SECTION X

RELEASE OF CAPTIVE-BRED SPECIES INTO THE WILD

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PROLOGUE

IVEM has an interest in three major aspects of the subject matter to be addressed within this contract, viz:-

1. It has been active in the fields of the ecology of insect baculoviruses (see Footnote) and the release into the environment of 'captive bred' viruses as biopesticides for in the region of fifeteen years.

2. For about six years IVEM has been in the forefront of developing insect baculoviruses as fast acting biopesticides, through genetic engineering, in a cautious but vigorous programme.

3. At the same time the complex questions posed by the putative environmental release of genetically engineered baculovirus pesticides have been and are being addressed in a risk assessment context.

Examples of the Release of Captive Bred Insect Viruses in the UK.

1A. the Nuclear Polyhedrosis Virus of *Mamestra brassicae* (MbNPV), Cabbage Moth.

NPV's are largely cultured *in vivo* using insectary-bred insects. Laboratory production of MbNPV has been in progess in IVEM for at least 15 years. Recently this virus has been cloned and the clones compared using restriction endonuclease (REN) digests. There were no REN differences between 4 clones inspected thus. However, a preliminary set of bioassays suggested one clone to be ~ x10 more infective that the 'wild' type of origin. These bioassays are currently being repeated. The infectivity of the 'wild type' has been carefully tested in 65 species of Lepidoptera, divided between 14 familes. Fifty one percent were susceptible but often only at high dosage levels (between $>10^3$-10^6 virus inclusion bodies).

After many (the number has not been systematically recorded) MbNPV passages in the laboratory this virus has been released into the field from which it has never previously been recorded in the UK. The releases have been in two main areas:-

(i) Wytham, near Oxford in 1979. Infected larvae were placed on cabbage plants for the purpose of studying the ecology of the NPV in an agroecosystem. MbNPV would certainly persist in the soil and might even now be detectable. No attempt has been made to see if caterpillars of other species of Lepidoptera in the area of this release subsequently showed MbNPV infections.

(ii) Sutherland, Scotland. 1984-88. MbNVP is highly cross-infective to larvae of Pine Beauty Moth, *Panolis flammea*. *P. flammea* has its own naturally occurring NPV. However, *P. flammea* is difficult to culture in the laboratory whilst *M. brassicae* is easy and so has been used for mass productions of these NPV's. PfNPV produced in *M. brassicae* has often turned out to be contaminated with MbNPV (reasons unknown) - these NPV's have very close homology. As it was easier to produce pure MbNPV than PfNPV it was decided to compare the two as control agents of *P. flammea* in aerial spray trials. Consequently, MbNPV was sprayed at several sites and, in one year, quite extensively (1986, on ~300 ha). There is little doubt that since this period MbNPV has spread in *P. flammea* populations. It has been detected in infected larvae ~2Km from a sprayed site and also as a latent infection (in eggs) 20-30 km from other sprayed sites. Currently studies are in progess to measure the value of the gradient of primary dispersal, a parameter we think likely to have a constant and unique value for any one host/virus 'system' and to be at least one descriptor of spread capacity.

Nine of 18 hosts species which are part of the 114 detected as part of the general Lodgepole pine forset ecosystem in Sutherland were susceptible to MbNPV. However, continuous weekly sampling of Lepidoptera on vegetation in and near an MbNPV-sprayed forest in 1986 did not reveal any infected larvae. It must be said, though, that the population density of Lepidoptera feeding on plants other than pines is usually very low, so minimising the chances of infection spreading, or of a single worker encountering an infection.

In addition to the MbNPV isolate (which was of continental origin) a pre-commerical preparation of MbNPV was obtained from a French company (Calliope) and in 1986 sprayed on ~30 ha giving quite good control of *P. flammea*. Again, this isolate was not subsequently detected in possible heterologous host species sampled in the forest concerned.

1B. The NPV of *Panolis flammea* (PfNPV).

This virus has been maintained in the laboratory at IVEM since at least 1980 being sometimes passaged through *M. brassicae* and less frequently through its homologous host. From this stock it has been mass produced for field spray trials during the years 1982-87 and to this extent it represents a captive bred species liberated into the wild. Despite the fact that cloning reveals PfNPV to be genetically very variable (at least to the extent of a lot of REN differences, which may or may not be reflected in biological differences) and though lab tests showed 36% of the pine forest ecosystem Lepidoptera to be susceptible, again it has not been detected in field survey of other species nor by vigilant (!)

entomologists from 1981-89. Currently the LD50 and host range of four clones is being studied.

2. Pines Sawfly, *Neodiprion sertifer* NPV (NsNPV)

It is for the general editor of this report to decide if, on the basis of the following account, this example fits the needs of the review.

