UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

WASHINGTON, D.C. 20460

2 June 1997

OFFICE OF POLLUTION PREVENTION AND TOXICS

POINTS TO CONSIDER IN THE PREPARATION OF TSCA BIOTECHNOLOGY SUBMISSIONS FOR MICROORGANISMS

Prepared by: EPA Office of Pollution Prevention and Toxics
401 M. Street, S.W.
Washington, D.C. 20460

For further information contact: New Chemicals Notice Management Branch Chemical Control Division,(202) 260-3749

I. PURPOSE

Living microorganisms are sufficiently different from the traditional chemical substances reviewed under §5 of the Toxic Substances Control Act (TSCA) to warrant development of separate regulations and guidance. The new regulations under TSCA applicable to biotechnology submissions were published in the Federal Register on April 11, 1997 (62 FR 17910-17958), and guidance for these regulations is contained in this "Points to Consider" document. These new TSCA regulations (as well as the proposed TSCA regulations for biotechnology), and this "Points to Consider" document, are accessible through the EPA Office of Pollution Prevention and Toxics' Home Page at http://www.epa.gov/opptintr/biotech/.

The Agency expects the submitter to provide information, as explained in this document, for two purposes: (l) to identify and list the microorganism on the TSCA Chemical Substances Inventory, and (2) to assess the risks of the microorganism to human health and the environment, given its intended use. Information regarding new microorganisms should not be submitted on the standard TSCA Premanufacture Notice (PMN) form for new chemicals, as this form is not applicable to microbial products. Rather, the format for the submission for microorganisms subject to TSCA is outlined in the April 11 Federal Register document. The guidance discussed within this "Points to Consider" document is designed to elicit this information and is meant to present points to consider rather than requirements to fulfill.

II. OVERVIEW OF THE FINAL BIOTECHNOLOGY RULE UNDER SECTION 5 OF TSCA

Section 5(a)(1) of TSCA requires any person who intends to manufacture or import a new chemical substance to submit information to EPA at least 90 days before manufacture or import commences. The final rule establishes all reporting requirements under §5 of TSCA for manufacturers and processors of microorganisms subject to TSCA jurisdiction, that are manufactured for commercial purposes, including research and development (R&D) for commercial purposes.

Manufacturers are required to report certain information to EPA before commencing the manufacture of new microorganisms. "New microorganisms" are intergeneric microorganisms that are not listed on the TSCA Inventory. Intergeneric microorganisms are those which have been formed by the deliberate combination of genetic material originally isolated from organisms of different taxonomic genera. The term "intergeneric microorganism" includes a microorganism which contains a mobile genetic element which was first identified in a microorganism in a genus different from the recipient microorganism. A further explanation of how "intergeneric" should be interpreted for mobile genetic elements is contained in Attachment 1. The rule also defines "small quantities for research and development" for microorganisms, the effect of which is to require §5 reporting for certain R&D activities.

TSCA §5 only applies to microorganisms that are manufactured, imported, or processed for commercial purposes. EPA has defined manufacture or process for commercial purposes as "manufacture or process for purposes of obtaining an immediate or eventual commercial advantage." Whether an activity has an immediate or eventual commercial advantage is determined by indicia of commercial intent. R&D activities are for commercial purposes, and thus subject to reporting, if tests are directly funded, in whole or in part, by a commercial entity; or, if the R&D activities are not directly funded by a commercial entity, if the researcher intends to obtain an immediate or eventual commercial advantage (see 40 C.F.R. §725.205). In addition, all post-R&D activities are considered manufacture or processing for a commercial purpose.

An examination of the major sections of the final rule, and the associated submissions noted under each section, is helpful in understanding the rule and how the "Points to Consider" compliments the new rule:

Subpart B of part 725 contains administrative procedures that have been adapted with little change from provisions in 40 CFR parts 720 and 721 of TSCA.

Subpart C of part 725 contains requirements for claiming confidential business information (CBI). These requirements were largely adapted from provisions in part 720.

Subpart D of part 725 establishes the reporting program for new microorganisms manufactured or imported for distribution into commerce and requires submission of a Microbial Commercial Activity Notice (MCAN)90 days prior to initiating manufacture or import of the new microorganism. This subpart codifies the requirements for information to be included in the MCAN at §725.155 and §725.160. Any manufacturer, importer, or processor of a living microorganism, who is required to report under §5 of TSCA must file a MCAN with EPA, unless the activity is eligible for one of the specific exemptions.

Subpart E of part 725 establishes the exemptions from full MCAN reporting for R&D activities. At §725.205(b), EPA defines "commercial purposes" for R&D activities to include all R&D directly funded in whole or in part by a commercial entity, and all R&D activities, regardless of funding source, for which the researcher intends to obtain immediate or eventual commercial advantage. Subpart E establishes, at §725.232, a complete exemption from TSCA §5 for certain R&D activities which are conducted in contained structures, and are subject to regulation by another Federal agency. Under §725.232, EPA has established a complete exemption from EPA review, reporting and record keeping requirements for contained research conducted by researchers who are required to comply with the NIH guidelines. All other manufacturers conducting contained TSCA research and development activities may qualify for a more limited exemption under §725.234 and §725.235. The exemption for R&D in contained structures specifies factors which the technically qualified individual must consider in selecting the appropriate containment. The manufacturer is required to keep records to document both compliance with the containment requirements and compliance with the notification process for employees involved in the R&D process.

Subpart E also establishes at §725.238 and §725.239 the TSCA Experimental Release Application (TERA) exemption process for R&D activities, primarily those involving intentional environmental release. EPA has revised requirements in §725.239 to limit the antibiotic resistance markers that may be used in the microorganisms eligible for the TERA exemption. For researchers conducting small-scale field tests with the two eligible microorganisms, Bradyrhizobium japonicum and Rhizobium meliloti, the final rule creates an exemption from EPA review, providing certain conditions are met. The field testing must occur on no more than 10 terrestrial acres; the introduced genetic material must comply with certain restrictions, and appropriate containment measures must be selected to limit dissemination.

If a submitter does not meet the requirements for the field test exemption under §725.238 and §725.239, he or she may submit a TERA under Subpart E. The TERA is essentially an abbreviated MCAN submission which is submitted for an individual field test application. EPA's review period for these applications is reduced to 60 days, although EPA may extend the period for good cause. EPA must approve the test before the researcher may proceed, even if the 60-day period expires. EPA's approval is limited to the conditions outlined in the TERA notice or approval. The requirements for information that must be included in the TERA are codified at §725.255 and §725.260.

Although a manufacturer may submit a MCAN for any R&D activity, EPA expects that most researchers will instead choose to submit a TERA. In addition to the longer review period for a MCAN, EPA expects that, because of the limited information at the R&D stage, the Agency would likely issue a §5(e) consent order with such an MCAN to impose conditions to address the uncertainties. These MCAN consent orders would need to be modified each time the manufacturer wanted to vary the terms of the order.

Subpart F contains the requirements for exemptions for test marketing activities. These requirements have been adapted, with little change, from provisions in part 720.

Subpart G establishes two exemptions from MCAN reporting for certain microorganisms used in closed systems. To qualify for the Tier I exemption, a manufacturer must use one of the recipient organisms listed at §725.420, and implement specific physical containment and control technologies as noted in § 725.422. In addition, the DNA introduced into the recipient microorganisms must be well-characterized, limited in size, poorly mobilizable, and free of certain sequences (see §725.421). In the Tier I exemption, if these three requirements are met, manufacturers only need to notify EPA that they are manufacturing a new microorganism that qualifies for this exemption 10 days before commencing manufacture, and to keep certain records. A manufacturer is not required to wait for EPA approval before commencing manufacture.

A manufacturer, who otherwise meets the conditions of the Tier I exemption, may modify the specified containment restrictions, but must submit a Tier II exemption notice under subpart G. The Tier II exemption requires manufacturers to submit an abbreviated notice describing the modified containment, and provides for a 45 day period, during which EPA would review the proposed containment. The manufacturer may not proceed under this exemption until EPA approves the exemption.

Subpart L establishes procedures for reporting significant new uses of microorganisms. These requirements have been adapted, with little change, from provisions in part 721. Subpart M is reserved for requirements for significant new uses for specific microorganisms; however, none are being promulgated at this time.

The regulatory text also amends existing regulations regarding the collection of fees from submitters of notices under §5 of TSCA (40 CFR part 700), to reflect the fee structure for the notices and applications that have been developed in the final rule. Additional amendments to parts 720, 721, and 723 consolidate TSCA §5 review of microorganisms into part 725.

Further information on the types of submissions under TSCA can be found in § III of this document.

III. ADMINISTRATIVE DETAILS FOR MICROORGANISM SUBMISSIONS

III.A. Prenotice Consultations:

EPA recommends that persons planning to provide EPA with any of the following submissions discuss their plans in advance with a Biotechnology Program Manager in the New Chemicals Notice Management Branch, Chemical Control Division of the Office of Pollution Prevention and Toxics (202-260-3749): a Microbial Commercial Activity Notice (MCAN), a TSCA Experimental Release Application (TERA) for Research and Development activities, a Test Marketing Exemption (TME), a Tier II exemption for a fermentation application, a petition to add microorganisms to the list of recipient microorganisms at 725.420, or a bona fide submission. It is important to begin discussions with EPA staff early in the submission planning process to identify data requests and preliminary concerns associated with the specific microorganism. Any meetings and relevant written communications may be claimed confidential. Persons who are unsure as to whether their microorganisms are subject to TSCA should consult with EPA before preparing any submission, including a bona fide.

In many cases submitters' questions can be addressed in telephone conversations. However, it is often useful for potential submitters to meet with a broader range of Agency staff prior to submission of documents for Agency review. This is true if the microorganism product is novel in construction or in intended use, or if the submitter is unfamiliar with the TSCA submission process. Although the overall format for the submissions noted in the above paragraph should follow the structure outlined in this document, a prenotice meeting should result in guidance which is more specifically tailored to the preparation of a document for the submitter's microorganism. Such specific guidance often results from discussion of section IV of this document with Agency scientific, legal, and policy staff. This may save significant time later in the review process.

With reference to research and development activities (R&D), EPA recognizes that research proceeds through various stages. There are a number of exemptions for research and development noted in Subpart E of the 1997 Rule (62 FR 17910-17958).

For more information on prenotice consultations, call the New Chemicals Notice Management Branch, Chemical Control Division at (202) 260-3749.

III.B. Microorganism Bona Fide submissions

Companies planning to manufacture, import, or process intergeneric microorganisms for commercial purposes subject to TSCA may submit a bona fide notice to determine their reporting obligations under §5 of TSCA. This is because confidential, specific identities and/or uses of microorganisms are not listed on the public version of the TSCA Inventory. If a company's microorganism is listed on the confidential version of the Inventory and is not subject to a Significant New Use Rule (SNUR), then no §5 submission would be necessary. The review process for a bona fide notice generally takes 30 days after receipt of sufficient information from the submitter. For more information on bona fide submissions, see Attachment 2.

III.C. Confidential Business Information (CBI)

EPA's confidential business information (CBI) policy is designed to provide effective public participation by making meaningful information available. CBI claims may be made and must be substantiated at the time of submission for MCANs, TMEs, Tier I certifications, and Tier II exemption requests. Upfront substantiation is not required for TERAs. In developing confidentiality provisions for submissions, EPA has balanced the need to provide nonconfidential information to the public in a reasonable period of time, to obtain the information it needs to respond to Freedom of Information Act (FOIA) requests, and to allow persons to assert CBI claims with the minimum burden. A claim of confidentiality may be asserted for any information submitted to EPA, with certain exceptions. However, submitters are encouraged to minimize the amount of CBI in biotechnology submissions, so that the public may participate as fully as possible in the review process. All CBI claims must be asserted at the time that the information is submitted.

C.1. Submission of confidential business claims

a. Indicate what information in the submission is claimed as confidential on a cover sheet (i.e., submitter, identity, microorganism identity, volume, process, etc.)
b. Circle the specific information which is claimed as confidential and mark the page on which that information appears with an appropriate designation such as "trade secret," "TSCA CBI," or "confidential business information."
c. Attachment 3 provides the questions a company should answer to substantiate CBI.

C.2. Submission of a Non-confidential 'sanitized' version

If information being submitted is claimed as CBI and is being submitted, then the company must also provide a sanitized copy of the submission (and any attachments). This copy is complete except that all information claimed as confidential must be deleted and the places marked where the deletions occurred. The sanitized copy will be placed in the public files.

C.3. Generic information

Submitters who claim microorganism identity and/or use as CBI also must provide generic information for release to the public. By requiring generic identity and use information, EPA would meet its obligation to provide the public with important information related to the potential risks of new microorganisms without revealing CBI. Generic information would also be used to prepare Federal Register notices which announce EPA's receipt of submissions and to develop generic Inventory listings. For more information on preparing generic designations, see Attachment 3.

III.D. Microorganism Reporting Mechanisms Under TSCA

If a microorganism is subject to TSCA and is not considered to be on the TSCA Inventory, the manufacturer or importer is required under TSCA to notify EPA from 45 to 90 days before they manufacture or import for commercial purposes a “new” chemical substance or manufacture, import, or process a chemical substance for a “significant new use”. Specific time frames for individual submissions are noted in Sections III.D.1 through III.D.5 of this document. The Final Rule under §5 of TSCA (62 FR 17910-17958), establishes all reporting requirements for manufacturers and processors of microorganisms subject to TSCA jurisdiction, that are manufactured for commercial purposes, including research and development for commercial purposes. The Rule establishes a

number of mechanisms for reporting to EPA, including a number of specific exemptions.

Certain microorganisms which are subject to TSCA do not require reporting to EPA prior to manufacture, and are not addressed by this document: research and development conducted solely within a contained structure by researchers required to comply with the NIH guidelines (§725.232), other research and development conducted solely within a contained structure (§ 725.234), and certain types of R&D field tests involving Rhizobium meliloti and Bradyrhizobium japonicum under the TERA exemption (§s 725.238 and 725.239).

In addition to the guidance provided for bona fide submissions under Item III.B., this document provides guidance on how to meet the reporting needs for the following seven types of MCAN and 5(h)(4) Exemption submissions: the Microbial Commercial Activity Notice (MCAN), including MCANs for significant new uses of microorganisms (Item III.D.2.), the TSCA Experimental Release Application (TERA) and the TERA exemption for field tests with certain rhizobia (Item III.D.3), the test marketing exemption (TME; Item III.D.4.), the Tier I and II exemptions for fermentation applications (item III.D.5), and petitions to add microorganisms to the list of recipient microorganisms at § 725.420 applicable to both Tier I and Tier II exemptions (item III.D.5).

