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A Discussion of Water Pollution in the United States and Mexico; with High School Laboratory Activities for Analysis of Lead, Atrazine, and Nitrate

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Water pollution has its roots in our desire to meet the agricultural, energy, and commercial needs of an ever-growing world population. The causes of water pollution are as diverse as the countries that struggle with it. It is as deeply rooted in economic and social conditions as it is in chemical interactions. In the first part of this paper we present an overview of water pollution in the United States and Mexico, including sources, federal regulations and standards, and standard methods of analysis for overall water quality. The second part of the paper focuses on three water pollutants—lead, atrazine, and nitrate—chosen as representative examples of heavy metals, organic herbicides and inorganic pollutants. In addition to a background discussion of each substance and analysis methods for it, we describe related laboratory activities that are suitable for the high school audience.

An Overview

Water pollution in urban areas was recognized as early as the 1850s—when in London, whose population was then 2.7 million, growing urbanization and industrialization led to noticeable pollution in the Thames River. The chemist and physicist Michael Faraday voiced his concern in a July 7, 1855, letter to the editor of the *London Times* newspaper (*1*):

> "Near the bridges the feculence rolled up in clouds so dense they were visible at the surface, even in water of this kind. The smell was very bad, and common to the whole of the water. It was the same as that which now comes up from the fully-holes in the streets."

He then issued a call for action (*1*):

> "If there be sufficient authority to remove a putrescent pond from the neighborhood of a few simple dwellings, surely the river which flows for so many miles through London ought not to become a fermenting sewer."

About the same time, Louis Pasteur and others were learning about the importance of microorganisms as causes of disease (*2*). Microorganisms responsible for many diseases, such as typhoid and cholera, were carried by water supplies. Water for household use was carried one bucket at a time from neighborhood wells on top of which waste was dumped.

A consequence of our industrial progress during the past 140 years is that the sources of water pollution are now much more varied and have expanded to affect rural, suburban, and urban areas throughout the world. Water supplies are now disinfected using oxidation by chlorine or ozone; solid material is removed by filtration; hardness ions (principally Ca^{2+}) are removed by pH control; and other objectionable substances are removed by a variety of specific reagents.

Sources of Water Pollution

The Federal Clean Water Act of 1972 defines water pollution as "the man-made or man-induced alteration of the chemical, physical, biological and radiological integrity of water." More generally, whenever some substance is added that leads to a poorer water quality, the water is said to be polluted. There are four major sources of pollution (*2*).

Point Sources. These include sources concentrated at a specific place, or "point". Direct inputs, such as waste spewing from drainpipes into lakes and streams, are point sources.

Diffuse (Nonpoint) Sources. Such things as runoff from farms into streams during a heavy rain are diffuse—that is, they do not come from a single source. Pesticides and fertilizers commonly enter the water supply from diffuse sources.

Indirect Pathways. Wastes, such as coal-burning residues and organic solvents, have long been stored in metal drums and buried just under the surface. These containers can rust and their contents leak out through soils and groundwater until they percolate into a lake, stream, or river. This indirect pathway to water pollution is fed by the millions of such drums buried throughout the United States.

Atmospheric Sources. Acid deposition is the key concern here, not only because of the lowering of waterway pH, but also because of the increased leaching of metal ions from the soil that results from a lower water pH.

Health Impact of Water Pollutants

A vast array of water pollutants can interact with the body in all kinds of ways. Table 1 lists the Federal Agency for Toxic Substances and Disease Registry (ATSDR) "Top 20" pollutants for 1995, including their uses and effects of overexposure (*3*). Many (denoted with *) are of concern as water pollutants.

In the United States there are well-established guidelines for the maximum contaminant level (MCL) for many water pollutants. (See levels given in Table 2) (*4*). These MCL values are generally comparable to the Mexican "Maximum Limits" (*5*). There have been many water pollution acts legislated in the United States; some of the more important ones are given in Table 3 (*6, 7*). Mexico also has had legislative initiatives; however, in spite of similar pollution limits and legislated acts (also listed in Table 3), the water pollution situation in Mexico is generally more serious than in the United States.

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Differences between Next-Door Neighbors

Although the U.S. chemical industry is about 20 times bigger than the Mexican chemical industry, it generates only 10 times more toxic waste (U.S. toxic waste generated in 1994 = 1.13 million tons [*8*]). This is due to better toxic waste management in the United States. Roughly only 5% of Mexico's waste is treated and about 90% has an unknown destination. About 80% of this waste is liquid and is assumed to go into the water supply without treatment. Only about 3% (33,000 tons) of the U.S. toxic waste went into surface water. Mexico also imports large amounts of toxic waste from the United States. (Castro-Acuña calls it "being a good neighbor"). The amount that was imported grew 700% between 1987 and 1992, or from 10,719 tons to 72,178 tons (*9*).

The largest city in Mexico is Mexico City, whose population is more than 9 million. It is part of the larger Zona Metropolitana del Valle de Mexico area that is home to 20 million people. In this vast region, roughly 75% (22,593) of the industries are so-called "micro-industries", which are family operated by persons who do not have the education or money to follow environmental regulations. They are part of the 30,100 industries in the Metropolitan Area of the Valley of Mexico that manufacture everything from calculators and batteries to steel and textiles. A study of the electric and electronic parts industries (*10*) found that many toxic organic compounds, such as benzene, carbon tetrachloride, vinyl chloride, and xylene, were going completely untreated into the sewer system. Thus water pollution remains a serious problem in Mexico.

