EUROPEAN COMMISSION
HEALTH & CONSUMER PROTECTION DIRECTORATE-GENERAL

Directorate E - Safety of the food chain
E3 - Chemicals,contaminants,pesticides

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Guidance Document on the assessment of new isolates of baculovirus species already included in Annex I of Council Directive 91/414/EEC

Content

1. Introduction

It is proposed to include Baculoviruses on species level in Annex I, and to add new isolates (after they have been evaluated) in a separate list, to be maintained in the Review Report. This list shall be amended by taking note in the Standing Committee on the Food Chain and Animal Health. New isolates shall be applied for at Member state level. The applicant asks for a national authorisation. The Member State shall assess the properties of the new isolate and compare this with the reference isolate.

This guidance document is intended to establish a harmonised procedure assessing new isolates of baculoviruses already included in Annex I.

2. Legal basis/Status quo

According to the Sanco document: *Guidance developed within the Standing Committee on the Food Chain and Animal Health on the taxonomic level of micro-organisms to be included in Annex I to Directive 91/414/EEC* (Sanco/10754/rev. 5) it has been concluded that micro-organisms should be included into Annex I at the strain level. However, the document also contains the following sentence:

General view:

"*In other cases in which the species is known to be relatively homogeneous and well studied it may be decided by experts if certain questions may be handled on a species/subspecies rather than on a strain level.*"

3. Background / Justification

Baculoviruses represent a family of double stranded DNA viruses that exclusively infect Arthropoda. The vast majority of the known species are confined to insects, predominantly *Lepidoptera*, with fewer species in *Diptera* and *Hymenoptera*. Some baculoviruses are used in plant protection products for the biological control of insect pests in agriculture, horticulture and forestry. Baculoviruses used as active substances in plant protection products in the EU are regulated as micro-organisms according to Council Directive 91/414/EEC. Data requirements for the registration of baculoviruses as active substances and of products based on baculoviruses are laid down in Council Directive 91/414/EEC, amended by Commission Directive 2001/36/EC. The Uniform Principles for evaluation and authorisation of plant protection products containing micro-organisms are laid down in Council Directive 2005/25/EC.

The OECD Consensus Document (ENV/JM/MONO (2002)1)

In 2002, the OECD released the "Consensus Document on information used in the assessment of environmental applications involving Baculoviruses". This document reviewed all information publicly available at that time which was considered relevant for safety assessments of baculoviruses. This includes the biology of baculoviruses, infection mechanisms in the host, host range determination, methods for molecular characterisation of isolates, and the history of use in plant protection products. Extensive information was gathered on effects of baculoviruses on human health including infectivity, replication in vertebrate cells, genotoxicity and carcinogenicity. Ecological information summarized in the OECD consensus document includes persistence and dissemination in the environment, host specificity and effects on non-target organisms.

The following characteristics of baculoviruses were outlined:

- Baculovirus species are extremely host-specific, with their host range limited to one or a few species of the same genus. Larger host ranges covering different genera or even different families are rare (e.g. *Autographa californica* NPV). Baculoviruses probably represent the most specific pesticidal agents, biologicals and chemicals taken together.
- Baculoviruses occur only in arthropods, predominantly in the insect orders *Lepidoptera*, *Diptera*, and *Hymenoptera*.
- Baculoviruses are not infective for mammals and replication does not occur in mammalian cells.
- No pathogenic, genotoxic, mutagenic, or carcinogenic effect of baculoviruses was ever observed in mammals.
- Baculoviruses do not produce metabolites since they have no independent metabolism.
- Effects on non-target species can be excluded, especially for vertebrates, micro-organisms, and plants.

It should be noted that the document was developed under the OECD Working Group on Biotechnology and not all countries may have involved specialists for risk assessment concerning plant protection products during the development of the document. Nevertheless, this document was reviewed by a number of OECD member states. Taken together, the OECD consensus document concludes, "the use of baculoviruses is safe". Even if the document does not specify which uses are considered safe, human safety is reasonably specified in the document (page 45): "safety tests of more than 51 entomopathogenic viruses including more than 30 baculoviruses resulted in a long and complete safety record. No adverse effect on human health has been observed in any of these investigations indicating that the use of baculoviruses is safe and does not cause any health hazard."

This OECD conclusion has been confirmed through the first evaluations of baculoviruses under 91/414/EEC (e. g. *Spodoptera exigua* Nucleopolyhedrovirus (SeNPV), *Cydia pomonella* Granulovirus (CpGV), *Adoxophyes orana* Granulovirus (AoGV)).