This document also includes guidance on how to submit information provided to other agencies related to the TSCA submission (Item III.D.6) and guidance for the preparation of 5(d)2 Notices (Item III.D.7).

D.1. General Administrative Requirements, Fees, and Notification in the Federal Register Applicable to MCANs and Exemptions

The Administrative requirements for the seven types of MCAN and Exemption submissions noted above can be found in Subpart B of the Final Rule. This Subpart contains information specific to the submission of MCANs and Exemptions, including information on the general format of these submissions (§s 725.25 and 725.27) and recordkeeping for these submissions (§725.65).

A fee of $2,500 is required for each MCAN or consolidated MCAN submitted, unless the company submitting the MCAN is a small business (see §700.45). A small business concern must remit $100 with each MCAN or consolidated MCAN. Persons are exempt from remitting any fee for exemptions (the TERA, TME, the Tier I and Tier II fermentation exemptions, and petitions for adding to the

recipient list for the Tier I and Tier II fermentation exemptions).

The Toxic Substances Control Act requires the Agency to publish a notice in the Federal Register announcing the receipt of each MCAN and Exemption submission. See item III.D.7 for further information on preparation of this notice.

D.2. Information Requirements for MCANs

Any manufacturer, importer, or processor of a living microorganism, who is required to report under §5 of TSCA must file a Microbial Commercial Activity Notice (MCAN) with EPA under §725.105, unless the activity is eligible for one of the specific exemptions noted in item III.D. above. Further, a MCAN submission is required under §725.105(c) for any person who intends to manufacture, import, or process for commercial purposes a microorganism identified as having one or more significant new uses, and who intends to engage in a designated significant new use of the microorganism or intends to distribute it in commerce. Microorganisms subject to MCAN reporting could be either for fermentation applications or for intentional release to the environment. A MCAN must be submitted 90 days before manufacture, import, or processing of the microorganism for commercial purposes.

In addition to the general administrative items and fees noted in item III.D.1 above, certain technical information is required for each MCAN submission as noted in §s 725.155 and 725.160. In order to provide complete information for EPA review, the following portions of item IV of the Points to Consider can be used as a guide for MCAN information submitted under §s 725.155 and 725.160:

Item 725.155(d)(1)Description of the recipient microorganism and the new microorganism subject to TSCA: see applicable portions of item IV.A.

Item 725.155(d)(2) Genetic construction of the new microorganism subject to TSCA: see item IV.B.

Item 725.155(d)(3) Phenotypic and ecological characteristics: see item IV.D., and include information as suggested in item IV.G. if the MCAN is for a field test.

Item 725.155(e) Byproducts: see item IV.E.2.

Item 725.155(f) Total production volume: see item IV.E.1.

Item 725.155(g) Use information: see item IV.E.3.

Item 725.155(h) Worker exposure and environmental release: for worker exposure, see either items IV.F.1.b. or IV.F.2.b.; for environmental release, see either item IV.F.1.c. or IV.F.2.b.

Item 725.160 Health Effects Information: see item IV.C.

D.3. Information Requirements for the TSCA Experimental Release Application (TERA) Exemption for Research and Development

As noted in III.D. above, there are two types of TERA exemptions which can be submitted instead of a MCAN. First, there is a TERA exemption for certain types of R&D field tests involving Rhizobium meliloti and Bradyrhizobium japonicum (see §s 725.238 and 725.239). This exemption requires no upfront reporting to EPA, although a certification statement and recordkeeping are required. Since guidance on how to submit a certification statement to EPA, and on the recordkeeping requirements for field tests with these bacteria, is provided under §725.238, additional guidance is not offered here.

Second, there is a TERA exemption from MCAN reporting for field tests with microorganisms which allows for a 60-day review by EPA, following receipt of the information described in § 725.250. The documentation provided by the submitter for this TERA exemption is similar in format and content to an MCAN submission for an individual field test, but is abbreviated relative to an MCAN submission. For example, persons who submit a TERA would not have to include information on all commercial manufacture, processing, transport, use, and disposal activities that may involve the new microorganism as is the case for a MCAN. EPA will, however, still review any general commercial uses of these new microorganisms through the MCAN process.

If a submitter believes sufficient information is available which could justify adding another recipient to the list exempted microorganisms at §725.239, please consult with the Agency.

In addition to the general administrative items noted in item III.D.1 above, certain technical information is required for each TERA submission as noted in 725.238, 725.250, and 725.255. The following portions of item IV of the Points to Consider can

be used as a guide for TERA information submitted under 725.255. The information should be organized in accordance with 725.255.

Item 725.255(c) Microorganism identity information required for MCANs: see items IV.A and IV.B.1

Item 725.255(d) Phenotypic and ecological characteristics as they relate directly to the conditions of the proposed research and development activity:

- Habitat, geographical distribution, and source of the recipient: See item IV.D.2.a. for habitat and geographical distribution, and item IV.A.2.b. for source of the recipient.

- Survival, dissemination, and detection of the subject microorganism: See item IV.D.2. for survival and dissemination; see item IV.G.7. for detection methodology descriptions.

- Anticipated adverse ecological effects: See item IV.D.1.

- Involvement in biogeochemical cycling processes: See item IV.D.1.b.

Item 725.255(e)(1) A detailed description of the proposed research and development activity:

Item (e)(1)(ii): Methods of application or release: see applicable portions of IV.G.1, IV.G.3., IV.G.5, and IV.G.8.

Item (e)(1)(iii): Characteristics of the test site: see applicable portions of items IV.G.2. and IV.G.4.

Item 725.255(e)(2) Information on monitoring, confinement, mitigation, and emergency termination procedures: see items IV.G.4, IV.G.5., IV.G.6, and IV.G.7.

D.4. Information Requirements for Test Marketing Exemptions

Test marketing activities usually involve limited sale or distribution of a substance within a predetermined period of time to determine its competitive value when its market is uncertain. In general, EPA suggests that manufacturers who intend to test market a new microorganism file a MCAN rather than request a Test Marketing Exemption(TME; see §725.300). However, there may be

situations in which this exemption is appropriate, such as for microorganisms which were previously reviewed by EPA at the R&D stage.

In addition to the general administrative requirements noted in item III.D.1 above, certain technical information is required for each TME submission as noted in 725.350 and 725.355. The following portions of item IV of the Points to Consider can be used as a guide for TME information submitted under 725.355, and the information should be organized in accordance with 725.355.

Item 725.355(c) Microorganism identity information: see applicable portions of item IV.A. for description of the recipient and subject microorganisms, and item IV.B. for genetic construction of the subject microorganism.

Item 725.355(d) Phenotypic and ecological characteristics:

- Habitat, geographical distribution, and source of the recipient: See item IV.D.2.a. for habitat and geographical distribution, and item IV.A.2.b. for source of the recipient.

- Survival, dissemination, and detection of the subject microorganism: See item IV.D.2. for survival and dissemination; see item IV.G.7. for detection methodology descriptions.

- Anticipated adverse ecological effects: See item IV.D.1.

- Involvement in biogeochemical cycling processes: See item IV.D.1.b.

Item 725.355(e)(1) Maximum quantity of microorganism, and duration and route of exposure of persons to microorganism: see items IV.E.1. on quantity of microorganism, either item IV.F.1.b or IV.F.2.b. for worker exposure, and IV.E.3. for consumer exposure.

Item 725.355(e)(2) Health and environmental effects information: see item IV.C. for health effects information.

D.5. Information Requirements for Tier I and II Exemptions for Closed Fermentation Systems

Certain microorganism applications for closed fermentation systems can fall under a TSCA §5(h)(4) exemption from MCAN reporting as described under §725.400. These exemptions can fall under one of three categories: (1) a Tier I exemption for an intergeneric microorganism based on a recipient microorganism listed under §725.420, (2) a petition to add a recipient microorganism to the list at §725.420, and (3) a Tier II exemption for an intergeneric microorganism submitted under § 725.428. The Tier I exemption requires only a brief certification and information statement to be forwarded to EPA. Since guidance on how to submit these certification statements for Tier I exemptions is provided under §725.424, additional guidance is not offered here.

The process for filing a petition to add a recipient microorganism to the list at 725.420 is addressed under item V.B.2.b. of the Preamble of the Final Rule (and further under Unit III.C.2 of the Response to Comments document). EPA expects that petitions to add microorganisms to the list of recipient microorganisms at 725.420 will be preceded by several MCANs so that sufficient information is available to determine whether the microorganism should be added. EPA would make this determination after evaluating information which addresses the six criteria for recipients noted in the Proposed Rule: (1) clear taxonomic identification of the microorganism, (2) availability of information to evaluate the relationship between the recipient and closely related microorganisms which have a potential for adverse effects, (3) a history of safe commercial use for the recipient, (4) commercial uses which indicate that the recipient’s potential products might be subject to TSCA, (5) studies are available which indicate the potential for the microorganism to cause adverse effects, and (6) studies are available which indicate the survival characteristics of the recipient in the environment.

The third microorganism submission under §725.428 is one in which (1) the recipient microorganism is listed in §725.420; (2) the introduced genetic material meets the criteria under §725.421; and (3)adequate physical containment is proposed, even though the containment does not meet the requirements at §725.422. The review period for a Tier II exemption is 45 days, beginning from the date the Agency determines that the submission is complete; the Agency may extend the review period for good cause. The following portions of item IV of the Points to Consider can be used as a guide for Tier II information submitted

under 725.455. The information should be organized in accordance with 725.455.

Item 725.455(b)(1) Microorganism identity information which indicates that its taxonomic name is listed under 725.420: see item IV.A., and provide a brief summary of the information as noted under IV.A.

Item 725.455(b)(2) Type of genetic modification and the function of the introduced genetic material: see item IV.B.2. for preparation of a final construct illustration, and Item IV.B.3. which assists in describing the function of the introduced genetic material. Provide a brief summary (a one-paragraph description of construct), using these sections of item IV.

Item 725.455(b)(3) Site of insertion: see item IV.B.

Item 725.455(c)Production volume: see item IV.E.1.

Item 725.455(d)(1)Process and containment information: See item IV.E. No additional guidance is offered in this document on justifications for lower levels of containment than that specified for the Tier I exemption. If the containment anticipated is less than that of the Tier I exemption, see EPA reference No. 4 in the Final Rule and consult with the Agency for presentation of containment information.

D.6. Submissions to Other Agencies

EPA requests that a company submit to EPA any information it has provided to other U.S. federal agencies or foreign governments to obtain regulatory approval of the microorganisms in question. Information submitted as nonconfidential to other U.S. Federal Agencies (e.g., the Food and Drug Administration) cannot be claimed as confidential when submitted to EPA.

D.7. 5(d)2 Notice:

The Toxic Substances Control Act requires the Agency to publish a notice announcing to the public the receipt of each MCAN or TSCA §5(h)(4) exemption submission that requires reporting (see item III.D. above). This is accomplished through a 5(d)2 notice published in the Federal Register. A sample 5(d)2 notice can be found at 52 FR 24527 (Wednesday, July 1, 1987). The submitter may consider summarizing this information in the MCAN or exemption submission.

IV. POINTS TO CONSIDER FOR MICROORGANISM SUBMISSIONS

This section presents a suggested format and information to be included, where applicable, for the seven kinds of microorganism submissions noted in Section III.D.: The Microbial Commercial Activity Notice (MCAN; including MCANs for significant new uses of microorganisms), the TSCA Experimental Release Application (TERA), the TERA exemption for field tests with certain rhizobia, the Test Marketing Exemption (TME), the Tier I and Tier II exemptions for fermentation applications, and petitions to add microorganisms to the list of recipient microorganisms for Tier I and Tier II exemptions. The information items listed under Section IV. are intended to provide data that would assist EPA in the review of these seven submission types. Since not all information items listed under Section IV. are applicable to each of the submission types, Section III.D. should be examined prior to reviewing item IV. so that only the necessary information under item IV. is submitted to EPA. Section III.D. links the specific sections of the Final Rule (62 FR 17910-17958) applicable to each of the seven submission types with the relevant portions of item IV. of the Points to Consider document.

If the information in a TSCA submission is inadequate for the Agency's review process, EPA staff will contact the submitter for clarification or further information. Prior to preparation of a microorganism submission, it may be beneficial for the company to consult with EPA staff (see Section III.A.).

TERMINOLOGY

The following terms are used to describe different microorganisms and their roles in the development of a microorganism which may be reportable under TSCA section 5. Information about these microorganisms is essential to EPA's review and will be discussed under the appropriate headings below.

Subject microorganism is the subject of the microbial submission. The seven types of submissions which persons provide for EPA review/documentation are noted in III.D.

Recipient microorganism is the strain into which the intergeneric DNA is introduced and which generally determines the taxonomic designation of the subject microorganism.

Donor microorganisms are those which contribute DNA to the subject microorganism, or those which contribute DNA to intermediate microorganisms used to construct the final subject microorganism.

Intermediate microorganisms do not contribute DNA, but may be used to temporarily contain a vector used to construct the subject microorganism, to aid in a triparental mating, etc.

Final construct is the term which describes the DNA sequences contributed by the donor microorganisms, and where applicable, the recipient's DNA sequences immediately flanking the inserted donor microorganisms' DNA. The construct includes such sequences as structural genes (usually genes which encode an enzyme that is of commercial importance), vector DNA, and marker genes (such as antibiotic resistance). Information on the final construct should focus on the intergeneric DNA, and intrageneric DNA that affects the expression, stability, and mobility of the intergeneric DNA.

TABLE OF CONTENTS

IV.A RECIPIENT ORGANISM CHARACTERIZATION

Section IV.A. provides guidance on information useful to the Agency in identifying the recipient microorganism (used to produce the subject microorganism). Many of the traits associated with the recipient will be considered in assessing the potential for the subject microorganism to cause adverse effects in humans and/or the environment. Hence, taxonomic classification is a key component in enabling an accurate assessment of the potential concerns for the subject microorganism. Item IV.A.1. provides general guidance for microbial classification, while item IV.A.2 suggests more specific information directly applicable to microbial submissions under TSCA.

A.1. Taxonomy: General

The following provides some general information relative to the use of taxonomy in the TSCA biotechnology review process:

(1) Taxonomy is a means of organizing items and showing their relative relatedness. (Vandamme et al., 1996) illustrate that taxonomy has three components: (I.) classification, or the ordering of organisms in groups, (ii.) nomenclature, or the labeling of units in those groups and (iii.) identification, or the determination that an unknown belongs in one of the labeled groups. For the purposes of this document and submission of notices under TSCA, the last meaning has the greatest relevance.