Standard Methods of Analysis for Water Samples

Although both countries have water quality concerns, they are unified in their analytic procedures. Even when the concentrations of pollutants in water samples grossly exceed government guidelines, their measurement is still correctly characterized as "trace analysis". To be considered a reliable method for analysis at the parts-per-million or parts-per-billion level, a method should ideally (i) have a suitably low limit of detection (LOD); (ii) be precise and accurate; (iii) have a wide dynamic range (i.e., be able to accurately measure the sample concentration over a wide range of concentrations); (iv) involve easy sample preparation; (v) be inexpensive; (vi) be able to determine many different kinds of substances; and (vii) be approved by regulatory authorities (*11*). This is quite

Table 1. Health Impact of "Top 20" ATSDR Pollutants (*2*)

ATSDR #	Pollutant[a]	Formula or Symbol	Sources	Some Health Effects of Overexposure
1	Lead*	Pb	plumbing, coal, gasoline	anemia, kidney disease, blindness, mental retardation
2	Arsenic*	As	pesticides	liver, bladder, kidney, lung cancer
3	Mercury	Hg	fish, heavy industries	central nervous system damage, mental retardation
4	Vinyl chloride*	C_2H_3Cl	polymer manufacture	liver cancer
5	Benzene	C_6H_6	crude oil, gasoline, industrial synthesis	blood cell damage, leukemia
6	Polychlorinated biphenyls* (PCBs) (ceased manufacture in 1977)	$C_{12}H_nCl_{10-n}$	coolants, lubricants, electrical equipment	cancer (?), reproductive effects (?)
7	Cadmium*	Cd	solder, batteries, lawn treatment chemicals	high blood pressure, kidney damage, destroys red blood cells
8	Benzo[*a*]pyrene	$C_{20}H_{12}$	smoke, soot, creosote	cancer
9	Chloroform	$CHCl_3$	paper mills, many others	impaired liver, kidney function
10	Benzo[*a*]fluoranthene	$C_{20}H_{12}$	wood, coal-burning stoves	cancer (?)
11	DDT* (ceased manufacture in 1972)	$C_{14}H_9Cl_5$	pesticides	nervous system damage, probable liver carcinogen
12	Aroclor 1260*	$C_{12}H_nCl_{10-n}$	coolants, lubricants, electrical equipment	cancer (?), reproductive effects (?)
13	Trichloroethylene	$C_2H_3Cl_3$	evaporation from adhesives, glues, paints	central nervous system, liver, kidney damage
14	Aroclor 1254*	$C_{12}H_nCl_{10-n}$	coolants, lubricants, electrical equipment	cancer (?), reproductive effects (?)
15	Chromium (6+)	Cr^{6+}	many chemical manufacturing processes; burning gas, oil, and coal	lung cancer
16	Chlordane*	$C_9H_5Cl_8$	pesticides	nervous and digestive system damage, impaired liver function
17	Dibenz[*a*,*h*]anthracene	$C_{22}H_{14}$	wood, coal-burning stoves	cancer (?)
18	Hexachlorobutadiene	C_4Cl_6		*no toxicology information available*
19	DDD*	$C_{14}H_{10}Cl_4$	breakdown of DDT	probable carcinogen
20	Dieldrin*	$C_{12}H_8Cl_6O$	insecticides	nervous system damage, cancer (?)

[a]An asterisk (*) designates a substance of concern as a water pollutant.

Table 2. Maximum Contaminant Level for Common Water Pollutants (*4*)

Substance	MCL[a] (mg L^{-1}; ppm)
Organic Chemicals	
Aldicarb	0.003
Aldicarb sulfone	0.002
Aldicarb sulfoxide	0.004
Atrazine	0.003
Carbofuran	0.04
Carbon tetrachloride	0.005
Chlordane	0.002
2,4-D	0.07
Dibromochloropropane (DBCP)	0.0002
p-Dichlorobenzene	0.075
o-Dichlorobenzene	0.6
1,2-Dichloroethane	0.005
1,1-Dichloroethylene	0.007
cis-1,2-Dichloroethylene	0.07
trans-1,2-Dichloroethylene	0.1
1,2-Dichloropropane	0.005
Endrin	0.0002
Ethylbenzene	0.7
Ethylene dibromide	0.00005
Heptachlor	0.0004
Heptachlorepoxide	0.0002
Lindane	0.0002
Methoxychlor	0.04
Monochlorobenzene	0.1
Pentachlorophenol	0.001 (0.001)
Polychlorinated biphenyls	0.0005
PCBs	0.0008
Styrene	0.1
Tetrachloroethylene	0.005
Toluene	1.0
Toxaphene	0.003
2-4-5-T	0.05
1,1,1-Trichloroethane	0.2
Trichloroethylene	0.005
Total trihalomethanes	0.1
Vinyl chloride	0.002
Xylenes	10.0
Inorganic Chemicals	
Arsenic	0.05
Barium	2.0 (1.0)
Cadmium	0.005 (0.005)
Chromium	0.1
Fluoride	4.0 (1.5)
Lead	0.015 (0.05)
Mercury	0.002 (0.001)
Nitrate	10.0 (5.0)
Nitrite	1.0 (0.05)
Nitrate + nitrite	10.0
Selenium	0.05 (0.05)

[a]Mexican "Maximum Limit" values of the MCL are shown in parentheses.

a large task for any one method, given the many types of compounds and microorganisms that can exist in drinking water. In fact, no one technique meets such criteria for all substances. Instead, several assay methods are used. A few of these approaches are discussed in some detail in this paper.

