

# **Organochlorine Pesticides in Fruits and Vegetables**



## **Instructor's Manual**

**Susan E. Kegley**

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Fruits and Vegetables  
Instructor's Manual**



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## INTRODUCTION

This module presents concepts related to structure/solubility relationships of organic compounds, risk assessment, gas chromatography, biodegradation, bioaccumulation, and organic extraction techniques. At the end of the module, students use the data collected by the class to write up a full report on the risks and benefits of pesticides in the food supply and/or debate the issue of pesticide use on our food supply. The full module can be carried out in four weeks, which includes one laboratory period for the debate on the use of pesticides in the food supply. Alternatively the instructor may choose to shorten the module by eliminating the debate or lengthen it by having students optimize the GC method of analysis.

This instructor's manual is provided to help someone new to pesticide analysis get started with this module. The following information is provided:

- Guidelines for material to be covered in the pre-lab lecture
- Suggested flow of lab
- Safety precautions
- Waste disposal
- Stockroom prep lists
- Suggested instrument procedures for setup and troubleshooting
- Answers to lab questions
- Example data

### **LEARNING GOALS FOR STUDENTS**

At the beginning of the module, it is important for you, the instructor, to present your students with a guide to what they should expect to have learned by the end of the module. This helps students gauge their progress and clarifies the important concepts covered by the module.

By the end of this module, **General** and **Organic Chemistry** students should have gained the following:

- An understanding of the solubility properties of organochlorine compounds and how the process of extraction works.
- An understanding of the basic principles of gas chromatographic analysis of organic compounds.
- Skepticism when interpreting computer-generated data.
- An introduction to the use of internal standards to gauge the percent recovery of extractables.
- An understanding of how risk assessment is carried out, including the complexities and vagaries of human perceptions of risk.
- An understanding of the problems and benefits associated with pesticide use and the difficulties inherent in regulating pesticides.

**Upper-Level Analytical** students should, in addition, have gained the following:

- A detailed understanding of gas chromatography as used in the analysis of organochlorine compounds, including the advantages and disadvantages of different detector systems, designing temperature programs to maximize accuracy and efficiency of the analysis, and the determination of response ratios.

## **LOGISTICS**

### **In What Courses Could the Module Be Used?**

This module can be used at a variety of levels throughout the undergraduate chemistry curriculum

#### **General Chemistry**

This module has been tested for seven semesters in a first semester general chemistry course at the University of California, Berkeley in special laboratory sections focusing on environmental chemistry. The experiments have been carried out successfully by students with a wide range of abilities, not just honors chemistry students. The rationale for teaching it in the first semester of freshman chemistry is based on the fact that it provides a broad range of students with evidence of the utility and importance of chemistry in our society. The major disadvantage of doing the module this early in the curriculum is students have had less exposure to some of the concepts and techniques presented. Equipment limitations might make it difficult to do this module at institutions with large (>350 students) general chemistry classes. Creative scheduling and/or use of more than one GC could circumvent this issue. In general, the response of freshmen to this module has been overwhelmingly positive.

#### **Organic Chemistry**

The module could also be used for an organic laboratory course, and is arguably better placed in a course where structure/solubility relationships, extraction techniques, and gas chromatography are more traditionally covered.

#### **Suggested Flow of the Module for General and Organic Chemistry**

Week #1:	Organic solubilities and risk assessment
Week #2:	Extraction of pesticide residues from produce
Week #3:	Data interpretation, data entry, and debate preparation
Week #4:	Pesticide debate

#### **Instrumental Methods of Analysis**

This module also works well in an upper level instrumental methods of analysis course and has been tested in this course three times at the University of California, Berkeley. With more advanced students, instructors are able to delve deeper into the theory and practice of gas chromatography. Students could determine response ratios and retention times for different pesticides and design their own temperature program that permits separation of all components of a pesticide mixture. They could then test the extraction efficiency of selected pesticides by processing a spiked sample. More time would need to be allotted for method development, but other parts of the experiment, such as the debate, could be trimmed if desired.

#### **Suggested Flow of the Module for Upper-Level Analytical Chemistry**

Week #1:	Determination of retention times and response ratios of selected pesticides.
Week #2:	Development of an appropriate temperature program for optimum separation and quantification of pesticide residues.
Week #3:	Extraction of pesticide residues from produce to determine

Week #4: extraction efficiency for selected pesticides.  
Data interpretation, risk assessment.

## Materials and Instrumentation

All materials and equipment not normally found in a chemistry stockroom are listed in the Stockroom Preparations sections with a source, catalog number, and approximate price. It is assumed that students have access to balances and the usual collection of flasks, beakers, clamps, etc. in their lab locker.

Analysis of organochlorine pesticide residues at trace levels requires a gas chromatograph with an electron capture detector. Alternatively, a mass spectrometric detector using single ion monitoring (SIM) techniques can be used with similar results. All development work was carried out using a capillary column, as recommended in the California Department of Food and Agriculture method.<sup>1</sup> An autosampler is **highly recommended**, particularly if class size is large. If an autosampler is available, up to 175 samples can be run in a week. If students work in pairs, a total of 350 students could easily do the experiment. Creative scheduling, where students rotate through several modules during the semester, would allow even larger classes to carry out the module. Alternatively, more than one GC could be used.

If a GC with an ECD/MSD or autosampler is not available at your institution and you would like to do this module, it might be worth considering the submission of a proposal to the National Science Foundation's Course Curriculum and Laboratory Improvement (CCLI) program (<http://www.nsf.gov>). Perkin-Elmer and Hewlett-Packard make excellent capillary instruments. Autosamplers and ECDs can usually be retrofitted onto existing instruments. The typical cost of an autosampler is ~\$10,000 and an ECD is ~\$3,000.

If you wish to carry out the supercritical fluid extraction process, you will need a supercritical fluid extraction system. An easy-to-use commercial system is the Hewlett-Packard 7690T, available for ~\$50-60K, depending on options. Be aware that with all SFE systems, you will likely need to spend a great deal of maintenance time to keep the instrument running well.

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1. California Department of Food and Agriculture, Division of Inspection Services, Chemistry Laboratory Services Branch, Pesticide Residue Program, *Multi-Residue Pesticide Screens*, Jan. 27, 1988.

## **RISK ASSESSMENT AND ORGANIC SOLUBILITIES**

On the first day of the module, it is important to set the stage for the entire 3-4-4 weeks of lab work. The first week focuses on the subtleties of risk assessment and structure/solubility relationships of organic compounds. In addition, the students will plan their experiment by creating a question they wish to know the answer to, then designing their produce sampling plan to best answer the question.

### **Reading in Laboratory Manual**

Laboratory Protocols and Safety: pages 3–6

Solubility of Organic Compounds: pages 20–29

Pre-lab Questions: page 25

Experimental Design: page 36

Risk Assessment: pages 64–74

### **Time Required**

- Risk Assessment: 30-40 minutes
- Solubility of Organic Compounds: 1.5-2 hours
- Experimental Design: 20-30 minutes

### **Pre-Lab Discussion**

Begin by spending a few minutes introducing the topic of pesticides in our environment. The structures of the pesticides analyzed for in this module are on page 13 in the text of the module. A transparency master of the pesticide structures is included in the Appendix to this Instructor's Manual. Points to bring out in your general overview are:

- The experimental techniques used in this module allow the detection of organochlorine pesticides, which are more long-lived than organophosphates (e.g., malathion) or carbamates (e.g., Sevin).
- Organochlorine pesticides are not very water soluble, so are not easily removed by washing produce.
- Organochlorine pesticides do not biodegrade readily. They often bioaccumulate in natural systems. Be sure to define biodegradation and bioaccumulation.
- Chronic, long-term exposure to organochlorine pesticides has been associated with an increased risk of cancer, as well as reproductive disorders that are thought to be due to the estrogenic effects of these pesticides. See the student manual for more information on these effects.

Solubility properties of organic compounds can be related to the issue of risk assessment by emphasizing how differences in the structure, and therefore solubility, of organic compounds provide the chemist with an easy way to separate pesticide residues from produce and allow for the determination of the exposure received by the average person.

### **Risk Assessment**

The main points of how risk assessment is done should be presented briefly. Discuss the methods used to construct a dose-response curve (animal tests, bacterial

tests, and epidemiological studies), and talk about perception of risk. Some additional reading on risk assessment can be found in the following books:

1. *Environmental Epidemiology and Risk Assessment*, Tim Aldrich and Jack Griffith, Van Nostrand Reinhold (New York, 1993), pp. 212-237.

2. *Principles of Environmental Toxicology*, Sigmund F. Zakrzewski, American Chemical Society (Washington, DC, 1991), pp. 11-25.

Students' task for this part of the experiment is a writing assignment, where they answer the questions on page 74 in the student manual. This assignment is a good place to have students work in groups to discuss possible answers, but it is best to have the expectation that each student do his or her own writing.

Because most of the answers are either in the reading or are an interpretation on the part of the student, this part of the experiment could also be a pre-lab exercise that students turn in when they arrive in class or lab. Once students have turned in their answers, it is helpful to discuss the answers with the entire class. Use the examples on pages 71-73 to get the students to discuss how the perception of risk often governs people's behavior.

### **Solubilities of organic substances**

Introduce the concept of organic extraction in the context of thinking about how it is possible to isolate a substance from a solution without getting too many other components with it. Some everyday examples of extraction the students will likely be familiar with include coffee from coffee beans, tea from tea leaves, proteins and flavor molecules from chicken bones, and spice extracts (vanilla, almond, etc). This part of the module provides an opportunity to discuss polarity, dipole moments, intermolecular forces, Lewis dot structures, and electronegativity. The old adage *like dissolves like* will be helpful for the students, but they should understand *why* that phrase is true. (see the lab manual on Organic Solubilities for more detailed background). The following demonstrations are helpful in presenting the concepts.

#### **DEMONSTRATION: The bending of water**

##### **Materials required**

- Two 50 mL burets, one full of water and the other full of hexane. Attach both burets to the same buret clip and have two wide diameter pans below the burets to catch wide-ranging drips. Label the burets as to their contents with labels that are large enough to be visible from the back of the room.
- One inflated balloon.
- A dark background in place behind the burets.

##### **Procedure**

Draw the structures or provide molecular models of water and hexane that clearly show the geometry around the atoms. Ask students to determine the expected polarity of each substance.

Demonstrate the polarity of water and the non-polarity of hexane by rubbing the balloon on your head (or have a student do it if you lack the hair!), then open the buret of water and bring the balloon close to the stream of water. The water will bend strongly towards the negatively charged balloon. (See "Why does a Stream

of Water Deflect in an Electric Field?" by G.K. Vemulapalli and S.G. Kukolich, *J. Chem. Ed.*, **1996**, *73*, 887 for a detailed explanation of the phenomenon). Try not to let the balloon get wet---once it's wet, the charge on the balloon is quickly neutralized.

Do the same with the hexane and you will observe no bending of the stream of liquid. Because hexane is not polar, there is no way to induce charge asymmetry in a collection of non-polar molecules.

### **DEMONSTRATION: Predicting solubilities of organic substances in water**

#### **Materials required**

- 5 labeled scintillation vials, half-full with the following solvents: acetone, hexane, methanol, methylene chloride, acetonitrile
- iodine crystals and a spatula
- salt (NaCl)

#### **Procedure**

Draw the chemical structures of these solvents up on the board or put them on the overhead projector (Transparency Master is available in the Appendix in this instructor's manual). Ask the students to make predictions about which compounds will be water soluble. Take a poll, tallying the predictions under each structure. This should happen with some discussion, i.e., ask students why they are predicting the result they are. This is a good opportunity to have them think about electronegativities of different atoms and the effects of electronegativity on the distribution of electrons in a molecule and its resulting polarity.

Add a speck of iodine to each solvent (for color), then fill the vial the rest of the way with water and let them see if their predictions hold. Add salt to the acetonitrile/water mixture and you will see the (now very polar) water separate from the acetonitrile to form two distinct layers. Emphasize that this is the procedure that will be used in the pesticide extraction to first dissolve all soluble compounds, then drive the non-polar pesticides into the acetonitrile layer, away from other impurities that are water soluble.

Finish by summarizing the effects of polar groups on the water solubility of chemical substances. Have students think about the need for using a fairly non-polar solvent to extract pesticide residues from produce by showing the structures of the pesticides being extracted (Transparency Master available). Emphasize "Like dissolves like."

#### **Experimental Design**

Students will need to come up with a question about pesticides in the food supply they wish to answer with their experiment, then decide the produce they will need to buy to answer the question. Give students about 10 minutes to discuss ideas in a group of 4-5 students, then open the discussion up to the class as a whole so the groups can share their ideas and decide on the **one** question they wish to answer. The instructor should guide this discussion and keep students from planning to do more than is feasible. The biggest mistake students make here is changing too many variables at once. This is a problem in pesticide analysis because the distribution of a pesticide on fruits or vegetables is not at all uniform. Because of this fact, it is necessary to have as many replicate samples as possible if the object of the study is to find out, for example, whether processed spinach (frozen, canned) has different concentrations of pesticides than fresh spinach. Students could also decide to analyze a wide variety of produce to

assess the extent of possible pesticide exposure from a typical adult or child diet. **Be careful** in interpreting results here---you really need more than one sample to draw any kind of conclusion about pesticides in the food supply. Be sure students think about this as they plan their experiment.

The list of eligible produce is on p. 36 in the module text. Remember that citrus fruits and members of the cabbage family (broccoli, cauliflower, cabbage, brussels sprouts) cannot be analyzed by this method due to the presence of compounds that interfere with the ECD. Carrots usually give some interfering peaks on the chromatogram as well. Remember that students will work in pairs, so this limits the number of different samples students can analyze to *half* the number of students in the class. Produce that most commonly contains detectable organochlorine pesticide residues includes grapes, spinach, strawberries, blueberries, peaches, nectarines, and plums. Less commonly, "hits" are obtained on apples, cucumbers, lettuce, tomatoes, potatoes, and squash.

### **Creating a flow chart for the extraction lab**

If you have additional time in the lab on this day, providing time for students to work with their partners to plan their work on the pesticide extraction helps immensely in increasing the students' efficiency during the week of the (long) extraction experiment. Have them create a flow chart plan for the extraction, paying particular attention to division of labor in the parts of the experiment partners do together. A sample flow chart is shown on the next page. This exercise could also be assigned as homework, but you should emphasize that it is **critical** for them to really do it.

### **Flow of lab**

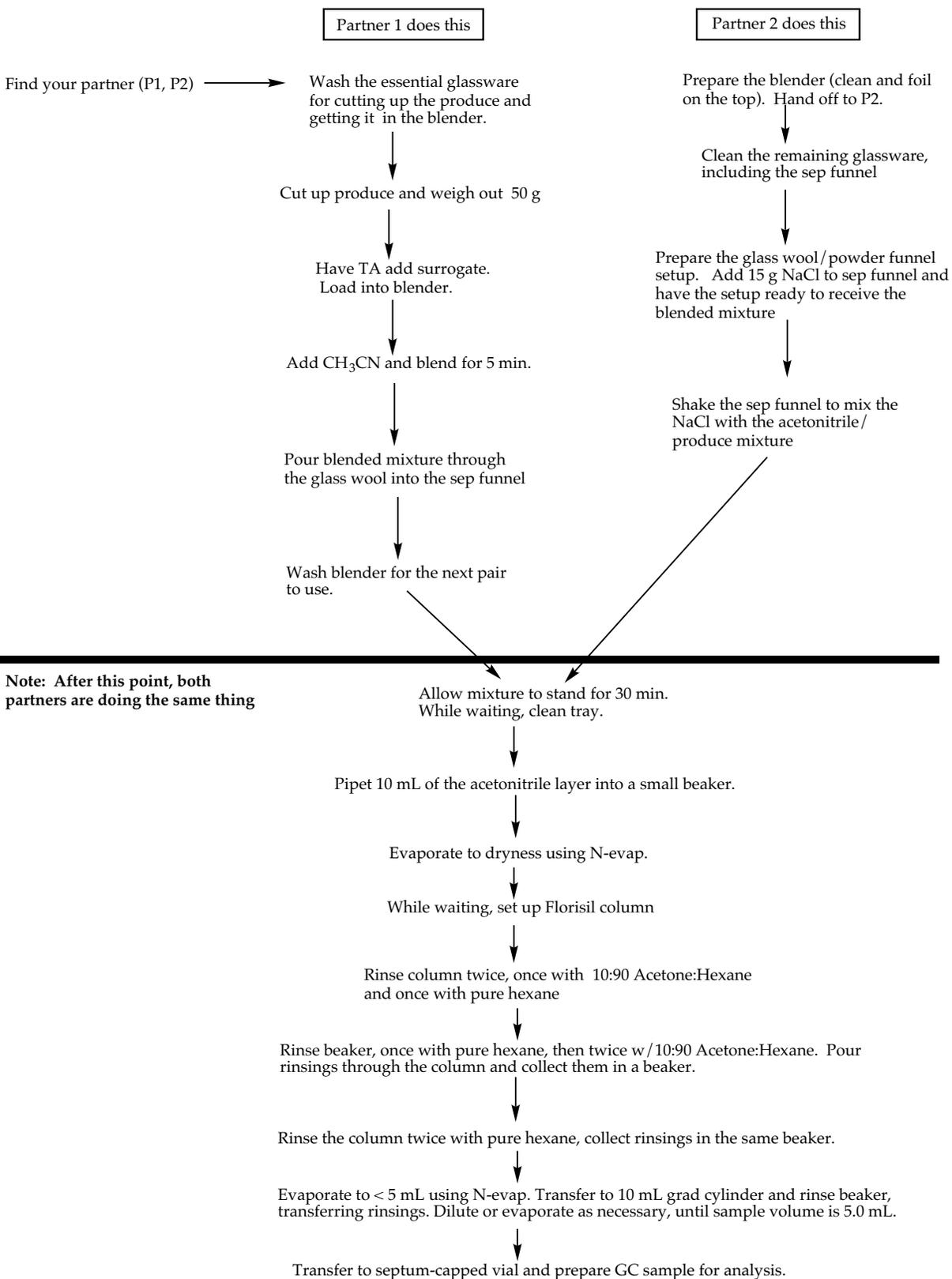
It is best to have students do the risk assessment writing exercise first, before they get involved in the details of carrying out the solubility tests. Students should work in pairs for the solubility tests. They should be encouraged to make copious notes on their observations to facilitate answering the post-lab questions. Planning the experiment should be done at the end of the laboratory period.

### **Safety Considerations**

Students should be aware that they will be working with flammable solvents. Remind them of the fire extinguisher and fire blanket locations and be sure there are no sources of open flames or sparks in the laboratory. Aniline should be kept in a fume hood. Provide rubber gloves for students to use in this experiment.

### **Waste Disposal**

This experiment generates about 50 mL of organic waste per pair of students, assuming they clean their test tubes with acetone. Some of the waste will contain the halogenated solvent methylene chloride. Have students place this waste in a separate container and do not mix it in with the other components, so only a small amount of the waste will need to be disposed of as halogenated waste.

**Sample Flow Chart for Pesticide Extraction**

## Answers to the Risk Assessment Exercise

1) List as many possible routes of exposure to pesticides as you can think of for an adult human and for a 2-year-old. Consider all forms of pesticides, not just food-use pesticides.

### Adult

- Ingestion of pesticides on food and in drinking water
- Inhalation or ingestion of pesticides while applying them in the home or garden
- Inhalation or ingestion of pesticides while applying them during work (home pest control, agriculture)
- Inhalation or ingestion of pesticides from spray drift if the person lives near an agricultural area
- From animal flea collars, foggers, and dip treatments

### Two-year-old

- Ingestion of pesticides on food and in drinking water. This may be more of a problem with children because they tend to eat a less varied diet and they eat and drink more than adults on a per kilogram basis.
- Inhalation or ingestion of pesticides from spray drift if the person lives near an agricultural area
- From touching animal flea collars and treated fur, then putting hands in mouth
- From crawling around in a yard that has been treated with lawn-care pesticides, then putting hands in mouth
- From crawling around on the floor of a house that has been fumigated for ants, roaches, termites, etc.
- From the clothes and hands of a parent who has been applying pesticides

2) If you eat a salad made with lettuce, carrots, and walnuts that have each been sprayed with a different type of pesticide, what factors would you need to take into consideration in order to estimate how much of each pesticide you actually ingest with this salad?

