liquid using a flow rate of approximately 0.3 liter/min of argon. This vapor is fed into the central core of the chimney interface where a further argon stream of approximately 0.3 litre/min is fed to a concentric ring of outlets around the central stream. This effectively constrains the mercury vapour in a laminar flow of argon past the fluorescence head of the monitor.

6.7 Drying tube - The drier tube is made by Perma Pure and is composed of silicon and nylon tubing. The drier tube has an internal tubular membrane through which the mercury and water vapor from the sample travels. Carrier argon pushes these vapors through. The water vapor permeates through the membrane to the outer tube. Drier argon gas is passed through the outer space to sweep out the water vapor that goes through the membrane. This process dries the mercury vapor which causes noise and matrix effects in detectors.

6.8 The Hydride vapor generator is assembled according to the manufacturer's instructions.

6.8.1 Carfully unpack the Hydride/Vapor generator.

6.8.2 Place the generator on the left side of the Merlin.

6.8.3 Ensure that voltage selector is switched to correct voltage selection.

6.8.4 Plug into mains supply and turn on switch at rear. Ensure Hydride motor starts and front LEDs light. Turn Vapore/Vapor Generator off.

6.8.5 Place Gas/liquid separator on right-hand side of Hydride unit in spring clips provided and carefully connect up the gas and liquid lines.

6.8.6 Connect drain line to Gas/Liquid Separator U-tube, ensure this is free running.

6.8.7 Connect sample out line from top of separator to rear of Merlin marked SAMPLE IN.

6.8.8 Connect the snap in connector to inputs of both rotameters and other end to Argon supply. Ensure Argon supply is regulated, a pressure setting of approximately 35 psi is required by the Merlin System.

6.8.9 Connect sheath gas rotameter to Merlin SHEATH GAS Input - via

suitable gas line.

6.8.10 Connect 15 way 'D' connector supplied to the rear of Hydride Gererator, connect other end of the cable to the 25 way 'D' connector at the rear of the computer.

Because mercury vapor is toxic, precaution must be taken to avoid its inhalation. Therefore, a bypass has been included in the system to vent the mercury into an exhaust hood after passing the vapor through ~~a~~ an absorbing medium (an activated charcoal filter).

6.9 Blue M oven - adjustable and capable of maintaining a temperature of 90-95°C.

6.10 Graduated cylinders — Class A, various sizes, 25 to 1000-mL.

6.11 Balance, capable of weighing to the nearest 0.001 g.

6.12 Acid-dispensing bottles, polyethylene, polypropylene or equivalent material, in various dispensing sizes (2.5, 3, 5, 10 and 15-mL).

6.13 250 mL Teflon bottles with caps.

6.14 Squeeze bottles — polyethylene or equivalent material, 500-mL size. Used for general rinsing purposes and to dispense reagent water.

6.15 Aluminum foil.

6.16 Thermometer — 0-100 °C, accurate to ± 1 °C.

6.17 Volumetric flasks, Class A, various sizes.

6.18 Electronic pipets - checked daily (acceptable ranges are 99-101%).

7.0 Reagents

7.1 Reagent water — Refer to SOP No. Q-008-ECC-GL, Reagent Water Generation and Quality Monitoring.

7.2 Acids must be analyzed to determine levels of impurities. If a method blank made with the acid is < MDL, the acid can be used. This should be verified with each new bottle of acid.

7.2.1 Concentrated sulfuric acid (H_2SO_4, JT Baker TraceMetal grade, Cat# 7664-93-9.

7.2.2 Concentrated nitric acid (HNO_3, Fisher TraceMetal grade, Cat# A-509.

7.2.3 Concentrated hydrochloric acid (HCl,Mallinckrodt TraceMetal grade, Cat# 5587.

7.3 Stannous chloride (2% w/v $SnCL_2$) - Using an acid dispensing bottle, transfer 80 ± 0.5 mL of concentrated HCl to a 1-L volumetric flask containing approximately 500 mL of reagent water and mix. Weigh 20 ± 0.5 g stannous chloride (Anhydrous stannous chloride from Aldrich Chemical catalog # 20,825-6 is recommended. If using an alternative source, please check that the solution is clear with no suspended white solid present before using) and transfer it to the volumetric flask. Bring to volume with reagent water and mix. This solution may be stored in a 1-L glass reagent bottle at room temperature.

7.4 Sodium chloride (NaCl) — ACS Reagent grade.

7.5 Hydroxylamine hydrochloride $(NH_2OH)\bullet HCl$ — ACS Certified.

7.6 Sodium chloride-hydroxylamine hydrochloride solution (0.73 M hydroxylamine sulfate hydrochloride). Weigh 120 ± 1 g of sodium chloride (Section 7.6) and 120 ± 1 g of hydroxylamine hydrochloride (Section 7.7) and transfer to a 1-L volumetric flask containing approximately 500 mL of reagent water. Mix to dissolve and bring to volume with reagent water. This solution may be stored in a 1-L glass reagent bottle at room temperature.

7.7 Potassium permanganate ($KMnO_4$), 5% solution (w/v) — Weigh 50 ± 0.5 g of potassium permanganate and transfer to a 1-L volumetric flask containing approximately 500 mL of reagent water. Mix to dissolve and bring to volume with reagent water. This solution may be stored in a 1-L glass bottle at room temperature.

7.8 Potassuim Persulfate - Dissolve 5 grams of potassium persulfate ($K_2S_2O_8$) in 100 mL of deionized water.

7.9 Stock Mercury Standard (10 mg/L Mercury) — Purchased from Alfa Aesar Stock #36747. Stock calibration solutions shall be purchased as certified solutions from commercial suppliers. Refer to SOP No. Q-003-ECC-QC, Standards Preparation, Traceability, and Storage for requirements. Certificates must be kept on file. Purchased stock solutions are stable for one year.

7.9.1 Primary source standards shall be used to prepare the initial 5-point calibration curve, continuing calibration verification (CCV) standard, and matrix spikes and their duplicates (MS/MSDs).

7.9.2 Secondary source standards shall be used to verify the initial calibration (ICV) curve only. These standards shall be purchased from a different vendor, unless the primary vendor can supply different lot numbers. See Section 7.16 for the preparation and final concentration of these standards. Secondary source is a 10 mg/L mercury standard from Claritas PPT catalog # CL-ICS-2.

7.10 Mercury working standard (0.2 mg/L Mercury) — Using an electronic pipetter,

transfer 2 mL of the intermediate mercury stock (Section 7.11) to a 100-mL volumetric flask containing approximately 50 mL reagent water and 2 ± 0.1 mL HNO_3. Mix and dilute to volume with reagent water. The final acid concentration will be approximately 2.0%. Prepare fresh each day.

7.11 Calibration Standards

7.11.1 Prepare a six point calibration curve which includes a calibration blank using the working standard from Section 7.10. The lowest standard shall be 3 to 5 times MDL. The highest standard shall not exceed the linear range of the instrument.

7.11.2 Transfer 0.0-, 0.05-, 0.10-, 0.20-, 0.50-, 1.0-, and 2.0- ml aliquots of the mercury working standard (0.2 mg/L) to a series of 200-ml class A volumetric flasks. Using an automatic pipetter, add a total volume of 100 ml of reagent water to the volumetric flask. Calibration standards are to be processed at the same time of the sample batch. Refer to Sections 8.1.2, 8.1.3 and 8.1.4 for additional processing information. Table 1 contains the default calibration curve.

Table 1. Default Calibration Curve

Volume of Working Standard (mL)	Hg per 10 mL of Standard (μg)
2.0	0.020
1.0	0.010
0.50	0.005*
0.20	0.002
0.10	0.001
0 (calibration blank)	0 (calibration blank)

* used for CCV

7.11.3 Client specific requirements may require a different calibration curve. DQOs should be consulted before sample analysis to ensure the calibration range is appropriate. In the absence of project specific requirements, the following default calibration curve in Table 1 will be used.

7.12 Laboratory Control Sample (LCS)

7.12.1 The LCS shall be prepared from the primary working standard in Section 7.10.

7.12.2 The LCS shall be prepared in the appropriate matrix (purified solid) and is processed at the same time as the sample batch. See Sections 8.1.2, 8.1.3, and 8.1.4.

7.12.2 The spiking level used should be at the same level as the site-specific action limit, with the exception that the concentration can never be

lower than the lowest standard used for the initial calibration.

7.12.3 If an action level is not known, the spiking level shall default between the low and mid-level calibration standards. The sample is spiked with working standard to give a final concentration equal to 50 % of the range of calibration curve.

7.13 Continuing Calibration Verification (CCV) standard

7.13.1 The CCV is prepared the same as the primary mid-level standard (Section 7.11.2) and is run after every 10 samples (sample includes duplicates and MS/MSD(s), when appropriate) and at the end of the analytical sequence.

7.13.2 A sufficient amount of CCV will need to be processed to meet the requirements of the batch size being used. They are processed in the same manner and at the same time of the sample batch. See Section 8.1.2, 8.1.3, and 8.1.4.

7.14 Matrix Spike (MS) Standard

7.14.1 The MS standard shall be prepared from the primary source calibration standard and processed at the same time as the sample batch. See Sections 8.1.2, 8.1.3, and 8.1.4.

7.14.2 The spike should be at the site action level, if applicable, or twice the expected concentration, if known. If an action level is not known, the spiking level would default between the low and mid-level calibration standards. The sample is spiked at either 0.75 or 1.0 ppb using the 0.2 mg/L working standard. For the 0.75 ppb spike use 56 ul of the 0.2 mg/L working standard, then dilute to 15 ml with the sample. For the 1.0 ppb spike use 75 ul of the 0.2 mg/L working standard, then dilute to 15 ml with the sample. The 0.75 ppb spike is usually used for sandy matrices and the 1 ppb spike is used for clay matrices.

8.0 Procedure

8.1 Sample preparation

8.1.1 Dry sample in oven at 60°C for approximately two hours (or until completely dry). Homogenize sample and sieve through a 40 mesh sieve. Weigh g, one aliquot of 0.5 g. to (to the nearest 0.001g). Place each aliquot in the bottom of a separate teflon bottle. Using an automatic dispenser, add 10 ± 0.1 mL of reagent water. Sample weights are to be recorded on the mercury digestion log (see Figure 1).

Note: A separate determination of percent solids must be performed. Refer to SOP No. M-2216-ECC-GC, Determination of Percent Solids (ASTM Method 2216).

8.1.2 Using an automatic pipetter, add 5 ± 0.5 mL of concentrated sulfuric acid and 2.5 mL ± 0.5 mL of concentrated nitric acid to each sample and standard, mixing after each addition. Place in an oven and heat from 90-95°C for at least 2 minutes from the time the oven reaches 90°C (about 15 minutes). Remove from oven and cool.

8.1.3 Using a automatic pipetter, add 53.5 ± 0.5 mL of reagent water to each bottle. Swirl. Using an automatic pipetter, add 15 ± 0.5 mL $KMnO_4$ solution (Section 7.7) and 8 ml ± 0.5 ml of potassium persulfate solution (Section 7.8 to each bottle, stirring after each addition. Mix thoroughly and return to the oven. Heat at 90-95°C for a minimum of 1 hour.

8.1.4 Remove from oven and allow to cool to room temperature. Using an automatic pipetter, add 6 ± 0.2 mL of sodium chloride- hydroxylamine hydrochloride (Section 7.6) to each bottle to reduce the excess permanganate. Swirl until all traces of purple color clears. Using a pipettor, add 30 ml of reagent water to each bottle. See Section 8.2.6 for the next applicable step for samples. The standards are diluted to volume (200 ml), allowed to cool, then transferred to 250 ml teflon bottles to which 60 ml of reagent water is added. The standards are now ready for analysis. Samples and standards should be capped until ready for analysis.

CAUTION: The addition of sodium chloride-hydroxylamine hydrochloride should be performed in a laboratory fume hood as Cl_2 could be evolved.

NOTE: Samples and standards must be at room temperature before the addition of reagents to avoid loss of mercury.

8.2 Instrument Calibration and Sample Analysis

8.2.1 Set up or zero mercury analyzer used according to manufacturer's recommended procedures. Mercury analysis is done manually after the instrument is allowed to warm up. The instrument zeros itself between each sample.

8.2.2 Set up the analytical run as presented in Table 2. If data system automates the analytical run, program samples into software.

Table 2. Analytical Sequence

Sample	Comment
Calibration Blank (CB)	Blank working standard
Standard 1 (S1)	Calibration standard #1
Standard 2 (S2)	Calibration standard #2

Sample	Comment
Standard 3 (S3)	Calibration standard #3
Standard 4 (S4)	Calibration standard #4
Standard 5 (S5)	Calibration standard #5
Standard 6 (S6)	Calibration standard #6
ICV	QC check--must be within ±10%
ICB	Instrument calibration blank
LCS	QC check--must be within control chart limits
MB/Calibration blank	No contamination above the MDL
1-9 Samples	Samples include field and QC (MS, MD, etc.)
CCV	QC check must be within 20% of known value
CCB	QC check must not indicate contamination above the MDL

Repeat box until samples are exhausted.