Environmental pollutants are usually present at low concentrations—often too low to be detected by many analytical methods. All methods are limited by their LOD. New methods with improved LOD are being developed continually, so analyses are now possible that were impossible only a few years ago.

A typical response curve of an analytical method is represented in Figure 1. Concentrations less than the LOD cannot be measured using the method. The slope of the rising part of the curve is called the method's sensitivity.

For multiresidue analysis, the first approach is usually to use one or more general screening methods. These are often based on gas chromatography (GC) or high-performance liquid chromatography (HPLC), which can separate and determine many organic substances of interest. Water contamination by sewage can be determined by a routine coliform bacterial count or by biological oxygen demand, which estimates the total amount of organic matter in water. Excellent methods are available for measuring the concentrations of many metals in a sample, including inductively coupled plasma spectroscopy and voltammetry. It is possible in the high school laboratory setting to quantitatively or qualitatively identify several water pollutants, including the three substances that are the focus of this paper.

Rationale for Focus on Lead, Nitrate, and Atrazine

Although the governments of Mexico and the United States have chosen to regulate many substances, high school and first-year college students can gain a broad understanding of substances that are water pollutants by choosing representative pollutants from three classes: heavy metals, inorganic compounds, and organic compounds. We chose lead as an example of a heavy metal pollutant for its historical importance, nitrate ion for its ease of analysis, and atrazine for its strong agricultural connection. In the sections that follow, we summarize the use and health effects of these substances and state-of-the-art methods for their analysis. Finally, we describe methods that can be used safely and within the time framework of the high school chemistry laboratory.

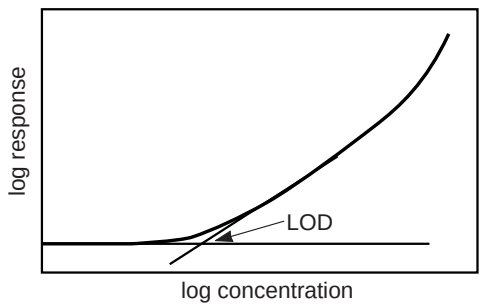

Figure 1. A typical response curve of an analytical method.

Table 3. Some Federal Water Pollution Control Acts (*6*)

Act	Acronym	Purpose
	United States	
Resource Conservation and Recovery Act (1976)	RCRA	Regulated reporting, handling storage, transport, and disposal of materials
Comprehensive Environmental Response, Compensation and Liability Act ("Superfund") (1980)	CERCLA	Regulated governmental response and clean-up of past hazardous waste sites
Superfund Amendments and Reauthorization Act (1986)	SARA	Reauthorized CERCLA and sharply increased clean-up funding
Safe Drinking Water Act (1974, 1986, 1996)	SDWA	Set standards for drinking water in public water systems
Clean Water Act (1972, 1986)	CWA	Continued federal aid for building municipal sewage treatment plants, postponed compliance deadlines for certain industry discharges
	Mexico	
Uso Urbano Público de la Ley de Aguas Nacionales		Regulates safe drinking water
Ley General de Salud en Materia de Control Sanitario de Actividades, Establecimientos, Productos y Servicios		Sanitary control regulations including maiximum permissible levels of substances in water

Lead in Water

Uses and Production

Lead is found in nature primarily in galena, or lead sulfide (PbS). It is easy to find inexpensive samples of galena in rock shops near such places as Rocky Mountain National Park. To obtain elemental lead, the ore is concentrated via flotation (see ref *12* for an excellent general discussion of the process), then roasted in air to form the corresponding oxide:

$$2PbS(s) + 3O_2(g) \rightarrow 2PbO(s) + 2SO_2(g)$$

The resulting oxide and any remaining PbS are then reduced by carbon monoxide, carbon, or iron in a blast furnace to give elemental lead:

$$PbO(s) + CO(s) \rightarrow Pb(l) + CO_2(g)$$

$$PbO(s) + C(s) \rightarrow Pb(l) + CO(g)$$

$$PbS(s) + Fe(s) \rightarrow Pb(l) + FeS(s)$$

Further processing (*12*) is used to separate the lead from small amounts of other metals (e.g., gold and silver) that are still present at this stage.

Lead is a soft, dense metal with a relatively low melting point of 327 °C. Because it can be worked with and shaped easily, it has been used for more than 5000 years. In ancient times (4000–3000 B.C.E.), it was part of eating utensils, cookware, statues, and pipes for plumbing (note similarity to the Latin name for lead, *Plumbum*, abbreviated as *Pb*). Lead has long been used as a pigment in paints (e.g., lead oxide was used in ancient Egypt as a pottery glaze.) Lead chromate, $PbCrO_4$, is yellow and is still used as a pigment (under the name of chrome yellow) to paint school buses and road stripes. White indoor paint contained $Pb_3(CO_3)_2(OH)_2$ until the danger of children eating paint flakes became apparent 40–50 years ago (*13*). Important current uses of lead include solder, which is a tin-lead alloy containing 50–95% lead, and lead storage batteries—the largest single use of lead at about 10^9 kg per year. Lead has also been used industrially in leather tanning, in manufacture of the pesticide lead arsenate, $Pb_3(AsO_4)_2$, and in lead-containing dyes (*14*).

