



Animal and Plant
Health Inspection
Service

Veterinary Services

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VETERINARY SERVICES MEMORANDUM NO. 800.51

TO: Veterinary Services Leadership Team
Directors, Center for Veterinary Biologics
Biologics Licensees, Permittees, and Applicants

FROM: for Jack A. Shere
Deputy Administrator

SUBJECT: Additives in Administered Animal Biological Products

I. PURPOSE

This memorandum clarifies policies and procedures to comply with the requirements of title 9, *Code of Federal Regulations* (9 CFR), parts 103.2(b) and (c), 103.3(f) and (g), 112.2(a)(8), and 114.10. These regulations cover slaughter withholding periods for food animals when those animals have been administered biological products formulated with additives, and requirements for ingredients of animal origin.

II. CANCELLATION

This memorandum cancels Veterinary Services Memorandum No. 800.51, dated November 1, 2016.

III. BACKGROUND

- A. Additives. Additives in veterinary biologicals may consist of adjuvants, carriers, inactivating agents, preservatives, or other ingredients added to cultures of microorganisms in the formulation of biological products used to treat animals.
- B. Adjuvants. Adjuvants are additives used to enhance the immunogenicity of the antigen.
- C. Authority and Consultation. 9 CFR 112.2(a)(8) prescribes a withholding period of not less than 21 days for food animals treated with veterinary biological products. APHIS may prescribe a longer withholding period on review of data regarding the use of additives in products used to treat animals.

IV. SPECIFIC GUIDANCE REGARDING ADDITIVE SOURCES

A. Ingredients of Animal Origin

1. Each lot of ingredient additive of animal origin used to prepare an administered biological product must be sterilized or tested as prescribed in 9 CFR 113.53.
2. Ingredients of animal origin are sourced from the United States or countries considered free, low risk, or not affected with foreign animal diseases of concern and with negligible or controlled risk of bovine spongiform encephalopathy (BSE), according to APHIS' Animal Disease Status designations, which may be viewed at:

<https://www.aphis.usda.gov/aphis/ourfocus/animalhealth/animal-and-animal-product-import-information/animal-health-status-of-regions>

The foreign animal diseases of concern to the Center for Veterinary Biologics (CVB) are:

- Foot-and-mouth disease (FMD),
- African swine fever (ASF),
- Classical swine fever (CSF),
- Highly pathogenic avian influenza (HPAI), and
- Newcastle disease (ND).

No animal ingredients of avian origin may be sourced from a country if HPAI or ND is present in any region of the country. No ingredients of mammalian origin may be sourced from a country if FMD, ASF, or CSF is present in any region of the country. If an outbreak of any of these diseases occurs in countries providing ingredients of animal origin, consult the CVB for an assessment of risk. A risk assessment, including an analysis of the risk of contamination to licensed biologics, must be submitted to the CVB for review to justify using an ingredient of animal origin from a country that has a foreign animal disease of concern.

This guidance does not abrogate any other import requirements; all National Import and Export Services restrictions apply.

3. Manufacturers must maintain a comprehensive list of all ingredients of animal origin used in production of biological products. This list should include the name of the material, the supplier, the country of origin, and the date of purchase of each lot. This list may be reviewed and certification of materials required at the time of inspection by the CVB-Inspection and Compliance (IC), or as requested by the CVB.

- B. Ingredients Not of Animal Origin. Ingredients or other substances used in biological products not of animal origin must meet acceptable standards for purity and quality as specified in 9 CFR 113.50 and the filed Outline of Production.

V. SPECIFIC GUIDANCE REGARDING PRESERVATIVES

- A. Antibiotics added to production media or at any subsequent stage of the manufacturing process are considered preservatives. The regulations at 9 CFR 114.10 specify the maximum permissible amounts and combinations of antibiotics authorized for use as preservatives in veterinary biologics. The maximum allowable concentrations of antibiotics listed are expected to inhibit growth of common susceptible bacterial contaminants, and a definite genetic determinant for resistance would be necessary for survival at these concentrations. Conventionally, amphotericin B has been dissolved in a small volume of dimethyl sulfoxide (DMSO). Amphotericin B dissolved in DMSO is permitted as stated in the Amphotericin B general requirements in 9 CFR 113.10.
- B. Antibiotics used in virus seed stock purification are not restricted as to amount if carryover into the final product is controlled and specified in the Outline of Production. The CVB considers carryover to be controlled if the antibiotics used in seed stock purification are added to the seed stock culture in concentrations no greater than generally accepted inhibitory levels for susceptible contaminants and/or the resultant culture media is decanted or filtered.
- C. A request to use an antibiotic or antibiotic combination not specified in 9 CFR 114.10(b) or (c) as a preservative may be considered under certain conditions. A request for use should include the reason why the antibiotic or antibiotic combination is needed, a risk assessment, the expected levels of the antibiotic in the final product, and data indicating the residual antibiotic level in tissues of animals to which the product is administered.

VI. SPECIFIC GUIDANCE REGARDING SLAUGHTER WITHDRAWAL PERIODS/ ADJUVANT APPROVALS FOR INJECTABLE PRODUCTS

- A. Describe the Adjuvant. All product licensing packages should include a detailed description of the formulation of any adjuvants included in the product. This information should be provided early in the licensing process. In general, all product license applications should contain the following information:
 - 1. Generic name of adjuvant (and trade name, if applicable).

2. Chemical composition of adjuvant (list all ingredients and proportions).
3. Amount of completed/total adjuvant per dose of product and dose volume of product. Include the percentage of adjuvant per dose.
4. Animal species in which product is to be used.
5. Route of administration.
6. Information regarding source, grade, and quality of each ingredient.
7. Any tests performed on ingredient lots and the finished adjuvant prior to use.
8. Proposed slaughter withdrawal period and supporting data.
9. Other products for which the adjuvant has been approved, if applicable. It is understood that in some cases, adjuvants are purchased from another firm; and, therefore, some of the data listed may be proprietary information of the supplier and may not be available to the recipient. In this case, it is acceptable for the recipient to request that the supplier submit the necessary information to the CVB in a manner that protects confidential business information.

B. CVB Review of Adjuvant Description. The adjuvant description and composition will be compared to historic data on file at the CVB. Comparison of the adjuvant description and composition with historic data may indicate that these data, in addition to satisfactory field safety trial results, are adequate to approve the use of the adjuvant in the new product.

C. Additional data may be required for previously-approved adjuvants used in new ways. To approve new adjuvants or previously-approved adjuvants used at increased levels, administered via different routes of administration, or proposed for shorter withdrawal periods, results of an injection site study in the host animal may be requested. The following guidelines should be considered when conducting an injection site study:

1. Submit a protocol for comment. The protocol should include key dates to allow observation by CVB personnel.
2. At least 10 animals of the minimum age of the target species should be included for adjuvants used in food-producing species other than fish and poultry.

- a. The proposed product should be administered as per the proposed label directions, and the withdrawal period should be evaluated after the last dose is administered.
 - b. Typically, the new adjuvant should be compared to the product-matched placebo by injecting the new product on one side of the animal, and the placebo at the same site on the other side of the animal. For each animal, assign the products randomly to the two sides. Comparing more than two products within an animal often calls for a more complex randomization plan that should be discussed with the CVB. Exceptions to using product-matched placebos may be considered. This issue will be addressed in the review of the protocol by the CVB.
 - c. The injection sites should be examined grossly by a veterinarian or veterinary pathologist blinded to the study, and results of the gross pathologic examination should be included in the report. The veterinarian/pathologist should also collect appropriate tissues for histopathology, including the injection sites (adjuvant and placebo). Samples for histopathology should be collected at the time of the proposed slaughter withdrawal period (i.e., if the proposed slaughter withdrawal period is 21 days, samples should be collected 21 days after injection). Photographs of any gross lesions at the injection site should be included in the final report.
 - d. Tissue samples should be examined histologically by a board certified pathologist who has no knowledge regarding the products used in the study; photomicrographs of histologic changes present at the injection site should be included in the final report. The histologic evaluation is conducted to evaluate and ensure that the local inflammatory response is consistent with resolution of the expected physiologic/immunologic response to foreign material in the respective tissue. For example, observations of ongoing inflammation that are inconsistent with resolution may require additional evaluation and/or longer withdrawal periods.
 - e. Collecting biopsies after local anesthesia, or using alternate methods to salvage study animals, may be acceptable.
3. For poultry, use at least 10 birds of minimum age of the target species. The new adjuvant should be compared to a product-matched placebo by injecting the new product on one side of the animal, and the product-matched placebo at the same site on the other side of the animal. Injecting the same bird with the placebo and the proposed product is not required if impractical or if two doses would cause deleterious effects not related to

adjuvant safety. (For example, if day-old chicks are inoculated, it would be acceptable to include a group of birds inoculated with product and a group of birds inoculated with matched placebo.) As with injection site studies for other species of animals, samples should be collected and evaluated as per sections VI.C.2.b and VI.C.2.c of this document.

4. At least 20 fish of minimum age/size of the species in which the product is to be used should be included for injection site studies in aquatic species meant for human consumption. The study may be done using the same dose as per label recommendations, or at double the dose recommended on the label.
 - a. Many adjuvants cause some degree of tissue adhesion and pigmentation in the abdominal cavity of fish when administered intraperitoneally. The Speilberg Scoring System, based on the size and density of the adhesion, should be used to analyze data from aquatic species. The study will be evaluated for degree of tissue pigmentation and adhesion in the abdominal cavity, as well as residual vaccine or vaccine components in the abdominal cavity.
 - b. Residual vaccine or vaccine components present at slaughter in edible portions of fish are not acceptable.
 - c. Injection sites should be examined grossly by a veterinarian, veterinary pathologist, or fish health specialist blinded to the study, and results of the gross pathologic examination should be included in the report. Samples for histopathology should also be collected and evaluated, as described in section VI.C.2.C of this document.
5. For animals not meant for human consumption (e.g., dogs, cats), the results of an acceptable field safety study are adequate to demonstrate safety of the adjuvant.
6. Summarize results from infection site studies with descriptive statistics. Avoid statistical inference that may not be supported by the study size or design.
7. Data generated from field safety or efficacy studies might support slaughter withdrawal periods of adjuvanted products when an injection site study has been accepted using a different antigen. Data will be considered on a case-by-case basis.
8. A single injection site study may be used to support the slaughter withdrawal period of not only a specific adjuvant, but also other adjuvants

formulated with lesser amounts of the same components, when used in the same species of animal and administered by the same route of inoculation.

9. Injection site study results such as the following would be considered unacceptable:
 - a. Significant gross lesions (e.g., purulent or caseous material) present at the proposed slaughter withdrawal time.
 - b. Indications of active inflammation at the time of slaughter.
 - c. Lesions observed histologically that are inconsistent with gross lesions observed.
- D. Additional information that may be included as supporting data in an injection site study report:
 1. Summary of other studies (e.g., peer-reviewed publications, relevant internal reports) that contain pathological assessments of the experimental product when administered to the target species.
 2. Data showing the time required for the outward resolution of any injection site reactions (obtained from the injection site study report, field safety report, and/or efficacy study report of the product to be licensed).
 3. Results of safety studies in laboratory animals.
- E. Additional considerations for novel adjuvants or other additives. For new additives, including unique adjuvants that have not been included in previously approved products, the following information may be required:
 1. Toxicological profile, which should include:
 - a. Any information relative to the listing of the additives on lists of approved additives (e.g., Generally Regarded as Safe (GRAS), Drinking Water Standards). Provide a copy of the Material Safety Data Sheet (MSDS) or reference the MSDS number for each additive, if available.
 - b. The results of toxicological studies to determine the local and/or systemic effects of the additive on laboratory animals, if available.
 - c. Summary of any oral/acute testing of the additive in target and non-target species.

- d. Summary of any information available regarding the metabolism of the additive.
 - e. Any information available regarding the carcinogenicity of the additive.
 - f. Any known reactivity of each additive.
 - g. Pharmacological activity of each additive.
2. Human exposure profile, which should include:
- a. Estimate of the total volume/mass of the additive that will be administered to the target animal under the proposed instructions for use.
 - b. Estimate of the human consumption/exposure to each additive.
 - c. Levels of residue in tissue at proposed withdrawal period. The levels should not exceed the Food and Drug Administration (FDA) tolerances for food, if available.
 - d. FDA tolerance level established for each additive (cite source).

VII. EXEMPTIONS TO SLAUGHTER WITHDRAWAL PERIOD REQUIREMENTS

- A. No slaughter withdrawal statement is needed for a non-parenteral (e.g., oral, intranasal, immersion) product used solely for neonatal animals that do not enter the food chain.
- B. Non-parenteral biologics used for food-producing animals entering the food chain typically are assigned a slaughter withdrawal period of 21 days.

VII. IMPLEMENTATION

APHIS expects to implement this guidance within 2 years from the date of this memorandum.