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TME must retain documentation of compliance with any restrictions imposed by EPA when it grants the TME. This information must be retained for 3 years from the final date of manufacture or import under the exemption.

§ 725.355 Information to be included in the TME application.

(a) To review a TME application, EPA must have sufficient information to permit a reasoned evaluation of the health and environmental effects of the planned test marketing activity. The person seeking EPA approval must submit all information known to or reasonably ascertainable by the person on the microorganism and the test marketing activity, including information not listed in paragraphs (c), (d), and (e) of this section that the person believes will demonstrate that the microorganism will not present an unreasonable risk of injury to health or the environment as a result of the test marketing. The TME application must be in writing and must include at least the information described in paragraphs (b), (c), (d), and (e) of this section.

(b) When specific information is not submitted, an explanation of why such information is not available or not applicable must be included.

(c) Persons applying for a TME must submit the submitter identification and microorganism identity information required for MCANs in § 725.155(c), (d)(1), and (d)(2).

(d) Persons applying for a TME must submit phenotypic and ecological characteristics information required in § 725.155(d)(3) as it relates directly to the conditions of the proposed test marketing activity.

(e) Persons applying for a TME must also submit the following information about the proposed test marketing activity:

(1) *Proposed test marketing activity.* (i) The maximum quantity of the microorganism which the applicant will manufacture or import for test marketing.

(ii) The maximum number of persons who may be provided the microorganism during test marketing.

(iii) The maximum number of persons who may be exposed to the microorganism as a result of test marketing, in-

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cluding information regarding duration and route of such exposures.

(iv) A description of the test marketing activity, including its duration and how it can be distinguished from full-scale commercial production and research and development activities.

(2) *Health and environmental effects data.* All existing data regarding health and environmental effects of the microorganism must be reported in accordance with § 725.160.

§ 725.370 EPA review of the TME application.

General procedures for review of all submissions under this part are contained in §§ 725.28 through 725.60. In addition, the following procedures apply to EPA review of TME applications submitted under this subpart:

(a) No later than 45 days after EPA receives a TME, the Agency will either approve or deny the application.

(b) A submitter may only proceed with test marketing activities after receipt of EPA approval.

(c) In approving a TME application, EPA may impose any restrictions necessary to ensure that the microorganism will not present an unreasonable risk of injury to health and the environment as a result of test marketing.

Subpart G—General Exemptions for New Microorganisms

§ 725.400 Scope and purpose.

(a) This subpart describes exemptions from reporting under subpart D of this part, and from review under this part altogether, for manufacturing and importing of certain new microorganisms for commercial purposes.

(b) Recipient microorganisms eligible for the tiered exemption from review under this part are listed in § 725.420.

(c) Criteria for the introduced genetic material contained in the new microorganisms are described in § 725.421.

(d) Physical containment and control technologies are described in § 725.422.

(e) The conditions for the Tier I exemption are listed in § 725.424.

(f) In lieu of complying with subpart D of this part, persons using recipient microorganisms eligible for the tiered

exemption may submit a Tier II exemption request. The limited reporting requirements for the Tier II exemption, including data requirements, are described in §§ 725.450 and 725.455.

(g) EPA review procedures for the Tier II exemption are set forth in § 725.470.

(h) Subparts A through C of this part apply to any submission under this subpart.

§ 725.420 Recipient microorganisms.

The following recipient microorganisms are eligible for either exemption under this subpart:

- (a) *Acetobacter aceti*.
- (b) *Aspergillus niger*.
- (c) *Aspergillus oryzae*.
- (d) *Bacillus licheniformis*.
- (e) *Bacillus subtilis*.
- (f) *Clostridium acetobutylicum*.
- (g) *Escherichia coli* K-12.
- (h) *Penicillium roqueforti*.
- (i) *Saccharomyces cerevisiae*.
- (j) *Saccharomyces uvarum*.

§ 725.421 Introduced genetic material.

For a new microorganism to qualify for either exemption under this subpart, introduced genetic material must meet all of the criteria listed in this section.