- how much of each vegetable you ate, in grams
- the average concentration of the pesticide typically found on each vegetable

Other factors you might wonder about include:

- How toxic are the pesticides?
- Do the different pesticides have synergistic effects that might amplify the toxic effects?

3) Consider the following statement by Lombardo and Yess<sup>2</sup> about weighing the risks and the benefits of pesticide use:

*“The concept of relative risk is a difficult one to communicate to the general public. Benefits are usually expressed in economic terms and are perceived as an advantage to business interests, while risks are expressed in life span, injury, or other physiological*

---

2. “The Food and Drug Administration Program on Pesticide Residues in Food,” P. Lombardo and N. J. Yess, *Pesticide Residues and Food Safety*, B. G. Tweedy, H. J. Dishburger, L. G. Ballantine, and J. McCarthy, eds., American Chemical Society (Washington, DC, 1991), p. 168.

*consequences that have emotional connotations and can be related to individual concerns. Economic benefits to the consumer from pesticide use in terms of a higher quality, more abundant, cheaper food supply have apparently not been effectively communicated.*

*“Despite the fact that the U.S. population is now the longest lived and best protected in history, the overall perception by most Americans is that they face more risks today than ever before and that the risks will continue to increase in the future.”*

*In the context of the reading in the section above, what do you think might be going on in the minds of the American people that would make them feel this way? Write a half page or so to explain your reasoning in some detail.*

Students should mention issues related to how different people perceive risk, regardless of the actual risk. They can also discuss the “risk-dread” diagram in the context of the fear of the unknown that most people have and comment on the uncertainty of the long-term effects on human health of the ingestion of small amounts of pesticides.

### Answers to Post-Lab Questions

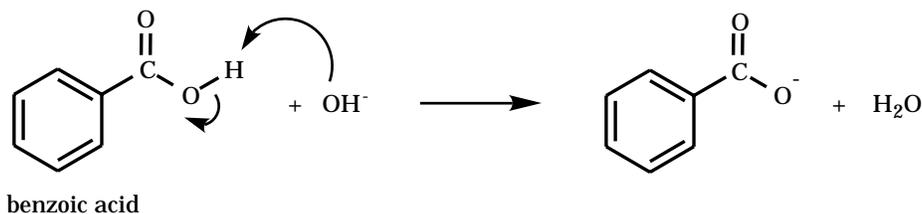
1) From a first glance at the structure and composition of methylene chloride, students might readily predict that methylene chloride is *not* soluble in hexane and *is* soluble in water. The two electronegative chlorine atoms bound to carbon create bond dipoles and a net molecular dipole, thus making methylene chloride a polar molecule. The results of the solubility tests will provide them with a real observation that methylene chloride is *soluble* in hexane. They are then left with the task of rationalizing this observation. What must be true is that the intermolecular forces between hexane molecules and methylene chloride molecules must be strong enough to overcome the intermolecular forces between methylene chloride molecules. The apparent paradox can be explained by noting that while chlorine is quite electronegative, it is large and polarizable, which reduces its tendency to concentrate charge in one area of the molecule.

2) Ammonia is more water soluble than triethylamine for two reasons:

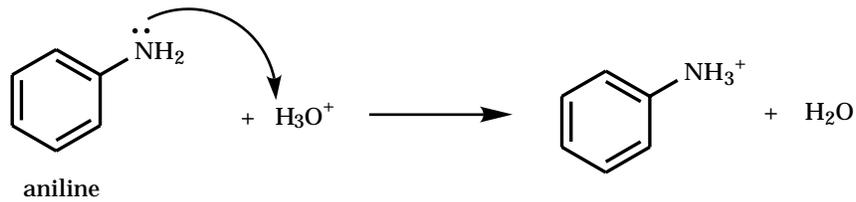
a) It can act as both a hydrogen bond *donor*, with an unshared pair of electrons, and a hydrogen bond *acceptor*, with the three hydrogens bound to the electronegative nitrogen.

b) Triethylamine has three very non-polar ethyl groups, rendering a significant fraction of the molecule hydrophobic. It is thus energetically unfavorable for triethylamine to participate in molecular interactions with water molecules.

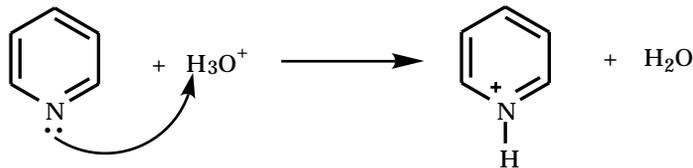
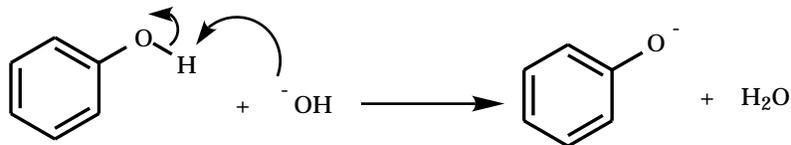
3) Benzoic acid is insoluble in acid, but soluble in base. The equation for the dissolution reaction is:



Aniline is insoluble in base, but soluble in acid. The equation for the dissolution reaction is: 4) Predict that phenol will be soluble in 6 M NaOH, pyridine will be soluble in 6M



HCl, and cyclohexane will be insoluble in both.



5) The following compounds would be able to participate in hydrogen bonding with water:

acetone	H-bond acceptor
benzoic acid	H-bond donor, H-bond acceptor
oleic acid	H-bond donor, H-bond acceptor
acetonitrile	H-bond acceptor
glucose	H-bond donor, H-bond acceptor
aniline	H-bond acceptor

Note that the ability to participate in hydrogen bonding with water does not guarantee water solubility. Molecules with an extensive hydrocarbon portion (oleic acid, aniline, and benzoic acid) are non-polar and will not dissolve appreciably in water.

## **STOCKROOM PREP: ORGANIC SOLUBILITIES**

This stockroom preparation list applies to a room of 25-30 students working in pairs. Have gloves available for students to use.

### **In the hood**

100 mL dropper bottles of these liquids:

- acetone
- hexane
- methylene chloride
- acetonitrile
- corn oil
- aniline

500 mL bottles of these solvents:

- hexane (cheapest grade possible)
- acetonitrile (cheapest grade possible)

3 dropper bottles (100 mL size) of 6 M NaOH

3 dropper bottles (100 mL size) of 6 M HCl

pH paper, range 1-12

Wash bottles of acetone for washing test tubes

### **Near balances**

Small bottles (< 100 g size) of the following solids:

- glucose
- benzoic acid

### **Waste Containers**

- 1 L waste container labeled "Organic Waste---NO methylene chloride"
- 500 mL waste container labeled "Methylene chloride waste ONLY"

# EXTRACTION OF PESTICIDES FROM PRODUCE

In this part of the module, students will extract pesticide residues from the produce they have chosen to analyze. The organic solubilities lab should have prepared them to think about why pesticides are more soluble in organic solvents than in water.

## **Reading in Laboratory Manual**

Organochlorine Pesticides: pages 9–18

Fundamentals of Extraction: pages 30–43

Supercritical Fluid Extraction (if your class is doing this): pages 44–50

Prelab Assignment: page 34

## *Review*

Solubility of Organic Compounds: pages 20–29

## **Time Required**

- Pesticide extraction: 3 hours
- NOTE: This is a long experiment and there is no good place to stop in the middle. The best way to get through it in a reasonable period of time is to do whatever you can to have students get organized ahead of time. The flow chart (part of the prelab assignment) is quite effective in requiring students to plan their lab work before they come into lab. You could also tell them to bring dinner if they don't read ahead. (This is usually quite effective in encouraging them to prepare!)

## **Before Lab**

Go shopping for some interesting fruits and vegetables in case the student responsible for shopping forgets to go. Alternatively, call and remind the student assigned to the task.

Be sure the GC is fully functional. See the Instructor's Manual section on preparing the GC for the experiment and plan to have the instrument ready about a week ahead of time, just in case something doesn't quite work as planned.

## **Pre-lab Discussion**

The instructor should re-emphasize structure/solubility relationships and remind the students of the purpose of the lab: to extract and quantify pesticide residues on produce with the intent of determining the average exposure consumers receive.

## **Solvent Extraction**

Extraction should be presented in the context of separating pesticide residues from the vegetable matter in a form that can be analyzed. Show the structures of some organic solvents on the board (draw out the structures---hexane, methylene chloride, acetonitrile, water), then have the students look at the structures of the pesticides they will test for on p. 13 in the module text and determine which solvent would be the best. There is no right answer for this question because the compounds have very different structures, but this exercise provides a good lead-in for reminders about polarity, electronegativity of elements, hydrogen bonding, and like-dissolves-like. Methylene chloride is probably the solvent that will best dissolve most of the chlorinated pesticides shown (like dissolves like); however, it is more hazardous to work with and more expensive to dispose of than a more benign solvent like acetonitrile, the solvent actually

used in the experiment. Methylene chloride is also inappropriate because it dissolves too many of the polar compounds found in fruits and vegetables, which results in a very messy chromatogram.

### Supercritical Fluid Extraction

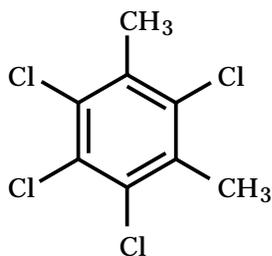
If you have access to a supercritical fluid extraction system, it is a nice comparison to have students run their samples using both extraction methods and then compare the results. The SFE process doesn't take much more time and can easily be fit into the 30 minute waiting period while students wait for the acetonitrile/water layers to separate. More advanced students can tinker with the extraction conditions to optimize the extraction for different pesticides.

Depending on the level of the class, the pre-lab discussion on SFE can touch on one or more of the following:

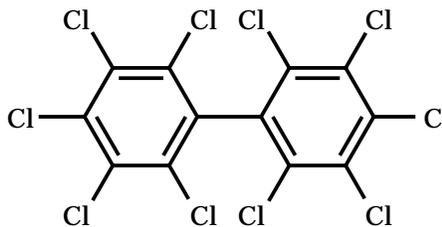
- Phase diagrams and the theoretical basis for supercritical phenomena
- The flexibility of SFE for fine tuning extraction conditions
- Advantages and disadvantages of SFE vs solvent-based extractions

### Using a surrogate spike

The use of an internal standard (the word **surrogate** is often used preferentially but interchangeably in environmental labs) to measure extraction efficiency is an important concept in environmental chemistry. In short, the surrogate provides information on the efficiency of the extraction by adding a compound of similar solubility characteristics to the mixture before extraction, then measuring the percent of the compound recovered from the sample. The two compounds tetrachloro-m-xylene (TCMX) and decachlorobiphenyl (DCBP) are used as the surrogate compounds to help estimate the recovery of pesticides from the extraction process. The major limitation of using these particular substances as surrogates is that they are not very similar in structure or boiling point to some of the pesticides. In practice, the results show that many students evaporate off the TCMX (and probably the lower-boiling pesticides as well) in the step in which solvents are removed using the nitrogen evaporator. Student recovery of the DCBP is usually higher than recovery of TCMX by at least a factor of two.



Tetrachloro-m-xylene  
TCMX



Decachlorobiphenyl  
DCBP

### Issues of contamination

Because the ECD is such a sensitive detector, it is important to keep potential sources of organic contaminants to a minimum. Solvents will dissolve contaminants from plastics (pipet tips, plastic bags), rubber (latex pipet bulbs or gloves, rubber

washers on blenders, rubber stoppers), vinyl (gloves), and stopcock grease. Teflon is the only non-contaminating plastic. The instructor should ensure that all separatory funnels have teflon stopcocks and that *no grease* is used in preparing the separatory funnels. If you have access to a machine shop, have them make teflon washers for the blenders. The students must be aware of potential sources of contamination and take special precautions to avoid contact of the extract with plastics or rubber. It is wise to make an extract of each contaminant that might possibly be encountered and run it through the GC to show the students the potential problems and to help them interpret the impurity peaks that may show up in their chromatograms. Make transparencies of the impurity chromatograms (or post the chromatograms in the lab room) to show them to the students before they begin their work so they know the importance of being careful. To make these contaminant extracts, simply place the object (rubber gloves, rubber stoppers, Pasteur pipet bulbs, blender washers, blender caps, etc.) in a small beaker of 10% acetone: 90% hexane (or even just pure hexane) for about 30 seconds. Obtain the chromatogram of these extracts using the same GC conditions as for the pesticide samples and standards.

### **Flow of lab**

The pre-lab lecture should be as short as possible. This is a long experiment and there is no good place to stop in the middle. Students work in pairs, using the blenders on a first-come, first-serve basis. While waiting 30 minutes for the acetonitrile layer to separate from the water layer, or while waiting for solutions to evaporate, students can take a tour of the GC to see how it works or, if you plan to also do an SFE extraction, students can load their extraction thimbles during this time.

### **Techniques and safety**

- 1) If students have not used a separatory funnel in their prior experiments, the instructor should demonstrate how it works. Show students how to vent the separatory funnel, reminding them to *hold the stopper on* when they turn it upside down.
- 2) The instructor should add the surrogate compound to each person's sample, using a gas-tight microliter syringe. Be sure to wash the syringe with acetone (4-5 times) at the end of the day.
- 3) The most important safety concern for this experiment is the mixture of flammable solvents and sparks generated by blender motors. In the lab room, the blenders should be set up in a geographically different location than the solvents. While we have never had a fire during this experiment, the instructor should know where the fire extinguisher is and not hesitate to use it.

### **Waste Disposal**

This experiment generates about 250 mL of waste per pair of students. Students should be encouraged to dispose of any acetonitrile/fruit/vegetable/water/acetone waste in a separate container from the waste containing hexane. In some areas, water soluble solvents such as acetonitrile and acetone can be drain-disposed in small quantities. Check your local regulations.

## STOCKROOM PREP: PESTICIDE EXTRACTION

This stockroom preparation list applies to a room of 25-30 students. Suppliers and catalog numbers for non-standard items are included here.

### **In one hood: Solvents**

- Acetonitrile, Pesticide-grade (Fisher Scientific, catalog # A999-4)  
Four 500 mL bottles
- 10% acetone:90% hexane (volume %), Pesticide-grade  
Two 500 mL bottles
- Hexane, Pesticide-grade (Fisher Scientific, catalog # H300-4)  
Two 500 mL bottles
- Acetone, Pesticide-grade (Fisher Scientific, catalog # A40-4), put out in *teflon* wash bottles (Fisher Scientific, catalog # 03-409-12A).

**NOTE:** All solvents should be put out in 500 mL *glass* bottles so they are easy to pour. Before the first use, the bottles should be cleaned and allowed to dry completely, then rinsed with Pesticide or Optima grade acetone. Labels should instruct students to never NEVER stick any implements (pipets, etc.) into the bottles of clean solvent and never NEVER pour solvent back into a bottle of clean solvent.

**SUPPLIERS:** Pesticide or Optima™ grade solvents can be ordered from Fisher Scientific. Although Optima™ grade is the highest quality, the cheaper Pesticide-grade solvents have never caused us any problems with contamination. The teflon wash bottles are essential because of contamination by the plastic from a normal wash bottle. Teflon wash bottles are also available from Fisher Scientific (Catalog # 03-409-12A).

### **In a second hood: Blenders**

- 5 blenders, with teflon washers if possible (machine shop can make these)

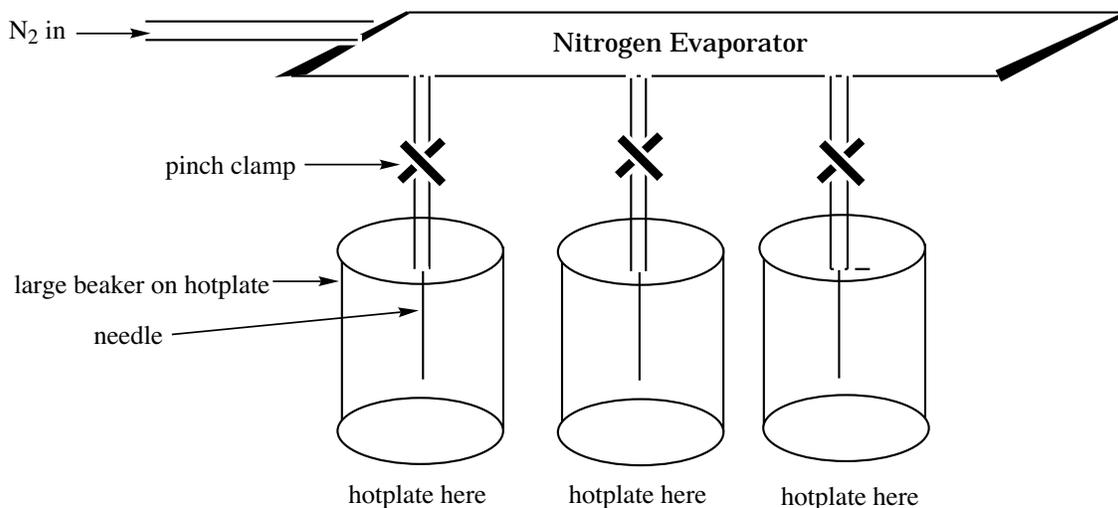
**NOTE:** If there is limited hood space, the blenders should be the first choice of equipment and/or supplies to put in a hood. If there is a fire caused by blender sparks, at least it will be localized in a small, contained, non-flammable space. Be sure no flammable materials (paper, Kimwipes, waste bottles, etc.) are in the hood with the blenders.

**SUPPLIERS:** Blenders can be purchased for a very reasonable price from a local discount store. There is no need to purchase them from a more expensive scientific supply house. Just be sure to purchase *glass* blenders (not plastic!) and wrap the plastic tops with foil to prevent contamination from dissolving plastic. These blenders are not spark-free, but acetonitrile is not flammable enough or volatile enough to flash at room temperature. Just be sure you do not use these blenders with very flammable solvents such as hexane or acetone.

### **In a third hood: Nitrogen Evaporator**

Set up the nitrogen evaporator in a different hood. This device can be constructed from some glass or plastic T-tubes, tygon tubing, pinch clamps, and 4-inch 16-gauge syringe needles. Alternatively, Pasteur pipets could be used instead of syringe needles. The gas

used does not necessarily have to be nitrogen, but does need to be clean and oil-free. If there is a clean air supply *and a hood* (remember, fumes floating around in the lab are a potential fire hazard) available at the students' individual desks, a better solution would be to have students construct their own evaporator, using air or house nitrogen as the gas. All needles (or Pasteur pipets) should be cleaned with an acetone-soaked Kimwipe before use and at the beginning and end of each lab day. Kimwipes should be placed near the nitrogen evaporator in the hood. A hot plate under the nitrogen evaporator that warms the water bath to just barely above body temperature ( $\sim 40^{\circ}\text{C}$ ) will speed the solvent evaporation process; however, if the water bath is *too* hot, pesticide residues will volatilize, resulting in low recoveries.



