Risk Assessment Outline
For Use in Preparing Risk Analyses
For Biotechnology-Derived Products

The Center for Veterinary Biologics
USDA/APHIS/VS

Risk assessment may be defined as determination of the likelihood of an adverse event occurring and the consequences if that adverse event occurs. An adverse event is defined as a safety hazard to animals, public health, or the environment. A safety hazard is defined as a danger, risk, or peril. There may be an absence of predictability associated with an adverse event.

When submitting a Risk Analysis to the Center for Veterinary Biologics (CVB) for prelicense evaluation of a biotechnology-derived product, the Risk Analysis should contain the most current version of the Summary of Information Format (SIF) and a Risk Assessment (RA) [Table 1]. The CVB requires that the applicant conduct a RA based on safety characteristics of the vaccine. The safety characteristics are based on empirical data and established scientific facts. The completion of a risk assessment requires that the vaccine microorganism is properly characterized. This information is included in Section III of the CVB SIF.

Risk Assessment Outline

A. Hazard Identification

Hazard identification consists of identifying all possible adverse events to animal safety, public health safety, and environment safety relative to recommended use of the product.

1. Animal Safety

a. Target animal safety

The safety of the experimental biologic in the target animal species is thoroughly evaluated. Several standard safety studies are available for veterinary vaccines. Thus, the safety of the experimental biologic in the target animal is based on direct scientific evidence.

i. Vaccination

The safety of the experimental biologic in the target animal species should be established. Adverse systemic reactions should be documented and described. Certain viruses are associated with

immunosuppression, e.g., feline leukemia and canine distemper. The impact of live vaccines that contain this type of virus should be thoroughly evaluated.

ii. Vaccination/Challenge

The safety of the experimental biologic when the target host is exposed to the challenge wild-type microorganism should be established. Although the induction of a specific immune response is essential for protection, its function can have adverse consequences, such as antibody-complement interaction, immune complex formation, and the generation of autoantibodies directed against normal tissues that have not themselves been infected. These examples demonstrate the importance of evaluating vaccine safety in the context of a challenge. Thus, experimental designs for immunogenicity studies should not be limited in their focus by concentrating exclusively on the efficacy of the product; the focus should be extended to include provisions for evaluating safety.

iii. Reversion to virulence

The inability of attenuated vaccine strains to revert to virulence should be established. In some cases, it may be determined that the vaccine microorganism does revert to virulence, but that the level of virulence is acceptable. This usually requires that the pathophysiology of the vaccine microorganism be thoroughly evaluated to ensure that the margin of safety will be acceptable for the target host.

iv. Purity testing

Contaminants in veterinary biologics are unacceptable. However, new techniques are providing levels of sensitivity not previously attainable. For instance, polymerase chain reaction (PCR) analysis may identify previously undetected contaminating retroviruses. In those cases, extraneous agents should be fully characterized. For example, it should be established that: 1) the detected agent is present in a replication competent form and is not just a genomic segment detected by PCR; 2) the agent is not a pathogen for the target host; 3) the agent cannot propagate in the target host; 4) the agent has no oncogenic properties; 5) the final product does not contain an infective dose of the extraneous agent(s); and 6) the margin of safety is sufficient so that the product will be safe when used in accordance with label recommendations.

v. Effect of gene manipulation on pathogenicity

Information on the genetic profile of the vaccine microorganism can contribute significantly to our understanding of its safety characteristics. This is not only applicable to genetically-engineered microorganisms, but to organisms that have been attenuated by conventional methods. The purpose is not to regulate the process used to construct the vaccine microorganism, but to identify potential safety hazards associated with any genetic modifications.

In the case of 'attenuated' vaccines, the actual attenuation may be the result of a single base pair substitution and the potential for reversion to virulence may be high.

Genetic markers may provide the means with which to monitor the dispersal or establishment of the vaccine microorganism upon its release in the environment. In some cases, genetic markers actually define the safety characteristics of the vaccine microorganism, as with *aro*A deletion mutants. The attenuation of these vaccine strains is attributable to their inability to produce para-aminobenzoic acid.