Genetic composition of baculovirus isolates

Micro-organisms are generally registered at strain level. Bacterial and fungal strains used in plant protection products derive from single colonies or spores and are consequently genetically homogenous. Different bacterial and fungal strains from the same species may have significant differences in their biology, especially in the production of secondary metabolites.

Baculoviruses, however, represent a unique case among micro-organisms used in plant protection products in that they consist of a mixture of different, often very similar genotypes. These variations may influence some biological properties, such as the virulence to their specific target host, but they appear to have no consequences on the safety towards non-target organisms or on the environment. The composition of this mixture depends among other factors on the genotype of the host used to multiply the baculovirus. Isolation of a single genotype is extremely difficult if not impossible and even not desired since genetic variation is needed to account for variation in the target organisms. Therefore, the demand to evaluate micro-organisms at strain level is not applicable for baculoviruses.

Potential risks from plant protection products containing baculoviruses

Due to the recorded safety of baculoviruses, potential risks from baculovirus products are minimal and can occur probably rather through product components than the baculovirus itself.

All baculoviruses have to be produced *in vivo* in order to be infective to larvae. Host insect or media components might be allergenic as any other biological molecule. Hairs from some lepidopteran larvae (caterpillars) are known for their irritating and sensitising potential. Sensitisation through baculovirus-containing products was tested and no effects were found in most cases for products containing CpGV (produced in *Cydia pomonella* larvae, non-hairy) and SpliNPV (*Spodoptera littoralis*, non-hairy larvae). However, some test results might indicate a sensitisation potential of viruses of non-hairy larvae which might depend on the virus content of the formulation (CpGV, one formulation).

Accordingly, the sensitisation potential of baculovirus products needs to be assessed in each case.

Also, microbial contaminants cannot be excluded in the products, but have to be monitored and controlled. It is desirable that maximum levels for certain contaminants be established on

EU or higher level. Currently two proposals exist, REBECA[1] and PMRA, Canada, which differ and which will be discussed during the peer review of the CpGV and AoGV DARs[2]. Relevant work is ongoing in the OECD Biopesticide Steering Group.

4. Conclusion

Based on the conclusions from the OECD consensus document (ENV/JM/MONO (2002)1), the REBECA Proposal on Facilitations in the Regulation of Plant Protection Products containing Baculoviruses (January 2007) and on the first results of the evaluation of dossiers of isolates of *Spodoptera exigua* NPV, CpGV and AoGV, experts of participating RMSs concluded in the List 4, Part C micro-organisms meeting (30 January – 1 February 2007) that the Baculoviruses themselves can be regarded as harmless concerning effects on human health and the environment.

Therefore it was proposed in that meeting to include Baculoviruses (not genetically modified) on species level in Annex I of Directive 91/414/EEC, and to add the different isolates (after they have been evaluated) to a separate list, to be maintained in the Review Report and to be amended by taking note in the Standing Committee. This approach has been confirmed by a decision in the Standing Committee on May 15, 2007 were *Spodoptera exigua* NPV was listed at species level in Annex I.

New isolates would be applied for at Member state level (application for national authorisation). The member state should prepare a report on the properties of the new isolate and to be taken note of by the Standing Committee.

5. Definitions

Reference Isolate

For each species a DAR (Draft Assessment Report) has to be prepared with one isolate as reference. Therefore the reference isolate is the first applied for inclusion into Annex I.

New Isolate

New Baculovirus isolates of species already included in annex I of directive 91/414/EEC are acceptable, if the new isolate presents a similar, or lesser hazard, compared to the reference isolate.

[1] Proposal on Facilitations in the Regulation of Plant Protection Products containing Baculoviruses, January 2007

[2] Draft OECD Issue Paper: Discussion on Microbial Contaminant Limits for Microbial Pest Control Products; Prepared on September 8, 2006

6. Evaluation of new isolates / procedure

- For each species a DAR with a reference isolate (the first applied for) must be available and the reference isolate must be listed on Annex I.
- New isolates will be applied for at Member state level. The applicant asks for a national authorisation. This is only possible if the reference isolate is already included in Annex I. Otherwise, either the normal EU procedure for a new active substance has to be followed, or the application be put on hold until the reference isolate has been included in Annex I.

6.1 Data requirements

For these applications for new isolates in principle a complete Dossier (II and III) has to be submitted on MS level. However for many data requirements reference could be made to the existing DARs. Notifiers are reminded that data requirements can be addressed not only by studies, but also by relevant literature and/or scientific justifications.