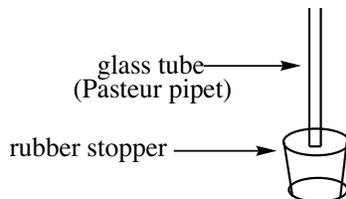
### In Control of the Instructor

- 15 mL of a 10,000  $\mu\text{g/L}$  TCMX-DCBP surrogate spiking solution, stored in a freezer when not in use (available from Supelco, Supelco Park, Bellefonte, PA 16823-0048, Phone: (800) 247-6628, as their PEST Surrogate Spike Mix, catalog #4-8460M). Prepare this standard using microliter syringes and very small (10 mL) volumetric flasks.
- 500  $\mu\text{L}$  gas-tight syringe for dispensing the surrogate (Fisher Scientific, catalog #13-684-106)

### Supplies and non-flammable chemicals

- 250g NaCl
- 30 Prep-Sep® Florisil™ funnels, 1 per student (Fisher Scientific, catalog # P476)
- 30 15-mL vials with teflon-coated septa, 1 per student (Fisher Scientific, catalog # 03-393C)
- 30 2-mL GC autosampler vials with teflon-coated septa (use the supplier recommended for your instrument). You will also need a crimping tool to seal the caps onto the autosampler vials.
- 10 knives (cheap serrated knives from a discount store work well)
- 10 aluminum pans for chopping produce (grocery store)
- Glass wool (about a golf ball sized lump for each **pair** of students)
- Pasteur pipets and latex pipet bulbs

- Latex gloves (contamination by plastics is minimized with latex gloves)
- 30 #5 one-hole rubber stoppers (*very clean*), fitted with a Pasteur pipet with the end removed, i.e., no glass protruding from the stopper (see picture below), 1 per student.



### **Waste Containers**

- Two 1 L waste bottles labeled "Acetonitrile/acetone/fruit/vegetable waste."
- One 1 L bottle labeled "Hexane waste"

## INSTRUMENT PREP: GC ANALYSIS

A capillary GC coupled to an electron capture detector (ECD) is especially well-suited for detection of halogenated organics and is essential for the sensitivity necessary to detect ppb levels of pesticide residues in environmental samples.

*Preparing the capillary gas chromatograph for the analysis of organochlorine pesticides should be started **the week before** the experiment begins. If you have not used an ECD before, you might want to start **two weeks before**.* The ECD is a tricky beast and requires special handling compared to an FID or TCD. Because of the long run time (~45 minutes), an autosampler is highly desirable for this experiment.

### **Instrument Setup**

This analysis is best carried out using a 25 m x 0.25 mm ID fused silica capillary column, loaded with a 0.25  $\mu\text{m}$  film thickness of 5% phenylmethyl silicone. This column is available as the PTE-5 column (catalog #2-4135M) from Supelco, Supelco Park, Bellefonte, PA 16823-0048, Phone: (800) 247-6628, FAX: (814) 359-3044). The detector makeup gas should be an ultra-pure mixture of 95% Ar/5% CH<sub>4</sub> (80-100 mL/min) and the carrier gas should be ultra-pure helium (~50 mL/min flow at split vent, 1-2 mL/min through the column). All gases should be connected to the GC using 1/8" copper tubing that has been pre-cleaned at initial setup to remove oils, by first rinsing tubing with methylene chloride, then with hexane and finally methanol. The gases should be passed through a final in-line moisture trap before entry into the GC. The output from all GC ports (split vent, purge vent, detector vent) should be exhausted to a hood. For maximum sensitivity, all injections should be carried out in the splitless mode using a special splitless capillary insert available from your GC manufacturer. Most new capillary GCs are shipped with both split and splitless inserts.

The retention times for the pesticides we tested for are given in Table 1. The temperature program and instrument parameters for pesticide analysis are given in Table 2. A sample chromatogram of a standard mixture is shown on page 54 in the student manual.

**Table 1: Typical Retention Times for Pesticides on a 5% Phenylmethyl Silicone Column**

<b>Pesticide</b>	<b>Retention time (minutes)</b>
Chlorothalonil	13.00
Vinclozolin	14.81
Kelthane	17.15, 30.30
DCPA	17.38
Anilazine	18.67
Captan	18.87
Endosulfan	20.23, 22.93
Dieldrin	21.47
Iprodione	29.37
Methoxychlor	30.54
Permethrin	38.28, 38.92

**Table 2: Instrument Parameters for Organochlorine Pesticide Analysis**

Carrier gas	He, 1-2 mL/min
Makeup gas	Ar/CH <sub>4</sub> , 80 mL/min
Detector	ECD
Injection port insert	Splitless
Flow from split valve	50 mL/min.
Injector temperature	225°C
Detector temperature	300-350°C
Sample volume	2-5 µL
Initial oven temperature: Ramp @ 4°C/min to: Ramp @ 2°C/min to: Ramp @45°C/min to:	140°C, hold 2 min. 220°C, hold 5 min. 250°C, hold 0 min. 265°C, hold 5 min.
Purge valve	Off, t = 0 min. On, t = 2 min. for the entire run
Total run time	47 min.

## Preparation of Pesticide Standards

A variety of pesticides are available from *Chem Service*, 660 Tower Lane, P.O. Box 3108, West Chester, PA 19381-3108, Phone: (215) 692-3026, FAX: (215) 692-8729. The *Chem Service* catalog is a valuable resource because of its extensive cross-referencing of trade names and common names for pesticides. For example, Kelthane is also known as Dicofol, and DCPA is also known as Chlorthal. We worked out the GC conditions for eleven of the organochlorine pesticides most commonly found on produce (see Table 3).

**Table 3: Pesticides Used in This Experiment with LD<sub>50</sub> and Order Number**

Pesticide	Alternate Names	LD <sub>50</sub> (mg/kg)	ChemService Catalog #
Anilazine	Triazine, Dyrene®, B-622®, Kemate®	2,710	PS-388
Captan	Orthocide 406®, Vancide 89®, SR-406®	9,000	PS-25
Chlorothalonil	Bravo®, Daconil 2787®	N/A	PS-1020
DCPA	Chlorthal, Dacthal®, Rid®, DAC 893®, Chlorthal-Methyl®, Fatal®	3,000	PS-33
Dieldrin	Compound 497®, HEOD, Octalox®	46	PS-76
Endosulfan	Chlorthiepin®, Malix®, Hoe 2671®, Thiodan®, Cyclodan®, Beosit®, Thimul®, Thifor®	18	PS-81
Iprodione	Rovral®, Glycophene, Chipco 26019®	3500	PS-1052
Kelthane	Dicofol, DTMC®, FW 293	668	PS-82
Methoxychlor	DMDT®, Marlate®, Methoxy-DDT®, Dimethoxy-DT®	6000	PS-83
Permethrin	Ambush	430	PS-758
Vinclozolin	Ronilan®	10,000	PS-1049

While other pesticides may be observed in an extract, it is not critical for students to know the identity of every peak on a chromatogram. You may choose to make up a standard containing only a few components. If so, it is best to choose pesticides most likely to be found on produce. These are: permethrin (spinach), captan (grapes, berries), iprodione (grapes, berries), vinclozolin (berries), and methoxychlor (apples). Data from a recent article from the *Journal of AOAC International*, Vol. 75, 1992, pp. 925-933 by H.J. Schattenberg, II, and J.-P Hsu entitled "Pesticide Residue Survey of Produce from 1989 to 1991" is included in Appendix B at the end of the student manual and provides a wealth of information on the occurrence of pesticide residues on produce.

Using care in handling the powdered pesticides (be sure to check the MSDS sheets accompanying the pesticide shipment), prepare primary stock solutions of

*individual* pesticides at concentrations of 1.00 g/L in Pesticide-grade acetone or methanol (weigh 100 mg of pesticide into a 100 mL volumetric flask or 50 mg into a 50 mL volumetric flask). These primary stock solutions should be diluted with hexane (*note change of solvent*) to the concentrations shown in Table 4 to give the secondary stock solutions. Because of the volatility of the organic solvents used to prepare the standards, gas-tight microliter syringes are the optimum method for transferring the solutions to prepare the more dilute standards. Fisher Scientific carries the Hamilton series 1700 syringes for volumes between 10-500  $\mu$ L and series 1000 syringes for volumes above 1 mL.

**Table 4: Preparation of Secondary Stock Solutions**

<b>Pesticide</b>	<b>Concentration of Secondary Stock Solution (<math>\mu</math>g/L)</b>	<b><math>\mu</math>L of Primary Standard Used to Prepare Secondary Stock Solution in a 10 mL Volumetric Flask</b>
Chlorothalonil	5,000	50
Vinclozolin	5,000	50
Kelthane	5,000	50
DCPA	5,000	50
Anilazine	5,000	50
Captan	5,000	50
Endosulfan	5,000	50
Dieldrin	5,000	50
Iprodione	50,000	500
Methoxychlor	5,000	50
Permethrin	50,000	500

All stock solutions should be stored in a freezer in clean glass vials with teflon-lined septum caps (Fisher Scientific, catalog # 03-393C). If the septum cap has been perforated, replace it immediately with a new one to avoid leaching of the silicone rubber into the solution. If stored properly, the lifetime of the 1.00 g/L standards is several years, and that of the 5,000 and 50,000  $\mu$ g/L standards is a year or so. Before storing any standard, draw a line on the outside of the vial at the level of the solvent. This will make it easy to spot a standard from which solvent has evaporated or spilled.

The multicomponent GC **working calibration standards** are prepared by using gas-tight microliter syringes to transfer the appropriate amounts of the secondary standards for each pesticide into a single 10 mL volumetric flask and diluting to volume with Pesticide-grade hexane. Because the detector response for each pesticide is different, it is essential to make up working standards that give a comparable detector response for each component. Table 5 lists the detector response ratio for each pesticide relative to anilazine (see p. 60 in the module text for more information on response ratios). Table 6 provides a guide to volumes that should be used to prepare a set of standards containing the eleven pesticides. This is only a guide,

as your detector may have slightly different sensitivity.

**Table 5: Response Ratios for Pesticides**

<b>Pesticide</b>	<b>Response Ratio (relative to anilazine)</b>
Chlorothalonil	3.48
Vinclozolin	1.88
Kelthane	0.16
DCPA	2.87
Anilazine	1.00
Captan	1.19
Endosulfan I	1.67
Dieldrin	1.75
Iprodione	0.14
Methoxychlor	0.20
Permethrin (cis)	0.11

**Table 6: Preparation of Working Standards**

<b>Pesticide</b>	<b>μL of Secondary Stock Solution in 10 mL volumetric flask (Standard #1)</b>	<b>μL of Secondary Stock Solution in 10 mL volumetric flask (Standard #2)</b>	<b>μL of Secondary Stock Solution in 10 mL volumetric flask (Standard #3)</b>
Chlorothalonil	10	25	50
Vinclozolin	25	50	100
Kelthane	250	500	1,000
DCPA	15	35	70
Anilazine	50	100	200
Captan	30	75	150
Endosulfan	30	60	120
Dieldrin	20	40	80
Iprodione	30	75	150
Methoxychlor	50	100	200
Permethrin	50	100	200

**Table 7: Final Concentration of Working Standards Prepared According to Table 6**

<b>Pesticide</b>	<b>Concentration of Components in Standard #1 (µg/L)</b>	<b>Concentration of Components in Standard #2 (µg/L)</b>	<b>Concentration of Components in Standard #3 (µg/L)</b>
Chlorothalonil	5	12	25
Vinclozolin	12	25	50
Kelthane	125	250	500
DCPA	8	17	35
Anilazine	25	50	100
Captan	15	32	75
Endosulfan	15	30	60
Dieldrin	10	20	40
Iprodione	150	320	750
Methoxychlor	25	50	100
Permethrin	250	500	1,000

Table 7 provides the final concentrations of the components in the working standards prepared according to Table 6. The lifetime of the working calibration standards is 3-4 weeks, if the vials are stored in the freezer when not in use and the septum caps are intact.

### **Preparation of Surrogate Standards**

The surrogate used in this analysis is a mixture of tetrachloro- meta-xylene (TCMX) and decachlorobiphenyl (DCBP), available from Supelco, Supelco Park, Bellefonte, PA 16823-0048, Phone: (800) 247-6628, as their PEST Surrogate Spike Mix, catalog #4-8460M. This commercially prepared primary standard is 200,000 µg/L. For GC surrogate analysis, 20, 50, 80, and 100 µg/L standards should be prepared. This can be done by carrying out an initial 100-fold dilution to give a secondary standard with a concentration of 2,000 µg/L. Dilution of 100µL of the primary standard to a final volume of 10.0 mL with hexane will provide this secondary standard. To prepare the working standards, use the amount of secondary standard indicated in Table 8 and dilute to 10.0 mL with hexane. Use gas-tight microliter syringes (Fisher Scientific) to carry out all transfers. While you have the concentrated surrogate solutions out, you may also want to prepare the 10,000 µg/L surrogate spiking solution to add to the students' samples before processing.

**Table 8: Preparation of Surrogate Standards**

<b>Working Standard Concentration (<math>\mu\text{g/L}</math>)</b>	<b>Volume of 2,000 <math>\mu\text{g/L}</math> standard to dilute to 10.0 mL with hexane (<math>\mu\text{L}</math>)</b>
100	500
80	400
50	250
20	100

A representative chromatogram of the standards and surrogates is shown on the following page.

### **Conditioning of the Column and Preparation of the ECD**

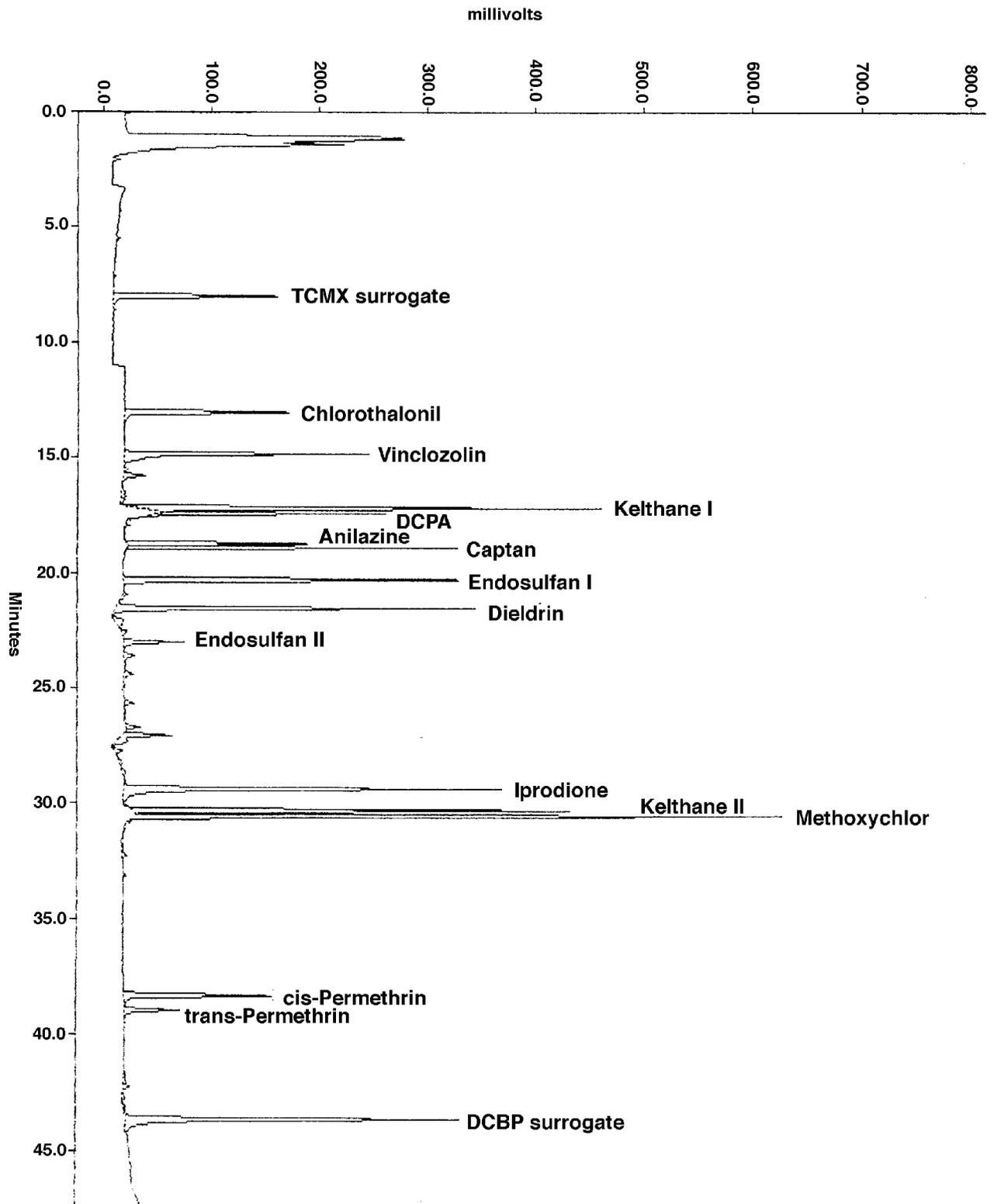
The column must be conditioned carefully to ensure saturation of the sites on the stationary phase and on the glass insert in the the injection port. This is done by injecting the most concentrated standard onto the column and allowing the temperature program to run its course. The number of times this must be repeated is related to the age and previous use of the column and detector. If the column is new, it will take longer to condition the system than for a previously-used column. If the column has been sitting unused for a more than a few days, but has been previously used for the analysis, fewer injections will be required. Best results will be obtained if the column is reserved for pesticide analysis only. The practice of "baking out" the column at the end of the day will drastically *reduce the sensitivity of the analysis and should not be done on a routine basis.*

The ECD is quite sensitive to temperature changes and functions best if it is left on at 300-350°C at all times, with the makeup gas flowing. Getting a good baseline after the detector has been turned off often requires cycling the GC through the temperature program a number of times. Conditioning of the column can be done at the same time by injecting the highest standard and cycling through the temperature program until a flat baseline is achieved throughout the run. With an autosampler, the easiest method is to set up the instrument to run the conditioning standard overnight (an out-of-date, high-concentration working standard works well for this purpose).

If the column and ECD have been used within a day or so and the instrument has been left on in the interim, it is usually only necessary to run the conditioning standard a few times (2-4) before analyzing real samples or standards. Interruption of a sequence of runs by more than 2-3 hours may result in deterioration of the accuracy of the standard curve, so it is best to run all samples with no interruption. The mid-range standard should be run periodically between samples to ascertain the continuing accuracy of the standard curve.

To avoid contamination of the ECD, don't use it for other experiments where significant concentrations of halogenated compounds are present. One shot of as little as 1 $\mu\text{L}$  of methylene chloride will decommission the ECD for several days!!





## Using an MSD with Single Ion Monitoring (SIM)

While we did not use an MSD in our development work, people do routinely use an MSD for detection of organohalogen compounds. Sensitivity will likely be somewhat less than with an ECD, but can be enhanced by using the technique of single ion monitoring, which can be programmed easily with most commercial GC-MS instruments. The technique allows you to focus the spectrometer scan on several high-intensity ions characteristic of each pesticide. The four most intense ions of the pesticides analyzed in this experiment are given in Table 9 below. An advantage of this technique is that most impurities do not show up because they do not (except by coincidence) have fragment ions of the same mass as the pesticides. Sensitivity can also be enhanced in the SIM analysis by tuning the MSD for maximum response in the 100-300 mass range.

**Table 9: Intense Ions of Pesticides for Single Ion Monitoring by Mass Spec**

Pesticide	m/z of Intense Ions in Mass Spectrum <sup>a,b</sup>
Anilazine	239 (100), 241 (66), 178 (35), 143 (33)
Captan	79 (100), 77 (42), 80 (25), 149 (21)
Chlorothalonil	266 (100), 264 (85), 268 (52), 109 (30)
D CPA	N/A <sup>c</sup>
Dieldrin	79 (100), 82 (42), 81 (35), 108 (21)
Endosulfan	195 (100), 241 (80), 197 (77), 237 (70)
Iprodione	56 (100), 43 (93), 58 (61), 314 (41)
Kelthane	139 (100), 111 (36), 141 (29), 250 (27)
Methoxychlor	227 (100), 228 (23), 152 (10), 138 (10)
Permethrin	183 (100), 163 (16), 165 (12), 184 (9)
Vinclozolin	N/A <sup>c</sup>

<sup>a</sup>The intensity of the peak is given in parentheses as a percentage of the base peak.

<sup>b</sup>Data from *Mass Spectra of Environmental Contaminants*, 2nd edition, Ronald A. Hites, Lewis Publishers (Boca Raton, FL, 1992).

<sup>c</sup>Not available.

