Environmental Biotechnology: An Overview

As industrial biotechnology continues to expand in many sectors around the world, it has the potential to be both disruptive and transformative, offering opportunities for industries to reap unprecedented benefits through pollution prevention.

Brent Erickson (2005) [1]

Two of the important topics at the threshold of the 21st century have been the environment and biotechnology. Erickson, representing BIO, the largest biotechnology organization, with more than 1200 members worldwide, succinctly yet optimistically characterized the marriage of environmental issues with the advances in biotechnology. Considered together, they present some of the greatest opportunities and challenges to the scientific community. Biotechnologies offer glimpses to solutions to some very difficult environmental problems, such as improved energy sources (e.g. literally “green” sources like genetically modified algae), elimination and treatment of toxic wastes (e.g. genetically modified bacteria to break down persistent organic compounds in sediments and oil spills), and better ways to detect pollution (e.g. transgenic fish used as indicators by changing different colors in the presence of specific pollutants in a drinking water plant).

Tethered to these arrays of opportunities are some still unresolved and perplexing environmental challenges. Many would say that advances in medical, industrial, agricultural, aquatic, and environmental biotechnologies have been worth the risks. Others may agree, only with the addition of the caveat, “so far.”

This text is not arguing whether biotechnologies are necessary. Indeed, humans have been manipulating genetic material for centuries. The main objective here is that thought be given to possible, often unexpected, environmental outcomes from well-meaning, important, and even necessary biotechnologies. Environmental biotechnology, then, is all about the balance between the applications that provide for a cleaner environment and the implications of manipulating genetic material.

In some ways, this is no different than any environmental assessment. An assessment is only as good as the assumptions and information from which it draws. Good science must underpin environmental decisions. The sciences are widely varied in environmental biotechnology, including most disciplines of physics, chemistry, and biology. Thus, to characterize the risks
and opportunities of environmental biotechnology, we must enlist the expertise of engineers, microbiologists, botanists, zoologists, geneticists, medical researchers, geologists, geographers, land use planners, hydrologists, meteorologists, computational experts, systems biologists, and ecologists.

**BIOCHEMODYNAMICS**

The only way to properly characterize biological systems is by simultaneously addressing chemical reactions, motion, and biological processes. Mass and energy exchanges are taking place constantly within and between cells, and at every scale of an ecosystem or a human population. Thus, biochemodynamics addresses energy and matter as they move (dynamics), change (chemical transformation), and cycle through organisms (biology). A single chemical or organism undergoes biochemodynamics, from its release to its environmental fate (see Figure 1.1).

Since biotechnologies apply the principles of science, the only way to assess them properly is by considering them biochemodynamically. Recently, the environmental community has become increasingly proficient in using biomonitoring to assess ecosystem condition or to determine pathways that have led to xenobiotic body burdens in humans. This has come to be known as exposure reconstruction. In other words, by analyzing concentrations of substances in tissue, the route that led to these concentrations can retrace the pathways, such as those in Figure 1.1.

Reconstruction of body burden in an organism that follows the release of a substance to the environment is an example of the biochemodynamic approach. To date, the use of biomonitoring data for environmental assessment has been limited to relatively straightforward...
exposure scenarios, such as those involving inert and persistent chemicals with relatively long biological half-lives and well-defined sources and pathways of exposure (e.g. the metal lead that is inhaled or ingested). More complex scenarios, including multiple chemical, multiple route of entry to the body and multiple pathway exposures, will need to complement biological information with large amounts of chemical and physical data (e.g. multimedia dynamics of the chemical). Table 1.1 provides examples of available population biomarker databases that can complement biomonitoring data.

Assessing biological doses and their effects using exposure measurements constitutes a “forward” analytical approach, whereas estimating or reconstructing exposures from biomarkers invokes an “inverse” methodology. The forward analysis can be accomplished through the direct application of exposure, toxicokinetic, and toxicodynamic models (discussed in Chapter 2), which can be either empirical or mechanistic (i.e. biologically based). Reconstruction requires application of both numerical model inversion techniques and toxicokinetic and/or toxicodynamic models. Physical, chemical, and biological information must be merged into biochemodynamic information to underpin a systematic, environmental assessment.

Physiologically based toxicokinetic (PBTK) and biologically based dose-response (BBDR) models combined with numerical inversion techniques and optimization methods form a biochemodynamic framework to support environmental risk assessment (see Figure 1.2). The inversion approach contrasts with so-called “brute-force sampling,” wherein possible factors as evaluated one-by-one. The biochemodynamic approach calls for a systematic evaluation of available methods and computational tools that can be used to “merge” existing forward models and biomarker data [2].

**ASSESSING BIOTECHNOLOGICAL IMPACTS**

Any consideration of present or future environmental problems requires a systematic perspective. Everything in the environment is interconnected. If we do not ask questions about the possible environmental impacts of biotechnologies and we have no data from which to answer these questions, we may be unpleasantly surprised in time when ecological and human health problems occur. This is doubly bad if such problems could have been prevented with a modicum of foresight. Since this is actually the rationale for environmental impact statements (EISs), they provide a worthwhile framework for the application of biochemodynamics in environmental assessments.

The National Environmental Policy Act (NEPA) was the first of the major pieces of legislation in the United States to ask that the environment be viewed systematically. It was signed into law in 1970 after contentious hearings in the US Congress. NEPA is not really a technical law, but created the environmental impact statement (EIS) and established the Council on Environmental Quality (CEQ) in the Office of the President. Of the two, the EIS represented a sea change in how the federal government was to conduct business. Agencies were required to prepare EISs on any major action that they were considering that could “significantly” affect the quality of the environment. From the outset, the agencies had to reconcile often-competing values, i.e. their mission and the protection of the environment. This ushered in a new environmental ethos that continues today.

Biotechnologies are tailor-made for the assessment process, owing to their complexities and the difficulty of predicting side effects and unexpected outcomes. For example, the US Department of Agriculture’s Biotechnology Regulatory Services program and Animal and Plant Health Inspection Service regulate the importation, movement, and potential releases of genetically engineered (GE) organisms, especially plants, insects, and microorganisms that may pose a plant pest risk [3]. The USDA works with the US Environmental Protection Agency (EPA) and the Food and Drug Administration (FDA), since GE organisms are also used for environmental, medical, and industrial applications. Thus, in the United States, the federal
### Table 1.1 Examples of biomarker databases available to conduct exposure reconstructions

<table>
<thead>
<tr>
<th>Program/Study</th>
<th>OP</th>
<th>PYR</th>
<th>Metals</th>
<th>Location: Number of subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHAMACOS (1999–2000)</td>
<td>bd</td>
<td>bd</td>
<td>bd</td>
<td>CA: 600 pregnant women</td>
</tr>
<tr>
<td>Castornia et al., 2003</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CTEPP (2000–01)</td>
<td>ac</td>
<td>ac</td>
<td>ad</td>
<td>NC: OH: 257 children (1.5-5 yr)</td>
</tr>
<tr>
<td>Wilson et al., 2004 (*)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MNCPES (1997)</td>
<td>ac</td>
<td>ac</td>
<td>ac</td>
<td>MN: 102 children (3-12 yr)</td>
</tr>
<tr>
<td>Quackenboss et al., 2000 (*)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NHANES-III (1988–94)</td>
<td>c</td>
<td>c</td>
<td>bc</td>
<td>US: 1000 adults (20-59 yr)</td>
</tr>
<tr>
<td>Hill et al., 1995</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NHANES (1999–2000)</td>
<td>cd</td>
<td>cd</td>
<td>cd</td>
<td>bc US: 9,282 subjects (all ages)</td>
</tr>
<tr>
<td>CDC, 2005 (*)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NHANES 2001–02 CDC, 2005 (*)</td>
<td>cd</td>
<td>cd</td>
<td>cd</td>
<td>bc US: 10,477 subjects (all ages)</td>
</tr>
<tr>
<td>NHANES 2003–04 (*)</td>
<td>cd</td>
<td>cd</td>
<td>cd</td>
<td>bc US: 9,643 subjects (all ages)</td>
</tr>
<tr>
<td>NHEXAS-AZ (1995–97)</td>
<td>ac</td>
<td>ac</td>
<td>ac</td>
<td>AZ: 179 subjects (all ages)</td>
</tr>
<tr>
<td>Robertson et al., 1999</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NHEXAS-MD (1995–96)</td>
<td>ac</td>
<td>ac</td>
<td>ac</td>
<td>MD: 80 subjects (above 10 yr)</td>
</tr>
<tr>
<td>----------------------</td>
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<td>-----------------------------</td>
</tr>
<tr>
<td>NHEXAS-V (1995–97)</td>
<td>ac</td>
<td>ac</td>
<td>ac</td>
<td>c</td>
</tr>
</tbody>
</table>

Notes:

- Measurements of multimedia concentrations (indoor, outdoor, and personal air; drinking water; duplicate diet; dust; and soil).
- Partial measurements of environmental concentrations (e.g., outdoor air concentrations; pesticide use; etc.).
- Specific metabolites.
- Non-specific metabolites.

Abbreviations: OP: organophosphates; PYR: pyrethroids. CHAMACOS = Center for the Health Assessment of Mothers and Children of Salinas; CTEPP = Children’s Total Exposures to Persistent Pesticides and Other Persistent Organic Pollutants; MNCPES = Minnesota Children’s Pesticide Exposure Study; NHANES = National Health and Nutrition Examination Survey; and NHEXAS = National Human Exposure Assessment Survey.

Referenced studies:


government seems to be aware of the need to look at possible implications in a systematic way. Under the biotechnology regulations, transgenic plants, insects, mollusks, and microbes are subject to regulation if they potentially pose a plant pest risk. A large number of organisms are included.

Any major action by a federal agency that may significantly affect the human environment falls under NEPA, which means that environmental impacts must be considered prior to undertaking the action. Unless an agency action is categorically excluded from a NEPA-mandated environmental analysis, the agency must analyze the action through the preparation of an environmental assessment (EA), and if needed, an EIS. An action that would result in "less-than-significant" or no environmental impacts can be categorically excluded. For example, a categorical exclusion would apply to the permitting of the "confined release of a GE organism involving a well-known species that does not raise any new issues." However, a categorical exclusion is not an "exemption" from NEPA, merely a determination that an EA or EIS is not necessary.

Many ecologists would argue that any action will impact ecosystems, since they are entirely interconnected to other systems and the systems within the ecosystem are influenced by changes among these systems. However, the NEPA catchword is "significant." Scientists, especially statisticians, have difficulty with the use of this term outside of prescribed boundaries, e.g. significant at the 0.05 level (5% likelihood that the outcome occurred due to chance). However, in general use, the term seems to indicate importance or that the action’s impacts are substantial.

Environmental assessments consider the need for the proposed action, especially highlighting and evaluating possible alternatives, including a "no-action" alternative. In other words, would the environment be better off if the action is not taken compared to all of the other alternative actions? All of these options are viewed in terms of potential impacts, with a ranking or comparison of the alternatives, and a recommendation to decision makers on how best to implement the proposed program with the least environmental implications. The EA is mainly a step to determine, in a publicly available document, whether to prepare an EIS. If the proposed action lacks a significant impact on the environment, the government agency will

FIGURE 1.2
Hypothetical event tree of possible outcomes from the initial action (e.g. using genetically modified microbes to breakdown a chemical waste in an aquifer).
issue a Finding of No Significant Impact (FONSI) [4]. If it determines that an aspect of the
quality of the human environment may be “significantly affected” by the proposed action,
then agency is required to prepare an EIS, which involves a more in-depth inquiry into the
proposal and any “reasonable” alternatives to it. For example, the USDA writes an EA before
granting permits for introductions of GE organisms that are considered new or novel (the crop
species, the trait, or both), with an opportunity for public comment before a permit is granted.
The agency also prepares an EA when it decides that a GE plant or microorganism will no
longer be regulated. The steps in the EA process are:

- Consultation and coordination with other federal, tribal, state, or local agencies;
- Public scoping;
- Federal Register notices;
- Public comments on a draft EA;
- Public meetings on a draft EA;
- Publication of final EA and FONSI; and
- Supplements to a previous EA.

An EIS is more detailed and comprehensive than the EA. Agencies often strive to receive
a so-called FONSI, so that they may proceed unencumbered on a mission-oriented project [5].
The process assists in deciding whether to use the NEPA process to improve decision making
behind projects of a more narrow scope, such as the deregulation of a specific GE crop. The
evaluation includes a discussion of direct, indirect, and cumulative impacts resulting from the
adoption of one of several reasonable alternatives, including the no-action alternative. Again,
this is evidence of the need for a systematic view of biotechnological environmental impacts.

The EIS may also specify actions that would mitigate any impact of the biotechnology product,
that is, possible measures that could reduce any potential impact would be put into place prior
to project implementation. Thus, an EIS can only be written by a multidisciplinary team of
experts.

The EIS process includes:

- Consultation and coordination with other federal, tribal, state, or local agencies, when
  appropriate;
- Scoping;
- Federal Register notices;
- Public comment on draft EIS;
- Public meetings on draft EIS when appropriate;
- Publication of a final EIS; and
- Supplements to an inadequate EIS, when necessary.

States also have their own environmental assessment processes (see Table 1.2). Like the federal
EIS process, the states have their own emphases and concerns about environmental impacts.

The EIS process, when followed properly, is an example of underpinning environmental
decisions with reliable biochemodynamic information. For example, in the USDA process,
decisions about field-testing of GE crops must ensure that these tests neither pose plant pest
risks nor pose significant impacts to the human environment [6]. An incomplete or inadequate
assessment will lead to delays and increase the chance of an unsuccessful project, so sound
science is needed from the outset of the project design. Even worse, a substandard assessment
may allow for hazards and risks down the road.

