Protocols for NADP Litterfall Mercury Monitoring Initiative
Version 1.1 July 2012

1. Site liaison and laboratory. The USGS site liaison will communicate with the NADP site sponsors, site operators, NADP Program Office, NADP Committees, and the USGS Mercury Research Laboratory. The laboratory will not communicate routinely with these entities.

2. Study plot location. The NADP site sponsor and site operator will consult with the USGS site liaison when selecting the location for the forest study plot at a NADP site to assure it is representative of the forest land cover near the site. The center of the sample plot should be less than 300 m (984 ft) from the NADP wet deposition collector. The study plot should be in a representative forest type for the area around the NADP site. For example, if the majority of the natural forest land cover in the vicinity of the site is deciduous oak-hickory forest, but a stand of planted white pine is also nearby, the forest study plot should be located in the oak-hickory forest, not the white pine stand. Similarly, if the majority of the natural forest land cover in the vicinity of the site consists of relatively mature trees more than 12 inches in breast height diameter, but a stand of relatively young trees of the same species less than 4 inches in diameter is also nearby, the forest study plot should be located in the stand of mature trees. Ideally, the forest study plot should be located where it is unlikely to be disturbed by foot and vehicle traffic and is away from game trails.

3. Site information. The site operator will receive a sample kit with a field form for recording descriptive information about the forest study plot, including a site diagram. Figure 1 shows an example diagram of a typical forest study plot. Figure 2 is the site information form.

4. Sample area. The litterfall sample collectors will be deployed in a 16-m by 16-m sample area within the forest study plot. The sample area should be more than 32 m from the edge of the forest study plot and should not include a creek, gulley, ravine or other feature of uneven terrain. It should be an area that looks like most of the study plot.

Supplies included in the sample kit for defining the sample area boundaries are an 8-m piece of line on two stakes and 4 marker flags. Pick the center of the sample area, such as a prominent tree that can serve as a future reference mark. Using the 8-m line and the other 4 flags, mark the middle of the 4 sides of the sample area. For a plan view of the sample area, refer to figure 3.

5. Sample collectors. A total of 8 litterfall sample collectors are deployed in a sample area. The sample collector is intended to passively capture and hold litterfall coming to the forest floor. Each sample collector has a removable, plastic sample box supported by a wooden base. The sample box is 0.25 m^2 and has 15 cm side walls. The box has a replaceable 0.6-mm mesh screen bottom to retain small particles while allowing water to drain. Each sample box has an ID number etched on the sides. Sample boxes are shipped to a site, pre-cleaned, and each one in a plastic bag.

Each sample collector has a wooden base that holds the bottom of the box 3 cm off the ground to avoid soil contact and to allow the sample box to drain water through the mesh bottom. The wooden base holds the sample box tightly and is heavy enough to provide stability in the wind or if a small animal investigates. Each wooden base is placed in the sample area once and not moved during the sampling season.

One shipping carton with the 8 sample boxes, sample area supplies, and instructions, plus two shipping cartons with 4 bases each are shipped to a site at the start of the sampling season. Sites do not return the bases at the end of the season if they plan to participate in the litterfall mercury monitoring the next year.

6. Placement of sample collectors. The locations for placement of the 8 sample collectors in the sample area are randomly selected for each forest study plot. The sample area has a grid of 16 cells, each with 1-m by 1-m sides and numbered as shown in figure 3. A random number generator assigns a number for each cell. The eight lowest random numbers designate the cells in the sample area where the collectors

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will be placed. An alternate cell is identified with the ninth lowest random number. A diagram with the assigned cells for the sample collectors is included with the instructions in the sample kit. Typically, a collector will be placed somewhere within the assigned cells. Collectors should not be placed in a cell with thick understory if it would prevent litterfall from entering the collector. A cell that is obstructed by a fallen log, stump, or boulder should be avoided. If possible, place the sample collectors on a Tuesday, consistent with the NADP schedule. Place the 8 wooden bases in the assigned cells of the sample area.