(2) Usually, the bulk of the genetic information in any subject microorganism is derived from the recipient microorganism. It is therefore likely that any added features from a donor organism, will be insufficient to warrant a species name for the subject microorganism different than that of the recipient parent. Thus, the following discussions concerning reporting of the taxonomic designation of the recipient microorganism are usually also relevant for the subject microorganism itself. An exception would be for products of cell fusion, where the differentiation between donor and recipient is not obvious and the resultant chimeric organism may not resemble either parent. In such cases, identification of both contributors to the genome of the resultant organism is needed.

(3) For a few taxa, the choice of identification methods, and the process of identification may be simple. For many others, this task is far from trivial. As there are no universally applicable methods for identifying microorganisms, it is up to the submitter, or its agent, to select the most appropriate ones for submitted organism(s).

(4) Traditional methods based on phenotype may be the best for some taxa. Numeric taxonomy, which uses statistical analyses of an array of characteristics to determine the similarities and differences between a given isolate and closely related taxa, has been successfully used for identifying a submitted organism to the species level. However, some common genera do not lend themselves to unequivocal application of these techniques.

(5) Modern classification schemes for bacteria rely on nucleic acid analyses to a great extent and, for many taxa, they are slowly supplanting phenotypic analysis as the primary means of identification. Recent developments in the use of genetic methods have resulted in the successful application of some of these to otherwise difficult-to-identify taxa. Several of these methods were first applied to the classification of groups of taxa, and are only recently being employed to identify individual isolates. Since many of these newer tools have not yet received widespread commercial application, and because there are some taxa for which they may not be most appropriate, EPA cannot recommend specific genetic methods at this time. Nevertheless, EPA believes that these methods warrant close consideration for difficult taxa. EPA is currently cosponsoring research in this area, and hopes to provide more definitive guidance with future revisions of these points-to-consider.

A.2. Taxonomy: Specific issues.

The following provides specific observations on the kinds of information needed to support the identification of the recipient microorganism associated with the subject microorganism. Except as indicated, the following applies primarily to bacteria. These data are not expected to be provided for exempt organisms under §5(h)4 of TSCA. Such information that is relevant to establishing that the recipient organism belongs in the class of exempt recipients should be retained in the submitter’s records.

A.2.a. §725.12 requires that a taxonomic designation for both donor and recipient be provided. For the recipient microorganism, this identification would include genus, species, and strain designations, when known. If applicable, description at a lower taxonomic rank (subspecies, biovar, pathovar, serovar, etc.) should be provided. The most current nomenclature should be used.

(1) For bacteria and viruses, the name should be the most current.

The international bacteriological community has adopted a naming convention based on the existence of bacterial names on the Approved List of Bacterial Names (1980), or on subsequent lists of validly published names as found in current issues of the International Journal of Systematic Bacteriology. All other names for a bacterium are considered synonyms.

In a similar way, the international virology community has adopted guidance for naming viruses. While not a formal code, guidance on naming has been provided in the International Code of Virus Classification and Nomenclature (1990) and the Sixth Report of the International Committee on Taxonomy of Viruses (1995).

(2) It is better to identify the organism to the taxonomic level for which data support a positive name assignment and indicate the alternative subtaxa believed to be nearest the isolate, than to attempt to provide a name at a lower level that is unsupported by data.

Not all microorganisms may be identifiable to the species level. Some isolates may be intermediate between members of accepted taxa, but do not fit the descriptions of a named species. In this case, it is appropriate to provide information on the features, phenotypic or genetic or both, which illustrate the relationships between the isolate and the alternative named organisms to which it appears closely related. EPA will take this into account when describing the subject organism for the TSCA Inventory.

(3) For fungi, the most common currently used names should be listed and a reference to a readily available source that will provide a cross-reference to other synonyms, if they exist, should be provided. Where

applicable, names of both teleomorphic and anamorphic forms should be given.

There is no comparable taxonomic authority for fungi, as for bacteria and viruses. Please contact the Agency for the most current guidance on accepted taxonomy for fungi.

(4) For other eukaryotic organisms, commonly used names should be provided.

As for fungi, naming conventions have not been codified for higher microorganisms as they have for bacteria and viruses. Please contact the Agency for the most current guidance on accepted taxonomy for microorganisms such as protozoa and algae.

(5) For any subject organism, provide taxonomic synonyms commonly used for the recipient.

A.2.b. Information substantiating the taxonomy of the recipient is requested.

A submitter is responsible for understanding the underlying assumptions behind any identification techniques they, or their identification supplier, use. A description of the source from which the recipient was originally isolated (environmental medium such as lake water, particular plant, etc.) is helpful in understanding the nature and ecology of the recipient. Please provide details on the source, such as location of lake where water sample was obtained, if known.

The submitter should be able to explain why one of several alterntative approaches to identification was chosen. §725.12 provides two means of supplying substantiating information. This information can take two forms: (1) a letter from a culture collection establishing an organism’s identification or (2) data/analyses used by the submitter or its agent in establishing the identity of the submitted organism. For the latter, this may take the form of a report from an expert on the taxon of the submitted organism, confirming the identification of the organism. In the cases where either a service culture collection or an expert provide the identification, EPA assumes that the submitter has access to, and could provide relevant

data utilized by the collection or expert, if requested by EPA.

A.2.c. When phenotypic methods are most appropriate, analysis methods should be described and data from these methods should be provided, either directly, or by easily accessible reference.

Types of data appropriate for these methods can include morphological, biochemical, immunological, or physiological characteristics. Method description can also be supplied by reference if these are readily accessible.

The selection of specific tests in a battery used for numeric taxonomy for bacteria is generally dependent on the suspected taxon of the organism. Such tests could comprise a wide range of traits including, but not limited to, presence of flagella, optimum temperature for growth, oxygen requirements, fatty acid profiles, serology, energy sources utilized, and life cycle information such as dormant stages. Experts have compiled these test matrices for specific purposes and, thus, they should not be used without a complete understanding of the function of each test within the matrix, and its individual utility in differentiating taxa. This information is usually available in publications regarding the specific test batteries, or from the supplier of the identification based on such tests. It is not necessary to supply EPA with a rationale for the selection of each test in a battery unless the test system has been modified by the submitter from one that has been published, or for which the relevant information could not be otherwise readily obtained by EPA. Rather, EPA suggests that the submitter be familiar with the rationale for choice of a specific identification matrix and statistical method, in order to have confidence that the identification of its organism was appropriate. In a similar way submitters should be aware of alternative typing methods for fungi, which often are based on interpretations of morphological features.

A.2.d. If the results of phenotypic analyses are submitted in support of the taxonomic designation, the test system used should be referenced in such a way that the individual tests used can be clearly determined.

Phenotypic information is usually comprised of either “binary” or numeric data. Binary data are results of tests where the organism either exhibits the trait or it does not; in numeric taxonomy, certain statistical treatments can also take into account equivocal or absent data of this type. Numeric data are results of tests where a graduated response is expected. “Grows at 37°C” is binary; ”minimum inhibitory concentration (MIC) of ampicillin is ___µg/ml.” is numeric. Different laboratories often use different sets of tests for identifying similar organisms. Occasionally, different test methods will receive identical descriptive names due to these interlaboratory differences. To avoid this problem and aid in describing individual tests used in phenotypic analyses, there exists an international system for encoding these data in a uniform shorthand way. The RKC format (Rogosa et al., 1986) would encode the above binary data example as “017015" and the numeric example as “535081". The individual codes for this system can be found in searchable format at the following sites on the World Wide Web:

http://bioinfo.ernet.in/cgi-bin/asearch/rkc/rkc_wais_read
http://fragrans/riken/go/jp/htbin/RKCCODE.pl
http://sun/im.ac.cn/cgi-bin/RKC.pl

A.2.e. In a similar way, when genetic methods are used, analysis methods should be described and data from these methods should be provided, either directly, or by easily accessible reference.

An alternative approach to traditional phenotypic analysis consists of analysis of relatedness among microbial taxa by comparing their nucleic acids rather than, or in addition to, comparing phenotypic information. Many different types of nucleic acid analysis have been utilized in research settings. For the purpose of classifying groups of bacteria, DNA:DNA homology has long been established as a standard. However, this has not been considered an appropriate method for routine bacterial species identification. Newer methods have moved away from homology methods based on annealing of DNA strands from different organisms, to sequence comparisons and restriction analyses. Some of these are just beginning to become available as commercial methods as well. The most promising techniques for bacteria are variations on the theme of ribosomal RNA (rRNA) analysis. Variability in certain conserved regions of rRNA sequences has become

well established as a means to reveal genus level differences among isolates. Recent work utilizing other portions of the rRNA or related DNA sequences has evolved into a variety of tools for separation of bacteria at species or lower taxonomic levels of organization. EPA cannot recommend any one method as appropriate for all species. EPA will continue to monitor the progress of development of these newer identification tools and may issue additional guidance when these methods become established as generally available and reliable tools.

A.2.f. Genetic data should also be submitted in a manner that allows comparison with similar data. Sequences should be provided where applicable. Relevant references should also be submitted. If the sequence is publicly available from the data bank, accession numbers may be provided in lieu of the sequence data.

Genetic information may take a variety of forms. See "Illustrations of Construct Information" below for details of graphical representations of these data. Sequence data are commonly presented as linear strings of base, or amino acid units, usually arrayed as a matrix. Sequences submitted to generally available sequence data banks will be given specific accession numbers by the curators of those data banks.

REFERENCES.

A. Vandamme, P. Pot, M. Gillis, P. De Vos, K. Kersters and J. Swings. 1996. Polyphasic taxonomy, a consensus approach to bacterial systematics. Microbiol. Rev. 60:407-438]

Franki, R., C.M. Fauquet, D. L. Knudson, and F. Brown. 1990. Classification and nomenclature of viruses. Arch. Virology Suppl. 2:1-445.

Murphy, F.A., C.M. Fauquet, D.H.L. Bishop, S.A. Ghabrial, A.W. Jarvis, G. Marinelli, M. A. Mayo, and M. D. Summers (eds.). 1995. Arch Virology Suppl. 10 Springer Verlag ,N. Y. 586 pp.).

Rogosa, M., M.I. Krichevsky, R. Colwell. 1986. Coding Microbiological Data for Computers. Springer Verlag, N.Y. 299 pp.

IV.B. SUBJECT ORGANISM CHARACTERIZATION

Item IV.B. is intended to provide information to the Agency that is useful in identifying the taxonomies of the subject and donor organisms (IV.B.1.), the final construct (item IV.B.2), the genetic manipulations done to derive the final construct (item IV.B.3.), and properties of the subject organism (item IV.B.4.). This information is useful to the Agency for listing of the organism on the TSCA Inventory and for assessment of the potential risks of the organism.

Examples of illustrations and text which describe the final construct and the genetic manipulations done to arrive at the final construct are provided in Attachment 4 of this document, and referenced in the text of item IV.B. These illustrations and their accompanying detailed text descriptions are intended to aid in the identification of major sources of DNA used to construct and/or introduce the intergeneric DNA sequence(s) contained in subject microorganisms.

Major sources of DNA may include the recipient microorganism and donor microorganism(s). Although the Agency will focus its risk assessment on the intergeneric DNA, all introduced DNA sequences (both intra- and intergeneric sequences which have been placed in the subject microorganism by using genetic manipulations) need to be identified. This allows the Agency to fully compare the subject microorganism to the recipient in order to assess any altered risk posed by the subject microorganism. Identification of all introduced DNA allows the Agency, for example, to determine how the intrageneric introduced DNA will affect the expression, mobility, and transfer potential of the intergeneric DNA.

In order to conduct the most efficient review of the genetic manipulations done, full reference citations noted in association with the construction schemes and final construct illustrations should be provided. It is also helpful if the full text of references cited is provided for documents such as internal company documents and other which do not appear in peer-reviewed journals, foreign journals, hard-to-access theses, and patents, all of which may be difficult for the Agency to obtain.

B.1. Taxonomies of the Subject and Donor Microorganisms

B.1.a. If genetic manipulation is so extensive that a subject microorganism might be more appropriately assigned to a different taxon than the recipient parent, the submitter should provide equivalent support for the designation that applies to the final construct as that

provided for the designation of the recipient parent. Such support should apply to the microorganism intended to be used as the final production strain.

B.1.b. A generic name for the subject microorganism is needed if the name of the subject microorganism is to be considered as Confidential Business Information (CBI). Any trade name under which the subject microorganism might be marketed should also be supplied.

B.1.c. Taxonomic characterization of donor microorganisms which contribute intergeneric DNA to the subject microorganism, or provide intrageneric DNA that may affect the expression or stability/transfer of the intergeneric DNA is needed. Characterization could include identification of a genus, species, and strain designation for each donor microorganism. Generally, support information for these taxonomic designations need not be provided in detail as suggested for the recipient microorganism under item IV.A. Taxonomic information on the microorganisms from which the nucleic acid sequences were first isolated may also be helpful, if their taxonomies differ from that of the donor microorganisms used to construct the subject microorganism.

B.2. Final Construct

Provide an illustration of the final construct which is in the subject microorganism identified in item IV.B.1., and again note the taxonomy of the subject microorganism. Provide a detailed legend to support the final construct illustration.

Often, the final construct can be classified into one of three different general types: (1) an intergeneric plasmid which is extrachromosomal, (2) an intergeneric plasmid which is integrated into the chromosome, or (3) an intergeneric insert, possibly with associated vector material, which is integrated into the recipient's genome. Combinations of these general types of constructs, as well as other construct types, are possible. Two examples of final construct diagrams accompanied by respective legends, are illustrated in Attachment 4 (Figure 1 refers to the pPCB plasmid and illustrates the first general construct type; Figure 2 refers to the dct/omega/nifA insertion and illustrates the third general construct type).

The legend which accompanies the final construct illustration should focus on the intergeneric DNA, and intrageneric sequences which could affect expression or genetic transfer of the

intergeneric DNA. For example, for general construct types (1) and (2) above, the introduced DNA should be described in terms of the original host source, followed by size and function of the DNA. Introduced intrageneric structural genes, promoters, leaders, repressors, antibiotic markers, transposon or transposon fragments, other gene fragments, and cloning sites (which affect the expression, stability, or mobility of the intergeneric DNA) should be identified in the introduced DNA. The level of detail for the legend which accompanies the final construct is illustrated by the legends for Figures 1 and 2 of Attachment 4.