The final EIS step is the Record of Decision (ROD). This means that someone in the agency will
be held accountable for the decisions made and the actions taken. The ROD describes the
alternatives and the rationale for final selection of the best alternative. It also summarizes the
comments received during the public reviews and how the comments were addressed. Many
states have adopted similar requirements for RODs.
The EIS documents were supposed to be a type of “full disclosure” of actual or possible problems if a federal project is carried out. Fully disclosing possible impacts can be likened to Lorenz’s view of chaos (see Discussion Box: Little Things Matter in a Chaotic World). For example, even a low probability outcome must be considered. In fact, most environmental risk calculations deal with low probabilities (e.g. a one-in-a-million risk of cancer following a lifetime exposure to a certain carcinogen). As shown in Figure 1.2, the fact that the vast majority of outcomes will be the desired effect does not obviate the need to consider all potential outcomes. This hypothetical example has four possible outcomes (in most situations there are myriad outcomes) from one initial event (e.g. the use of a genetically modified microbe to break down a persistent chemical). The good news is that 97.5% of the time, the beneficial outcome is achieved. And, sometimes an unplanned benefit is realized (0.2%). Most of the rest of the outcomes are neither good nor bad (2%). However, on rare occasions, given the complexities and variable environmental conditions, the beneficial outcomes do not occur and negative impacts ensue (0.3%). NEPA and other systematic decision support

<table>
<thead>
<tr>
<th>Table 1.2 North Carolina’s State Environmental Policy Act (SEPA) review process</th>
</tr>
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<tbody>
<tr>
<td><strong>Step I:</strong> Applicant consults/meets with Department of Environment and Natural Resources (DENR) about potential need for SEPA document and to identify/scope issues of concern.</td>
</tr>
<tr>
<td><strong>Step II:</strong> Applicant submits draft environmental document to DENR.</td>
</tr>
<tr>
<td>Environmental document is either an environmental assessment (EA) or an environmental impact statement (EIS).</td>
</tr>
<tr>
<td><strong>Step III:</strong> DENR-Lead Division reviews environmental document.</td>
</tr>
<tr>
<td><strong>Step IV:</strong> DENR-Other Divisions review environmental document.</td>
</tr>
<tr>
<td>15–25 calendar days. DENR issues must be resolved prior to sending to the Department of Administration – State Clearinghouse (SCH) review.</td>
</tr>
<tr>
<td><strong>Step V:</strong> DENR-Lead Division sends environmental document and FONSI (a) to SCH.</td>
</tr>
<tr>
<td><strong>Step VI:</strong> SCH publishes Notice of Availability for environmental document in NC Environmental Bulletin. Copies of environmental document and FONSI are sent to appropriate state agencies and regional clearinghouses for comments.</td>
</tr>
<tr>
<td>Interested parties have either 30 (EA) or 45 (EIS) calendar days from the Bulletin publication date to provide comments.</td>
</tr>
<tr>
<td><strong>Step VII:</strong> SCH forwards copies of environmental document comments to DENR-Lead Division who ensures that applicant addresses comments.</td>
</tr>
<tr>
<td>SCH reviews applicant’s responses to comments and recommends whether environmental document is adequate to meet SEPA.</td>
</tr>
<tr>
<td>Substantial comments may cause applicant to submit revised environmental document to DENR-Lead Division. This will result in repeating of Steps III–VI.</td>
</tr>
<tr>
<td><strong>Step VIII:</strong> Applicant submits final environmental document to DENR-Lead Division.</td>
</tr>
<tr>
<td><strong>Step IX:</strong> DENR-Lead Division sends final environmental document and FONSI (in case of EA and if not previously prepared) to SCH.</td>
</tr>
<tr>
<td>Environmental Assessment (EA)</td>
</tr>
<tr>
<td><strong>Step X:</strong> SCH provides letter stating one of the following:</td>
</tr>
<tr>
<td>■ Document needs supplemental information, or</td>
</tr>
<tr>
<td>■ Document does not satisfy a FONSI, and an EIS should be prepared, or</td>
</tr>
<tr>
<td>■ Document is adequate; SEPA is complete.</td>
</tr>
<tr>
<td>Environmental Impact Statement (EIS)</td>
</tr>
<tr>
<td><strong>Step XI:</strong> After lead agency determines the FEIS is adequate, SCH publishes a Record of Decision (ROD) in the NC Environmental Bulletin.</td>
</tr>
</tbody>
</table>

Notes:  
PUBLIC HEARING(S) ARE RECOMMENDED (BUT NOT REQUIRED) DURING THE DRAFT STAGE OF DOCUMENT PREPARATION FOR BOTH EA AND EIS.  
For an EA, if no significant environmental impacts are predicted, the lead agency (or sometimes the applicant) will submit both the EA and the Finding of No Significant Impact (FONSI) to SCH for review (either early or later in the process).  
Finding of No Significant Impact (FONSI): Statement prepared by Lead Division that states proposed project will have only minimal impact on the environment.
systems must help to determine whether 0.3% risk of a negative outcome is acceptable. This depends on the severity, persistence, and extent of the negative impact. For example, if the microbial population of an ecosystem is not in danger of irreversible or long-term damage and the scope of the problem is contained, then this probability of harm may be acceptable. However, if the microbial population changes and there is long-term loss of biodiversity, the risk may not be worth it. For these scenarios, other alternatives must be sought.

Figure 1.3 shows the same hypothetical scenario as that in Figure 1.2, but in this case actions are taken to prevent some of the adverse outcomes. Such mitigating measures [7] can include better matches of microbes to the specific environmental conditions, more frequent and reliable monitoring of the project, and using safer (e.g. non-genetically modified microbes) methods. The likelihood of the adverse outcome has fallen to 0.1%, but the likelihood of the desired outcome has decreased to 97.3% (including the fortuitous benefits).

These may seem like small differences, but environmental decisions often hinge on a few parts per billion or a risk difference of 0.00001 on whether a project is acceptable. Thus, in this hypothetical case, the measure of success has decreased from 97.5% to 97.3%, or a success rate decrease of 0.2%. Sometimes, such a drop affects cleanup rates (e.g. the 0.2% rate translates into another three months before a target cleanup level is achieved). It may also translate into the inability for some microbes to break down certain recalcitrant pollutants. Conversely, the better adverse outcomes may well be worth it, if the 0.1% improvement means less ecosystem effects and fewer releases and exposures to toxic substances.

The reasons given for not taking mitigating measures often have to do with costs and efficiencies. For example, in the scenario described in Figure 1.3, the naturally available microbes may be slower to degrade the compound, so at the same point in time in the future, less of the waste has been detoxified. Even though the possibility of negative outcomes has been cut, so has the removal of the toxic waste. This is an example of a contravening risk and risk tradeoff; that is, we must decide whether a less efficient contaminant cleanup (human health risk) is more important than ecosystem integrity (ecological risk).

In a complete event tree, all of the events following the initial event would need to be considered. This is the only way that the probability of the final outcome (positive, neutral or
negative) can be calculated as the result of contingent probabilities down the line. In fact, each of the mitigating measures shown in Figure 1.3 has a specific effect on the ultimate probability of the outcome. Thus, mitigating measures can be seen as interim events with their own contingent probability (e.g., choosing natural attenuation versus enhanced biodegradation lowers the probability of the desired rate of biodegradation, but also lowers the probability of adverse genetic effects on the ecosystem).

The systematic approach considers all of the potential impacts to the environment from any of the proposed alternatives, and compares those outcomes to a “no action” alternative. In the first years following the passage of NEPA many agencies tried to demonstrate that their “business as usual” was in fact very environmentally sound. In other words, the environment would be better off with the project than without it (action is better than no action). Too often, however, an EIS was written to justify the agency’s mission-oriented project. One of the key advocates for the need for a national environmental policy, Lynton Caldwell, is said to have referred to this as the federal agencies using an EIS to “make an environmental silk purse from a mission-oriented sow’s ear!” [8].

The courts adjudicated some very important laws along the way, requiring federal agencies to take NEPA seriously. Some of the aspects of the “give and take” and evolution of federal agencies’ growing commitment to environmental protection was the acceptance of the need for sound science in assessing environmental conditions and possible impacts, and the very large role of the public in deciding on the environmental worth of a highway, airport, dam, waterworks, treatment plant, or any other major project sponsored by or regulated by the federal government. This was a major impetus in the growth of the environmental disciplines since the 1970s. Experts were needed who could not only conduct sound science but who could communicate what their science means to the public.

All federal agencies must adhere to a common set of regulations [9] to “adopt procedures to ensure that decisions are made in accordance with the policies and purposes of the Act.” Agencies are required to identify the major decisions called for by their principal programs and make certain that the NEPA process addresses them. This process must be set up in advance, early in the agency’s planning stages. For example, if waste remediation or reclamation is a possible action, the NEPA process must be woven into the remedial action planning processes from the beginning with the identification of the need for and possible kinds of actions being considered.

Noncompliance or inadequate compliance with NEPA rules and regulations can lead to severe consequences, including lawsuits, increased project costs, delays, and the loss of the public’s trust and confidence, even if the project is designed to improve the environment, and even if the compliance problems seem to be only “procedural.” The US EPA is responsible for reviewing the environmental effects of all federal agencies’ actions. This authority was written as Section 309 of the Clean Air Act (CAA). The review must be followed with the EPA’s public comments on the environmental impacts of any matter related to the duties, responsibilities, and authorities of EPA’s administrator, including EISs. The EPA’s rating system (see Appendix 1) is designed to determine whether a proposed action by a federal agency is unsatisfactory from the standpoint of public health, environmental quality, or public welfare. This determination is published in the Federal Register and referred to the CEQ.

**BIOTECHNOLOGY AND BIOENGINEERING**

Biotechnology as an endeavor is not new. Even the term itself is almost a century old. Karl Ereky, a Hungarian engineer, is credited with coining the word “biotechnology” in 1919 when he referred to approaches that recruited the help of living organisms to produce materials. More recently, 1992, the Convention on Biological Diversity settled on defining biotechnology as “any technological application that uses biological systems, living organisms or derivatives thereof, to make or modify products and processes for specific use” [10]. This goes well beyond
microbiology, thus, an understanding of biotechnology entails a need to consider the chemical processes at work in living systems.

One of the challenges of this book is to get a sense of the extent to which existing engineering analytical tools can support biotechnological decision making, especially as this applies to potential environmental impacts. One indispensable tool used to assess the complete environmental footprint of a process is the life cycle analysis (LCA). We will address this in much greater detail in subsequent chapters, but it is worth noting now that LCA is more than a particular software package or set of engineering diagrams and charts. It is a way of considering the history and future of a biotechnological enterprise as a complete system with respect to inputs and outputs. As such, it provides a means of demonstrating and evaluating the physical, chemical, and biological systems within a system. That is, the LCA considers the environmental worthiness of any endeavor, including biotechnologies, within the context of first principles of thermodynamics, motion and the other laws and theories that underpin the system. All of the energy and matter inputs must balance with outputs. As such, the outcomes can be studied rationally and objectively.

Numerous cases demonstrate the lack of a life cycle perspective (see Discussion Box: Little Things Matter in a Chaotic World). The systematic nature of LCA extends from these first physical principles to biological principles. Arguably, the two bioengineering disciplines are biomedical and environmental engineering. Both deal directly and indirectly with living things and, as such, with biotechnologies. Both approach biology as a means of understanding and managing risks to living organisms, especially humans. Often, however, the information that goes into LCAs is qualitative, such as the hazards posed directly or indirectly by organisms, especially genetically modified microorganisms (see Tables 1.3 and 1.4). Note that such hazards extend to both human populations and ecosystems [11].

<table>
<thead>
<tr>
<th>Hazard level</th>
<th>Description of microbial hazard</th>
</tr>
</thead>
<tbody>
<tr>
<td>Least</td>
<td>Never identified as causative agents of disease in humans nor offer any threat to the environment</td>
</tr>
<tr>
<td>Hazardous when contained, low human population risk</td>
<td>May cause disease in human and might, therefore, offer a hazard to laboratory workers. They are unlikely to spread in the environment. Prophylactics are available and treatment is effective</td>
</tr>
<tr>
<td>Severe when contained, moderate human population risk</td>
<td>Severe threat to the health of laboratory workers, but a comparatively small risk to the population at large. Prophylactics are available and treatment is effective</td>
</tr>
<tr>
<td>High human population risk</td>
<td>Severe illness in humans and serious hazard to laboratory workers and to people at large. In general, effective prophylactics are not available and no effective treatment is known</td>
</tr>
<tr>
<td>Greatest ecological and human population risk</td>
<td>Most severe threat to the environment, beyond humans. May be responsible for heavy economic losses. Includes several classes, Ep1, Ep2, Ep3 (see Table 1.4 for description) to accommodate plant pathogens</td>
</tr>
</tbody>
</table>


*a LCA can also be shorthand for life cycle assessment, which for the sake of this discussion, is synonymous with life cycle analysis.
DISCUSSION BOX

Little Things Matter in a Chaotic World

Most would agree that the Monarch butterfly is a beautiful creature. What if we lost it because of biotechnology, as suggested by a recent report? The report stated that pollen from corn that had been genetically engineered with genetic material from soil bacterium *Bacillus thuringiensis* (Bt) posed a threat of killing Monarch butterfly larvae [12].

The Bt produces a protein that targets insect pests. Scientists “borrow” the genetic material that expresses this protein and insert it into plant species, including corn.

The original report showed only that Bt-containing pollen fed directly to Monarch larvae is toxic but did not include realistic field exposures. Since then, more intensive studies suggest the risk is low enough to be acceptable, given the benefits of insect resistance.

Some studies indicated that corn pollen normally travels in limited distances and that the pollen has a tendency not to accumulate on the favored Monarch food, i.e. milkweed leaves. Also, pollen production usually does not occur at the same time as the active feeding by Monarch larvae. These factors supported the US EPA decision to continue to approve the planting of Bt corn. The question in such decisions is whether the decision was based on sufficient field studies and the possibility of the combination of rare events.

Biotechnology puts living things to work for certain purposes. An excellent example is *phytoremediation*, which utilizes biochemodynamic processes to remove, degrade, transform, or stabilize contaminants that reside in soil and groundwater (see Figure 1.4). Subtle changes in any of these processes can make the difference between a successful remediation effort and a failure. Phytoremediation uses plants to capture the water from plumes of contaminated aquifers. The plants take up the water by the capillary action of their roots, transport it upward through the plant until the water is transpired to the atmosphere. The good news is that many of the contaminants have been biochemically transformed or at least sequestered in the plant tissue.

