SUMMARY

The Center for Veterinary Medicine requires strain/construct-specific data for recombinant fermentation organisms used in the production of animal drugs and feed additives. Fermentation plant biocontainment schemes are chosen based, in part, upon the ability of the organism to survive and persist in the environment and to transfer genetic information to indigenous organisms. Survival and persistence study methods may include one of the following ecosystems: activated sludge, mammalian gut, soil or river water. Gene transfer protocols can be incorporated into a persistence study. These studies are designed to show that the recombinant construct behaves similarly to the host in a representative ecosystem where the organism could be introduced inadvertently. The studies need to provide repeatable results and reflect current state-of-art design and methods. Data verification is conducted by FDA investigators during Good Laboratory Practice inspections. Biocontainment guidelines, such as those developed by the NIH Recombinant DNA Advisory Committee, set general biocontainment goals for large groupings of recombinant organisms. The FDA, as required under the National Environmental Policy Act, must base its decision making on verifiable scientific data specific to each application. Therefore, in addition to using these guidelines as benchmarks, sponsors are required to submit strain/construct-specific data to support the selection of an appropriate biocontainment level. Once additional well-controlled studies for a variety of constructs are available, broader generalizations as to biocontainment may be drawn.
the accuracy and the appropriateness of the information contained in the EA. Upon acceptance of environmental documents prepared by applicants, the agency becomes responsible for the accuracy and objectivity of the EA. When the product is approved, the EA is released to the public.

In summary, NEPA establishes a framework for public oversight of FDA’s environmental decision making. Since that decision making includes the potential impact of the manufacture of FDA-regulated products, biocontainment decisions for recombinant fermentation organisms are reviewed by the FDA and the public. Failure to consider appropriate information in those decisions is reviewable by the courts. What then, is the appropriate information used to formulate and support biocontainment decisions?

**BIOLOGICAL CHARACTERISTICS**

FDA considers individual animal drug products for approval. Therefore, it would appear logical to consider information specific to the biology of the individual production strain used to produce that product. However, it is not practical to attempt to completely characterize every biological aspect of the production strain. Which characteristics make the most sense to evaluate?

The overall environmental fate considerations associated with recombinant DNA-derived fermentation microorganisms that the Center for Veterinary Medicine (CVM) primarily considers are: (i) survivability and colonization potential of the recombinant organism in the environment; and (ii) the ability of the recombinant organism to transfer some or all parts of its genome to indigenous organisms in the environment, dependent or independent of its survival or colonization ability.

There are environmental effects considerations associated with the fate considerations. For example, a potential effect on the environment resulting from colonization would include the displacement of environmentally important microorganisms by recombinant microbes with consequential effects on microbe-mediated ecological processes (e.g., disruption of nutrient cycling, important symbiotic relationships, etc.). Another potential effect involved with an organism that displays any pathogenic characteristics could be the inadvertent release of and establishment of organisms pathogenic to humans, other animals or plants.

The discussion that follows uses as examples *E. coli* K-12 constructs with well-known plasmid expression systems. The characteristics we will discuss should not be thought of as unique to or specific hazards associated with *E. coli* strains. In fact, *E. coli* K-12 strains containing pBR322 expression systems incorporate many of the known safe biological characteristics that would be expected in a environmentally responsible construct. These basic biological characteristics may be used to evaluate entirely different bacterial constructs for use in commercial fermentations.

**INFORMATION INDEPENDENT OF THE RECOMBINANT CONSTRUCT**

The host, vector, and inserted genetic material in the recombinant organism and how that organism will be manipulated to produce indigenous proteins or their close derivatives need to be described in detail. The scientific literature must be searched for basic information concerning the GEM to provide: (i) the characteristics of the unaltered parental bacterium; (ii) the characteristics of the unaltered plasmid vector; and (iii) the presence of transposable elements within the parental bacterium and unaltered plasmid vector.

**CHARACTERISTICS OF THE UNALTERED PARENTAL BACTERIUM**

Let us now examine the previously mentioned biological characteristics individually, in a bit more detail, remaining within the boundary of *E. coli* K-12 batch fermentations. Within this fairly innocuous and relatively widely studied organism exists certain characteristics: pathogenicity, colonization ability, and gene transfer ability which can be identified as to being associated with a greater or lesser degree of potential risks to man and the environment.

Human and animal pathogenicity of the parent strain is an important initial characteristic that requires evaluation at the earliest stage in production strain development. Distinct types of *E. coli* are known to cause urinary tract infections, gastroenteritis, septicemia, wound infections and meningitis. Enteropathogenic, enteroinvasive, and enterotoxigenic *E. coli* are involved in *Salmonella*-type diarrhea, bacillary dysentery, and travelers diarrhea, respectively [2]. Mastitis can be caused by *E. coli* types as well. Piliation, which often contributes to adhesive tendencies, has been associated with virulence in *E. coli* and is a characteristic to be avoided in the selection of a suitable parent strain. In short, any characteristic that increases the potential pathogenicity of the parent strain increases the risk associated with using that strain.

Associated with pathogenicity is the colonization potential of the parent strain. Organisms that demonstrate the potential to establish and grow to sufficiently high cell density in a given environment can be associated with a greater potential to colonize humans and animals.

Colonization ability or survival potential is related to various characteristics of the organism. The competitive