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United States Department of Agriculture

Animal and Plant Health Inspection Service

Veterinary Services

Washington, DC 20250

VETERINARY SERVICES MEMORANDUM NO. 800.51

TO: VS Management Team
Directors, Center for Veterinary Biologics
Biologics Licensees, Permittees, and Applicants

FROM: John R. Clifford /s/John R. Clifford
Deputy Administrator

SUBJECT: Additives in Animal Biological Products

I. PURPOSE

The purpose of this memorandum is to clarify policies and procedures to comply with the requirements of 9 CFR 103.2(b) and (c), 103.3(f) and (g), and 112.2(a)(8). These regulations pertain to slaughter withholding periods for animals used for food purposes after those animals have been treated with biological products formulated with additives.

II. CANCELLATION

This memorandum cancels Veterinary Services Memorandum No. 800.51, dated April 27, 2000.

III. BACKGROUND

A. Additives—Additives in veterinary biologicals may consist of adjuvants, carriers, inactivating agents, preservatives, or other substances or ingredients that are added to cultures of microorganisms in the formulation of biological products that are used to treat animals.

B. Adjuvants—Adjuvants are additives that may be added to a biological product to enhance the immunogenicity of the antigen.

C. Authority and Consultation—The regulations in 9 CFR 112.2(a)(8) prescribe a withholding period of not less than 21 days for animals that may be used for food purposes that have been treated with veterinary biological products containing additives. APHIS may prescribe a longer withholding period upon review of data regarding the use of additives in products used to treat animals.

IV. SOURCE OF ADDITIVES

A. Ingredients of Animal Origin

1. Each lot of ingredient additive of animal origin used to prepare a biological product must be sterilized, or tested as prescribed in 9 CFR 113.53.

2. Ingredients of animal origin should be sourced from the United States or countries whose Bovine Spongiform Encephalopathy (BSE) status is either no risk or minimal risk, as defined by the National Center for Import and Export (NCIE) and 9 CFR 94.18. A statement certifying that ingredients of animal origin are sourced from countries where BSE is not known to exist or from countries considered to be minimal risk as defined by the NCIE and 9 CFR 94.18 should be included in Section II of each Outline of Production.

3. A comprehensive list of all ingredients of animal origin used in production of biological products should be maintained. 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 Center for Veterinary Biologics-Inspection and Compliance (CVB-IC), or at other times as requested by CVB.

B. Ingredients Not of Animal Origin—Ingredients or other substances used in biological products that are 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

A. Describe the Adjuvant - All product licensing packages should include a thorough description of any adjuvants included in the product. Ideally, 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,

4. Animal species in which product is to be used,

5. Route of administration (and specific anatomical site, if designated),

6. Information regarding source, grade, and quality of each adjuvant lot,

7. Any tests performed on adjuvant lots prior to use,

8. Proposed slaughter withdrawal period [at least 21 days is required as per 9 CFR 112.2(a)(8)], and

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 the proprietary information of the supplier and may not be available to an applicant buying the materials from that source. In this case, it is acceptable for the applicant to arrange to have the adjuvant supplier submit adjuvant 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 regarding adjuvants on file at the CVB. The review process may be expedited if data regarding previously approved adjuvants have already been submitted to 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.

Additional data may be required, however, for previously approved adjuvants that are to be included in products in unique ways. In order to approve new adjuvants, or to approve 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 in order to develop a complete pathology profile.

Any adjuvant may be reevaluated if field safety trial results indicate this is warranted.

C. Injection Site Study - In order to approve new adjuvants, or to approve 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 if results of an injection site study are required.

1. A protocol should be submitted prior to conducting the study so that CVB may comment on the study design. Critical study dates should be included to allow observation by CVB personnel, if deemed necessary.

2. At least 10 animals of the minimum age of the species in which the product is to be used should be included for adjuvants used in food-producing species other than fish and poultry.

   a. Compare the injection site of the new adjuvant to a 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 CVB. For new products, it may be acceptable to not include the placebo. This issue will be addressed in the review of the protocol by the CVB.

b. The injection sites should be examined grossly by a blinded veterinarian or veterinary pathologist and results of the gross pathologic examination should be included in the report. The veterinarian/pathologist should also collect appropriate tissues for histopathology. Histopathologic sections should be taken at the time of the proposed slaughter withdrawal period (i.e. if the proposed slaughter withdrawal period is 21 days, histopathologic sections should be taken 21 days after injection). Photographs of any gross pathologic lesions should be included in the final report.

c. Tissue samples should be analyzed histopathologically by a board certified pathologist who has no knowledge regarding the products used in the study and photographs of the histopathologic sections of the injection site should be included in the final report. The histological evaluation is conducted to evaluate and ensure that the local inflammatory response is consistent with the expected physiological/immunological response to foreign material in the respective tissue. Observations that are inconsistent with the expected process may require additional evaluation.

3. At least 10 birds of minimum age of the species in which the product is to be used should be included for adjuvants to be used in poultry. The injection site of 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 (see Section IV.C.1.a). Comparison of two products is not required if impractical or if two doses would cause deleterious effects not related to adjuvant safety or clearance.

4. For non-food-producing animals like dogs and cats that are not meant for human consumption, the results of an acceptable field safety study are adequate to demonstrate safety of the adjuvant.

5. At least 20 fish of minimum age/size of the species the product is to be used in 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,[1] 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 in edible portions of fish present at slaughter are not acceptable.

6. *Additional information that may be included as supporting data in an injection site study report:*

a. Summary of any available studies (i.e. peer reviewed publications, relevant internal reports) that contain pathologic assessments of the experimental product when administered to the target species.

b. 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).

c. Results of safety studies in laboratory animals

D. Additional Considerations for novel adjuvants or other additives - For new additives, including adjuvants, that are unique and 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 (i.e. Generally Regarded as Safe (GRAS), Annex II, Drinking Water Standards, etc.). 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 additive. Cite source.

VI. EXEMPTIONS TO SLAUGHTER WITHDRAWAL PERIOD REQUIREMENTS

No slaughter withdrawal statement is needed for a noninjectable (i.e. oral, intranasal, immersion, etc.) product clearly labeled solely for neonatal animals because they do not enter the food chain.

Noninjectable biologics used for food producing animals entering the food chain must have a slaughter withdrawal period of not less than 21 days, as per 9 CFR 112.2(a)(8).

VII. REFERENCE

[1] Midtylyng, et al, *Experimental studies on the efficacy and side-effects of intraperitoneal vaccination of Atlantic salmon (Salmo salar) against Furunculosis*, in "Fish and Shellfish Immunology," (1996) 6, 335-350.