NsNPV was first detected in Sweden and was then used in Canada for control in ~1955 where *N. sertifer* was deemed to be an exotic species. Subsequently (~1965) NsNPV was brought to the UK and sprayed in control trials in Scotland. It is, however, uncertain that this or a similar virus type was not already here. Restriction endonuclease comparisons of a British and a North American isolate in 1982 revealed genomic differences. The method of NsNPV production (it has been a marketed pesticide since 1983, trade marked 'Virox') is that quite small but high population density areas on young pine trees are sprayed with a virus suspension in June/July and that later on the infected larvae are harvested. A subsection of this material is then used agains in the following year to produce more virus in the same way. Hence there is apparently a continous genetic line from year to year - but the virus is hardly captive bred in any normal sense. In any event no other hosts are known in the UK whilst in Canada cross-infectivity to two other diprionid sawflies is not easily achieved.

3. The granulosis virus (GV) of Codling Moth, *Cydia pomonella* (CpGV).

This virus belongs to the same family as the NPV's - the Baculoviridae. It has been isoalted from the 'wild' on only a very few occasions and never in the UK. The original isolate, employed extensively in field control trials on apples, pears and walnuts throughout the world, came from Mexico. Despite its low innate frequency CpGV is an extremely promising biopesticide. Such an apparently contradictory situation is not unique in virus control of pests and probably is an example of how a virus adapted to a K-strategist host can be 'manipulated' as a control agent. Left alone, however, it is likely to revert to its original low incidence status. In the UK it is known to be cross-infective to at least two other moths, Pine Shoot moth (*Rhyacionia* sp.) and Pea moth (*Cydia nigricana*), though it has never been detected in these in the field. CpGV has been sprayed in orchard trials in the south of the UK in several recent years. As far as I know there have been no attempts to look at its prevalence in the wild Lepidoptera in orchards though, as part of a safely study, antibodies were sought (without success) in small mammals trapped in sprayed areas.

4. Brown-tail Moth *Euproctis chrysorrhoea*, NPV (EcNPV).

In recent years (1985-88) there has been a series of control trials, all on very small areas of hedgrow/bramble, in the south of England on NPV control of *E. chrysorrhoea*. The virus used came from a single isolate from the Isle of Grain, Kent, discovered in 1977/78 and subsequently replicated in the lab sometimes in bred and sometimes in field-collected host larvae. Hence virus used in trials has direct continuity with the original isolate: no new field infections have been found since 1977/78. About 40 other species of Lepidoptera have been tested, but none is susceptible: this situation is generally regarded as surprising and no explanation has yet been found.

5. Releases of Genetically Engineered *Autographa claifornica* NPV (AcNPV).

AcNPV is quite famous on two counts:

(i) It has a very wide host range, stretching across 13 families of Lepidoptera. (However, this is to be regarded with at least some caution since in very few instances has progeny virus been checked for identity; some hosts could simply be expressing a latent infection.)

(ii) It is the central subject of baculovirus genetic investigation and engineering world wide. Laboratories everywhere seem to employ AcNPV of the same origin and frequently exchange clones. It is a truly captive bred 'species'.

It is not known if AcNPV occurs naturally in the UK - it has never been sought. The source of the original isolate is California but it is probably widespread in North America, at least, and the epizootics which build up in populations *Trichoplusia ni* on brassica crops on the eastern seaboard in the fall are caused by an NPV with common identity. An NPV isolated from Greater Wax moth (*Galleria mellonella*) in the USSR is also said to be AcNPV.

A programme of genetic investigation and engineering of AcNPV has been underway in IVEM for ~5 years. Some of this work concerns the development of AcNPV as an expression vector for gene products of medical and veterinary importance (e.g. vaccines of hepatitis B, blue tongue of sheep, and etc.) and there is, of course, no intention that such 'variants' should ever be released. Their escape has not so far been considered, but would probably be deemed very unlikely. However, there is a programme to enhance the lethality, etc of NPV's to insects as improved biopesticides and in this connection the following steps have been taken in the UK with AcNPV:

(i) Insert a junk gene (a non-coding oligonucleotide sequence) into the genome at a non-disruptive site. This would then act as an unique tag permitting

this population of AcNPV to be traced following release using appropriate genetic probes.

(ii) Do the same but attempt to destabilise the virus environmentally by deleting the polyhedrin gene which codes for the protective virus inclusion body protein but which is inessential to replication *per se*. This is a step towards the concept of an engineered improved biopesticide with limited environmental persistence and hence enhanced 'safety'.

(iii) Do similar things to (i) and (ii) but insert other marker sequences, such as the well known β-galactosidase gene which permits a colorimetric method of virus detection.

(iv) Introduce genes of putative insecticidal value. The delta-endotoxin gene from *Bacillus thuringiensis* is a now very well publicised example but others in which there is interest are genes coding for insect growth regulators and arachnoid venins.