(a) *Limited in size*. The introduced genetic material must consist only of the following:

- (1) The structural gene(s) of interest.
- (2) The regulatory sequences permitting the expression of solely the gene(s) of interest.
- (3) Associated nucleotide sequences needed to move genetic material, including linkers, homopolymers, adaptors, transposons, insertion sequences, and restriction enzyme sites.
- (4) The nucleotide sequences needed for vector transfer.
- (5) The nucleotide sequences needed for vector maintenance.

(b) *Well-characterized*. For introduced genetic material, well-characterized means that the following have been determined:

- (1) The function of all of the products expressed from the structural gene(s).
- (2) The function of sequences that participate in the regulation of expression of the structural gene(s).

(3) The presence or absence of associated nucleotide sequences and their associated functions, where associated nucleotide sequences are those sequences needed to move genetic material including linkers, homopolymers, adaptors, transposons, insertion sequences, and restriction enzyme sites.

(c) *Poorly mobilizable*. The ability of the introduced genetic material to be transferred and mobilized is inactivated, with a resulting frequency of transfer of less than 10^{-8} transfer events per recipient.

(d) *Free of certain sequences*. (1) The introduced genetic material must not contain a functional portion of any of the toxin-encoding sequences described in this paragraph (d).

(i) For the purposes of this section, a functional portion of a toxin-encoding sequence means any sequence which codes for a polypeptide that has one of the following effects:

(A) It directly or indirectly contributes to toxic effects in humans. Directly contributes to toxic effects in humans means those sequences encoding polypeptides that have direct toxicity to target cells. An example of a sequence which directly contributes to toxic effects in humans is one which encodes the portion of diphtheria toxin, listed in paragraph (d)(2) of this section, capable of interacting with elongation factor 2, leading to inhibition of protein synthesis in target respiratory, heart, kidney, and nerve tissues. Indirectly contributes to toxic effects in humans means a sequence whose encoded polypeptide is not directly toxic to target cells, yet still adversely affects humans. An example of a sequence which indirectly contributes to toxic effects is the sequence which encodes the portion of the botulinum toxin, listed in paragraph (d)(3) of this section, capable of blocking the release of acetylcholine from gangliosides. Botulinum toxin affects neuromuscular junctions by its blockage of acetylcholine release, leading to irreversible relaxation of muscles and respiratory arrest.

(B) It binds a toxin or toxin precursor to target human cells.

(C) It facilitates intracellular transport of a toxin in target human cells.

(ii) While these toxins are listed (with synonyms in parentheses) in paragraphs (d)(2) through (d)(7) of this section according to the source organism, it is use of the nucleotide sequences that encode the toxins that is being restricted and not the use of the source organisms. The source organisms are listed to provide specificity in identification of sequences whose use is restricted. Although similar or identical sequences may be isolated from organisms other than those listed below in paragraphs (d)(2) through (d)(7) of this section, these comparable toxin sequences, regardless of the organism from which they are derived, must not be included in the introduced genetic material.

(2) *Sequences for protein synthesis inhibitor.*

Sequence Source	Toxin Name
<i>Corynebacterium diphtheriae</i> & <i>C. ulcerans</i>	Diphtheria toxin
<i>Pseudomonas aeruginosa</i>	Exotoxin A
<i>Shigella dysenteriae</i>	Shigella toxin (Shiga toxin, Shigella dysenteriae type I toxin, Vero cell toxin)
<i>Abrus precatorius</i> , seeds	Abrin
<i>Ricinus communis</i> , seeds	Ricin

(3) *Sequences for neurotoxins.*

Sequence Source	Toxin Name
<i>Clostridium botulinum</i>	Neurotoxins A, B, C1, D, E, F, G (Botulinum toxins, botulin toxins)
<i>Clostridium tetani</i>	Tetanus toxin (tetanospasmin)
<i>Proteus mirabilis</i>	Neurotoxin
<i>Staphylococcus aureus</i>	Alpha toxin (alpha lysin)
<i>Yersinia pestis</i>	Murine toxin
Snake toxins	
<i>Bungarus caeruleus</i>	Caeruleotoxin
<i>Bungarus multicinctus</i>	Beta-bungarotoxin (phospholipase)
<i>Crotalus</i> spp.	Crotoxin (phospholipase)
<i>Dendroaspis viridis</i>	Neurotoxin
<i>Naja naja</i> varieties	Neurotoxin
<i>Notechia scutatus</i>	Notexin (phospholipase)
<i>Oxyuranus scutellatus</i>	Taipoxin
Invertebrate toxins	
<i>Chironex fleckeri</i>	Neurotoxin
<i>Androctonus australis</i>	Neurotoxin
<i>Centruroides sculpturatus</i>	Neurotoxin