<table>
<thead>
<tr>
<th>European Federation of Biotechnology Class</th>
<th>Description of Microbes in Class</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ep 1</td>
<td>May cause diseases in plants but have only local significance. They may be mentioned in a list of pathogens for the individual countries concerned. Very often they are endemic plant pathogens and do not require any special physical containment. However, it may be advisable to employ good microbiological techniques</td>
</tr>
<tr>
<td>Ep 2</td>
<td>Known to cause outbreaks of disease in crops as well as in ornamental plants. These pathogens are subject to regulations for species listed by authorities in the country concerned</td>
</tr>
<tr>
<td>Ep 3</td>
<td>Mentioned in quarantine lists. Importation and handling are generally forbidden. The regulatory authorities must be consulted by prospective users</td>
</tr>
</tbody>
</table>

Plants do not metabolize organic contaminants to carbon dioxide and water as microbes do. Rather they transform parent compounds into non-phytotoxic metabolites. After uptake by the plant, the contaminant undergoes a series of reactions to convert, conjugate, and compartmentalize the metabolites. Conversion includes oxidation, reduction, and hydrolysis. Conjugation reactions chemically link these converted products (i.e. phase 1 metabolites) to glutathione, sugars, or amino acids, so that the metabolites (i.e. phase 2 metabolites) have increased aqueous solubility and, hopefully, less toxicity than the parent compound. After this conjugation, the compounds are easier for the plant to eliminate or compartmentalize to other tissues. Compartmentalization (phase 3) causes the chemicals to be segregated into vacuoles or bound to the cell wall material, such as the polymers lignin and hemicellulose. Phase 3 conjugates are considered to be bound residues in that laboratory extraction methods have difficulty finding the original parent compounds [13].

Enter the butterfly. It turns out that some of the phytoremediation products of conversion reactions can become more toxic than the parent contaminants when consumed by animals or potentially leached to the environment from fallen leaves. For example, the release of contaminants from conjugated complexes or compartmentalization could occur in the gut of a worm, a snail, or a butterfly [14]. This means that there is a distinct possibility of reintroducing the pollutant, by means of the butterfly, into the food chain.

Ironically, the butterfly is also the metaphor for chaos. Edward Lorenz’s Butterfly Effect postulates “sensitive dependence upon initial conditions” [15] as a postulate of chaos theory: a small change for good or bad can reap exponential rewards and costs. Lorenz, at a 1963 New York Academy of Sciences meeting, related the comments of a “meteorologist who had remarked that if the theory were correct, one flap of a seagull’s wings would be enough to alter the course of the weather forever.” Lorenz later revised the seagull example to be that of a butterfly in his 1972 paper “Predictability: Does the Flap of a Butterfly’s Wings in Brazil Set Off a Tornado in Texas?” at a meeting of the American Association for the Advancement (Continued)
of Science in Washington, DC. In both instances Lorenz argued that future outcomes are determined by seemingly small events cascading through time. Engineers and mathematicians struggle with means to explain, let alone predict, such outcomes of so-called “ill-posed” problems. Engineers generally prefer orderly systems and a well-posed problem, that is, one that is uniquely solvable (i.e. a unique solution exists) and one that is dependent upon a continuous application of data. By contrast, an ill-posed problem does not have a unique solution and can only be solved by discontinuous applications of data, meaning that even very small errors or perturbations can lead to large deviations in possible solutions [16].

The importance of seemingly small things within a systematic approach is demonstrated by what happened near the Iron Gate Dam in Europe. This case demonstrates the enormous ecological price that must be paid when biodiversity is destroyed. The case is very interesting in that something we do not ordinarily consider to be a pollutant or even a limiting ecological factor, silicates, led to major problems.

The Black Sea is the largest enclosed catchment basin, receiving freshwater and sediment inputs from rivers draining half of Europe and parts of Asia. The Danube River, which flows into the Black Sea, receives effluents from eight European countries, and is the largest source of stream-borne nutrients. The sea is highly sensitive to eutrophication, i.e. nutrient enrichment leading to adverse trophic changes, and has experienced change numerous times in recent decades. In less than a decade, the system changed from an extremely biologically diverse one to a system dominated by jellyfish (*Aurelia* and the comb jelly *Mnemiopsis*) [17].

These invaders were unintentionally introduced in the mid-1980s, culminating in the fisheries almost completely vanishing by the early 1990s. This collapse was first attributed to unpalatable carnivores that fed on plankton, roe, and larvae. Subsequently, however, the jellyfish takeover was found to result from human perturbations in the coastal ecosystems and in the drainage basins of the rivers, including changing the hydrologic character of out-flowing rivers. The biggest of these was the damming of the Danube in 1972 by the Iron Gates, approximately 1000 km upstream from the Black Sea. In addition, urban and industrial development, heavy use of commercial fertilizers, over-fishing, and the introduction of exotic, invasive organisms (e.g., *Mnemiopsis*) contributed to the problem.

After 1970 this change in nutrient concentrations induced phytoplankton blooms during the warm months and changed the dominance to nonsiliceous species that were not a first choice as food for meso-zooplankton. The decreased fish stocks further increased the dominance of the jellyfish, since they competed better than the game fish for the same food. Ironically, since the mid-1990s, the ecosystems have begun to improve, mainly due to increased nutrient (phosphorus and nitrogen) loading. In most situations, we are looking to decrease this loading, to prevent eutrophication. But in this system, the added nutrients have allowed certain plankton and benthic (bottom dwelling) organisms to re-colonize. The abundance of jellyfish has also stabilized, with a concomitant increase in anchovy eggs and larvae.

Nutrient limitation occurs when the presence of a chemical, such as phosphorus or nitrogen, is insufficient to sustain the growth of community or species. Usually, marine systems are nitrogen-limited whereas freshwater plankton systems are phosphorus-limited. Numerous freshwater organisms can fix atmospheric nitrogen but, with minor exceptions, the nitrogen is impeded in marine water. The nutrient requirements differ by species. A disturbance in the ratio of nitrogen, phosphorus, silica, and even iron changes the biotic composition of a particular plankton community.

Often, all four nutrients can be considered as limiting. For instance, the lack of silica limits diatoms. This was observed first in natural blooms off Cape Mendocino in the United States and subsequently observed in the northwest part of the Black Sea, after closing the Iron Gates dam. The case also demonstrates that economics is crucial, since the marine ecosystem improvement directly corresponds to the decline of the economies of Central and Eastern European nations in the 1990s.

So what is the lesson from the butterfly and jellyfish? Small changes for good or bad can produce unexpectedly large effects. Ignoring the biochemodynamic details can lead to big problems down the road.
The smallest living systems, the viruses, bacteria, and other microbes, are amazing biochemical factories. For much of human history, we have treated them as marvelous black boxes, wherein mysterious and elegant processes take place. These processes not only keep the microbes alive, but they provide remarkable proficiencies to adapt to various hostile environments. Some produce spores; many have durability and protracted latency periods; all have the ability to reproduce in large numbers until environmental conditions become more favorable. The various systems that allow for this efficient survival have become increasingly better understood in recent decades, to the point that cellular and subcellular processes of uptake and absorption, nutrient distribution, metabolism and product elimination have been characterized, at least empirically. More recently, the genetic materials of deoxyribonucleic acid (DNA) and the various forms of ribonucleic acids (RNA) have been mapped. As genes have become better understood, so has the likelihood of their being manipulated. Such manipulation is the stuff of biotechnology.

Biotechnology began as a passive and adaptive approach. For example, sanitary engineers noted that natural systems, such as surface waters and soil, were able to break down organic materials. Such processes are now known as biodegradation. When engineers put microbial populations (usually bacteria and fungi) to work to clean up the soil or groundwater, this is called bioremediation. In other words, biological processes are providing a remedy for some very important societal problems, i.e. polluted resources.

As scientists studied these processes, they realized that various genera of microbes had the ability to use detritus on forest floors, suspended organic material in water and organic material adsorbed onto soil particles as sources of energy needed for growth, metabolism and reproduction. The engineers [18] correctly hypothesized that a more concentrated system could be fabricated to do the same thing with society's organic wastes. Thus, trickling filters, oxidation ponds, and other wastewater treatment systems are merely supercharged versions of natural systems.

In a passive biotechnological system, the microbes used are those found in nature, but that have been allowed to acclimate to the organic material that needs to be broken down. The microbial population's preference for more easily and directly derived electron transfer (i.e. energy sources) is overcome only permitting them to come into contact with the chemicals in the waste. In the presence of oxygen the stoichiometry stated simply is:

\[
\text{organic matter} + O_2 \xrightarrow{\text{microbes}} \text{CO}_2 + \text{H}_2\text{O} + \text{cell matter biomass} + \text{other end products} \quad (1.1)
\]

Thus, the microbes adapt their biological processes to use these formerly unfamiliar compounds as their energy sources and, in the process, break them down into less toxic substances. Ultimately the microbes degrade complex organic wastes to carbon dioxide and water in the presence of molecular oxygen, or methane and water when molecular oxygen is absent, known as aerobic and anaerobic digestion, respectively.

Numerous examples of passive systems have been put to use with the evolution of complex societies. For example, passive biotechnologies were needed to allow for large-scale agriculture, including hybrid crops and nutrient cycling in agriculture and vaccines in medicine.

Very recently, more active systems have been used increasingly to achieve such societal gains, but at an exponentially faster pace. In addition, scientists have developed biotechnologies that bestow products that simply would not exist using passive systems.

ENVIRONMENTAL BIOTECHNOLOGY AS A DISCIPLINE

If having a professional society is an indication that something is a scientific discipline, then environmental biotechnology meets that requirement. The International Society of Environmental Biotechnology [19] promotes interest in environmental biotechnology and offers the exchange of information regarding the development, use, and regulation of
biological systems for remediation of contaminated environments (land, air, water), and for environment-friendly processes (green manufacturing technologies and sustainable development). This definition mainly focuses on the application of biotechnology to the natural environment, but is not nearly broad enough to encompass the comprehensive and complex relationship between biotechnology and the environment.

**BIOTECHNOLOGY AND SOCIETY**

Even minor tampering with nature is apt to bring serious consequences, as did the introduction of a single chemical (DDT). Genetic engineering is tampering on a monumental scale, and nature will surely exact a heavy toll for this trespass.

Eva Novotny [20]

Society will judge the success of all engineering research, including biotechnology, based on its results. If devices and systems are designed that improve and protect life, then engineers are successful. Conversely, if the risks outweigh the benefits, we have failed. Engineering research is largely evaluated based on its risks and reliability. We will discuss these topics in greater detail in Chapter 4.

As scientists, we are asked if we have appropriately considered all of the possible human and ecological impacts, not merely from the way we conduct research, but also in how that research is or will be applied. Movies and books have challenged researchers to consider the possible future impacts of even seemingly benign research, when such research is emergent. It does not particularly matter that dire consequences are only remote and unlikely outcomes. Biotechnology and other emerging technologies present a particular challenge, owing to the numerous areas of uncertainty. Could the design lead to environmental risk and will this risk be distributed proportionately throughout society? Will the consequences be irreversible and widespread? In short, biotechnological research requires a healthy, honest, and objective perspective and a level of commitment to the future, including sustaining and improving environmental quality. Sometimes scientists become so committed to the possible and actual beneficial aspects of their research that they overlook or at least give less weight to possible, negative outcomes (see Figure 1.5).

Biotechnological research embodies practicality. Of course, bioengineering researchers are interested in advancing knowledge, but always with an eye toward practice. Society demands that the state-of-the-science be advanced as rapidly as possible and that no dangerous side effects ensue. The engineering practitioner and researcher are also adept at optimizing among numerous variables for the best design outcomes. Emergent areas are associated with some degree of peril. A recent international query of top scientists [21] asked about what are the most pressing technologies needed to help developing countries (see Table 1.5). Each expert was asked the following questions about the specific technologies:

- Impact. How much difference will the technology make in improving health?
- Appropriateness. Will it be affordable, robust, and adjustable to health care settings in developing countries, and will it be socially, culturally, and politically acceptable?
- Burden. Will it address the most pressing health needs?
- Feasibility. Can it realistically be developed and deployed in a time frame of 5–10 years?
- Knowledge gap. Does the technology advance health by creating new knowledge?
- Indirect benefits. Does it address issues such as environmental improvement and income generation that have indirect, positive effects on health?

All of the areas of need identified in Table 1.5 involve biotechnologies, either directly (as in numbers 5 and 8, the need for improved sequencing and genetically modified organisms) or indirectly (e.g. improved environmental tools. Thus, bioengineers are at the forefront of technological progress and will continue to play an increasingly important role in the future.
The concomitant societal challenges require that every engineer fully understand the implications and possible drawbacks of these technological breakthroughs. Key among them will be biotechnical advances at smaller scales, well below the cell and approaching the molecular level.

Technological processes at these scales require that engineers improve their grasp of the potential environmental implications. As biotechnologies advance, so will the concomitant societal challenges. Many engineering and science disciplines will be involved, requiring a better appreciation for and ability to predict the implications and possible drawbacks of technological developments. Key among them will be biotechnical advances at smaller scales that approach the molecular level. Biotechnological processes at these scales, for example, require that engineers improve their grasp of the potential environmental implications.

**RISKS AND RELIABILITY OF NEW BIOTECHNOLOGIES**

There are two major schools of thought on determining whether to pursue biotechnologies: the precautionary approach and the evidence-based approach. The precautionary principle states:

> When an activity raises threats of harm to human health or the environment, precautionary measures should be taken even if some cause-and-effect relationships are not fully established scientifically. [22]
The precautionary perspective requires that a threshold of certainty be met before allowing a new technology. The evidence-based perspective allows the technology so long as the evidence supports it. This may seem similar, but in fact, the two viewpoints are quite different with respect to onus. The precautionary approach clearly places the onus on the technologist who wants to use the biotechnology to prove that it is safe under every possible scenario, whereas the evidence-based approach allows the technology if it undergoes a risk assessment that shows that the risks, even those that are unknown, are acceptable.

