SURFACTANTS

What they are, how they work, why we care

Learning objectives and classroom demonstrations

I. Overview of the issues

Surfactants are among the most widely used synthetic chemical compounds in our society. They are not only used in cleaning products (shampoos, bar soaps, laundry soap, dish and dishwasher soaps, hand sanitizers) and cosmetics, but as wetting agents (sticker-spreaders) and emulsifiers in spray formulations of pesticides, herbicides, fertilizers, and paints, as well as the fuels, lubricants, and antifreeze used in our cars and boats. Soap is a multi-billion dollar industry, and chemists are inventing new kinds of surfactants every year. Hundreds of millions of tons of surfactants are released into the environment every year in North America alone. Lakes, streams and ponds in the San Juan Islands generally have measurable residual surfactants that are close to 0.5 part per million, a level that the Environmental Protection Agency regards as affecting water quality.

Surfactants are not regulated as strictly as the toxic chemicals, such as pesticides, with which they are often combined as "adjuvants". Manufacturers are only required to declare the surfactants contained in cosmetics and cleaning products, and can satisfy this requirement by referring to chemical families rather than individual chemical species. In other products surfactant adjuvants are currently classified as inactive or inert ingredients, which means that they do not have to be identified on product labels – and do not have to be tested for ecotoxicity (as opposed to effects on human health) before being sold. As a result, most new surfactants are manufactured and used for years before their impacts on fish and wildlife are evaluated.

By their very nature all surfactants harm aquatic animals and insects. The amount of harm done, and whether it is lethal, depends on the dose (concentration of surfactant x duration of exposure). Surfactants can plug the breathing pores or spiracles in insects and other small arthropods, suffocating them: this is why ordinary soapy water has been used for generations as a homemade insecticide. Surfactants impair gas exchange through cell membranes, plugging the molecular trapdoors cells use to selectively admit nutrients and expel waste products. At high concentrations, surfactants can disrupt cell membranes and kill cells. This is a big problem for aquatic animals that breath through their skin, and for the eggs of aquatic animals, which are simply large cells with exposed membranes. Gills in fish and crustaceans are ruptured by high surfactant concentrations, suffocating them.

People are much less susceptible to surfactant damage than aquatic organisms for a very simple reason. Our skin has an outer layer of dead cells that protect our living skin cells from chemical damage. Our stomach is lined with tough-walled cells, moreover, so that we can use acids to help digest our food. Most of our body is well protected—except

for our lungs, which have sensitive exposed cell membranes like the gills of fish. If soap gets in our lungs, it can be very bad indeed. But unlike fish, we don't "breathe" the water that contains our discarded soaps, garden products and motor vehicle leaks.

Surfactants also affect the distribution and toxicity of other contaminants, such as pesticides and polycyclic aromatic hydrocarbons (PAHs). These toxic chemicals are very waxy and hydrophobic. They tend to accumulate in oil or adsorb to silt. Add surfactants and lipophilic contaminants disperse, suspended in the water column, making them more mobile and more likely to encounter the exposed cell membranes of aquatic animals such as the gill tissues of fish. When we dispose of toxic lipophilic chemicals and surfactants in the same watersheds, draining or running off into the same wetlands and seashores, the effect is synergistic.

Using products with less surfactants, using surfactants more sparingly, and using them in ways that prevent them from being washed into wetlands and shorelines by rain showers, are all ways of reducing surfactant concentrations in aquatic ecosystems. But a growing number of surfactant species have an additional toxic effect, even at very small concentrations. They are Endocrine Disrupting Compounds or EDCs. Their structure is similar to some of the hormones that animals use to regulate their growth and behavior. For example, alkyl phenyl ethoxylates or APEOs, illustrated in the slideshow that comes with this curriculum, break down in the environment to nonyl phenol (NP), which looks and behaves a lot like estrogen. Animals such as salmon that have been exposed to only a few parts per billion of NP can grow two nonfunctional sets of reproductive organs, or simply stop mating. So while all surfactants should be used carefully and in moderation, it is especially important to minimize the use of surfactants that are EDCs.

Surfactants are not new in nature, however. Many millions of years before people learned to make soap from animal fat and ashes—and before chemists assembled the first synthetic detergent, sodium lauryl sulfate—many plant species produced surfactants as a weapon in their never-ending struggle with insects and other herbivores. Plant-produced surfactants are mostly saponins, chemically, and many non-industrial societies used these naturally occurring surfactants as soap: for example, the saponin-rich sap of yucca in the American Southwest. Most saponins taste awful to people and other mammals, which is a deterrent to herbivory by (for instance) deer or cattle. Some are oddly sweet, however, such as the saponin that we use to flavor licorice. Humans can eat and enjoy very small quantities of these unusual, flavorful saponins, but they still kill insects.

Most surfactants are biodegradable. That is, bacteria (and in the soil, also fungi) can break some of the chemical bonds holding surfactant molecules together. Bacterial colonies can use this metabolic process to harvest energy, and it breaks surfactants into smaller molecules that may be less toxic because they no longer behave like surfactants or as EDCs. Biodegradation takes time, and some chemical groups are not biodegradable at all. Bacteria easily split the carbon-carbon bonds in aliphatic hydrocarbons such as the alkyl groups in the slideshow. It's just a matter of time. The longer the alkyl chain, the longer it takes to biodegrade. Phenyl groups (six-carbon rings) are much more persistent, which is why when APEOs break up they leave NP in the environment. The persistence of a surfactant species can vary from days to centuries depending on its structure.

Naturally occurring surfactants such as saponins tend to biodegrade rather quickly into non-toxic byproducts. The reason is simple. Most plants need pollinators as well as an effective defense against herbivores. If a plant's chemical defenses against herbivores are too mobile and persistent—if they get around and stick around—they will accumulate in the plant's habitat and poison its pollinators. Indeed, saponins can also disrupt and kill the soil organisms (mycorhizal fungi, nitrogen-fixing bacteria, soil arthropods) on which they depend for many nutrients. Hence the chemical weapons plants have developed tend to stay put in leaves, bark and buds—where they are likely to be ingested by unwelcome sucking and chewing—and when released into the soil or water, feed bacteria and fungi.