(4) *Sequences for oxygen labile cytolysins.*

Sequence Source	Toxin Name
<i>Bacillus alve</i>	Alveolysin
<i>Bacillus cereus</i>	Cereolysin
<i>Bacillus laterosporus</i>	Laterosporolysin
<i>Bacillus thuringiensis</i>	Thuringiolysin
<i>Clostridium bifermentans</i>	Lysin
<i>Clostridium botulinum</i>	Lysin

Sequence Source	Toxin Name
<i>Clostridium caproicum</i>	Lysin
<i>Clostridium chauvoei</i>	Delta-toxin
<i>Clostridium histolyticum</i>	Epsilon-toxin
<i>Clostridium novyi</i>	Gamma-toxin
<i>Clostridium oedematiens</i>	Delta-toxin
<i>Clostridium perfringens</i>	Theta-toxin (Perfringolysin)
<i>Clostridium septicum</i>	Delta-toxin
<i>Clostridium sordellii</i>	Lysin
<i>Clostridium tetani</i>	Tetanolysin
<i>Listeria monocytogenes</i>	Listeriolysin (A B)
<i>Streptococcus pneumoniae</i>	Pneumolysin
<i>Streptococcus pyogenes</i>	Streptolysin O (SLO)

(5) *Sequences for toxins affecting membrane function.*

Sequence Source	Toxin Name
<i>Bacillus anthracis</i>	Edema factor (Factors I II); Lethal factor (Factors II III)
<i>Bacillus cereus</i>	Enterotoxin (diarrheagenic toxin, mouse lethal factor)
<i>Bordetella pertussis</i>	Adenylate cyclase (Heat-labile factor); Pertussigen (pertussis toxin, islet activating factor, histamine sensitizing factor, lymphocytosis promoting factor)
<i>Clostridium botulinum</i>	C2 toxin
<i>Clostridium difficile</i>	Enterotoxin (toxin A)
<i>Clostridium perfringens</i>	Beta-toxin; Delta-toxin
<i>Escherichia coli</i> & other Enterobacteriaceae spp.	Heat-labile enterotoxins (LT); Heat-stable enterotoxins (STa, ST1 subtypes ST1a ST1b; also STb, STII)
<i>Legionella pneumophila</i>	Cytolysin
<i>Vibrio cholerae</i> & <i>Vibrio mimicus</i>	Cholera toxin (cholera toxin)

(6) *Sequences that affect membrane integrity.*

Sequence Source	Toxin Name
<i>Clostridium bifermentans</i> & other <i>Clostridium</i> spp	Lecithinase
<i>Clostridium perfringens</i>	Alpha-toxin (phospholipase C, lecithinase); Enterotoxin C, lecithinase (phospholipase C), Ovis toxin (sphingomyelinase D)
<i>Corynebacterium pyogenes</i> & other <i>Corynebacterium</i> spp.	Beta-lysin (beta toxin)
<i>Staphylococcus aureus</i>	Beta-lysin (beta toxin)

(7) *Sequences that are general cytotoxins.*

Sequence Source	Toxin Name
<i>Adenia digitata</i>	Modeccin
<i>Aeromonas hydrophila</i>	Aerolysin (beta-lysin, cytotoxic lysin)
<i>Clostridium difficile</i>	Cytotoxin (toxin B)
<i>Clostridium perfringens</i>	Beta-toxin; Epsilon-toxin; Kappa-toxin
<i>Escherichia coli</i> & other Enterobacteriaceae spp.	Cytotoxin (Shiga-like toxin, Vero cell toxin)
<i>Pseudomonas aeruginosa</i>	Proteases
<i>Staphylococcus aureus</i>	Gamma lysin (Gamma toxin); Enterotoxins (SEA, SEB, SEC, SED SEE); Pyrogenic exotoxins A B; Toxic shock syndrome toxins (TSST-1)