B.3. Construction of the Subject Microorganism

In this section, the methods and sources used to produce the final construct in the subject microorganism should be described. The description should consist of a flow diagram and its associated text in which the flow diagram is discussed. The flow diagram and associated text should describe the names, functions, and sources of donor, recipient, and vector DNA which have been manipulated to produce the subject microorganism. Source microorganisms for the introduced DNA are those microorganisms from which the DNA sequences were first identified in the literature. Again, the flow diagram should focus on the original sources for the intergeneric DNA, and other sequences associated with its expression and stability/transfer, including the items listed below. Figure 3 of Attachment 4 and its associated text provide an example of the level of detail helpful to the EPA evaluation of the cloning techniques involved in production of the subject microorganism.

3.a. A brief summary of the construction strategy should be presented. The summary should indicate why the genetic manipulations were done and their effect(s) relative to the recipient microorganism. The strategy, as indicated by the text associated with the construction of plasmid pPCB in Attachment 4, should provide an adequate level of detail. This summary should allow the Agency to follow the manipulations done, beginning with the original sources of the introduced DNA, to arrive at the final construct.

3.b. Final recipient microorganism characterization:

(1.) Prior modifications (deletions, additions)
(2.) Presence of plasmids and their ability to promote mobility/transfer, or affect the expression, of the introduced DNA
(3.) Location of endogenous gene(s) homologous to the introduced DNA that could promote mobility/transfer of the introduced DNA

(4.) Characterization of the insertion site for the introduced DNA

3.c. Reference to any prior submission to EPA (or other Federal Agencies) which is directly related to construction of the subject microorganism (provide submission number if available).

3.d. A key which contains full names for abbreviations used in the diagram.

3.e. As many circular plasmid/vector maps of intermediate constructs as necessary to clearly show genetic manipulations and gene modification. A linear portion of a plasmid representing only the changes is adequate for plasmids which have been illustrated in their entirety earlier in the diagram. These intermediate construct illustrations should be sufficient to trace and verify the origins of intergeneric DNA shown in the final construct illustration. The level of detail of the flow diagram should be similar to Figure 3 of Attachment 4.

3.f. Sizes of important gene fragments retained and lost, sequences altered, and addition and/or deletion of restriction sites.

3.g. Restriction enzymes used, including whether resulting cuts are full or partial.

3.h. Detailed map of the final cloning vector if the vector, or portions of that vector, are to be retained in the subject microorganism.

3.i. Methods for isolating and identifying the DNA used to modify the recipient microorganism.

3.j. Preparation or modification of DNA including procedures such as deletion, insertion, directed mutagenesis, and rearrangement.

3.k. Procedures for selection of intermediate hosts including methods and results for determining the success of insertion, deletion, and/or rearrangement.

3.l. Characterization of vectors so that their function (cloning, expression, or shuttle) is noted.

3.m. Transfer or integration techniques such as transformation, filter matings, triparental matings, and recombination events critical to the construction of the subject microorganism.

3.n. Catalogue references for commercial systems used such as special recipient strains, plasmids, cosmids, etc.

3.o. Literature references to original sources of important sequences used in both intermediate and final constructs.

B.4. Properties of the Subject Microorganism

In this section, please provide a brief description of the characteristics of the subject microorganism. Such characteristics may be available for the recipient microorganism, but applicable to the subject microorganism. Such information could include the items below.

4.a. Methods and results used to verify the final construct, including verification of the location of the intergeneric DNA and its copy number.

4.b. Nucleotide and protein sequences of major structural gene(s), if appropriate.

4.c. Description of gene regulation and expression in the subject microorganism and the characteristics of the product encoded for the intended use.

4.d. Possibility of unexpected gene expression or suppression in the subject microorganism as a result of the genetic modification, both during use and subsequent to release to the environment.

4.e. Indication of whether the major gene product is extracellular or intracellular.

4.f. Methods and results for determining stability of the introduced DNA.

4.g. Likelihood of genetic transfer of the introduced DNA by transformation, conjugation, transduction, and/or transfection during use and subsequent to release to the environment.

4.h. Growth characteristics in laboratory and environmental media such as generation time, growth temperature (optimum and range),

pH (optimum and range), oxygen requirements (optimum and range), preferred energy and carbon sources, etc.

4.i. Factors limiting growth, survival, or reproduction (such as auxotrophy, asporogenicity, debilitation from continuous culturing, etc.).

4.j. Subject microorganism antibiotic profile.

IV.C. POTENTIAL HUMAN HEALTH EFFECTS OF THE SUBJECT MICROORGANISM

Potential effects on fermentation workers, those who apply the microorganisms in the field, and/or consumers are assessed by the Agency for some of the categories of submissions reviewed under TSCA. In order to examine these potential effects, it is helpful to provide a review of the ability of the subject microorganism (or the recipient) to cause diseases, or be associated with disease, in humans. This section identifies information which is helpful in assessing both the potential for the subject microorganism to cause pathogenicity (IV.C.1) and toxicity (IV.C.2.) in humans.

C.1. Pathogenicity of Subject Microorganism

1.a. Nature and degree of pathogenicity, virulence, or infectivity, in humans.

1.b. Results of pathological tests on effects of the subject microorganism in mammalian species, if relevant and available.

1.c. Ability to colonize humans (e.g., the skin, the gut, etc.).

1.d. Ability to grow at human body temperature, 37 ^{o}C.

1.e. Susceptibility to control measures such as antibiotics or disinfectants, substrate requirements, or physical and chemical control methods.

C.2. Toxicity and Immunological Effects of Subject Microorganism or Its Products

a. Nature and degree of toxicity to humans.

b. Results of toxicological tests on effects of the subject microorganism or its products in mammalian species, such as allergenicity, or immunological responses after exposure via ingestion, inhalation, or dermal contact.

IV.D. PREDICTED ENVIRONMENTAL EFFECTS AND FATE OF SUBJECT MICROORGANISM

Potential effects on organisms other than humans outside the fermentation facility, and/or in the vicinity where the microorganism or its products are applied for commercial purposes need to be assessed by the Agency for some of the categories of submissions under TSCA. The potential for exposure to organisms in these areas may also need to be assessed. In order to examine these potential effects, it is helpful to provide a review of the ability of the subject microorganism (or the recipient) to cause diseases, or be associated with disease, in organisms other than humans. A review of the known ability for the subject (or recipient) microorganism to survive and spread in the environment is also helpful. This section identifies information which is helpful in assessing both the potential for the subject organism to cause adverse ecological effects (item IV.D.1.) and its ability to survive and spread in the environment (item IV.D.2.)

D.1. Ecological Effects

1.a. Nature and degree of pathogenicity, virulence, or infectivity to mammals, fish, insects and other invertebrates, and plants; including host range.

1.b. Toxicity of microbially-produced toxins to mammals, fish, insects and other invertebrates, and plants.

1.c. Involvement in or effects on biogeochemical processes (e.g., effects on nutrient cycling, particularly C,N,P, and S; effects on primary production (CO_2 fixation); utilization of complex carbon substrates, such as cellulose and lignin degradation; effects on nitrogen fixation; effects on nitrification).

1.d. Known or predicted effects on other organisms including microorganisms in the environment, including effects on competitors, prey, hosts, symbionts, predators, parasites, pathogens; effects on community structure or species diversity.

1.e. Identification and description of target organisms, e.g. taxonomy, agricultural uses, and the anticipated mechanism of interaction between microorganism and target organism.

1.f. Existence of nontarget organisms or alternate hosts (e.g., nitrogen fixing bacterial inoculants often have an intended legume host, but may be able to infect other leguminous plants).

1.g. Known or expected substrate range of degradative gene protein products including both contaminant compounds and environmental substrates (e.g., lignin).

1.h. Known or expected metabolic pathways of xenobiotic contaminant(s) present.

1.i. Nature and degree of toxicity of metabolites (dead-end or intermediate metabolites produced by metabolism of a xenobiotic contaminant) to mammals, fish, insects and other invertebrates, and plants. Toxicity should be compared with the toxicity of the parent contaminant compound.

1.j. Resident antibiotic production levels.

D.2. Survival and Fate

2.a. Natural habitats and geographical distribution of the recipient microorganism.

2.b. Laboratory studies comparing survival of the subject microorganism and the unmodified parental recipient strain in soil or water samples taken from the release site.

2.c. Survival/persistence in other environmental media aside from that found at the release site (e.g. other types of soil, water, and/or air).

2.d. Known and predicted environmental conditions that may affect survival, multiplication, dissemination.

2.e. Method of detection and detection limits of microorganism in soil and/or water (particularly in the intended environmental media or release site).

2.f. Prevalence of gene exchange in natural populations.

IV.E. PREDICTED PRODUCTION VOLUME, BYPRODUCTS, USE, AND CONSUMER EXPOSURE

This section provides guidance on submission of information on production volume of microorganisms, the types of byproducts from microbial production which may pose hazards, the ultimate commercial use of the subject microorganism or its products, and likely consumer exposures to the subject microorganism or its products.

E.1. Information on production volume

1.a. For batch processes, the batch volume, the maximum cells/batch, and the maximum CFU/batch that are likely to be produced in the first year of production; and the maximum cells/batch and the maximum CFU/batch that are likely to be produced during any 12-month period during the first three years of production. Provide also the maximum cell density of the fermentation broth in CFU/ml, the number of hours needed to produce each batch, and the number of batches per year.

1.b. For continuous processes, the maximum cells/day and CFU/day that are likely to be produced during any 12-month period during the first three years of production, the # hrs/day and # days/yr, and the maximum cell density of the fermentation broth in CFU/ml.

E.2. Information on Byproducts

In addition to information on the amounts of viable cells produced per year, it may also be helpful to know about the concentration of proteins, DNA, or other materials produced as byproducts of the manufacturing process, if such material may pose hazards to humans or the environment. Please indicate amounts of byproducts which may pose hazards to man or the environment as was done for viable cells under item E.1.

E.3. Use Information and Consumer Exposure

3.a. Describe the intended use(s) of the microorganism for the particular processes for which it is intended (such as waste degradation) or products it is intended to produce (such as enzymes for detergent use).

3.b. Estimate the percent of the production volume for each use.

3.c. Estimate the concentration of the microorganism in the formulations for each use identified.

3.d. Generic use if the use is claimed as CBI.

3.e. Based on the intended use(s), identify products in which the subject microorganism or its product will be present.

3.f. Presence of subject microorganism in consumer products (estimate amount if possible).

IV.F. PREDICTED RELEASES DUE TO MANUFACTURING OF THE SUBJECT MICROORGANISM, AND WORKER AND CONSUMER EXPOSURES TO THE SUBJECT MICROORGANISM

This section provides guidance on information useful to the Agency in developing a quantitative assessment of the potential for releases and exposures of the microorganism. This assessment is used to develop EPA's risk assessment for the microorganism

Most of the information listed below is requested of any submitter of a pre-manufacture notice (PMN) for a new chemical substance under the traditional chemical program. This information has also been requested of previous submitters of biotechnology PMNs under EPA's 1986 policy and was found to be helpful in developing a quantitative exposure and release assessment. As per the standard PMN guidance for chemical PMNs, the information requested is organized according to whether the manufacturing site is owned by (a) the company which is submitting information on the subject microorganism or (b) someone other than the company submitting information on the subject microorganism.

F.1. Industrial Sites Controlled by the Submitter

1.a. Operation description

1.a.1. Identity - identity of the site at which the operation will occur to include the name, site address and city, county, state and zip code.

1.a.2. An indication of whether the operation is best described as manufacturing, processing, or use

1.a.3. Process description

- A diagram of the major unit operation steps such as provided in the attached example (Attachment 5).

- As part of the diagram, an indication of the identity, the approximate weight (by kg/day or kg/batch) and entry point of all starting materials or feedstocks, and of all products, recycle streams, wastes and any chemicals used for inactivation or cleaning.

- Identification of the points of release of the subject microorganism, including small or intermittent releases, to the environment outside the fermentation facility.

1.b. Occupational Exposure

1.b.1. Description of activities in which workers may be exposed to the recombinant microorganisms

1.b.2. A description of any protective equipment and engineering controls used to protect workers during these activities

1.b.3. Estimates of the maximum number of workers involved in each activity

1.b.4. Estimates of the maximum duration of the activity in hours per day and days/year

1.b.5. An example of a summary table for items 1b.1.-1.b.4. is provided below.

Worker Activity	Protective Equipment/ Engineering Controls	# of Workers Exposed	Maximum Duration (hrs/day)	Maximum Duration (days/yr)

1.b.6. Results of any personnel or area monitoring conducted during the production process.

1.c. Environmental Release and Disposal

For each release point identified in the process description:

1.c.1. An estimate of the amount of the subject microorganism released (a) directly to the environment and (b) into control technology, in CFU/day or CFU/batch

Note: a control technology is any combination of engineering, mechanical, procedural, or biological method designed and operated to restrict environmental release of viable microorganisms from a structure. For air waste streams such as the off gas from the fermentor, examples include: HEPA filters, mist eliminators, caustic scrubbers, and ozone treatment. For liquid and solid waste streams such as the biomass from separation processes, examples include: steam sterilization, treatment with caustic or acidic solutions, or other antimicrobial chemicals.

1.c.2. Indication of the media (air, water, or land) to which the new substance will be released from that release point

1.c.3. For each release point released into control technology:

- A description of the control technology that will be used to limit the release of the subject microorganisms into the environment

- An estimate of the efficiency of the control technology

- An estimate of the amount of subject microorganisms released to the environment after control technology in CFU/day, and CFU/unit volume of liquid or gas

- An example of a summary table for the items listed above is provided below.

Release Number	Amount of new substance released (CFU/day or CFU/bt)		Media of Release	Control Technology	Efficiency
	To Environment	To Control Technology			

1.c.4. The basis for the estimates of release and control technology efficiency.

1.c.5. For air modeling purposes, information on the configuration of off-gassing vents if applicable, e.g. height above ground level would be helpful.

1.c.6. For environmental fate and transport assessment, information on the location of the waste disposal site for solid wastes; and for aqueous wastes, determination of whether releases are direct to surface waters or indirect to surface waters via POTW, name of receiving stream, and NPDES numbers (for manufacturing site and/or POTW), where applicable.

1.c.7 For the purpose of modeling potential exposures to the general population resulting from releases outside the plant, an indication of the proximity of the site to population areas, ground water aquifers, surface water and drinking water sources would be helpful.

F.2. Industrial Sites Controlled by Others

2.a. Operation Description

2.a.1. A diagram of the major unit operation steps, including interim storage and transport containers. On the diagram, indicate where the major unit operations are by labeling with a letter, etc and provide a brief description of worker activities associated with each one of these labeled operations.

2.a.2. An estimate of the number of sites at which the operation will occur.