Risk management is an example of optimization. However, optimizing among variables is not usually straightforward for biotechnology and bio-engineering applications. Optimization models often apply algorithms to arrive at a net benefit/cost ratio, with the selected option being the one with the largest value, i.e. greatest quantity of benefits compared to costs. Steven Kelman of Harvard University was one of the first to articulate the weaknesses and dangers of taking a purely utilitarian approach in managing environmental, safety and health risks [23]. Kelman asserts that in such risk management decisions, a larger benefit/cost ratio does not always point to the correct decision. He also opposes the use of dollars, i.e. monetization of non-marketed benefits or costs, to place a value on environmental resources, health and quality of life. He uses a logical technique of reductio ad absurdum (from Greek, ἡ ἀπεισοδίων πείσαρα “reduction to the impossible”) where an assumption is made for the sake of argument, a result found, but it is so absurd that the original assumption must have been wrong [24]. For example, the consequences of an act, whether positive or negative, can extend far beyond the act itself. Kelman gives the example of telling a lie. Using the pure benefit/cost ratio, if the person telling the lie has much greater satisfaction (however that is quantified) than the dissatisfaction of the lie’s victim, the benefits would outweigh the cost and the decision would be morally

<table>
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<tr>
<th>Final ranking</th>
<th>Biotechnology</th>
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<tbody>
<tr>
<td>1</td>
<td>Modified molecular technologies for affordable, simple diagnosis of infectious diseases</td>
</tr>
<tr>
<td>2</td>
<td>Recombinant technologies to develop vaccines against infectious diseases</td>
</tr>
<tr>
<td>3</td>
<td>Technologies for more efficient drug and vaccine delivery systems</td>
</tr>
<tr>
<td>4</td>
<td>Technologies for environmental improvement (sanitation, clean water, bioremediation)</td>
</tr>
<tr>
<td>5</td>
<td>Sequencing pathogen genomes to understand their biology and to identify new antimicrobials</td>
</tr>
<tr>
<td>6</td>
<td>Female-controlled protection against sexually transmitted diseases, both with and without contraceptive effect</td>
</tr>
<tr>
<td>7</td>
<td>Bioinformatics to identify drug targets and to examine pathogen–host interactions</td>
</tr>
<tr>
<td>8</td>
<td>Genetically modified crops with increased nutrients to counter specific deficiencies</td>
</tr>
<tr>
<td>9</td>
<td>Recombinant technology to make therapeutic products (for example, insulin, interferons) more affordable</td>
</tr>
<tr>
<td>10</td>
<td>Combinatorial chemistry for drug discovery</td>
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</tbody>
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acceptable. At a minimum, the effect of the lie on future lie-telling would have to be factored into the ratio, as would other cultural norms.

Another of Kelman’s examples of flaws of utilitarianism is the story of two friends on an Arctic expedition, wherein one becomes fatally ill. Before dying, he asks that the friend return to that very spot on the Arctic ice in 10 years to light a candle in remembrance. The friend promises to do so. If no one else knows of the promise and the trip would be a great inconvenience, the benefit/cost approach instructs him not to go (i.e. the costs of inconvenience outweigh the benefit of the promise because no one else knows of the promise). These examples illustrate that benefit/cost information is valuable, but care must be taken in choosing the factors that go into the ratio, properly weighing subjective and non-quantifiable data, ensuring that the views of those affected by the decision are properly considered, and being mindful of possible conflicts of interest and undue influence of special interests.

From either the precautionary or evidence-based scientific perspective, environmental systems must be viewed comprehensively. There is no way to evaluate a biotechnology without considering the concept of risk [25]. Managing risks to human and ecosystem health is one of the principal bioengineering mandates. Reliability lets us know how well the natural and engineered biosystems are working, not only in their delivery of manufactured products, but also in ensuring that wastes are not released, that exposures are prevented and minimized and that ecosystems are protected now and in the future.

Risk, as it is generally understood, is the chance that some unwelcome event will occur. The operation of an automobile, for example, introduces the driver and passengers to the risk of a crash that can cause damage, injuries, and even death. Understanding the factors that lead to a risk is known as risk analysis. The reduction of this risk (for example, by installing airbags and wearing seat belts in the driving example) is risk management. Risk management is often differentiated from risk assessment, which is comprised of the scientific considerations of a risk. Risk management includes the policies, laws, and other societal aspects of risk.

Enlisting the biological sciences to address society’s medical, industrial, agricultural, and environmental challenges must be accompanied by considerations of the interrelationships among factors that put people and the environment at risk, suggesting that biotechnologists are indeed risk analysts. Technologies must be based on the sound application of the physical and social sciences. The public expects safe products and processes, and the public holds biotechnologists accountable for its health, safety, and welfare. Engineers employ systems to reduce and to manage risks. So, bioengineers employ biosystems, to reduce and to manage environmental risks. Risk is indirectly proportional to reliability. Thus, bioengineers seek ways to enhance the reliability of these systems. Like all engineering disciplines, biotechnological design deals directly or indirectly with risk by improving system reliability.

Both risk and reliability are expressed as probabilities. Most everyone, at least intuitively, assesses the risks of new technologies before they use them and, when presented solutions by engineers, makes decisions about the reliability of the designs. People, for good reason, want to be assured that every aspect of a new biotechnology is and will be “safe.” But, safety is a relative term. Calling something “safe” integrates a value judgment that is invariably accompanied by uncertainties. The safety of a product or process can be described, at least to some extent, in objective and quantitative terms. Factors of safety are a part of every design. Most of the time, environmental safety is expressed by its opposite term, risk. As such, risk is a good metric against which biotechnological safety is judged [26].

Bioengineering is indeed an “applied social science,” so bioengineering must be seen less as a profession that builds things than one that provides useful outcomes. It is tempting at this point to count only those factors that are quantifiable. This includes measures and interventions that protect and enhance human and ecological resources and assets; those goods and
services that are valued. Even deciding on value is laden with perspective, (anthropocentric, biocentric, and ecocentric viewpoints are discussed in chapter 12). Here is where the social sciences can help the bioengineer. Social science is concerned with human society and its individual members. Although, most engineers are steeply trained in the physical sciences (those that explain natural phenomena), their professional codes are written as social science mandates. For example, the stated mission of the Biomedical Engineering Society (BES) consists of five key areas, three of which directly address the application of biological sciences to solve and to prevent societal problems:

- Fostering translation of biomedical engineering and technology to industrial and clinical applications through enhancing interactions with industry and clinical medicine …
- Holding top-quality scientific meetings and publishing the best possible journal for the communication and exchange of state-of-the-art knowledge at the frontier of biomedical engineering and bioengineering.
- Enhancing the impact of biomedical engineering on economy and human health and maximizing the performance of the discipline … [27]

Biosystem engineering success or failure is in large measure determined by what the engineer does with respect to what the profession “expects.” As mentioned, safety is always a fundamental facet of our professional duties. Thus, we need a set of criteria that tells us when designs and projects are sufficiently safe. Four main safety criteria should be applied to test a biotechnology’s worthiness [28]:

- The design must comply with applicable laws.
- The design must adhere to “acceptable engineering practice.”
- Alternative designs must be sought to see if there are safer practices.
- Possible misuse of the product or process must be foreseen.

The first two criteria are easier to follow than the third and fourth. The well-trained bioengineer can look up the physical, chemical, and biological factors to calculate tolerances and factors of safety for specific designs. Laws have authorized the thousands of pages of regulations and guidance that codify when acceptable risk and safety thresholds are crossed; meaning that the design has failed to provide adequate protection. Bioengineering standards of practice go a step further. Biosystematic success or failure here can be difficult to recognize, since the biosciences being applied are rather esoteric and specific to the technology. Only other engineers with explicit expertise in this biology specialty can adequately ascertain the ample margin of safety as dictated by sound engineering principles and practice. Identifying alternatives and predicting misuse requires quite a bit of creativity and imagination. This type of failure falls within the domain of social science.

BEYOND BIOTECHNOLOGICAL APPLICATIONS

Indeed, numerous applications of biotechnology are effectively addressing environmental problems, but implications of using living systems to solve society’s ills also constitute some of the greatest challenges. Living organisms can be used to sense and to indicate changes in the environment (so-called bioindicators). Also, living organisms are being put to work to clean up chemically contaminated waste sites (e.g. microbes that break down hazardous chemicals into less toxic byproducts). Yet, merely presenting the positive sides of environmental biotechnology leaves the impression that it is entirely a beneficial resource, without hazards. Conversely, only worrying about the environmental pollution associated with emerging biotechnological advances also paints an unrealistic, overly negative image of biotechnology’s impact on society. Therefore, it is important and crucial to treat every aspect of biotechnology objectively and in a scientifically credible manner. There are many sources that either support or condemn biotechnology. The attempt here
is to provide a balanced and comprehensive consideration of all aspects of environmental biotechnology.

This begs the question then of what we mean when we say environmental biotechnology. Both words are important. In fact, in spite of the growth of environmental science and engineering over recent decades, there is not complete unanimity as to what is meant by important environmental terms. Pollution, contamination, hazard, risk, and other key environmental terms have various meanings within the environmental scientific community. Thus, after a brief introduction of biotechnologies, we will discuss a number of environmental concepts so that at least in this work, we have agreement on vernacular and basic science.

Terminology

Distinguishing “environmental” science and engineering from other scientific endeavors often hinges on what is important. For example, the general definition of biology is that it encompasses everything to do with the study of living things. Thus, to see what a biologist considers important must come from his or her specialization. Biology must be subdivided into more specific fields. Moving toward our specific focus, we are predominantly interested in environmental biology, which is the study of living things with respect to their surroundings. To most engineers and technologists, any field of biology, no matter the adjective in front of it, is less about application than about understanding the principles and concepts of that field. Therefore, even a more specific focus is still mainly interested in description than in application. That is, biologists tend to be more interested in living things as subject matter, whereas bioengineers are more concerned in living things with respect to solving problems. The bioengineering danger is the view that all things, including living things, are merely objects that should be manipulated to achieve some end. So, a biological process may only be seen as unacceptable if it does not meet the stated objective, e.g. converting a given mass of polychlorinated biphenyls to a given mass of carbon dioxide, water, and chlorine. If an ecosystem’s other characteristics are not also part of this measure of success, even a successful bioremediation can still be a failure (intermediate chemical products released to the environment, changes in microbial populations that harm ecosystem diversity, etc.).

Environmental protection is another way to provide focus. For example, the United States Environment Protection Agency (US EPA) has as its mission the protection of public health and the environment, and goes about that mission by addressing problems that can either be categorized as human health or ecological. Some see the need to protect ecosystems due to their inherent value, and others see the need to protect ecosystems due to their instrumental value, i.e. the goods and services that they provide to humans. Either way, introducing possible stressors to these systems must be considered from an ecological, systematic perspective. After all, ecology is the subdiscipline of biology that concerns itself with how organisms interact with their environments.

In addition to the various perspectives on the environment, there are also varying views about biotechnology. Biotechnology means many things to many people. Every part of the word, “biotechnology” is important. The suffix, “bio” indicates that we are exclusively concerned about living things. However, the role that living things play in this technology varies considerably among even scientists working in various fields of biotechnology. For example, some consider biotechnology to include every aspect of the application of living systems to solving any societal problem. This is the broadest of definitions and arguably tells you the least of all.

Therefore, at least with regard to environmental biotechnology, the definitions need to be more specific. In fact, the areas of bioscience, bioengineering, and biotechnology need to be distinguished from each other: science is the explanation of natural phenomena; engineering is the application of the sciences to addressing societal problems; technology results from scientific and engineering advances.
Eureka!

Hērēkā!

Archimedes, as recorded by Marcus Vitruvius Pollio in De architectura, c.25 BC

Discovery is the lifeblood of science. It brings excitement, not to mention investment, to those involved and to the broader society. Biotechnology has brought with it actual and promising improvements in almost every economic sector, from the food supply to medicine to environmental remedies. Although it is tempting to think of biotechnology as a uniquely modern and systematic enterprise, many of the discoveries have been incidental and accidental over centuries. The story of Archimedes’ discovering the phenomenon of volumetric displacement demonstrates that discovery takes place within and outside of the laboratory. Or, it may demonstrate that the laboratory extends well beyond the bench (as it did to Archimedes’ bathtub). Thus, biotechnologies have found their way through numerous medical, industrial, agricultural and defense systems, not just from earnest scientists trying to find new applications of the biosciences.

Indeed, although biotechnology is a modern term, many of the human advances over the past few millennia can be categorized as biotechnological. For example, humans greatly improved crops by selecting various seed types with characteristics preferred for their taste, quality, and disease resistance. The same processes are used in modern times, only now genetic engineering and other scientifically advanced approaches have speeded up the time needed for selection and have enabled a more precise focus on specific selected attributes of the plants.

Biological systems range in complexity and scale, from subcellular to planetary. The two fields of engineering which are most intimately engaged in the life systems are biomedical and environmental engineering. Nevertheless, all fields of engineering to some extent must address living systems. Indeed, any engineering is the practice whereby scientific information is applied in order to improve the world. And this improvement is measured according to where human society places its values. One big area that is not completely addressed in either environmental textbooks or engineering textbooks is the effect that biotechnological sources have on the environment. This can range in scale from changes in cellular chemical messaging systems to possible long-range transport of biological agents with possible risks at the biome, or even global, scale. This entire domain is the subject of environmental biotechnology.

The biomedical, agricultural science, industrial hygiene, and engineering disciplines must apply science and technology to understand and to enhance a myriad of biological systems. In fact, the systematic approach is the best way to address biological challenges, from an understanding of nano-scale changes to a living cell that leads to cancer to the cycling of greenhouse gases that affect global climate.

“System” is a widely used, yet misapplied term in both scientific and lay communities. In fact, it has currency in various venues, from thermodynamics to fluid dynamics to motion to pharmacokinetics. Thus, scientists and engineers who engage in environmental processes must have a common understanding and application of living systems, i.e. biosystems. Biotechnologies present both challenges and opportunities for environmental science and engineering.

This book will present a comprehensive treatment of actual and potential biotechnologies at the full range of environmental scales. It is also a valuable companion and link to biomedical applications, such as computational toxicology and physiologically based, compartmental models. Such emergent and promising tools are being used in both medical and engineering disciplines. The two principal engineering disciplines engaging in biosystems are biomedical and environmental. Unfortunately, most textbooks do not bridge these perspectives. The author’s research and teaching at Duke University has provided him with an all-too-rare opportunity to work in both areas. This book will take
advantage of this unique perspective to link and to contrast the biosystem approach in these diverse and essential fields.

Oh no!
The most exciting phrase to hear in science, the one that heralds new discoveries, is not “Eureka!” (I found it!) but “That’s funny…”

Isaac Asimov, 1920–92

Scientific discoveries that involve only one experimental or independent variable are the easiest to explain. One can manipulate that single factor, keeping others constant, and watch for changes in the dependent variable. Biological systems seldom allow researchers to hold these other variables constant. In fact, many variables are not even known; only outward manifestations or indicators can be observed. Thus, in a static system, i.e. one that is not changing in space and time, the interaction of variables is complex, but in a dynamic system, the uncertainties can increase dramatically. Thus, in characterizing and applying the biological sciences, biotechnologists are challenged to document what is going on. The outcome of the experiment is explained within the context of its constraints. For dynamic systems, things get astronomically more complicated in time and space, as more variables are introduced and environmental conditions change.