Then end run with:

CCV

CCB

8.2.3 The instrument is programmed to automatically mix the proper amount of stannous chloride to reduce the mercury to metallic mercury which is pumped to the liquid gas separator where it is separated into the gaseous phase. The program will continue aerating until the absorbance reaches a maximum reading (usually within 30 seconds). The program saves the reading. The program continues aerating until the absorbance reading falls back to zero or baseline reading.

Note: Between each standard, sample, or QC sample, check the instrument zero with reagent water.

8.2.4 Construct a calibration curve by plotting the absorbances of the standards versus the true mercury concentration (ppb of mercury). The instrument software is programmed to automatically construct a calibration curve by plotting the fluorescence of the standards versus the true mercury concentration.

8.2.5 The results of the ICV must be within control limits of ±10% for the curve to be acceptable for sample analysis. Verify the LCS is within control chart, or default limits.

8.2.6 Proceed with sample analysis as in Section 8.2.3. Check the calibration curve with the midpoint standard (CCV) after every 10 samples and at the end of the analytical sequence.

8.2.7 Determine sample concentrations (in ppb of mercury). Calculate the concentration in mg/kg as presented in Section 9.1. For sample absorbances that exceed the highest calibration standard, dilute and rerun the sample.

9.0 Calculations

9.1 Calculate the mercury concentration for each sample on a dry weight basis as follows:

$$Concentration\ (mg/Kg) = \frac{A}{W} \times 100$$

where:

A = ppm mercury read from standard curve
W = weight in g of sample (dry weight basis)
100 = the extract volume used

9.2 Percent Recovery

9.2.1 For LCS, recovery is calculated using:

$$\%\ Recovery = \frac{A}{B} \times 100$$

where:

A = µg/L mercury measured
B = known true value

9.2.2 For MS/MSDs, recovery is calculated using:

$$\%\ Recovery = \frac{|Sample + Spike\ Result - Sample\ Result|}{Spike\ Added}$$

9.3 Relative percent difference

$$RPD = \frac{|S_1 - S_2|}{(S_1 + S_2)/2} \times 100$$

where:

S_1 and S_2 represent sample and duplicate sample results, or matrix spike and matrix spike duplicate results.

10.0 Quality Assurance/Quality Control

10.1 A preparation batch of samples is defined as a group of up to twenty field samples of similar matrix type that have been prepared at the same time or time sequence with the same lots of reagents for the same analysis. In addition to the twenty samples, each preparatory batch will contain at a minimum, a method blank, a laboratory control sample, a matrix spike, and a matrix duplicate. A matrix spike duplicate may be included if required in the project specific DQOs. An analytical, or instrumental batch is defined as samples that are analyzed together within the same time period or in continuous sequential time periods. Within the analytical batch are included individual QC requirements as defined by the analytical (determinative) method. For instance, each analytical batch would begin with the 6 point calibration plus a calibration blank, an ICV, ICB, an LCS, followed by the up to ten QC (normally a method blank, MS, (MSD), MD, etc.) and field samples, then continuing calibration verification, an instrument blank, and so on. Preparation batches of samples may be continuously strung together in these run sequences, as long as the analytical batch QC requirements meet the acceptance criteria established within the appropriate SOP. At the conclusion of the last sequence, a CCV and CCB are required. Each analytical sequence must be documented using the run log in Figure 1.

10.2 Run a 6-point initial calibration curve plus a calibration blank, using the primary source standards with each sample preparation batch. Acceptance criteria are presented in Section 11.1.

10.3 A method blank must be prepared with each batch of samples. The method blank should be prepared with reagent water. Acceptance criteria for these blanks are listed in Section 11.2.

10.4 Run an ICV standard using second source standards following the 6-point calibration curve Acceptance criteria are presented in Section 11.3.

10.5 A laboratory control sample must be prepared and analyzed with each batch of samples. The LCS would be prepared using the primary source standard. Control charts will be maintained for the LCS. Acceptance criteria are presented in Section 11.4.

10.6 Run a mid-point Continuing Calibration Verification (CCV) using the primary source standards after every 10 samples (bottles), and at the end of the analysis. Acceptance criteria are listed in Section 11.5.

10.7 The use of the matrix spike and matrix spike duplicate will depend in part on what role the laboratory is playing. When the laboratory serves the role as the primary laboratory, then site-specific documents should be consulted. The

sample to be used for the MS/MSD may be specified in the field. This previously designated sample would then be spiked a concentration equivalent to the site action level. The MS/MSD would be prepared using the primary source standards. If this information was not specified or unknown, then the laboratory would choose a representative sample from each batch of samples analyzed. If samples from multiple sites were to be analyzed in the same batch, then multiple sets of MS/MSDs may be required. When the laboratory serves the role as the QA laboratory, the above scenario may not be practical to implement. If the site-specific requirements are unknown and samples from multiple sites are analyzed in the same batch, then the laboratory should select a single sample for spiking. Each batch of samples would then contain at least one MS/MSD pair. Acceptance criteria are presented in Section 11.6 for percent recovery and RPD.

10.8 The use of the matrix duplicate may also depend in part on the role the laboratory is playing. The selection of a matrix duplicate will be performed as described for the MS/MSD in Section 10.7. Acceptance criteria are presented in Section 11.7 for RPD.

10.9 Data shall be checked to ascertain if it conforms to accepted practices for data reporting. All sample analytical results used for final data reporting must be above that of the low standard used during the initial calibration. Results which fall below the low standard are to be reported as estimated values. Corrective actions are described in Section 11. Other reporting limits can be used based upon site specific criteria.

10.10 MDLs are determined in reagent water and verified annually. (Project-specific requirements may require that the MDL study be performed in the site-specific matrix.) Refer to SOP No. Q-019-ECC-QC, Method Detection Limits (MDLs), Method Quantitation Limits (MQLs), and Laboratory Reporting Limits (LRLs).

10.11 The analyst must demonstrate proficiency in performing the analysis as outlined in SOP No. Q-016-ECC-QC, Technical Training. Method proficiency must be redemonstrated anytime a major method modification is made, a major software revision is added, or a major instrument modification is made. Demonstration of method proficiency may also be required after major instrument maintenance. This is decided on a case by case basis through discussions with the Section Chief, Laboratory Director, and Laboratory QA Officer.

11.0 Data Validation

Site-specific requirements must be checked and used, if known, for data review. The criteria presented in this section should be used as a default list in the absence of site specific requirements. The items shall be verified and documented using the data review checklist in Figure 2.

11.1 The calibration curve shall be linear. The correlation coefficient of the curve shall be $\geq$ 0.995.

11.2 Assess the method blanks. The analyst shall confirm that these blanks were analyzed at the required frequency.

The method blank should not exhibit any mercury contamination above the MDL. Corrective action should be performed any time mercury is detected above the MDL to reduce and control contamination. Corrective action will be required if mercury is detected at greater than 5% of the regulatory limit or greater than 5% of the sample result, or if detected above the low standard used during initial calibration. Corrective action would include reanalysis of field and QC samples in the batch if some or all of the samples also contained mercury levels that exceeded the above criteria. If none of the field samples had values above the stated criteria, then reanalysis may not be necessary. The source of contamination should still be investigated and reduced/eliminated. Any time contamination is noted in the method blank, the situation and impact on the data should be discussed in the case narrative.

11.3 After the 5-point initial calibration, verify the initial calibration verification (ICV) was performed. The recovery of the ICV must be within 90 - 110%. If not, reanalyze or prepare a new calibration curve as necessary.

11.4 Assess that LCSs were prepared at the required frequency. Plot on the appropriate control charts. (Refer to SOP No. Q-009-ECC-QC, Control Chart Generation, Maintenance, and Usage.) If not, control limits, redigestion and reanalysis of the sample preparation batch is necessary.

Note: Even though control charts must be maintained for the LCSs, the acceptance criteria derived from these charts may not be appropriate if the charted ranges are too wide. A maximum default range should be used. It is recommended that the maximum default range be set to 80-120%. The maximum default ranges for the LCS is set at the recommended range of 80-120%.

The RPD of interbatch LCSs should fall within the control limits determined from the precision control charts. However, if the RPD is outside these control limits, the batch will not be rejected, as long as the LCS recovery is acceptable. This precision information should be evaluated to see if systematic problems can be identified. If problems are suspected, the method should be fully evaluated.

11.5 Verify that the continuing calibration verification (CCV) standard was run at the required frequency (after every 10 samples and at the end of the analytical sequence). The acceptance criteria is that the CCV must be within 20% of the true value.

If a CCV fails during an analytical sequence, the previous ten samples need to be reprocessed from calibration through analysis.

11.6 Assess that matrix spike/matrix spike duplicates were analyzed at required frequency. The analyst shall also verify that the samples were spiked at the appropriate level. The order of preference for spiking levels is as follows; 1) If

the target analyte concentrations are known, spike to increase the background concentration by a factor of approximately two, 2) if an action level exists, spike at this level, or 3) if neither of the first two conditions apply, spike at a level that corresponds between the low and mid-level calibration standards. Acceptance criteria are that all % Recovery and/or RPD results meet project established goals. If no project goals are specified, then results must be within the indicated control limits on the appropriate LCS control charts. If these conditions are not met, perform the following corrective actions as appropriate.

- If both LCS and MS/MSD recoveries are unacceptable, then the entire batch of field and QC samples must be redigested and reanalyzed.

- If the MS/MSD is unacceptable, but the LCS is acceptable, then a potential matrix effect has been identified. Redigest and reanalyze the MS/MSD to verify matrix effect. If a matrix effect is still suspected then the project manager must be contacted to discuss further alternatives and the potential impact on the project. Reasonable attempts must be made to address a matrix interference. Reported data should be flagged.

11.7 Assess matrix duplicates were analyzed at required frequency. Acceptance criteria are that all RPD results meet project established goals. If no project goals are specified, then results must be within the indicated control limits on the appropriate LCS precision control charts. If these conditions are not met, perform the following corrective actions as appropriate.

- Reanalyze that sample to verify a matrix effect.

- If the duplicate precision is still unacceptable, then a potential matrix effect has been identified. The project manager must be contacted to discuss further alternatives and the potential impact on the project.

11.8 The analyst must verify all reported results are derived from analytical results that are below the highest standard of the initial calibration curve and above the low standard. Values reported below the low standard are to be reported as estimated values (J values). For samples that exceed the calibration curve, reanalyze using a smaller sample size.

11.9 Besides the items listed in Sections 11.1 through 11.8, the analyst should also verify the additional items as noted in Figure 2.

11.10 Additional levels of review are performed as described in SOP No. Q-024-ECC-DR, Data Reduction and Review/Validation (In-House/Contractor Data).

12.0 Waste Disposal

This procedure generates corrosive and metallic wastes that must be disposed of in accordance to all local regulations. Refer to the ERDC WES site Waste Management

Plan.

13.0 References

13.1 Test Methods for Evaluating Solid Waste Methods, SW-846, Third Edition, Update II, July 1992, Method 7471A.

13.2 SOP No. Q-003-ECC-QC, Standards Preparation, Traceability, and Storage.

13.3 SOP No. Q-008-ECC-QC, Reagent Water Generation and Quality Monitoring.

13.4 SOP No. Q-005-ECC-SH, Sample Receipt, Login, and Storage.

13.5 SOP No. Q-019-ECC-QC, Method Detection Limits (MDLs), Method Quantitation Limits (MQLs), and Laboratory Reporting Limits (LRLs).

13.6 SOP No. Q-016-ECC-QC, Technical Training.

13.7 SOP No. Q-024-ECC-DR, Data Reduction and Review/Validation (In-House/Contractor Data)

13.8 SOP No. Q-009-ECC-QC, Control Chart Generation, Maintenance, and Usage.

13.9 SOP No. M-2216-ECC-GC, Determination of Water (Moisture) Content of Soil (ASTM Method 2216).

13.10 ERDC WES site Waste Management Plan.

MERCURY ANALYTICAL LOG
ERDC EL EEC

Inst.:	File Name:
OID No.:	Project:
Parameters Analyzed:	Method:

AZ			
S1			
S2			
S3			
S4			
S5			
S6			
RS			

	Sample ID	spike	DILUT
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	Sample ID	SPIKE	DILUT
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FIGURE 1

Data Review Check List for Mercury
ERDC EL EEC

Project Number(s)	
Batch Number(s)	
SOP No.	

	Review Item	Yes (x)	No (x)	N/A (x)	2nd Level Review (x)
1.	Does the daily standard curve consist of a Calibration Blank and the required 5 calibration standards?				
2.	Is the low standard near, but above, the MDL?				
3.	Is the LCS from a second source and is its percent recovery within QC limits?				
4.	Are the CCV standards analyzed at required frequency and at the end of the analytical sequence and meet QC limits?				
5.	Is the Matrix Spike/Matrix Spike Duplicate run at the desired frequency and is the percent recovery/RPD within QC limits?				
6.	Is the Matrix Duplicate run at the desired frequency and is the RPD within QC limits?				
7.	Are all samples with concentrations > the highest standard used for initial calibration reprocessed and reanalyzed?				
8.	Are all sample holding times met?				
9.	Are all nonconformances included and noted?				
10.	Is the correct methodology used for sample prep and analysis?				
11.	Are all calculations checked at the minimum frequency?				
12.	Did analyst sign/date the appropriate printouts and report sheets?				
13.	Are all sample ID and units checked for transcription errors?				

