WORLD HEALTH ORGANIZATION
TECHNICAL REPORT SERIES
No. 531

FAO AGRICULTURAL STUDIES
No. 91

THE USE OF VIRUSES FOR THE CONTROL OF INSECT PESTS AND DISEASE VECTORS

Report of a Joint FAO/WHO Meeting on Insect Viruses

Geneva, 22-27 November 1972

Published by
FAO and WHO

WORLD HEALTH ORGANIZATION
GENEVA
1973

PRINTED IN SWITZERLAND

CONTENTS

		Page
1.	Introduction	5
2.	Current problems and priorities for the use of insect viruses to control pests and disease vectors	6
2.1	Agriculture and forestry	7
2.2	Human and veterinary public health	17
2.3	Production and application of insect viruses	17
2.4	Training of personnel	19
3.	Characteristics and specificity of insect viruses	19
3.1	Identification and characterization	19
3.2	*In vivo* specificity for insects	20
3.2.1	Infection by ingestion	20
3.2.2	Infection by intrahaemocoelic injection	21
3.3	*In vitro* specificity for tissue cultures	21
3.3.1	Cell cultures derived from invertebrates	22
3.3.2	Cell cultures derived from vertebrates	22
3.4	Possible interactions between insect viruses and vertebrates	23
4.	Safety considerations and possible hazards in the use of insect viruses	24
4.1	Present evidence of safety	24
4.2	Possible hazards	24
4.2.1	Man and other vertebrates	25
4.2.2	The environment and insect ecosystems	29
5.	Stages of development and regulation of an insect virus as a control agent	30
5.1	Discovery and characterization of a candidate virus	30
5.2	Safety tests and limited field trials	31
5.3	Large-scale testing	32
6.	Conclusions	32
7.	Recommendations	34
7.1	Priorities	34
7.2	Conditions for safe use	35
7.3	Technology of production and application	35
7.4	Field monitoring	35
7.5	Information	36
7.6	Special recommendations	36
Annex 1.	Guidelines proposed by the United States Environmental Protection Agency for safety evaluation of microbial agents in the USA	38
Annex 2.	Protocols followed by the Insect Pathology Laboratory, United States Department of Agriculture, for safety testing of insect viruses	40

JOINT FAO/WHO MEETING ON INSECT VIRUSES

Geneva, 22–27 November 1972

Members :

Dr R. Engler, Pesticides Tolerance Division, United States Environmental Protection Agency, Washington, D.C., USA (*Rapporteur*)

Dr L. A. Falcon, Department of Entomology, University of California, Berkeley, Calif., USA

Dr C. E. Gordon Smith, London School of Hygiene and Tropical Medicine, London, England (*Chairman*)

Dr A. M. Heimpel, Insect Pathology Laboratory, Plant Protection Institute, United States Department of Agriculture, Beltsville, Md., USA

Dr C. M. Ignoffo, Biological Control of Insects Research Laboratory, United States Department of Agriculture, Columbia, Mo., USA

Dr E. Kurstak, Department of Microbiology and Immunology, Faculty of Medicine, University of Montreal, Montreal, Quebec, Canada

Dr T. R. E. Southwood, Department of Zoology and Applied Entomology, Imperial College, London, England

Dr T. W. Tinsley, Unit of Invertebrate Virology, Natural Environmental Research Council, Oxford, England

Dr C. Vago, Cytopathological Research Station, Montpellier University, Montpellier, France (*Vice-Chairman*)

Secretariat :

Dr P. Brès, Medical Officer, Virus Diseases, WHO, Geneva, Switzerland (*Joint Secretary*)

Dr A. W. A. Brown, Vector Biology and Control, WHO, Geneva, Switzerland (*Joint Secretary*)

Dr W. Chas. Cockburn, Chief Medical Officer, Virus Diseases, WHO, Geneva, Switzerland

Dr R. H. González, Plant Protection Service, FAO, Rome, Italy (*Joint Secretary*)