When scientific, engineering, and technological advances occur relatively rapidly, unintended consequences can result. More complex approaches are often associated with a lack of understanding of how they work, at least in the early stages of development. Such “black boxes” can contain steps and processes with unforeseen hazards. In addition to complexity, the rapid dissemination of new approaches in modern times can lead to trouble on a grand scale.

Thus, the complexity and scale of possible environmental impacts must be considered as early as possible in decisions involving emerging biotechnologies. Otherwise, numerous countervailing and downstream risks can present themselves. Such risks may not be readily apparent and may even be unprecedented. For example, the new technology may supplant or completely change design and manufacturing processes. Biotechnology-derived products may impact the use of other products.

Agricultural materials, such as hybrid seeds of cash crops may alter farming practices which, in turn, may adversely affect water and soil quality. For instance, if the biologically altered plant’s root has a very different capillary function than the non-genetically modified plant nutrients may be held and released at a rate that is not sustainable. Or, the new capillarity may improve sustainability by enhanced root–soil interactions. Further, the shallower or deeper root system may upset the delicate balance in the water cycle. A shallower root system may translate into a diminished ability of the plants to hold soil in place, thus leading to a loss of top soil. A deeper root system may extract water from lower strata of an aquifer, leading to drawdown.

An interesting example of the complications and connectedness between slight changes to an ecosystem and human health is the effect of change in flora and the Ross River virus. Ross River virus causes a severe illness in human populations, manifested in muscle and joint pain, polyarthritis and lethargy. In Australia, for example, the salt marsh mosquito Aedes vigilax and the freshwater Culex annulirostris are the main vectors of Ross River, but the virus is also carried by Aedes notoscriptus and the brackish water species Aedes funereus, with marsupials serving as the main reservoir hosts. The connection between ecosystems and human disease is often indirect and complicated [29] (see Figure 1.6).

Most ecologists rightfully rue the fact that wetlands have been dwindling globally. In fact, the civil engineering profession has been quite successful in advancing wetland construction efforts. Historically, however, wetlands were seen as problematic. Major public and private swamp drainage efforts were undertaken in the 20th century to prevent disease.
Whether a wetland is a contributor to a human disease is a function of location. If the wetland is within mosquito flight range from the nearest human population the risk of an outbreak is increased. This can vary from a few hundred meters to greater than 10 kilometers [30]. Shallow wetlands with shrubs and trees usually support higher mosquito populations than deep pools with steep edges. This may be due to the latter's support of mosquito predators. Thus, if water levels do not fluctuate sizably, mosquito populations can flourish, along with exposures to the virus. Thus, when farmers remove trees with deep roots to grow shallow-rooted crops, these crops do not siphon the water and transpire it through their leaves like trees and shrubs do. Thus, the farmers actually create wetlands, but unlike many wetland construction efforts, these cause problems. The problem is not so much an ecological problem, but an ecosystem event that leads to a human health problem. This highlights that, depending on the circumstances, a change may be adverse or beneficial. Either way, a decision on whether to apply a biotechnology is complex.

**THE SCIENCE OF ENVIRONMENTAL BIOTECHNOLOGY**

In a span of just a few decades advances and new environmental applications of science, engineering, and their associated technologies have coalesced into a whole new way to see the world. Science is the explanation of the physical world, while engineering encompasses applications of science to achieve results. Thus, what we have learned about the environment by trial and error has incrementally grown into what is now standard practice of environmental science and engineering. This heuristically attained knowledge has come at a great cost in terms of the loss of lives and diseases associated with mistakes, poor decisions (at least in retrospect), and the lack of appreciation of environmental effects.

The “environmental movement” is a relatively young one. The emblematic works of Rachel Carson, Barry Commoner, and others in the 1960s were seen by many as mere straws in the wind. The growing environmental awareness was certainly not limited to the academic and scientific communities. Popular culture was also coming to appreciate the concept of “spaceship earth,” i.e. that our planet consisted of a finite life support system and that our air, water, food, soil, and ecosystems were not infinitely elastic in their ability to absorb humanity’s willful disregard. The poetry and music of the time expressed these fears and called for a new respect for the environment. The environmental movement was not a unique enterprise, but was interwoven into growing protests about the war in Vietnam, civil rights, and...
a general discomfort with the "establishment." The petrochemical industry, the military, and capitalism were coming under increased scrutiny and skepticism.

The momentum of the petrochemical revolution following World War II was seemingly unstoppable. However, much of the progress we now take as given was the result of those who agitated against the status quo and refused to accept the paradigms of their time. In fact, this book provides evidence of the validity of some of these early environmentalists' causes. A handful of cases were defining moments in the progress in protecting public health and the environment. It seems that every major piece of environmental legislation was preceded by an environmental disaster precipitated from mistakes, mishaps, and misdeeds. Amendments to the Clean Air Act resulted from deadly episodes such as were experienced in Donora, Pennsylvania and London, UK. Hazardous waste legislation came about after public outcries concerning Love Canal in New York state. "Right-to-Know" legislation worldwide grew from the disaster at Bhopal, India. Oil spill and waste contingency plans were strengthened following the Exxon Valdez spill in Alaska. International energy policies changed, with growing anti-nuclear power sentiments, following the near disaster at Three Mile Island in the United States and the actual catastrophe at Chernobyl in Ukraine. Most recently, engineering and public health emergency response planning has been completely revamped in response to the events of September 11, 2001.

Certainly these can all be classified as "environmental" problems, but they represent new, societal paradigms as well. That is the tricky part of dealing with emerging technologies, including biotechnologies. Contemporary society has a way of thrusting problems upon us. Ironically, society demands the promotion of new and better things and processes, simultaneously demanding that scientists, engineers, physicians, and others in the scientific community sufficiently control the consequences of the very same technologies that members of society insist we use. For example, society may demand, reasonably or unreasonably, certain food characteristics (higher nutrition, less fat, attractive color). They may be quite happy with a product, until it is found to have actual or perceived negative characteristics. For example, the public may be pleased that the price of strawberries remains low and the texture of a high quality until they find out that the plants have been genetically altered to resist frost damage. However, this engineered characteristic could have been the principal driver for the lower price and better texture. Likewise, cleanup of polluted waters and sediments can benefit from genetically altered bacteria and fungi to break down some very persistent contaminants, but the public may fear potential problems if these microbes somehow escape their intended use and find their way into unplanned components of the food chain. Prominent and infamous environmental problems have emerged as byproducts of some useful, high-demand enterprise.

**BOXES AND ENVELOPES: PUSHING THE BOUNDARIES, CONTAINING THE RISKS**

The engineer, more than ever, must balance creativity and innovation with caution and deliberation. Preventing and reducing the risks posed by hazardous substances involves discovery, brilliance, and due diligence. Engineers are simultaneously asked to provide proven products in high and low technologies, while being at the same time encouraged to think outside the box and to push the envelope to find better ways to address the health and ecological perils imposed by these wastes.

The "box" and "envelope" are illustrative metaphors. Bioreactors are boxes in a thermodynamic sense. Physicists would envision a bioreactor as a box (known as a control volume) in which mass and energy enter, within which substances change in terms of energy and mass, and from which the parents and byproducts of these substances exit. The bioreactor is different from other reactors in the fact that biological processes are at the entrance to, within, and at the exit from the control volume. Chemical engineers see this box as any other reactor,
nonetheless; that is, the facility is the enclosure in which all the physical and chemical processes are (or should be) controlled. The envelope is also an appropriate bioengineering term. It is the maximum capacity or capability of a system. As new substances are generated or existing mass and energy are released to the environment, the engineer is expected to push beyond existing solutions to reduce the potential for adverse effects that can result from exposures to these wastes. But, prudence dictates that the box and envelope not be contorted in ways that may lead to unacceptable risks. As such, engineering the risks of biotechnology is always in the balance between flux and stability.

Biotechnologies must be addressed across many scales, ranging from the atom to the planet. At the so-called nano-scale, we simultaneously fear the new environmental challenges of nano-materials (e.g. nanoparticles and fullerenes that display properties not as well understood as those of larger scales), while enthusiastically embracing these same nanotechnologies as improvements in ways to measure contaminants and to improve treatment of conventional and toxic wastes. At the planetary scale, we must consider the cumulative effect of contaminants on the atmosphere, the oceans, sensitive biosystems, and human populations. We must also consider economic and political solutions to the problems presented, including multinational perspectives to reduce the need to produce the wastes in the first place. Not only are engineers charged with a mandate to solve and to prevent environmental problems, but we must do such things in a way that does not lead to unacceptable side effects, especially those that affect our vital life systems on this planet. This is embodied in the first canon of engineering practice. We must hold paramount the public’s safety, health, and welfare. Engineers have become increasingly adept at distinguishing between what we can do and what we should do.

**RESPONSIBLE BIOENGINEERING**

By three methods we may learn wisdom: First, by reflection, which is noblest; second, by imitation, which is easiest; and third by experience, which is the bitterest.

*Confucius, c.551–479 BC*

Confucius’ quote is genuinely applicable to biotechnologies, since it encapsulates the incrementalism of engineering knowledge and wisdom. Engineers spend most of the preparation for the profession learning the scientific principles and applying them to problems; what Confucius might have called “reflection.” Next, the newly minted engineer observes and applies lessons from seasoned mentors in increasingly less “safe” venues. And, the engineer hopes not to experience the bitterness of direct failure by adopting practices that have worked for others in the past.

The bioengineering growth relies on a framework of texts, manuals, and handbooks, not only from engineering, but also from allied biological and chemical sciences. Only when experience is added to the mix can wise decisions be made [31]. Engineers who intend to practice must first submit to a rigorous curriculum (approved and accredited by the Accreditation Board for Engineering and Technology), then must sit for the Future Engineers (FE) examination. After some years in the profession (assuming tutelage by and ongoing intellectual osmosis with more experienced professionals), the engineer has sufficiently demonstrated professional strength [32] and sits for the Professional Engineers (PE) exam. Only after passing the PE exam does the National Society for Professional Engineering certify that the engineer is a “professional engineer” and eligible to use the initials PE after his or her name. The engineer is, supposedly, now schooled beyond textbook knowledge. The professional status demands a transition from knowing the “what” and the “how” to knowing the “why” and the “when.” The engineer knows more about why technical and ethical problems require a complete understanding of the facts and possible outcomes (i.e. conditional probabilities). Details and timing are critical attributes of a good engineer. The wise engineer grows to appreciate that the correct answer to many engineering problems is “It depends.”
Emergent research, such as that of biotechnology and nanotechnology, continues to become smaller in scale. Many research institutions have numerous nano-scale projects (within a range of a few angstroms). Nascent areas of research include ways to link protein engineering with cellular and tissue biomedical engineering applications (e.g. drug delivery and new devices); ultra-dense computer memory; nonlinear dynamics and the mechanisms governing emergent phenomena in complex systems; and state-of-the-art nano-scale sensors (including photonic ones). Complicating the potential societal risks, much of this research frequently employs biological materials and self-assembly devices to design and build some strikingly different kinds of devices. Some of the worst-case scenarios have to do with the replication of the “nano-machines.” We need to advance the state-of-the-science to improve the quality of life (e.g. treating cancer, Parkinson’s disease, Alzheimer’s disease, and improving life expectancies, or cleaning up contaminated hazardous wastes), but in so doing are we introducing new societal risks? In his recent book Catastrophe: Risk and Response, Richard Posner, a judge of the US Court of Appeals for the Second Circuit, describes this paradox succinctly:

*Modern science and technology have enormous potential for harm. But they are also bounteous sources of social benefits. The one most pertinent is the contribution technology has made to averting both natural and man-made catastrophes, including the man-made catastrophes that technology itself enables or exacerbates.* [33]

Posner gives the example of the looming threat of global climate change, caused in part by technological and industrial progress (mainly the internal combustion engine and energy production tied to fossil fuels). Emergent technologies can help to assuage these problems by using alternative sources of energy, such as wind and solar, to reduce global demand for fossil fuels. We will discuss other pending problems, such as the low-probability but highly important outcomes of genetic engineering, e.g. genetically modified organisms (GMOs) used to produce food. There is both a fear that the new organisms will carry with them unforeseen ruin, such as in some way affecting living cells’ natural regulatory systems. An extreme viewpoint, as articulated by the renowned physicist Martin Rees, is the growing apprehension about nanotechnology, particularly its current trend toward producing “nanomachines.” Biological systems, at the subcellular and molecular levels, could very efficiently produce proteins, as they already do for the own purposes. By tweaking some genetic material at a scale of a few angstroms, parts of the cell (e.g. the ribosome) that manufacture molecules could start producing myriad molecules designed by scientists, such as pharmaceuticals and nanoprocessors for computing. However, Rees is concerned that such assemblers could start self-replicating (like they always have), but without any “shut-off.” Some have called this the “gray goo” scenario, i.e. accidentally creating an “extinction technology” from the cell’s unchecked ability to exponentially replicate itself if part of their design is to be completely “omnivorous,” using all matter as food! No other “life” on earth would exist if this “doomsday” scenario were to occur [34].

Certainly, this is the stuff of science fiction, but it does call attention to the need for vigilance, especially since our track record for becoming aware of the dangers of technologies is so frequently tardy. It also points out that “rare” does not equal “impossible.” The events that lead to a rare outcome are all possible. In environmental situations, messing with genetic materials may harm biodiversity, i.e. the delicate balance among species, including trophic states (producer–consumer–decomposer) and predator–prey relationships. Engineers and scientists are expected to push the envelopes of knowledge. We are rewarded for our eagerness and boldness. The Nobel Prize, for example, is not given to the chemist or physicist who has aptly calculated important scientific phenomena, with no new paradigms. It would be rare indeed for engineering societies only to bestow awards on the engineer who for an entire career has used none but proven technologies to design and build structures. This stems from our general approach to contemporary scientific research. We are rugged individualists in a quest to add new knowledge. For example, aspirants seeking PhDs must endeavor to add
knowledge to their specific scientific discipline. Scientific journals are unlikely to publish articles that do not at least contain some modicum of originality and newly found information [35]. We award and reward innovation. Unfortunately, there is not a lot of natural incentive for the innovators to stop what they are doing to “think about” possible ethical dilemmas propagated by their discoveries [36].

Thus, biotechnologies call both for pushing the envelopes of possible applications and simultaneously for a rigorous approach to investigate likely scenarios, from the very beneficial to the worst-case (“doomsday”) outcomes. This link between fundamental work and outcomes becomes increasingly crucial as such research reaches the marketplace relatively quickly and cannot be confined to the “safety” and rigor of the laboratory and highly controlled scale-ups.