Comments on any "No" response:

_____

_____

_____

_____

Analyst: _____ Date: _____

2nd Level Reviewer: _____ Date: _____

Figure 2.

ENVIRONMENTAL CHEMISTRY CENTER
ENVIRONMENTAL LABORATORY
ERDC

CEERD-EE-C
3909 HALLS FERRY ROAD
VICKSBURG,MS 39180

TITLE: Explosives
(SW-846 Method 8330)

SOP NUMBER: M-8330-ECC-OP/A, Ver. 1.1

PREPARED BY: B. Lynn Cocalon 6/28/00
Date

REVIEWED BY: Karen J Myers 6/29/00
Technical Specialist Date

Karen J Myers 6/29/00
QA Officer Date

Richard A. Kaun 6/29/00
Laboratory Director Date

SOP MANUAL CONTROL NO.: _____

1.0 Scope and Application

1.1 The procedures in this SOP are used for the extraction and trace analysis of explosive residue in water and soil/sediment matrices by high performance liquid chromatography (HPLC) using a UV detector. Table 1 lists the target compounds. The listing in Table 1 will be used in the absence of project-specific requirements.

Table 1. Target Compounds

Compound	Abbr.	CAS No.
Octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine	HMX	2691-41-0
Hexahydro-1,3,5-trinitro-1,3,5-triazine	RDX	121-82-4
1,3,5-Trinitrobenzene	TNB	99-35-4
1,3-Dinitrobenzene	DNB	99-65-0
Methyl-2,4,6-trinitrophenylnitramine	Tetryl	479-45-8
Nitrobenzene	NB	98-95-3
2,4,6-Trinitrotoluene	TNT	118-96-7
4-Amino-2,6-dinitrotoluene	4-A-DNT	1946-51-0
2-Amino-4,6-dinitrotoluene	2-A-DNT	355-72-78-2
2,4-Dinitrotoluene	2,4-DNT	121-14-2
2,6-Dinitrotoluene	2,6-DNT	606-20-2
2-Nitrotoluene	2-NT	88-72-2
3-Nitrotoluene	3-NT	99-08-1
4-Nitrotoluene	4-NT	99-99-0

1.2 Appendix 1 provides MDLs and reporting limits for target analyte compounds in low- and high-level waters as well as soils.

2.0 Method Summary

2.1 This SOP provides high performance liquid chromatographic (HPLC) conditions for the detection of ppb (µg/L) levels of certain explosives residues in water, soil and sediment. Samples must be appropriately extracted prior to HPLC analysis.

2.2 There are two appropriate sample preparation techniques for aqueous samples.

2.2.1 **Low-level Salting-out Method with No Evaporation**: Aqueous samples of low concentration are extracted by a salting-out extraction procedure using acetonitrile and sodium chloride. The small volume of acetonitrile which remains undissolved above the salt water is drawn off and transferred to a smaller volumetric flask. It is then back-extracted by vigorous stirring with a specific volume of salt water. After equilibration, the phases are allowed to separate and the small volume of acetonitrile residing in the narrow neck of the volumetric flask is removed using a Pasteur pipet. The concentrated extract is diluted 1:1 (v/v) with reagent grade water. An aliquot is separated on a Supelco C-18 reverse phase column, determined at 245 nm, and confirmed on a Supelco CN reverse phase column at 245 nm.

2.2.2 Low-**level Solid Phase Method With No Evaporation**: Aqueous samples of low concentration are extracted by a solid phase extraction procedure using a vacuum manifold and solid phase cartridges (Waters SepPak Vac cc (500 mg) Porapak RDX, catalog # WAT047220). The water samples are concentrated onto the cartridges and are eluted off using a small volume of acetonitrile. The concentrated extract is diluted 1:1 (v/v) with reagent grade water. An aliquot is separated on a Supelco C- 18 reverse phase column, determined at 245 nm, and confirmed on a Supelco CN reverse phase column at 245 nm.

2.2.3 High-level Direct Injection Method: Aqueous samples of higher concentration can be diluted 1:1 (v/v) with methanol or acetonitrile, filtered, separated on a Supelco C-18 reverse phase column, determined at 245 nm, and confirmed on a Supelco CN reverse phase column at 245 nm. If HMX is an important target analyte, methanol is preferred.

2.3 Soil and sediment samples are extracted using acetonitrile in an ultrasonic bath, filtered, and treated as described in the high-level direct injection method.

3.0 Health and Safety

3.1 Soil samples as high as 2% 2,4,6-TNT have been safely ground. Samples containing higher concentrations must not be ground in the mortar and pestle.

3.2 A visual inspection of the sample shall be performed prior to analysis.

3.2.1 Lumps of material having a chemical appearance are considered suspect and shall not be ground.

3.2.2 Explosives are generally a very finely ground grayish-white material.

3.2.3 Pure TNT crystals can be straw-colored or dark red or brown when exposed to sun light.

3.3 The toxicity or carcinogenicity of each compound or reagent used in this procedure has not been precisely determined. Each chemical should be treated as a potential health hazard and be handled in a fume hood. Each laboratory is responsible for maintaining awareness of OSHA regulations regarding safe handling of chemicals used in this method. MSDS sheets are available in Room # 111 and Room # 122 and should be consulted as needed.

3.4 This procedure employs organic solvents that are flammable and/or may pose a risk through inhalation. Therefore, exposure to these solvents should be reduced to the lowest possible level and must be handled in a hood with the analyst wearing the appropriate personal safety equipment (i.e., lab coat, gloves, goggles, etc.) at all times.

3.5 When preparing standard solutions from neat material, all weighing tools must be Teflon coated. Weighing shall be performed behind an explosion-proof shield.

4.0 Sample Preservation, Containers, Handling, and Storage

4.1 Requirements for sample preservation, sample containers, and sample storage are detailed in SOP No. Q-005- ECC-SH, Sample Receipt, Login, and Storage.

4.2 Sample extracts must be stored in the dark at 4 ± 2°C. Soil or sediment samples must be extracted within 14 days of collection, and analyzed within 40 days of extraction. Water samples must be extracted within 7 days of collection, and analyzed within 40 days of extraction.

5.0 Interferences and Potential Problems

5.1 Solvents, reagents, glassware and other sample processing hardware may yield discrete artifacts and/or elevated baselines, causing the misinterpretation of chromatograms. All of these materials must demonstrate freedom from interference. Refer to SOP Nos. Q-012-ECC-GL, Glassware Cleaning and Q-015-ECC-QC, Reagent Control.

5.2 2,4-DNT and 2,6-DNT elute at similar retention times (retention time difference of 0.2 minutes). A large concentration of one isomer may mask the response of the other isomer. If it is not apparent that both isomers are present (or are not detected), an isomeric mixture must be reported.

5.3 Tetryl decomposes rapidly in methanol/water solutions, and also with heat. All aqueous samples expected to contain Tetryl must be diluted with acetonitrile prior to filtration. All samples expected to contain Tetryl must not be exposed to temperatures above 30°C.

5.4 Tetryl's degradation products appear as a shoulder on the 2,4,6-TNT peak. Peak

heights rather than peak areas should be used when Tetryl is present in concentrations that are significant relative to the concentration of 2,4,6-TNT.

6.0 Equipment/Apparatus

6.1 HPLC system

6.1.1 HPLC — an analytical system equipped with a Waters 610 Fluid Unit pump capable of achieving 6000 psi , a Waters 717plus Autosampler including a 200 µl loop injector, a Waters 486 Tunable UV Absorbance detector monitored at 245 nm and Millenium 2.1 Chromatography Software (Waters Chromatography Division, Milford, MA).

6.1.2 Primary column — Supelco LC-18 reverse phase HPLC column 25 cm x 4.6 mm (5 µm), Catalog # 5-8298.

6.1.3 Secondary (confirmatory) column — Supelco LC-CN reverse phase HPLC column 25 cm x 4.6 mm (5 µm), Catalog # 5-8231.

6.1.4 Filtration system to filter and degas HPLC mobile phase—Millipore Solvent and Degassing Unit (catalog # XX1504700)with 0.22 µm filter (catalog # GVWP04700) (attaches to vacuum) and Waters In-Line Degasser.

6.1.5 Pre-column — Novapak C-18 catalog # WAT015220 and Novapak CN catalog # WAT020800 (Waters Chromatography Division, Milford, MA)

6.1.6 Column heaters — Cera Column Heater 250 set at 30° C, catalog # 282-0252 (Cera, Inc., Baldwin Park, CA)

6.2 Refrigerator, Explosion-proof, capable of maintaining 4 ± 2°C.

6.3 Temperature-controlled ultrasonic bath, not to exceed 30° C. — Cole-Pamer Instrument Co., model # 8855-00.

6.4 Vortex mixer (Glas-Col model VB2 or equivalent) — Scientific Industries Vortex Genie 2, model G-560

6.5 Balance capable of measuring to ± 0.0001g.

6.6 Magnetic stirrer with stirring pellets.

6.7 Tube Rotator — Scientific Equipment Products, catalog # 60448

6.8 Oven — Forced air, without heating — GS Blue M Electric, Model 256

6.9 Filtration system and disposable cartridge filters (0.45 µm Teflon filter). Vacuum Filtration System from Millipore (catalog # XX1504700) with .22 µm filters (catalog #GVWP04700) to degas mobile phase. Millipore Millex-SR .50 µm

Teflon filters (catalog # SLSR025NB) for all samples.

6.10 Volumetric pipets — Class A 1-mL, 2.5-mL, 10-mL, or appropriate volume.

6.11 Disposable glass pasteur pipets.

6.12 Vials, scintillation, 20 mL.

6.13 Vials — 10-mL, 20-mL, 40-mL glass, Teflon-lined cap, or appropriate volume.

6.14 Volumetric flasks — Class A, round bottom, 10-mL, 25-mL, 100-mL, and 1L, or appropriate volume with tight fitting plastic snap caps, for use for the low level salting out procedure.

6.15 Volumetric flasks — Class A, 10-mL, 25-mL, 100-mL, and 1L, or appropriate volume for use for in standards preparation.

6.16 Vacuum desiccator.

6.17 Mortar and pestle — Ceramic. When processing a large number of samples at a time, it is better to have several mortar and pestle sets available to facilitate sample processing.

6.18 Sieve — 30 mesh.

6.19 Graduated cylinders — Class A, 10-mL, 25-mL, 250-mL, 1-L, or appropriate volume.

6.20 Disposable Syringes — 10 cc Luer-Lok syringe, VWR catalog # BD30-9604

6.21 Automatic pipets with disposable tips.

6.22 Automatic diluter or dispenser — Hamilton MicroLab 500 Diluter

6.23 Freezer, Explosion-proof, capable of maintaining -15° ± 5°C.

6.24 Solid Phase Cartridges — Waters SepPak Vac cc (500 mg) Porapak RDX cartridges, catalog # WAT047220

6.25 Solid Phase Extraction Vacuum Manifold (Supelco, catalog # 5-7250) with attached 4000 mL vacuum trap

6.26 15 mL Graduated conical bottomed centrifuge tubes (Fisher, catalog # 05-495R)

6.27 500 mL fleakers (Fisher, catalog # 02-599-19)

6.28 SepPak adapters (Supelco, catalog # 5-7020)

6.29 60 cc LuerLok syringe (used as reservoirs for solid phase extraction), VWR,

catalog # BD30-9663

6.30 FreeZone 4.5 liter benchtop Freeze Dry System, Model 77500

6.31 Omni Mixer ES Homogenizer, catalog # ES-115

6.32 Glass wool — muffle furnace extracted glass wool, Fisherbrand, catalog # 11-390

6.33 Florisil- Fisher Scientific, catalog # F100-500

6.34 Alumina- Fisher Scientific, catalog # A540-500

7.0 Reagents

7.1 Reagent grade inorganic chemicals must be used in all tests. Unless otherwise indicated, it is intended that all reagents shall conform to the specifications of the Committee on Analytical Reagents of the American Chemical Society, where such specifications are available. Other grades may be used, provided it is first proven the reagent is of sufficiently high purity to permit its use without lowering the accuracy of the determination.

7.2 Acetonitrile, CH_3CH — HPLC grade.

7.3 Methanol, CH_3OH — HPLC grade.

7.4 Calcium chloride, $CaCl_2$ — Prepare an aqueous solution of 5 g/L. Weigh 5 g to the nearest milligram and place in a 1-L volumetric flask. Dilute the solution to 1-L using reagent water. Stopper until use.

7.5 Sodium chloride, NaCl (salt) — Prepare a solution to contain 325 g NaCl per 1000 mL reagent water. Dissolve 325 g NaCl in 1 L of water to make a saturated sodium chloride. Place salt solution onto a magnetic stir plate, add a stir bar, and mix at maximum speed (using no heat) until NaCl is completely dissolved. Make enough for all samples to be extracted (~90 mL each).

7.6 Organic-free reagent water — as defined in SOP No. Q-008-ECC-GL, Reagent Water Generation and Quality Monitoring.

7.7 Stock Standard Solutions — Both primary and secondary source stock standards shall be stored in the dark at < 6°C. Follow supplier's instructions. These stock solutions may be used for up to one year.