## TROUBLESHOOTING GUIDE FOR GC ANALYSIS

There are a number of possible problems that might be encountered in any GC analysis of trace level components. Some of them have to do with the particular analysis and others relate only to the GC conditions. What follows is a list of common problems and some potential solutions. If you really get stuck, you should probably call your GC manufacturer's technical service department.

### No peaks observed when sample injected

- Check that there is enough sample in the autosampler vial.
- Check to be sure the syringe is not clogged.
- Check that the injection port insert is splitless and the split ratio is no higher

than 50:50.

- Check that purge valve is *off* for at least two minutes after injection of sample.
- If the column or detector has just been changed, the positioning of the capillary column in the detector or injector might be off. Check this and reposition the column if necessary.
- If the column is new, be sure it is properly conditioned.
- Is the detector on?

### **Too many peaks observed during a run**

- Are the gas lines and gases clean? To check this, run the temperature program without injecting a sample. If you observe extra peaks, suspect the purity of your gases. If you don't have in-line oxygen and water filters, get them. You may need to buy higher purity gases.
- Is the syringe clean? If you are using an autosampler, check to be sure the wash vials are filled. Try a new syringe.
- Are there contaminants in the solvent? Inject pure solvent to check. Have only Pesticide-grade solvents been used in all analyses? Are there known impurities that may be present from the procedures? If so, extract the suspected contaminant and obtain a chromatogram for cross-checking.
- Are there contaminants or incompatible compounds in the sample? Some samples contain compounds that interfere with ECD pesticide analysis. These include broccoli, brussel sprouts, cabbage, cauliflower, citrus fruits, avocados, and carrots.

### **Wandering baseline**

- Is the instrument just warming up? It may take several injections and over a day for the ECD to stabilize and give a good baseline. If this is the problem, be patient and keep injecting samples and cycling the instrument through the temperature program.
- Is there methylene chloride contamination? If so, it may take several days to get the detector to calm down.
- If the baseline rises during the analysis, you can still get useful data by putting in additional autozero commands at unimportant times (i.e., no peaks come out at these times) in the method.

### **Irreproducible Retention Times**

- Is the carrier gas tank turned on?
- Is there sufficient gas pressure in the tank?
- Is the capillary column partially clogged? You can check this by detaching the detector end of the column and placing it in a beaker of water. If the column is clear, you should see a steady stream of bubbles coming out of the end.

### **Detector signal off-scale and won't come back on scale**

- Check to be sure the make-up gas is on and there is sufficient pressure in the tank.
- Is the detector sufficiently warmed up and stabilized? Depending on your detector, this may take 30 minutes to a few hours.
- Is it time for a new detector?

## **INSTRUMENT PREP: SFE EXTRACTION**

This method was developed using a Hewlett-Packard 7690T SFE system. Your system is likely to be somewhat different; however, the conditions given in Table 10 below should be readily transferable between instruments.

**Table 10: Conditions for SFE Pesticide Extraction**

<b>Parameter</b>	<b>Value</b>
Extractant	Ultrapure CO <sub>2</sub>
Pressure/Density	
Extraction temperature	
Extraction time Static Flow	
Nozzle temperature	
Trap packing	Stainless steel or C18
Trap temperature	
Rinse solvent #1	Pesticide-grade hexane 2 mL
Rinse solvent #2 (cleanup solvent)	Pesticide-grade acetone 5 mL??

The parameters above provide conditions for a good general extraction program, but the conditions don't work equally well for all pesticides. If you are only going to analyze for a select few pesticides, you may want to experiment with different times, temperatures, and pressures to optimize the conditions for those particular pesticides.

### **Instrument Supplies**

- Ultrapure CO<sub>2</sub> with eductor tube to use as the supercritical extraction fluid (from your local gas supplier)
- xxx-grade CO<sub>2</sub> to use for cooling (from your local gas supplier)
- Extraction thimbles, thimble caps, funnels, etc. (from SFE system manufacturer)
- Pesticide-grade hexane
- Pesticide-grade acetone
- 2-mL GC autosampler vials with teflon-coated septa (use the supplier recommended for your instrument). You will also need a crimping tool to seal the caps onto the autosampler vials.

### **Some Precautions for Best Performance**

Leaks are common in these systems and are usually caused by solid materials that find their way into the threaded part of the extraction thimble/thimble cap. The best way to avoid this problem is to clean the threads on both the thimble and the thimble cap before use and using a thimble funnel (screws into the thimble so threads

are not contaminated) to load the produce. Be sure to stuff all the loose ends of the plant material down into the thimble before removing the funnel. Wipe the threads clean if necessary, then put on the thimble cap.

The thimble caps clog easily---proper maintenance is essential if the caps are to last for more than one extraction. After each extraction, the caps should be immediately placed in a beaker of water or acetone to prevent the plant material stuck on the caps from hardening. The caps should then be sonicated, first for 15 minutes in distilled water, then for 15 minutes in Pesticide-grade acetone. The use of an additional filter within the funnel (the Kimwipe™ in the procedure) helps to keep solid matter from contaminating the thimble caps in the first place.

## DATA ANALYSIS AND DEBATE PREPARATION

The goal of this day's lab work is to teach the students about interpreting the data they acquire from the GC and to prepare them for the upcoming debate. It is important to communicate to the students the human factor in data interpretation. The setting of tolerance levels should also be discussed in order to show students the connection between instrument accuracy and sensitivity, and laws regulating pesticide use.

### Reading in Laboratory Manual

Gas Chromatography: pages 51–63

Pesticide Regulations: pages 76–80

The Greate Debate: pages 82–85

### Time Required

- Chromatogram interpretation: 30-40 minutes
- Calculations and data entry: 20-30 minutes
- Discussion of data: 20-30 minutes
- Preparation for Pesticide Debate: 20-30 minutes

### Before Lab

- Select students to be in the Debate Groups, Agribusiness and Environmentalists. Be sure to divide the strong and/or articulate students equally, so both groups are on a fairly even playing field.
- Have the GC files available in case students need to reintegrate their chromatograms.
- Print out chromatograms and report printouts for each student's sample(s). Prepare a spread sheet for students to enter their data into such as the one sketched out below.

Name (Partner)	Produce tested	mass (g)	Pesticide(s) found	Conc. $\mu\text{g/g}$	Tolerance ( $\mu\text{g/g}$ )	$\mu\text{g/L}$ TCM X	% recovery	$\mu\text{g/L}$ DCBP	% recovery
Gilbert (Elizabeth)	Apples washed/unpeeled	50	Methoxychlor Kelthane	0.14 0.046	14 5	17	19	17	19

A sample data set from our class can be downloaded from the web at:  
<http://www.cchem.berkeley.edu/~chem1a/echem.S97/Pesticides/Pest2.html>

### Pre-lab Discussion

Begin by spending a few minutes introducing the importance of tolerance levels in pesticide regulation, defining them and discussing the rather random way they have been set in the past (see student manual). Emphasize that ultimately, the tolerance level depends on the technology available for detection. As detection limits have plummeted in the last 10-15 years with advances in GC and detector technology,

tolerance levels that were once set at the detection limits are now being revised.

This part of the module is also a chance to convince students of the value of skepticism in chromatogram interpretation and data analysis. Students tend to believe something if it is on a computer printout, regardless of the quality of the data. This laboratory period will give them a chance to practice with critical analysis of data.

### Gas chromatography

It is useful to emphasize the differences between organic and inorganic substances in terms of volatility and use this to lead in to GC as a separation technique. Only *volatile* substances can be analyzed this way, and the analysis is for substances in the *gas phase*. Pages 51–61 provide detailed background information on gas chromatography. Most freshman students have no trouble understanding basic chromatographic concepts. The table below is a good way to present chromatography basics in a way that allows comparison between different types of chromatography (thin layer chromatography, paper chromatography, ion chromatography, high pressure liquid chromatography). For an advanced class, this will help the students see the similarities between the different types of chromatography.

mobile phase	helium gas
stationary phase	organic resin that compounds stick to (dipole-dipole interactions)
column	0.25 mm x 30 m (capillary)
chromatographic "force"	high pressure gas
temperature of analysis	30-400°C, as needed
detector	Electron Capture (ECD)

Depending on the level of the class, discussion of the ECD or the MSD as a detector can range from a simple explanation of its advantages in quantifying organohalogen compounds to a complete description of the electronics involved in making it work. If possible, have your students take a "GC tour" of the instrument, so they can see it and explore how it is put together.

### Interpreting a chromatogram

Return the chromatograms to the students. Remind them how important it is to look at the chromatogram carefully and critically, not just to read off numbers from the report. Instill in them that they should never trust a computer! Common computer mistakes students should know about include:

- Sometimes the GC will label a peak as a pesticide, even though the retention time is far off from the expected retention time. Students must compare actual and expected retention time of a suspected peak. If it is within 0.15 minutes, there is a good chance it really is the component in question. If not, it is worth being suspicious.
- Sometimes the GC will label a peak as a pesticide, even though the peak is not really shaped like a peak. Shapeless blobs will sometimes be labeled as peaks and students must *look* at the chromatogram to determine if the peak is real.
- The integration of the peaks may not be correct, due to computer error in drawing the baseline. This explains a surrogate recovery greater than 100% that is sometimes observed. To fix this problem, students will need to go back to the

computer on the GC, fix the baseline, and reintegrate the peak. It is good to leave some extra time to do reintegrations during this class period.

- Remind them of interference peaks. Chromatograms of expected interferences (rubber gloves, rubber stoppers, blender washers, pipet bulbs, etc.) should be posted in the lab. Overheads of these chromatograms (available in the Appendix of this Instructor's Manual) are useful for the pre-lab lecture.

### **Method Detection Limits and Tolerance Levels**

The concept of method detection limit (MDL) is an important one for students. Because of the different response ratios for the different pesticides, the MDLs are different for each pesticide. The table on p. 62 in the module text provides an estimate of the MDL (25% of the lowest standard concentration for each pesticide) for our instrument and conditions, assuming the peak shape and and retention time are acceptable. *Your instrument will likely have different MDLs, so you should experiment with very low concentrations of standards.*

Discuss tolerance levels and point out that the differences in MDLs for the different pesticides have implications for setting tolerance levels, since they cannot be set lower than measurable levels. The students may wish to use this information in preparing their debate presentation.

### **Data Analysis**

Give students 10-15 minutes to work with their extraction partners to compare the chromatograms and decide which peaks are real and should be entered into the database. Have them fill out the table on p. 63 in the module text for their chromatogram and be sure the instructor checks the results. The instructor should be actively working with the student groups to help them with any questions.

### **Calculations**

Spend a few minutes going over the calculations to determine the concentration of pesticides in produce and for surrogate recovery. This part is difficult for students. An overhead transparency of Figure 3-2 in the module text will be helpful in presenting this material, but it is a good computational exercise to let them work with each other and struggle a bit to figure it out.

#### **• Calculation of Concentration of Pesticides in Produce**

Use the concentration of pesticides in the sample analyzed by the GC and the total volume of the GC-ready extract (5 mL) to calculate the number of  $\mu\text{g}$  in the entire extract. For an example, look at a pesticide with a concentration of 426  $\mu\text{g}/\text{L}$  in the GC-ready extract.

$$\frac{426\mu\text{g}}{\text{L}} \times 0.005 \text{ L} = 2.13\mu\text{g} \text{ of pesticide in GC extract}$$

Knowing that only a fraction of the sample was carried through the entire analysis, correct for the actual volume of sample analyzed (the 10 mL aliquot out of 110 mL total volume of acetonitrile). Continuing with the above example:

$$\frac{2.13\mu\text{g}}{10 \text{ mL}} \times 110 \text{ mL} = 23.43\mu\text{g} \text{ in the entire acetonitrile extract}$$

The final step is to calculate the concentration of the pesticide residue on the 50 g of produce analyzed. This number can be directly compared to the tolerance levels,

as given in Appendix A in the module text.

$$\frac{23.43 \mu\text{g of pesticide}}{50.0 \text{ g of produce}} = 0.47 \mu\text{g/g} = 0.47 \text{ ppm}$$

- **Calculation of Surrogate Recovery**

The surrogate recovery provides an estimate of the efficiency of the extraction procedure. First, determine the theoretical maximum percent recovery of surrogate.

$$500 \mu\text{L} \times \frac{10,000 \mu\text{g}}{\text{L}} \times \frac{1 \text{ L}}{10^6 \mu\text{L}} = 5 \mu\text{g of surrogate added}$$

Correct for the actual amount of sample taken through the entire procedure.

$$\frac{5 \mu\text{g}}{110 \text{ mL}} \times 10 \text{ mL} = 0.45 \mu\text{g of surrogate in GC extract}$$

Determine the theoretical maximum concentration of surrogate in the 5 mL GC extract.

$$\frac{0.45 \mu\text{g}}{0.005 \text{ L}} = 91 \mu\text{g/L surrogate in GC extract}$$

Finally, compare this number to the actual number for surrogate recovery obtained for a real sample and multiply by 100% to obtain the percent recovery.

If students get low surrogate recoveries, they may have lost some of the surrogate compounds and pesticides either by physical processes (spilling, not rinsing the Florisil™ column enough) or by evaporation due to heating the sample too much in the nitrogen evaporation step. Loss by evaporation is more significant for TCMX (typically 30-40% recovery) than DCBP (typically 70-80% recovery) because it boils at a much lower temperature than DCBP and is “blown-off” in the nitrogen evaporation step. There will even be differences in lab partners’ sample handling that will show up here. This difference in recovery for the two surrogates also points out that neither of the two numbers are necessarily accurate for all pesticides. Those pesticides that have boiling points similar to DCBP (and correspondingly elute later on the chromatogram) will have percent recoveries similar to DCBP. The same is true for pesticides with boiling points closer to TCMX. It should be pointed out that surrogate recoveries are only a guide to how efficient the extraction is and no exact corrections can be made. In the environmental analysis business, surrogate recoveries are only reported, not used to calculate “corrected concentrations.”

### Results and Discussion

Have students share their raw data (pesticide and surrogate concentrations in the GC extract in  $\mu\text{g/L}$ ) with the class, either by putting the results up on the blackboard or entering them into a spreadsheet or graphing program. Once all the data is entered and everyone can look at it, have the students critique the data set. Do all duplicates match? Are all reported results above the MDL? Look at the “hits,” where measurable pesticide residues were found in the produce samples. Is there Dieldrin (banned 20 years ago)? What pesticides occur commonly on different fruits and vegetables? Are there peaks in the student chromatograms that look like pesticides but are not in the standard mixture (these may be real)? These questions will generate discussion about the results.

### Preparing Students for the Pesticide Debate

If the class is doing the debate, the remainder of the class period should be used

to divide students into two groups of 8-12 students each, Agribusiness and Environmentalists. It is best for the instructor to choose the groups before the lab period. The students should be put into groups based on abilities, with an attempt to equally divide students of different abilities. The groups should meet during this lab period to look over the procedures for the debate and the data they collected, and determine who in the group will play the different roles described on p. 82 in the module text.

There are 10 sets of individual readings, each set containing one or more related articles. References to these web-based readings can be found at <http://mc2.cchem.berkeley.edu/MCSquared/UCB/Echem/Pesticides/Pestshort.html>. Additional references are cited in the module text. On the web, the reading sets are color-coded depending on the slant the group publishing the article brings to the debate, red for the Agribusiness side, green for the Environmental side, and black for (apparently) neutral sources. The stronger students should be made responsible for the readings in Sets #7 and #8 on the regulation of pesticides, since these articles are essential for making a good argument about the existing pesticide regulations.

Give each student his or her individual reading assignment and emphasize that everyone will be expected to have read not only their individual readings, but also all of the module text on pesticides and risk assessment. Fully explain the grading scheme and the fact that they must bring to class a one-page summary of their readings (and copies to hand out to all) the next week. The one-page summary is quite effective in ensuring that students take the reading assignment seriously and come prepared to present their knowledge to the group.

NOTE: It is **critical** for students to do this assignment; otherwise the debate will be a flop because they won't know enough to put together a good set of arguments. Be sure to make this clear to the students.

### **Flow of Lab**

Begin with an overview of data analysis and chromatogram interpretation, then have the students meet with their partners to review their data and determine which peaks are real on their chromatograms. If they need to re-integrate their chromatograms, this is a good time to do it and provide students with an opportunity to learn more about the hands-on use of the gas chromatograph. Have students write their GC data on the board, then lead a class discussion about the data and its validity. Describe how to do the calculations to convert the GC data in  $\mu\text{g}$  per liter of extract to  $\mu\text{g}$  per gram of produce. Go over the expectations for the final lab report, then break the students into their debate groups to plan their debate strategy.

# THE GREAT DEBATE

The goal of this day's work is for students to use the data they acquired to debate the issue of pesticides in the food supply and to examine the effectiveness and fairness of the Food Quality Protection Act of 1996 from different points of view.

## **Web Readings**

Each student should have read his or her assigned web reading and prepared a summary of the article to hand out to fellow students in preparation for the debate.

## **Reading in Laboratory Manual**

*Review:*

Organochlorine Pesticides: pages 9–18

Risk Assessment: pages 64–74

Pesticide Regulations: pages 76–80

The Great Debate: pages 82–85

## **Time Required**

- Debate preparation: 60-90 minutes
- The Debate: 30-45 minutes
- Peer Grading: 10 minutes

## **Before Lab**

- Print out a copy of the data set for students to use in their debate.
- If you have an overhead projector available for students to use, bring blank transparencies and pens for students to write on them. They can give more effective presentations with visual aids.
- Prepare a list of the students in the class to help you keep track of participation in the discussion.
- Make copies of the Peer Grading sheet on p. 40 in this Instructor's Manual, one for each student.

## **Flow of Lab**

Remind students about report due dates and expectations. See following section in this Instructor's Manual.

Hand out pesticide data to all students and tell them that in order to make an effective argument, they will need to use the data they obtained. Any knowledge about uncertainty in the measurements, capability of the instrument and the detector used, and other experimental details might help (or hinder!) their arguments.

Students get into their groups and spend about 60-90 minutes formulating their debate plan, according to the questions in the lab manual. You should tell students they have slightly less time than you have actually allotted. Even with a timekeeper, students are usually not prepared at the end of the designated time. At this point, it is useful for the instructor to remind them to be sure to address the questions on page 83 in the module text. The group activities during this time period should flow as outlined below.

Debate preparation begins with each student giving a 2-minute summary of each reading to the group (~25 minutes). The **timekeeper** should be sure everyone stays on track. Students who have the same set of readings (most sets contain more

than one article) should alternate being the first presenter to the group. The **summarizer** should summarize what the group has heard, once in the middle and once at the end of the individual reports, reminding the group of the main questions they need to address. The **recorder** should write down the main points. The **spokespeople** should listen closely for any facts they might want to use in their arguments.

Using the information presented, the group should now begin to formulate a position consistent with their interests (Agribusiness, Environment). The **director** will play a major role in reminding the group of its mission, which is to address the questions on p. 83 in the module text. The **summarizer** should speak every 10-15 minutes to clarify what has been discussed. The **timekeeper** should keep an eye on the remaining time and move the discussion along if it gets bogged down on one particular point. At the end of the discussion, the **recorder** will have the group go back over the questions and write down the consensus of the points to be made in the presentation by the **spokespeople**.

The debate will follow and should last ~30-45 minutes. You, the instructor, are the director of the debate, with your mission being to ensure that as many people as possible have a chance to contribute to the debate. You should also be sure to cut it off before the students begin going in circles or just reiterating points already made. Here are some rules students must follow (write these on the board or hand them out ahead of time).

- The preliminary speeches by the **spokespeople** may not exceed 10 minutes.
- No interrupting is allowed. Students must be acknowledged by the instructor before speaking.
- Any comment during the general discussion is limited to ~1 minute. No filibustering!
- Courtesy is of utmost importance! Just because you disagree with someone does not mean you have to be rude to them.