In addition to surfactants, plants' chemical arsenals include a wide variety of toxic oils that function as insect repellants and insecticides. Many of them evaporate slowly in warm weather, producing an invisible protective cloud or haze surrounding the plant that insects can smell and tend to avoid. Humans find some of these plant chemical weapons very pleasing to smell and taste, and we use them in perfumes and food. It would be hard to list them all. Lavender, thyme, mint, lemon peel oil, cedar bark oil (thujol), cinnamon, and cloves are only a few. Many of these natural "essential oils" are being marketed now as "earth friendly," "safe" or even (paradoxically) "non-toxic" pesticides. They do work as insecticides, applied to plants before insects munch them; and they are also toxic to us, if we consume too much of them.

Essential oils are added to the surfactants in cosmetics and cleaning products such as body soaps and shampoos to give them a pleasant fragrance, and because people think that essential oils are good for them. But the combination of surfactants and essential oils makes products more toxic to insects and aquatic organisms. Indeed, the surfactants help emulsify and disperse the essential oils, making them far more mobile and effective than they can ever be when they exist naturally in the tissues of plants. So when people switch to "natural" cosmetics and cleaners, they are often choosing *more* toxic products than the old-fashioned soaps and detergents they used before.

There is still much that scientists want to learn about the behavior and toxicity of surfactants. Most surfactant chemical species remain largely unstudied. But we do have options for protecting the waters and wildlife of the San Juan Islands. Here are some tips to discuss with students that relate to this curriculum. Ask them to think of more.

- Saponins extracted from plants, and old-fashioned soaps (saponified vegetable oils without fragrances or other additives), are relatively non-toxic and tend to biodegrade quickly in soils and sediments. Choose them if they are available.
- Nonionic surfactants are much more likely to be endocrine disrupters than the older anionic detergents such as sulfonates and phosphonates. Avoid them, to the extent possible, because they can be toxic at such low concentrations.
- Avoid products that combine essentials oils with surfactants.
- If you cannot identify all of the ingredients on a product label, or if all of the ingredients are not disclosed, avoid the product as a precaution.
- A well-maintained on-site septic system can biodegrade a lot of surfactants—but surfactants can also kill the bacteria that make the septic system work. Be gentle with your septic, and do not assume that can eliminate surfactants.

- Do not assume that our islands' sewage treatment systems can remove anionic or nonionic surfactants from wastewater. Many persist.
- Avoid using any water-emulsion sprays outdoors, in your garden, lawn, or the foundations of your house. Nearly all of them contain nonionic surfactants as emulsifiers or wetting agents. If you must use chemicals, spot apply them on a brush or sponge to minimize collateral damage and keep chemicals off soils. Physically remove insect pests or weeds if feasible.
- Use less soap. Substitute hot water and elbow grease. Try plain ground clay, used for centuries in North Africa, as an alternative to soap and shampoo.

II. Learning objectives and demonstrations

1st Class session

Theme: Composition of soils and sediments

Principle: Soils and sediments consist of mineral grains of different sizes

Contaminants interact differently with water and with the mineral grains in soils and sediments. Some dissolve easily in water, while others dissolve only a little, or not at all, preferring to stick (adsorb) to minerals. The size and composition of particles making up sand and silt influence the behavior of toxic chemicals. Let's begin by looking inside some local soils and sediments to see what they're made of…

1. Have students bring samples of sediments from lakes or ponds, or soil from home. An 8-ounce (half-pint) jelly jar is just about right for collecting samples.
2. Spread samples on newspaper or cardboard to dry them thoroughly. Remove any seashells or plant debris such as visible leaves, roots or seeds.
3. Crush samples carefully with a mortar and pestle. Clay silt tends to form pellets if not thoroughly dried and crushed.
4. Use a Keck-type sand shaker (or similar graduated set of sieves) to sort each soil sample into grain-size classes. We recommend using 1-mm, 0.5-mm, 0.25-mm, and 0.1-mm mesh sizes, which correspond to very coarse sand, coarse sand, fine sand, very fine sand, and clay silt.
5. Examine each fraction of sand and silt under a microscope (at least 20X). Sand is composed mainly of distinct mineral grains, rounded or broken, most commonly milky or clear glassy quartz. Silt particles (clay minerals) are too small to see individually with most ordinary microscopes, and tend to clump together in tiny grayish masses, often coating sand grains.

Questions for discussion: How do soils differ from sediments? Do their minerals appear to come from the same sources? Can you match up the minerals in sediment with nearby soils in an exposed shoreline bluff or bank? Can you match the minerals with any large glacially grooved rock outcrops or glacial erratics nearby?

Technical notes: Most soils in San Juan County are glacial in origin and therefore quite recently formed (less than 10,000 years old). Glaciers crushed and scrape bedrock, leaving "tills" composed of broken boulders, gravels, and sand grains that are "prismatic" with irregular outlines and sharp edges. Sands washed down streams or transported along shorelines by tides and currents tend instead to be sorted by size, and to be more rounded. Long, heavy glacial loading produces very fine gray "rock flour" and beds of slick sandy gray clays that oxidize to orange if later exposed to oxygenated fresh water. Most island

soils are "heavy" with clay and not very permeable so that winter rains quickly turn fields and meadows into mud and standing water.

Granitic glacial erractics (glacially transported boulders) are common in San Juan County. They appear white from a distance, but on closer examination they have a "salt-and-pepper" appearance, with fused crystals of white or clear quartz, black hornblende (a group of iron-magnesium-aluminum silicate minerals), gray to pink feldspar (a group of potassium-aluminum-silicate minerals), and thin golden or silvery flecks of mica (another group of aluminum silicates). Weathering and hydration turns feldspars into clays, while quartz and iron silicates tend to resist weathering and survive as distinct crystal grains for a very long time. Salt-and-pepper sands and gray clays in the islands owe their origins to granites in the north Cascades and British Columbia's Coast Range, scraped and ground by continental glaciers.