2.a.3. As part of the diagram, an indication of the identity, the approximate weight (by kg/day or kg/batch) and entry point of all starting materials or feedstocks, and of all products, recycle streams, wastes. Include cleaning chemicals.

2.a.4. Identification of the points of release, including small or intermittent releases, to the environment of the new microorganism.

2.b. Worker Exposure/Environmental Release

2.b.1. Completion of items 2.b.1 to 2.b.4. for each worker activity described.

2.b.2. An estimate of the number of workers exposed for all sites combined.

2.b.3. An estimate of the typical duration of exposure per worker in (a) hours per day and (b) days/year

2.b.4. A description of any protective equipment and engineering controls used to protect the workers.

2.b.5. Completion of items 2.b.6 and 2.b.7 for each release point identified in the diagram.

2.b.6. An estimate of the amount of subject microorganisms released (a) directly into the environment or (b) into control technology

2.b.7. A description of the media of release i.e., stack air, fugitive air, surface water, on-site or off-site land or incineration, POTW or other (specify) and control technology, if any, that will be used to limit the release of the subject microorganisms into the environment.

2.b.8. An example of a summary table for items 2.b.1 to 2.b.7 is provided below.

Letter of Act-ivity	# of Workers Exposed	Duration of Exposure	Protective Equipment/Eng Controls	Release Number	Amount of New Substance Released	Control Technology

2.b.8. The basis for the estimates of release and control technology efficiency

IV.G. INFORMATION APPLICABLE TO FIELD TESTS OF THE SUBJECT MICROORGANISM

G.1. Objectives

1.a. List the objectives of the field trial and describe the rationale which requires the environmental release of the subject microorganism.

1.b. Describe the possible benefits and risks of the proposed field test.

1.c. Approximate start date and the duration of the field test.

G.2. Nature of the site

2.a. Location and size of the test area

2.b. Describe why this site was selected (relying on items such as those listed in 2.c. - 2.g. below).

2.c. Describe the history of site use. Describe any prior agricultural uses of the site, if applicable. Include items such as cropping history, tillage management systems, fertilizer and pesticide applications, and other factors, conditions, or practices which might influence characteristics of the site. If the site is not an agricultural site, describe other prior uses of the site

that would influence survival, distribution, and effects of the subject microorganism such as wastes present in surface or subsurface soils, surface or underground installations, etc.

2.d. Describe physical characteristics of the site related to surface and ground water such as distance to surface or ground water including public and private drinking water sources. For groundwater include temperature, flow velocity, dissolved oxygen, dissolved organic carbon, suspended solids, direction of flow, volume, depth, width of aquifer, and pH.

2.e. Describe physical/chemical characteristics of the soil for agricultural and other applications such as its pH, organic carbon content, texture (silt loam, clay loam, sand, etc) and cation exchange capacity. Include, if available, information on the soil series and associated soil classification employing current soil taxonomic schemes (see USDA references at the end of Section IV.G.).

2.f. Evaluate the possibility of dissemination to adjacent ecosystems and other characteristics of the site that would influence containment or dispersal e.g. relation to flood plain, slope, average wind speed and direction, annual rainfall.

2.g. Identification and description of nontarget human and nonhuman populations of concern that may be exposed, e.g. distance to nearest dwellings and population density around site.

G.3. Field test design

3.a. Rationale for field test design, description of proposed statistical analyses, and explanation of how the statistical analysis will answer the field test objectives.
3.b. Describe the control treatments being used for comparison purposes.
3.c. Submit a diagram of the plot layout and describe the procedure for randomization of the test plots.
3.d. In a table: summarize the types of samples that will be collected, where the samples will be taken, how frequently each type of sample will be taken, and how it will be stored prior to analysis.
3.e. Describe the proposed management of the site. If the subject microorganism is intended for an agricultural application, describe the planting and spacing of the test crop, width

and placement of border rows, and, pesticide applications, crop rotation, crop harvesting schedule, etc.

G.4. On-site containment practices

4.a. Describe the procedures that will be followed for packaging and transporting the subject microorganism to the site.

4.b. Describe the procedures that will be followed for the packaging and transport of samples from the field site to the laboratory for processing, including the labeling of such containers

4.c. Describe procedures for cleaning or disinfecting of planting, sampling, earthmoving and excavation equipment.

4.d. Describe procedures for the disposal of field test samples and materials that contain the subject microorganism strain(s).

4.e. Describe physical containment features such as the disposal of crop plants or dikes to contain water runoff.

4.f. Biological containment features of the field test (e.g. the use of trap plants) and the subject microorganism(s).

4.g. Describe access and security measures to be observed during the field test.

4.h. Describe containment procedures and training of personnel allowed on site.

4.i. Describe frequency and type of observations (e.g. ambient conditions or adverse effects) that will be made on site, submit sample observation forms.

G.5. Application methods

5.a. Describe the application methods and precautions that will be followed to control dissemination during the initial release.

5.b. Describe any personal protective measures that will be followed to reduce human exposure.

5.c. Describe waste materials handling and disposal procedures.

5.d. Describe method, amount, frequency, and duration of application of microorganisms.

5.e. Describe methods of cultivation after application if any.

5.f. Describe number of workers involved in application or subsequent activities, duration and routes of exposure.

5.g. Describe worker safety procedures during application and cultivation.

G.6. Termination and mitigation procedures

6.a. Procedure to be followed after the field test is completed such as: level of the subject microorganism population at which no containment measures are necessary, use of plots after the field test is terminated.

6.b. Define the type of unexpected effects and the quantitative level (if possible) which would necessitate the emergency termination of the field test.

6.c. Describe the emergency termination procedures to be followed if adverse environmental effects are observed during the course of the field test.

6.d. Describe how spills or leaks will be handled.

G.7. Monitoring endpoints & procedures for isolating subject microorganism

7.a. Relate the monitoring endpoints that will be evaluated to samples that are collected, such as population trends in soil or rhizosphere samples, or based on aerial dissemination during application as indicated by gravity plate samples.

7.b. Describe comparisons between the subject microorganism strain and the unmodified parent that will be monitored in the field.

7.c. Describe the techniques used to isolate the subject microorganism from test samples and the rationale for this procedure. Include information on positive or negative controls used with sampling technique, if applicable.

7.d. Describe the selectivity or specificity of the monitoring technique based on experimental observations under conditions similar to the field test site.

7.e. Describe the sensitivity and reliability of the isolation and identification procedure based on experimental data.

7.f. Include experimental data on the efficiency of recovery for each of the sampling techniques.

G.8. Sampling procedure

8.a. For each objective or monitoring endpoint identify the following: how, where and when samples will be taken.

8.b. Describe how the samples will be labeled so that they can later be traced back to their source.

8.c. Include the standard procedures for preserving, processing, and analyzing samples.

8.d. Describe methods of measurement, equipment used, the precision and accuracy of the method of analysis.

8.e. Describe methods for the statistical analysis of field data.

G.9. Record keeping & reporting test results

9.a. Describe the frequency of reports on the field experiment and the proposed format of the reports.

9.b. Outline the contents of the progress reports including a summary statement, statistical analysis procedures and presentation of the raw data.

9.c. Describe procedures for filing raw data and information on procedures followed for the analysis of the samples.

9.d. Describe compliance with Good Laboratory Practices.

REFERENCES FOR IV.G.

Soil Survey Division Staff. 1993. Soil survey manual. USDA Handbook number 18. USDA-SCS. U.S. Government Printing Office, Washington, D.C.

Soil Survey Staff. 1975. Soil taxonomy: A basic system of soil classification for making and interpreting soil surveys. USDA-SCA Agricultural Handbook 436. U.S. Government Printing Office, Washington, D.C.

ATTACHMENT 1

DEFINITION OF NEW MICROORGANISM
MOBILE GENETIC ELEMENTS POLICY

Excluded from the definition of “new” microorganism are naturally occurring microorganisms and genetically modified microorganisms other than intergenerics. EPA also specifically excluded from the definition of “new” microorganisms, those microorganisms that have resulted from addition of intergeneric material consisting of well-characterized non-coding regulatory regions such as operators, promoters, origins of replication, terminators, and ribosome-binding regions.

In the course of implementing the Agency’s regulatory policies for microbial products of biotechnology, it became apparent that a policy was needed to address certain genetic elements which can be transferred between microorganisms of different genera. These are termed mobile genetic elements (MGEs) and include plasmids, transposons, and viruses.

For MGEs, the major consideration was the source of the original isolation of the MGE. The genus of the original source microorganism for the MGE was identified as that genus which was first noted (in the literature, etc.) as bearing the MGE. Microorganisms were considered “new” and thus subject to reporting requirements, if the MGE was originally isolated from a microorganism in a genus different from the recipient genus. Microorganisms were considered intrageneric, and hence not subject to reporting requirements, if the MGE was originally isolated from a microorganism in the same genus as the recipient. This policy, which distinguishes which microorganisms with deliberately introduced MGEs are subject to reporting under TSCA, has been retained in the 1997 Rule (62 FR 17910-17958). Microorganisms subject to TSCA with such intergeneric MGEs should be reported using one of the seven submission types noted in item III.D., or are subject to recordkeeping and other requirements of TSCA if exempted from reporting.

EPA has adopted this interpretation for reasons of regulatory clarity and uncertainty about the possibility of the subject microorganism exhibiting new traits. For example, some MGEs may contain genetic material that normally is not expressed in one microorganism but, when inserted into another microorganism, may be expressed and result in a new trait.

ATTACHMENT 2

GUIDANCE FOR BONA FIDE NOTICES OF INTENT TO MANUFACTURE/IMPORT INTERGENERIC MICROORGANISMS

INTRODUCTION

A "new" microorganism is one that is not included on the TSCA Inventory. In the final rule (62 FR 17910-17958) the EPA defines a "new" microorganism as one which is constructed to contain genetic material originally isolated from a different genus (intergeneric). This includes microorganisms which are constructed to contain material from a mobile genetic element (MGE) which originally was isolated from a host of a genus different from that of the recipient microorganism.

To determine reporting obligations for unreported "new" microorganisms, manufacturers/importers usually consult the public version of the TSCA Inventory. There, microorganisms which are on the Inventory are listed. However, when any portion of a microorganism's identity or use has been claimed as TSCA confidential business information (CBI), that information does not appear on the public version of the Inventory. Generic identity or use information is listed instead to identify the microorganism. In fact, several of the new microorganisms which have been added to the Inventory since 1986 will have generic listings on the public version of the Inventory. This is because portions of the specific identity information, e.g., taxonomic designation, genotypic traits or phenotypic traits, are claimed as CBI. When generic information is listed on the public Inventory, manufacturers/importers may request that the Agency search the confidential version of the Inventory.

To assist manufacturers/importers in determining their reporting obligations, they may file a submission establishing a bona fide intent to manufacture, process or import a new microorganism and request that EPA determine whether their particular, unreported microorganism is listed on the confidential version of the Inventory. The Agency will determine whether a microorganism or a specific use which is identified in a bona fide notice is the same as, or is equivalent to, a microorganism/specific use that is listed on the confidential TSCA Inventory.

The Agency will process bona fide notices and conduct a search of the Master Inventory File only if the submitter demonstrates a bona fide intent to manufacture or import the microorganism for a TSCA purpose, and if sufficient information is provided to indicate the specific identity and use of the microorganism. The Agency will make every effort to complete its review and notify the submitter of the Inventory status of an unreported microorganism within 30 days.

Information provided in a bona fide notice may be claimed as CBI.

INFORMATION REQUIREMENTS

The information which should be included in a bona fide notice is described in the Final Rule. These requirements were developed specifically for microorganisms. However, due to the complex nature of microorganisms, EPA may request additional information to document the unique identity of a microorganism described in a bona fide notice. In those cases, a notice will be considered incomplete until the requested information is provided.

The following information should be included:

1. A signed statement certifying that the submitter intends to manufacture, process or import the microorganism for commercial purposes.

Certification statements must be made by the person (company) who intends to manufacture, process or import an intergeneric microorganism. Consultants or law firms retained by the company may not sign the certification statement; cover letters may be written by such legal representatives.

The submitter must be incorporated, licensed, or doing business in the United States. If the microorganism is to be imported, the authorized agent or importing company must submit the notice. Bona fide notices submitted by foreign companies with no identifiable domestic subsidiary or agent will not be accepted or processed.

2. Taxonomic designation and supplemental information for the subject microorganism, the recipient (host) microorganism and the source(s) organism (donor(s) of introduced genetic material).

Information on taxonomic designations for the recipient and donor(s) organisms should include the following:

- Taxonomic designation (including strain designations, if possible) for recipient and donor organisms, including synonyms for designations;

- Information substantiating taxonomic designations, especially that of the recipient microorganism, which may include a letter from a culture collection which verifies the taxonomic designation, literature references, or the results of tests conducted to determine phenotypic characteristics for purposes of taxonomic classification; and

- For commercially available plasmids, identify the source from which it was originally isolated, provide a genetic map that shows both coding and regulatory sequences, and provide the name of the commercial supplier.

Information that provides the specific identity of the microorganism(s), and the development methods used should include the following:

- Analytical data which describe and document the genotypic and phenotypic traits of the new microorganism, e.g., genetic map(s) of the vectors used to introduce genetic material into the recipient microorganism (see Attachment 4). A vector map should clearly indicate DNA sequences, their sources, and the functions they encode. The cellular location (chromosomal or extrachromosomal) and number of copies of introduced genetic material maintained in the subject microorganism should also be noted.

- An indication of those traits known to be added, modified, or deleted;

- A description of genotypic and phenotypic characteristics of the source microorganisms that are known/determined by the submitter; and

- A description of methods used to construct the expression and/or selection vector(s), to introduce the vectors into the recipient microorganism (e.g., cell fusion, injection of DNA, transformation), and any mutagenesis, selection, chemical treatment, and/or processing (e.g., covalent attachment of new microorganism to other organisms, chemicals, or physical structures) used to manipulate the subject microorganism.

3. Indication of whether a related microorganism has been reviewed by the Agency.

A submitter may think that the microorganism is related to one previously reviewed by the Agency as a part of Premanufacture (PMN) or Microbial Commercial Application Notifications (MCAN). In such cases, the bona fide notice should include the following information:

- The PMN or MCAN submission number; and

- A description of ways in which the bona fide microorganism is similar to and/or differs from the microorganism described in a previous PMN/MCAN submission, e. g., similarities/differences in source strains and vectors used and in steps and methods used in the development of the bona fide microorganism.

4. Description of Research and Development (R&D) conducted with the microorganism described in the bona fide notice, and a demonstration of the submitter's ability to produce or obtain the intergeneric microorganism from a foreign manufacturer.

