APPENDIX XV

Sampling/Measurement Protocol for Airborne Dust Levels

Field Operations Manual
NIOSH Contract No. 210-76-0175

APPENDIX XV

Sampling/Measurement Protocol.

for Airborne Dust Levels

Environmental Studies:

Airborne dust levels: These studies were performed by NIOSH Personnel. Method of collection of respirable and total personal dust samples were:

1) "Respirable, personal dust samples were collected utilizing an air sampling train consisting of a 10 mm nylon cyclone respirable dust sample assembly connected to a personal air pump by a 2 ft. length of 1/4" diameter tygon tubing. Each pump was calibrated to provide an air sampling flow rate of 1.7 ± 0.1 L/min. over a full work shift. The dust samples were collected in a two-piece filter cassette holder (supplied with the cyclone containing a 37 mm diameter pore size DM-800 Gelman filter supported on a cellulose back-up pad. The samplers were placed on each worker studied immediately after his pre-shift medical examination and removed just prior to his post-shift medical examination conducted by the University of Wisconsin laboratory for gravimetric analysis and mycological evaluation. All gravimetric analyses (including pre- and post-weights) and filter cassette assemblies were conducted by the University of Wisconsin, Department of Plant Pathology Laboratory personnel."

"Personal total dust samples were collected in the same manner as the respirable dust samples except the cyclones were not used and FWSB 5.0 um MSA filters were used instead of DM-800's. In addition, the Utah Biomedical Test Laboratory (UBTL) provided the two-piece filter cassette used and conducted pre- and post-weighing for analysis. Pump flow rates for all total dust samples were 2.0 ± 0.1 L/min."

"Sampling error is 0-5% for both respirable and total air sampling."

2) For Controls

b. "Respirable dust concentrations of Superior city workers were determined using a 37 millimeter diameter acrylic copolymer 0.8 micrometer pore size filter (DM-800, Gelman) desiccated and preweighed to the nearest 0.001 milligram, supported by a cellulose backup pad and sealed with cellulose bands into a two-piece 37 mm filter cassette. Prepared filter cassettes were uncapped and securely placed into a 10 mm nylon cyclone assembly attached by 0.75 m long tygon tubing to personal sampling pumps equipped with pulsation flow dampers (Model G, MSA). Sampling pumps were periodically monitored over the shift to insure a flow rate of 1.7 Lpm $\pm$ 0.1 Lpm. At the end of sampling, filter cassettes were removed from the cyclones, capped, taped and hand carried back to the laboratories for desiccation and re-weighing."

"Total dust sampling was conducted in a similar manner to respirable dust sampling except cyclones were not used, and the sampling pumps were calibrated at the flow rate of 2 Lpm $\pm$ 0.1 Lpm."

"Ten percent of the filters used in a sampling day were used as controls and treated identically to sampling filters except no air was drawn through the filters."

Notice of Related Work:

The mycological and entomological contaminations of grain and grain Dust were examined independently under a separate NIOSH contract (No. 210-77-0150) entitled "Combined Mycological/Entomological Evaluation of Grain dust Components"; University of Wisconsin-Contractor.

Appendix XVI

Chest Radiograph Reading Form
Field Operations Manual
NIOSH Contract No. 210-76-0175

CHEST ROENTGENOGRAM

Date:
Reader:

A. QUALITY
0. Not done (reason_____)
1. Adequate
2. Deficient, but acceptable
3. Inadequate

B. THORACIC CAGE (May be more than one)
1. Normal
2. Kyphoscoliosis
3. Abnormal rib fracture (specify)
a) old b) new
4. Abnormal degenerative arthritis of spine
5. Abnormal - other (specify_____)

C. HEART
1. Normal
2. Abnormal - enlarged
3. Abnormal - other (specify_____)

D. AORTA
1. Normal
2. Abnormal

E. LUNGS
1. Normal
2. Abnormal
3. Questionable abnormality

E-1 Type of Lesion (only one)