During the debate, *it is most important for the instructor to be watching all students in the room, not just the ones who are dominating the discussion.* The debate is most successful if there is participation from as many students as possible. You may wish to have a list of students on which you make a mark next to a student's name each time she or he speaks. This will provide a concrete reminder of who has contributed more or less than their share, and, while you should not try to stifle creative comments from the talkative students, you can choose to call on other students besides the "talkers." Watch out for gender bias in which students you call on. Studies have shown that males are more aggressive than females at being recognized in a group where they must compete for "permission" to talk. It is easy for the hand-wavers to obscure your notice of the rest of the students. Again, keeping a list of students and how often they have responded will be useful.

At times during the debate, you may need to ask for clarification from a student if a question or comment is worded in a way that is difficult to understand. Keep students to the 1 minute limit for comments.

As the end of the time approaches (or as the discussion slows down or students start to repeat themselves), you should bring the debate to a close by summarizing the evolution of student opinion throughout the course of the debate. Be sure to highlight the good points the two groups have made (you should write these down as you hear them), with particular emphasis on *solutions* to the problem, not just differences of opinion. Often students have come to some middle ground by this time and it is useful to remind them that the problem of pesticides and food production is not just black or white and they have illustrated this first hand. Finally, compliment everyone on their participation and effort.

After the debate is over, the students should spend 5-10 minutes assigning

grades to the other students in their group. Students do not grade themselves. It is important that they give this careful thought, so be sure everyone takes this seriously and does it with quiet and in private.

### **Potential problems and How to Handle Them**

- **Overly aggressive students:**

A gentle way to handle these students is to compliment all talkative students for their wonderful insights, but you want to be sure that others have a chance to state their opinions and to remind them that you must call on them first before they can speak. Don't dwell on it, but move things along by immediately calling on someone else to continue the debate. If this doesn't work, you may need to stop the debate and remind all students of the rules. At worst, you may have to ask a particular student to refrain from participating.

- **Overly passive students:**

There are many students who are reluctant to speak their mind for fear of saying something "stupid" in the eyes of their classmates or just because they are uncomfortable speaking in front of a large group. Often it is possible to bring these students out of their shells if the issue excites them enough to motivate a response. As director of the debate, you should watch closely for indications that these shy students might be willing to contribute to the discussion. If you see someone with a half-raised hand or an eager look, be sure to call on him or her as soon as possible. If you wait and let another "eager beaver" student speak first, you will have lost them. If you missed the boat on getting them to contribute, you might be able to bring them back by saying something like, "Tina, I noticed you had your hand up a minute ago---would you like to say something?"

- **The debate is going in circles:**

Sometimes the students will get into a line of argument that appears to be going nowhere. At this point, it is useful to re-focus them on the questions of p. 83, perhaps saying: "But what effect does this point you're making have on the way pesticides are regulated?" or something to that effect that brings the discussion back to the broader issues.

- **Students who have not done the assignment:**

These students are a problem for the rest of the group because there will be a hole in the group's knowledge base. The best solution is prevention. Be sure all students know what will be expected of them the week before and how their performance will be graded.

### **Peer Grading**

On the following page is a sheet to be handed out for students to use in grading each other for their work in preparing the debate.



## THE REPORT

You may wish to customize the instructions for the students' lab reports (see pages 94–98 in the module text). The existing instructions request a full written report, with students expected to explore the issue of pesticides in the food supply in some detail. An alternative format that is less demanding for students would be a simple report of the class data, showing detailed calculations and including comments on the significance and validity of the data. Several post-lab questions are provided as well. Answers to these questions can be found below. If instrumentation is the focus of the course, the report could be more related to the subtleties of GC analysis of organochlorine pesticides.

### Reading in Laboratory Manual

Final Report for the Pesticide Module: pages 94–98

### Answers to Post-Lab Questions

1) The yearly exposure to Alar can be calculated as follows:  
First, obtain an average concentration of Alar in apples and apple products by taking into account the assumptions that the concentration of Alar in apples is 30% of the tolerance level and only 5% of the pesticide measured at the farm gate is contained in the food.

$$5 \mu\text{g/g} \times 0.30 \times 0.05 = 0.075 \mu\text{g/g}$$

Next, calculate the absolute amount of Alar ingested in a year. For a child who eats 4 oz of applesauce and 8 oz of apple juice:

$$12 \text{ oz/day} \times 28 \text{ g/oz} \times 365 \text{ days/yr} \times 0.075 \mu\text{g/g} = 9,198 \mu\text{g/yr}$$

For an adult who eats three 6 oz apples per week:

$$18 \text{ oz/week} \times 28 \text{ g/oz} \times 52 \text{ weeks/yr} \times 0.075 \mu\text{g/g} = 1,966 \mu\text{g/yr}$$

Finally, calculate the amount of pesticide ingested per kg of body weight:

For a 10 kg child:  

$$9.20 \text{ mg}/10 \text{ kg} = 0.92 \text{ mg/kg}$$

For a 70 kg adult:  

$$1.97 \text{ mg}/70 \text{ kg} = 0.028 \text{ mg/kg}$$

The amount of pesticide ingested per kg of body weight is *320 times greater* for a child than for an adult. Clearly, the tolerance levels should reflect this difference in exposure between adults and children.

2) Set I

Chlorpyrifos, 1.12 mg/L in water at 24°C

Dicamba, 5,600 mg/L at 20°C

Dicamba is more water soluble because it can form hydrogen bonds to water through the carboxyl group.

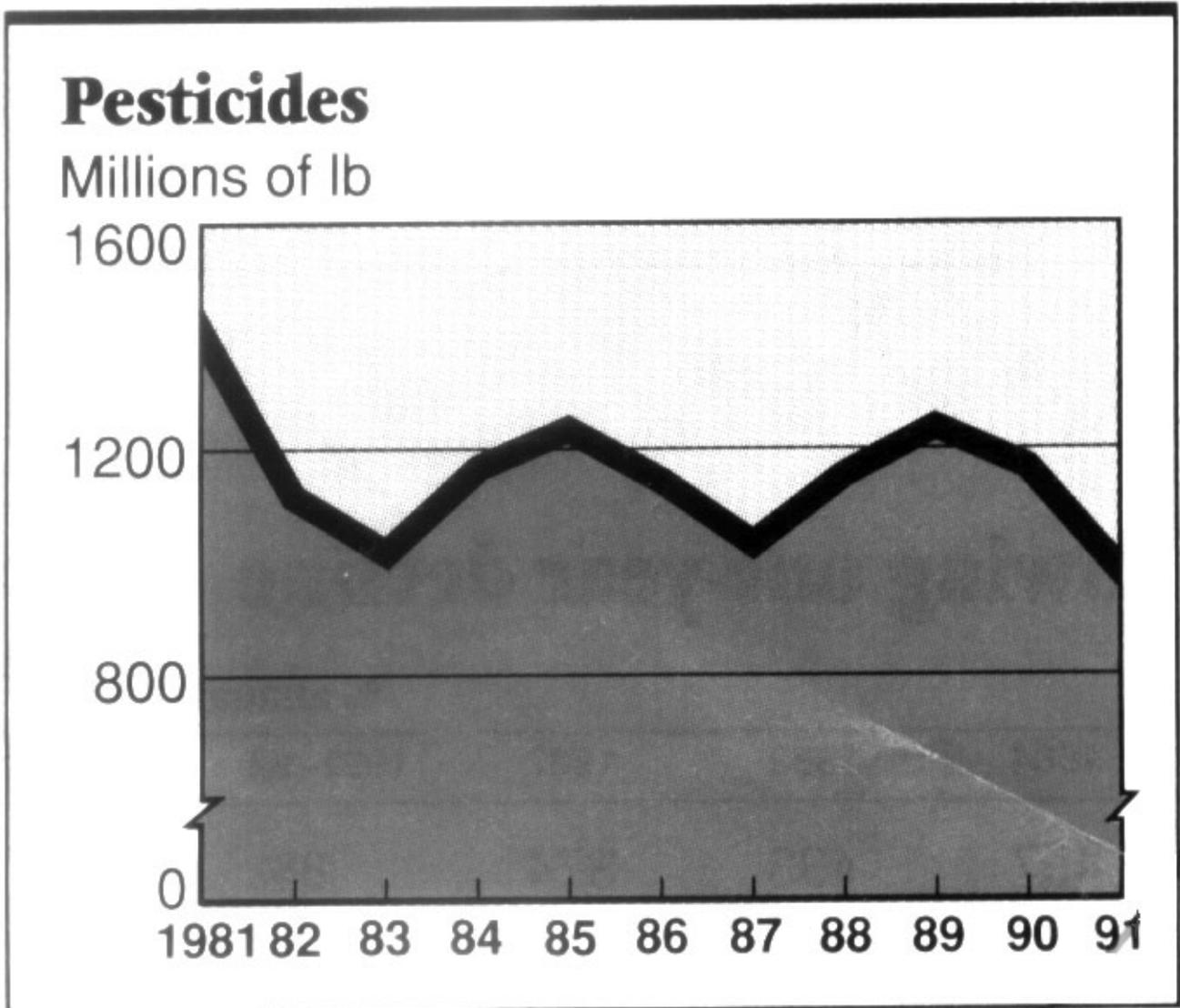
Set II

Aldicarb, 6,000 mg/L at 25°C

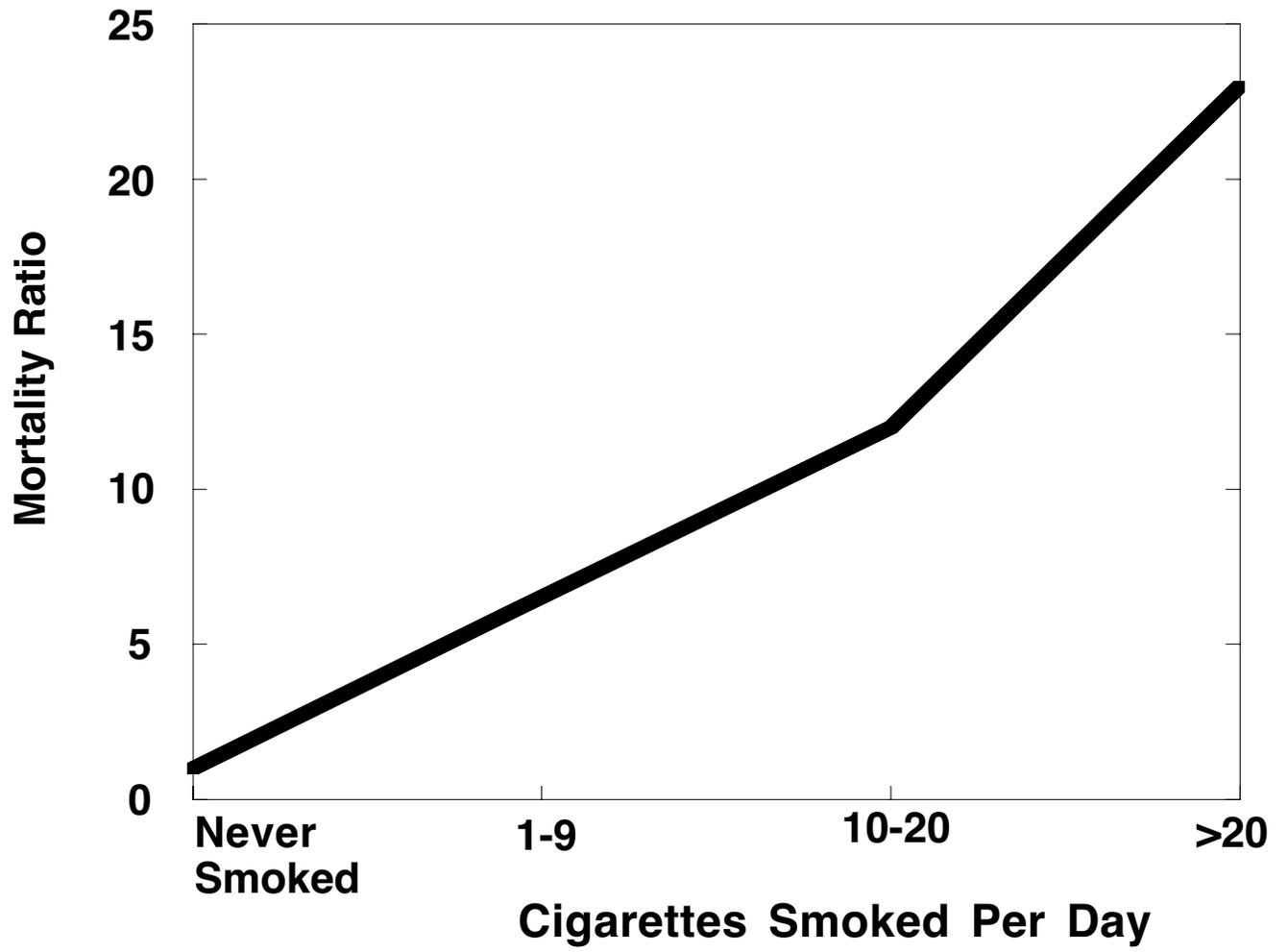
Kepone, 7.6 mg/L at 25°C

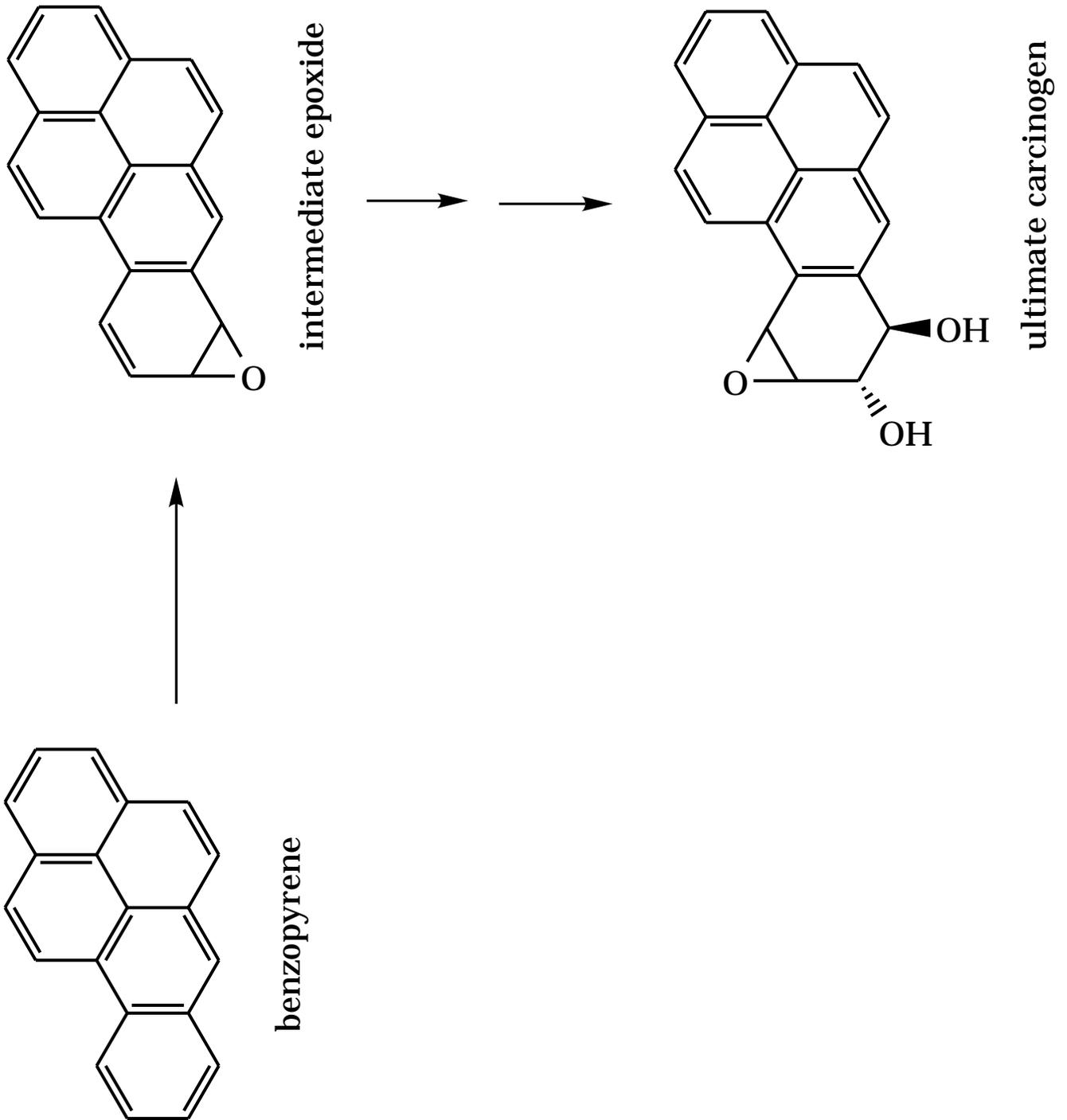
Aldicarb is more water soluble because it can form hydrogen bonds to water through the amido group. In addition, there are multiple electronegative or highly polarizable heteroatoms with unshared pairs of electrons (N, O, S) present in the aldicarb molecule. Organochlorines are typically not very water soluble. Kepone is particularly insoluble because it is a symmetric molecule, and the bond dipoles between carbon and chlorine atoms tend to cancel out.

**Appendix A:**  
**Transparency Masters**

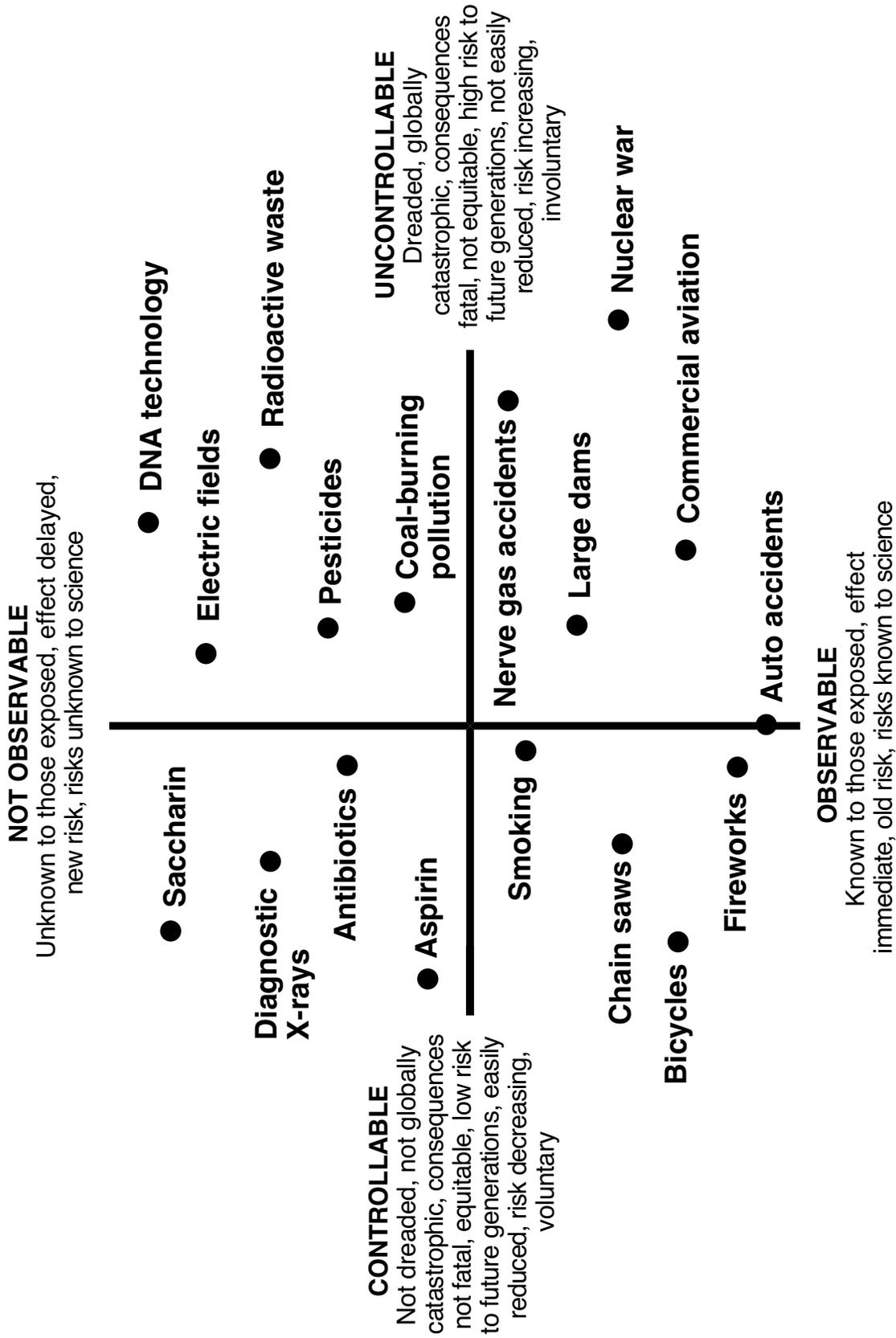




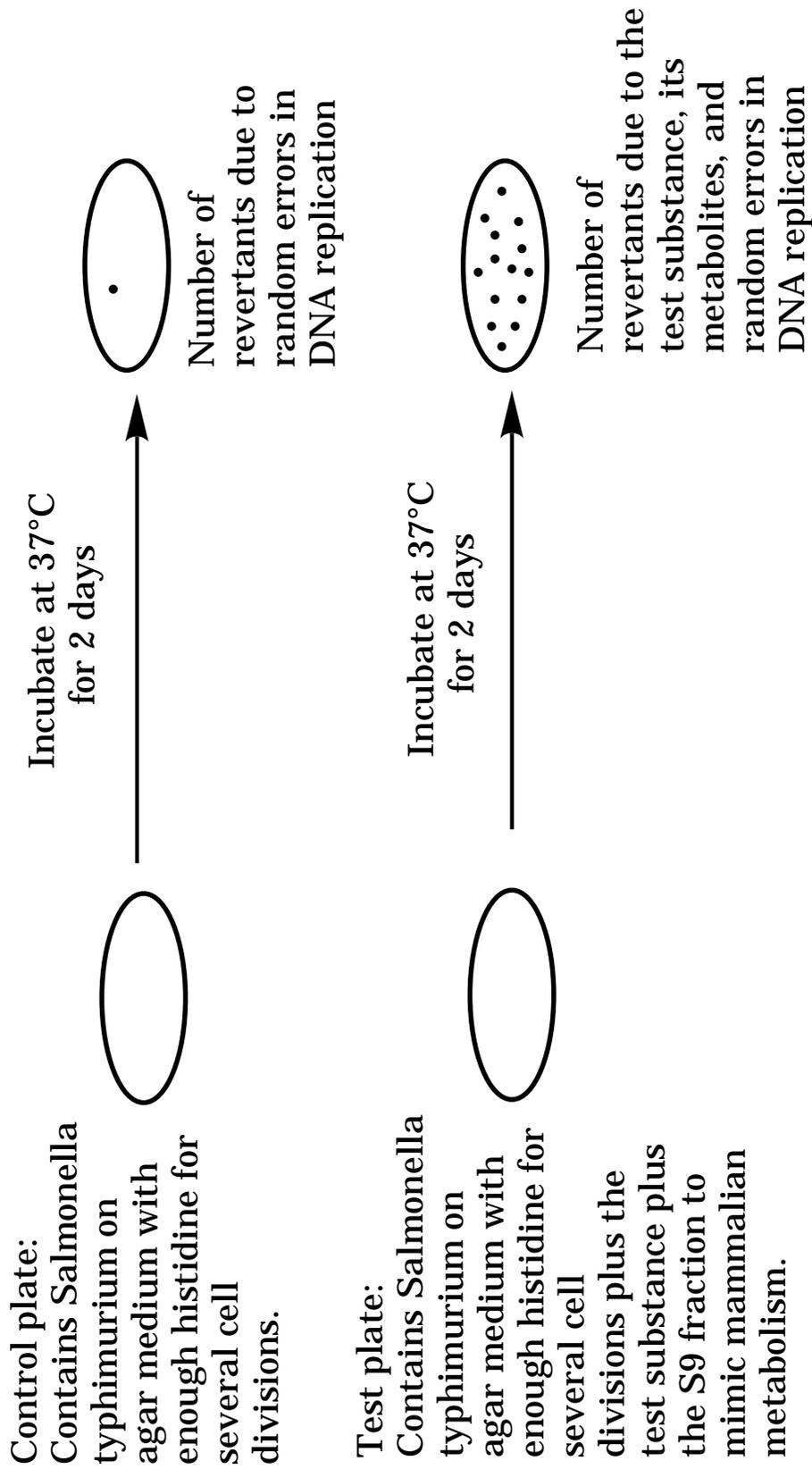




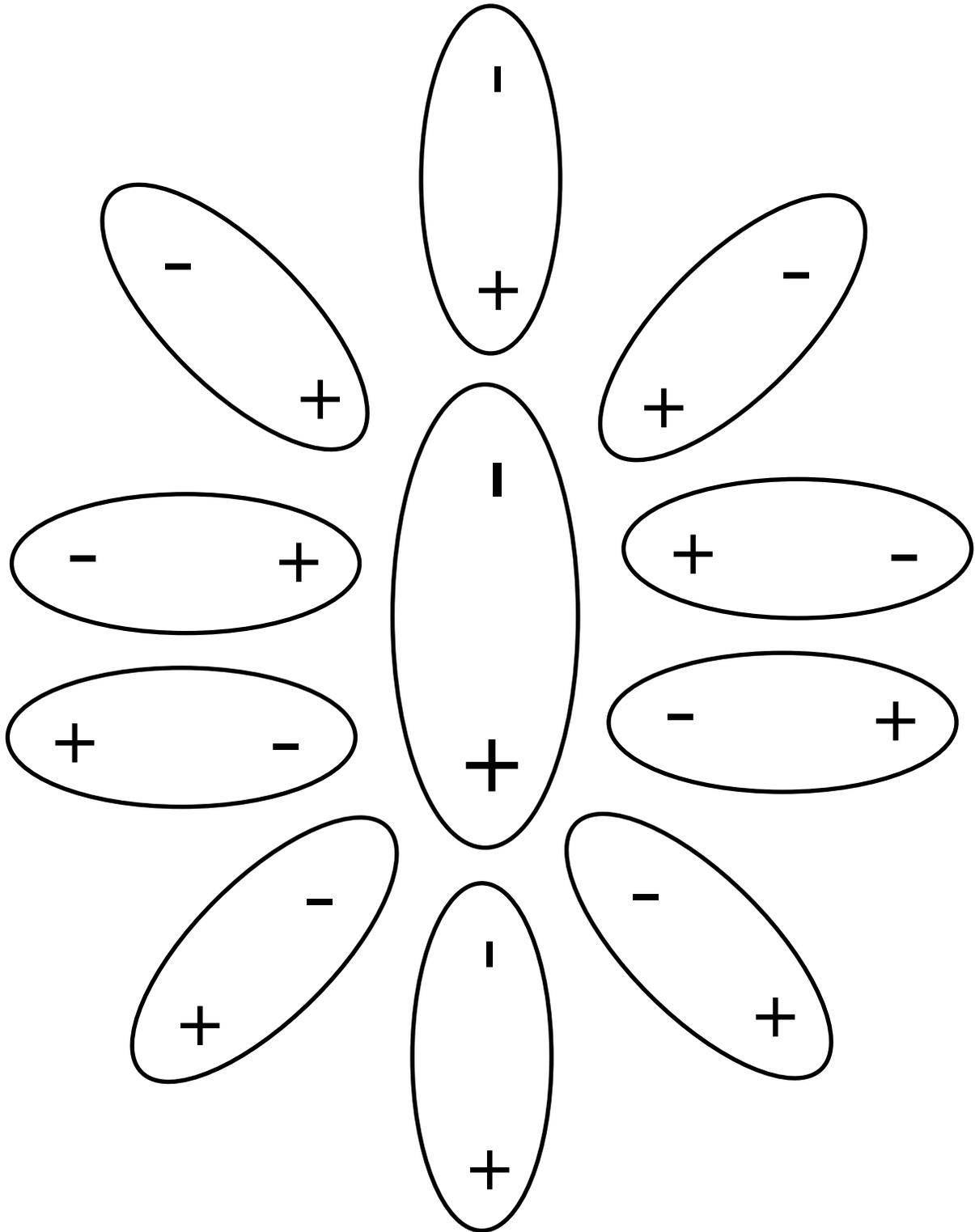




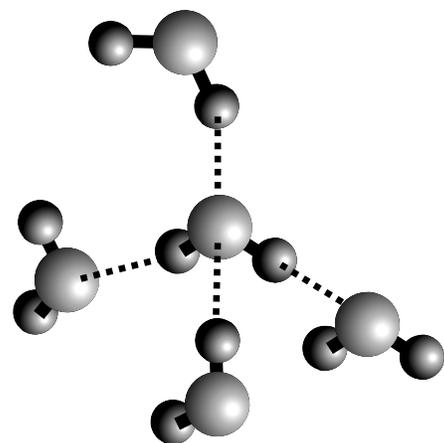
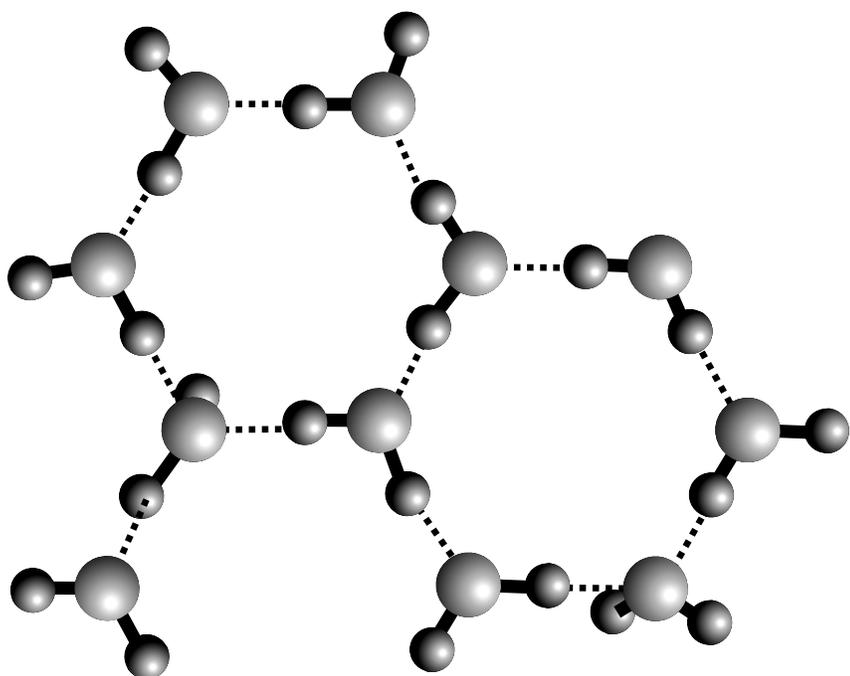




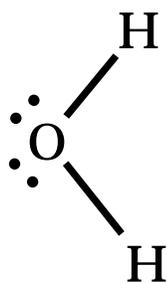




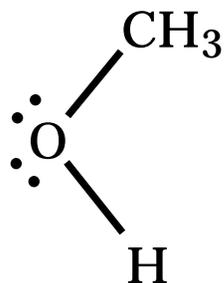
# **WATER MOLECULES FORM HYDROGEN-BONDED CLUSTERS**







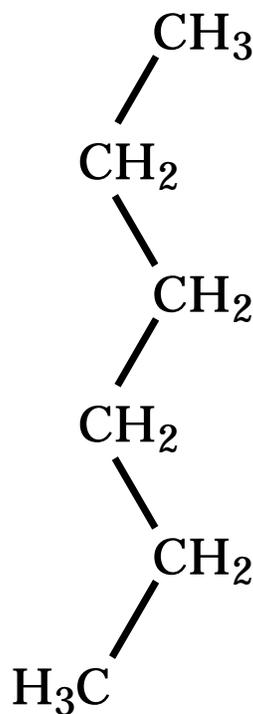
water



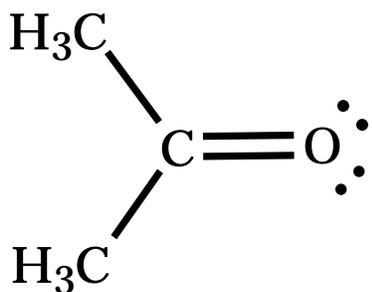
methanol



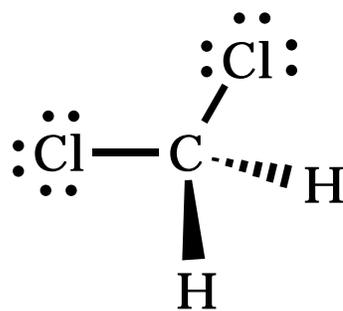
acetonitrile



hexane

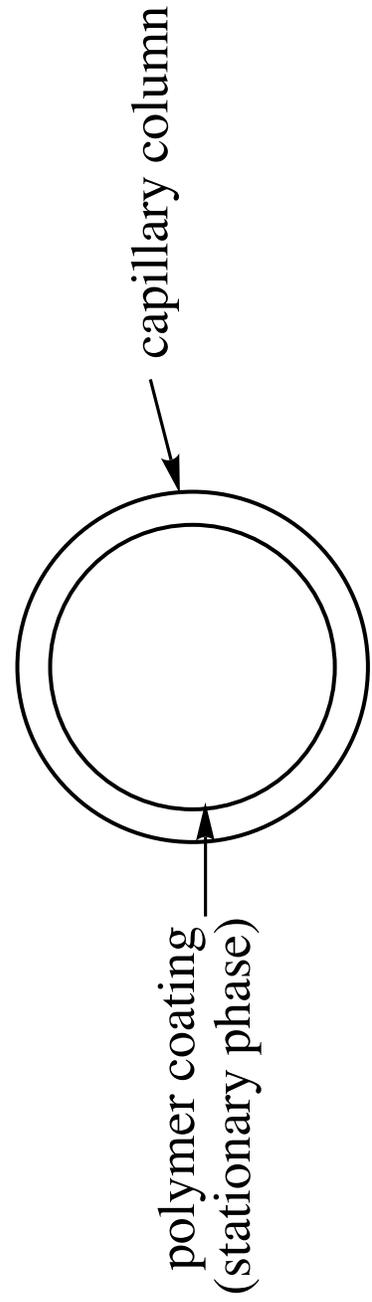
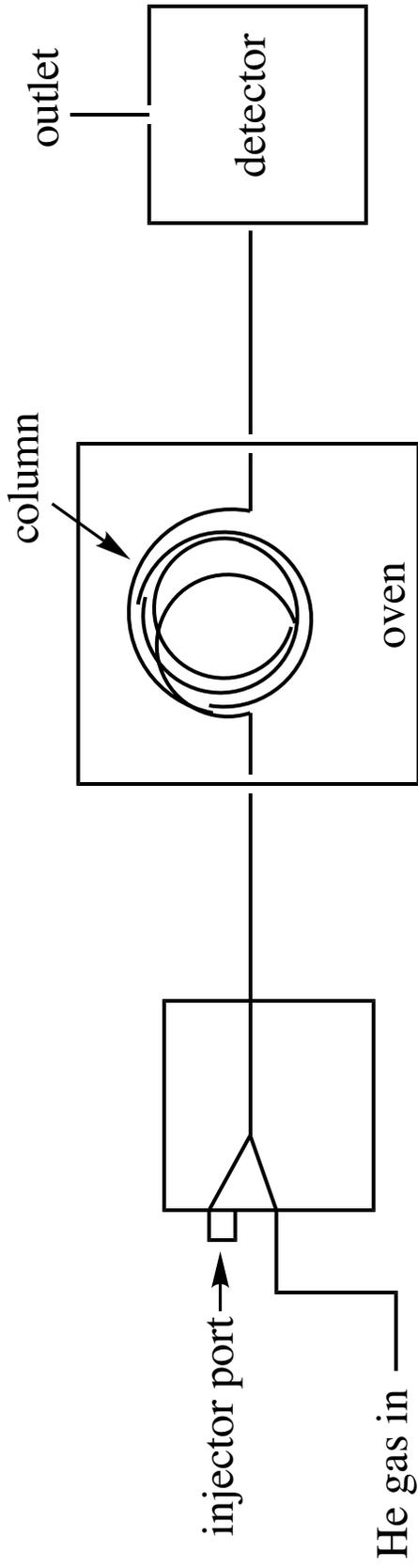