Each data point for the active substance and the product has to be addressed by data or justifications. However it is not necessary to submit isolate - specific information for many data points. Most of the data required are published and relevant for all baculoviruses and already assessed by MS and EU authorities. Based on published or own data, the applicant shall make a reasoned case why the new isolate presents a similar, or lesser hazard, compared to the reference isolate. Isolate - specific data have to be submitted for data points concerning the individual baculovirus isolate.

The following isolate-specific information has to be provided for the active substance (according to Annex II data requirements):

- Origin of the isolate (Annex IIM 2.1)
- Deposition of the new isolate in a recognised culture collection (Annex IIM 1.3.2)
- A molecular identification and characterisation, e.g. by genom sequencing or by restriction length polymorphism (RFLP) analysis of DNA (Annex IIM 1.3.3)
- The manufacturing process including threshold levels for contaminants (Annex IIM 1.4.3)
- Quality control data (Analytical profile of batches; particularly levels of contaminating micro-organisms) (Annex IIM 1.4.4)
- Analytical methods for the detection of the new isolate as well as methods for the detection of microbial contaminants (Annex IIM 4.3)
- Biological properties, especially the host range (Annex IIM 2^3)

Product-specific data (according to Annex III data requirements) have to be provided including the production method (medium components, larvae hairy or not), information on the amount of non-pathogenic and pathogenic bacteria and fungi, and composition of the product. Changes when compared to methods already submitted for other products have to be declared. Data on toxicology and ecotoxicology should be based on the composition of the product. The health and environmental hazards of a preparation should be assessed as described in article 6 and 7 of 99/45/EEC, hence by a conventional (calculation) method or by providing (eco)toxicological data on the preparation or its individual components. If the composition of the product is similar to an already evaluated product, the applicant may refer to this product (with appropriate justification and, if necessary, bridging studies). Efficacy data have to be submitted for a product containing a new isolate according to national regulations.

6.2 Data Protection

Unpublished data submitted for the inclusion of a baculovirus species in Annex I are in principle protected for a certain period. This means that all notifiers applying for national authorisation of a plant protection product containing an active substance which was included in Annex I must either prove legal access to all protected data that were necessary for the Annex I inclusion, or provide equivalent own data. This refers only to data still under data protection (i.e., not to published literature). For submitted studies, for which the notifier claims data protection, the standard EU rules for data protection apply. Likewise, notifiers of products containing a new isolate have to provide own data or legal access to the protected parts of an already submitted dossier (the reference isolate dossier).

6.3 Evaluation process

The responsible MS shall carry out a risk assessment and compare the outcome with the risk of the already included isolate and inform COM and MSs with a report of its assessment.

6.4 Decision making

- If the assessment of the new isolate concludes that this isolate is comparable to the already listed isolate (i.e. similar or posses less hazard) the new isolate can, after noting by the Standing Committee, be added to the Review Report which is publicly available. Thus, all MSs are adequately informed and involved in a decision which will have direct implications for them (e.g., via mutual recognition). The legal basis for mutual recognitions would be clearly established.

[3] As the host range of baculovirus species is in most cases restricted to few species within the same family (OECD Consensus document, p. 15), investigations should be focused first on other permissive, and also on semipermissive species and species which are closely related to them.

- If the assessment of the new isolate concludes that this isolate is not comparable to the already listed isolate (i.e. not similar or posses further hazards) the Standing Committee has to take note of the report. The consequence of such a conclusion is that the notifier has to follow the normal EU procedure for inclusion as new active substance in Annex I.

6.5 Reporting

A report must be prepared in the format of Appendix I and II.

7. References

OECD consensus document (ENV/JM/MONO (2002)1), Consensus Document on Information used in the Assessment of Environmental Applications involving Baculoviruses. *Series on Harmonization of Regulatory Oversight in Biotechnology, No. 20, OECD Environment, Health and Safety Publications,* 90p.

REBECA proposal on facilitations in the regulation of plant protection products containing baculoviruses (REBECA Conference 2006, 18 - 22 September)

Report of List 4, Part C micro-organisms meeting (30 January – 1 February 2007)

European Commission

Evaluation report of a new isolate of

XXXXXXXXXXX

Part I

Member State:

Month/year

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1. PURPOSE FOR WHICH THE REPORT WAS PREPARED

This report was prepared in accordance with the guidance document SANCO/XX/XX *(Guidance Document on the assessment of new isolates of baculovirus species already included in Annex I of Council Directive 91/414/EEC).*

2. SUMMARY, EVALUATION AND ASSESSMENT OF DATA (DOSSIER DOCUMENTS J, K-II AND L-II)

SECTION A: IDENTITY OF THE MICRO-ORGANISM (Annex IIM 1)

A.1 NAME AND ADDRESS OF APPLICANT(S) (ANNEX IIM 1.1)