Lead-containing gasoline—now in use only in older vehicles, some older two-cycle engines, and racing cars—contains tetraethyl lead, $Pb(C_2H_5)_4$, or TEL, in order to increase the octane rating of the fuel. In its period of greatest use (the 1950s), between 0.75 mL and 3.0 mL of TEL was added per gallon of fuel (*15*), which converts to about 0.7–2.7 g of lead per gallon. Tetravalent lead compounds are generally covalent rather than ionic. When leaded gasoline is burned, it ultimately forms lead oxide.

Actions of Lead in the Body

Many of the compounds listed in this section contain the ionic form of lead, Pb^{2+}. This is the form that is a health hazard to humans. Lead can enter the body through the lungs, skin, or stomach. The bioavailability of lead (i.e., the fraction of ingested lead that is absorbed into the systemic circulation) can vary from 5 to 50% (*16*) and depends on many factors, including (i) the particles that lead is bound to when it enters the body (e.g., silicon, peat, or oxides of aluminum, iron, or manganese [*17*]); (ii) the surface area of the particles; and (iii) other variables such as the nutritional status of the individual. Different sources of lead will produce different levels in the blood. As might be expected, stomach acidity also plays a significant role: the dissolution of lead increases significantly at lower pH (*16*).

The Pb^{2+} ion, along with other "heavy metal" ions like Hg^{2+} and Ag^+, forms strong bonds with the carboxylate (–COOH) and sulfhydryl (–SH) groups of certain amino acids in proteins. The metal–amino acid bonds cause two things to happen: other bonds necessary for the proper functioning of proteins (polymers of amino acids) are broken, and the metal–protein complexes precipitate as insoluble metal–protein salts. This is why protein-containing substances such as milk and egg whites can be used as antidotes to heavy metal poisoning—they bind to the metal ions, preventing them from binding to the body's own proteins (*2*).

There appears to be no "safe" level of lead in the bloodstream, although its effects become more severe as the concentration increases. Health concerns in children include lowering of IQ, interference with the formation of red blood cells, and delayed physical development (*18*). At high blood levels (greater than about 100 parts per billion [ppb]; note: 1 μg/L or 1 ppb = 1 ounce in 7.8 million gallons), anemia, kidney damage, and mental retardation can result. Because of these effects and because lead accumulates in the body over time, the Environmental Protection Administration (EPA) considers lead a major public health threat. This concern is by no means restricted to the United States. High lead levels in the lungs of women and children living in Mexico City (*19*) and in the breast milk of nursing mothers in Mexico City (where leaded gasoline is still in use) (*20*)

and a recent report of lead poisoning in children in Trinidad who lived near a battery recycling facility (*21*) attest to the international scope of the problem.

Mitigation of Lead in Public Water Supplies

The EPA estimates that up to 20% of our exposure to lead comes from drinking water (*22*), the rest coming from dust, soil particles, and—in severe cases—latex paints. The reduction of human exposure to lead can be accomplished on two fronts: via social (legislative) action and by personal prevention. The maximum allowable lead content of household plumbing materials, including faucets and pipes, is 8.0%, although some commercially available solders do not contain lead (*23*). In 1991, the EPA set a Maximum Contaminant Level Goal (MCLG) of *zero* for lead in drinking water. Knowing that this is both practically unattainable and instrumentally unmeasurable, the EPA instituted a Primary Drinking Water Maximum Standard "Action Level" of 15 ppb. This means that action must be taken if 10% of tested homes have water exceeding this concentration. This standard affects nearly 80,000 public water suppliers in the United States. Some more personal methods of minimizing exposure to lead in drinking water are familiar: letting water run from the tap before drinking is one common and effective method. Several home-based lead-reduction technologies have also proven practical, including distillation, reverse osmosis, and using various filters (*18, 22*).

Determination of Lead

State-of-the-Art Methods

Lead is generally present at low concentrations in waterways. One recent analysis (*24*) found the lead concentration in three of the Great Lakes (Superior, Erie, and Ontario) to be between 3 and 11 ng L^{-1} (parts per trillion). Lead in soil samples can be much higher—up to 3000 mg/kg of soil at one particularly bad site north of Boston (*14*). Current analytic methods must therefore be selective for lead and have suitably low limits of detection.

Atomic absorption spectrophotometry (AAS) exploits the absorption of light of characteristic wavelengths by free atoms. Energy levels of electrons in atoms differ in energy by an amount that is often equal to the energy of photons in visible light. These differences are distinctive for each element and can be used to determine that element unequivocally. For example, copper's energy level difference corresponds to 324.7 nm and lead has energy differences corresponding to 405.8, 283, and 217 nm. If light of the correct wavelength shines on such atoms, a fraction of the light will be absorbed and this fraction is related to the number of atoms of the particular element in the sample.

Free atoms like lead must be separated from the matrix in order to do AAS. A conventional method for doing this, called flame atomic absorption spectrophotometry (FAAS), is to aspire a dilute aqueous solution of the element into a flame. The flame destroys the compound (e.g., lead(IV) chloride) and the constituent lead atoms are uncombined for a short period. The flame is lined up directly in the light path (see Fig. 2) so that the absorption of the light can be measured immediately. FAAS is often incapable of detecting the very low concentration of lead in environmental water samples. In these cases, a measured portion of the sample is placed in a graphite furnace having windows on each end. The sample is rapidly heated to a high temperature by electrical heating. The sudden high temperature volatilizes the sample and converts lead into free atoms in the light path. This method, called graphite furnace atomic absorption spectrophotometry (GFAAS), allows detection of much lower concentrations than FAAS.