Take the sample boxes to the study plot in their plastic bags and fit each sample box snugly in the wooden bases. Save the plastic bags and shipping carton for returning the sample boxes with the last samples of the season. Save the other shipping cartons to store or return the wooden bases between sampling seasons.

7. Site record. The site information form (figure 2) included in the kit has a numbered grid of the sample area that should be used to record the ID numbers of the sample boxes placed in the 8 cells.

8. Sample collection. Litterfall samples will be retrieved once every 4 weeks on a Tuesday. Depending on the latitude and altitude of the NADP site, the length of the litterfall sampling season will require that samples will be collected 2 to 6 times at 4-week intervals until litterfall is over. The sample kit contains a set of large ziploc bags, one for each 4-week sample. In each large bag are 8 small ziploc bags, one for each of the 8 sample collectors. Each small bag is labeled with the ID number on the sample box.

Take the large bag to the sample area. At each collector, match the bag ID with the collector ID. Either pour the contents of the sample box into its labeled ziploc bag, or use a gloved hand to transfer the contents into the bag. Disposable gloves are in the sample kit. Wet litterfall samples can be submitted. Avoid including substantial amounts of frozen precipitation. If a sample box is empty, keep its ID-labeled, empty ziploc bag with the other sample bags. Whether they are empty or contain litterfall, place the 8 small ziploc bags in the large ziploc bag and label the large bag with the sample date. The sampling kit includes shipping pouches with a pre-addressed shipping label. Soon after they are collected, use one of the pouches to send the 4-week samples to the USGS Indiana Water Science Center, 5957 Lakeside Blvd. Indianapolis, IN 46278. The cost for shipping samples in the responsibility of the site sponsor.

9. Sample information. The sample information form (figure 4) included in the kit should be used for recording information about the litterfall samples. Include the completed form with the sample boxes shipped at the end of the season.

10. Retrieval of sample collectors. For the last sample of the season, remove the sample box and place it in one of the clean plastic bags in which it was shipped. Close the bag with a rubber band or tape so it won't open during shipping. Return the sample box in a bag, even if the sample box is empty. It is expected that the plastic bag will retain the litterfall collected by the sampler during shipment. Remove the wooden base at the end of the sampling season. Mark the center of the sample area to use next season. Place the 8 sample boxes with the litterfall samples into the shipping carton that was provided. Make a copy of the field information form. Enclose the completed field form in a plastic bag and put it in the carton. Use the pre-addressed Federal Express label for shipping. Store the wooden bases in their two shipping cartons until next season.

11. Annual litterfall samples. An annual litterfall sample consists of all the 4-week samples from the 8 collectors at an NADP site. The samples shipped to USGS, including the last one of the season, are stored in a freezer until they are inventoried, weighed, labeled, and shipped to the laboratory. Empty bags will not be included in the lab shipment. Each small bag of litterfall obtained from each sample collector every 4 weeks will be freeze dried, weighed, and the weight recorded. These data will be used to compute the total litterfall dry mass and the variability of the litterfall sample catch from 2 m^2 (8 collectors x 0.25 m^2) of the sample area. Empty sample collectors and missing samples will be included in the data computation.

12. Sample inventory. The inventory of the samples is based on the sample box ID and includes the sample date, wet weight, litterfall description, and dry weight.

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13. Sample analysis. In a sample area, 8 sample collectors are used so that in an ideal situation, data on the total amount and variability of the litterfall sample catch will be obtained from all 8 collectors. The dried litterfall from all of the 4-week samples from 4 of the collectors will be composited, ground, homogenized, and analyzed for mercury. The 4 collectors whose samples will be composited for analysis will be based on the four lowest random numbers assigned to the 8 collectors. If some of the 4-week subsamples from one or more collectors are lost or incomplete, all of the samples from at least 4 of the 8 collectors will be complete and suitable for analysis. Collectors with missing samples will be skipped and the collector with the next lowest random number will be used for mercury analysis. A proportional subsample from the 4 litterfall samples analyzed for mercury will be composited and analyzed for methylmercury. A proportional subsample accounts for differences in the litterfall mass among the four samples and obtains a proportionally higher amount from the sample with the higher mass.

14. Quality assurance. Sample boxes are pre-cleaned before they are shipped to a NADP site. Cleaning is a detergent solution scrub, deionized water rinse, and dilute hydrochloric acid rinse. Boxes are dried in a HEPA work station and placed in a new plastic bag.

The USGS Mercury Research Laboratory will follow its Quality Assurance Plan for mercury and methylmercury analysis. Certified reference materials will be analyzed for mercury to evaluate analytical accuracy for litterfall, based on percent recovery. At least 1 of every 5 litterfall samples will have two laboratory split replicate samples prepared and analyzed for mercury to evaluate analytical precision, based on relative percent difference. Spatial variability in litterfall mercury concentrations and litterfall sample catch at a site will be evaluated using data from the 4 sample collectors (concentration) or 8 sample collectors (catch) at a NADP site, based on relative standard deviation. At least 1 of every 20 sample boxes will have a field blank prepared for mercury analysis by rinsing the inside with mercury-free blank water from the USGS Laboratory and collecting the rinseate in a sample container.

15. Data management. Analytical data for mercury and methylmercury analysis will be combined with data on litterfall sample catch in an Excel spreadsheet. The USGS site liaison will assign each sample a quality rating of A, B, or C that is similar to the MDN system. For each site, three values will be calculated: mean annual litterfall mercury concentration for a representative 1-m^2 sample; total annual sample catch for a representative 1-m^2 sample; annual litterfall mercury deposition per m^2. In addition, the litterfall methylmercury concentration will be included.

16. Data access. The Excel spreadsheet of annual sample and mercury data will be available from a password-protected page of the NADP web site. An example of the spreadsheet information is in figure 5.

List of figures.

Figure1. Diagram of a typical forest study plot
Figure 2. Litterfall monitoring site information form
Figure 3. Diagram of litterfall sample area
Figure 4. Litterfall sample information form
Figure 5. Litterfall mercury data spreadsheet example