Statements about R&D conducted with the microorganism described in the bona fide notice should contain any of the following information which applies:

- A statement that testing, e.g., genetic, biochemical, etc., of the microorganism has been conducted;

- A statement that the microorganism has been produced on laboratory scale;

- Description of research on possible uses for the microorganism; and

- A statement regarding whether the microorganism has already been manufactured outside the United States, and, if so, by whom, the quantity (volume, cell number, or colony forming units / year), and for how long.

5. A specific description of the major intended application or use of the microorganism.

If a microorganism will be imported and the foreign supplier considers the identity of the microorganism proprietary, then the

foreign supplier should submit identity information directly to the Agency. This constitutes a joint submission of a bona fide notice. However, the U. S. importer still must submit the bona fide notice, indicating that information concerning the identity of the microorganism is proprietary, and that the foreign supplier will provide this information to the Agency. It is the responsibility of the importer to ensure that this information is submitted to the Agency.

In response to joint submissions, the Agency informs only the submitter of a bona fide notice of the TSCA Inventory status of the microorganism. Proprietary information is treated as confidential business information. The Agency will not disclose proprietary information submitted by a foreign supplier to the submitter of the bona fide notice.

ATTACHMENT 3

CONFIDENTIAL BUSINESS INFORMATION (CBI) CLAIMS
SUBSTANTIATION QUESTIONS &
GUIDANCE FOR PREPARING GENERIC INFORMATION

I. CBI SUBSTANTIATION QUESTIONS

EPA's general procedures for processing and reviewing confidentiality claims are published at 40 CFR Part 2. The basic points that should be covered in CBI substantiation are set out at 40 CFR §2.204(e)(4)(I) through (ix). To ensure that substantiation responses are appropriate for submissions involving microorganisms, EPA has developed a set of questions based on the points in 40 CFR Part 2. These questions are designed to reduce the burden of substantiation by focussing the inquiry on points relevant to a biotechnology product. Upfront substantiation is required for CBI claims in MCANs, TMEs, Tier I certifications, and Tier II exemption requests.

A. Prior to commencement of manufacture or import.

1. MCAN, TME, Tier I, Tier II Submissions. Any person who submits a MCAN, TME, Tier I exemption request, or Tier II exemption request should limit confidentiality claims to that information which is confidential and proprietary to the business. If any information in the submission is claimed as confidential business information, the submitter must substantiate each claim by submitting written answers to the questions in sections C., D., and E. at the time the information is submitted. If the submitter does not provide written substantiation, the submission will be considered incomplete and the review period will not begin.

2. TERA Submission. Any person who submits a TERA should limit confidentiality claims to that information which is confidential and proprietary to the business. If any information in such a submission is claimed as confidential business information, the submitter must substantiate each claim by preparing written answers to the questions in sections C., D., and E. and submitting them to the Agency upon EPA's request.

B. After commencement of manufacture or import.

If a submitter claimed portions of the microorganism identity confidential in the MCAN and does not wish to have the specific identity listed on the public version of the Inventory, the claim

must be reasserted and substantiated at the time the Notice of Commencement is submitted. Otherwise, EPA will list the specific microorganism identity on the public Inventory. The submitter must substantiate the claim for confidentiality of the microorganism identity by answering all of the questions in sections C., D., and E. below. In addition, the following questions must be answered:

1. What harmful effects to the company or institution's competitive position, if any, would result if EPA publishes the identity of the microorganism on the public Inventory? How could a competitor use such information given the fact that the identity of the microorganism otherwise would appear on the Inventory with no link between the microorganism and the company or industry? What is the causal relationship between the disclosure and the harmful effects? How substantial would the harmful effects of disclosure be?

2. Has the identity of the microorganism been kept confidential to the extent that competitors do not know it is being manufactured or imported for general commercial use by anyone?

C. General questions

The following questions must be answered in detail for each confidentiality claim:

1. For what period of time is a claim of confidentiality being asserted? If the claim is to extend until a certain event or point in time, indicate that event or time period. Explain why the information should remain confidential until such point.

2. Briefly describe any physical or procedural restrictions within the company or institution relating to the use and storage of the information claimed as confidential. What other steps, if any, apply to use or further disclosure of the information?

3. Has the information claimed as confidential been disclosed to individuals outside of the company or institution? Will it be disclosed to such persons in the future? If so, what restrictions, if any, apply to use or further disclosure of the information?

4. Does the information claimed as confidential appear, or is it referred to, in any of the following:

(1) Advertising or promotional materials for the microorganism or the resulting end product.

(2) Material safety data sheets or other similar materials for the microorganism or the resulting end product.

(3) Professional or trade publications.

(4) Any other media available to the public or to your competitors.

(5) Patents.

(6) Local, State, or Federal agency public files.

If the answer is yes to any of these questions, indicate where the information appears and explain why it should nonetheless be treated as confidential.

5. Has EPA, another Federal agency, a Federal court, or a State made any confidentiality determination regarding the information claimed as confidential? If so, provide copies of such determinations.

6. For each type of information claimed confidential, describe the harm to the company or institution's competitive position that would result if this information were disclosed. Why would this harm be substantial? How could a competitor use such information? What is the causal connection between the disclosure and harm?

7. If EPA disclosed to the public the information claimed as confidential, how difficult would it be for the competitor to enter the market for the resulting product? Consider such constraints as capital and marketing cost, specialized technical expertise, or unusual processes.

D. Microorganism identity and production method.

If confidentiality claims are asserted for the specific identity of the microorganism or information on how the microorganism is produced, the following questions must be answered:

1. Has the microorganism or method of production been patented in the U.S. or elsewhere? If so, why is confidentiality necessary?

2. Does the microorganism leave the site of production or testing in a form which is accessible to the public or to competitors? What is the cost to a competitor, in time and money, to develop appropriate use conditions? What factors facilitate or impede product analysis?

3. For each additional type of information claimed as confidential, explain what harm would result from disclosure of each type of information if the identity of the microorganism were to remain confidential.

E. Health and safety studies of microorganisms.

If confidentiality claims are asserted for information in a health and safety study of a microorganism, the following questions must be answered:

1. Would the disclosure of the information claimed confidential reveal: (I) Confidential process information, or (ii) information unrelated to the effects of the microorganism on human health and the environment? Describe the causal connection between the disclosure and harm.

2. Does the company or institution assert that disclosure of the microorganism identity is not necessary to interpret any health and safety studies which have been submitted? If so, explain how a less specific identity would be sufficient to interpret the studies.

II. GENERIC INFORMATION FOR CBI CLAIMS

The generic information must reveal the identity and use of the microorganism to the maximum extent possible without revealing proprietary information. Submitters are encouraged to review the guidelines for preparing generic descriptions listed below and consult with EPA regarding appropriate generic information prior to submitting a notice. A description for a microorganism must be specific enough to allow clear interpretation of any accompanying health and safety data. When the location of the release site is claimed as CBI, a generic description for use must include information regarding the type of environment into which the microorganism will be released.

A. MICROORGANISM IDENTITY

Subject microorganisms cannot be specifically identified by a taxonomic designation alone. The microorganism identity used to describe such microorganisms on the Inventory will be based, in part, on the supplemental information provided which defines the specific identity of the subject microorganism. The description used to identify microorganisms on the TSCA Inventory may include the following information:

1. Taxonomic designation of genetically modified microorganism: genus, species, strain.

2. Description of the key phenotypic traits of the microorganism, including a description of anticipated behavior of microorganism.

3. Description of the key genotypic traits and modifications of the microorganism, including the following. Discussion of genotypic traits should focus on the intergeneric DNA in the final construct, and the intrageneric DNA that affects the expression, stability, and mobility of the intergeneric DNA:

   b. Introduced genes: genes introduced into, and retained in, the recipient microorganism.

   c. Vector construct: introduced genetic material used to transfer intergeneric DNA into recipient microorganism.

   d. Cellular location of introduced genes: stability of introduced genes and location of introduced genetic material in recipient (on chromosomes or on extra-chromosomal material).

   e. Number of genes introduced: number of copies of introduced genes present in recipient microorganism.

   f. Method of construction: how the genes were assembled and introduced into the recipient microorganism.

Generic names for microorganism identity should be created by masking only the portion of the information above which the submitter considers to be proprietary. Generic microorganism identity should be specific enough to allow clear interpretation of any accompanying health and safety data.

Taxonomic Designation. Submitters are encouraged always to reveal the taxonomic designation of the microorganism, since that information alone is not a sufficient generic description for a subject microorganism, unless a strain designation has been developed to describe that microorganism and its new traits. If the submitter claims the taxonomic designation as CBI, it can be masked by substituting a description of the microorganism that is as specific as possible without revealing CBI.

Description of the Microorganism. In most cases, changes in taxonomic designation of the subject microorganism have not been necessary scientifically as a result of such modifications. As

indicated above, some description of the microorganism, beyond the taxonomic designation, will be required for the specific and generic names. Given that a new microorganism is the result of the introduction of genetic material, EPA has determined that the generic description of the microorganism identity must, at a minimum, include a statement regarding the function and stability of the genetic construct. This includes an indication of whether the introduced genes are present on the chromosome or remain extra-chromosomal. Additional descriptive information should only be masked or not included in the generic description insofar as the submitter is claiming this as CBI.

B. MICROORGANISM USE

A generic description of use should describe the use or proposed use as precisely as possible without revealing proprietary information. However, the description should indicate to the maximum extent possible how the use may result in human exposure to the microorganism or its release to the environment. Alternatively, the generic use description may provide an indication of potential exposure or can describe the degree of containment of the microorganism.

When a subject microorganism is intended for use in the environment, a generic description for use must include information regarding the type of environment into which the microorganism will be released (e.g., description of field test site). If the exact location of the proposed environmental release is claimed as confidential, the submitter should provide a description of the site that will allow for an ecological analysis. While the exact information necessary for such an analysis will vary, such information could include: elevation, climate, slope and aspect, proximity to open water, groundwater level, vegetation type, and proximity to habitations and roads.

C. <u>Identity in health and safety studies</u>.

TSCA section 14(b) states that EPA is not prohibited from disclosing health and safety studies of substances for which TSCA §5 notification is required, unless disclosure reveals confidential information on process or mixture. EPA has determined that the regulations developed to address chemical identity in health and safety studies can also be applied to microorganism identity. In this regard, if any health and safety information has been submitted for the microorganism in question, the specific microorganism identity will be held confidential only if disclosure would reveal confidential process or mixture information or if the specific microorganism identity is not necessary to interpret any of the information. Under this approach, companies that claim

specific microorganism identity confidential in their submissions and wish to argue that knowledge of the specific identity is not necessary to interpret their health and safety information are encouraged to choose generic names which are sufficiently specific to allow interpretation of such information. Sufficiently specific generic names will tend to support arguments that disclosure of the specific microorganism identity is not necessary to understand the health and safety information.

ATTACHMENT 4

SUMMARY ILLUSTRATIONS FOR CONSTRUCT ANALYSIS

Guidance on how to prepare construct analysis is provided below so that only key information needed to fully characterize the genetic manipulations is provided. Illustrations provided include a text discussion and accompanying flow diagram and two illustrations for final construct. Flow diagram and final constructs are central to the description of the genetic manipulations.

The diagrams can be made using appropriate software, or legibly hand-drawn if such software is not available. For sequence information, such as analysis of open reading frames (ORFs), computerized references such as a GenBank sequence accession number, or electronic files of sequences, could be supplied.

This Attachment provides guidance under Item 1. on preparation of a final construct illustration and description. See Figures 1 and 2 and its associated legends for more information on final construct illustration and text preparation. Item 2 provides guidance on preparation of a flow diagram and its associated descriptive text. See Figure 3 and its associated text for examples of information useful in the review of the construction strategy for the subject microorganism.

1. Final Constructs (Figures 1 and 2)

Figure 1 provides an example of the plasmid construct pPCB where the entire plasmid is retained in the recipient to produce the subject microorganism. Chromosomal final construct illustrations (such as Fig. 2) would contain the same level of detail and include information on the sequences flanking the site of the chromosomal insertion. Figure 2 provides a dct/omega/nifA cassette integration into a recipient's megaplasmid by homologous recombination in which the vector sequence was not inserted.

(1) Figure 1 (plasmid final construct) and its legend provide an example of the level of detail necessary for a plasmid final construct illustration. The plasmid map should have a scale so that approximate location and size of DNA segments on the plasmid can be located on the map. First, the molecule itself has a number of details relevant to the EPA review. The coding genes of primary commercial interest are the bph genes, driven by the kanamycin promoter (Kp). The bph sequence also contains 3 open reading frames (ORFs). The third one, ORF2, has been determined recently

to encode four enzymes. The technique used to identify ORFs should be noted, along with any possible functions for these putative genes. The marker gene (Tc) and direction of transcription are also identified. Vector material originally from plasmid pRK2 with a description of which genes are present that serve plasmid maintenance and transfer functions is noted. Also, key restriction enzymes are noted on the molecule (5 EcoRI sites on the bph gene insert). The PCB degradative genetic unit (bphA to bphD) is within a 12.4 kb A. eutrophus EcoRI restriction fragment. It has a 5' flanking sequence of about 1.4 kb containing an open reading frame (ORF0) but the 3' flanking sequence (<500 bp) can be considered as part of bphD gene since this sequence is essential for the expression of bphD.

Second, brackets and labels surround the molecule which along with the Figure 1 legend, identify the original sequence sources used to construct the plasmid. Since the entire plasmid pPCB remains in the recipient, every gene segment on the plasmid should be identified by its original host, size, and function. These segments refer to the EcoRI fragment containing the bph genes, the two kanamycin fragments (one contains the promoter), the vector sequence carrying the tetracycline antibiotic marker, and the MCS sites. In this case, intergeneric DNA comes from two sources: The bph genes from A. eutrophus and the vector sequence/antibiotic marker from K. aerogenes. The source of promoter, although it is intergeneric, is excluded since it is a noncoding regulatory gene and is well characterized. For plasmids and transposons, the original host is defined as that organism from which the DNA was isolated first, according to the literature. For example, many broad host range plasmids can be maintained in any number of bacteria, but the original host is the one from which that plasmid was first obtained according to the literature, submitter records, etc. The legend contains additional information including the full name of the gene identified in Figure 2, as well as their size and function. Since the entire plasmid pPCB is retained in the new microorganism, a similar detailed plasmid map of vector, plasmid pCL1, is also required in the submission.

(2) Figure 2 (gene cassette final construct without plasmid vector sequence) and its legend is an example of another type of final construct illustration. Chromosomal insertion would contain the same level of detail and include information on sequences flanking the site of the chromosomal insertion. In this figure, the dct genes are from an intrageneric source (another species of Rhizobium) and nifA, from Rhizobium meliloti, (same as the recipient). The omega marker makes this new microorganism intergeneric. The omega marker lacks the direction of

transcription in this example, although this information would be useful.