Also used for the trace analysis of lead and other metals is voltammetry, the measurement of current when a voltage is applied to two electrodes without stirring. When a reducible ion strikes an electrode with too little voltage for it to be reduced, it merely diffuses away into the solution. However, when a sufficient voltage is applied to the electrode system, the ion will be reduced. The electron needed for the reduction comes from the externally applied voltage, so a current flows. The size of the current is a measure of the concentration of ions being reduced. Since there is no stirring, ions move to the electrode only through diffusion, hence the current is called the "diffusion current".

The most common and traditional form of voltammetry uses a voltage that changes from zero to increasingly negative values. This means that easily reduced ions will be reduced early in the experiment and ions more difficult to reduce are observed later, when the potential has become sufficiently negative. Concentrations as low as 10^{-4} M can be determined readily by this method. Use of more complicated voltage programs allows lower concentrations to be detected.

A specific voltammetric technique suitable for detecting much lower concentrations is anodic stripping voltammetry. In this form of voltammetry, the ions of interest are reduced over a considerable period of time, usually several minutes. Following this, these cations are then reoxidized in a few seconds. The same charge (in the opposite direction) in a shorter time gives rise to a considerably larger anodic current, which is therefore easier to measure. Anodic stripping voltammetry can readily detect 10^{-8} M lead ion.

Determination of Lead—
Methods Suitable for the High School Laboratory

Lead is a difficult substance to measure at low levels without an instrumental method of some type. This is not economically feasible for most high schools. The way around this is to emphasize a qualitative test for lead, which has the advantages that it does not necessarily require working at trace levels and would generally be much cheaper and easier. Additionally, it is possible to analyze lead in many common and interesting samples. A good method to determine lead in pottery glaze is to put white vinegar into the vessel, let it sit overnight, and then add sodium sulfide. A black precipitate of PbS indicates lead (*25*). Lead chromate was used for years as a yellow dye in paint. A drop of sodium dichromate or chromate in a solution containing lead (pH not too acidic) gives a beautiful yellow precipitate. A white precipitate of lead sulfate is also a confirmatory test for the presence of lead. The test solution should have a milligram or so of lead to get a good result from any of these three qualitative tests. Little waste is left over when a minimum amount of lead is used. The waste can be stored for many years.

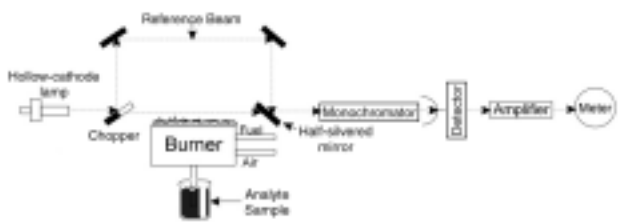

Figure 2. Essential components in a flame atomic absorption spectrometer (FAAS): hollow cathode lamp for the element of interest; burner, which atomizes the sample; and monochromator, which focuses a single wavelength on the detector. A graphite furnace atomic absorption spectrophotometer (GFAAS) uses the same components, except that a graphite furnace replaces the burner.

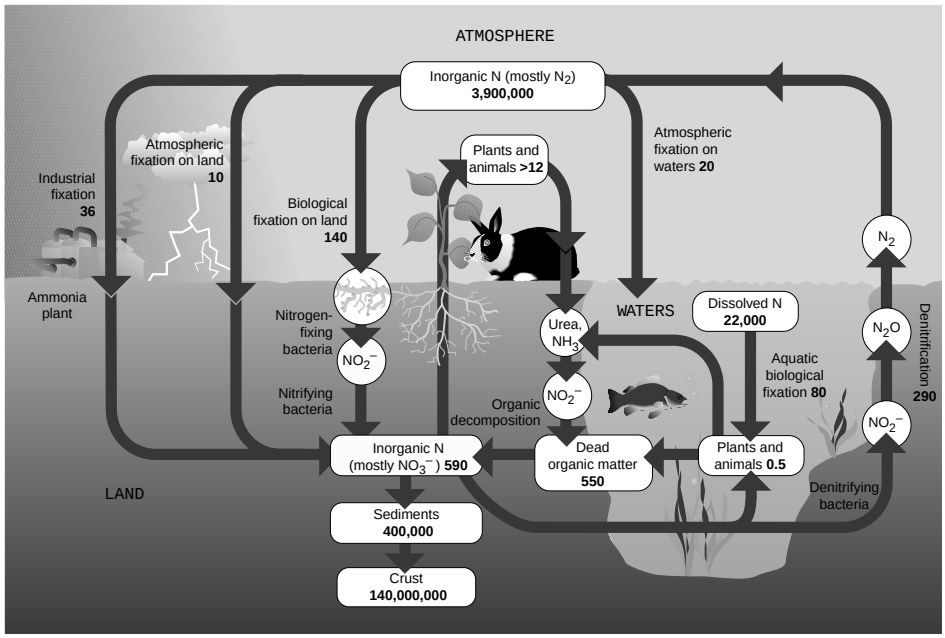

Figure 3. The nitrogen cycle. From Martin Silberberg, *Chemistry: The Molecular Nature of Matter and Change*; ©1996 by Mosby–Year Book, Inc., Boston; p 1021. Reprinted by permission of The McGraw-Hill Companies.