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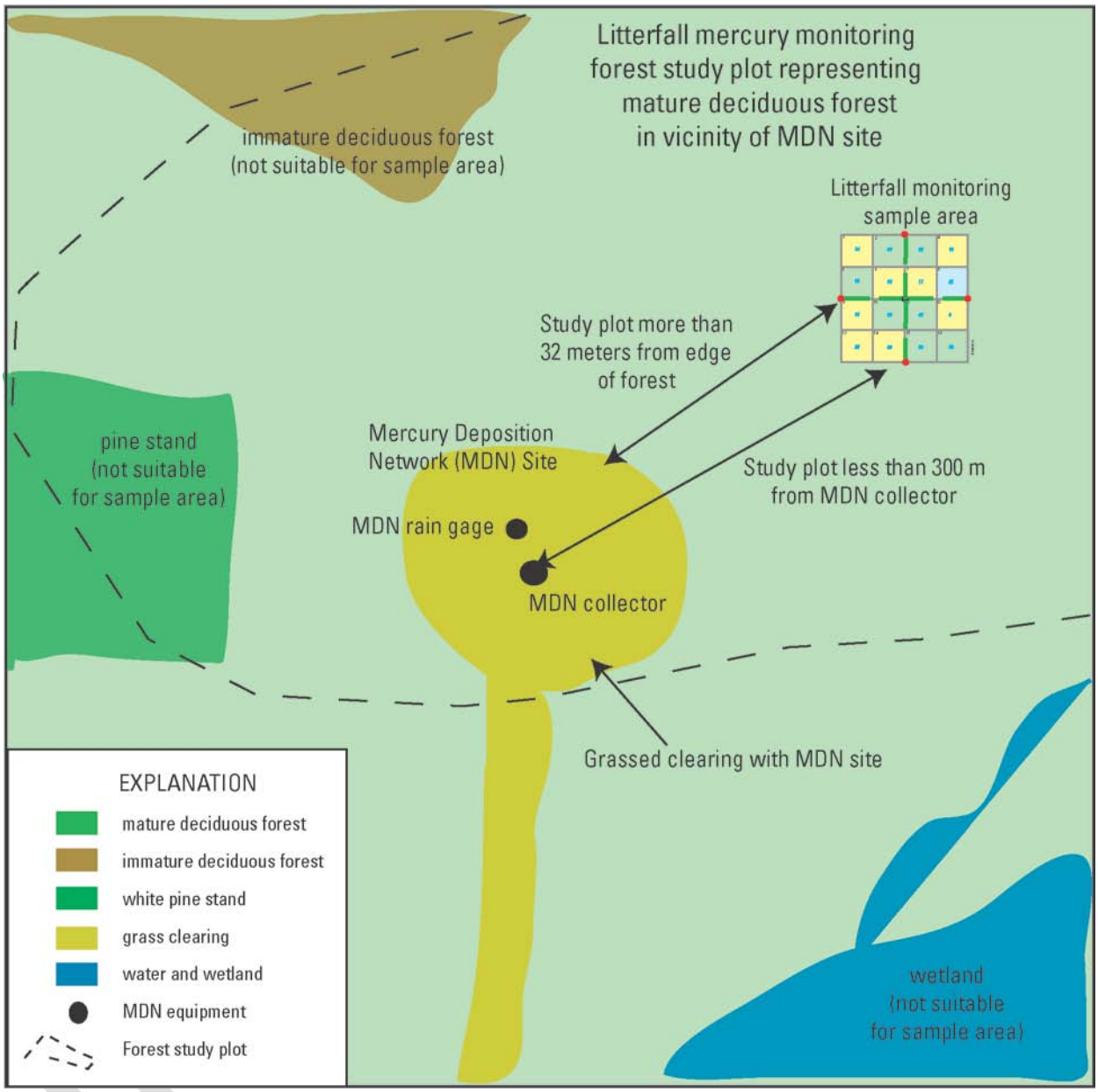

Figure1. Diagram of a typical forest study plot at an NADP site, here an MDN site.

Litterfall Mercury Monitoring Initiative Site Information Form

NADP Site ID _____ NADP Site Name _____

Site operator name_____

Site operator phone _____ Site operator email_____

Other atmospheric mercury monitoring at or near NADP site (such as MDN, AMNet, surrogate surface, throughfall) _____

Classify the dominant forest cover within 1 km (0.6 mi) of MDN collector (the forest study plot)

__Deciduous __Coniferous __Mixed Deciduous/Coniferous

Other (describe) _____

Pick the dominant forest type within 1 km (0.6 mi) of MDN collector (the forest study plot)

__oak-hickory __maple-beech-birch __aspen-birch __oak-gum-cypress

__white-red-jack pine __loblolly-shortleaf pine

The sample area is where the litterfall sample collectors are placed. List the tree species in the sample area (in order or abundance, use common or scientific name)

_____

_____

Understory species in sample are that contribute to litterfall sample (if any)

_____

Starting at the NADP wet-deposition collector, identify

The compass direction to the center of litterfall sample area _____

The distance to the center of the litterfall sample area _____

Date litterfall collectors installed _____

Date litterfall collectors removed _____

Please see opposite side.

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Figure 2. Litterfall mercury monitoring site information form