The exposed native bedrock of San Juan County, by comparison, is mainly basalt (fine-grained "mafic" or hornblende-rich dark lava rocks) and old metamorphosed shale, turbidite (sedimentary siltstones) and sandstone. Some island sands have tiny red garnets or small grains of greenish olivine (a metamorphic magnesium iron silicate) – a good sign that they have a local "parent" rock.

The mineral grains in different soils and sediments seem surprisingly alike when examined microscopically, differing in size and shape rather than color. Remember that quartz minerals and feldspars comprise most of the earth's crust. Hence we can assume, for our next class session, that most of the mineral grains in soils and sediments have the same chemical composition and specific gravity (density) as quartz and feldspars. Quartz has a specific gravity of about 2.65, and feldspars of about 2.56 – very close.

2nd Class session

Theme: Sediment transport and sediment sorting

Principle: Silts are suspended longer in water and travel farther than sands

The size and density of mineral grains in soils and sediments determine their mass and their mobility. Water has a specific gravity of 1.0, so anything with a greater specific gravity than 1.0 will eventually sink. But there are ways to keep heavy things floating. A ball of solid iron will quickly sink. Hammer it into a very thin sheet (like aluminum foil), and it may float for a little while. Shape the thin foil into a little boat, and it will continue float unless you load it with more mass than the weight of the water it displaces. When a solid sinks, it must push water out of its way, and because water has mass, it resists. Air is less massive than water and resists a lot less, so an object must be much lighter to float on air. At the same time, water and air move—they have currents—and they can push a very large object like a boat or balloon. Even a sinking object can be pushed quite a long distance while it's sinking. The faster than water is moving—the more kinetic energy it has—the more mass it can push along.

What does this have to do with sediment transport? A grain of sand or silt, while heavier than water, can be pushed a long way before it sinks to the bottom. Faster water

can keep more massive objects in motion than slower water. Likewise, a stream of water of given velocity (and force) can move all mineral grains below a certain size (and mass). And if it starts to slow down, which is what happens when a stream widens or enters the sea, water will drop the heaviest or most massive objects first. It sorts materials by their mass (or if they all have the same specific gravity, by their size, which is the same). The largest or heaviest are dropped first, then smaller and smaller ones, until only the tiniest are left: in the case of natural sediments, fine silts can travel miles from shore before they settle to the seafloor.

1. Take 10 cubic centimeters (=10 mL) of coarse sand and 10 cc of fine silt from the sediment sorted into size fractions as part of the 1st class.
2. Mix the sand and silt in a 500 mL Erlenmeyer flask or bottle, then add 250 mL of water and shake vigorously.
3. Observe the settling of the solids. What falls out first? How long does it take for the sand to settle completely? The silt?
4. Leave the flask or bottle for 24 hours, then use a turbidimeter or nephelometer to measure any residual turbidity that is invisible to the unaided eye. Make another turbidity measurement every day for several days and plot the data on a graph to produce a decay curve, from which students can extrapolate the length of time it will take for all of the silt to settle.
5. A further experiment can be carried out with a slightly tilted wooden board and a pitcher of water. Mix silt, fine sand, and coarse sand in a bowl. Wet the mixture so that it just sticks together, and move it to the upper end of the board. Begin to pour water onto the upper end of the board, just above the sediment. Tipping the pitcher more will increase the rate of flow. Challenge students to pour the water just fast enough to be cloudy (silty) but not fast enough to move the sand.

Questions for discussion: As water velocity increases, what sediments get washed away first? What sediments stay behind until the rate of flow is increased? If we ground up the silt even finer, how long might we be able to keep it suspended in standing water? Clay mineral particles can be as small as 2 nanometers thick. How small is that? If clay is roughly 2.5 times denser than water, how heavy is a clay particle 4 nm wide, 6 nm long and 2 nm thick? (Remember that one cc of water weighs 1 gram at room temperature.)

Technical notes: Turbidity is usually measured with a special instrument that uses light scattering as a way of "seeing" and counting extremely tiny suspended particles. An ordinary spectrophotometer measures absorbance of specific wavelengths of light as they travel through a liquid to seek clues as to the identity of dissolved compounds. Turbidity instruments look for the reflection or blocking of light by suspended solids. If you do not have a turbidimeter or nephelometer, you can use a sensitive spectrophotometer to get the same kind of results with any wavelength, although best results will be with red light.

Turbidimeters report results in Nephelometric Turbidity Units (NTUs), which are an arbitrary scale that does not distinguish between larger and small particles. Very good nephelometers allow the operator to probe for different size ranges of particles. This can

be useful, of course, if measurements are being made in the field—for example, in water dipped from a moving stream—rather than a container of water left to stand long enough for larger silt particles to settle.

As an extra credit exercise, consider a discussion of how water transport creates different kinds of sediments that become different kinds of rocks: from conglomerate that looks like pebbly concrete in fast-moving rivers, to sandstone in smaller streams, shale in lakes and lagoons, and very fine-grained turbidites on sea bottoms within a few miles of land. Geologists can use the sizes and shapes of grains ("clasts") in sedimentary rocks to identify the "depositional environment" in which those rocks were formed. Students can pursue a treasure hunt for rock samples that represent different depositional conditions in their island's distant past. Around Point Colville on Lopez, for example, there are shales, slates (metamorphosed shales) and turbidites; while at the ferry landing there is a deposit of conglomerate.

Only one sedimentary rock is not a product of sediment transport: limestone. It is created by the precipitation of carbonate minerals, mainly dissolved shells of invertebrate animals—and sometimes also includes fossilized animals that sank to the seafloor.