1. Increase in lung markings a) localized b) diffuse-bilateral
2. Reticulonodular pattern a) localized b) diffuse-bilateral
3. Reticulo-linear pattern a) localized b) diffuse-bilateral
4. Nodular (many rounded opacities less than 3mm)
a) localized-unilateral
b) diffuse-bilateral
IF localized, circle location: RU LU
RM or LM or R hilum area (RHA)
RL LL L hilum area (LHA)

IF yes to 1, 2, 3 or 4:
Severity of above lesions (degree of profusion of lesions)
0. Minimal or questionable
1. Definitely present, but few
2. Numerous opacities, but lung markings still present
3. Very numerous, obscuring vascular pattern

5. Nodule, non-calcified (3 mm - 2.5 cm)

   a) single _____

   b) more than one _____, circle location: RU, RM, RL, LU, LM, LL, RHA, LHA

6. Nodule calcified (3 mm - 2.5 cm)

   a) single _____

   b) more than one _____, circle location:RU, RM, RL, LU, LL, RHA, LHA

7. Mass 2.5 cm, circle location: RU, RM, RL, LU, LM, LL, RHA, LHA

F. PLEURA

1. Normal

2. Abnormal a) unilateral

   b) bilateral

   Describe _____

G. DIAPHRAGM

1. Normal

2. Abnormal - flat (hyperinflated lungs)

3. Abnormal - other (specify _____)

H. OTHER FINDINGS AND NARRATIVE: _____

_____

_____

_____

_____

APPENDIX XVII
Blood Chemistries

A Manual and Automated Procedure for Measuring Serum Cholinesterase Activity and Identifying Enzyme Variants

ALANINE AMINOTRANSFERASE (ALT)
GLUTAMATE-PYRUVATE TRANSAMINASE - (GPT)

GAMMA-GLUTAMYL=TRANSPEPTIDASE (GGTP)

CREATININE

ALANINE AMINOTRANSFERASE (ALT)

(Glutamate-pyruvate transaminase - GPT)

Principle

This procedure utilizes the Calbiochem Single Vial Reagent (S.V.R.) system. (Catalog number 869302)

In this reaction, α-ketoglutarate and L-alanine, in the presence of ALT, yield L-glutamate and pyruvate. The latter is reduced by lactate dehydrogenase (LDH) to L-lactate; simultaneously a mmolar equivalent of NADH is oxidized. The rate of change in absorbence at 340 nm is proportional to the activity of the ALT in the sample.

Specimen:

Serum. Hemolysis does not interfere but do not use specimen with appreciable hemolysis. GPT (ALT) activity is 7 times higher in RBC's than serum.

Reagents: (Note A)

1.	TES buffer	0.08 moles/L
2.	L-Alanine	0.56 moles/L
3.	α-ketoglutarate	2.0×10^{-2} moles/L
4.	NADH	2.0×10^{-4} moles/L
5.	LDH (animal)	720 IU/L
6.	pH	7.5

7. Non-reactive stabilizers

Reconstitute by adding 15 ml double distilled water to vial. One vial will be sufficient for 5 tests. Substrate is stable 72 hours when stored between 2° and $8^{\circ}C$.

If reagent shows initial absorbence reading of less than 1:1, or evidence of bacterial contamination, discard.

Procedure:

1. Set up Coleman 124D spectrophotometer; (see general instructions under Coleman 124D). Variable settings are as follows:

Lamp:	D_2
Wavelength:	340 nm
Mode:	ultraviolet
Scale:	0-1
Reference Cell:	dichromate (Note C)

2. Determine total number of assays. Each patient is done in duplicate and in separate runs. There must be at least one control per run. A "run" is comprised of 4 assays monitored sequentially at 15 second intervals.
3. Reconstitute appropriate number of GPT vials. Mix vials gently by inversion to dissolve but DON'T SHAKE. You will need 3 ml substrate per assay.
4. After solution is complete, pour all vials (if more than one is reconstituted) into a larger container and swirl to mix. This will eliminate vial-to-vial variation in the run.
5. Pipette 3 ml pooled substrate solution (step 4) into disposable cuvettes and place in a 30°C water bath for 5-8 minutes to bring to reaction temperature. Do not pre-incubate more than eight cuvettes at a time.
6. Add 0.200 ml of control or specimen (Eppendorf Pipet). Cap cuvet. Mix well by inversion, tap to remove bubbles. Remove cap. Wipe cuvet and place in holder #1 making sure clear sides of cuvet are in the light path.
7. Repeat step 6 until all four positions in the cell chamber are filled, inserting 2nd and 3rd and 4th cuvets in a counter-clockwise manner. (See notes B & C)
8. When the final cuvet is in place, activate cell programmer by switching from manual to auto mode. Switch recorder to "chart" position.

9. Allow recorder to chart change in absorbence for several minutes. Refer to section on calculations to determine patient and control results. (See note E & F)

Notes:

A. If bottle does not have a vacuum or shows evidence of moisture, do not use.

B. Remove cover only long enough to place cuvette in position in order to maintain 30°C in well.

C. Offset may have to be used to get some of the samples on the chart. If that still doesn't work, switch to 0-2 scale settings and see note in calculation section.

D. This test should be run only after one is familiar with kit information supplied by Calbiochem (Document No. L03426, 4/1/78).

E. If ΔA is greater than .390/min, the activity is greater than 1000 mU/ml and the sample should be diluted with saline and rerun.

F. Elevated levels of ALT (GPT) may be substantially reduce NADH before initial absorbence is recorded. If a sample gives an initial reading of 0.6 or less dilute with saline and rerun.

G. Dichromate solution: Use reagent #PD3 from Oxford Spectrocheck set. Dilute 1:100 as directed on vial. Cover reference cuvette with parafilm so that it may be reused.

Calculations:

$$\frac{\Delta A/\text{min} \times \text{total volume} \times 1000}{\text{mM absorptivity} \times \text{sample vol.} \times \text{light path} \times \text{min}} = \text{mU/ml}$$

mM absorptivity = 6.22 @ 340 nm for NADH

This reduces to:

$$\frac{\Delta A/min \times 3.2\ ml \times 1000}{6.22 \times 0.2\ ml \times 1 \times 1} = 2572 \times \Delta A/min = mU/ml$$

When reading from the chart let each square represent a unit of absorbence; then

$$\Delta A \times 2.572 = mU/ml$$

when a line is extrapolated to cover a 10 minute period.

Note: If using 0-2 scale because of lipemic specimens, use:

$$\Delta A \times 5.144 = mU/ml$$

(also use a 10 minute line)

Expected Values: (taken from kit literature)

Male: 1-25 mU/ml @ 30°C

Female: 2-24 mU/ml @ 30°C

References:

See kit insert, Calbiochem Doc. No. LO3426.

GAMMA-GLUTAMYL-TRANSPEPTIDASE (GGTP)

Principle:

The assay is based on the transfer of the glutamyl group from L-γ-glutamyl-p-nitroanilide to glycyl-glycine in the presence of GGTP. The rate of p-nitroaniline formation measured at 405 nm is proportional to the GGTP concentration in the sample. (Sigma Technal Bulletin #415, 1/77).

Specimen:

Plasma: Blood is drawn into a tube containing either heparin or EDTA and centrifuged to obtain plasma. (See note D)

Serum: Blood is drawn into a plain tube and allowed to clot. The serum is separated from the clot as soon as possible. (See note D)

Storage: GGTP is stable in serum for at least 1 week at $4^{o}C$ and 2 months at $-18^{o}C$. A minimum of 1 ml is needed for analysis.

Instrument Settings:

Allow a 30 minute warm-up for instrumental system (Coleman 124D spectrophotometer and attachments).

1. Spectrophotometer:
    a. Wavelength: 405 nm
    b. Slit width: 1.0 nm
    c. Read absorbence on 0-1 scale.
    d. Place dichromate solution in reference. Set zero to keep readings on chart (1:100 dilution of stock #PD-3) (Note A).
    e. Tungsten lamp: on
    f. Mirror towards tungsten lamp
2. Recorder:
    a. Chart speed (20)
    b. Range (10)
    c. Power on Servo, then to chart when reading absorbence.

3. Cell Programmer:

   a. Power on

   b. Measurement period - 15 seconds

   c. Manual, initially, then to auto for readings

4. Scale Expander:

   a. All offset dials to 0

   b. High readings can be offset by turning appropriate knob to make readings stay on recorder.

5. Constant Temperature Circulating Water Bath:

   a. Set temperature 30.0°C (31°C on thermometer) (See Note E).

   b. Turn tap water on slowly.

6. Water Bath for Incubation

   Set at 30°C.

Reagent Composition:

GGTP substrate: (Sigma stock #445-5

L-γ-glutamyl-p-nitro anilide	70 µmol/L
Glycylglycine	600 µmol/L

A.M.P.D. buffer (Sigma stock #415-8)

2-amino-2-methyl-1, 3-propanediol	0.2 mol/L
pH 8.6	

Reagent Preparation:

Add 15.5 ml A.M.P.D. buffer to substrate vial. Shake vigorously for a few seconds and place in 37°C water bath 2-3 minutes until substrate is dissolved. Each vial contains enough substrate for 5 tests. Reagent is stable 2-3 hours at R.T.

Procedure:

1. Determine total number of assays: each patient is done in duplicate and at least one control in each run; a "run" consists of four assays being monitored sequentially at 15 second intervals.
2. Prepare the appropriate number of GGTP substrate vials. Pour all vials into a larger container and swirl to mix. This eliminates any vial-to-vial variation.
3. Pipet 3 ml of the pooled substrate into disposable square cuvettes and place in a 30°C water bath 5-8 minutes to bring to reaction temperature. Do not pre-incubate more than 8 vials at one time.
4. Add 200 µL sample to a cuvet with an Eppendorf pipet. Cap and invert several times to mix. Tap to remove any air bubbles. Remove cap, wipe cuvet and place in holder #1. (Clear sides of cuvet in light path.)
5. Repeat step 4 with 2nd, 3rd & 4th cuvettes, placing them in holders in a counter-clockwise manner. (Note B)
6. Switch cell programmer from manual to auto mode and immediately switch recorder to "chart" position.
7. Allow recorder to trace changes in absorbence for several minutes. Refer to calculations for how to determine patient and control results. (Note C)

Notes:

A. This is the same blank used in the ALT & AST procedures. If a sample is unusually lipemic or icteric, 200 µL of sample should be added to a cuvet containing the dichromate solution and this mixture should be used as a blank for that sample (to keep the readings on the chart).

B. Remove top from cuvet well only long enough to place each cuvet in its holder in order to maintain temperature in well.

C. If A is greater than 0.125/min, dilute the sample with saline and rerun.

D. Fluoride, oxalate and citrate have been found to inhibit GGTP activity. Falsely elevated levels occur in patients taking antiepileptic drugs, such as phenytoin and barbiturates.

E. The heating unit of the constant temperature water bath is usually set at 31°C depending on room temperature. The circulating water will cool as it warms the cell chamber. The temperature of the cell chamber can be checked periodically by placing tight fitting styrofoam material on the top of the cell chamber and then pushing a thermometer through this material.

Calculations:

$$\text{mU/ml} = \frac{\Delta A/\text{min} \times \text{total volume} \times \text{temp. correction factor}}{\text{micromolar extinction factor} \times \text{sample volume}}$$

$$\text{mU/ml} = \frac{\Delta A/\text{min} \times 3.2 \times 0.8}{.0099 \times 0.2}$$

$$\text{mU/ml} = \Delta A \times 1293$$

Note: When reading from chart, let each square represent a unit of absorbence. Extrapolate a line for a 5 minute reading, then ΔA (5 minutes) x 2586 = mU/ml.

Controls:

Controls consist of two levels of unassayed control material (Hyland Scan I & II). Control limits are ±2.0 standard deviations or other range as indicated in the current Scan Control Data Book. Unknowns are to be assayed in duplicate but not within the same run of four. There must be one control

in each run of four. If control values are not acceptable, check wavelength, slit width, cuvets, reaction temperature, pipetting, age of reagents, storage of reconstituted reagents, manual reading of reaction curves, and finally reconstitute new controls.

Normal Range:

Adults: up to 30 mU/ml

Reference:

Sigma Technical Bulletin No. 415, January, 1977.

CREATININE

Principle:

Creatinine reacts with picrate under alkaline conditions (Jaffe reaction) to give a yellow-red solution which is measured photometrically at 505 nm.

The determination is made on diluted urine or on protein-free filtrate (dialysate) of plasma or serum.

The method employed is a modification of the procedure of Folin and Wu taken from the text "Hawk's Physiological Chemistry."

Creatinine clearance is a sensitive measure of glomerular filtration rate. Relatively minor changes in serum creatinine are accompanied by changes in creatinine clearance which are more dramatic, especially in the early phase of kidney disease.

Specimen:

Creatinine may be determined in any biological fluid, but plasma, serum, amniotic fluid, and urine are the specimens most commonly employed. Plasma and serum are preferred to whole blood since considerable amounts of noncreatinine chromogens are present in red cells. If kept for a few days, specimens for creatinine are best stored at refrigerator temperatures; if kept for longer periods, they should be frozen. Aqueous solutions of creatine and creatinine very slowly approach a state of equilibrium with respect to each other. Creatinine is formed rather quickly from creatine in either alkaline or acid solutions.

When performing a creatinine clearance, a precisely timed urine specimen and serum sample is required. The blood is generally collected in the middle of the urine collection period. Submit a 50-100 ml aliquot of the well-mixed 24 hour urine collection with a record of the total volume.

Controls:

1. Hyland Scan I and II (serum)
2. 2 levels of frozen serum pools
3. 2 levels of frozen urine pools
4. Occasional assayed lyophilized urine material from Hyland

Reagents:

1. Saline, 9.0 gm NaCl 1000 ml double distilled water

   Add 0.5 ml Brij-35-mix

   Stable indefinitely at room temperature.

2. Sodium Hydroxide, 0.5 N

   20 gm/1000 ml double distilled water. Stable indefinitely at room temperature.

3. Saturated Picric Acid

   13 gm/1000 double distilled water. Stable indefinitely. (See note 1)

   a. To 13 gm of reagent grade picric acid in a one liter volume flask. Add distilled water to the mark.

   b. Allow the excess picric acid to remain in contact with the water and shake occasionally.

   c. Filter and store in a polyethylene bottle:

4. Stock creatinine standard (1 mg/ml)

   1000 gm/1000 ml 0.1 N HCl (Stable 1 year at room temperature)

5. Working creatinine standards:

   Dilute stock creatinine standard with 0.2 N HCl.

ml stock	Dilute to:	mg creatinine/100 ml
0.5	100 ml	0.5
1.0	"	1.0
2.0	"	2.0
3.0	"	3.0
4.0	"	4.0
5.0	"	5.0
7.0	"	7.0
10.0	"	10.0

Stable 3 months at room temperature.

Procedure:

Equipment Needed

Autoanalyzer I

1. Sampler II - (run at 60 per hour)
2. Proportioning Pump
3. Dialyzer (37°C - Type C Membrane)
4. 40 ft. time delay coil (Room temperature)
5. Colorimeter (505 mu - 15 mm tubular flow cell)
6. Recorder

(See attached flow diagram)

The sample stream segmented with air, is diluted with 0.9% sodium chloride. This combined stream enters the sample side of the dialyzer. The recipient stream consists of water segmented with air. (See notes 3, 4, & 5) After emerging from the dialyzer it is joined with a stream formed by a combination of saturated picric acid and 0.5 normal sodium hydroxide. The streams are mixed, sent through a time delay coil and then go into the colorimeter. The developed color is read at 505 nm using a 15 mm tubular flow cell.

-Warm up time of colorimeter - 20 min.

-Time to bring up reagents - 20 min.

-Set Baseline at 95% T

-Keep washline separate from picric acid and NaOH line.

-Serum samples should be mixed and centrifuged before being placed on sampler.

-Results that are higher than the 10 mg/dl standard should be diluted and repeated. Multiply result by appropriate dilution factor.

Plate format:

1. 0.5 mg/dl
2. 1.0 "
3. 2.0 "
4. 3.0 "
5. 4.0 "
6. 5.0 "
7. 7.0 "
8. 10.0 "
9. Water
10. S I
11. S II
12. Water
13. Frozen Pool #1
14. Frozen Pool #2
15. Water
16. Serum specimens
17. Water
18. Urine Pool #1
19. Urine Pool #2
20. Water
21. Urine specimens
22. Water

Notes:

1. Sigma Stock #925-40 (As a safety precaution, aqueous picric acid should be purchased, as the dry powder can be explosive)
2. A is obtained from a surface-area monogram. (p. 916, Hawk's Physiological Chemistry. See attached monogram).
3. For optimal bubble pattern and low noise use 0.5 ml of Brij-35 per liter of saline and distilled water recipient.

4. The noise with serum may sometimes be due to the formation of a precipitate. If this occurs, it is advisable to try a different lot of picric acid. It may also be helpful to clean the picric -sodium hydroxide lines and coils as well as the flow cell with 10% acetic acid.
5. When running the creatinine determination a check should be made of the noise. This can be done by continually aspirating a 5 mg/100 ml creatinine standard. The noise level should be no greater than ± 0.5 transmission line. If the noise level is greater, a check of the manifold and dialyzer should be made to insure that a good bubble pattern is being obtained. Noise is generally related to a poor bubble pattern which gives poor proportioning of reagents.

Calculations:

1. Serum or Plasma - these are read directly from a standard curve. Results are reported in mg/dl.
2. Urine results are reported in gm/24 hr vol. Samples are generally diluted 1:30 before analysis.

$$\text{mg/100 ml} \times 30 \times \frac{\text{aliquot vol}}{\text{24 hr vol}} \times \frac{1 \text{ gm}}{1000 \text{ mg}} = \text{gm/24 hr vol}$$

where mg/100 ml is the reading from standard curve and 30 is the dilution factor

3. Creatinine clearance is calculated as follows:

$$C = \frac{UV}{P} \times \frac{1.73}{A}$$

where U = mg creatinine/ml urine

V = ml urine/min

P = mg creatinine/ml serum

A = body surface area of individual being tested.

(See Note 2 or attached nomogram)

$\dot{C}$ = ml serum cleared/min/std surface area

Normal Ranges:

1. Serum or plasma:

Female	0.8-1.2 mg/dl
Male	0.9-1.4 mg/dl

2. Urine

Female	0.8-1.8 gm/24 hr vol
Male	1.0-2.0 gm/24 hr vol

3. Creatinine clearance

Female	75-115 ml/min
Male	85-125 ml/min

References:

1. Technicon Autoanalyzer Methodology Method File N-11B

2. Fundamentals of Clinical Chemistry, Norbert Tietz, 1976 p.996-998

3. Hawk's Physiological Chemistry, Edited by Bernard L. Oser, Fourteenth Edition 1965.

*U.S. GOVERNMENT PRINTING OFFICE: 1986-646-117-40930