**Acceptable risk**

Environmental biotechnology thrusts the engineer into uncomfortable places. The frustration for engineers lies in the fact that there is seldom a simple answer to the questions “How healthy is healthy enough?” and “How protected is protected enough?” Managing environmental risks consists of balancing among alternatives. Usually, no single solution to an environmental problem is available. Whether a risk is acceptable is determined by a process of making decisions and implementing actions that flow from these decisions to reduce the adverse outcomes or, at least to lower the chance that negative consequences will occur [37].

Risk managers can expect that whatever risk remains after their project is implemented, those potentially affected will not necessarily be satisfied with that risk. It is difficult to think of any situation where anyone would prefer a project with more risk than with one with less, all other things being equal. It has been said that “acceptable risk is the risk associated with the best of the available alternatives, not with the best of the alternatives which we would hope to have available” [38].

Since risk involves chance, risk calculations are inherently constrained by three conditions:

- The actual values of all important variables cannot be known completely and, thus cannot be projected into the future with complete certainty.
- The physical and biological sciences of the processes leading to the risk can never be fully understood, so the physical, chemical, and biological algorithms written into predictive models will propagate errors in the model.
- Risk prediction using models depends on probabilistic and highly complex processes that make it infeasible to predict many outcomes. [39]

The “go or no go” decision for most engineering designs or projects is based upon some sort of “risk–reward” paradigm, and should be a balance between benefits and costs [40]. This creates the need to have costs and risks significantly outweighed by some societal good. The adverb “significantly” reflects two problems: the uncertainty resulting from the three constraints described above; and the “margin” between good versus bad. Significance is the province of statistics, i.e. it tells us just how certain we are that the relationship between variables cannot be attributed to chance. But, when comparing benefits to costs, we are not all that sure that any value we calculate is accurate. For example, a benefit/cost ratio of 1.3 with confidence levels that give at a range between 1.1 and 1.5 is very different from a benefit/cost ratio of 1.3 with a confidence range between 0.9 and 1.7. The former does not include any values below 1, while the latter does (i.e. 0.9). This value means that even with all of the uncertainties, our calculation shows that the project could be unacceptable. This situation is compounded by the second problem of not knowing the proper margin of safety. That is, we do not know the overall factor of safety to ensure that the decision is prudent. Even a benefit/cost ratio that appears to be mathematically high, i.e. well above 1, may not provide an ample margin of safety given the risks involved.
The likelihood of unacceptable consequences can result from exposure processes, from effects processes or both processes acting together. So, four possible permutations can exist:

- Probabilistic exposure with a subsequent probabilistic effect;
- Deterministic exposure with a subsequent probabilistic effect;
- Probabilistic exposure with a subsequent deterministic effect; or
- Deterministic exposure with a subsequent deterministic effect. [41]

A risk outcome is deterministic if the output is uniquely determined by the input. A risk outcome is probabilistic if it is generated by a statistical method, e.g. randomly. Thus, the accuracy of a deterministic model depends on choosing the correct conditions, i.e. those that will actually exist during a project’s life, and correctly applying the principles of physics, chemistry, and biology. The accuracy of the probabilistic model depends on choosing the right statistical tools and correctly characterizing the outcomes in terms of how closely the subpopulation being studied (e.g. a community or an ecosystem) resembles those of the population (e.g. do they have the same factors or will there be sufficient confounders to make any statistical inference incorrect?). A way of looking at the difference is that deterministic conditions depend on how well one understands the science underpinning the system, while probabilistic conditions depend on how well one understands the chance of various outcomes (see Table 1.6).

Actually, the deterministic exposure/deterministic effect scenario is not really a risk scenario because there is no “chance” involved. It would be like saying that releasing a 50 kg steel anvil from 1 meter above the earth’s surface runs the risk of falling toward the ground! The risk only comes into play when we must determine external consequences of the anvil falling. For example, if an anvil is suspended at the height of one meter by steel wire and used by workers to perform some task (i.e. a deterministic exposure) there is some probability that it may fall (e.g. studies have shown that the wires fail to hold one in 10,000 events, i.e. failure probability of 0.0001), so this would be an example of a deterministic exposure followed by a probabilistic effect (wire failure), i.e. permutation number 2. A biotechnological example would be the potential release of microorganisms. If microbial spores are included in a release from a facility’s stack due to a chain of events (e.g. the tanks hold in all but one in 10,000 events), then the failure probability is 0.0001 or $10^{-4}$, i.e. the deterministic exposure to the spores could then be calculated from the likelihood that populations would come into contact with them. If that likelihood of contact to a spore suspended in air, re-entrained after setting, from dermal contact, or from food or drinking water, is 0.01 or $10^{-2}$, then the overall exposure probability of this scenario is $10^{-4} \times 10^{-2} = 10^{-6}$. Note that this is not the risk, but the exposure. The risk is a function of the exposure and the hazard, e.g. the effect from receiving a dose of spores.

Estimating risk using a deterministic approach requires the application of various scenarios, e.g. a very likely scenario, an average scenario or a worst-case scenario. Very likely scenarios are valuable in some situations when the outcome is not life-threatening, or one of severe effects, like cancer. The debate in the public health arena is often between a mean exposure and a worst-case exposure (i.e. maximally exposed and highly sensitive individuals). The latter is more protective, but almost always more expensive and difficult to attain. For example, lowering the emissions of particulate matter (PM) from a power plant stack to protect the mean population of the state from the effects of PM exposure is much easier to achieve than lowering the PM emissions to protect an asthmatic, elderly person living just outside of the power plant property line (see Figure 1.7).

So, then, why not require zero risk from biotechnological materials, or from any hazard for that matter. It becomes an exercise in optimization. Of course, the most protective standards are best, but the feasibility of achieving them can be a challenge. The regulated standard can be very close to zero, especially if one assumes a worst-case scenario for exposure and provides adequate or even more conservative factors of safety. For example, preventing the
Table 1.6 Exposure and effect risk management approaches

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<tr>
<th>Probabilistic exposure</th>
<th>Deterministic exposure</th>
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<tr>
<td><strong>Probabilistic effect</strong></td>
<td>Occupational exposure to asbestos</td>
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<tr>
<td>Contracting the West Nile virus</td>
<td>Exposure to asbestos from vermiculite workers is deterministic because the worker chooses to work at a plant that processes asbestos-containing substances. This is not the same as the person choosing to be exposed to asbestos, only that the exposure results from an identifiable activity. The potential health effects from the exposures are probabilistic, ranging from no effect to death from lung cancer and mesothelioma. These probabilistic effects increase with increased exposures that can be characterized (e.g. number of years in certain jobs, availability of protective equipment and amount of friable asbestos fibers in the air)</td>
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<tr>
<td>Although many people are bitten by mosquitoes, most mosquitoes do not carry the West Nile virus. There is a probability that a person will be bitten and another, much lower probability, that the bite will transmit the virus. A third probability of this bitten group may be rather high that once bitten by the West Nile virus-bearing mosquito the bite will lead to the actual disease. Another conditional probability exists that a person will die from the disease. So, death from a mosquito bite (probabilistic exposure) leads to a very unlikely death (probabilistic effect)</td>
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<th>Deterministic effect</th>
<th>Death from methyl isocyanate exposure</th>
<th>Generating carbon dioxide from combusting methane</th>
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<tr>
<td>Deterministic effect</td>
<td>Death from methyl isocyanate exposure</td>
<td>Generating carbon dioxide from combusting methane</td>
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<td>Exposure to a toxic cloud of high concentrations of the gas methyl isocyanate (MIC) is a probabilistic exposure, which is very low for most people. But, for people in the highest MIC concentration plume, such as those in the Bhopal, India, tragedy, death was 100% certain. Lower doses led to other effects, some acute (e.g. blindness) and others chronic (e.g. debilitation that led to death after months or years). The chronic deaths may well be characterized probabilistically, but the immediate poisonings were deterministic (i.e. they were completely predictable based on the physics, chemistry and biology of MIC)</td>
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<td>The laws of thermodynamics dictate that a decision to oxidize methane, e.g. escaping from a landfill where anaerobic digestion is taking place, will lead to the production of carbon dioxide and water (i.e. the final products of complete combustion). Therefore, the engineer should never be surprised when a deterministic exposure (heat source, methane, and oxygen) leads to a deterministic effect (carbon dioxide release to the atmosphere). In other words, the production of carbon dioxide is 100% predictable from the conditions. The debate on what happens after the carbon dioxide is released (e.g. global warming) is the province of probabilistic and deterministic models of these effects</td>
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escape of 99% of microbes from a reactor is much easier and cheaper than preventing the escape of 99.999% microbes. Stated differently, putting controls and fail-safe measures in place to prevent an accidental release 99% of the time is much more feasible than the controls and fail-safe measures needed to prevent an accidental release 99.999% of the time (see Figure 1.8). Depending on the hazard, however, even the 99.999% may not meet engineering and risk standards. Some substances are so toxic, and some biological agents so poorly understood, that prevention of their releases may require numerous redundancies. The risk reduction measures are not only dependent on initial design, but on retrofits, operation and maintenance, and decommissioning of a bioreactor or other biotechnological equipment.

Actual or realistic values are input into the deterministic model. For example, to estimate the risk of tank explosion from rail cars moving through and parked in a community, the number of cars, the flammability and vapor pressure of contents, the ambient temperature, the vulnerability of the tank materials to rupture, and the likelihood of derailment would be assigned numerical values, from which the risk of explosion is calculated. Similarly, a biotechnological deterministic example may estimate the risk of a breach of physical contaminant of a microbe based on adaptability and spore-forming ability of the microbe and environmental conditions, e.g. available nutrients, routes and modes of physical movement by air and water and the physical characteristics of barriers leading to rupture or otherwise fail. A probabilistic approach would require the identification of the initiating events and the plant operational states to be considered; analysis of the adverse outcome using statistical analysis tools, including event trees; application of fault trees for the systems analyzed using the event trees (i.e. reliability analyses – see Chapter 3), collection of probabilistic data (e.g. probabilities of failure and the frequencies of initiating events); and the interpretation of results.

Human beings engage in risk management decisions every day. They must decide throughout whether the risk from particular behaviors is acceptable or whether the potential benefits of a behavior do not sufficiently outweigh the hazards associated with that behavior. In engineering terms, they are optimizing their behaviors based upon a complex set of variables that lead to numerous possible outcomes. A person wakes up and must decide whether to drink

FIGURE 1.7
Difference in control strategies based on concentration of the allowable emissions of a substance to reduce risks to maximally exposed versus mean population and a very low exposure scenario. Hypothetical data for fictitious microbe, Clostridium “difficulty”, which produces a spore that is an allergen. The concentration would be even lower if the risks are based on a highly sensitive subpopulation (e.g. elderly, infants, asthmatic, or immunocompromised), depending upon the effects elicited by the emitted substance. For example, if the spores are expected to cause cardiorespiratory effects in babies, an additional factor of safety may push the risk-based controls downward by a factor of 10 to achieve 0.1 µg m⁻³ to protect the maximally exposed, sensitive population.
coffee that contains the alkaloid caffeine. The benefits include the morning “jump-start,” but the potential hazards include induced cardiovascular changes in the short term, and possible longer-term hazards from chronic caffeine intake. The decision is also optimized according to other factors, such as sensory input (e.g. a spouse waking earlier and starting the coffee could be a strong positive determinate pushing the decision toward “yes”), habit (more likely to drink a cup if it is part of the morning routine, less likely if it is not), and external influences (e.g. seeing or hearing a commercial suggesting how nice a cup of coffee would be, or conversely reading an article in the morning paper suggesting a coffee-related health risk). This decision includes a “no-action” alternative, along with a number of other available actions.

One may choose not to drink coffee or tea. Other examples may include a number of actions, with concomitant risk.

FIGURE 1.8
Prototypical contaminant removal cost-effectiveness curve. In the top diagram, during the first phase a relatively large amount of the contaminant is removed at comparatively low costs. As the concentration in the environmental media decreases, the removal and control costs increase substantially. At an inflexion point, the costs begin to increase exponentially for each unit of contaminant removed, until the curve nearly reaches a steady state where the increment needed to reach complete removal is very costly. The top curve does not recognize innovations that, when implemented, as shown in the bottom diagram, can make a new curve that will again allow for a steep removal of the contaminant until its cost-effectiveness decreases. This concept is known to economists as the law of diminishing returns.


The no-action alternative is not always innocuous. For example, if a person knows that exercise is beneficial but does not act upon this knowledge, the potential for adverse cardiovascular problems is increased. If a person does not ingest an optimal amount of vitamins and minerals, disease resistance may be jeopardized. If a person always stays home to avoid the crowds, no social interaction is possible and the psyche suffers. The management decision in this case may be that the person decided that human-to-human contact, correctly, is a means of transmitting pathogens. But, implementing that decision carries with it another hazard, i.e. social isolation. Likewise, the engineer must take an action only if it provides the optimal solution to the environmental problem, while avoiding unwarranted financial costs, without causing unnecessary disruption to normal activities, and in a manner that is socially acceptable to the community.
In addition, the engineer must weigh and balance any responsibility to represent the client with environmental due diligence. This diligence must be applied to designs and plans for manufacturing processes that limit, reduce or prevent pollution, to ways to reduce risks in operating systems, to the assessment of sites and systems for possible human exposures to hazardous and toxic substances, and to the evaluation of designs systems to reduce or eliminate these exposures. Ultimately, the engineer participates in means to remedy the problem, i.e. to ameliorate health, environmental, and welfare damages. The remedy process varies according to the particular environmental compartment of concern (e.g. water, air, or soil), the characteristics of contaminant of concern (e.g. toxicity, persistence in the environment and likelihood to be accumulate in living tissues), and the specific legislation and regulations covering the project. However, it generally follows a sequence of preliminary studies, screening of possible remedies, selecting the optimal remedy from the reasonable options, and implementing the selected remedy (see Figure 1.9). The evaluation and selection of the best alternative is the stuff of risk management.

Given this seemingly familiar role of the engineer, why then do disasters and injustices occur on our watch? What factors cause the engineer to improperly optimize for the best outcome? In part, failures in risk decision making and management are ethical in nature. Sometimes organizational problems and demands put engineers in situations where the best and most moral decision must be made against the mission as perceived by management. Working within an organization has a way of inculcating the “corporate culture” into professionals. The process is incremental and can “desensitize” employees to acts and policies that an outsider would readily see to be wrong. Much like the proverbial frog placed in water that gradually increases to the boiling point, an engineer can work in gradual isolation, specialization, and compartmentalization that ultimately changes to immoral or improper behavior, such as ignoring key warning signs that a decision to locate the facilitate will have an unfair and disparate impact on certain neighborhoods, that health and safety are being compromised, and that political influence or the “bottom line” of profitability is disproportionately weighted in an engineer’s recommendation [42].

Another reason that optimization is difficult is that an engineer must deal with factors and information that may have not been adequately addressed during formal engineering training or even during career development. Although environmental and public health decisions must always give sufficient attention to the toxicity and exposure calculations, these quantitative

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**FIGURE 1.9**
Steps in cleaning up a contaminated site.
results are tempered with feasibility considerations. Thus, engineers’ strengths lie to the far left and right of Figure 1.9, but the middle steps, i.e. feasibility and selecting the best alternative, require information that is not “well behaved” in the minds of many engineers. For example, in 1980 the US Congress passed the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA), commonly referred to as Superfund [43]. The Superfund law authorizes the federal government to respond directly to releases or to the threat of releases of hazardous substances and enables the US Environmental Protection Agency (EPA) to take legal action to force parties responsible for causing the contamination to clean up those sites or reimburse the Superfund for the costs of cleanup. If the responsible parties for site contamination cannot be found or are unwilling or unable to clean up a site, the EPA can use funds from the Superfund to clean up a site.

Bioengineering is “utilitarian”; that is, we are called upon “to produce the most good for the most people” [44]. Bioengineers are also bound to codes of ethics, design criteria, regulations, and standards of practice. Engineers must, to the best of their abilities, consider all possible design outcomes, planned or otherwise. But this is not easy when the most appropriate “benchmarks” for success in biotechnology are moving targets. There are seldom, if ever, clear measures of success and failure. At the beginning of any engineering endeavor, there is no absolute certainty that the project will be a complete success, even if it meets the specifications laid out at the beginning. Sometimes, failure can result in implementing the plan, but other times the failure results from the confluence of events during construction and during the useful life of the project. For example, environmental decisions regarding the level of cleanup at a hazardous waste site can be based on the target health risk expected after cleanup. This remediation target can be based on what has been called the “residential standard.” In the United States this was a common measure of success for hazardous waste laws passed in the late 1970s and early 1980s. That is, immediately following the passage of key hazardous waste laws, regulators held the general view that a polluted site needed to be cleaned up to the point that no undue risk remained and the site could be returned to productive use, as if it had never been polluted.

On its face, the residential standard closely follows the steps of hazard identification, dose-response relationships, exposure analysis and effects assessment to characterize risks. However, this could actually lead to a lower level of cleanup in a previously polluted area, which would be unfair to the people living there compared to those living in more pristine areas. Thus, had the engineers designed a cleanup based on the residential standard, their plans may well have been considered failures by today’s standards. This also provides an example of why cleanups often must be adaptive to changing conditions [45].

In the United States, the cleanup of a hazardous waste begins with a feasibility study to address nine criteria that will determine the best approach for addressing the contamination, as well as the ultimate level of cleanup:

- Overall protection of human health and environment
- Compliance with applicable or relevant and appropriate requirements
- Long-term effectiveness and permanence
- Reduction of toxicity, mobility or volume through treatment
- Short-term effectiveness
- Ease of implementation
- Cost
- State acceptance
- Community acceptance

The first and fourth criteria are clearly the product of a sound, quantitative risk assessment. The other criteria must also include semi-qualitative and qualitative information. This illustrates the variety of data and information used in evaluating the environmental implications of a biotechnology.
When considering biotechnological advances, or any emerging technology, concerns about downstream impacts are crucial. Possible problems may not present themselves until sufficient time and space has been allowed to elapse. Products that contain dangerous materials like asbestos, lead, mercury, polybrominated compounds, and polychlorinated biphenyls (PCBs) were once considered acceptable and were even required by law or policy to protect the public safety and health, such as asbestos-containing and polybrominated materials to prevent fires, DDT and other persistent pesticides to kill mosquitoes in an effort to prevent disease and methyl tert-butyl ether (MTBE) as a fuel additive to prevent air pollution. Subsequently, these products were all found to cause adverse environmental and health problems, notwithstanding ongoing disagreements within the scientific community about the extent and severity of these and other contaminants. Countless environmental problems are yet to be resolved and others are plagued with incomplete or nonexistent unanimity of thought as to their importance or even whether indeed they are problems, such as the cumulative health and environmental impacts of confined animal feeding operations on microbial populations.

The tradeoff in such cases may be between two competing societal needs, such as a reliable food source and clean water. Sometimes the two needs are mutually exclusive, but others may be met by modifications of one or both solutions (unconfined, local, and more spatially distributed animal operations, along with the public’s willingness to accept higher prices for meat). The key to good environmental decision making is that it be informed with reliable information.

**SEMINAR TOPIC**

**Antibiotic Resistance and Dual Use**

Molecular biology has both advanced the state of the science of antibiotics and caused them to present vexing problems. Antibiotics are chemicals that interfere with metabolic processes that inhibit the growth of or kill microbes, especially bacteria. The mechanisms of antibiotics vary. Penicillin and vancomycin, for example, cause lysis in gram-positive bacteria (i.e. they are narrow spectrum) by obstructing their ability to synthesize cell walls. Conversely, tetracyclines affect both gram-positive and gram-negative bacteria (i.e. broad spectrum) by impeding their binding to ribosomes, thereby impeding the production of proteins and limiting their activity [46].

The dilemma of dual use illustrates the various scientific perspectives involved in a real-world problem. Bacterial resistance to antibiotics is a real and growing problem in the medical community. Tuberculosis, malaria, and ear infections and numerous other diseases are becoming increasingly difficult to treat. For example, tuberculosis cases in the United States were almost completely eliminated shortly after discovery of the pharmaceutical isoniazid in 1940, but the emergence of resistant strains that can only be treated with less effective drugs is very dangerous to public health. The sources are varied. For example, about 2 million patients contract bacterial infections in hospitals each year, known as nosocomial infections. Immunocompromised patients are particularly vulnerable to infections of *Staphylococcus aureus*, which is commonly found in hospitals, leading to pneumonia and other ailments. Isolated strains of *S. aureus* are now found to be resistant to previously efficacious antibiotics, including methicillin, oxacillin, penicillin, and amoxicillin. Some strains are even resistant to vancomycin, the antibiotic prescribed by physicians after exhausting other options [47].

A gene in *Yersinia pestis*, the causative agent of plague, was isolated in 2006 and was found to be similar to an *Escherichia coli* gene known to cause multiple types of antibiotic resistance [48]. This non-virulent strain of *Y. pestis* that over-expresses the gene is resistant to numerous common antibiotics, including those commonly prescribed to treat plague infection. Spontaneous antibiotic resistant bacteria have been found to mutate to forms that affect the expression of this gene, indicating a mechanism by which bacteria can acquire resistance [49].

Antibiotic resistance is genetically encoded. Numerous microbes produce antibiotic compounds to protect themselves from other microbes, and as a result, some of these microbes have evolved to be resistant to them. Spontaneous mutations can also produce resistance genes which can be passed on to future generations via genetic exchange processes. For instance, bacteria may transfer a circular strand of DNA (i.e. plasmid) external to its chromosome to another bacterium through conjugation. Bacteria may also get genes that have been released from dead bacteria, incorporating them into their chromosome or plasmid through transformation. A third means is transduction, whereby a bacterial virus (i.e. bacteriophage) invades the bacterial cell and removes genetic material. When the bacteriophage infects another cell, that gene may be incorporated into the other cell’s chromosome or plasmid.

(Continued)
It is quite common in biological laboratories to make antibiotic-resistant bacterial strains by plasmid-insertion of genes to express resistance to a known antibiotic. A major problem with this practice is that this resistance can be transferred from one type of bacteria to another by way of a single gene. For example, two antibiotics, tetracycline and chloramphenicol, are currently used to treat plague infections. This brings us to the dual use problem. Biological weapons could be developed using these antibiotic-resistant pathogens. This is not science fiction. For example, during the 1980s the Soviet Union developed antibiotic-resistant strains of plague, anthrax, tularemia, and glanders bacteria. The Iraqi regime of Saddam Hussein focused on anthrax, botulinum toxin, and aflatoxin [50]. Biological researchers must consider that there are possible uses, possibly not even considered in the quest to provide needed medical advances that may have profound and disastrous dual uses. In addition, the combination of molecular biology and physical sciences is needed. For example, not only the biological practice of gene insertion and ancillary medical research presents problems in security and disease prevention, but so do the means by which the bacteria and other biological materials are transported.

Aerosol science is a major part of most environmental research programs. It addresses the ways that particulate matter moves and changes in the atmosphere and in other environmental compartments. Nebulizers are devices that are used to deliver drugs. Aerosolization has become an important part of biomedical engineering with increasingly sophisticated methods adding to the effectiveness of medical treatment of asthma and other pulmonary diseases. Nebulizers are needed for immediate delivery of deep doses of medicines to the lungs, with research matching the deposition sites in the lung with the type of drug being delivered. Stokes’ law states:

\[ F_d = 6\pi\mu RV \]

where, \( F_d \) is the frictional force (Newtons), \( \mu \) is the fluid’s dynamic viscosity (Pascal seconds), \( R \) is the radius of the spherical object (meters), and \( V \) is the particle’s velocity (in m s \(^{-1}\)).

Thus, Stokes’ law explains the radius of a sphere and the viscosity of a fluid together predict the force needed to move it without settling. So, then, drug delivery to the lungs can be optimized by accounting for the density, morphology, and dynamics of the particle.

Particulate matter (PM) is a common physical classification of particles [51]. The size of a particle is determined by how the particle is formed. For example, combustion can generate very small particles, while coarse particles are often formed by mechanical processes (such as the particles to the left of the dashed line in Figure 1.10 and micrographs in Figures 1.11 and 1.12) and from vehicle exhausts. If particles are sufficiently small and of low mass, they can be suspended in the air.
for long periods of time. Larger particles (e.g. >10 μm aerodynamic diameter) are found in smoke or soot (see Figure 1.11), while very small particles (< 2.5 μm) may be apparent only indirectly, such as when they diffuse, diffract, absorb, and reflect light (see Figure 1.12).

The term “aerosol” is often used synonymously with PM. An aerosol can be a suspension of solid or liquid particles in air, and an aerosol includes both the particles and all vapor or gas phase components of air.

Since very small particles may remain suspended for some time, they can be particularly problematic from a pollutant transport perspective because their buoyancy allows them to travel longer distances. Smaller particles are also challenging because they are associated with numerous health effects (mainly because they can penetrate more deeply into the respiratory system than larger particles).

Generally, the mass of PM falling in two size categories is measured, i.e. ≤2.5 μm diameter, and >2.5 μm ≤ 10 μm diameter. These measurements are taken by instruments (see Figure 1.13) with inlets using size exclusion mechanisms to segregate the mass of each size fraction (i.e. “dichotomous” samplers). Particles with diameters >10 μm are generally of less concern, however they are occasionally measured if a large particulate-emitting source (e.g. a coal mine) is nearby, since these particles rarely travel long distances.

Mass can be determined for a predominantly spherical particle by microscopy, either optical or electron, by light scattering and Mie theory, by the particle's electrical mobility, or by its aerodynamic behavior. However, since most particles are not spherical, PM diameters are often described using an equivalent diameter, i.e., the diameter of a sphere that would have the same fluid properties. Another term, optical diameter, is the diameter of a spherical particle that has an identical refractive index as the particle. Optical diameters are used to calibrate the optical particle sizing instruments, which scatter the same amount of light into the solid angle measured. Diffusion and gravitational settling are also fundamental fluid phenomena used to estimate the efficiencies of PM transport, collection, and removal processes, such as in designing PM monitoring equipment and ascertaining the rates and mechanisms of how particles infiltrate and deposit in the respiratory tract.

Only for very small diameter particles is diffusion sufficiently important that the Stokes diameter is often used. The Stokes diameter for a particle is the diameter of a sphere with the same density and settling velocity as the particle. The Stokes diameter is derived from the aerodynamic drag force caused by the difference in velocity of the particle and the surrounding fluid. Thus, for smooth, spherical particles, the Stokes diameter is identical to the physical or actual diameter. The aerodynamic diameter ($D_{pa}$) for all particles greater than 0.5 μm can be approximated [52, 53] as the product of the Stokes particle diameter ($D_{ps}$) and the square root of the particle density ($\rho_p$):

$$D_{pa} = D_{ps}\sqrt{\rho_p}$$  \hspace{1cm} (1.3)

If the units of the diameters are in μm, the units of density are g cm$^{-3}$.

Fine particles (<2.5 μm) generally come from industrial combustion processes (such as the particles in Figure 1.12) and from vehicle exhaust. As mentioned, this smaller sized fraction has been closely associated with increased respiratory disease, decreased lung functioning, and even premature death, probably due to their ability to bypass the body’s trapping mechanisms, such as cilia in the lungs, and nasal hair filtering. Some of the diseases linked to PM exposure include aggravation of asthma, chronic bronchitis, and decreased lung function.

In addition to health impacts, PM is also a major contributor to reduced visibility, including near national parks and monuments. Also, particles can be transported long distances and serve as vehicles on which contaminants are able to reach water bodies and soils. Acid deposition, for example, can be as dry or wet. Either way, particles play a part in acid rain. In the first, the dry particles enter ecosystems and potentially reduce the pH of receiving waters. In the latter,
FIGURE 1.12
Scanning electron micrograph of spherical aluminosilicate fly ash particle emitted from an oil-fired power plant. Diameter of the particle is approximately 2.5 μm. Photo courtesy of R. Willis, Man Tech Environmental Technology, Inc., 2004; used with permission.

FIGURE 1.13
Photo and schematic of sampling device used to measure particles with aerodynamic diameters ≤2.5 μm. Each sampler has an inlet (top) that takes in particles ≤10 μm. An impactor downstream in the instrument cuts the size fraction to ≤2.5 μm, which is collected on Teflon filter. The filter is weighed before and after collection. The Teflon construction allows for other analyses, e.g. X-ray fluorescence to determine inorganic composition of the particles. Quartz filters would be used if any subsequent carbon analyses are needed. Photo and schematic courtesy of US EPA.

particles are washed out of the atmosphere and, in the process, lower the pH of the rain. The same transport and deposition mechanisms can also lead to exposures to persistent organic contaminants like dioxins and organochlorine pesticides, and heavy metals like mercury that have sorbed in or on particles.