Nitrate in Water

Occurrence in Nature

Nitrate (NO_3^-) is a natural component of the earth's nitrogen cycle (Fig. 3). As part of this cycle, molecular nitrogen in the air is *fixed* (converted to nitrogen-containing compounds), thus providing important nutrients for plant growth. There are several ways to fix nitrogen. Biological fixation of nitrogen to ammonia is accomplished by several bacteria, including *Rhizobium* in the roots of legumes, *Azotobacter* and *Clostridium* in the soil, and *Nostoc* and *Calothrix* in soil and water. Subsequent oxidation of ammonia to nitrite (NO_2^-) and water (a process called *nitrification*) is accomplished by the bacterium *Nitrosomonas*; *Nitrobacter* then oxidizes the nitrite to nitrate (*26*). Along with these processes, about 10% of nitrogen fixation to nitrate occurs directly in the atmosphere, mediated by high-energy sources such as lightning. The nitrate combines with water and comes to Earth's surface as highly diluted nitric acid. Nitrogen enters the soil via application of ammonia or composite fertilizers prepared by addition of nitric acid and ammonia to phosphate rock and potash. Once in the soil, nitrate can leach into groundwater, eventually to be ingested by humans and other animals.

Health Concerns

Although nitrate itself is not toxic, its conversion to nitrite is of concern. In the relatively high-pH (5–7) environment in the stomach of a human infant 6 months old or less or in the rumen of a cow, bacteria flourish that can reduce nitrate to nitrite. In the body, nitrite can oxidize the iron(II) in hemoglobin to iron(III), forming *methemoglobin,* which binds oxygen less effectively than normal hemoglobin. The resulting decrease in oxygen levels in young children leads to shortness of breath, diarrhea, and vomiting, and in extreme cases, even death. An occasional blue color around the mouth has led to the name "blue baby syndrome" for this condition, formally known as *methemoglobinemia.* The latter term is also used with other substances that oxidize iron in hemoglobin. Cattle can be similarly affected by nitrite poisoning (*27*).

In combination, the availability of analytical methods for nitrate, diligent water supply monitoring, improved water purification, and education of the general public regarding risks associated with high nitrate levels in water have greatly reduced the incidence of nitrite poisoning in industrial nations. For example, there have been very few cases of blue baby syndrome in the United States during the past three decades and no known cases in Great Britain since 1972 (*13*). Nitrite poisoning is generally considered not to be a problem when the nitrate-nitrogen concentration in the water supply is below 10 mg L^{-1} (10 ppm). Fortunately, this criterion is easily met in public water supplies. Well water in farm communities is of greater concern, but even here nitrite poisoning can be avoided by having expectant mothers and infants use alternative sources of drinking water.

Determination of Nitrate

State-of-the-Art Methods

Over the years, the importance of monitoring nitrate in water has led to the development of numerous testing methods. The simplest involves acidifying the sample and measuring nitrate by taking advantage of its strong absorbance at 220 and 275 nm. This method is easy to perform and can detect as little as 1 mg L^{-1} of nitrate (*28*), but it may suffer from interferences from other absorbing species in the sample (nitrite, phosphate, etc.). It is the general procedure, described below, that we recommend for the high school laboratory. An alternative method involves reduction of nitrate in a cadmium–copper column followed by combination of the resulting nitrite with sulfanilamide and *N*-(1-napthyl)-ethylenediamine hydrochloride to form a highly colored product that can be measured at 540 nm (*29, 30*). This method also responds to nitrite (*6, 7*), again giving rise to possible interferences. Another popular technique for analyzing nitrate is ion chromatography (*31*). It uses a column contain-

ing an anion-exchange resin to separate nitrate from other ions in the sample, followed by detection of the eluting nitrate by means of an on-line conductivity or absorbance detector. This approach has fewer potential interferences than the colorimetric methods and can allow both nitrate and nitrite to be determined simultaneously (*8*).

Capillary electrophoresis (CE) is a relatively new method for monitoring nitrate and nitrite in water (*32, 33*). It makes use of the differential migration of charged solutes in a small-diameter capillary (typically 25–100 μm i.d.) in the presence of a strong electric field (e.g., an applied potential of 10–30 kV across a 50–100-cm capillary). Under these conditions, the sample ions (nitrate and nitrite) will travel through the capillary and be separated on the basis of their different charge-to-mass ratios. The migration of these ions and their corresponding peak sizes are monitored by an on-line absorbance or conductance detector to determine the amount of each species present in the original sample. The advantages of the method include its speed and specificity and the small amount of sample required (*32, 33*).

Method Suitable for the High School Laboratory

Spectrophotometry in the UV range is a simple and effective procedure that permits detection in the parts-per-million range. One excellent procedure includes preparation of potassium nitrate standards and takes advantage of the absorption of nitrate at 220 and 275 nm. Much dissolved organic matter also absorbs at 220 nm but not at 275 nm, so the effect of interferants is somewhat reduced by using two wavelengths. The general procedure (*28*) is:

1. Prepare a 50-ppm stock solution of KNO_3 (36.1 mg of KNO_3 per 100 mL of solution).
2. Prepare standards ranging from 0.5 to 10 ppm by adding 2 mL of 1 M HCl to appropriate aliquots of the stock standard and diluting to 100 mL.
3. Add 2 mL of 1 M HCl to a known volume of the sample and dilute to 100 mL; similarly, prepare a blank solution.
4. Measure absorbance at 220 and 275 nm for the standards, sample, and blank.
5. Determine the sample concentration using an experimentally determined absorbance vs. concentration plot made with data from the standards (using the difference between the 220- and 275-nm absorbances).