3rd Class

Theme: Adsorption of contaminants to sediments

Principle: Oils and the contaminants dissolved in them adsorb to fine sediments

Now that we know something about silts and their behavior in water, it is time to explore their role as transporters of pollution. Some toxic chemicals dissolve in water—they are "hydrophilic," or "water loving". But most contaminates are waxy, or oily, and they are hydrophobic: they do not dissolve easily in water, although they can dissolve in organic (carbon-based) solvents such as hexane, turpentine, or "mineral oil." Hence they are not hydrophilic, but rather lipophilic ("oil loving"). If exposed to petroleum or one of its derivative fractions, such as gasoline or kerosene, they will dissolve in it and go where it goes. Otherwise, lipophilic compounds have only one alternative: to adsorb, or stick to the mineral grains in sediments. Sediments can be very oily. Recent studies have found that sediments in San Juan County working harbors can be 1-4 percent oil by weight, and this is where a disproportionate amount of toxic chemicals such as PAHs and PCBs may be found. Many aquatic animals make their livelihood by filtering water and sediments, and digesting the food particles and fats and oils adsorbed to sand and silt. They become contaminated—and in turn contaminate the animals that eat them.

1. Add 1 gram of annatto seeds to 10 mL of mineral oil and heat gently (do not boil) in a beaker or flask until the oil has turned deep yellow to red in color.
2. Mix 10 cc each of silt and coarse sand from the 1st Class in a bowl. Transfer to a 250 mL glass flask or jar, and then add 100 mL of water.
3. Add 5 mL of dyed oil to the flask or jar, cap tightly, and shake vigorously. Allow the contents to settle for only 10 seconds. Then quickly pour all the liquid off into a second jar, and allow the solids (silt) to settle overnight. While a centrifuge can

be used to reduce silt separation to a few minutes, we recommend allowing solids, water and oil to separate on their own, as they would in the environment.

4. Pour 50 mL of distilled water into the first (sand) jar, swirl it gently, and pour off as much of the liquid as possible leaving only the sediment. Pour off all the liquid from the second (silt) jar also, using a pipette if available to first remove any dyed oil that is floating on the water. If time permits, leave both jars overnight to allow any remaining water to evaporate.
5. Observe the colors of the sediments in the two jars. Where does it appear that the dyed oil went?
6. Confirm the actual distribution of the oil as follows. Add 5 mL of methyl alcohol to each jar of sediment and shake the contents vigorously for about one minute to dissolve any dyed oil that may be present. Allow all the solids to settle again, for at least an hour, and then pour off the alcohol into two pre-weighed watch glasses or foil "boats". Centrifugation will improve recovery from the silt.
7. Allow the alcohol to evaporate at room temperature. Observe the amount of dyed oil remaining in each watch glass or boat. If practical, weigh each watch glass or boat dish, and calculate the net weight of oil recovered.

Questions for discussion: In principle the amount of oil adsorbed should be a function of sediment size, since dividing something into smaller and smaller pieces increases total surface area. If we divide a cube into eight smaller cubes, how much more surface area is created? What about dividing a cube into a thousand (10^3) smaller cubes? That is almost exactly the difference in size and surface area between the sand and silt in this classroom demonstration. Did the silt actually adsorb that much more oil? If not, what other factors may affect adsorption rates? Are we certain that we recovered all the oil from the silt? Is it possible that a stronger solvent than methanol would give us different results? This can be tried using laboratory solvents (such as hexane) or paint thinners, but exercise caution: they are more volatile and toxic than methyl alcohol!

Technical notes: The chemical structures of different clay minerals affects the surface electrochemistry of clay particles, which do tend to be relatively thin and flat with a high ratio of surface area to mass. The chemical structures of lipophilic contaminants differ in ways that can affect solubility, adsorption rates, and the "partitioning" of each compound between sediment and water. Water temperature and acidity also affect adsorption, as do other chemical compounds that may be present—so it can be quite complicated to predict partitioning in real environments. Silts can transport toxics a long way before they settle, in any case; and they can transport proportionally more toxics than coarser sediments.

Please refer to the Teacher Prep Notes attached to this curriculum for information on annatto and mineral oil, which we recommend as relatively non-toxic materials for class use that mimic the behavior of lipophilic contaminants dissolved in petroleum.

4th Class

Theme: Surfactant transport and effects

Principle: Surfactants emulsify by lowering the surface tension of water

The mobility of lipophilic contaminants is affected by environmental conditions such as the kinds of sediments available as transporters, and the temperature and acidity of water. Surfactants such as household soaps and detergents can become contaminants, and they can also modify the behavior of other contaminants in aquatic ecosystems. By emulsifying and dispersing oils and the toxic chemicals dissolved in oils, surfactants can make these contaminants more mobile and spread them farther and faster. Adsorption of contaminants to silt, the bodies of aquatic animals, and other solids can also be enhanced by surfactants. The term "surfactant" is shorthand for "surface acting agent," and this is a clue to how they work. By reducing the surface tension of water, surfactants can make it "wetter," and can slip little droplets of oil into water where they behave like silt particles.

1. Mix a few drops of green food coloring in 20 mL of water.
2. Divide the colored water into two jars. Add 1 mL detergent (shampoo, dish soap or liquid laundry soap) to one jar and gently stir until it is completely mixed.
3. Insert a thin glass capillary tube into each jar, and compare how high the colored water rises. How has the surfactant changed the behavior of the water? Can you manipulate this behavior by changing the concentration of the surfactant?
4. Now add 2 mL of the red-dyed oil from the 3rd Class to a large glass test tube (at least 25 mL capacity), followed by 15 mL of distilled water. Observe the "phase separation" between oil and the water. Shake vigorously. Allow the aqueous and oily phases to separate again.
5. Add a few drops of detergent (shampoo, dish soap or liquid laundry soap) to the test tube and shake vigorously. Where did the layer of red-dyed oil go? How do surfactants make oil and water miscible?
6. Add another 2 mL of red-dyed oil to 5 cc of silt from the 1st Class in the bottom of a second glass test tube and blend thoroughly with a glass rod. Gently add 15 mL of distilled water to the tube. Cap and shake vigorously. Does the dyed oil leave the sediment? Does the sediment go into suspension or stay on the bottom?
7. Add a few drops of detergent to this test tube and shake vigorously. Observe the results. What has happened to the dyed oil? What has happened to the silt?