In addition to their inherent toxicity, particles can function as vehicles for transporting and transforming chemical contaminants. For example, compounds that are highly sorptive and that have an affinity for organic matter can use particles as a means for long-range transport. Also, charge differences between the particle and ions (particularly metal cations) will also make particles a means by which contaminants are transported.

The human body and other biological systems have a tremendous capacity for the uptake of myriad types of chemicals and either utilize them to support some bodily function or eliminate them. As analytical capabilities have improved, increasingly lower concentrations of chemicals have been observed in various parts of the body. Some of these chemicals enter the body by inhalation.

The primary function of the human respiratory system is to deliver O₂ to the bloodstream and to remove CO₂ from the body. These two processes occur concurrently as the breathing cycle is repeated. Air containing O₂ flows into the nose and/or mouth and down through the upper airway to the alveolar region, where O₂ diffuses across the lung wall to the bloodstream. The counterflow involves transfer of CO₂ from the blood to the alveolar region and then up the airways and out the nose. Because of the extensive interaction of the respiratory system with the surrounding atmosphere, air pollutants or trace gases can be delivered to the respiratory system.

The anatomy of the respiratory system is shown in Figure 1.14. This system may be divided into three regions: the nasal, tracheobronchial, and pulmonary. The nasal region is composed of the nose and mouth cavities and the throat. The tracheobronchial region begins with the
trachea and extends through the bronchial tubes to the alveolar sacs. The pulmonary region is composed of the terminal bronchi and alveolar sacs, where gas exchange with the circulatory system occurs. Figure 1.14 illustrates the continued bifurcation of the trachea to form many branching pathways of increasingly smaller diameter by which air moves to the pulmonary region. The trachea branches into the right and left bronchi. Each bronchus divides and subdivides at least 20 times; the smallest units, bronchioles, are located deep in the lungs. The bronchioles end in about 3 million air sacs, the alveoli.

The behavior of particles and gases in the respiratory system is greatly influenced by the region of the lung in which they are located [54]. Air passes through the upper region and is humidified and brought to body temperature by gaining or losing heat. After the air is channeled through the trachea to the first bronchi, the flow is divided at each subsequent bronchial bifurcation until very little apparent flow is occurring within the alveolar sacs. Mass transfer is controlled by molecular diffusion in this final region. Because of the very different flows in the various sections of the respiratory region, particles suspended in air and gaseous air pollutants are treated differently in the lung.

Particle behavior in the lung is dependent on the aerodynamic characteristics of particles in flow streams. In contrast, the major factor for gases is the solubility of the gaseous molecules in the linings of the different regions of the respiratory system. The aerodynamic properties of particles are related to their size, shape, and density. The behavior of a chain type or fiber may also be dependent on its orientation to the direction of flow. The deposition of particles in different regions of the respiratory system depends on their size. The nasal openings permit very large dust particles to enter the nasal region, along with much finer airborne particulate matter. Particles in the atmosphere can range from less than 0.01 μm to more than 50 μm in diameter. The relationship between the aerodynamic size of particles and the regions where they are deposited is shown in Figure 1.15. Larger particles are deposited in the nasal region by impaction on the hairs of the nose or at the bends of the nasal passages. Smaller particles pass through the nasal region and are deposited in the tracheobronchial and pulmonary regions. Particles are removed by impacts with the walls of the bronchi when they are unable to follow the gaseous streamline flow through subsequent bifurcations of the bronchial tree. As the airflow decreases near the terminal bronchi, the smallest particles are removed by Brownian motion, which pushes them to the alveolar membrane.

The respiratory system has several mechanisms for removing deposited aerosols. The walls of the nasal and tracheobronchial regions are coated with a mucous fluid. Nose blowing, sneezing, coughing, and swallowing help remove particles from the upper airways. The tracheobronchial walls have fiber cilia which sweep the mucous fluid upward, transporting particles to the top of the trachea, where they are swallowed. This mechanism is often referred to as the mucociliary escalator. In the pulmonary region of the respiratory
system, foreign particles can move across the epithelial lining of the alveolar sac to the lymph or blood systems, or they may be engulfed by scavenger cells called alveolar macrophages. The macrophages can move to the mucociliary escalator for removal. For gases, solubility controls removal from the airstream. Highly soluble gases such as SO₂ are absorbed in the upper airways, whereas less soluble gases such as NO₂ and ozone (O₃) may penetrate to the pulmonary region. Irritant gases are thought to stimulate neuroreceptors in the respiratory walls and cause a variety of responses, including sneezing, coughing, bronchoconstriction, and rapid, shallow breathing. The dissolved gas may be eliminated by biochemical processes or may diffuse to the circulatory system.

Since the location of particle deposition in the lungs is a function of aerodynamic diameter and density, then changing the characteristics of aerosols can greatly affect their likelihood to elicit an effect. Larger particles (>5 μm) tend to deposit before reaching the lungs, especially being captured by ciliated cells that line the upper airway. Moderately sized particles (1–5 μm) are more likely to deposit in the central and peripheral airways and in the alveoli but are often scavenged by macrophages. Particles with an aerodynamic diameter less than 1 μm remain suspended in air and are generally exhaled. Recent studies have shown that large drug particles may be able to evade macrophages past the ciliated cells of the upper respiratory tract and deep into the lungs” [55]. Medicine particle design has usually sought a standard size range of 1–5 μm, but a recent study used particles of non-standardized density and aerodynamic diameter. Specifically, the researchers believed that large porous particles would have the mass and dynamics of smaller particles but since they are bigger they would more effectively evade scavenging macrophages in the alveoli. Thus, doses would be less frequent, since more of the medicine would penetrate to the desired, deeper locations in the lungs [56].

Indeed, as the researchers postulated:

large porous particles of insulin stayed active in rat lungs for 96 hours, 15 times longer than the longest-acting aerosol on the market. They also found that porous particles embedded with testosterone effectively raised blood hormone levels for extended periods. In the case of estradiol (a potent female sex hormone) delivered as an aerosol into the lungs of rats, bioavailability approached 87%, a much higher percentage than previously achieved. Such enhanced efficiencies permit prescribed medicines to be taken at less frequent intervals and at lower doses, thereby improving convenience. Future applications may include not only obvious pulmonary disorders such as asthma, but also inhalant delivery of insulin, testosterone, estradiols, and monoclonal antibodies for treating viral diseases. [57]

It did not seem to occur to the researchers that while the porous particle technology holds tremendous promise for improving drug inhalants, they may have introduced a mechanism by which the natural defenses of respiratory system could be overridden. Specifically, any deep lung penetration of spores (e.g. anthrax) would increase their lethality since a greater number of spores would penetrate more deeply into the lungs. Pathogenic microbes would also become

**FIGURE 1.15**

Particle deposition as a function of particle diameter in various regions of the lung. The nasopharyngeal region consists of the nose and throat; the tracheobronchial (T-bronchial) region consists of the windpipe and large airways; and the pulmonary region consists of the small bronchi and the alveolar sacs.

more virulent (e.g. pneumonic plague, tularemia, Q fever, smallpox, viral encephalitis, viral hemorrhagic fevers, and botulism). Subsequently, the researchers have recognized the new opportunity for “reverse engineering” of an inhaled drug delivery system, which would increase vulnerabilities in public health and national security.

Seminar Questions
Considering the two biochemodynamic factors of gene transfer and aerosol transport, what are the important steps that should be taken to prevent dual use problems?
What other biochemodynamic factors should be considered for public health protection? … for preventing terrorist acts?

How can the lessons of antibiotic resistance and aerosol delivery be applied to environmental biotechnology? In particular, what lessons can be learned from these chaotic biomedical systems to prevent a benign entity from evoking an environmental problem?

What are the similarities and differences in considerations of dual use as it applies to environmental engineers, biomedical engineers, agricultural scientists, microbiologists, and medical researchers?

How might a reductionist view differ from a systems biology view in addressing this dual use problem and preventing possible negative outcomes from environmental biotechnologies?

NOTES AND COMMENTARY

5. This is understandable if the agency is in the business of something not directly related to environmental work, but even the natural resources and environmental agencies have asserted that there is no significant impact to their projects. It causes the cynic to ask, then, why are they engaged in any project that has no significant impact? The answer is that the term “significant impact” is really understood to mean “significant adverse impact” to the human environment.
6. Ibid.
7. I learned the meaning of mitigation in my first professional job, i.e. writing an environmental impact statement for a large, coal-fired power plant that needed an EPA permit to release turbine cooling water to the Missouri River. I asked why other cooling approaches, especially cooling towers and lakes, were not being designed into this large, 600+ megawatt facility. After all of the risk assessment and management decisions were made, the power company had to add some features, such as sloughs and co-generation and heated water sharing with a nearby chemical company. Actually, in retrospect, the advice that I received from my senior colleagues was soon vindicated by the federal decision to eliminate all once-through cooling systems shortly after the acceptance of my EIS.
My second EIS was also somewhat controversial, but for different reasons. The EIS was again called for because of wastewater discharge issues, but this time it was a city’s wastewater treatment funding under the so-called “Construction Grants” program pursuant to Section 201 of the Federal Water Pollution Control Act Amendments. The city also needed a discharge permit. I sometimes wonder to this day why this facility of the hundreds being constructed in the mid-1970s with federal dollars (often 75% of the total project costs) rose to meet the two EIS metrics; i.e., being a “major federal action” and having “significant environmental impact.” It must have been the innovation of land application of the wastewater. It was not a typical way of releasing wastes to the environment. The paradigm was, and still is in most instances, that if the waste came to the plant in the form of a wastewater, it was to be released to a water body. This was a classic case of pleasing the technologists and professionals, and alienating the rest of the public. After all, the nutrients in the wastewater are really fertilizers, so the plants growing in the sprayed fields would benefit. The engineers were happy, the agricultural scientists were happy, and the city planners were happy. We were turning a waste into a resource, after all!

That was my take up to my first public hearing. What a surprise! The questions and indictments went something like this: “What about drift?” “This is sewage, and sewage is loaded with pathogens, can you guarantee that they will not find their way to the air that I breathe or the water that I drink?” “Don’t things like viruses pass through systems untreated? So, aren’t you just putting this waste out there to infect me?”

I cannot speak for my colleagues, but I was stunned. Why couldn’t they see? As the EIS progressed and I learned more about land application, many if not all of the concerns were also being voiced around the world. And the scientific community was ill-equipped to allay the fears. I learned many valuable lessons about environmental assessments in that relatively small town on the High Plains. One was that risk communications should be ever on the mind of the environmental professional. And beyond communications, one should learn to listen to the community members. They live there and they will be affected by the “expert” decisions long after we leave.

Elmo Roper had it right back in 1942. The famous pollster said: “many of us make two mistakes in our judgment of the common man. We overestimate the amount of information he has; and underestimate his intelligence.” Roper seemed to be surprised that the general public frequently has too little information to decide on important matters. Roper was even more surprised that in spite of this lack of sufficient information, the common person’s “native intelligence generally brings him to a sound conclusion. ” This is important to keep in mind in dealing with people who will potentially be affected by the recommendations of environmental experts.

8. Quote attributed to Timothy Kubiak, one of Professor Caldwell’s former graduate students in Indiana University’s Environmental Policy Program. Kubiak has since gone on to become a successful environmental policy maker in his own right, first at EPA and then at the US Fish and Wildlife Service.

9. 40 CFR 1507.3.
18. Such engineers were either formally trained in microbiology, like Ross McKinney at MIT, or learned about the microbial world on-the-job.
24. This is also known as proof by contradiction.
25. For example, Love Canal, Times Beach, Missouri and the Valley of the Drums in Kentucky are major cases that led to regulatory changes.
26. This is not to suggest that there is unanimity, or even consensus, within the scientific community on the role of risk in evaluating new technologies. For example, many scientists and policy makers prefer precaution to evidence-based decision making. Precaution would more likely ban or tightly control a new technology when key
evidence is missing, whereas risk-based decisions tend to allow a technology to go forward so long as the existing evidence supports its safety and healthy use.


31. For example, in Acts 24:25 and II Peter 1:6 St Peter associates maturation with greater “self-control” or “temperance” (Greek kratos “strength”). Interestingly, he considered knowledge as a prerequisite for temperance. Thus, from a professional point of view, we could take his argument to mean that one can really only understand and appropriately apply scientific theory and principles after one practices them. This is, in fact, the path taken toward the preparation of most professionals. For example, graduates of undergraduate engineering programs may have mastered the mathematical and scientific requirements of the curriculum and, as such, can sit for a Future Engineer (FE) or Engineer-in-Training (EIT) examination. After passing the exam, however, they must also work under the mentorship of a licensed engineer for a specified number of years before being allowed to sit for the Professional Engineering (PE) exam.

32. Ibid., from the Greek kratos “strength”. Biosystems engineering is complex and complicated, so the nurtured kratos has an added measure of importance.


35. Depending on the journal, this can contradict another tenet of scientific research, i.e. the research should be able to be conducted by other researchers, following the methodology described in the article, and derive the same results. However, there is little incentive to replicate research if the likelihood of publication is low. That is, the research is no longer “new” because it was conducted by the original researcher, so the journal may well reject the second, replicate research.

36. However, the engineering profession is beginning to come to grips with this issue. For example, in emergent “macroethical” areas like nanotechnology, neurotechnology, and even sustainable design approaches. For example, see: National Academy of Engineering, 2004, Emerging Technologies and Ethical Issues in Engineering. The National Academies Press, Washington, DC.


41. Morgan, Probing the question of technology-induced risk.

42. For case analyses where engineers have made such unethical decisions, see: W.M. Evan and M. Manion (2002). Minding the Machines: Preventing Technological Disasters. Prentice-Hall PTR, Upper Saddle River, NJ.


44. J.S. Mill, 1863, Utilitarianism.


48. Ibid.


51. Ibid.


53. Aerosol textbooks provide methods to determine the aerodynamic diameter of particles less than 0.5 micrometers. For larger particles gravitational settling is more important and the aerodynamic diameter is often used.
55. Federation of American Scientists, Aerosol Delivery Case Study.
57. Federation of American Scientists, Aerosol Delivery Case Study.