Numerous colorimetric methods for detecting nitrate exist. Most involve reducing nitrate to nitrite and allowing the nitrite to react with an organic compound to form a characteristically colored substance. One such kit, permitting multiple tests, is available for $12.50 (*34*).

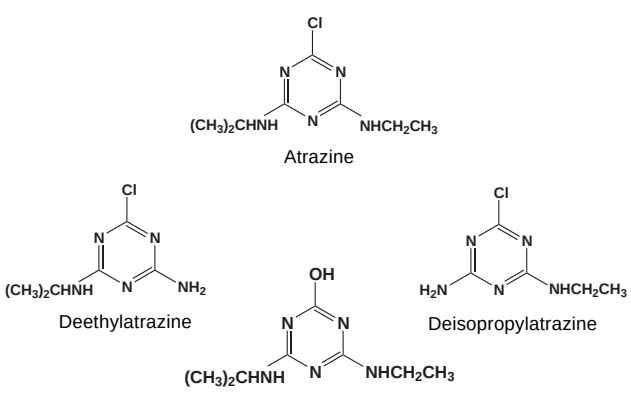

Figure 4. Atrazine and its major degradation products.

Atrazine

Occurrence and Uses

Atrazine (2-chloro-4-ethylamino-6-isopropylamino-*s*-triazine) and related triazine herbicides are used throughout the world to protect crops from broadleaf weeds and for such nonagricultural purposes as soil sterilization and road maintenance. Because of its widespread application, solubility in water, and persistence in the environment, atrazine has become a common pollutant in the United States (*35*) and Europe (*36*). Since atrazine carry-over is known to reduce yields when crop rotation is practiced, fields are often monitored for residual atrazine before vulnerable crops are planted (*37*). Although the threat to humans is less clear, the U.S. Safe Drinking Water Act (see Table 3) currently sets the maximum allowable level of atrazine in drinking water at 3 μg/L (3 ppb) and requires that public water supplies be monitored for its presence (*38*).

Figure 4 shows the structures of atrazine and its major degradation products in the environment. After application, atrazine that enters the soil, groundwater, and surface water is degraded by biological and nonbiological processes that lead to the formation of dealkylated triazines (e.g., deethylatrazine and deisopropylatrazine) and hydroxylated products (e.g., hydroxyatrazine) (*39*).

Herbicides such as atrazine are especially interesting for use as a cross-curricular exercise because their concentration in water can be seasonal, owing to their use at specific times of the planting and harvesting season. Figure 5 shows the results of atrazine analysis (by GC-MS) on the Platte River system of eastern Nebraska between 1989 and 1996. The annual patterns are similar, but the details depend on the rainfall conditions and time of the year. For example, the first rainfall after corn planting (usually late April to early May) brings atrazine out of agricultural fields and into the Platte River system. In 1989, a near-drought year, the maximum atrazine concentration wasn't found until early July when the dominant rainfall of the summer occurred, whereas another major rainstorm in early August had no effect. By that time all the atrazine had either percolated deep into the soil or undergone decomposition. A general conclusion is that the intensity, timing, and geographical area of rainstorms in the Platte River basin governs the amount of atrazine entering the river. Widespread, intense storms soon after the first of May lead to higher concentrations of atrazine in river. The great rains of 1993 that caused widespread flooding also resulted in lower maximum atrazine concentrations than in previous years. There was simply so much water that the atrazine was more dilute. These rains

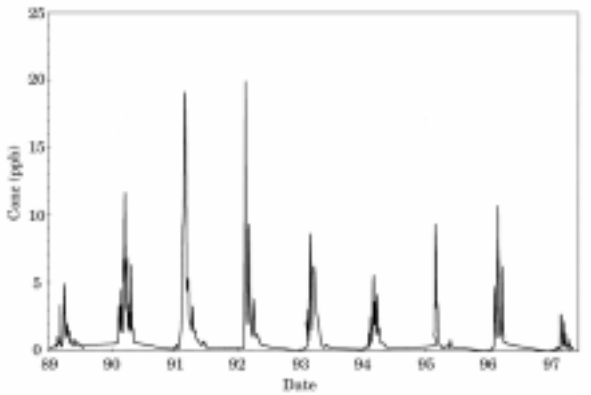

Figure 5. Atrazine in the Platte River, 1989–1997. Note the seasonal variations in atrazine concentration.

also delayed or prevented planting, so that less atrazine was applied to fields in that year. An especially useful application of such data in the high school classroom is the importance of sampling technique.

Determination of Atrazine

State-of-the-Art Methods

The task of examining the environmental fate of atrazine and related triazines is complex and expensive. It requires analysis of many samples from a variety of sources for a host of metabolites and degradation products that differ widely in chemical, physical, and biological properties. There are many well-established procedures for determination of these analytes based on HPLC or GC (*40*). They achieve low detection limits by taking a large volume of sample (100 mL to 1 L) through several extraction and sample cleanup steps. However, this type of sample pretreatment is time consuming and labor intensive; it is subject to emulsion formation and generates waste organic solvents that may be dangerous or have high disposal costs.