7.7.1 Stock primary source standard solutions shall be made from neat or crystalline stock explosives standards obtained from the Army Environmental Center at Aberdeen Proving Ground. Stock solutions will be prepared as single analyte solutions of 1,000 μg/mL in acetonitrile.

These stock solutions will be used to prepare the initial 5-point calibration curve, continuing calibration verification (CCV) standard, surrogates, laboratory control sample (LCS), and matrix spikes and their duplicates (MS/MSDs). See Sections 7.8, 7.9, 7.10, 7.11, and 7.12.

Each analyte is to be dried (at ambient temperature) to a constant weight in a vacuum dessicator in the dark. Weigh 0.100 g ± 0.001 g of the analyte into a 100 mL volumetric flask and dilute to volume with acetonitrile. Invert several times to dissolve. Store in an explosion proof refrigerator at < 6°C in the dark. Calculate the concentration from the actual weight used (see Section 9.1).

7.7.2 Secondary source standards shall be purchased as certified solutions for use as the initial calibration verification (ICV) standard. This standard shall be at a concentration near the mid-level calibration standard. (When these standards are purchased, flexibility from this criterion may be exercised.) Ultra Scientific, catalog # NAIM833C, contains 1000 µg/ml of HMX, RDX, TNB, NB, TNT, and 2,4-DNT (Diluted to 1.0 ppm for working secondary source).

7.8 Intermediate Standards

7.8.1 Intermediate standards are prepared from the above stock solution(s). These standards are then used to create the calibration standards for the initial 5-point calibration curve and the ICV.

7.8.2 If both 2,4-DNT and 2,6-DNT are to be determined, prepare two separate solutions; the first containing HMX, RDX, 1,3,5-TNB, 1,3-DNB, NB, 2,4,6-TNT, 4-A-DNT, and 2,4-DNT, and the second containing Tetryl, 2-A-DNT, 2,6-DNT, 2-NT, 3-NT, 4-A-DNT, and 4-NT. When analyzing soil samples, prepare solution in acetonitrile; use methanol when analyzing aqueous samples. Refer to Table 2.

Table 2. Intermediate Standard Preparation

Compound	Intermediate 1			Intermediate 2		
	mL Stock	Final vol., mL	Conc., mg/L	mL Stock	Final vol., mL	Conc., mg/L
HMX	1	10	1			
RDX	1	10	1			
TNB	1	10	1			
DNB	1	10	1			
Tetryl				1	10	1
NB	1	10	1			

Compound	Intermediate 1			Intermediate 2		
	mL Stock	Final vol., mL	Conc., mg/L	mL Stock	Final vol., mL	Conc., mg/L
TNT	1	10	1			
4-A-DNT	1	10	1			
2-A-DNT				1	10	1
2,4-DNT	1	10	1			
2,6-DNT				1	10	1
2-NT				1	10	1
3-NT				1	10	1
4-NT				1	10	1

7.8.3 Intermediate Standards are to be stored at < 6°C in the dark. Standards should be allowed to come to room temperature and be thoroughly mixed prior to use. Standards can be used beyond 30 days if instrument response of CCV is monitored and compared to response of freshly prepared standard. Intermediate standards should be replaced if degradation occurs such that the CCV response exceeds ±15% of the original response.

7.9 Calibration Standards

7.9.1 The two intermediate standards in Section 7.8.2 are diluted to generate two sets of calibration standards at five concentration levels. The low standard is set at a concentration approximately 3 to 5 times the MDL and the high standard is typically chosen to correspond to the linear range of the instrument. The calibration curve presented in Section 8.5.3 is used in the absence of project-specific information. Specific client requirements may dictate that a different curve be constructed.

7.9.2 Table 3 summarizes the solvents and diluents used in the preparation of the calibration standards.

Table 3. Diluents for Calibration Standards

Sample Type	Sample Matrix	Diluent	Factor	Std. Matrix	Diluent	Factor
Low Level Water	Acetonitrile	Reagent Water	1:1 (v:v)	Acetonitrile	Reagent Water	1:1 (v:v)
High-Level Water	Water	Acetonitrile	1:1 (v:v)	Acetonitrile	Reagent Water	1:1 (v:v)

Soil	Acetonitrile	5 g/L $CaCl_2$	1:1 (v:v)	Acetonitrile	5 g/L $CaCl_2$	1:1 (v:v)

Depending on the columns and eluent used in the HPLC analysis, as well as the types of samples received, the lab may vary the solvents and dilution ratios in order to achieve acceptable separation.

7.10 Surrogate Spiking Solution

Surrogate solutions are prepared from the primary stock standards. The use of one surrogate is mandatory; the use of additional surrogates is optional. See Table 4.

Table 4. Surrogate Spikes

Method	Surrogate Used	Surrogate Solution Concentration (mg/L)	Volume Surrogate Added (mL)	Per Sample Volume	Final Surrogate Concentration
Low-Level Water	3,5-DNA	50	0.050	700 mL	0.0036 mg/L
High-Level Water	3,5-DNA	50	0.050	5 mL	0.5 mg/L
Soil	3,5-DNA	50	0.050	2 g	1.25 mg/kg

7.11 Matrix spike (MS) standards

7.11.1 The matrix spike standard shall be prepared from the primary Stock standards. Using Class A volumetrics, measure 5 mL from the 1000 ppm stock solutions of HMX, RDX, TNB, TNT, 4-A-DNT, and 2,4-DNT into a 50 mL volumetric (final solution is 100 ppm). Add acetonitrile to the line and vortex. Keep in the freezer at -15° ± 5°C.

7.11.2 The spike should be at a level that would approximately double the concentration of the target analytes present, if known. In the absence of target analytes, the spike would be made at the site action level, assuming that this level did not also correspond to the value of the low standard used. If the action level is the same as the low standard used, then the spiking would occur at a slightly higher level, i.e., at the value of the next calibration standard used. If the action level is not known, the spiking level would then default between the low and mid-level initial calibration standards.

7.11.3 Ideally, all target analytes should be contained in the MS spike. Subset target analytes may be used based on project specific requirements. In the absence of project related information, a representative subset

of the target analytes may be used. The following is a recommended default subset — HMX, RDX, TNB, TNT, 4-A-DNT, and 2,4-DNT. See Table 5.

Table 5. Matrix Spiking Solutions

MS Std. Compounds	Method	MS Std. Conc. mg/L	Vol. MS Added mL	Sample Aliquot	Final MS Conc.
HMX RDX TNB TNT 4-A-DNT 2,4-DNT	Salt-Out	10	0.200	1 L	0.0020 mg/L
	SPE	5	0.100	500 mL	0.0020 mg/L
	High-Level Water	100	0.100	5mL	2.0 mg/L
	Soil	100	0.100	2 g	5 mg/kg

7.12 Laboratory Control Sample (LCS)

7.12.1 The LCS shall be prepared from the primary stock standard. The LCS is the same as the MS solution in section 7.11.1

7.12.2 The LCS shall be prepared in the appropriate matrix (organic-free reagent water, or purified solid) depending upon the matrix within the batch; and contains all of the method target analytes. A subset of target analytes could be used based on the project specific requirements. The spiking level used would be at the same level as the site-specific action limit. If site-specific action limits are not available, the spiking level shall be at a concentration between the low and the mid-level calibration standard.

7.13 HPLC Mobile Phase

To prepare 1 L of mobile phase (1:1 (v/v) methanol/reagent water), measure 500 mL of each using graduated cylinders and combine. Mix thoroughly. Filter the mixture through a 0.45 micron filter and degas prior to use.

8.0 Procedure

The low-level method is used for aqueous samples with expected single component explosive concentrations below 50 μg/L. If expectant concentrations are not known, project-specific DQOs should be consulted. Project-specific minimum reporting limits will help determine whether the low or high-level method should be used. Process waste samples should be screened to determine which approach is appropriate.

Extraction information should be recorded using Figure 1 or 2, as appropriate.

8.1 Preparation Of Aqueous Samples by Low-Level Method (salting-out extraction).

8.1.1 Using a 1-L graduated cylinder, measure out 770 mL of sample, and add it to a 1-L volumetric flask.

8.1.2 Spike samples and associated QC samples with surrogate spiking solution and matrix spike solution, (see Sections 7.10 and 7.11). After addition of the surrogate and matrix spiking solutions, the samples are to be swirled or shaken to allow complete mixing of the solutions within the sample.

8.1.3 Using a disposable weigh dish, weigh out 251.3 ± 0.5 g of sodium chloride (NaCl) and add to the sample.

8.1.4 Place sample on a magnetic stir plate, add a stir bar, and mix at maximum speed (using no heat) until NaCl is completely dissolved.

8.1.5 Using a 250-mL graduated cylinder, add 164 ± 2 mL of acetonitrile while the solution is being stirred and stir for an additional 15 min. Turn off the stirrer and allow the phases to separate for 10 min. Remove the acetonitrile (upper) layer (approximately 8 mL) with a disposable glass pasteur pipet and transfer it to a 100-mL volumetric flask with a plastic snap lid.

CAUTION: Incomplete mixing will result in low extraction efficiencies. In some cases the vortex doesn't reach the top of the liquid column in the neck of the flask. When that happens the acetonitrile sits on top of the stirring sample and little to no extraction takes place. If the sample prep technician walks away for the 15 minutes, they may not notice this. They need to establish a complete mix and then monitor it, adjusting the flask as necessary in order to get a complete extraction.

8.1.6 Add 10 mL of fresh acetonitrile to the water sample in the 1-L flask. Again, stir the contents of the flask for 15 min., followed by 10 min. phase separation. Combine the second acetonitrile portion with the initial extract. The inclusion of a few drops of salt water at this point is unimportant.

8.1.7 Using a 100-mL graduated cylinder, add 84 mL ± 2 mL of NaCl solution (see Section 7.5) to the acetonitrile extract in the 100-mL volumetric flask. Cap the volumetric, clip onto the tube rotator, and mix for 15 min. Allow 10 min. for phase separation. Using a disposable glass pasteur pipet, carefully transfer the acetonitrile phase to a 15 mL graduated centrifuge tube. At this stage, the amount of water transferred with the acetonitrile must be minimized. The water contains

a high concentration of NaCl producing a large peak at the beginning of the chromatogram where it could interfere with the HMX determination.

8.1.8 Add an additional 1.0 mL (using a 1-mL volumetric pipet) of acetonitrile to the 100-mL volumetric flask, recap and return to the tube rotator for 15 min., followed by 10 min. for phase separation. Combine the second acetonitrile portion with the initial extract in the 15 mL graduated centrifuge tube. Record the total volume of acetonitrile extract to the nearest 0.1 mL in the Explosive extraction log book . (Use this as the volume of total extract [V(t)] in the calculation of concentration after converting to uL). The resulting extract, about 5 - 6 mL, is then diluted 1:1 (v/v) with organic-free reagent water prior to analysis.

8.1.9 Filter the extract through a 0.45 µm Teflon filter using a plastic disposable syringe. Discard the first 0.5 mL of filtrate, and retain the remainder in a vial with a Teflon cap for HPLC analysis as in Section 8.6.

8.2 Preparation of Aqueous Samples by Low-Level Method (Solid Phase Extraction)

8.2.1 Using a 500 mL graduated cylinder, measure out 500 mL of sample, and add it to a 500 mL fleaker.

8.2.2 Spike samples and associated QC samples with surrogate spiking solution and matrix spike solution, (see Sections 7.10 and 7.11). After addition of the surrogate and matrix spiking solutions, the samples are to be swirled or shaken to allow complete mixing of the solutions within the sample.

8.2.3 Condition the cartridges:

8.2.3.1 Remove the internal rack from the vacuum manifold.

8.2.3.2 Insert a Sep-Pak adapter into each Propak RDX cartridge.

8.2.3.3 Connect the cartridges to the vacuum manifold.

8.2.3.4 Connect the 60 cc syringe reservoirs to the adapters.

8.2.3.5 Fill each reservoir with 15 mL acetonitrile. Pulse the vacuum pump to start the flow, then let the acetonitrile drip through under gravity alone.

8.2.3.6 Just before each reservoir runs dry, refill the reservoir with 30 mL organic free reagent water.

8.2.3.7 Turn on the vacuum pump and adjust the flow rate with the stopcock valves to draw the water through the cartridge. The

flow rate should not exceed 10 mL/min. Close valves.

CAUTION: Once the packing material has been activated, do not expose it to air until the entire sample has been loaded. If the cartridge runs dry, repeat the conditioning procedure.

8.2.4 To load the sample onto the cartridge, fill the reservoirs with the water sample that is to be concentrated. Turn on the vacuum. Use the stopcocks to adjust the flow rate to about 10 mL/min. Refill the reservoir and empty the vacuum trap as needed. Be sure the bed does not run dry during loading.

8.2.5 After the 500 mL sample is loaded, remove the adapter and reservoir from the cartridge. Set up the vacuum manifold with clean 15mL graduated centrifuge tubes and needles.

8.2.6 Put 5 mL acetonitrile into the cartridge. Pulse the vacuum pump to start the flow, then let the acetonitrile drip through under gravity alone. The flow rate should be about 1 mL/min. After acetonitrile stops dripping, turn on the vacuum briefly (too much vacuum will cause the sample to splatter) to draw the remaining acetonitrile through the cartridge.