Name of the person responsible for the submission of the dossier:

Contact:
Telephone:
Facsimile No:
E-mail:

A.2 PRODUCER (ANNEX IIM 1.2)

XXXXXXX
Contact point:
Telephone:
Facsimile No:
E-mail:

Location of the production plant for the micro-organsim:

XXXX

A.3 NAME AND SPECIES DESCRIPTION; STRAIN CHARACTERISATION (ANNEX IIM 1.3)

A.3.1 ACCESSION NUMBER IN CULTURE COLLECTION

A.3.2 SCIENTIFIC NAME AND TAXONOMIC GROUPING

A.3.3 TEST PROCEDURES AND CRITERIA USED FOR IDENTIFICATION

A.3.4 COMMON NAME OR ALTERNATIVE AND SUPERSEDED NAMES AND CODE NAMES USED DURING THE DEVELOPMENT

Synonyms:

Common name:

Tradename:

Development code:

A.4 SPECIFICATION OF THE MATERIAL USED FOR MANUFACTURING OF FORMULATED PRODUCTS (ANNEX IIM 1.4.1 - 1.4.2 AND OECD IIM 1.4.4)

A.4.1 Content of the micro-organism

A.4.2 Identity and content of relevant impurities in the technical grade micro-organism

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B.1 ORIGIN AND NATURAL OCCURRENCE:

B.2 DESCRIPTION OF THE TARGET ORGANISM*, *focus on differences in comparison to the reference isolate

B.2 MODE OF ACTION, focus on differences in comparison to the reference isolate

B.3 HOST SPECIFICITY RANGE AND EFFECTS ON SPECIES OTHER THAN THE TARGET HARMFUL ORGANISM, focus on differences in comparison to the reference isolate

B.4 INFECTIVENESS AND DISPERSAL ABILITY, focus on differences in comparison to the reference isolate

B.5 GENETIC STABILITY AND FACTORS AFFECTING IT, focus on differences in comparison to the reference isolate

SECTION C: ANALYTICAL METHODS

C.1 ANALYTICAL METHODS FOR IDENTIFICATION AND DETERMINATION OF THE MICRO-ORGANISM (ANNEX IIM 4.3.1, IIM 4.3.4)

C.2 METHODS FOR THE DETERMINATION OF (RELEVANT) IMPURITIES AND CONTAMINATING MICRO-ORGANISMS IN MANUFACTURED MATERIAL (ANNEX 4.3.5, 4.3.6)

D. ASSESSMENT

D.1 TOXICOLOGY

D.2 ECOTOXICOLOGY

3. Conclusions and recommendations

4. References relied on

A. IDENTITY (Annex IIM 1)

Author(s)	Annex point/ reference number	Year	Title Source (where different from company) Company, Report No GLP or GEP status (where relevant) Published or not	Owner

B. BIOLOGICAL PROPERTIES (Annex IIM 2)

Author(s)	Annex point/ reference number	Year	Title Source (where different from company) Company, Report No GLP or GEP status (where relevant) Published or not	Owner

C. METHODS OF ANALYSIS (Annex IIM 4)

Author(s)	Annex point/ reference number	Year	Title Source (where different from company) Company, Report No GLP or GEP status (where relevant) Published or not	Owner

3.1. TOXICOLOGY AND METABOLISM (Annex IIM 5)

Author(s)	Annex point/ reference number	Year	Title Source (where different from company) Company, Report No GLP or GEP status (where relevant) Published or not	Owner

3.2. ECOTOXICOLOGY (Annex IIM, Point 8)

Author(s)	Annex point/ reference number	Year	Title Source (where different from company) Company, Report No GLP or GEP status (where relevant) Published or not	Owner

APPENDIX II Confidential report of a new isolate

European Commission

Evaluation report of a new isolate of

XXXXXXXXXXX

Part II

Confidential information

Rapporteur:

Month/year

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XXXX

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A.3 INFORMATION ON THE MANUFACTURING PROCESS OR PROCESSES (ANNEX IIM 4.1, 4.2)

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B.1 ANALYTICAL METHODS FOR THE DETERMINATION OF IMPURITIES AND ADDITIVES (ANNEX IIM 1.4.2)

XXXXXXXXX

References relied on

A. IDENTITY (Annex IIM 1)

Author(s)	Annex point/ reference number	Year	Title Source (where different from company) Company, Report No GLP or GEP status (where relevant) Published or not	Owner

B. METHODS OF ANALYSIS (Annex IIM 4)

Author(s)	Annex point/ reference number	Year	Title Source (where different from company) Company, Report No GLP or GEP status (where relevant) Published or not	Owner