Some of these disadvantages can be reduced by using solid phase extraction (SPE). This can be performed either before GC analysis (*41, 42*) or on-line with HPLC by using automated column-switching methods (*43, 44*). However, this approach still has several problems. First, solid-phase extractions based on simple polar or nonpolar stationary phases are generally nonselective, and this leads to difficulties with coextracted interferants in both GC and LC techniques. The problem is magnified when the quest for low detection limits demands that large volumes of sample be preconcentrated. Moreover, the solid-phase extraction cartridges or disks themselves can contribute interferants, such as phthalate esters and various silicon compounds (*41, 45*), unless scrupulously cleaned before sample application.

Recent studies have examined the use of immunoassays for simple and inexpensive routine atrazine measurements. In these assays, a sample containing atrazine is combined with a limited amount of atrazine-binding antibody and a fixed amount of a labeled atrazine analog. The amount of atrazine in the sample is determined by measuring how much of the labeled analog binds to the antibodies (*46–49*). Immunoassay methods have good detection limits and correlate well with reference methods (*41*), but they also have a number of disadvantages. For example, the manual techniques on which they are based are not easily automated, nor do they provide sufficient precision and accuracy for quantitative work. More seriously, since the antibodies used cross-react with similar compounds such as atrazine metabolites and related triazine herbicides, most available immunoassays respond to an entire class of compounds and cannot differentiate the individual members of the class. These shortcomings limit the use of available atrazine immunoassays to the on-site screening of field samples before quantitation by GC-MS (*50*).

Method Suitable for the High School Laboratory

Semiquantitative atrazine analysis kits containing enough reagent for class use are available for $190. Each kit allows 20–25 tests, making it practical for demonstration purposes.[1] This kit is based on a typical immunoassay, which uses antibodies as reagents for selectively binding atrazine and related compounds. (Antibodies are proteins that make up part of the immune system. There are between a million and a billion different antibodies in the body, each type having the ability to bind to a particular foreign agent—bacterium, virus, unfamiliar protein, etc.) For the atrazine kit, an analog of atrazine was injected into a laboratory animal, which generated antibodies that bound specifically to atrazine-like compounds. These antibodies were collected and isolated for use in immunoassays.

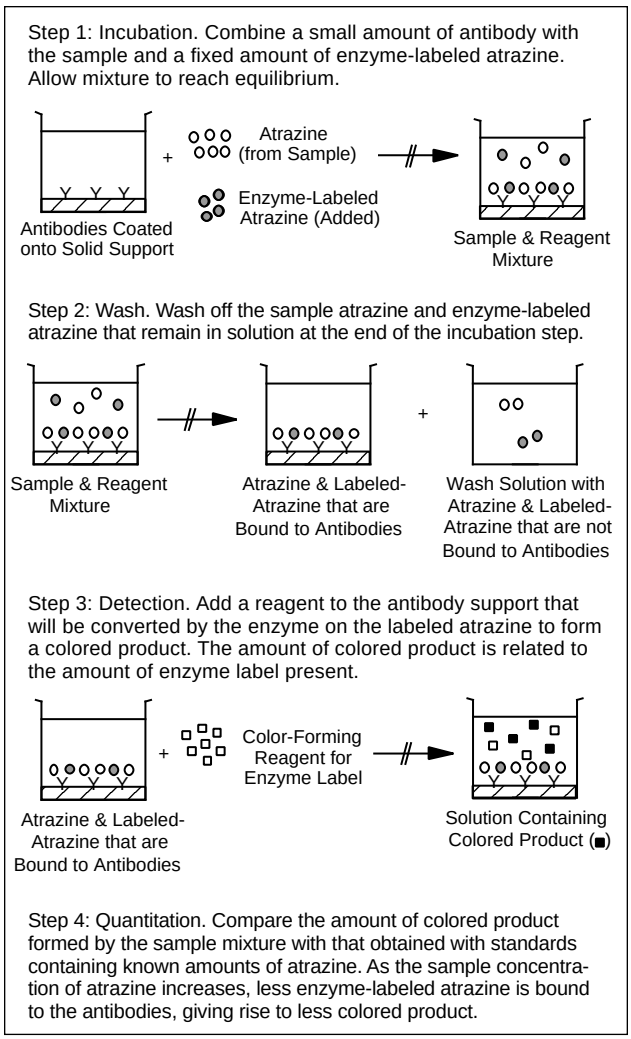

Figure 6. Typical competitive binding immunoassay for the analysis of atrazine, based on an enzyme label for detection.

One common immunoassay format for detecting atrazine is a competitive binding assay (Fig. 6). Here, a small amount of antibody is combined with the atrazine-containing sample and a fixed amount of atrazine analog bearing an easily detectable label, such as an enzyme. After incubation of this mixture, the atrazine and labeled analog that remain in solution are separated from the portion bound to the antibodies. The amount of the label in each fraction is determined and used as an indirect measure of the amount of atrazine in the original sample. If no atrazine was present in the sample, then the amount of labeled analog bound will be a maximum and the largest signal will be obtained for the bound fraction. If there was a large amount of atrazine in the sample, then very little of the labeled analog will be bound to the antibodies and a small signal will be measured for the bound fraction. Intermediate levels of atrazine give intermediate signals.

Conclusion

The nature, occurrence, and analysis of water pollutants is interesting chemically and relates well to the interdisciplinary nature of the modern chemistry curriculum. The issue is of worldwide significance, affecting both wealthy and poor nations. We have focused on three representative pollutants, but many other substances can be easily and safely analyzed in the high school laboratory.

Note

1. Atrazine kits can be obtained from Strategic Diagnostics Inc. (SDI), 128 Sandy Dr., Newark, DE 19713; phone: 302/456-6789; fax: 302/456-6770.

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