8.2.7 Open the manifold and remove the centrifuge tubes. Fill the centrifuge tubes to the 5 mL mark with acetonitrile and vortex to mix thoroughly. Record the total volume of acetonitrile extract to the nearest 0.1 mL in the Explosive Extraction Log Book. (Use this as the volume of total extract [V(t)] in the calculation of concentration after converting to µL). The resulting extract, about 5 mL, is then diluted 1:1 (v/v) with organic-free reagent water prior to analysis.

8.2.8 Filter the extract through a 0.50 µm Teflon filter using a plastic disposable syringe. Discard the first 0.5 mL of filtrate, and retain the remainder in a vial with a Teflon cap for HPLC analysis as in Section 8.6.

8.3 Preparation of Aqueous Samples by High-Level Method

8.3.1 Transfer 5-mL of sample volumetrically into a 20-mL glass vial.

8.3.2 Spike samples and associated QC samples with surrogate spiking solution, and matrix spike solution (see Sections 7.10 and 7.11). After addition of surrogate and matrix spiking solutions, the sample is to be shaken (by hand) to allow complete mixing of the solutions within the sample.

8.3.3 Volumetrically add 5 mL of acetonitrile. (HMX quantitation can be improved with the use of methanol rather than acetonitrile for dilution.)

8.3.4 Using a vortex mixer, mix sample for 2 minutes.

8.3.5 Filter through a 0.45 µm Teflon filter using a plastic disposable syringe. Discard the first 3 mL of filtrate, and retain the remainder in a 10-mL glass vial with Teflon-cap for HPLC analysis as in Section 8.6.

8.3.6 All radioactive samples will be prepared according to Section 8.3. The samples will be contained only in areas that are designated by the ECB Safety Officer for radioactive prep work.

8.4 Soil and Sediment Samples

8.4.1 Thoroughly mix sample as defined in SOP No. Q-021-ECC-GL, Subsampling of Containers. Using disposable weigh dishes, weigh out 10 -20 grams of wet weight sample (that would yield a sample weight of 2.0 g after drying) and allow to air dry. A forced-air oven at room temperature may also be used.

8.4.2 After drying, grind and homogenize thoroughly in an acetonitrile-rinsed mortar (Section 6.17) to pass a 30-mesh sieve.

Weigh out 2.0 ± 0.5 g of each ground soil sample (record weight in the Explosive Prep Lab book into a 20-mL glass vial with a Teflon lined cap.

8.4.3 Spike samples and associated QC samples with surrogate and matrix spike spiking solutions (see Sections 7.10 and 7.11).

8.4.4 Volumetrically add 10.0 mL of acetonitrile.

8.4.5 Using a vortex mixer, swirl for one minute, and place in a cooled ultrasonic bath for 18 hours.

8.4.6 After sonication, allow sample to settle for 30 minutes. Remove 5 mL of supernatant, using a 5-mL pipet with disposable tips, and put in a 20-mL vial. Volumetrically add 5 mL of calcium chloride solution (Section 7.4) to the 5 mL of supernatant. Vortex for 2 minutes and let stand for 15 minutes.

8.4.7 Using a disposable glass pasteur pipet, place the supernatant in a disposable plastic syringe and filter through a 0.45 µm Teflon filter attached to the syringe. Discard the first 3 mL and retain remainder in a 10 mL glass Teflon-capped vial for HPLC analysis as in Section 8.6.

8.4.8 Sample Dilution - Samples are to be diluted in the same matrix as the prepared, filtered sample. Standards must be prepared in this same matrix (see Table 3). Samples are diluted using the automated Hamilton MicroLab 500 diluter for 1:10, 1:100, and 1:1000 dilutions. The

accuracy of the diluter is checked periodically. Standards are diluted using class A volumetrics.

8.4.9 All radioactive samples will be prepared according to Section 8.4. The samples will be contained only in areas that are designated by the ECB Safety Officer for radioactive prep work.

8.4.10 See Appendix 3 for Preparation of Compost and Plant Samples.

8.5 Calibration of HPLC

8.5.1 Chromatographic Conditions: The room is maintained at a constant temperature (between 65-70°F). The equipment is kept in a low humidity and relatively dust free environment.

Primary column: Supelco LC-18 reverse phase HPLC column 25 cm x 4.6 mm (5 µm), Catalog # 5-8231 with a Novapak C-18 pre-column, Catalog # WAT020800. Secondary column:Supelco LC-CN reverse phase HPLC column 25 cm x 4.6 mm (5 µm), Catalog # 5-8231 with a Novapak CN pre-column, Catalog # WAT020800

Mobile Phase: 1:1 (v/v) methanol/organic-free reagent water

Flow Rate: 1.2 mL/min

Injection volume: 50-µL

UV Detector: 245 nm

8.5.2 All electronic equipment must warm up for 30 minutes. During this period, at least 15 void volumes of mobile phase are passed through the column (approximately 30 min at 1.2 mL/min) and continued until the baseline is level at the UV detector's greatest sensitivity.

8.5.3 Initial Calibration — Prepare the calibration curve using the intermediate standards (see Section 7.8) as described in Table 6 below using volumetric flasks and either methanol or acetonitrile as the dilution solvent. . The five calibration standards are project specific (standards bracket the estimated sample concentrations). Calibration is to be performed in singlet. A second source standard (ICV) is analyzed to verify the acceptability of the initial curve. Acceptance criteria are as discussed in Sections 11.1 and 11.2 for the initial curve and ICV, respectively.

Table 6. Initial Calibration Standards

Standard No.	Intermediate Std. Used	Volume of Std. Used	Final Volume	Final Standard Conc.

1	20 mg/L	10 mL	10 mL	20 mg/L
2	20 mg/L	5 mL	10 mL	10 mg/L
3	20 mg/L	2 mL	10 mL	4 mg/L
4	20 mg/L	0.5 mL	10 mL	1 mg/L
5	4 mg/L	1 mL	10 mL	0.40 mg/L
6	1 mg/L	1 mL	10 mL	0.10 mg/L
7	1 mg/L	0.5 mL	10 mL	0.050 mg/L
8	1 mg/L	0.2 mL	10 mL	0.020 mg/L

8.5.4 Peak heights are obtained for each analyte. Calculate the calibration factor (CF) for each analyte as described in Section 9.2. Calculate the % RSD as presented in Section 9.3.

8.5.5 Retention Time Windows

8.5.5.1 Before establishing windows, make sure the HPLC system is within optimum operating conditions. Make three injections of all standard mixtures throughout the course of a 72-hour period. Serial injections over less than a 72-hour period result in retention time windows that are too tight.

8.5.5.2 Plus or minus three times the standard deviation of the retention times for each analyte will be used to define the width of the retention time window; however, the experience of the analyst should weigh heavily in the interpretation of chromatographs.

8.5.5.3 In those cases where the standard deviation (SD) for a particular standard is zero, the analyst should refer to SW-846 Method 8000B for instruction. A zero SD is not acceptable.

8.5.5.4 The laboratory must calculate retention time windows for each analyte on each HPLC column and whenever a new HPLC column is installed. The laboratory must retain the data.

8.5.5.5 Establish the midpoint of the retention time window for each analyte and surrogate by using the absolute retention time established from the mid-level standard of the initial calibration. The absolute retention time window equals the midpoint ± 3SD (as determined above).

8.5.6 Daily Calibration — At a minimum, midpoint calibration standards

(CCVs) are to analyzed in singlet, at the beginning of the analytical run, every 8 hours of continuing analysis, and after the last sample of the day. It is recommended that a CCV be analyzed after every 10 samples.

8.5.6.1 Obtain the calibration factor for each analyte from the peak height and compare it with the calibration factor obtained for the initial calibration. The % difference between the calibration factor for the daily calibration and the calibration factor of the initial calibration must be < 15%, or a new initial calibration must be performed (see Section 9.4). (The laboratory may perform a complete 5 point calibration each day if the analyst determines it is appropriate.)

8.5.6.2 The retention time of all target analytes and surrogates in the CCVs must fall within the absolute retention time windows calculated in 8.5.5.5. If the retention time of any target analyte does not fall within the ±3SD window, then a new initial calibration curve for the failed analytes is necessary unless system maintenance corrects the problem. Reanalysis of all affected sample extracts must also be performed for those failed analytes. Appendix 2 summarizes the estimated retention times on the two columns for a number of analytes analyzable using this method.

8.6 HPLC Analysis

Document appropriate information using the Explosive instrument logbook.

8.6.1 Samples are analyzed in a set referred to as an analytical sequence. The sequence begins with instrument calibration, ICV standard, method blank, and sample extracts interspersed with continuing calibration checks every 10 samples and at the end of the analysis. An analytical sequence is shown in Table 7.

Table 7. Analytical Sequence

Analysis	Purpose
Calibration standard(s)	Initial 5-point calibration or single-point calibration verification.
ICV	For the verification of the initial calibration curve only.
Method blank	Verify that carry over has not occurred from the

	calibration standard, and that the extraction and analytical system do not exhibit contamination above the detection limits of the procedure.
Samples	"Samples" includes all field samples, spiked samples, laboratory control samples, and dilutions.
Calibration verification (CCV) (See Section 8.5.6)	Single-point calibration verification standard, (mid-point standard).
Samples	"Samples" includes all field samples, spiked samples, laboratory control samples, and dilutions.
Calibration verification (CCV) (See Section 8.5.6)	Single-point calibration verification standard, (mid-point standard).

8.6.1.1 All samples analyzed must be bracketed by in-control calibration verification standards (± 15%). If the calibration verification standard is outside the control limits, all the samples analyzed within the out-of control brackets must be reanalyzed.

8.6.1.2 The analytical sequence in Section 8.6.1 may be continued indefinitely, as long as QC acceptance criteria are met.

8.6.2 Each sample is injected by an auto-injector onto a 200 µl loop using 50 µl volume injection. All radioactive samples will be injected by an auto-injector (onto a 200 µl loop using 50 µl volume injection) which has been designated as "radioactive" by the ECB Safety Officer.

8.6.3 If any analyte response exceeds the linear range of the system, dilute the extract and reanalyze. It is recommended that extracts be diluted so that all peaks are on scale. Overlapping peaks are not always evident when peaks are not on scale. Chromatograms should be reviewed at different attenuations via the computer screen after the analysis is complete, to ensure the validity all peaks.

8.6.4 Tentative identification of an analyte occurs when a peak from a sample extract falls within the absolute retention time window. Confirmation is required on a second HPLC column (see Section 8.7). Analyst experience is important in confirming the presence of target compounds.

8.6.5 Sample concentrations are calculated by comparing the sample responses with the initial calibration of the system. Record the resulting peak sizes in peak heights. Refer to Section 9.5 for calculation of analyte concentrations.

8.7 Second Column Confirmation

Any time target analytes are detected on the primary column, second column confirmation must be performed. These confirmation analyses are subject to the same requirements as the primary analyses (i.e., MDL studies, retention time windows, initial and continuing calibrations, etc.). Confirmation shall be based on both qualitative and quantitative agreement. Requirements for qualitative agreement are the same as for the primary analysis (Section 8.6.4). Regarding quantitative agreement, the concentrations determined on the two columns should be within 40% RPD. Refer to Section 11.13 for additional information on confirmation requirements.

9.0 Calculations

$$\text{mg/L Standard} = \%\ \text{Purity x} \left(\frac{\text{mg Standard}}{\text{Solute vol, L}} \right)$$

9.1 The standard is calculated as:

9.2 The calibration factor (CF) is calculated as:

$$CF = \frac{\text{Peak Height}}{\text{Std conc, ng/}\mu\text{L}}$$

9.3 To evaluate the linearity of the initial calibration, calculate the mean CF, the standard deviation (SD), and the RSD as follows:

$$\text{mean CF} = \overline{CF} = \frac{\sum_{i=1}^{n} CF_i}{n}$$

$$SD = \sqrt{\frac{\sum_{i=1}^{n} (CF_i - \overline{CF})^2}{n-1}}$$

$$RSD = \frac{SD}{\overline{CF}} \text{ x } 100$$

where n is the number of calibration standards and RSD is expressed as a percentage (%).

9.4 To evaluate the calibration verification, calculate the % difference as follows:

$$\% \text{ Difference} = \frac{|\overline{CF} - CF_v|}{\overline{CF}} \times 100$$

where CF_v is the calibration factor from the analysis of the verification standard, and $\overline{CF}$ is the mean calibration factor from the initial calibration.

9.5 Target Analyte Concentrations

9.5.1 For aqueous samples

$$\text{Concentration (ug/L)} = \frac{(A_x)(V_t)(D)}{(\overline{CF})(V_s)}$$

where:

A_x = Height of the peak for the analyte in the sample.

V_t = Total volume of the concentrated extract (µL) before diluting 1:1 (v/v) as appropriate.

D = Dilution factor, if the sample or extract was diluted prior to analysis. If no dilution was made, D = 1. The dilution factor is always dimensionless.

CF = Mean calibration factor from the initial calibration (height/ng/µL).

V_s = Volume of the aqueous sample extracted in mL. If units of liters are used for this term, multiply the results by 1000.

Use of the units specified here for these terms will result in a concentration in units of ng/mL, which is equivalent to µg/L. Concentrations may be converted to mg/L by dividing by 1000.

9.5.2 For non-aqueous samples

$$\text{Concentration (ug/kg)} = \frac{(A_x)(V_t)(D)}{(\overline{CF})(W_s)}$$

where A_x, V_t, D, and CF are the same as for aqueous samples, and
W_s = Weight of sample extracted (g). The wet weight or dry weight may be used, depending upon the specific application of the data.

Use of the units specified here for these terms will result in a concentration in units of ng/g, which is equivalent to µg/kg. Concentrations may be converted to µg/g by dividing by 1000.

10.0 Quality Assurance/Quality Control

10.1 A preparation batch of samples is defined as a group of up to twenty field samples of similar matrix type that have been prepared at the same time or time sequence with the same lots of reagents for the same analysis. In addition to the twenty samples, each preparatory batch will contain at a minimum, a method blank, a laboratory control sample, a matrix spike, a matrix spike duplicate and a matrix duplicate. An analytical or instrumental batch is defined as samples that are analyzed together within the same time period or in continuous sequential time periods. Within the analytical batch time are included individual QC requirements as defined by the analytical (determinative) method. For instance, each injection sequence would begin with a CCV (or initial 5-point calibration and ICV), followed by a instrument, and up to ten QC (normally a method blank, LCS, MS, MSD, MD, etc.) and field samples, then calibration verification, instrument blank, and so on. Preparation batches of samples may be continuously strung together in these run sequences, as long as the analytical batch QC requirements meet the acceptance criteria established within the appropriate SOP. At the conclusion of the last sequence, a CCV is required. Each analytical sequence must be documented.

10.2 Run a 5-point calibration curve using the primary source standards initially, each time major instrument maintenance occurs, or if the CCV does not meet acceptance criteria. Acceptance criteria are presented in Section 11.1.

10.3 After the analysis of an acceptable 5-point calibration curve, run a Initial Calibration Verification (ICV) standard (single injection) before sample analysis. The ICV standard must be prepared from a second source standard. Acceptance criteria are listed in Section 11.2.

10.4 Run a mid-point Continuing Calibration Verification (CCV) on a daily basis before sample analysis. Also run a CCV every 8 hours of continuous analysis, and at the end of the analytical sequence. It is recommended that a CCV be analyzed after every 10 samples. Acceptance criteria are listed in Section 11.3 and 11.9.1.

10.5 A method blank must be extracted with each batch of samples. The method blank should be prepared from either organic-free water or sand. Method blanks may be injected at any time in the sequence to verify absence of contamination. Acceptance criteria for these blanks are listed in Section 11.4.

10.6 A laboratory control sample must be prepared and analyzed with each batch of samples. The LCS would be prepared using the primary source standard and would contain all method target analytes. Monitoring of the LCS for all target analytes and surrogate spikes. (Refer to SOP No. Q-009-ECC-QC, Control Chart Generation, Maintenance, and Usage.) Acceptance criteria are presented in Section 11.5.

10.7 The use of the matrix spike and matrix spike duplicate will depend in part on what role the laboratory is playing. When the laboratory serves the role as the primary laboratory, then site-specific documents should be consulted. The sample to be used for the MS/MSD may be specified in the field. This previously designated sample would then be spiked with the site-specific target analytes at a concentration equivalent to the site action level. The MS/MSD would be prepared using the primary source standards. If this information was not specified or unknown, then the laboratory would choose a representative sample from each batch of samples analyzed. If samples from multiple sites were to be analyzed in the same batch, then multiple sets of MS/MSDs may be required. When the laboratory serves the role as the QA laboratory, the above scenario may not be practical to implement. If the site-specific requirements are unknown and samples from multiple sites are analyzed in the same batch, then the laboratory should select at least one sample for spiking. Each batch of samples would then contain at least one MS/MSD pair. Best professional judgement should be used to determine whether or not additional matrix spikes are appropriate. Acceptance criteria are presented in Section 11.6 for percent recovery and RPD.

10.8 The use of the matrix duplicate may also depend in part on the role the laboratory is playing. The selection of a matrix duplicate will be performed as described for the MS/MSD in Section 10.7. Acceptance criteria are presented in Section 11.7 for RPD.

10.9 Surrogate recoveries are calculated for each LCS, method blank, matrix spike, matrix duplicates, and field sample analyzed. Acceptance criteria are listed in Section 11.9.

10.10 The retention times (RTs) of identified compounds need to be checked for each identified compound in samples, and compared to absolute RTs. Refer to Section 11.9.

10.11 All sample analytical results used for final data reporting must be between the low standard and the high standard of the calibration curve. Results which fall below the low standard are to be reported as estimated (J value). Corrective actions are described in Section 11.10.

10.12 MDLs are determined in either reagent water or organic-free sand / soil and verified annually. Project specific requirements might require that an MDL study be performed in the site-specific matrix. Refer to SOP No. Q-019-ECC-QC,

Method Detection Limits (MDLs), Method Quantitation Limits (MQLs), and Laboratory Reporting Limits (LRLs). Whenever MDLs are updated, Appendix 1 values should also be updated.

10.13 The analyst must demonstrate proficiency in performing the analysis as outlined in SOP No. Q-016-ECC-QC, Technical Training. Method proficiency must be redemonstrated anytime a major method modification is made, a major software revision is added, or a major instrument modification is made. Demonstration of method proficiency may also be required after major instrument maintenance. This is decided on a case by case basis through discussions with the Section Chief, Laboratory Director, and Laboratory QA Officer.

10.14 All target analytes detected on the primary column must be confirmed on a second column. Acceptance criteria are presented in Section 11.13.

11.0 Data Validation

Site-specific requirements must be checked and used, if known, for data review. The criteria presented in this section should be used as a default in the absence of site-specific requirements. The following items shall be verified and documented using the data review checklist in Figure 4 and 5.

11.1 After a five-point initial calibration curve is analyzed, ensure that the following criteria were met. For the CFs, the %RSD must be less than 20% for all target analytes. Alternatively, the correlation coefficient (r) for the calibration line must be > 0.995.

- If the %RSD of any target analyte is 20% or less, then the CF is assumed to be constant over the calibration range, and the average CF may be used for quantitation.

- If these acceptance criteria are not met, then the following corrective actions should be performed: (1) adjust the instrument and/or perform instrument maintenance; or (2) narrow the calibration range using five standards at different concentrations. The low end of the calibration curves must be carefully watched.

If an alternative calibration technique is necessary, refer to SOP No. M-8081-ECC-OA, Organochlorine Pesticides and PCBs, for further information.

11.2 If a 5-point calibration was performed, verify that an ICV was analyzed. The ICV standard compound results must be within 15% of the known values for each compound. If this criterion is not met, perform the following corrective actions:

11.2.1 Check ICV preparation and standard curve preparation for accuracy. If either preparation is found to be in error, redo the appropriate standard preparation and reanalyze. Also, if the ICV does not check due to possible instrumental or injection errors, reanalyze and reassess.

Note: If either of the sources for the standards is being used for the first time, the analyst may gain useful information by referring to a third source standard.

11.2.2 If the ICV is still out of compliance, perform minor instrument maintenance and reinject and reassess.

11.2.3 If ICV is still out of compliance, perform new 5-point curve and ICV verification, remaking all standards from the beginning. If this still does not correct the problem, perform major instrument maintenance or call instrument manufacturer for instruction.

11.3 After the continuing calibration verification (CCV) standard has been analyzed, ensure it was run at the required frequency (every 8 hours, at the end of the sequence, or initially before daily analysis). Calculate the percent difference as in Section 9.4.
If the percent difference for any compound is less than or equal to 15%, the initial calibration is assumed to be valid. If the criterion is not met (> 15% difference) for any compound, corrective action must be taken. For example:

- Assess the shape and size/area of the peaks and compare to historical data (if applicable)
- Change the guard column
- Check injection volumes
- Remake standards
- Ensure samples and standards are both cut and are in the same matrix.

If this still does not correct the situation, change column and recalibrate instrument with all 5 standards. This above criteria must be met before sample analysis begins and/or reanalysis of all samples up to the last acceptable CCV standard.

11.4 Assess the method blanks. The analyst shall confirm that this blank was extracted at the required frequency. The method blank should not exhibit any contamination above the MDL for any of the method target analytes. Corrective action should be performed any time method target analytes are detected above the MDL to reduce and control contamination. Corrective action will be required if site-specific target analytes are detected at greater than 5% of the regulatory limit for that analyte or if the concentration in the blank is greater than 5% of that in the sample. The first step of corrective action is to assess the effect on the samples. Corrective action would include reanalysis of field and QC samples in the batch if some or all of the samples also contained levels of target analytes that exceeded the above criteria. If none of the field samples had values above the stated criteria, then reanalysis may not be necessary. The source of contamination should still be investigated and reduced/eliminated.

11.5 Assess that LCSs were extracted at the required frequency. Plot the target compounds on appropriate control charts. (Refer to SOP No. Q-009-ECC-QC, Control Chart Generation, Maintenance, and Usage.) If recoveries of all target compounds are not within control limits, the LCS extract is to be reanalyzed for verification. If acceptable, all affected sample extracts should be reanalyzed. If it is still out of control limits, then all field and QC samples in the batch must be reextracted and reanalyzed.

Note: Even though control charts must be maintained for the LCSs, the acceptance criteria derived from these charts may not be appropriate if the charted ranges are too wide. A maximum default range should be used. It is recommended that the maximum default range be set to 80-120%.

The RPD of interbatch LCSs should fall within the control limits determined from the precision control charts. However, if the RPD is outside these control limits, the batch will not be rejected, as long as the LCS recovery is acceptable. This precision information should be evaluated to see if systematic problems can be identified. If problems are suspected, the method should be fully evaluated.

11.6 Assess that matrix spike/matrix spike duplicates were analyzed at required frequency. The analyst shall also verify that the samples were spiked at the appropriate level. The order of preference for spiking levels is as follows; 1) If the target analyte concentrations are known, spike to increase the background concentration by a factor of approximately two, 2) if an action level exists, spike at this level, or 3) if neither of the first two conditions apply, spike at a level that corresponds between the low and mid-level calibration standards. Acceptance criteria are that all % Recovery and/or RPD results meet project established goals. If no project goals are specified, then results must be within the indicated control limits on the appropriate LCS control charts (if the spike concentration increased the native analyte concentration by a factor of 2 or more). If these conditions are not met, perform the following corrective actions as appropriate.

- If both LCS and MS/MSD recoveries are unacceptable, then the entire batch of field and QC samples must be re-extracted.

- If the MS/MSD is unacceptable, but the LCS is acceptable, then a potential matrix effect has been identified. Review the surrogate recovery data. If surrogates are acceptable, reanalyze the MS/MSD extracts to verify a matrix effect. If a matrix effect is still suspected, then the project manager must be contacted to discuss further alternatives and the potential impact on the project. Further alternatives may include re-extraction and reanalysis. Reasonable attempts must be made to address matrix interferences.

Note: If the MS/MSD % recovery is outside LCS limits because the native analyte concentration is greater than the spike by a factor of 2 or more

and the sample had to be diluted, a matrix effect is not demonstrated.

11.7 Assess matrix duplicates were analyzed at required frequency. Acceptance criteria are that all RPD results meet project established goals. If no project goals are specified, then results must be within the indicated control limits on the appropriate LCS precision control charts. Refer to SOP No. Q-009-ECC-QC, Control Chart Generation, Maintenance, and Usage. The acceptance criteria derived from these charts may not be appropriate if the charted ranges are too wide. A maximum default range of 25% RPD should be used for samples with target analyte concentrations ≥ 10 times the MDL. If these conditions are not met, perform the following corrective actions as appropriate.

- Reanalyze the sample extract to verify a matrix effect.

- If the duplicate precision is still unacceptable, then a potential matrix effect has been identified. The project manager must be contacted to discuss further alternatives and the potential impact on the project. Further alternatives may include re-extraction and reanalysis.

11.8 Check the surrogate calculations for correctness for all samples, blanks, LCS, MS, MSD, and MD. Check that the surrogate recoveries are properly control-charted for the LCS. The following acceptance criteria apply to surrogate recoveries.

- The surrogate recoveries for the method blank(s) and LCS(s) must be within control limits on the LCS control charts. If it is suspected that failure was due to instrumental malfunction, the sample extracts are reanalyzed. If recoveries fail reanalysis, then re-extraction is necessary.

- Sample, MS, MSD, and MD surrogate recoveries are compared to the LCS control chart. A maximum default range of 60-125% should be used. If results are outside these limits but the LCS surrogate recoveries are acceptable, the extracts of the sample(s), MS, MSD, or MD with the unacceptable surrogate recoveries shall be reanalyzed. If still out, the project manager is to be contacted to discuss further alternatives and the impact on the project. Further alternatives may include re-extraction and reanalysis.