acetone

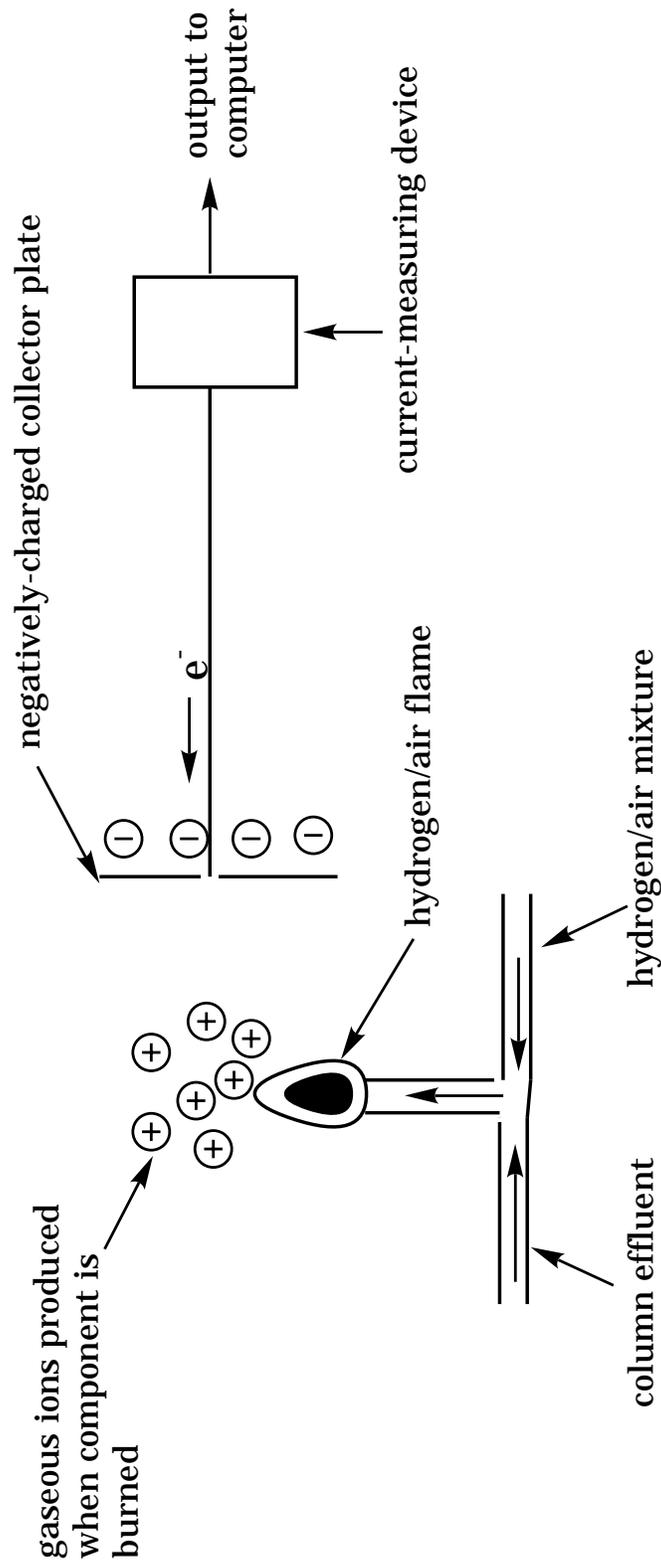


methylene chloride

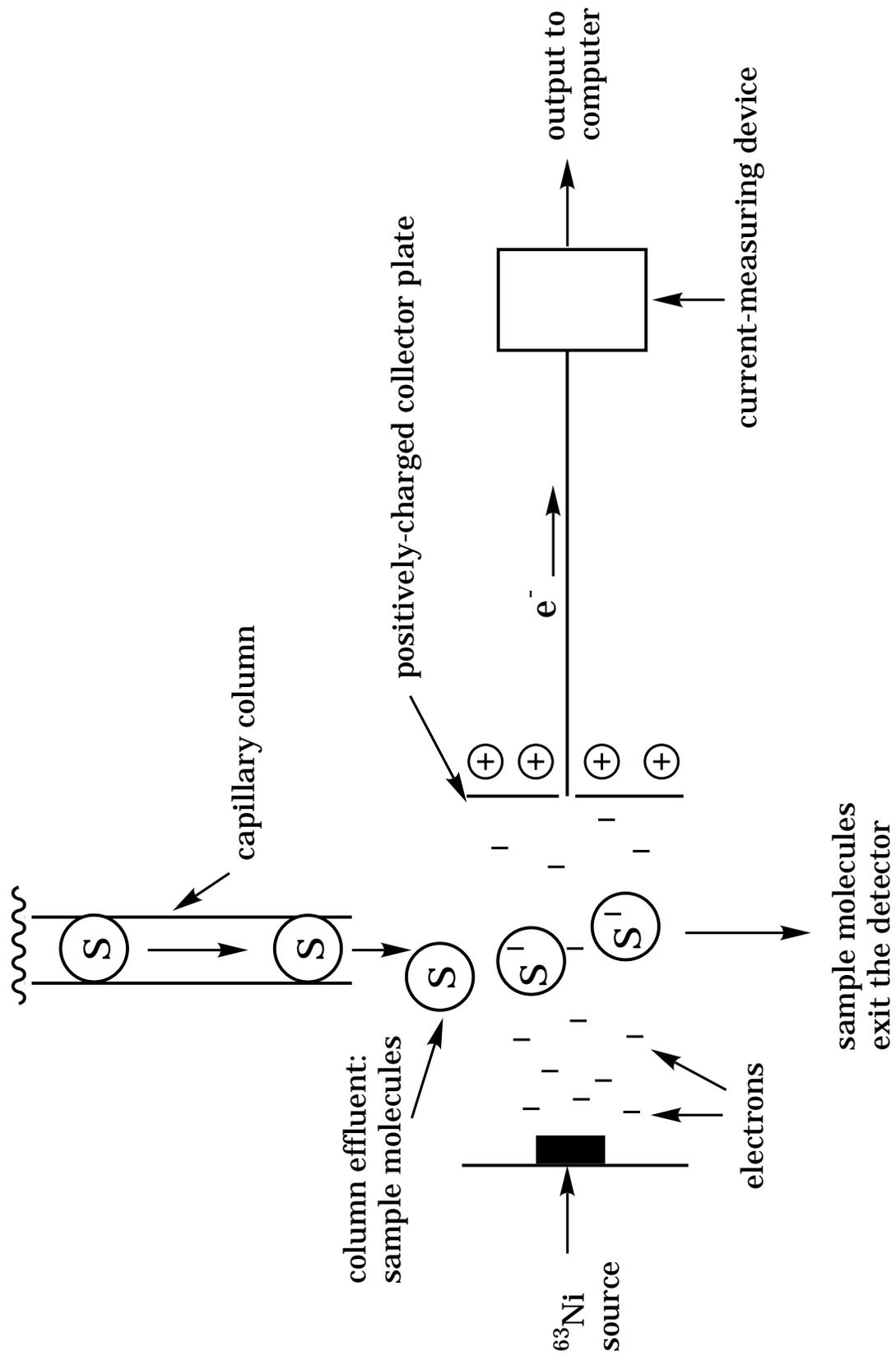




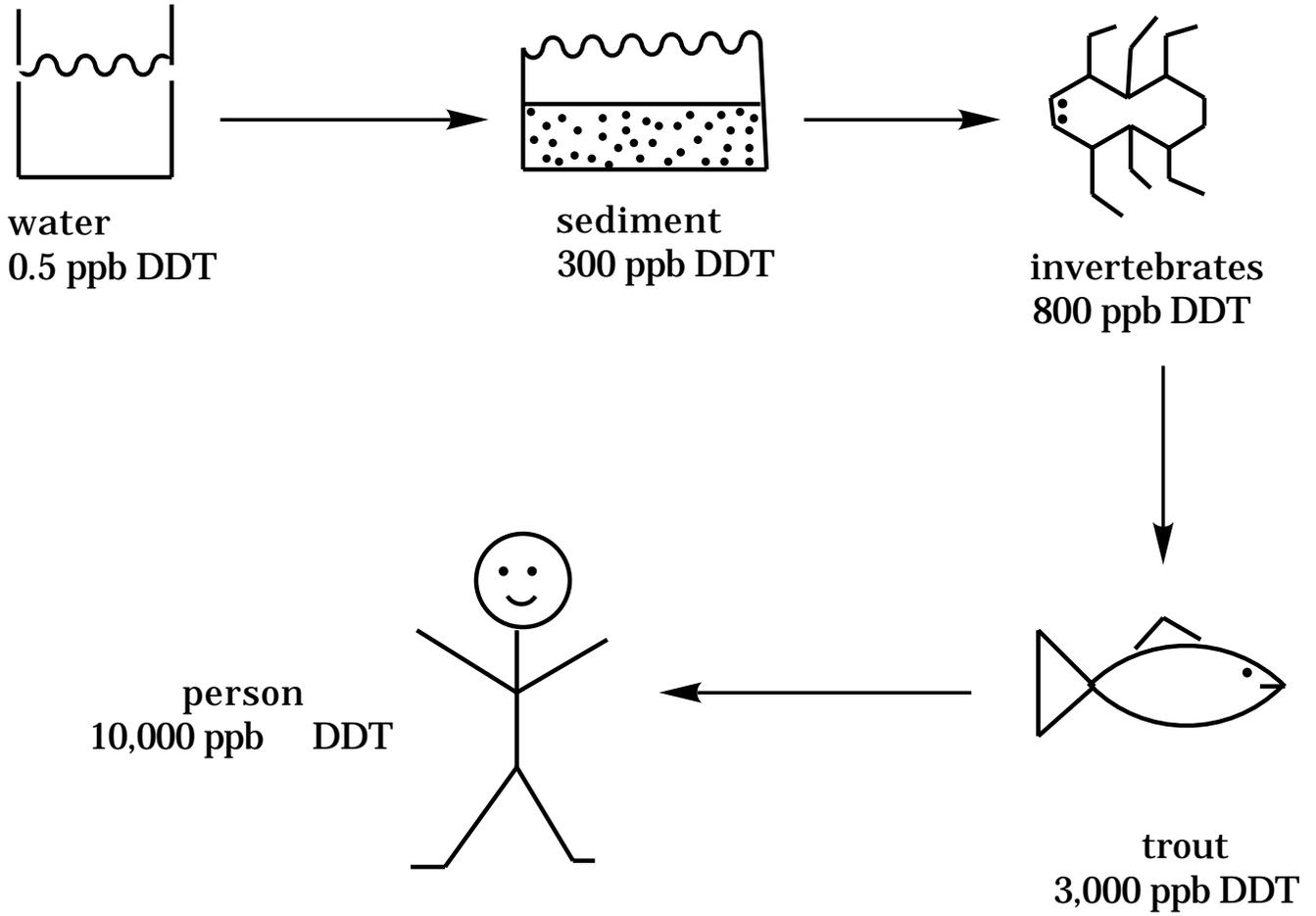


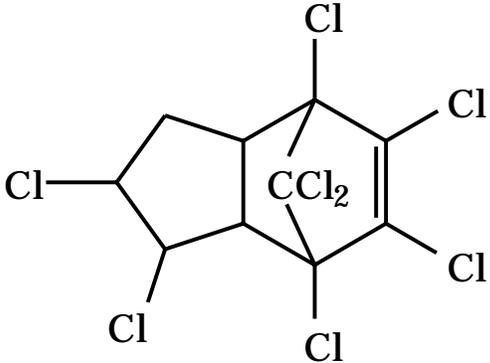
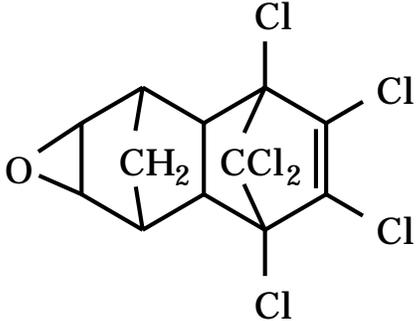
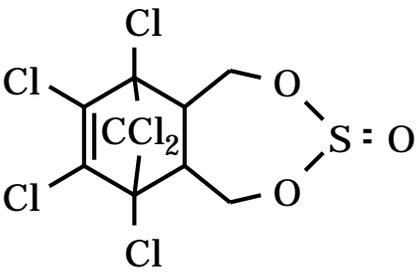


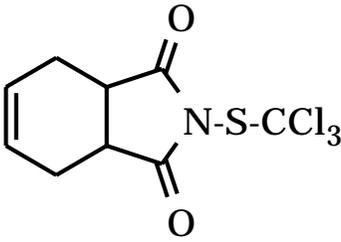
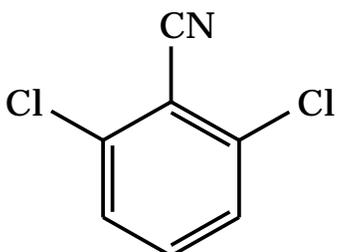
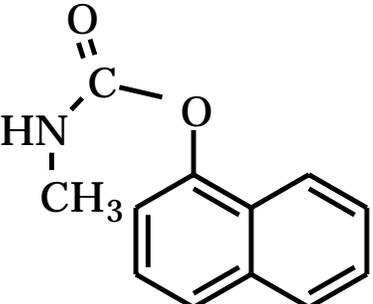


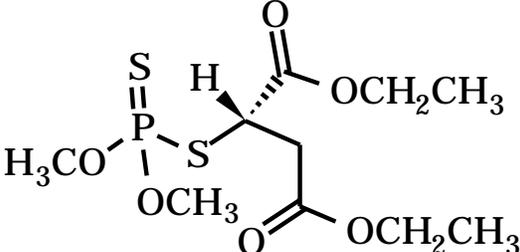
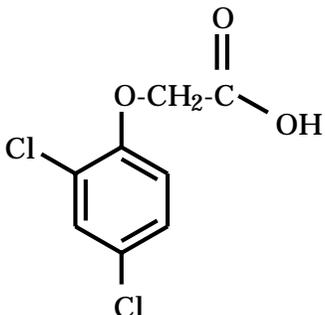


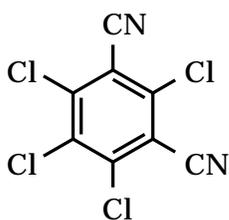
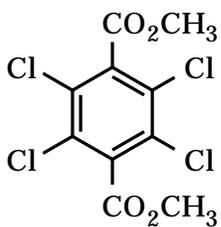
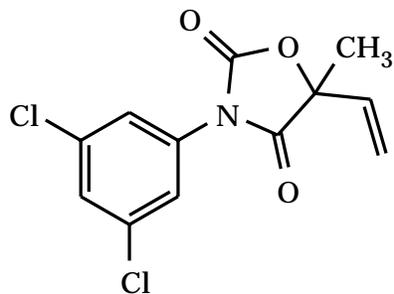
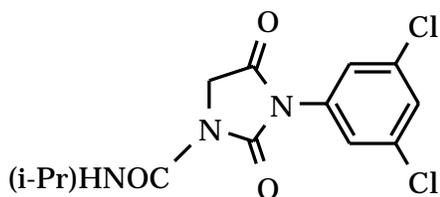
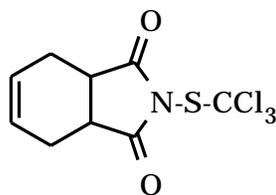
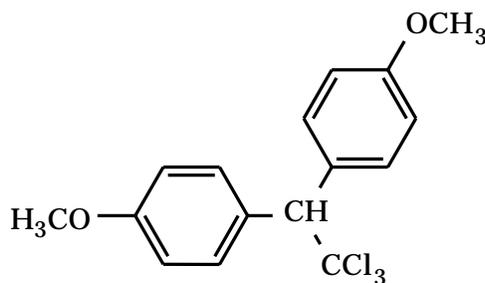
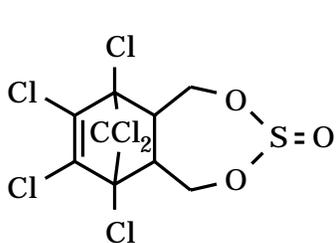
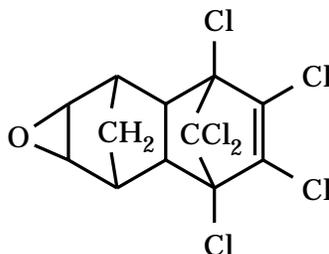
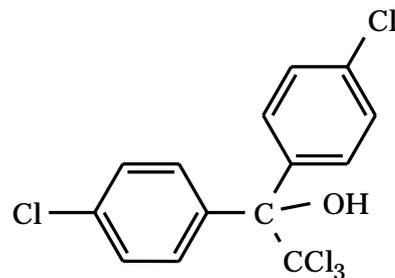
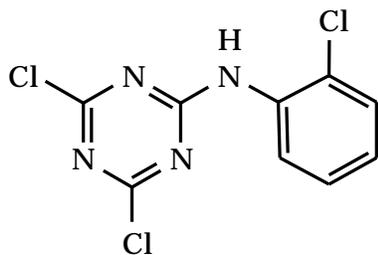
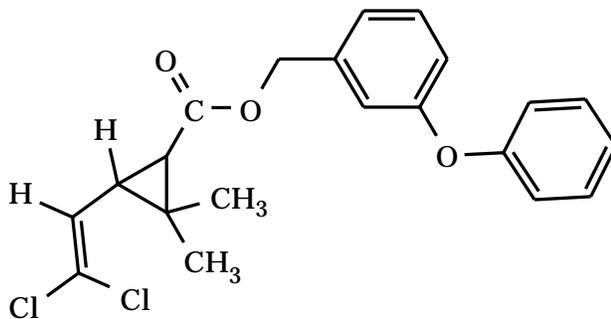
# BIOACCUMULATION



<b>Pesticide</b>	<b>Solubility in Water (mg/L) at 20°C</b>	<b>Half-Life</b>
<p><b>Chlordane</b></p> 	<b>0.1</b>	<b>3.3 years</b>
<p><b>Dieldrin</b></p> 	<b>0.17</b>	<b>&gt;7 years</b>
<p><b>Endosulfan</b></p> 	<b>0.5</b>	<b>35-190 days</b>

<b>Pesticide</b>	<b>Solubility in Water (mg/L) at 20°C</b>	<b>Half-Life</b>
<b>Captan</b> 	<b>0.5</b>	<b>1-12 days</b>
<b>Dichlobenil</b> 	<b>18</b>	<b>1 year</b>
<b>Carbaryl</b> 	<b>32</b>	<b>4-29 days</b>

<b>Pesticide</b>	<b>Solubility in Water (mg/L) at 20°C</b>	<b>Half-Life</b>
<b>Malathion</b> 	<b>143</b>	<b>4-6 days</b>
<b>2,4-Dichlorophenoxyacetic acid</b> 	<b>682</b>	<b>1-14 days</b>

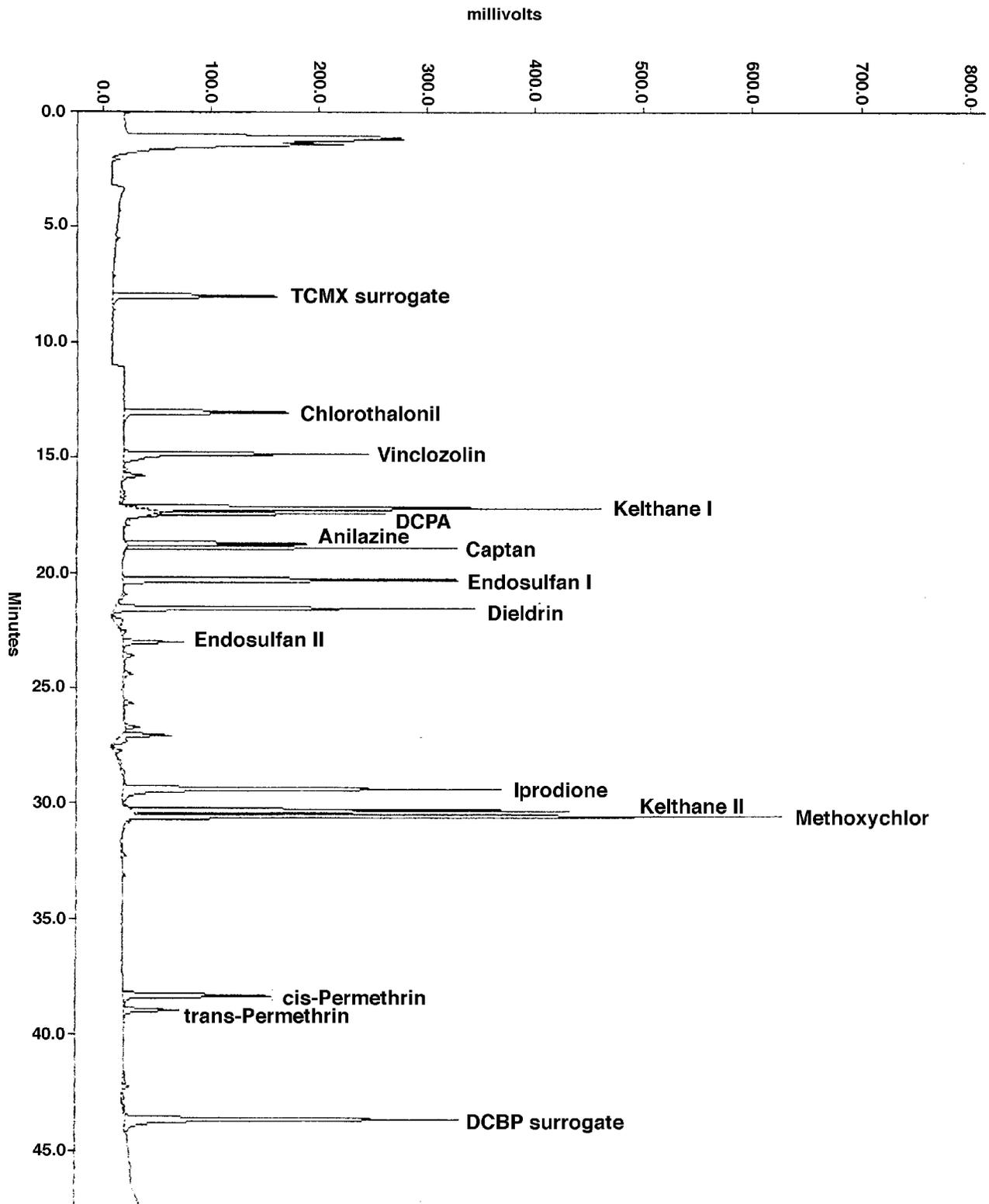
**Chlorothalonil****DCPA****Vinclozolin****Iprodione****Captan****Methoxychlor****Endosulfan****Dieldrin****Kelthane****Anilazine****Permethrin**

## § 180.378 Permethrin; tolerances for residues.

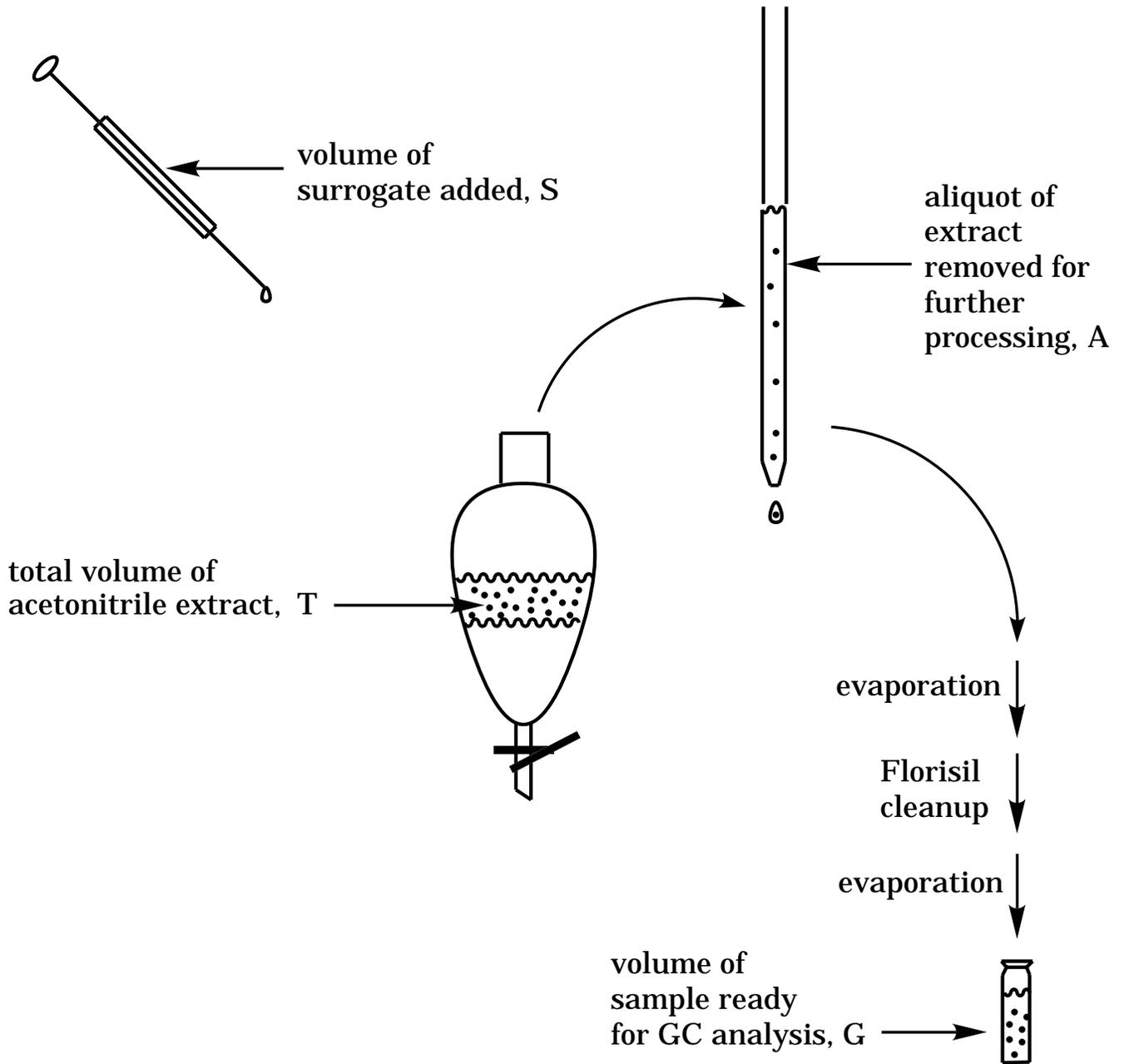
<b>Commodity</b>	<b>ppm</b>
<b>Apples</b>	<b>0.05</b>
<b>Artichokes</b>	<b>10.0</b>
<b>Asparagus</b>	<b>1.0</b>
<b>Avocados</b>	<b>1.0</b>
<b>Broccoli</b>	<b>1.0</b>
<b>Cabbage</b>	<b>6.0</b>
<b>Celery</b>	<b>5.0</b>
<b>Cauliflower</b>	<b>1.0</b>
<b>Cherries</b>	<b>3.0</b>
<b>Corn, forage</b>	<b>60.0</b>
<b>Eggplant</b>	<b>1.0</b>
<b>Filberts</b>	<b>0.05</b>
<b>Garlic</b>	<b>0.1</b>
<b>Lettuce (head)</b>	<b>20.0</b>
<b>Mushrooms</b>	<b>6.0</b>
<b>Onions</b>	<b>0.1</b>
<b>Peaches</b>	<b>5.0</b>
<b>Pears</b>	<b>3.0</b>
<b>Potatoes</b>	<b>0.05</b>
<b>Soybeans</b>	<b>0.05</b>
<b>Spinach</b>	<b>20.0</b>
<b>Tomatoes</b>	<b>2</b>











**Appendix B:**

**Evaluation Data on the  
Pesticide Debate**

What follows is evaluation data collected over the course of 5 semesters at the University of California, Berkeley on freshmen students taking special environmental laboratory sections as an alternative to the traditional laboratory offered with the large freshman chemistry course. The data presented here indicate the effects of the debate on the students' opinions of the exercise, in addition to an evaluation of the effect of the debate on students' views towards pesticide use.

## Overview

In the pesticide lab, students extract organochlorine pesticides from fruits and vegetables and use their data in a role-playing exercise. The students are divided into two groups, one representing the Agribusiness and Chemical Manufacturing Industry and the other representing the "die-hard" Environmentalists. A week before the lab, they are provided with readings (each reading had two students responsible for reporting on it) that supports their sides of the story (somewhat---a few of the readings were the same) and their mission is to debate the issue, after formulating plans within their groups. Students in each group are assigned the tasks of **spokesperson**, **timekeeper**, **recorder**, **summarizer**, and **director**, a structure which helps the students stay on track. The individual group discussions begin with each student summarizing his or her own particular article to the group. After all the summaries are given, the groups then tackle the issues below and use the information to make a case for their side. The specific issues debated are:

- Students begin by presenting the class data on pesticide residues in produce. They are expected to use this data to support their point of view and comment on whether their group perceives there is a problem with pesticides in the food supply.
- Do you support or reject the new Food Quality Protection Act of 1996 that regulates pesticide use?
- Should we:
  - a) regulate pesticides more heavily than the new law dictates?
  - b) forbid them entirely?
  - c) decrease the regulatory burden on pesticide producers and users?
  - d) re-write the laws for pesticide regulations? If so, how?
  - e) keep the status quo?
  - f) some combination of the above?
- Is our present method of regulation effective? Over burdensome?

While the groups formulate their debate plan, the instructor is available to help clarify issues, but does not guide the discussion. When the debate begins, each group has 10 minutes to present its case, then the floor is opened for general discussion. The instructor's role is one of referee (for the outgoing students) and encourager (for the less aggressive students).

The Agribusiness versus Environmentalists debate was a success with most students, with words like "exciting," "fun," and "interesting" dominating the comments. Students who were very pro-environment found themselves arguing for the side of the Agribusiness interests, which required them to see a different side of the issue and forced a realization that not all environmental problems have clear-cut solutions. With very few exceptions, all students came to class prepared and participated fully in the discussion. Students graded each other on participation. The detailed instructions students received for the debate and for the peer-grading scheme can be found on pp. 82–85 in the module text.

## Evaluation Data

Over the five semesters the course was evaluated, the debate was carried out by 9 lab sections of students; a total of 184 students. At the end of the course, students were asked several questions about the debate and the grading scheme and to provide additional comments if they desired. The results of these questions follow.

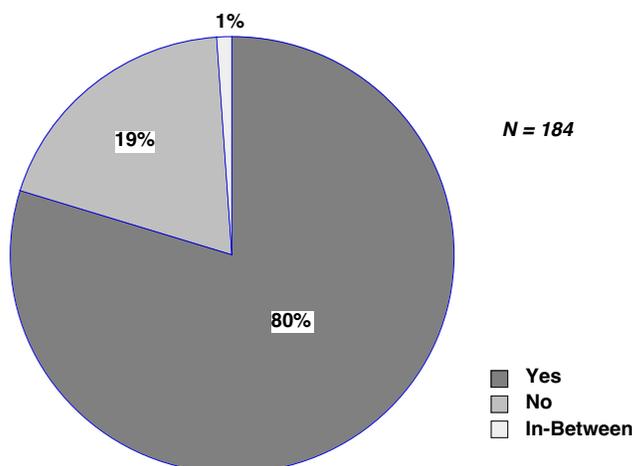
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### Did you like the discussion/debate on the pesticide module?

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The debate was popular with the students, with a large fraction (80%) giving it a "thumbs-up." Nineteen percent of the students did not like this alternative approach.

#### DID STUDENTS LIKE THE DEBATE?

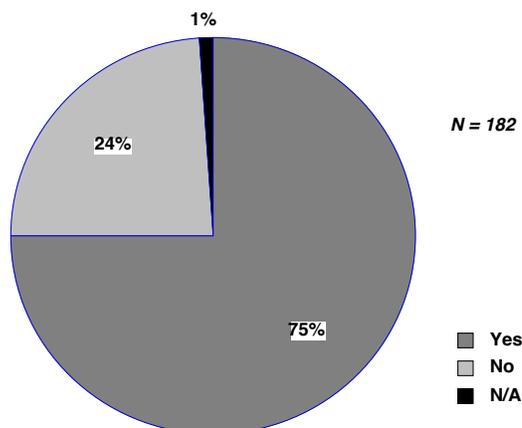



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### Did you think the grading scheme for the debate was fair?

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The debate assignment was peer-graded, with students grading each other on both the quality of the summary of their article and their level of participation in the pre-debate discussion. Students were provided with the guidelines shown on p. 85 in the student laboratory manual to rate the performance of their fellow students. The teaching assistants were given discretionary points to use if they felt it necessary, but these were used only rarely. Grades given to students by their peers were generally B or better, with only those students who did not come prepared graded down significantly. In light of the full participation of all of the students observed, the teaching staff in the course would have given very similar grades for the most part. Students were generally accepting of this method of grading, with 75% in favor and 24% opposed.

**DID STUDENTS THINK THE PEER GRADING APPROACH WAS FAIR?**

---

**If you did not think the grading scheme was fair, why not?**

---

The 25% of the students who did not like the grading scheme cited several reasons. They thought their peers lacked the maturity to do a good job and were concerned that competitiveness and self-interest might influence the grading. Some students felt that their peers would give higher grades than a TA might have. It was also clear that some students were uncomfortable grading their peers, remarking that they were not qualified or that it was not their job.

In the last semester the class was surveyed, we asked additional questions to find out the effect of the debate on shaping students' opinions on the issue of pesticide use. Because of the small number of students ( $N = 41$ ), it is difficult to obtain precise conclusions, only a general overview.

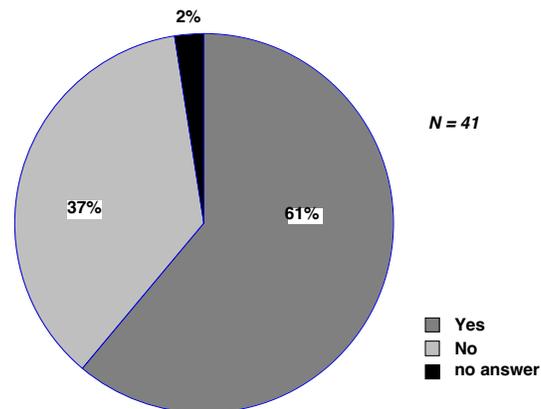
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**Did the debate serve to reinforce your present views on issues related to pesticide use? If so, which issues?**

---

More than half of the students (61%) indicated that the debate reinforced their present views on pesticide use, while 37% indicated the opposite.

## DID THE DEBATE REINFORCE STUDENTS' VIEWS ON PESTICIDE USE?



When asked to cite on which issues their views were reinforced, students didn't really seem to answer the question that was asked; instead, they mostly cited new revelations they had arrived at during the debate. Sixty-one percent (N = 24) of the students provided comments which fell into several general categories. Percentages are expressed as the number of comments out of 24 total comments.

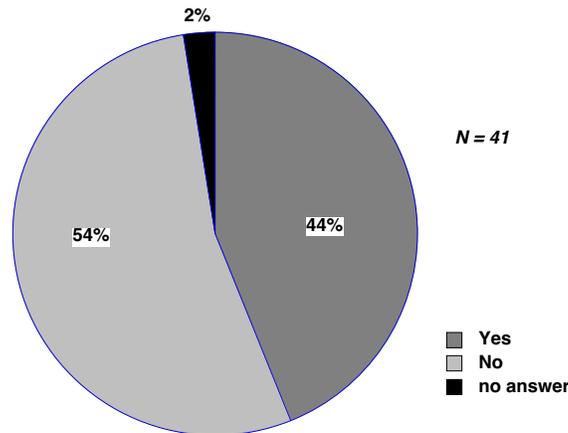
- Students became more aware of the societal costs of pesticides (33%).
- Students became more aware of the health risks of pesticides (29%).
- Students became convinced that pesticide use should be minimized and alternatives explored (21%).
- Students decided that the pesticide problem was not as bad as they had thought (8%).
- Miscellaneous, not related to any of the above (8%).

---

### Did the debate serve to change your views on issues related to pesticide use? If so, which issues?

---

Nearly half of the students (44%) were influenced by the debate to change their opinions related to pesticide use.

**DID THE DEBATE CHANGE STUDENTS' VIEWS ON PESTICIDE USE?**

Forty-four percent (N = 18) of the students provided comments which fell into several general categories. Percentages are expressed as the number of comments out of 18 total comments.

- Students became convinced that it would be difficult to completely do away with pesticides (33%).
- Students decided that the pesticide problem was not as bad as they had thought (22%).
- Students became convinced that pesticide use should be minimized and alternatives explored (17%).
- Students decided that the pesticide problem was worse than they had thought (11%).
- Students were confused about the issue (6%).
- Miscellaneous--not related to any of the above (11%).

---

**Additional comments on the debate.**


---

Space was provided on the questionnaire for additional comments on the debate. Forty-six percent (N = 84) of the 184 students responded over the three semesters the debate was included in the curriculum. The categories of responses and their percentages are shown below. Percentages are expressed as the number of comments out of 84 total comments. The comments fell into six major categories:

- Some students were dissatisfied with the organization and format of the debate (32%).
- The pesticide issue is not black and white. Some students appreciated this and others did not (24%).

- Nonspecific positive comments (20%).
- The relationship of chemistry to the debate (9%).
- Students felt like they learned a great deal (7%)
- Students liked the change of pace (7%).

### **I. Some students were dissatisfied with the organization and format of the debate (32%)**

The largest percentage of students (32%) were dissatisfied with some aspect of the organization or format of the debate. Fifteen percent thought the debate could be more organized or that more time should be provided for constructing their arguments.

"The discussion was good, but needed more structure and organization to it. There was little resolved in the end. A good debate experience and good practice working in groups."

"We needed more preparation time and less time to debate."

These comments were well-placed and matched our own assessment of a problem area. We changed the structure of the debate over the course of the project, imposing more structure and providing more time for discussion and debate planning. Thorough TA preparation and greater attention to moderating the debate solved many of the organizational problems.

Some students (7%) wanted more information on the topic. Since the two sides (Agribusiness and Environmentalists) were given a combination of identical readings and other readings specific to only their own group, students felt as if information was being withheld. In the first two semesters, we did make all readings available on reserve at the library, but students didn't remember this in-class announcement or they didn't take the time to go to the reserve section. We initially addressed this problem by making a course "reader" available for purchase that contained all of the readings. Because permissions for reprints are expensive, this was a costly route for the students, with readers selling for \$22. We have now modified this part of the module so students can access the readings for the debate on the world wide web. This appears to be the best solution to this problem, with many good articles available from reliable sources.

Students also commented that the debate was too hostile and competitive (4%). These comments came exclusively from two different occasions (out of a total of nine times the debate was staged), where one aggressive individual from the class behaved in a way that created outright hostility between the two sides. As might be anticipated, this was not well-received by the students. We countered this problem by providing the instructors with some tactics to deal with aggressive students. These suggestions are included on p. 38 in this Instructor's Manual.

A small group of students was dissatisfied with the fact that grades were based on participation. The shy students felt as if they were being penalized for being quiet (4%) and the outgoing students felt like they were not getting enough credit for being leaders (2%).

## II. The pesticide issue is not black and white. Some students appreciated this and others did not (24%).

A discussion about the use of pesticides in the food supply is a true debate. There are good points to be made on both sides and there are many issues which are not clear-cut. Trade-offs must be made to reach a workable compromise in our present political and economic climate. The debate was successful in making students think about the multiple issues involved in regulating pesticides. While 6% of the students were dissatisfied that they had not been able to choose the side of the issue they were debating, 9% of the comments indicated that students thought the act of debating was particularly effective in educating them to the issues faced by "the other side."

"I thought the set-up was thought-provoking---some of us had to seriously argue the "other" side of the picture, thus it was enormously beneficial to step into the shoes of an agribusiness woman."

"It was very nice to take "hard core" science and apply it socially with human nature clouding the facts---as it does in real society."

"I think it is a gateway to get people involved in the issues we study in lab. It forces people to take a side and argue that point of view. What better way is than to make people think about both sides of an issue? I think that debate is a great idea."

Students (5%) also commented that others were not really taking sides during the debate. It is true that a class full of environmentalists (as is typical for this class) are usually all on the same side. We had the most success and the most lively debate when students were encouraged (either by an instructor or by peers) to really play their roles as much as possible.

Some students (4%) were very uncomfortable with the fuzzy nature of the problem. They wanted a *right* answer to exist and be presented to them.

"The pesticide debate was very biased. If there was going to be a true debate, there should be a few people (preferably the TAs and standing on neutral ground) overlooking the debate without pointing out either sides's faults but only the *right* (italics added) statements."

## III. Nonspecific Positive Comments (20%)

A significant fraction (20%) of the students simply made enthusiastic comments about the debate.

"It was one of the best things I've had this year."

"It was fun and helpful!"

"I loved it!!!"

"It's cool!"

## IV. The relationship of chemistry to the debate (9%)

Students also brought out the relationship of chemistry to the debate, with 5% of the comments indicating that students felt that the debate had shown them the relevance of chemistry to the real world.

"Made the rest of the lab more interesting because I understood the impact and relevance of what we are studying. Helped me to notice connections in other labs. Made me think!"

Four percent of the students felt there was not enough chemistry involved in the debate.

"The discussion for the pesticide module was good because it was a different approach to the material and I learned a lot about the debate, but strictly in terms of chemistry, it wasn't as effective. I was forced to really learn about the stuff in the lab manual because I had to do those big old lab reports. For the pesticide one I didn't learn so much about organic compounds, polar/non-polar substances, etc. --- but keep it anyway. Because it was a refreshing aspect of the material."

The lack of chemistry discussed in the debate was a concern for the instructors in the course as well. While the goal of the debate was for the students to use their data to help make their points, discussion of data did not typically dominate the students' debate. Indeed, students more often resorted to anecdotal evidence to support their statements rather than real data they had collected. It is very clear that if students are given an opportunity to skirt the real science, they will take it and rely more heavily on ways of thinking and arguing they are more accustomed to. Because the students in our class were freshmen, this is not surprising, since their level of scientific sophistication was still low. It would be interesting to see how upper-level chemistry students handle this aspect of the debate. Our solution to this problem was to change the debate format so the presentation of data is *required* as the initial part of the debate presentation, as indicated in the instructions for the debate.

## **V. Students felt like they learned a great deal (7%)**

Some students (7%) commented on the fact that they learned and remembered a great deal from the debate experience.

"I learned more from that discussion than I did from the other labs."

"I learned a lot from the discussion, and more importantly, I remember a lot."

"I learned a great deal. I also feel more at ease about the issue now that I understand it a little better. I can recognize media hype of the issue has expanded the proportions of the issue. Pesticides are lousy and do lousy things to the soil, but I don't believe we are at a serious risk right now and I learned this through the discussion."

## **VI. Students liked the change of pace (7%)**

We planned the debate to take place at the end of the semester to partially take the place of the large lab reports expected of the students for the other three modules earlier in the semester. The students had worked hard the entire semester, and this activity was meant to create a bit of a break and a change of pace for the students at this stress-filled time in the semester. The students noticed this and were grateful for a chance to do something different.

"Three modules and the debate were more realistic than 4 modules."

" . . . It also eased the final crunch by pardoning us from another massive report."