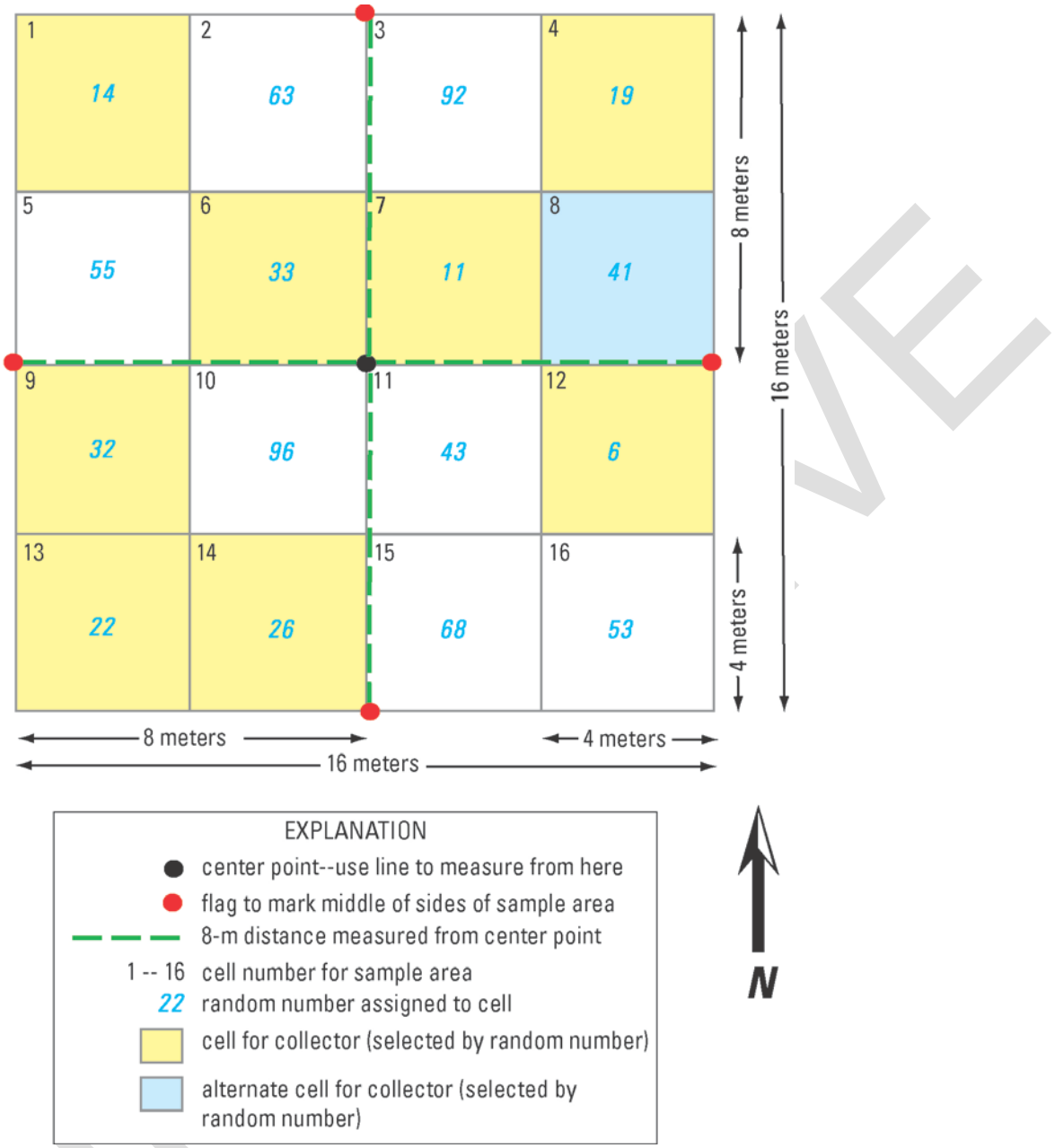

Figure 3. Diagram of litterfall sample area at a NADP site

Diagram the NADP wet deposition collector, sample area, and other important features within 1 km (0.6 km)

List the litterfall **sample box ID number** in the cell where it was placed.

SITE ID/NAME:_____ DATE: _____

1	2	3	4
5	6	7	8
9	10	11	12
13	14	15	16

N

OPERATOR:
The yellow cells are where the litterfall samplers should be installed.
The blue cell is an alternate, if needed.
Please mark the litterfall sample box ID number in the cell where it was installed.