Questions for discussion: Apply the results of this demonstration to an imaginary oil spill. If there were no surfactants in the water, where would most of the oil go? If we add surfactants (or there are already surfactants in the water, as contaminants from homes and businesses), where would most of the oil go? Would surfactants increase or decrease the time it takes for contaminants to affect fish that people eat such as salmon, rockfish or tuna? Would surfactants increase or decrease the time it takes for contaminants to come into contact with naturally occurring bacteria that can biodegrade (*i.e.* metabolize) them?

Where do most marine bacteria live—in sediments, mid-water, or nearer the sea surface? What kinds of molecular bonds can bacteria metabolize most easily… or not at all?

Technical notes: The key to understanding the physical properties of surfactants is the nature of micelles—the extremely small droplets of lipophilic molecules suspended in water by a halo of surfactant molecules. Micelles are too small to be seen with an optical microscope, but you can use a nephelometer to demonstrate their nature as droplets rather than dissolved molecules. Be sure to mix only water, oil, and a surfactant – no silts – for this purpose, since any silt particles in the mixture will produce a misleading signal.

Surfactants are sometimes described and used in industry as "wetting agents," for example in garden-sprayer pesticides. What does that mean in practical terms? You can demonstrate this phenomenon with a potted plant, a few drops of distilled water, and just a few drops of a 20 percent soap-and-water mixture. Glue two fresh green leaves flat on a board or tray, using a water-based paste or glue-stick. As soon as the leaves are secure use a dropper to apply a little distilled water to one lead, and soap solution to the other. The soap solution will not "bead" as much, and should be a bit less likely to completely drain or spill off the leaf. After the 6th Class, ask students why surfactants can be used in garden sprays without killing the plants to which they are applied! The answer: vascular plants have thick cuticles protecting the cells in their leaves and steam, much like the thin layer of dead cells protecting human skin cells. And leaves "breathe" through large pores called stomata, which can close up tight and prevent chemical damage—whereas insects breathe through tiny holes in their chitinous exoskeleton (spiracles) that cannot be closed!

5th Class

Theme: Surfactants have biophysical effects on most organisms

Principle: Surfactants affect the physiology and behavior of membranes

Because they can exert strong physical forces on other molecules, surfactants can alter the behavior of membranes including cell walls, as well as multi-cellular membranes such as the linings of our lungs and intestines and the gills of fish. Biological membranes function as selective molecular-exchange machines. They allow some molecules to enter or leave, and exclude others. Our intestinal wall (for example) admits water, a variety of carbohydrates and fatty acids we need as food, some amino acids, and other molecules it recognizes. Our lung lining allows oxygen in, and carbon dioxide out. Properly working membranes are essential to all organisms, even bacteria. Membranes may have "passive" doorways—tiny holes that simply exclude any larger molecules—or "active" ones, which only admit molecules with particular chemical structures that fit into a complexly folded protein keyhole. Surfactants can stick into membrane pores and keyholes, plugging them completely—or can hold these doorways open, making membranes *more* permeable even to toxic compounds.

1. Cover the wide end of a glass thistle tube or funnel with an osmotic membrane such as dialysis tubing—or better yet, actual animal membrane, available from scientific suppliers (*e.g.* Carolina Biological Supply part number DH-684030). Secure the membrane with a clean rubber band or o-ring.

2. Immerse the membrane in distilled water if necessary to soften it prior to use.
3. Place the tube or funnel wide-end down, and fill it to the bottom of its narrow stem with water dyed green with a few drops of food coloring.
4. Suspend the tube or funnel wide-end down in a beaker of distilled water. The colored water will slowly rise as water from the beaker is admitted selectively through the membrane. Why does the food coloring remain inside the tube or funnel?
5. Set up five more membrane-covered tubes or funnels in beakers of water, and explore the effects of five different surfactant products (such as shampoos), or five different concentrations of a single product. Which products enhance and which products impair the passage of water through the membrane? Is there a demonstrable relationship between surfactant concentrations and the height of the colored water column?

Questions for discussion: Can you think of a way of using this process to measure the concentration of surfactants in water? Would you be able to measure total surfactant loads, or only the loading of particular chemical species of surfactants? How does using osmosis to measure surfactant concentrations compare with simply using capillary tubes and surface tension (2nd Class)? Can osmosis be used to tell which surfactant species are most likely to be harmful to animals? Design an experiment to screen surfactant species for potential toxicity.

Technical notes: Many prescription drugs contain surfactants in order to enhance uptake through the human gut. Ordinarily, our gut membranes admit only a small part of the drugs we ingest; most of the active ingredients in our pills simply go right through our digestive system and are excreted in our urine. One solution is to raise the concentrations of active ingredients in pills. Higher concentrations increase absorption rates. The other solution is to use a surfactant that alters the permeability of gut membranes. This tends to be less costly to the manufacturer. However, it is unclear whether it is better or worse for the environment. Using surfactants reduces the amount of medicines in our urine, so less gets into our septic systems, sewage treatment plants, and—ultimately—our streams and nearshore habitats. But the surfactants used to achieve this are also potentially harmful to aquatic organisms.

6th Class

Theme: Surfactant toxicity in aquatic ecosystems

Principle: Surfactants are more toxic to some organisms than others

We have seen that surfactants affect the behavior of membranes, and that different surfactants can have different effects on membranes. This suggests that some surfactants may be broadly toxic to aquatic organisms, while others affect some species of organisms much more than others. Indeed, membrane disruption is not the only way that surfactants can be toxic. Many kinds of surfactants, such as alkyl phenyl ethoxylates (APEOs), have chemical structures that mimic endocrine hormones like estrogen. If they pass into cells,

they can switch key biological processes on or off. Endocrine hormones are information carriers or signals, like text messages between different organs inside an animal's body. A false or counterfeit message can stop an animal from growing larger, or make it grow extra organs, or shut down its reproductive drive. Because some surfactants are not only membrane disrupters but also endocrine disrupters, they are especially toxic at very small concentrations. At the same time, endocrine disrupters only affect certain animal species, such as fish, that have endocrine glands, or other internal signaling systems using similar chemical messengers.