11.9 The retention times must be checked for all identified compounds in both standards and samples. The calibration standard absolute retention times should also be checked for all initial and continuing calibrations. Retention time noncompliances are attributed to changes in temperature during analysis if a column heater is not used, and to subtle changes in the ratio of aqueous phase to organic phases of the mobile phase over the course of the analysis. Unless a gradient is used or there is a leak, the flow rate seldom changes. The corrective actions are to isolate the cause of retention time noncompliances, correct the situation, and reinject the sample. Acceptance criteria are as

follows:

11.9.1 The retention time of all target analytes and surrogates in the CCVs must fall within the absolute retention time windows calculated in 8.5.5.5. If the retention time of any target analyte does not fall within the ±3SD window, then a new initial calibration curve for the failed analytes is necessary unless system maintenance corrects the problem. Reanalysis of all affected sample extracts must also be performed for those failed analytes.

11.9.2 The analyte RT must fall within the absolute retention time window within (± 3 standard deviations of the RTs for each standard will be used to define the retention time window) (see Section 8.5.5). However, the experience of the analyst should weigh heavily in the interpretation of the chromatographs.

11.10 The analyst must verify all reported results are derived from analytical results that are either above the LRL or below the highest standard of the initial calibration curve. Verify the results are reported as follows:

- Sample concentrations that have been analyzed using the extract in its most concentrated form, and are below the MDL, should report the result as less than the LRL.

- Sample results (again using the extract in its most concentrated form) that are above the MDL but below the low standard need to be flagged as estimates, (J) values, when reported.

- For samples that exceed the calibration curve, dilute and analyze an appropriate sample aliquot.

11.11 Besides the items listed in Sections 11.1 through 11.10, the analyst should also verify the additional items as noted in Figures 3 and 5.

11.12 Additional levels of review are performed as described in SOP No. Q-024-ECC-DR, Data Reduction and Review/Validation (In-House/Contractor Data) and can be documented on forms such as those presented in Figures 4 and 5.

11.13 For confirmation purposes, target analytes on the second column must fall within established retention time windows. Analyte concentrations on the two columns must be within 40% RPD. It should be noted that coeluting compounds (e.g., TNB/DNB, 2,6-DNT, 2-A-DNT/4-A-DNT) may not have an accurate concentration for both columns when both compounds are present. Analyst experience is important when confirming the presence of target compounds.

12.0 Waste Disposal

This procedure generates organic solvent wastes. All waste disposal procedures must comply with all federal and local regulations. Refer to the ERDC WES site Waste Management Plan. Solvent wastes are collected in DOT approved containers in the Explosives Laboratory. When a container is full, a Hazardous Waste Profile Sheet (WES Form 1930) is completed and the waste is turned over to the ECB Hazardous Waste Officer. The waste is then transferred to the WES Logistics Management Office, Material Control Section. All radioactive waste is collected in contained areas that are designated by the ECB Safety Officer. The radioactive waste is given to the Radio-Isotope Lab for proper disposal.

13.0 References

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13.2 Bauer, C.F., T.F. Jenkins, S.M. Koza, P.W. Schumacher, P.H. Miyares and M.E. Walsh (1989). Development of an analytical method for the determination of explosive residues in soil. Part 3. Collaborative test results and final performance evaluation. USA Cold Regions Research and Engineering Laboratory, CRREL Report 89-9.

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13.10 Miyares, P.H. and T.F. Jenkins (1990) Salting-out solvent extraction for determining low levels of nitroaromatics and nitramines in water. USA Cold Regions Research and Engineering Laboratory, Special Report 90-30.

13.11 Gas Chromatography. Method 8000B. (Test Method for Evaluating Solid Waste Physical/Chemical Methods), SW-846, 3rd Edition, proposed Update III, January 1995.

13.12 SOP No. Q-005-ECC-SH, Sample Receipt, Login, and Storage.

13.13 SOP No. Q-008-ECC-GL, Reagent Water Generation and Quality Monitoring.

13.14 SOP No. Q-021-ECC-GL, Subsampling of Containers.

13.15 SOP No. Q-019-ECC-QC, Method Detection Limits (MDLs), Method Quantitation Limits (MQLs), and Laboratory Reporting Limits (LRLs).

13.16 SOP No. Q-016-ECC-QC, Technical Training.

13.17 SOP No. Q-009-ECC-QC, Control Chart Generation, Maintenance, and Usage.

13.18 SOP No. Q-024-ECC-DR, Data Reduction and Review/Validation (In-House/Contractor Data).

13.19 SOP No. M-8081-ECC-OA, Organochlorine Pesticides and PCBs.

13.20 ERDC WES site Waste Management Plan.

Appendix 1
MDLs and Laboratory Reporting Limits (LRLs)

Compound	Water (mg/L)						Soil (mg/kg)	
	Low-Level Salt-Out		Low-Level SPE		High Level			
	MDL	LRL	MDL	LRL	MDL	LRL	MDL	LRL
HMX	0.00006	0.00030	0.00005	0.00018	0.004	0.02	0.02	0.2
RDX	0.00010	0.00050	0.00009	0.00031	0.005	0.02	0.02	0.2
TNB	0.00005	0.00025	0.00005	0.00017	0.004	0.02	0.02	0.1
DNB	0.00005	0.00025	0.00005	0.00016	0.003	0.02	0.02	0.1
Tetryl	0.00008	0.00040	0.00007	0.00033	0.006	0.02	0.03	0.2
NB	0.00013	0.00065	0.00012	0.00041	0.002	0.02	0.01	0.2
TNT	0.00005	0.00025	0.00005	0.00017	0.003	0.02	0.02	0.1
4-A-DNT	0.00006	0.00030	0.00006	0.00020	0.002	0.02	0.02	0.2
2-A-DNT	0.00007	0.00035	0.00005	0.00017	0.002	0.02	0.01	0.25
2,4-DNT	0.00009	0.00045	0.00009	0.00021	0.005	0.02	0.02	0.15
2,6-DNT	0.00014	0.00070	0.00013	0.00043	0.005	0.02	0.02	0.2
2-NT	0.00009	0.00045			0.004	0.02	0.04	0.2
3-NT	0.00012	0.00060			0.004	0.02	0.04	0.2
4-NT	0.00014	0.00070			0.004	0.02	0.04	0.2

Appendix 2
Retention Times

Compound	Column 1	Column 2
HMX	3.25	12.12
RDX	4.63	8.05
TNB	3.13	5.15
DNB	7.32	5.15
Tetryl	8.08	9.87
NB	8.52	4.65
TNT	9.80	6.20
4-A-DNT	10.45	6.67
2-A-DNT	10.45	7.15
2,6-DNT	11.33	5.73
2,4-DNT	11.57	5.98
2-NT	14.05	5.22
4-NT	15.01	5.32
3-NT	15.89	5.22

Soil Extraction Log
ERDC EL EEC

Batch No(s):	Extraction: ☐ SOP #
Extracted Date:	Clean-up: ☐ SOP #

Type	Lab ID No.	% Dry Weight	Sample Aliquot (g)	Final Vol. mL (pre-cleanup)	Clean Up Aliquot (mL)	Final Vol. mL (postcleanup)	Sample Comments
MB							
LCS							
1							
2							
3							
4							
5							
6							
7							
8							
9							
10							
11							
12							
13							
14							
15							
16							
17							
18							
19							
20							
MD							
MS							
MSD							

Surrogate Std #: _____ Amount: _____

LCS Std #: _____ Amount: _____

MS/MSD Std #: _____ Amount: _____

Witness: _____ Date: _____

Solvents/Reagent Lots:

_____ _____

_____ _____

Extraction Analyst(s): _____ KD Analyst(s): _____ Clean Up Analyst(s): _____

Reviewed by: _____ Date: _____

Figure 1

Aqueous Extraction Log
ERDC EL EEC

Batch No(s):	Extraction: ☐ SOP #
Extracted Date:	Clean-up: ☐ SOP #

Type	Lab ID No.	Sample Aliquot (g)	Final Vol. mL (pre-cleanup)	Clean Up Aliquot (mL)	Final Vol. mL (postcleanup)	Sample Comments
MB						
LCS						
1						
2						
3						
4						
5						
6						
7						
8						
9						
10						
11						
12						
13						
14						
15						
16						
17						
18						
19						
20						
MD						
MS						
MSD						

Surrogate Std #: _____ Amount: _____

LCS Std #: _____ Amount: _____

MS/MSD Std #: _____ Amount: _____

Witness: _____ Date: _____

Solvents/Reagent Lots:

_____ _____

_____ _____

Extraction Analyst(s): _____ KD Analyst(s): _____ Clean Up Analyst(s): _____

Reviewed by: _____ Date: _____

Figure 2.

HPLC Instrument Log (Explosives)
ERDC EL EEC

Instrument ID:	Flow Rate:
Column ID:	Detector:
Injection Volume:	
Batch No(s):	Method:
Start Date:	SOP Number:

Lab ID	Instrument File ID	Low Level Method	High Level Method	Dilutions	Comments

CCV Lot #: _____ Conc. _____ Amount _____

ICV Lot #: _____ Conc. _____ Amount _____

Witness: _____

HPLC Analyst: _____

Reviewer: _____ Date: _____

Figure 3.

Lab Data Review Check List
Organic Extractions
ERDC EL EEC

Project Number(s)	
Batch Number(s)	
Extraction SOP No.	Clean-up SOP No. (if applicable)

Review Item	Yes (x)	No (x)	N/A (x)	2nd Level Review (x)
Were the project number, batch number, and sample identification number(s) properly recorded?				
Were the weight/volume of sample extracted and the final extract volume properly recorded?				
Were extraction sample dilutions or concentration factors properly recorded?				
Was a method blank prepared at the required frequency using a blank solid/liquid matrix?				
Was a Laboratory Control Sample (LCS) prepared at the required frequency?				
Were a matrix spike (MS) and matrix spike duplicate (MSD) prepared at the proper frequency?				
Were the matrix duplicates (MD) prepared at the proper frequency?				
Were correct surrogates used?				
Are there any Corrective Action Reports associated with this sample batch?				
Are copies of Corrective Action Reports attached?				
Were the spiking volume, stock source, and spike concentration properly recorded for the MS/MSD and LCS?				

NOTES: _____

_____

_____

Analyst: _____ Date: _____

2nd Level Reviewer: _____ Date: _____

Figure 4.

HPLC Data Review Check List
ERDC EL EEC

Project Number(s):
Batch Number(s):
Method:

Review Item	Yes (x)	No (x)	N/A (x)	2nd Level Review (x)
A. Initial Calibration 1. Does the curve consist of five Calibration Standards?				
2. Is the low standard near, but above, the MDL?				
3. Are calibration factors updated or were curves drawn and are CFs and %RSDs within QC limits?				
4. Are retention time windows established and updated?				
B. Continuing Calibration 1. Are the Continuing Calibration Verification (CCV) standards run every 8 hours and at the end of the analytical sequence?				
2. Is the % difference within QC limits?				
C. Sample Analysis 1. Are all sample holding times met?				
2. Are all samples with concentrations > the highest standard used for initial calibration diluted and reanalyzed?				
3. Are reported compounds within retention time windows?				
4. Are all hits confirmed on a second column?				
5. Are surrogate recoveries within QC limits?				
D. QC Samples 1. Is the Method Blank run at the desired frequency and is its concentration for target analytes less than the MDLs?				
2. Is the ICV from a second source and its recovery within QC limits?				
3. Is the Laboratory Control Sample percent recovery within QC limits?				
4. Is the Matrix Spike/Matrix Spike Duplicate run at the desired frequency and is the percent recovery/RPD within QC limits?				

Figure 5.

HPLC Data Review Check List
CE ERD EE-C

Review Item	Yes (x)	No (x)	N/A (x)	2nd Level Review (x)
5. Is the Matrix Duplicate run at the desired frequency and is the RPD within QC limits?				
E. Others 1. Are all nonconformances included and noted?				
2. Is the correct methodology used for sample prep and analysis?				
3. Are all calculations checked at the minimum frequency?				
4. Did analyst sign/date the appropriate printouts and report sheets?				
5. Are all sample ID and units checked for transcription errors?				

Comments on any "No" response:

_____

_____

_____

_____

_____

_____

Analyst: _____ Date: _____

Second-Level Review: _____ Date: _____

Figure 5. (Cont.)

Appendix 3

Preparation of Compost and Plant Samples

1.0 Compost Samples

1.1 Thoroughly mix sample as defined in SOP No. Q-021-WES-GL, Subsampling of Containers. Weigh a disposable weigh dish and record the weight in the laboratory sample prep logbook. Weigh out 2-4 grams of the wet sample into the disposable weigh dish. Record the combined weight of the sample and disposable weight dish into the sample prep logbook. Subtract the weight of the disposable weigh dish and record the wet weight of the sample. Allow the sample to dry. A forced-air oven at ambient temperature may also be used.

1.2 After drying, weigh the sample in the disposable weigh dish and record the combined weight in the sample prep logbook. Subtract the weight of the pan and record the weight of the dry sample. Divide the weight of the dry sample by the weight of the wet sample to obtain the percent solid. Record the percent solid in the sample prep logbook.

1.3 Weigh out approximately 5.0 grams ± 0.5 g of each wet compost sample (record the weight in the laboratory sample prep logbook) into a 20-mL glass vial with a Teflon lined cap.

1.4 Calculate the dry weight of the compost by multiplying the weight wet and the percent solid. Subtract the calculated dry weight from the wet weight of the compost to obtain the percent moisture in the sample.