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Sequence Source	Toxin Name
<i>Staphylococcus aureus</i> & <i>Pseudomonas aeruginosa</i>	Leucocidin (leukocidin, cytotoxin)
<i>Streptococcus pyogenes</i>	Streptolysin S (SLS); Erythrogenic toxins (scarlet fever toxins, pyrogenic exotoxins)
<i>Yersinia enterocolitica</i>	Heat-stable enterotoxins (ST)

§ 725.422 Physical containment and control technologies.

The manufacturer must meet all of the following criteria for physical containment and control technologies for any facility in which the new microorganism will be used for a Tier I exemption; these criteria also serve as guidance for a Tier II exemption.

- (a) Use a structure that is designed and operated to contain the new microorganism.
- (b) Control access to the structure.
- (c) Provide written, published, and implemented procedures for the safety of personnel and control of hygiene.
- (d) Use inactivation procedures demonstrated and documented to be effective against the new microorganism contained in liquid and solid wastes prior to disposal of the wastes. The inactivation procedures must reduce viable microbial populations by at least 6 logs in liquid and solid wastes.
- (e) Use features known to be effective in minimizing viable microbial populations in aerosols and exhaust gases released from the structure, and document use of such features.
- (f) Use systems for controlling dissemination of the new microorganism through other routes, and document use of such features.
- (g) Have in place emergency clean-up procedures.

§ 725.424 Requirements for the Tier I exemption.

(a) *Conditions of exemption.* The manufacture or import of a new microorganism for commercial purposes is not subject to review under this part if all of the following conditions are met for all activities involving the new microorganism:

- (1) The recipient microorganism is listed in and meets any requirements specified in § 725.420.
- (2) The introduced genetic material meets the criteria under § 725.421.

(3) The physical containment and control technologies of any facility in which the microorganism will be manufactured, processed, or used meet the criteria under § 725.422.

(4) The manufacturer or importer submits a certification described in paragraph (b) of this section to EPA at least 10 days before commencing initial manufacture or import of a new microorganism derived from a recipient microorganism listed in § 725.420.

(5) The manufacturer or importer complies with the recordkeeping requirements of § 725.65 and maintains records for the initial and subsequent uses of the new microorganism that verify compliance with the following:

- (i) The certifications made in paragraph (b) of this section.
- (ii) All the eligibility criteria for the Tier I exemption including the criteria for the recipient microorganism, the introduced genetic material, the physical containment and control technologies.

(b) *Certification.* To be eligible for the Tier I exemption under this subpart, the manufacturer or importer must submit to EPA a document signed by a responsible company official containing the information listed in this paragraph.

- (1) Name and address of manufacturer or importer.
- (2) Date when manufacture or import is expected to begin.
- (3) The identification (genus, species) of the recipient microorganism listed in § 725.420 which is being used to create the new microorganism which will be used under the conditions of the Tier I exemption.
- (4) Certification of the following:
 - (i) Compliance with the introduced genetic material criteria described in § 725.421.
 - (ii) Compliance with the containment requirements described in § 725.422, including the provision in paragraph (a)(3) of this section.
 - (5) The site of waste disposal and the type of permits for disposal, the permit numbers and the institutions issuing the permits.
 - (6) The certification statement required in § 725.25(b). Certification of submission of test data is not required for the Tier I exemption.

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§ 725.426 Applicability of the Tier I exemption.

The Tier I exemption under § 725.424 applies only to a manufacturer or importer of a new microorganism that certifies that the microorganism will be used in all cases in compliance with §§ 725.420, 725.421, and 725.422.

§ 725.428 Requirements for the Tier II exemption.

The manufacturer or importer of a new microorganism for commercial purposes may submit to EPA a Tier II exemption request in lieu of a MCAN under subpart D of this part if all of the following conditions are met:

(a) The recipient microorganism is listed in and meets any requirements specified in § 725.420.

(b) The introduced genetic material meets the criteria under § 725.421.