1. Create three aquatic microcosms by reconstituting the dormant bacteria, algae and small unicellular and multicellular animals in Neo/SCI's MicroLife® mix in clear glass 1000 mL Erlenmeyer flasks or glass bottles with clean filtered water. Two microscosms will be treated with diluted surfactants. The third will be a control.
2. Allow the microcosms to habituate to ambient conditions inside the classroom for 48 hours. Be sure that they have adequate and equal lighting, but are not left on a windowsill or other place where they are in direct sunlight or may overheat.
3. Transfer about 5 mL from each microcosm to a small petri dish and observe with a dissecting microscope at 20X – 40X. What organisms can be observed? Which ones or primary producers? Which ones are herbivores? Which rely on eating other animals? Which are detritivores? Draw a food web to represent the trophic relationships between at least 10 different organisms that are abundant and easy to identify in the three microcosm communities. And carefully count their numbers in each of the three petri dishes, to estimate their abundance in each microcosm.
4. Prepare two dilutions of a cleaning product selected by students. We recommend final concentrations within the microcosms of 5 parts per million and 50 parts per million. For a final concentration of 5 ppm in one liter of microcosm water, first measure 1mL of the product with a disposable pipette, and mix it thoroughly into 200 mL distilled water. Then add 1 mL of diluted mixture to a microcosm. For a 50 ppm final concentration, begin again with 1 mL of the product but mix into 20 mL distilled water before adding 1 mL of diluted mixture to a microcosm.
5. Once surfactants have been added to the two "treatment" microcosms, allow all of the microcosms (treatments and control) to rest, undisturbed, for at least 24 hours, before sampling them again and examining the composition of the community in each flask or bottle. Once again, focus on the 10 or more indicator species used to describe the microcosms' food web, and carefully count their numbers in the three petri dish samples drawn from the treatment and control flasks.

Questions: Which indicator species fared the best and the worst? Did they belong to similar taxonomic groupings (for example, were they predominantly crustaceans), or to the same guilds (for example, were most of them predators)? Can similar body structures or similar "livelihoods" help explain the pattern of survival or surfactant-toleration? Was there a concentration effect? Did the higher concentration simply kill a larger proportion of the same relatively vulnerable organisms, or did it also result in a different pattern of survival? Did exposure to surfactants alter the structure of the microcosm food web?

Technical notes: Ambient concentrations of surfactants in the islands' freshwater ecosystems range from roughly 0.5 to 15 parts per million. The lower concentration used in this class demonstration corresponds to conditions periodically observed in road runoff from the San Juan Islands' more developed and populous Urban Growth Areas, such as Friday Harbor and Eastsound. When road runoff enters salt water through storm drains it continues to disperse and dilute. However, the marine waters of San Juan County already bear a heavy load of surfactants and other contaminants from the region's cities—Seattle, Tacoma, Vancouver, Victoria—and commercial agricultural areas. The combined effect of local-source and regional-source pollution can be considerable in the islands' shallow bays and other low-energy nearshore habitats, which are also the areas of highest nutrient concentrations, the greatest primary ocean productivity, and nurseries for salmon, smelt, herring, rockfish, pacific cod and other fish valued by our communities.

Education enrichment options: The microcosm method described here can be used to explore the ecotoxicology of other products or a wider range of product concentrations. We recommend using this method to explore the synergistic effects of adding fragrances, usually "essential oils," to soaps and cleaners to make them more appealing, especially to "organic" or "alternative" consumers. Essential oils are plant chemical defenses, and can be extremely toxic to insects and other invertebrates. As oily, lipophilic compounds they are not very mobile on their own. But if surfactants are added to essential oils, they form micelles, and can be transported long distances by water before they adsorb to sediments. Scented soaps and shampoos probably have a much greater impact on aquatic ecosystems than unscented products containing the same surfactants—and alternative products based on vegetable-oil-derived surfactants may actually be more toxic than conventional soaps, if they contain essential oils. To investigate these issues use three microcosms where one is treated with an unscented product, the second is treated with the same product with the addition of an essential oil at the same concentration, and the third is an untreated control. Individual essential oils can be purchased as specialty body-care products. Or make your own by macerating lavender, thyme, cloves or other herbs or spices in a small quantity of drugstore mineral oil with a few drops of alcohol.

7th Class

Theme: Quantifying the toxicity of surfactants to specific organisms

Principle: Acute and chronic toxicities are usually expressed as "LC50"

Studies of the impacts of contaminants on entire ecosystems, even as microcosms, involve many variables that are difficult to control experimentally. Toxicologists usually prefer to test products on a single representative (or "model") organism so that results are strictly comparable from laboratory to laboratory. Widely used model organisms include rainbow trout, gammarid amphipods ("beach bugs" or "sand fleas"), and the cladoceran *Daphnia magna*, a planktonic crustacean large enough to be visible with the unaided eye, which we recommend for this demonstration. Large numbers of the chosen organism are exposed to serial dilutions of the compound or product to be tested for both a short period of time (30 minutes, an hour, or a day) and then a longer period of time (days to weeks). From a graph of the percentage of organisms killed by each dilution, we can interpolate

the concentration of the tested product that would kill exactly 50 percent of them. This is the LC50 ("lethal concentration 50 percent"), and is reported as "acute" and "chronic" for short- and long-duration exposures. A toxicology study might say, for example, that Joy Dish Soap has an LC50 (acute 30 minutes) to *Daphnia magna* of 7.3 parts per million, and an LC50 (chronic 7 days) of 5.8 parts per million. In the language of ecotoxicology, this is the way that toxicities are reported and compared. Let's measure the LC50 of one or more locally popular soaps or shampoos.