1.5 Spike samples and associated QC samples with surrogate and matrix spike spiking solutions (see Sections 7.10 and 7.11).

1.6 Volumetrically add 10.0 mL of acetonitrile.

1.7 Using a vortex mixer, swirl for one minute, and place in a cooled ultrasonic bath for 18 hours.

1.8 After sonication, allow sample to settle for 30 minutes. If the sample has not completely settled, place in the centrifuge at 2500 rpm for 5 minutes. Remove 5 mL of supernatant, using a 5-mL pipet with disposable tips, and put in a 20 mL vial. Volumetrically add 5 mL of calcium chloride solution (Section 7.4) to the 5 mL of supernatant. Vortex for 2 minutes and let stand for 15 minutes.

1.9 Using a disposable glass pasteur pipet, place the supernatant in a disposable plastic syringe and filter through a 0.45 µm Teflon filter attached to the syringe. Discard the first 3 mL and retain the remainder in a 10 mL glass Teflon-capped vial for HPLC analysis as in Section 8.6.

1.10 Sample Dilution - See Section 8.4.8.

1.11 All radioactive samples will be prepared according to Appendix 3, Section 1. The samples will be contained only in areas which are designated by the ECB Safety Officer for radioactive prep

work.

1.12 HPLC Calibration and Analysis - See Section 8.0.

1.13 Calculations of Analyte Concentration - See Section 9.5.2.

2.0 Plant Samples

2.1 Allow the samples to come to room temperature. Take a subsample of the plant and place it onto a clean paper towel to remove any excess moisture.

2.2 Using Table 8, weigh out the appropriate fresh weight ± 0.5 g of the plant species and record in the plant prep logbook.

2.3 Using scissors and/or knife, cut the fresh samples into small pieces.

2.4 Place the cut up sample into a homogenizing chamber. Add enough Milli-Q water to just cover the top of the sample. Homogenize using a sawtooth generator probe, beginning at 500 rpm. Once 500 rpm has been reached, increase in intervals of 2500 rpm, 5000 rpm, and 7500. Homogenize at 10000 rpm if the sample is not a frothy paste.

2.5 After homogenizing the sample to a frothy paste, pour the sample into the 120 mL Labconco freeze drier flasks. Cover the flask with parafilm and place in the freezer until frozen (approximately 1-2 hours).

2.6 Rinse the generator probe between samples by homogenizing Milli-Q water in a flask.

2.7 Insert a freeze drier filter between the freeze drier adapter and rubber top. Place the rubber top onto the freeze drier flask containing the frozen sample. Insert the exposed adapter end into the valve on the freeze drier condenser.

2.8 Turn the plastic valve stem to the "vacuum" position. Once the vacuum reading has dropped to below 200, add the next sample.

2.9 Remove the sample from the freeze drier when the flask is no longer cool to touch and no ice chunks or crystals are left in the sample (appoximately 2 days to dry most samples). Remove flask from freeze drier by turning the plastic valve stem to the "vent" position. Remove the rubber top from the sample flask.

2.10 Place a disposable weigh dish onto the balance and tare. Scoop out the freeze dried sample into the disposable weigh dish. Record the dry weight in the plant prep logbook.

2.11 Weigh out 0.25 grams of each freeze dried sample (record weight in plant prep logbook) into a 20-mL glass vial with a Teflon lined cap.

2.12 Spike samples and associated QC samples with surrogate and matrix spike spiking solutions (see Sections 7.10 and 7.11).

2.13 Volumetrically add 10.0 mL of acetonitrile.

2.14 Using a vortex mixer, swirl for one minute, and place in a cooled ultrasonic bath for 18 hours.

2.15 After sonication, place sample into the centrifuge at 2500 rpm for 5 minutes. Allow the sample to sit for approximately 1 hour.

2.16 Remove 5 mL of supernatant, using a 5-mL pipet with disposable tips, and put in a 20-mL vial.

2.17 Prepare enough filter columns to clean-up all of the samples.

2.17.1 Place a small piece of glass wool into a 5 3/4" glass disposable pipette.

2.17.2 Place 0.5 g of florisil into the pipette.

2.17.3 Place 0.5 g of alumina into pipette (on top of florisil).

2.18 Rinse the filter column with 5 mL of acetonitrile. Discard the filtrate.

2.19 Supernatant is added to the filter column using a glass disposable pipette. Collect the filtrate in a 20-mL glass vial.

2.20 After the supernatant has completely filtered through, filter 5 mL of acetonitrile through the column, collecting in the 20-mL glass vial containing the filtered supernatant.

2.21 Vortex the filtered supernatant for one minute.

2.22 Volumetrically transfer 2 mL of the filtered supernatant, using a 5-mL pipet with disposable tips, and put in a 20 mL vial. Volumetrically transfer 2 mL of Milli-Q water into the same 20 mL vial.

2.23 Using a disposable glass pasteur pipet, place the supernatant in a disposable syringe and filter through a 0.45 µm Teflon filter attached to the syringe. Discard the first 1 mL and retain the remainder in a 10 mL glass Teflon-capped vial for HPLC analysis as in Section 8.6.

2.24 Sample Dilution - See Section 8.4.8.

2.25 All radioactive samples will be prepared according to Appendix 3, Section 2. The samples will be contained only in areas that are designated by the ECB Safety Officer for radioactive prep work.

2.26 HPLC Calibration and Analysis - See Section 8.0.

2.27 Calculations of Analyte Concentrations - See Section 9.5.2.

Table 8. Plant Species

PLANT SPECIES	FRESH WEIGHT
Cyperus	5.00g
Aquatics	5.00g
Corn Kernels	10.0g
Corn Silage	10.0g
Tomato	20.0g
Lettuce	20.0g
Radish	20.0g

Appendix 4
Preparation of Insect Tissue

1. Freeze drying
   1.1. Place tissue sample into a pre-weighed freeze drier flask sized appropriately for the volume of the sample.
   1.2. Re-weigh the flask and sample and record the weight in the explosives prep logbook. Seal the flask with a sheet of parafilm and place in the freezer a minimum of 2 hours or until the tissue is frozen.
   1.3. Insert a freeze drier filter between the freezer drier adapter and rubber top. Place the rubber top onto the freeze drier flask containing the frozen sample. Insert the exposed adapter end into the valve on the freeze drier condenser.
   1.4. Turn the plastic vale stem to the "vacuum" position. Once the vacuum reading has dropped below 200 mm Hg, add the next sample.
   1.5. Remove the flask from the freeze drier when the flask is no longer cool to the touch and no ice chunks or crystals are left in the sample. Remove the flask from the freeze direr by turning the plastic valve stem to the "vent" position. Remove the rubber top from the sample flask.
   1.6. Re-weigh the sample and flask, record the weight, and calculate the percent solids. Remove the dried material from the flask. Grind and homogenize the tissue by passing through a clean 40-mesh sieve. Store the sample in a pre-cleaned glass vial in the freezer at −10°C or lower.
2. Sample extraction
   2.1. Weigh out 0.25 grams of each freeze-dried sample (record weight in explosives prep logbook) into a 20-ml glass vial with a Teflon lined cap.
   2.2. Spiked samples and associated QC samples with surrogate and matrix spiking solutions (see Sections 7.10 and 7.11).
   2.3. Volumetrically add 5.0 ml of acetonitrile.
   2.4. Using a vortex mixed, swirl for one minute, then place in a cooled ultrasonic bath for 18 hours.
   2.5. After sonication, allow sample to sit for at least 30 minutes while solids settle to bottom of vial. The sample may be centrifuged at 2500 rpm for 5 minutes if necessary.
   2.6. Remove 5.0 ml of supernate, using a 5-ml volumetric pipet with disposable tip. Place the 2.5 ml of supernate into a clean 20-ml vial.
   2.7. Volumetrically add 2.5 ml of calcium chloride solution (Section 7.4) to the 5 ml of sample supernate. Vortex for 2 minutes and let stand for 15 minutes.
   2.8. Using a disposable glass Pasteur pipet, place the sample mixture into a disposable plastic syringe and filter through a 0.50 µm Teflon filter attached to the syringe. Discard the first 1.5 ml and retain the remainder of the sample in a 10 ml glass Teflon capped vial for HPLC analysis.
3. HPLC analysis
   3.1. See Section 8.0.
   3.2. Note: the insect matrix used to develop this method and the MDLs given below did not exhibit interferences across the UV across the UV chromatogram. If other insect extracts were to contain interferences, the extracts would be cleaned up according to the method in Appendix 3, Paragraph 2.
4. Calculations of analyte concentrations
   4.1. See Section 9.5.2.

Detection limits

5.1. Detection limits for insect tissue were calculated using crickets. Table 9 contains MDLs and LRLs in mg/kg based upon dry weight.

Table 9.
Example of reporting limits for insect tissue.

Explosive	MDL (mg/kg)	LRL (mg/kg)
HMX	1.0	3.2
RDX	0.3	0.8
TNB	0.4	1.2
DNB	0.3	1.0
Tetryl *		
NB	0.8	1.7
TNT	0.4	1.3
4-A-DNT	0.8	2.5
2-A-DNT	0.4	1.2
2,4-DNT	0.2	0.7
2,6-DNT	0.4	1.3
2-NT	0.6	1.9
3-NT	0.5	1.6
4-NT	0.6	2.1

Appendix 5
Sample Preparation and Analysis of 8330 Degradation Products

1.0 Scope and Application

1.1 The procedures in this Appendix are used for the extraction and trace analysis of SW846 Method 8330 degradation products in water and soil/sediment matrices by high performance liquid chromatography (HPLC) using an UV detector. Table 1 lists the degradation products. These products are analyzed depending upon project-specific requirements.

Table 1. SW846 Method 8330 Degradation Compounds

Compound	Abbr.	Estimated RT (1)
1,3,5-Trinitroso-1,3,5-triazacyclohexane	TNX	3.44
1-Nitroso-3,5-dinitrohexahydro-1,3,5-triazine	MNX	4.26
1-Nitro-3,5-dinitroso-1,3,5-triazacyclohexane	DNX	3.73
1-Nitroso-3,5,7-trinitro-1,3,5,7-tetraazacyclooctane	MN-HMX	3.14
3,5-Dinitroaniline	DNA	8.28
N-Methyl-2,4,6-trinitroaniline	R-TET	(2)
2,4-Diamino-6-nitrotoluene	24-DANT	4.05
2,6-Diamino-4-nitrotoluene	26-DANT	3.66
2,2-Dinitro-44-azoxytoluene	44-AZOXY	6.81 (3)
4,4-Dinitro-22-azoxytoluene	22-AZOXY	4.82 (3)

(1) RTs are column specific. These RTs are for information purposes and might not reflect elution times on future columns.
(2) omitted because we are omitting tetryl
(3) from Nova Pak C18 column

2.0 Procedure

2.1 The compounds listed in Appendix 5, Table 1 are extracted using the appropriate procedures that are located in Sections 8.1,8.2,8.3, and 8.4 of the SOP.

3.0 Analysis

3.1 The compounds listed above (except for 44-Azoxy and 22-Azoxy) are analyzed with the SW846 Method 8330 compounds. The chromatographic conditions are listed in Section 8.5.1 of the SOP.

3.2 Coelution and Interferences: 26-DANT and 24-DANT coelute on the CN column. If there are high concentrations of 4A-DNT and 2A-DNT, then MNX, TNX, and DNX have the

potential to coelute on the CN column. High concentrations of 26-DANT, 24-DANT, MNX, DNX, and TNX may coelute on the C18 column. (This has never been seen in actual sample analysis because the degradation products are usually found in low concentrations.)

Coelution problems due to high concentrations are resolved by diluting and reanalyzing the sample. In instances where there is no confirmation due to high concentrations of Method 8330 analytes and a dilution is not feasible due to low concentrations of the degradation products, the samples are analyzed under the same conditions and are confirmed by spectal analysis using a photodiode array detector set to extract at 245 nm.

3.3 The following chromatographic conditions are for the analysis of 44-Azoxy and 22-Azoxy:

Column: Waters Nova-Pak C18 reverse phase HPLC column 3.9 mmX 150 mm (4μm)

Mobile Phase: 54:46 Acetonitrile/organic-free reagent water

Flow Rate: 1.5 ml/min

Injection volume: 50-μl

Detector: Photodiode Array Detector set to extract at 245 nm

These compounds are confirmed using the spectral analysis from the photodiode array detector.

4.0 Calibration

4.1 The calibration curve for each analyte is performed in singlet. There are no second sources available for confirmation of these compounds. Each calibration is a five point curve with the lowest standard being the laboratory reporting limit (see Appendix 5, Table 2). Peak heights are obtained for each analyte. A check standard is analyzed after every tenth analytical sample.

TABLE 2

Reporting Limits for SW846 Method 8330 Degradation Products

Degradation Product	Water LRL (mg/L)	Soil LRL (mg/kg)
TNX	0.020	0.100
MNX	0.020	0.100
DNX	0.050	0.100
MN-HMX	0.250	0.500
DNA	0.020	0.100
R-TET	****	****
24-DANT	0.200	1.00
26-DANT	0.100	0.500
44-AZOXY	0.500	1.00

22-AZOXY	0.500	1.00

****No LRL's have been generated for this compound.