(c) Adequate physical containment and control technologies are used. The criteria listed under § 725.422 for physical containment and control technologies of facilities should be used as guidance to satisfy the Tier II exemption request data requirements listed at § 725.455(d). EPA will review proposed process and containment procedures as part of the submission for a Tier II exemption under this section.

§ 725.450 Procedural requirements for the Tier II exemption.

General requirements for all submissions under this part are contained in § 725.25. In addition, the following requirements apply to requests submitted under this subpart:

(a) *Prenotice consultation.* EPA strongly suggests that for a Tier II exemption, the submitter contact the Agency for a prenotice consultation regarding eligibility for the exemption.

(b) *When to submit the Tier II exemption request.* Each person who is eligible to submit a Tier II exemption request under this subpart must submit the request at least 45 calendar days before the person intends to commence manufacture or import.

(c) *Contents of the Tier II exemption request.* Each person who submits a request under this subpart must provide the information described in §§ 725.428 and 725.455, as well as information known to or reasonably ascertainable

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by the person that would permit EPA to determine that use of the microorganism, under the conditions specified in the request, will not present an unreasonable risk of injury to health or the environment.

(d) *Recordkeeping.* Each person who submits a request under this subpart must comply with the recordkeeping requirements of § 725.65. In addition, the submitter should maintain records which contain information that verifies compliance with the following:

(1) The certifications made in the request.

(2) All the eligibility criteria for the Tier II exemption request including the criteria for the recipient microorganism, the introduced genetic material, the physical containment and control technologies.

§ 725.455 Information to be included in the Tier II exemption request.

The submitter must indicate clearly that the submission is a Tier II exemption request for a microorganism instead of the MCAN under subpart D of this part and must submit the following information:

(a) *Submitter identification.* (1) The name and headquarters address of the submitter.

(2) The name, address, and office telephone number (including area code) of the principal technical contact representing the submitter.

(b) *Microorganism identity information.* (1) Identification (genus, species, and strain) of the recipient microorganism. Genus, species designation should be substantiated by a letter from a culture collection or a brief summary of the results of tests conducted for taxonomic identification.

(2) Type of genetic modification and the function of the introduced genetic material.

(3) Site of insertion.

(4) Certification of compliance with the introduced genetic material criteria described in § 725.421.

(c) *Production volume.* Production volume, including total liters per year, and the maximum cell concentration achieved during the production process.

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(d) *Process and containment information.* (1) A description of the process including the following:

(i) Identity and location of the manufacturing site(s).

(ii) Process flow diagram illustrating the production process, including downstream separations, and indicating the containment envelope around the appropriate equipment.

(iii) Identities and quantities of feedstocks.

(iv) Sources and quantities of potential releases to both the workplace and environment, and a description of engineering controls, inactivation procedures, and other measures which will reduce worker exposure and environmental releases.

(v) A description of procedures which will be undertaken to prevent fugitive emissions, i.e. leak detection and repair program.

(vi) A description of procedures/safeguards to prevent and mitigate accidental releases to the workplace and the environment.

(2) Certification of those elements of the containment criteria described in § 725.422 with which the manufacturer is in compliance, including stating by number the elements with which the manufacturer is in full compliance.

(e) The site of waste disposal and the type of permits for disposal, the permit numbers and the institutions issuing the permits.

(f) The certification statement required in § 725.25(b). Certification of submission of test data is not required for the Tier II exemption.

§ 725.470 EPA review of the Tier II exemption request.

General procedures for review of all submissions under this part are contained in §§ 725.28 through 725.60. In addition, the following procedures apply to EPA review of Tier II exemption requests submitted under this subpart:

(a) *Length of the review period.* The review period for the request will be 45 days from the date the Document Control Officer for the Office of Pollution Prevention and Toxics receives a complete request, or the date EPA determines the request is complete under § 725.33, unless the Agency extends the

review period for good cause under § 725.56.

(b) *Criteria for review.* EPA will review the request to determine that the new microorganism complies with § 725.428 and that its manufacture, processing, use, and disposal as described in the request will not present an unreasonable risk of injury to health or the environment.

(c) *EPA decision regarding the Tier II exemption request.* A decision concerning a request under this subpart will be made by the Administrator, or a designee.