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Figure 4. Litterfall sample information form

Figure 5. Litterfall mercury data summary example (next 2 pages)

NADP Litterfall Mercury Monitoring Initiative Data

[example data spreadsheet for one NADP site for one sampling season]

1. Litterfall Sample Catch

NADP Site ID	Sample Box ID	Sample Date	Subsample number	Sample catch in bag	Sample bag weight	Sample catch wet weight	Sample catch dry weight	Percent moisture	Sample box annual catch dry weight	NADP site annual sample catch
XX-nn	10	9/4/2012	1	A	B	C	D	E		
		10/2/2012	2							
		10/30/2012	3							
		11/27/2012	4						F	
	11	9/4/2012	1							
		10/2/2012	2							
		10/30/2012	3							
		11/27/2012	4							
	12	9/4/2012	1							
		10/2/2012	2							
		10/30/2012	3							
		11/27/2012	4							
	13	9/4/2012	1							
		10/2/2012	2							
		10/30/2012	3							
		11/27/2012	4							
	14	9/4/2012	1							
		10/2/2012	2							
		10/30/2012	3							
		11/27/2012	4							
	15	9/4/2012	1							
		10/2/2012	2							
		10/30/2012	3							
		11/27/2012	4							
	16	9/4/2012	1							
		10/2/2012	2							
		10/30/2012	3							
		11/27/2012	4							
	17	9/4/2012	1							
		10/2/2012	2							
		10/30/2012	3							
		11/27/2012	4							
	18	9/4/2012	1							
		10/2/2012	2							
		10/30/2012	3							
		11/27/2012	4							
							I	J	H	G

Data item	Calculation/Explanation	Units
A	wet weight of 4 week sample and bag	grams
B	tared weight of empty bag	grams
C	A - B	grams
D	dry weight of sample without bag	grams
E	[(C -D) / C] x 100	percentage
F	D1 + D2 + D3 + D4 where D is subsample dry weight for subsample number	grams/year
G	(F10 + F11 + ….F18) /2 where F is annual catch for sample box ID	grams/square meter/year
	note that each box is 0.25 square meter and 8 boxes are 2 square meter;	
	divide by 2 to get unit of 1 square meter;	
	this value is used to compute annual litterfall mercury deposition	

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	supplementary statistics
H	mean and standard deviation of sample catch dry weight
	in 8 sample boxes in NADP site litterfall sample area for the season
I	accumulation of litterfall sample catch every 4 weeks in each box thru season
J	mean and standard deviation of sample catch moisture for the season
K	distribution of annual litterfall sample catch in 8 sample boxes
	in NADP litterfall sample area for the season (show in box plot)

2. Litterfall Mercury Concentration

NADP Site ID	Sample Box ID	Sample start date	Sample end date	Number of subsamples in composite	Total Hg concen-tration
XX-nn	10	9/4/2012	11/27/2012	4	L
	14	9/4/2012	11/27/2012	4	L
	15	9/4/2012	11/27/2012	4	L
	18	9/4/2012	11/27/2012	4	L
					M
					N

Data item	Calculation/Explanation
L	4 of the 8 sample boxes were randomly selected for Hg analysis of sample
	in this example, a total of (4) 4-week samples from a box were composited
	Hg concentration, nanograms per gram, dry weight (ng/g)
M	mean total Hg concentration in 4 sample boxes (ng/g) at NADP site
	standard deviation of Hg concentration at NADP litterfall sample area
N	methylmercury concentration (ng/g) in proportional composite of litterfall
	from 4 sample boxes at NADP site

3. Litterfall Mercury Deposition

NADP Site ID	Sample Box ID	Sample start date	Sample end date	Mean total Hg concen-tration	Annual litterfall sample catch	Annual litterfall Hg deposition
XX-nn	10	9/4/2012	11/27/2012	L	M	**O**

Data item	Calculation/Explanation
O	L * M is the annual litterfall Hg deposition at the NADP site
	litterfall sample area, in nanograms per square meter per year
	convert to micrograms per square meter per year for comparison
	with annual Hg wet deposition at NADP MDN site

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