The diagrams and descriptions in the two legends are not a fixed format but a guide to the submitter.

2. Construction of Subject Microorganism -- Text discussion and Flow diagram (Figure 3)

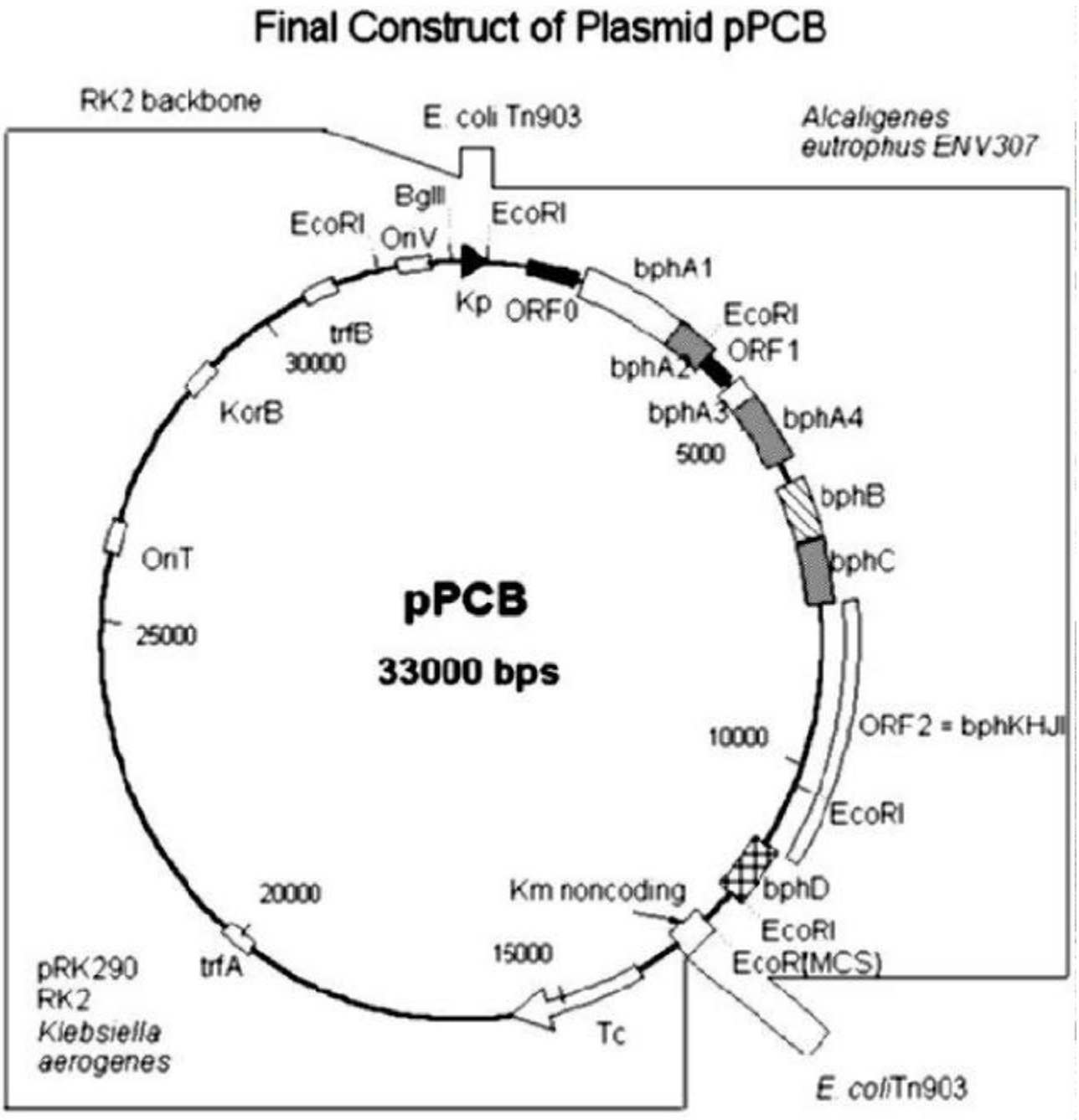
The original source of the final plasmid example in construct analysis (flow diagram in Figure 3 and final construct in Figure 1) is a Section 5 submission to OPPT of a microorganism containing a recombinant plasmid pPCB for degradation of polychlorinated biphenyls. The two diagram illustrations can be compared and contrasted to the information provided in the open literature citation by Lajoie, Layton and Sayler, 1994 (Appl. & Environm. Microbiol., V60(8):2826-2833).

In the flow diagram, sufficient circular plasmid maps have been presented to clearly show the genetic manipulations through the intermediate plasmids and vectors. Particularly important is the size of intermediate plasmids/vectors and final plasmid (pPCB)/cloning vector (pCL1). Since the map of vector pRK248 and the restriction enzyme used to cut the vector for the construction of pRK2501 are not available, the submitter should verify that the EcoRI/SalI kanamycin marker fragment from pRK2501 used in the construction of pRK293 contains only E. coli and RK2 sequences. The lines on pRK290 and pRK2501 mark the location of restriction enzyme fragment replacement to obtain the next plasmid, pRK293. Note the size of the XhoI/HindIII fragment removed from E. coli Tn903 kanamycin resistance gene. In this case, having the nucleotide sequence of the kanamycin resistance transposon Tn903 is very helpful to verify the size of the kanamycin fragments deleted and retained. The restriction enzymes for the 40 bp multiple restriction site were identified. The partial EcoRI fragment containing the A. eutrophus PCB genes cloned in plasmid pUC19 is about 22 kb and that on plasmid pCL1 is about 12.4 kb. The map of cloning vector, plasmid pCL1, is presented in the flow diagram but the final plasmid pPCB is not since it is identical to the plasmid final construct in Figure 2.

ATTACHMENT 4

SUMMARY ILLUSTRATIONS FOR CONSTRUCT ANALYSIS

Figure 1: Final plasmid construct illustration



Legend for Figure 1

Kp (360 bp) -- Region from Tn903 which includes kanamycin promoter, one 18 bp repeat sequence, and 29 bp of kanamycin structural gene (original host E. coli).

Bph genes -- A EcoRI Alcaligenes eutrophus DNA fragment, approximately 12.4 kb) -- responsible for degradation of polychlorinated biphenyls. The are 5 EcoRI restriction sites on the fragment, 3 of them at internal positions. The first EcoRI site starts at about 1.4 kb from bphA1 gene and the last EcoRI site ends at <500 bp beyond bphD gene. The 3' flanking sequence is essential for bphD gene function. Approx. size: bphA (bphA1 1376 bp, bphA2 566 bp, bphA3 318 bp, and bphA4 1225 bp); bphB 830 bp; bphC 897 bp; bphD 861 bp; encodes biphenyl dioxygenase; biphenyl-2,3-dihydrodiol-2,3-dehydrogenase; biphenyl-2,3-diol-1,2-dioxygenase; and 2-hydroxy-6-oxo-6-phenylhexa-2,3-dienoate hydrolase respectively.

ORFs -- approx. Size: ORF0 738 bp, may involve in the regulation of the bph operon; ORF1 416 bp, function unknown; ORF2 3.5 kb, this region was characterized in 1994 reference to contain closely spaced cistrons (bphKHJI) encoding a glutathione S-transferase (GST), a 2-hydroxypenta-2,4-dienoate hydrolase, an acetaldehyde dehydrogenase (acylating) and a 4-hydroxy-2-oxovalerate aldolase, respectively.

Km (549 bp) -- region from Tn903 which includes one 18 bp repeat, and 265 bp of kanamycin structural gene. Region also includes 34 bp of a synthetic MCS.

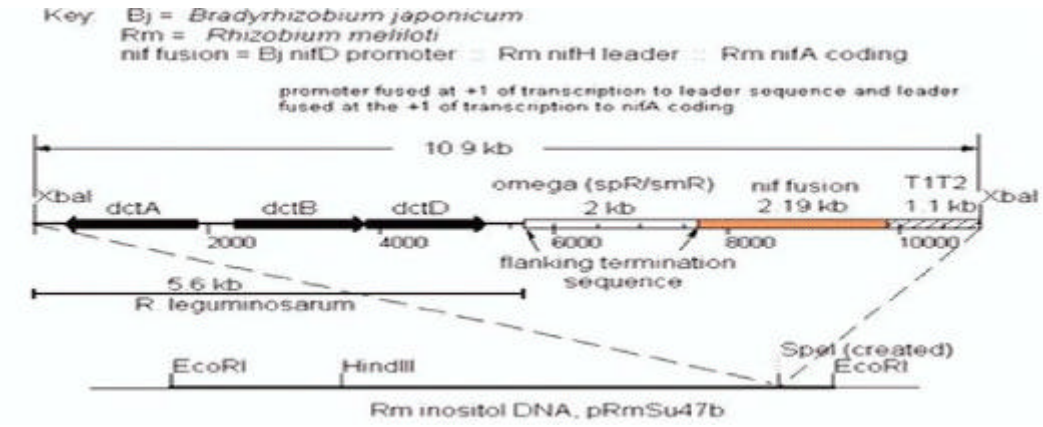
Tc (approx. 2000 bp) -- functional tetracycline resistance gene.

pRK290 genes (approx. 20000 bp) -- plasmid RK2 sequence including Tc^r gene and a number of genes for plasmid maintenance genes including the vegetative (OriV) and transfer (OriT) genes (pRK2 original host: K. Aerogenes).