1. For each surfactant or surfactant-containing product to be tested, you will need six glass 150-mL beakers or bottles, and 60 *Daphnia*.
2. Mark the six beakers as follows: *Negative Control*, *Positive Control*, *100 ppm*, *10 ppm*, *1 ppm*, and *0.1 ppm* and arrange them in that order on the tabletop.
3. Add 100 mL of distilled water to the first three beakers and 90 mL to the rest.
4. Carefully add 10 *Daphnia* to each beaker.
5. Add 1 mL of a 1% Sodium Lauryl Sulfate (SLS) standard solution to the *Positive Control*. The *Positive Control* is now a 100-ppm solution of SLS.
6. In a clean 150-mL beaker gently stir 1 mL of the product to be tested into 100 mL of distilled water. Avoid producing foam. This is now a 1% solution.
7. Add 1 mL of 1% product solution to the beaker marked *100 ppm* and stir gently.
8. Transfer 10 mL of liquid from the *100 ppm* beaker to the *10 ppm* beaker and stir.
9. Transfer 10 mL of liquid from the *10 ppm* beaker to the *1 ppm* beaker and stir.
10. Transfer 10 mL of liquid from the *1 ppm* beaker to the *0.1 ppm* beaker and stir.
11. Count the number of animals still active and swimming in each beaker after 30 minutes (acute toxicity) and after 24 hours (chronic toxicity).
12. Convert the data from number living to percent survival (if four are living and six have died the survival rate is 40 percent). Plot data for each product on log paper, percent survival against log concentration, and connect the data points with a line. At what concentration does the line cross 50 percent survival? This is the LC50 of the product tested.

Questions: Should chronic LC50 always be smaller than acute LC50? May some organisms adapt or habituate to particular contaminants over a long period of exposure? How could this be detected or tested? What are the ecosystem-scale implications of the *Daphnia* results? If a contaminant kills or reduces the *Daphnia* in the environment, what cascade of effects on the wider food web might we expect to see? Do you think *Daphnia* is a good model for the islands' aquatic ecosystems? What kinds of ecological data could help you make that decision?

Technical notes: Standardized strains of *Daphnia magna* and other model aquatic organisms are available from Carolina Biological Supply and should be ordered at least 2 weeks in advance of use. They will be shipped overnight, and should be used within two days of arrival. Since the laboratory strain of this animal is not native to the Salish Sea, it is essential to destroy these animals after use and never release them into the environment

alive—not even down the sink. A little bleach is an effective and quick way to kill them, as well as any other organisms, including bacteria that may have arrived with them in the same containers. A discussion of the ethics of sacrificing laboratory organisms would be appropriate as a part of this class session. You may also want to make comparisons with human toxicology, which uses other mammals (usually rats) as models. In medicine, we tend to speak of "dose" rather than LC50. Dose is simply the amount ingested divided by the mass of the animal: for example, the acute toxicity (24 hours) of a drug might be 175 milligrams per kilogram of body mass. A good discussion question is why aquatic toxics data is expressed as concentration rather than dose! It's simpler in water, of course.

Education enrichment options: Once students are acquainted with LC50 methods, many variations are possible, apart from simply testing a greater variety of products, and comparing them. Students can look for behavioral effects of products on *Daphnia*, which appear at lower concentrations than lethality. Maintaining two *Daphnia* colonies for over a month in aerated aquaria with plenty of food (Carolina Biological Supply will send you information on feeding when it ships the animals) will usually result in reproduction, so a treated colony can be compared with an untreated control. Do they both produce healthy eggs? Surfactants often affect reproductive physiology. Students may also enjoy looking for native *Daphnia* species in the local environment. They can best be found in lakes and ponds in March or April, as soon as the weather begins to warm. This class can also be a good opportunity to introduce students to statistics. Have five or more student teams test the same product on *Daphnia* and compute the standard deviation of the results (survival) for each product concentration in these replicate experiments. How consistent are results from trial to trial? How confident can we be in the mean LC50? How high or low might it really be? Are 10 animals per treatment enough, or would it be better to use more?

8th Class

Theme: Quantifying the concentration of surfactants in the environment

Principle: Ion pair exchange can be used to measure ionic surfactants in water

In earlier sessions we discussed the role of ionization and polarity in determining which chemical compounds dissolve in water; and we observed how surfactants emulsify lipophilic compounds and suspend them in water. We will now apply this knowledge to measuring the concentration in water of certain kinds of surfactants—strongly polar ones that form ions in water. Once again, we will begin with dyed oil. But this time the dye is Methylene Blue, a hydrophilic stain used in cellular biology. In the presence of an ionic surfactant such as Sodium Lauryl Sulfate, which we used as a standard in the last class exercise, Methylene Blue will migrate from water into an oily organic solvent, turning it blue in direct proportion to the amount of surfactants present.

1. Prepare a stock solution of 0.01 M Boric Acid by dissolving 0.62 grams dry Boric Acid in 500 mL of distilled water, then adding additional water to one liter.
2. Weigh 100 mg of dry Methylene Blue powder and transfer it to a 100 mL flask or graduated cylinder. Use a few drops of the Boric Acid stock solution as needed to

wet the dye, stirring gently with the tip of a glass rod or a stainless steel chemist's spatula. Treat this dye with respect—it is relatively non-toxic but the tiniest bit of it will stain hands, clothing, and tabletops! Once all of the dye is wet add distilled water to 100 mL, swirl until all of the dye has dissolved, and set the solution aside tightly capped and sealed.