For genetically-engineered microorganisms, the phenotypic effect of any genetic modification(s), either gene insertions or deletions, should be thoroughly assessed. This includes but is limited to the following: 1) the function of the gene located at the insertion site; 2) the modification to the donor genes; 3) the molecular properties of the regulatory elements; and 4) the phenotypic effect of any marker genes (e.g., the *E. coli lac*Z gene). The previous use of the recipient microorganism, the donor gene(s), insertion site(s), or any gene deletion(s) provides valuable information and should be documented. One should not assume that certain genetic modifications will result in the expected effect.

vi. Genetic stability

The genetic stability of the vaccine microorganism *in vitro* should be evaluated at the highest passage level to be used in production of the vaccine. If stable, it suggests that the safety characteristics of the vaccine microorganism will not be altered as a result of manipulations during the manufacturing process.

Studies to evaluate the genetic stability of the microorganism *in vivo* provide significant safety information, but are not generally required.

vii. Phenotypic stability

The phenotypic stability of the vaccine microorganism provides the definitive answer to the stability of the vaccine in production or when injected in the host animal.

viii. Alteration of tissue tropism

Any alteration in tissue tropism should be reported. Changes in tissue tropism may allow the organism to shed in new ways, or there may be an alteration in its pathogenicity.

ix. Effect of overdosing

Overdosing is a standard safety test that confirms the attenuation of the vaccine microorganism. Any pathogenic effects should be identified.

b. Non-Target animal safety

Safety studies in non-target animal species should be considered when the vaccine microorganism sheds and can potentially disperse in the environment.

i. Susceptible non-target animals and probability of their exposure

Susceptible non-target hosts should be identified. Any differences between the vaccine microorganism and the parent should be documented.

ii. Virulence in non-target animals (susceptible non-target animals)

iii. Possible outcome of non-target animal exposure

The possible outcome of non-target animal exposure should be assessed. If no experiments were conducted with the recombinant agent, information on the effect of the parent organism should be discussed.

2. Public Health Safety

a. Probability of human exposure

The potential for human exposure, through both direct and indirect routes should be identified.

b. Expected pathogenicity of the parent microorganism in humans

c. Expected virulence of the vaccine microorganism in humans:

d. Possible outcome of human exposure

Potential safety hazards to public health should be identified and assessed.

3. Environmental Safety

a. Shed/Spread capabilities

b. Horizontal gene transmission/recombination potential

c. Host/Range specificity

d. Survivability of the microorganism in the environment

e. Potential for transmission to invertebrates

f. Physical and/or chemical factors affecting dispersal in the environment

g. Adverse ecological effects

B. Release Assessment Prior to a Proposed Field Safety Trial

The safety characteristics of the vaccine microorganism must be evaluated within the context of the target environment. Thus, the release assessment consists of a comprehensive evaluation of the proposed release so as to determine: 1) the location and characteristics of the release site; 2) the test dose and total amount of the experimental biologic to be used in the proposed study; 3) the frequency and duration of exposure to the test material; 4) potential escapes into occupational, residential, or outdoor environments; and 5) the individuals, populations, or ecosystems that will be, or may be, exposed to the experimental biologic.

1. Environmental Release

a. Location of test site

The exact location of the test site is identified. For proposed commercial uses, the types of the conditions under which the vaccine will be used are identified; e.g., unlimited commercial distribution and use, restricted for use by veterinarians only, small animal veterinary hospitals, commercial poultry houses, etc.

b. Characteristics of the test site

A description of the test site is provided, including relevant geographical and environmental information. The area surrounding the test site is also described,

including the presence of non-target animal species. The condition of the test site should be documented, as well as previous studies conducted on the test site.

c. Personnel

The personnel conducting the study are identified, including their qualifications, training, and specific role in the study. Appropriate safeguards, education, and training are provided as needed.

d. Experimental design

The objectives of the release are identified. For small-scale field tests, the protocol of study should include the following information, as appropriate: 1) the number of animals; 2) a description of the animals; 3) the route of administration; 4) the dose; 5) the total amount of test material; 6) frequency and duration of exposure; 7) the method of disposing of waste; 8) decontamination of the test site.

i. animals

ii. vaccine

iii.vaccination

iv. total doses of test material

v. method of disposing of waste

vi. decontamination of the test site

e. Potential for escape and dispersal

The potential for escape and dispersal from the release site should be assessed. Possible exposure to the area surrounding the test site should be considered and evaluated, including the probability of non-target animal exposure.

f. Potential for establishment in the environment

The habitability of the test site and/or environments for the introduced vaccine microorganism is appraised. The following environmental characteristics are evaluated, as appropriate: 1) the presence of other biological organisms; 2) the nutrient status; 3) physicochemical factors; 4) the presence of toxic chemicals and metabolites.

g. Monitoring

Appropriate methods and procedures for monitoring the released vaccine microorganism in and around the test site should be identified prior to initiating the study. The monitoring methods should be sensitive and specific. The frequency of the monitoring should be identified. Provisions for recording the results of the monitoring should be in place.

h. Contingency plans in case of adverse event

The sponsor of the proposed study should identify contingency plans in case an adverse event occurs. Contingency plans should include procedures for terminating the study as quickly as possible, and identify methods to stop the shed, spread, or dispersal of the vaccine microorganism once released in the environment.

C. Risk Characterization

Risk Characterization integrates the results of the hazard identification and the release assessment into a risk statement that includes: 1) a **likelihood rating**; 2) a **consequence rating**; 3) a **risk rating**; and 4) a discussion of risk. Each likelihood and consequence rating is qualified by a **Degree of certainty rating** and includes a justification for the rating. The justifications for the ratings consist of identifying the applicable sections in the hazard identification and the release assessment that support the assigned rating. The **risk rating** is based upon the likelihood, consequence, and degrees of certainty ratings.

1. Likelihood Rating

Likelihood ratings are assigned for animal safety, public safety, and environmental safety based on the following criteria:

Low	=	An adverse event is unlikely to occur
Medium	=	An adverse event could possibly occur
High	=	An adverse event will most probably occur

2. Consequence Rating

Consequence ratings are also assigned for animal safety, public safety, and environmental safety based on the following criteria:

Low	=	The consequences if the adverse event occurs will not be severe (the adverse event is self-limiting and would have negligible impact).
Medium	=	The consequences if the adverse event occurs is moderately severe (the adverse event will have an impact, but it is not permanent, and can be treated).
High	=	The consequences if the adverse event occurs are severe (the adverse event will have an impact, is permanent, and can not be treated).

3. Degree of Certainty Rating

Each likelihood and consequence rating is qualified by a degree of certainty rating that is based on the following criteria:

Certain	=	The rating is supported by direct scientific evidence.
Moderately Certain	=	The rating is supported by indirect scientific evidence.
Uncertain	=	The rating is not supported by scientific evidence.

4. Calculating the Expected Risk

Numerical values have been assigned to the likelihood, consequence, and degree of certainty ratings described in Table 2 (page 12). Each numerical value rating was derived from the importance placed on the rating of each category. The assigned numerical values are weighed to place emphasis on the severity of expected risk. These values reflect the scientific and professional judgment of the applicant and will be reviewed by the CVB. To determine the expected risk, the numerical values are multiplied.

The numerical values assigned to the "*Degree of Certainty*" reflect the level of uncertainty associated with the risk ratings. The need for two different rating systems reflects the reality of how uncertainty is perceived when handing risk. A low risk with a high degree of certainty is of **less concern** than a low risk which shows a high degree of uncertainty; a high risk with a high degree of certainty is of **more concern** than a high risk which shows a high degree of uncertainty.

This is easy to understand by using an analogy about risk. A pedestrian is less likely to cross the road if he is convinced he is going to be hit by a car than if he is not sure that he will be hit by a car (*high risk with a high degree of certainty is of **more concern** than a high risk which shows a high degree of uncertainty*). However, the same pedestrian is more likely to cross the road if he is convinced that he is not going to be hit by a car than if he thinks that he might be hit by a car (*low risk with a high degree of certainty is of **less concern** that a low risk which shows a high degree of uncertainty*). The use of the two rating systems (one a reciprocal of the other) reflects this perception of risk.

5. Risk Ratings

The **risk ratings** are based upon the likelihood, consequence, and degrees of certainty ratings and the expected risk for each category (Table 2, Page 12). A total of 81 rating combinations are possible; e.g., Likelihood Low-Moderately Certain, Consequence Low-Moderately Certain (Table 3, Page 13). Each combination has been assigned a **risk rating** of low, medium, or high. The assigned ratings were weighed to place emphasis on the severity of the expected risk. Again, the severity of the risk reflects the professional judgment of the applicant and will be reviewed by the CVB. The low, medium, or high **risk ratings** are defined for the purpose of decision-making, as follows:

Low=Acceptable risk - very little concerns are associated with the proposal (does not justify denying the proposal)

Medium=Unacceptable risk - moderate concerns are associated with the proposal (either identify valid mitigative procedures or deny the proposal).

High=Unacceptable risk - major concerns are associated with the proposal (deny the proposal).

A. Animal safety – example

Likelihood Rating: Low (LL)
Degree of Certainty Rating: Certain (C)
Consequence Rating: Low (CL)
Degree of Certainty Rating: Certain (C)
Expected Risk Rating: 1.0000
Risk Characterization: LL.C.CL.C
Risk Rating: L
Justification for Rating: (cite relevant Sections of Risk Assessment)

(i) Conclusion and discussion of risk

B. Public health safety – example

Likelihood Rating: Low
Degree of Certainty Rating: Certain
Consequence Rating: Low
Degree of Certainty Rating: Certain
Expected Risk Rating: 1.0000
Risk Characterization: LL.C.CL.C
Risk Rating: L
Justification for Rating: (cite relevant Sections of Risk Assessment)

(i) Conclusion and discussion of risk

C. Environmental safety – example

Likelihood Rating: Low
Degree of Certainty Rating: Certain
Consequence Rating: Low
Degree of Certainty Rating: Moderately Certain
Expected Risk Rating: .7500
Risk Characterization: LL.C.CL.MC
Risk Rating: L
Justification for Rating: (cite relevant Sections of Risk Assessment)

(i) Conclusion and discussion of risk

Table 1: Veterinary Biologics Risk Analysis

RISK ANALYSIS FOR VETERINARY BIOLOGICS

I. Objective/Proposal

II. Summary of Information Format (SIF) providing characterization of the vaccine microorganism (http://www.aphis.usda.gov/vs/cvb/lpd/sifs.htm)

- A. Microbiological/Molecular properties
- B. Biological properties

III. Risk assessment

- A. Hazard identification
  1. Animal safety
  2. Public health safety
  3. Environmental safety
- B. Release assessment
- C. Risk characterization

IV. Risk management

- A. Contained release – not applicable for Field Safety Trials
- B. Environmental release

V. Risk communication

- A. Finding of No Significant Impact (FONSI)
- B. Publication in the Federal Register announcing availability of an environmental assessment and CBI-deleted risk analysis

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Table 2: Calculating the Expected Risk

VALUE RATINGS

Likelihood (L)

Low (L)LL = 1.00

Medium (M)LM = 0.50

High (H)LH = 0.10

Consequence (C)

Low (L)CL = 1.00

Medium (M)CM = 0.10

High (H)CH = 0.01

If the Likelihood rating is Medium or High and the Consequence rating is also Medium or High use *Degree of Certainty Ratings I*; for all other combinations use *Degree of Certainty Ratings II*.

Degree of Certainty Ratings I

Certain (C) C = 0.50

Moderately Certain (MC)MC = 0.75

Uncertain (U) U = 1.00

Degree of Certainty Ratings II

Certain (C) C = 1.00

Moderately Certain (MC)MC = 0.75

Uncertain (U) U = 0.50

EXPECTED RISK
[(likelihood) x (degree of certainty)] x [(consequence) x (degree of certainty)] = Risk Rating

Table 3: Risk Ratings

Risk Characterization	Expected Risk	Risk Rating	Risk Characterization	Expected Risk	Risk Rating
LL.C.CL.C.	1.0000	L	LM.C.CM.C.	.0125	M
LL.C.CL.MC.	.7500	L	LH.U.CM.U.	.0100	M
LL.MC.CL.C.	.7500	L	LL.C.CH.C.	.0100	M
LL.MC.CL.MC.	.5625	L	LH.MC.CM.U.	.0075	M
LL.C.CL.U.	.5000	L	LH.U.CM.MC.	.0075	M
LL.U.CL.C.	.5000	L	LL.C.CH.MC.	.0075	M
LM.C.CL.C.	.5000	L	LL.MC.CH.C.	.0075	M
LL.MC.CL.U.	.3750	M	LH.MC.CM.MC.	.0056	M
LL.U.CL.MC.	.3750	M	LL.MC.CH.MC.	.0056	M
LM.C.CL.MC.	.3750	M	LH.C.CM.U.	.0050	M
LM.MC.CL.C.	.3750	M	LH.U.CM.C.	.0050	M
LM.MC.CL.MC.	.2813	M	LL.C.CH.U.	.0050	M
LL.U.CL.U.	.2500	M	LL.U.CH.C.	.0050	M
LM.C.CL.U.	.2500	M	LM.U.CH.U.	.0050	M
LM.U.CL.C.	.2500	M	LH.C.CM.MC.	.0038	M
LM.MC.CL.U.	.1875	M	LH.MC.CM.C.	.0038	M
LM.U.CL.MC.	.1875	M	LL.MC.CH.U.	.0038	M
LM.U.CL.U.	.1250	M	LL.U.CH.MC.	.0038	M
LH.C.CL.C.	.1000	M	LM.MC.CH.U.	.0038	M
LL.C.CM.C.	.1000	M	LM.U.CH.MC.	.0038	M
LH.C.CL.MC.	.0750	M	LM.MC.CH.MC.	.0028	M
LH.MC.CL.C.	.0750	M	LH.C.CM.C.	.0025	M
LL.C.CM.MC.	.0750	M	LL.U.CH.U.	0025	M
LL.MC.CM.C.	.0750	M	LM.C.CH.U.	.0025	M
LH.MC.CL.MC.	.0563	M	LM.U.CH.C.	.0025	M
LL.MC.CM.MC.	.0563	M	LM.C.CH.MC.	.0019	H
LH.C.CL.U.	.0500	M	LM.MC.CH.C.	.0019	H
LH.U.CL.C.	.0500	M	LM.C.CH.C.	.0013	H
LL.C.CM.U.	.0500	M	LH.U.CH.U.	.0010	H
LL.U.CM.C.	.0500	M	LH.MC.CH.U.	.0008	H
LM.U.CM.U.	.0500	M	LH.U.CH.MC.	.0008	H
LH.MC.CL.U.	.0375	M	LH.MC.CH.MC.	.0006	H
LH.U.CL.MC.	.0375	M	LH.C.CH.U.	.0005	H
LL.MC.CM.U.	.0375	M	LH.U.CH.C.	0005	H
LL.U.CM.MC.	.0375	M	LH.C.CH.MC.	.0004	H
LM.MC.CM.U.	.0375	M	LH.MC.CH.C.	.0004	H
LM.U.CM.MC.	.0375	M	LH.C.CH.C.	.0003	H
LM.MC.CM.MC.	.0281	M			
LH.U.CL.U.	.0250	M			
LL.U.CM.U.	.0250	M			
LM.C.CM.U.	.0250	M			
LM.U.CM.C.	.0250	M			
LM.C.CM.MC.	.0188	M			
LM.MC.CM.C.	.0188	M			