(d) *Determination that the microorganism is ineligible for a Tier II review.* (1) EPA may determine that the manufacturer or importer is not eligible for Tier II review, because the microorganism does not meet the criteria under § 725.428 or the Administrator, or a designee, decides that there is insufficient information to determine that the conditions of manufacture, processing, use, or disposal of the microorganism as described in the request will not present an unreasonable risk to health or the environment.

(2) If the Agency makes this determination, the Administrator, or a designee will notify the manufacturer or importer by telephone, followed by a letter, that the request has been denied. The letter will explain reasons for the denial.

(3) If the request is denied, the manufacturer or importer may submit the information necessary to constitute a MCAN under subpart D of this part.

(e) *Approval or denial of the Tier II exemption request.* (1) No later than 45 days after EPA receives a request, the Agency will either approve or deny the request.

(2) In approving a request, EPA may impose any restrictions necessary to ensure that the microorganism will not present an unreasonable risk of injury to health and the environment as a result of general commercial use.

(f) EPA may seek to enjoin the manufacture or import of a microorganism in violation of this subpart, or act to seize any microorganism manufactured or imported in violation of this section or take other actions under the authority of sections 7 or 17 of the Act.

(g) A manufacturer or importer may only proceed after receipt of EPA approval.

Subparts H–K [Reserved]

Subpart L—Additional Procedures for Reporting on Significant New Uses of Microorganisms

§ 725.900 Scope and purpose.

(a) This subpart describes additional provisions governing submission of MCANs for microorganisms subject to significant new use rules identified in subpart M of this part.

(b) Manufacturers, importers, and processors described in § 725.105(c) must submit a MCAN under subpart D of this part for significant new uses of microorganisms described in subpart M of this part, unless they are excluded under §§ 725.910 or 725.912.

(c) Section 725.920 discusses exports and imports.

(d) Additional recordkeeping requirements specific to significant new uses of microorganisms are described in § 725.950.

(e) Section 725.975 describes how EPA will approve alternative means of complying with significant new use requirements designated in subpart M of this part.

(f) Expedited procedures for promulgating significant new use requirements under subpart M of this part for microorganisms subject to section 5(e) orders are discussed in §§ 725.980 and 725.984.

(g) This subpart L contains provisions governing submission and review of notices for the microorganisms and significant new uses identified in subpart M of this part. The provisions of this subpart L apply to the microorganisms and significant new uses identified in subpart M of this part, except to the extent that they are specifically modified or supplanted by specific requirements in subpart M of this part. In the event of a conflict between the provisions of this subpart L and the provisions of subpart M of this part, the provisions of subpart M of this part shall govern.

(h) The provisions of subparts A through F of this part also apply to

subparts L and M of this part. For purposes of subparts L and M of this part, wherever the words “microorganism” or “new microorganism” appear in subparts A through F of this part, it shall mean the microorganism subject to subparts L and M of this part. In the event of a conflict between the provisions of subparts A through F and the provisions of subparts L and M of this part, the provisions of subparts L and M of this part shall govern.

§ 725.910 Persons excluded from reporting significant new uses.

(a) A person who intends to manufacture, import, or process a microorganism identified in subpart M of this part and who intends to distribute it in commerce is not required to submit a MCAN under subpart D of this part, if that person can document one or more of the following as to each recipient of the microorganism from that person:

(1) That the person has notified the recipient, in writing, of the specific section in subpart M of this part which identifies the microorganism and its designated significant new uses, or

(2) That the recipient has knowledge of the specific section in subpart M of this part which identifies the microorganism and its designated significant new uses, or

(3) That the recipient cannot undertake any significant new use described in the specific section in subpart M of this part.

(b) The manufacturer, importer, or processor described in paragraph (a) of this section must submit a MCAN under subpart D of this part, if such person has knowledge at the time of commercial distribution of the microorganism identified in the specific section in subpart M of this part that a recipient intends to engage in a designated significant new use of that microorganism without submitting a MCAN under this part.

(c) A person who processes a microorganism identified in a specific section in subpart M of this part for a significant new use of that microorganism is not required to submit a MCAN if that person can document each of the following: