5.9 Appendix - Answer Key

5.2.2 Standard Solutions

1. **Which SOP is used in the laboratory to ensure primary standard purity and reliability? (**Quote local laboratory pesticide standard SOP.) PAM I section 205 addresses pesticide reference standards.

2. **What constitutes a stock solution? A working solution?** A stock solution is the initial solution from which other diluted solutions are prepared. Working solutions are prepared from the stock solutions and further diluted for use in quantitation. (PAM I, 205-3)

3. **Which SOP is used to ensure reliability of stock and working solutions?** (Quote local laboratory pesticide standard SOP.) PAM I section 205 addresses pesticide reference standards.

4. **What are the hazards associated with handling and storage of pesticide standards?** All pesticides are toxic, but the relative degree of toxicity varies greatly. Some are readily absorbed through the skin, especially the liquids. Skin absorption is enhanced by dilution in solvents when preparing standard solutions. Gloves should be used when handling and preparing pesticide standards. Another entry route is inhalation, therefore pesticide standards should be handled inside a safety hood. Studies with rats indicate that pregnant women have increased susceptibility to pesticide poisoning, therefore should not handle pesticide standards. The Material Safety Data Sheet supplied with each reference standard contains information about the toxicity, hazards, and safe handling of the compound. (LIB 663)

5. **What class of pesticides is particularly toxic by skin absorption as well as by inhalation and ingestion?** Organophosphate pesticides such as parathion are particularly toxic and absorb through the skin rapidly. (LIB 663)

6. **Where can information such as chemical structure for EPA standards be found?** Structural information for pesticides can be found in several resources including the Merck Index, Farm Chemicals Handbook, Agrochemicals Handbook, FDA Surveillance Index, Menzie's Metabolism of Pesticides, various chemical vendor catalogues, and via internet search.

7. **In general, what would be the correct solvent for organochlorine pesticides? For organophosphates? How would one prepare a mixed standard containing both organochlorine and organophosphate pesticides?** For organochlorine pesticides iso-octane is most commonly used, for organophosphates acetone is used. For a mixture dissolve the pesticides in 1-2 mL of acetone and dilute to volume with iso-octane. (PAM I, 205-4)

5.3.2 Injection Techniques

1. **What is the major consideration in using the "solvent flush" technique for GC injections?** This technique when performed manually is the most reproducible among analysts because the actual sample volume injected is measured and completely delivered regardless of individual differences. Another consideration is the amount of solvent used to flush the sample volume. Excessive solvent flush can distort the chromatography of the analytes, especially the earlier eluters. This effect can be eliminated using temperature programming to concentrate the analytes on the front of the column until the solvent is evaporated out of the injection port.

2. **Generally, what causes bubbles or non-smooth draw up of solvent into a syringe?** A vacuum is created in the syringe when the plunger is withdrawn too quickly.

3. **For acceptable practice, what are the practical volumes to inject using a 10 uL syringe? What is the desirable percent relative standard deviation for repeated injections?** For a 10 uL syringe, the practical injection volume is 3-8 uL. The relative standard deviation (RSD) of five injections of a 1 ppm standard should be 2 % or less. Greater RSD values can be expected for injections of lower concentrations.

4. **What effects can be expected from a leaky septum?** Leaky septa cause inaccuracies in quantitation, problems with chromatography, and exposure of the system to air. (PAM I, 502-18)

5. **Explain the "blow-by" phenomenon.** If a syringe plunger does not seal against the syringe body properly the carrier gas of a GC can “blow by” the plunger out the back of the syringe.

6. **How is a syringe checked for "blow-by?”** Draw up 50 % of the syringe volume followed by a small volume of air, then insert the syringe into a GC, holding steadily and not allowing the plunger to move. If the air bubble moves toward the back of the syringe, the integrity of the syringe is compromised and the syringe should be repaired or replaced.

7. **Explain automated injection techniques e.g. split, split/splitless, on-column, and temperature programming.** For split injections, the flow of the carrier gas is split at the injection port between the column and waste. The split ratio of column/waste is set with a valve in the injection port. This procedure is used for injections of highly concentrated analytes. Split/splitless injection ports are simply split injection ports with the ability to close the split so that the whole injection is introduced onto the column. For on-column injections, the injection needle is inserted into the column during injection. This technique calls for very low volume injections (1-2 uL) or temperature programming to prevent injection losses

during rapid expansion of the sample solvent to vapor. Temperature programming allows the analyst to program the temperature of the injection port. The primary advantage of this technique is the solvent can be evaporated out of the injection port before the analytes are introduced onto the column eliminating undesirable solvent broadening effects.

5.3.3 Columns

1. **How are retention times measured?** The time of an unretained analyte (solvent) is subtracted from the time of the peak maximum response. (PAM I, 502-4)

2. **What GC parameter has the greatest effect on relative retention times?** Column temperature has the greatest effect on relative retention time. (PAM I, 502-4)

3. **Why are relative retention times used rather than absolute retention times?** For the same (or equivalent) liquid phase, relative retention time of an analyte is independent of column type (packed vs. capillary), liquid load, column length, and carrier gas flow. (PAM I, 502-4)

4. **What GC conditions are to be met for acceptable performance using the DG-1 system for chlorinated compounds?** Adjust column temperature (about 200 ^{o}C isothermal) so that relative retention time of pp'-DDT is 3.10 ± 0.06 when chlorpyrifos elution is fixed at 4.0 ± 0.5 minutes. Set detector response for a 0.15 ng injection of chlorpyrifos to 50 % full scale deflection. Set injection port temperature to 220-250 ^{o}C. (PAM I, 302-33)

5. **Why is chlorpyrifos used as the reference pesticide for determining relative retention times?** Chlorpyrifos is used because it chromatographs well and contains all the heteroatoms to which selective GC detectors respond. (PAM I, 502-4)

6. **What are some of the indicators of a deteriorating column?** Peak broadening, tailing, reduced theoretical plates and capacity factors, and diminished resolution between adjacent analyte peaks are all signs of a deteriorating column. (PAM I, 502-4)

7. **Describe the effect of pesticide polarity on chromatography and on column selection. Describe how columns are conditioned to achieve linear response.** Polar pesticides tend to react with active silanol sites in the injection port and column causing peak broadening, tailing, and even splitting. Polar compounds chromatograph best on polar columns. Capillary columns are conditioned after installation, with carrier gas flow thoroughly purging and then set at a designated level, followed by setting the column temperature 20-30 ^{o}C higher than the highest operating temperature for 1 hour but below its upper temperature limit. (PAM I, 502-21)

5.4.1 Electron-Capture Detector

1. **Briefly describe the principles of the ECD.** High-energy beta particles (electrons) emitted by a ^{63}Ni source collide with carrier gas molecules to create low energy electrons. These electrons are collected at the cell anode generating a baseline current that is monitored continuously. When electrophylic molecules enter they absorb the low energy electrons. The drop in baseline current is proportional to the quantity of electrophylic molecules in the detector. (PAM I 503-3)

2. **What are the advantages and disadvantages of the ECD?** The ECD is extremely sensitive to most electrophylic molecules; responses to 1-10 pg of analyte are normal. Organohalogens are generally excellent candidates for ECD. The major disadvantage is that compounds containing oxygen, a weaker electrophile, also respond very strong, generating considerable interfering co-extractant responses. (PAM 503-5)

3. **What is the maximum operating temperature for the ^{63}Ni detector?** The maximum operating temperature is 400 ^{o}C. (PAM I 503-7)

4. **What class of pesticides is generally determined using the ECD? What other types of chemicals respond to an ECD?** The ECD is generally used for the analysis of organohalogens. (PAM 503-5)

5. **Name at least factors that can affect detector response.** Sensitivity varies for each compound. Lack of selectivity can result from interfering co-extractant responses, especially at low levels. The detector has a dynamic range of response over five orders of magnitude, however, it is not linear over the entire range so standard and sample response matching of ± 25 % is needed. Other factor include cell volume, carrier gas flow, type of carrier gas used, amount of sample injected, the cleanliness of the source, temperature of the cell, solvent used, and cleanup of the sample.

5.4.2 Nitrogen/Phosphorus Thermionic Detector

1. **Briefly describe the principles of the N/P-TD.** GC column effluent impinges onto the surface of an electrically heated and polarized alkali source in the presence of an air/hydrogen plasma; ionization occurs and the flow of ions between plasma and ion collector is amplified and recorded. Detector response to analytes results from the increased ionization that occurs when compounds containing nitrogen or phosphorous elute from the column. (PAM I 503-27)

2. **What type of compound is detected by the N/P-TD under normal operating conditions?** Organonitrogen and organophosphorous compounds are detected. (PAM I 503-27)

3. **How does increasing H_2 flow affect response of the N/P-TD?** N/P-TD response decreases with increased H_2 flow. (PAM I 503-29)

4. **What hazards are associated with H_2?** Hydrogen is extremely reactive with oxygen in the air.