3. Use 90 mL of the Boric Acid stock solution to dilute 10 ml of the Methylene Blue stock solution. This is the working reagent.
4. Use reagent grade Sodium Lauryl [docecyl] Sulfate powder to prepare standards. Make a 1000 parts-per-million stock solution by 1.00 gram of SDS into a one liter volumetric flask, filling to the mark with distilled water, and inverting until solids dissolve. From this stock solution prepare a 1:100 dilution in distilled water for a 10 part per million SDS standard, and then dilute the 10 part per million standard solution 1:10 for a 1 part per million standard.
5. Set up a test tube rack with 10 clean glass 10x13 test tubes. Label them in order: SB (solvent blank), RB (reagent blank), S1 (1-ppm SLC standard), S10 (10-ppm SLS standard), and 1 through 6 for six environmental water samples.
6. Add 2 mL of Dichloromethane to the SB tube and 2 mL of distilled water into the RB tube. Add 2 mL of the appropriate standard solutions to the S1 and S10 tubes.
7. Into each of the test tubes except SB, add 0.1 mL of the Methylene Blue working reagent. You will need a 1/10 serological pipette with a manual pipette filler to be most accurate. The best pipette filler for this purpose is the VWR "pipette pump" available from scientific suppliers.
8. Add 2 mL of Dichloromethane to every one of the 10 test tubes.
9. Cap and shake each tube for exactly 10 seconds. Use a vortexer if available to get more consistent results.
10. Centrifuge the 10 test tubes at high speed for exactly one minute. This will ensure complete separation of the oil and water phases inside the tubes.
11. Use a spectrophotometer to measure absorbance of the contents of each test tube at 650 nm. Plot the results of the four controls (SB, RB, S1 and S10) on a graph: Concentration in parts per million is the X axis, and Absorbance is the Y axis. SB and RB should be zero, and of course S1 should be 1 and S10 should be 10. Now interpolate the absorbance results for the six samples and estimate their surfactant concentrations.

Questions: Which phase (oil or water) is on the top in this process? Why does the dye "choose" the organic solvent over the water? What could interfere with this test, and reduce its accuracy or reliability? Why do the blanks have absorbance greater than zero, if they contain no surfactants? What can be done to avoid contamination of the glassware that may cause false positives? Based on your results, what is the smallest concentration of surfactants that this method can "see"—that is, its limit of detection or LOD?

Technical notes: Water-soluble anionic surfactants such as the linear alkyl sulfates (LAS) form a 1:1 ion pair with the water-soluble cationic dye Methylene Blue. Once the ion pairs have formed they are effectively no longer polar—and therefore extractable into an organic solvent such as Dichloromethane. Of course this means that it is essential that the test tubes be placed in the spectrophotometer well so that the beam passes through the organic solvent and not the water! Using only 10x13 test tubes and standard square 1-cm cuvette holder in your spectrophotometer will ensure this. (Round tubes of this size do fit into square holes.) Naturally this analytical method depends on high-purity reagents, and glassware that has been cleaned with acid rather than soaps. Hydrochloric acid diluted to 10% with distilled water is effective for cleaning, and use only distilled water for rinsing. And use Milli-Q or Nanopure water if available—they are cleaner than distilled water. Environmental samples can be collected in clean glass jars with Teflon lids. Rinse the jar three times in the water to be sampled, then fill and seal it. Process samples within a day or two—light, heat and bacteria break down many surfactants within days to weeks. Try not to get any silt in samples, and if possible, filter samples through an acetate (not paper) vacuum or syringe filter as soon as possible after collection to remove silt and bacteria.

This method is a refinement of EPA Method 5540C that uses less toxic reagents, and achieves comparable or higher sensitivity. Pre-packaged kits such as LaMotte's LAS Surfactants kit (Part number 4876) utilize toluene or chloroform as solvents and have less precision and poorer sensitivity, *e.g.* LOD of 1-2 parts per million compared to 0.1 parts per million for the method described here (and in Bell et al. 2008).

Teacher preparation notes

"Mineral" oil is simply a gravimetric fraction of petroleum (a "mineral") as opposed to a biological product (vegetable oils and animal fats).

Paraffinic oil is a good proxy for petroleum. It is a heavy fraction of crude oil, syrupy in texture like vegetable cooking oils hence visibly and obviously "oily", and relatively safe to handle. Unlike crude petroleum it is clear, because the heavy tars and carbon granules that give petroleum its black color have been removed.

The "mineral oil" sold in pharmacies is a somewhat lighter fraction of crude oil, and also can be used in these demonstrations. Check to make sure that there are no additives such as fragrances or surfactants, which could affect the behavior of the oil in demonstrations.

There are a number of ways to dye mineral oils, including the use of lipophilic vital dyes such as the Sudan reds. Vital dyes can be quite toxic, however, so their use in classrooms is not advisable. The annatto-derived red dye bixin is recommended because it is easy to extract and use, and non-toxic, although it can leave a very persistent stain. For advanced students, the structure of bixin is very interesting. It is a long chain of alternately singly- and doubly-bonded carbons with semi-polar ends. If it has weak polarity, why is bixin so hydrophibic? Compare the structures of bixin and alkyl phenyl ethoxylates (APEOs), the most common nonionic surfactants, which are also weakly polar.

To prepare bixin-dyed mineral oil, combine approximately five grams of annatto seeds—available in the spices or Hispanic foods sections of most supermarkets—with 10 mL oil. Warm the mixture gently in a glass beaker. Do not try to bring the mixture to a boil; just 50^{o} Celsius should suffice to begin to extract bixin from the seeds to the oil. Remove the mixture from heat when the oil has turned red and allow it to cool before pouring off the oil into a glass jar for safekeeping. The seeds can be disposed in compost or trash.

Use the Keck sand shaker demonstration to prepare silt and sand fractions for subsequent demonstrations. We recommend using a 0.5-mm fraction for sand and a 0.1-mm fraction for silt to get the clearest results. The closer the two fractions in size, of course, the less a difference in their adsorptive properties—assuming they have the same composition. The crystal surface electrodynamics of clay minerals and quartz are different enough to affect the strength and selectivity of adsorption. Most island marine sediments are rich in clay, but it is prudent to confirm the mineralogy of sediments before using them. For advanced students or extra credit, ask for an analysis of the kinds of compounds that preferentially adsorb to clay minerals, and why!

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