The host range of the engineered AcNPV at stages (i) and (iii) has been extensively investigated in the lab at IVEM. It appears to be curiously limited in view of the wide host spectrum of the 'wild' type from which it has derived and often where a species has proved susceptible the LD_{50} has been very high. This 'loss of activity' could be a consequence either of repeated passage *in vitro* or the chance selection of a clone of innately limited activity.

During the past three years very restricted field releases have been made near Oxford. These releases have taken the form of placing lab-infected larvae of *Spodoptera exigua*, a frequent migrant to the UK but not resident here, onto beet plants in a field containment facility. The function of the facility is to prevent ingress/egress of any but the most minute insects. The infected larvae are further confined to a central portion of the facility which itself is circumscribed with trap crop/healthy *S. exigua* larvae to assess movement of AcNPV within the contained area. After each experimental run the facility, especially its soil, has been carefully sterilised with formalin and this sterility checked through sensitive bioassays.

Under these stringent conditions the following releases have been made:

(i) AcNPV with a non-coding marker sequence - 1987

(ii) ditto, but with the polyhedrin gene deleted - 1988

(iii) β-galactosidase, polyhedrin gene deleted AcNPV

The general results have been as follows. There was no movement of AcNPV from the central area of the facility. Polyhedrin-minus AcNPV persisted for only a fraction of the time of the intact virus whether on leaf surface or in soil. No evidence of AcNPV spread, as active infections, was detected outside the facility.

GENERAL COMMENTS

1. Host Ranges. Many NPV's have quite wide host ranges - within the Lepidoptera, at least. Other, perhaps rare instances, appear to be host-specific (e.g. EcNPV). The captive bred baculoviruses so far released into the wild in the UK have not, apparently, had any adverse effects on non-target or heterologous, hosts. It cannot at this stage of our understanding be said that this is because the released viruses are out-competed by their wild counterparts in any particular host species because generally the detection of a virus depends upon an insect population having been sufficiently high for long enough for disease to become readily manifest. Insufficient funds/attention have been invested to follow such possible phenomena.

2. Genetic Recombination. Probably most lepidopteran species (and members of other insect taxa also) have naturally associated viruses. Because of this it seems quite possible that coinfection of an individual insect with two or more viruses is possible. In this situtation there could be genetic recombination, for which there is already some evidence. In the context of assessing the risks of environmental releases of genetically engineered baculoviruses this is of great interest and of concern because it asks the question "can engineered genes be 'caught' by other baculoviruses?". The much wider and more speculative question "can they also be caught by microorganisms in other major taxa" has yet to be given appropriate attention : it is known DNA can persist in soils where it can be acquried by bacteria in the so-called process of 'transformation'.

3. Risk Assessment. The risk assessment preoccupation of the last two decades has turned baculovirus ecology from an essentially academic study into a key topic. Much interest is now expressed in tracing environmental pathways, particularly of NPV's and there is now little doubt that some major questions, not already on the book, will soon be addressed. Salient amongst these are:-

(i) What is the potential impact of either captive bred wild type or genetically engineered viruses on major food chain insects, insects significant in other areas of ecological 'balance', insects of aesthetic value?

(ii) What is the likelihood of engineered genes being lost to other baculoviruses, and even to other microorganisms, and achieving an enhanced capacity to (a) affect wider segments of the biological environment and (b) in their new host, flow spatially through the environment.

Many aspects of questions such as these, and also ones concerning the comparative capacity of engineeered viruses to survive environmentally will, of course have to be addressed long before their release can be contemplated. A lot can be acheived through the use of simulated ecostsytem

components, microcosms, in severe containment conditions. IVEM is already developing appropriate microcosms in the context of EEC-funded Risk Assessment programmes.

Footnote. The Baculoviridae (BV's). This family of viruses is restricted to the Arthropoda, apprearing to be especially prevalent in insects. The genome is double-stranded DNA. The virion measures about 250 x 50 nm consisting of a core of DNA surrounded by the nucleocapsid protein and enveloped in a lipoprotein envelope. Three subgroups are recognised:

(i) A. Nuclear polyhedrosis viruses (NPV's). Here groups of virions are embedded in proteinaceous inclusion bodies of a size (1-5µm dia.) visible under the compound microscope. NPV's are especially well known in Lepidoptera (over 900 recorded) but also in diprionid sawflies, caddis flies and some Diptera.

(ii) B. Granulosis Viruses (GV's). Each virion has its own ovoid inclusion body (0.3 - 0.5 µm long). GV's are known only from Lepidoptera (over 100 discovered).

(iii) C. Non-occluded, of which the BV of *Oryctes rhinoceros* (Coconut Rhinoceros Beetle) is best known and characterised. Tentatively, about thirty viruses have been assigned to this subgroup. The host spectrum of the subgroup is probably wider than for subgroup 'A'.