ATTACHMENT 4

SUMMARY ILLUSTRATIONS FOR CONSTRUCT ANALYSIS

Figure 2: Final linear construct illustration



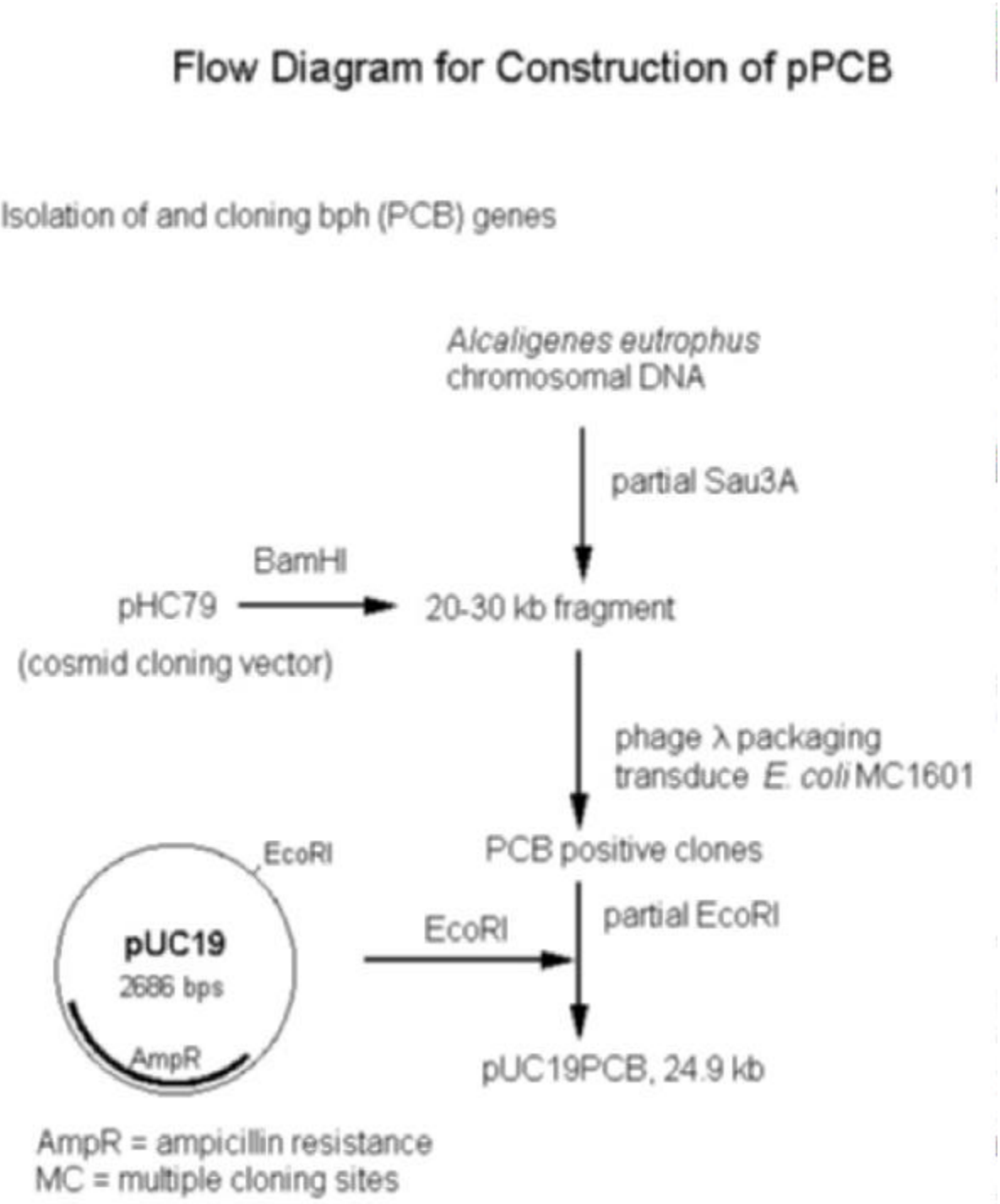
Legend for Figure 2

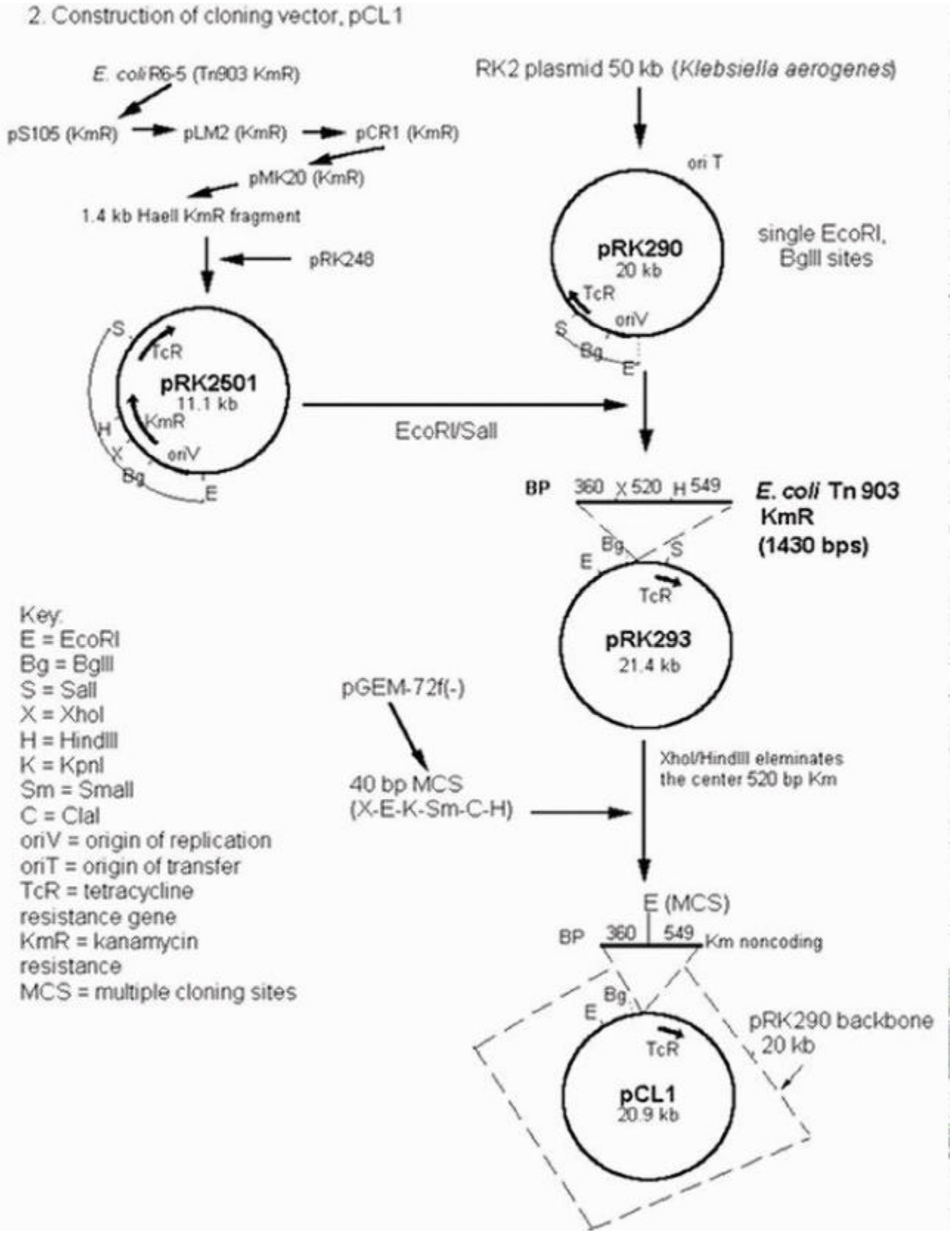
dctABD--	a 5.645 kb BglII/PstI Rhizobium leguminosarum chromosomal DNA. dctABD, 4.8 kb, involves in the C4 carboxylate transport important for nitrogen fixation. DctBD are regulatory genes.
Bj nifD promoter--	565 bp including approx. 200 bp E. coil pBR322 tetracycline resistance sequence upstream of promoter.
Rm nifH leader--	69 bp synthetic.
Rm nifA--	1.6 kb coding sequence for the gene which activates the production of nitrogenase enzyme which is required for symbiotic nitrogen fixation.
Omega fragment--	aadA gene, 2kb, specifying streptomycin/spectinomycin resistances, derived from antibiotic plasmid R100 of Shigella flexneri. The gene is flanked by transcription termination sequences from bacteriophage T4, synthetic translation terminators and polylinkers.
T1T2 terminator--	a 1.1 kb tandem repeat of two 500 bp DNA fragment from E. coli rrnB gene which encode 5S ribosomal RNA. The 1.1 kb includes a 100 bp pBR322.

ATTACHMENT 4

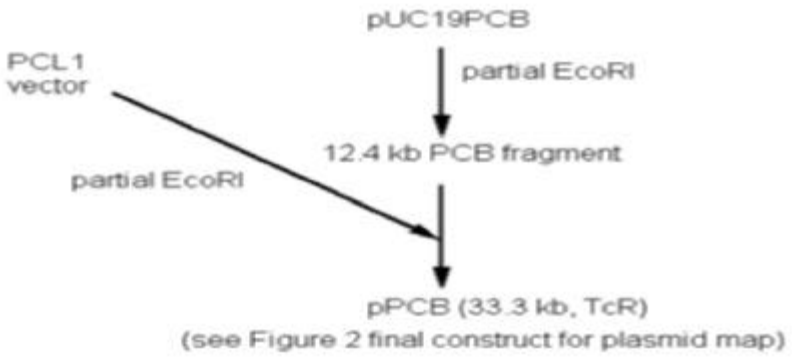
SUMMARY ILLUSTRATIONS FOR CONSTRUCT ANALYSIS

Figure 3: Flow diagram illustration




2. Construction of cloning vector, pCL1
E. coli R6-5 (Tn903 KmR)
pS105 (KmR)
pLM2 (KmR)
pCR1 (KmR)
pMK20 (KmR)
1.4 kb HaeII KmR fragment
pRK248
RK2 plasmid 50 kb (Klebsiella aerogenes)
ori T
pRK290
20 kb
TcR
oriV
single EcoRI, BglII sites
pRK2501
11.1 kb
KmR
oriV
EcoRI/SalI
BP 360 X 520 H 549
E. coli Tn 903 KmR (1430 bps)
pRK293
21.4 kb
pGEM-72f(-)
40 bp MCS
(X-E-K-Sm-C-H)
XhoI/HindIII eleminates the center 520 bp Km
E (MCS)
BP 360 549 Km noncoding
pRK290 backbone 20 kb
pCL1
20.9 kb
Key
E = EcoRI
Bg = BglII
S = SalI
X = XhoI
H = HindIII
K = KpnI
Sm = SmaII
C = ClaI
oriV = origin of replication
oriT = origin of transfer
TcR = tetracycline resistance gene
KmR = kanamycin resistance
MCS = multiple cloning sites

3. Construction of the pPCB



Construction of Pseudomonas putida IPL5 (pPCB)

Text-Discussion for Fig. 3

The PCB-degradative genes (bphABCD) were cloned from Alcaligenes eutrophus strain ENV307. Large fragments (20 to 30 kb) from Sau3A partial digests of chromosomal DNA from ENV307 were ligated into the BamHI site of the cosmid cloning vector pHC79 (Bethesda Research Laboratories, Gaithersburg, Md.). The cosmids were then packaged into bacteriophage lambda with an in vitro packaging system. The resulting lambda phage were used to transduce E. coli MC1061, and ampicillin positive transductant clones were selected. Clones containing PCB-degradative genes were selected on the basis of their ability to convert 2,3-dihydroxybiphenyl (DHB) to a yellow meta-cleavage product. The partial EcoRI restriction enzyme fragment containing the PCB degradative genes were subcloned into pUC19 which was cut with EcoRI to give pUC19PCB. The partial EcoRI fragment containing the subcloned PCB genes was about 22 kb.

The plasmid backbone of pPCB plasmid is plasmid pCL1 which is derived from cloning vector plasmid pRK290, a broad host plasmid. Plasmid pRK290 is derived from plasmid RK2, a 50 kb natural plasmid in Klebsiella aerogenes. Plasmid RK2 is identical to plasmid RP1 and RP4 carried in Pseudomonas aeruginosa. During the development of pRK290, the genes for plasmid self-transmission were removed so that the plasmid can only be transferred to a new host by triparental mating using a helper plasmid. The kanamycin resistance gene from Tn903 was inserted into plasmid pRK290 to create plasmid pRK293. This was achieved by replacing the small EcoRI/SalI fragment on plasmid pRK290 with EcoRI/SalI kanamycin fragment from plasmid pRK2501, another RK2-derived plasmid. Plasmid pRK2501 was obtained by cloning a 1.4 kb HaeII fragment, containing the Tn903 kanamycin gene from plasmid pMK20, into plasmid pRK248. Transposon Tn903 carrying the kanamycin resistance gene was originally isolated from plasmid R6-5 onto plasmid pSC101 to obtained plasmid pSC105). Subsequently, the kanamycin resistance gene was carried on pLM2, then on pCR1 , and finally on pMK20. E. coli R6-5 is a spontaneous mutant of E. coli R6. The complete sequence of Tn903 has been published. The 1.4 kb HaeII fragment contains all of the kanamycin resistance gene and promoter and 2 of the 4 18-bp inverted repeats. A 520 bp XhoI/HindIII fragment was eliminated from the central region of the kanamycin resistance gene on plasmid pRK293 and a 40-bp XhoI-HindIII fragment from the multiple cloning site (MCS) of plasmid pGEM-7f(-) (Promega Corp.) was inserted into the XhoI/HindIII site on plasmid pRK293 to create the cloning vector (plasmid pCL1). The insertion of the MCS leaves 360 bp Km fragment including one 18 bp repeat, the kanamycin promoter, and 29 bp of coding and 549 bp Km fragment

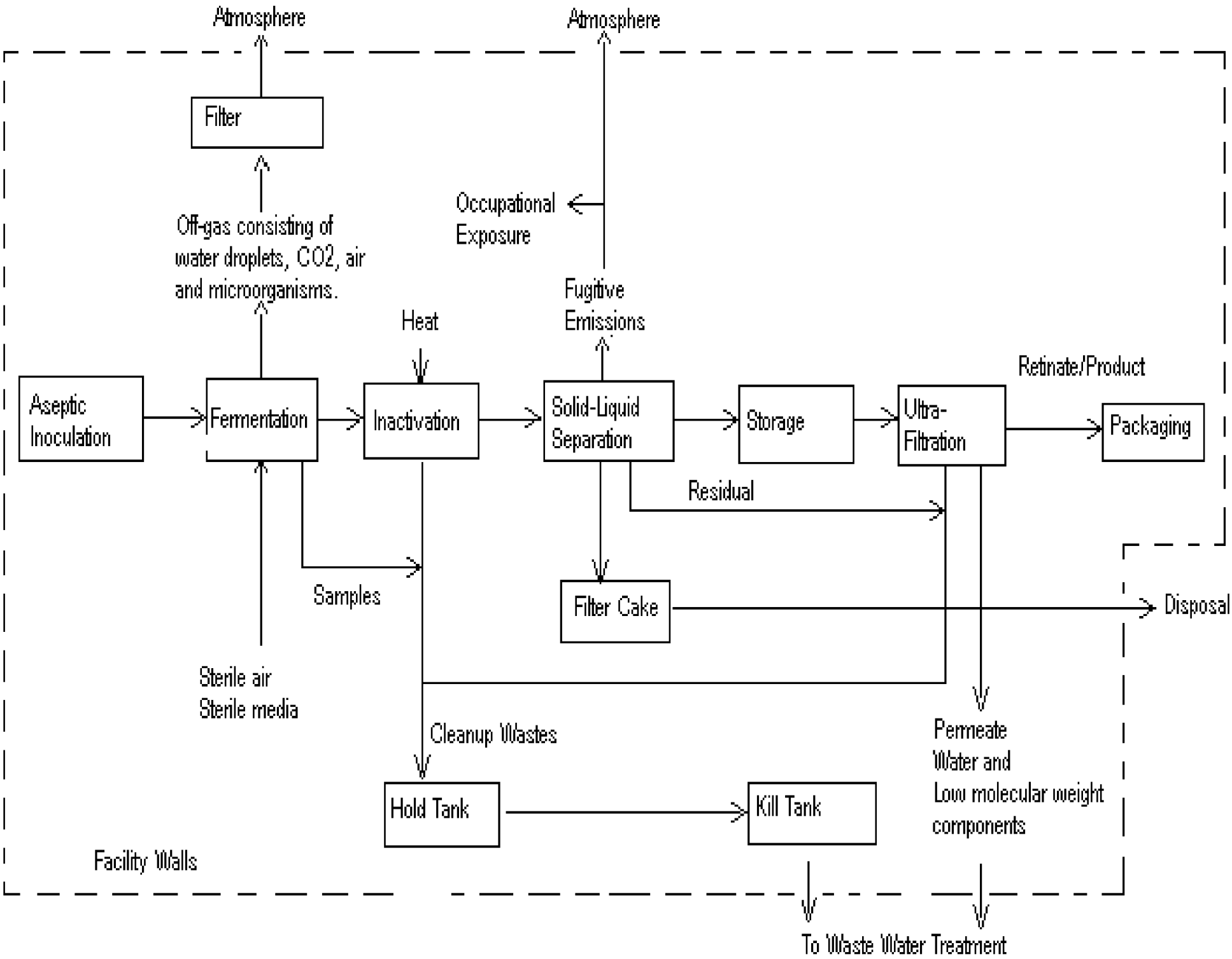
including another 18 bp repeat and 265 bp of coding sequence. Plasmid pCL1 contains two EcoRI sites, one of which is in the MCS downstream from the kanamycin promoter and is the site for the insertion of the PCB degradative genes. Thus, the bph genes are controlled by the kanamycin promoter. The other EcoRI site is on the plasmid pRK290 vector near the oriV.

Plasmid pPCB was obtained by inserting partially EcoRI-digested pUC19 containing the PCB genes into partially EcoRI digested plasmid pCL1. The size of Alcaligenes eutrophus DNA on pCL1 is approximate 12.4 kb. The cloned PCB genes from strain ENV307 have not been sequenced, but restriction mapping analysis indicates that these genes are extremely similar if not identical to the PCB genes from Pseudomonas LB400. The entire bph locus from strain LB400 has been sequenced.

Plasmid pPCB in E. coli DH5" was transferred to Pseudomonas putida IPL5 by triparental mating using E. coli containing the helper plasmid (plasmid pRK2013). The positive clones were identified by tetracycline resistance and by turning yellow when they were sprayed with DHB. Plasmid pPCB is maintained in the recipient as an extrachromosomal plasmid. The new strain is designated as Pseudomonas putida IPL5(pPCB).

ATTACHMENT 5

Diagram for Fermentation Facility Releases


Atmosphere
Atmosphere
Filter
Occupational
Exposure
Off-gas consisting of
water droplets, CO2, air
and microorganisms.
Fugitive
Emissions
Heat
Retinate/Product
Aseptic
Inoculation
Fermentation
Inactivation
Solid-Liquid
Separation
Storage
Ultra-
Filtration
Packaging
Residual
Samples
Filter Cake
Disposal
Sterile air
Sterile media
Cleanup Wastes
Permeate
Water and
Low molecular weight
components
Hold Tank
Kill Tank
Facility Walls
To Waste Water Treatment