5. **Describe the modes of operation of the N/P-TD.** The detector can be configured to detect organonitrogens and organophosphates or just organophosphates alone. (LIB 1904 and *J. Chrom. Sci*., Vol. 12., pp. 625-9).

6. **What is the approximate difference in response between nitrogen and phosphorus with the N/P-TD?** Phosphorous response is approximately 5 times greater than nitrogen response. (PAM I, 503-29)

5.4.3 Flame Photometric Detector

1. **What are the differences between the FPD and the N/P-TD?** The FPD uses a hydrogen/air flame to raise atomic energy levels, then emission is filtered for phosphorous or sulfur and photomultiplied. The detector is designed for the optical filter used with the emission. The N/P-TD detector uses a heated alkali bead with hydrogen/oxygen plasma to ionize nitrogen and phosphorous containing compounds. The N/P-TD is selective for those compounds. (PAM I, 503-09)

2. **Assuming optimum flame conditions, what FPD component is the next most likely to affect sensitivity?** Detector sensitivity is greatly affected by the condition of the photomultiplier tube. Also, light leaking into the photomultiplier will greatly increase detector noise and decrease sensitivity. (PAM I, 503-10)

3. **Which detector is to be placed first in a dual detection system containing an ECD and FPD in series? Why?** The analyte structure is consumed by combustion in a FPD detector; therefore the ECD is placed first. (PAM I, 503-10)

4. **Why is the ignition button depressed and hydrogen slowly introduced when igniting the FPD?** In some older FPD models the flame is lit by turning on the oxygen flow and depressing the ignition button, then slowly introducing hydrogen until the flame ignites. If the hydrogen flow is increased too quickly, the hydrogen/oxygen mixture reacts too violently (explodes) inside the detector.

5.4.4 Hall Electrolytic Conductivity

1. **Briefly describe the principles of the HECD in the halogen mode.** GC column effluent is pyrolized in a nickel reaction tube at 900 ^{o}C in the presence of hydrogen gas. Halogen heteroatoms (X) react with the hydrogen to form HX gas [Nickel is a catalyst for the formation of HX] that is dissolved in a scrubbed conductivity solvent circulating through a conductivity cell. In the conductivity cell, electrolyte conductivity is constantly monitored. The HX reaction products increase the conductivity inside the cell, which is converted to a signal. To prevent neutralization of the acid formed in the reaction tube, the pH of the electrolyte is slightly acidic, so the electrolyte is continuously scrubbed through an ion exchange resin. (PAM I, 503-16)

2. **What are two major advantages of the HECD over an ECD?** When the HECD is running properly, it is much more selective to halogen than ECD. Because the ECD uses a radioactive ^{63}Ni foil the restrictions and requirements of the Nuclear Regulatory Agency must be observed. (PAM I, 503-14)

3. **Are there any advantages of the HECD (sulfur mode) over the FPD?** HECD in the sulfur mode is seldom used; however it does have an advantage over FPD in the sulfur mode (FPD-S) because it is more linear.

4. **Explain the steps to prepare the HECD for operation in the halogen mode.** Use N-propanol as the conductivity solvent with the halogen mode scrubbing resin. Use hydrogen as the reactant gas with a nickel reactor tube. Set flows and other instrument parameters as directed by the instrument manufacturer. (PAM I 503-21)

5.4.5 Mass Selective Detector

1. **Why should a MSD with a diffusion pump be cooled below 100º C before venting?** The system can become contaminated if it is not cooled before venting.

2. **What can we learn from the ratio of air to water ions in the Air and Water Check?** When a system is initially pumped down the air pumped out more quickly than water; water tends to stick to the stainless steel. If the air is still high after a period of time, there may be a leak.

3. **What is the highest electron multiplier voltage setting?** Most electron multipliers have a limit of 3000 volts. They are normally operated in 1100 – 2500 volts range, with the voltage increasing during its lifetime to achieve good sensitivity. At higher voltages, system noise rises, limiting sensitivity.

4. **In the full scan mode, what mass to charge range (M/Z) is available?** The M/Z range can vary from instrument to instrument but is usually two to 800 atomic mass units (amu). It is

useful to limit the scan range of a full scan to above 40 amu (to avoid ions of argon in the system) and below the largest molecular weight expected to be encountered (for pesticides, below 500 amu).

5. **Describe the libraries available using the data system.** The NIST Spectral Library is usually purchased with the instrument. Other spectral libraries, such as a pesticide library, are available from vendors. Become familiar with all the libraries on your system.

6. **What are the trade offs between the following: dwell time, number of ions monitored in a SIM Method and the shape of the chromatographic peak?** Long dwell times or a large number of ions monitored will result in fewer data points taken across the GC eluting peak. This may result in misshapen peaks which can give poor quantization areas.

7. **Why is the start operation of the MSD delayed? (i.e. a few minutes after the sample is injected).** This will prevent the filament from burning out.

5.4.6 Halogen Specific Detector

1. **What gases are necessary for the operation of the Halogen specific detector? Explain the purpose of each gas**. There are two gases required for the proper operation of the Halogen Specific Detector. Oxygen or Air and Helium. Oxygen is required for the complete pyrolysis of the analytes. Helium is used as the carrier gas.

2. **What classes of compounds can be determined using the Halogen specific detector?** The Halogen Specific Detector (XSD) is selective for halogen containing analytes, predominantly the chloride atom. The detector is 10,000 times more sensitive to chloride containing compounds than to hydrocarbons.

3. **Why is the Halogen Specific Detector operated at elevated temperatures?** The detector is operated at these high temperatures to insure the rapid and complete pyrolysis of the sample including the analytes of interest.

4. **What are the advantages of the Halogen Specific (XSD) detector over other halogen selective detectors?** The ***simplicity*** of the Halogen Specific detector provides enhanced reliability and reproducibility. There are numerous maintenance issues related to the ECD and the ELCD detectors. In addition, the ***selectivity*** of the Halogen Specific Detector is greater than the ECD. The ECD which is very sensitive to halogens is also capable of detecting other classes of compounds such as those containing nitro and carbonyl groups. This lack of specificity can be problematic in the qualitative determination of organochloride pesticides using ECD. The XSD detector will not detect the nitro and

carbonyl compounds thus making identification of organochloride analytes much easier.

5. **Describe briefly the Principle of Operation of the Halogen Specific Detector (XSD).** The detector is operated in the oxidative mode using oxygen (air) and high temperature to completely pyrolize the effluent from a GC column. This process oxidizes the compounds to their oxidative by products releasing free halogen atoms. These free halogen atoms are then adsorbed onto an alkali sensitized cathode. This reaction at the cathode surface generates a current which is measured by the electrometer and converted to a 0-1 volt signal. This signal is compatible with most chromatographic data stations.

5.5 Quantification

1. **What are the two types of manual Quantitation discussed in the PAM-504?** The two type of manual quantitation are peak height and triangulation.

2. When is peak height the measurement technique of choice? When measuring early eluting peaks that are very small, peak height is often the peak measurement method of choice. This avoids potential problems some integrators have in the accurate measurement of extremely small area responses.

3. What can be a major pitfall with electronic integration techniques? A major pitfall with electronic integration can be the uncritical acceptance of results. It is crucial that competent analysts continually examine the integration results. Often if a peak is poorly shaped or incompletely resolved, the integration algorithm may not accurately measure the peak. This can give inaccurate results.

4. Explain the purpose of "peak width" and "peak threshold" settings in electronic integration. Peak width is an integration parameter used to aid the integration process. Generally, smaller numerical values are appropriate for narrow peaks while larger values are appropriate for larger wider peaks. Peak threshold or slope sensitivity is a parameter used by the integrator to indicate the slope at which the integrator starts and stops the peak measurement process. Low values of threshold are used for small peaks. Higher values may be used for large analyte responses. If set too low, the baseline may be integrated along with peaks. If set too high, the peak may not integrate at all. A balance of peak width and peak threshold values is determined for each analysis.

5. **According to PAM Section 504, briefly explain a strategy that gives good quantitation of PCB.**
    - Use an extraction technique that removes the PCBs from other co-extractives.
    - Select a GLC system that separates the peaks as completely as possible.
    - Select the reference standard Aroclor mixture that most closely matches the sample

chromatogram. (The responses and retention times of peaks in the reference standard matches closely those of the sample.)

- Quantitate the PCB by adding all of the standard responses together. Do the same for the sample responses. Use only the peaks in the sample that match exactly the peaks in the standards.

5.6.1 Sensitivity Levels for Pesticide Residue Determination

1. **What criteria is to be met before considering a peak of 10% FSD as a trace or non-significant peak?** The correct analytical procedure specified by PAM is used and documented. The sensitivity of the determinative step is established as specified by the PAM. The sample weight equivalence specified by the procedure is examined by the determinative step. (PAM I, 105-1)

2. **What is the identity and quantity of each pesticide injected in establishing detector response for chlorinated, organophosphorus, and early-eluter residue determinations?** For chlorinated, organophosphorous, and early-eluter compounds set detector response so 1.5 ng chlorpyrifos produces a 50 % full-scale deflection (FSD). (PAM I 105-4)

3. **What is the trace level (ng) for an organophosphorus compound that gives 75% FSD for 7.5 ng, assuming correct setup?** Lq = 7.5 ng/75 % FSD x 10 % FSD = 1 ng (PAM 105-2)

5.6.2 Sample Preparation and Compositing

1. **How would someone prepare a sample of 20 melons submitted to the laboratory?** Remove and discard stems and composite whole melons using food processor. For smaller food processors equal portions of the each melon may be used for compositing. (PAM I, 102-2, 203-3)

2. **How would someone prepare a sample of six l-gallon cartons of milk?** Composite by handmixing equal portions from each carton. (PAM I, 102-4)

3. **How would someone prepare a sample of 20 lb of wheat?** Mix sample thoroughly. Mill about 4 qts through 1-3 mm sieve. (PAM I, 102-3, 203-4)

4. **How would someone prepare a sample of slightly decomposed heads of lettuce? Very decomposed heads of lettuce?** Cut out decomposed portion of lettuce heads and composite remaining lettuce using food processor. Do not analyze very decomposed product. (PAM I, 102-2)

5. **What are the major considerations for a laboratory sample in regard to the sample**

received by the laboratory and storage of reserve portions? Sample integrity is to be maintained by storing the sample in locked cabinets or refrigerator/freezers. Composites are stored under conditions to preserve the residues and commodity as much as possible. (PAM I, 102-6)

5.6.3 Nonfatty Foods-Multiresidue Determination

1. **Briefly describe the extraction and cleanup principles involved in this assignment.** See PAM I, 302 & 303.

2. **What three types of samples call for the following blending techniques: acetonitrile, 35% water-acetonitrile, heated acetonitrile-additional water?** Acetonitrile is used to extract high moisture low-fat products such as fruits and vegetables. A mixture of 35 % water in acetonitrile is used to extract low moisture low-fat products such as grains. A heated mixture of acetonitrile and water is used to extract low-fat very high sugar items such as honey. (PAM I, 303-1)

3. **What extraction procedure would be recommended to obtain complete extraction?** The Soxhlet exhaustive extraction is used for obtaining complete extraction when the nature and importance of the sample and residue warrant the time and effort needed for this procedure. (PAM I, 301-6)

4. **Where are the sample extracts from assignment (2), above, stored?** Sample extracts should be stored in a refrigerator/freezer to minimize concentration through evaporation.

5.6.4 Fatty Foods-Multiresidue Determination

1. **Briefly describe the extraction and cleanup principles involved in the assignment.** For the analysis of cheese use PAM I 304 E4/C6 (Cheese method). Sample is liquefied and denatured with alcohol and oxalate. Sample solids are separated using centrifugation. Fat and residues are partitioned from the supernatant using petroleum and ethyl ethers. The ether extract is washed with water to remove polar co-extractants. (For the analysis of ground oilseeds, high fat feeds or feed materials, grains, or nuts use PAM I 304 E5. Fats and residues are removed by dissolving in petroleum ether and ethyl ether, followed by ethyl alcohol. The organic extract is washed with large quantities of water to remove co-extractives.) Gel permeation chromatography uses size exclusion to remove fat from the extracts. The larger fat molecules are discarded and the smaller residues are collected. Using Florisil chromatography very polar co-extractants are removed by irreversible binding to the very polar Florisil adsorbent and the remaining residues in sample extract are fractionated by polarity.

5.8 Pesticide Residue Confirmation

1. **Where do you find listed the official tolerances for pesticide residues.** Official residue tolerances can be found in the 40 CFR 180.

2. **When the level of a residue exceeds an official tolerance, what type of analysis is performed? How close must this agree with the original analysis?** A check analysis must be performed and the quantification values from the original and the check must agree within 30%.

3. **If there is no tolerance for a residue, what value must the residue level exceed?** The analytical level must exceed the Lq or Limit of Quantitation.

4. **In the identification of residues, what is required to "Confirm" the identity of the residue?** Normally, there must be retention data on two different column types to confirm a residue. However if a GC-mass spectrometer is used for the identification, only one column is required.

5. **When is recovery information required?** Recovery information for the food commodity and the residue is required in cases where a tolerance exists and has been exceeded.

6. **Is quantification always required on Original and Check analyses?** No, if there is no tolerance for the residue, then the check analysis can be confirmatory only, indicating that the residue has been confirmed in a second test portion of the commodity.