Dr F. W. Whittemore, Plant Protection Service, FAO, Rome, Italy

Mr J. W. Wright, Chief, Vector Biology and Control, WHO, Geneva, Switzerland

THE USE OF VIRUSES FOR THE CONTROL OF INSECT PESTS AND DISEASE VECTORS

Report of a Joint FAO/WHO Meeting

A joint meeting of the FAO Working Party of Experts on Pest Resistance to Pesticides and of an *ad hoc* WHO group on insect viruses[1] was held in Geneva from 22 to 27 November 1972. The meeting was opened by Dr L. Bernard, Assistant Director-General of the World Health Organization, on behalf of the Directors-General of the Food and Agriculture Organization of the United Nations and the World Health Organization.

1. INTRODUCTION

It has become necessary to consider the use of alternative methods of pest control because of the development of resistance to chemical pesticides and public concern about their contribution to the pollution of the environment—a concern that has been reflected in recent court decisions restricting or revoking the use of certain pesticides. The use of insect viruses, particularly the nuclear polyhedrosis viruses (NPVs) and granulosis viruses (GVs), at present appears to be the most promising alternative method.

The meeting was convened in order :

(*a*) to review the current situation with respect to insect control by insect viruses ;

(*b*) to examine data on the specificity and host range of insect viruses ;

(*c*) to identify the hazards that the use of insect viruses in pest control might present for man, animals, non-target insects, and the environment ;

(*d*) to examine the different stages in the development and regulation of an insect virus as a control agent.

The groups to which the viruses that have been found in insects belong are listed in Table 1, according to the terminology proposed by the International Committee on Nomenclature of Viruses,[2] together with indications of their relationships to vertebrate and plant viruses, based on morphological and biochemical criteria. A comprehensive review of insect viruses as control agents may be found in the book *Microbial Control of Insects and Mites*.[3]

---

[1] The expression " insect viruses " is used in this report as a term of convenience to describe viruses infecting insects and acarines. These are the two groups in the phylum Arthropoda that are candidates for biological control by these viruses.

[2] Wildy, P. (1971) *Classification and nomenclature of viruses*, Basel, Karger (Monographs in Virology, Vol. 5).

[3] Burges, H. D. & Hussey, N. W. (1971) *Microbial control of insects and mites*, London & New York, Academic Press.

TABLE 1. GROUPS OF VIRUSES IN WHICH INSECT VIRUSES OCCUR

Group	Nucleic acid	Inclusion body (— or +)	Symmetry of particle	Similarities to animal viruses and plant viruses based on morphological and biochemical criteria: Animal	Plant
Nuclear polyhedrosis viruses	DNA	+	rod	none — apparently restricted to insects	none
Granulosis viruses	DNA	+	rod	ditto	ditto
Cytoplasmic polyhedrosis viruses	RNA D/S	+	isometric	reovirus, bluetongue virus, a fish virus	wound tumour virus, rice dwarf virus, fungal viruses
Entomopoxviruses	DNA	+	brick	poxviruses of vertebrates	none
Cytoplasmic desoxyriboviruses (iridescent group)	DNA	—	isometric	viruses in fish, frogs, and lizards ; African swine fever virus	? fungal viruses
Parvoviruses	DNA S/S	—	isometric	small single-stranded DNA viruses of hamsters, mice, and rats ; probably also certain viruses of pigs, dogs, cats, and mink	none
Picornaviruses	RNA	—	isometric	enteroviruses of vertebrates	? small RNA viruses
Rhabdoviruses	RNA	—	bacilliform bullet-shaped	rabies virus, vesicular stomatitis virus, etc.	potato yellow dwarf virus, lettuce necrotic yellows, etc.

2. CURRENT PROBLEMS AND PRIORITIES FOR THE USE OF INSECT VIRUSES TO CONTROL PESTS AND DISEASE VECTORS

Chemical pesticides have been widely used for the control of insects of agricultural, veterinary, and public health importance for many years. Resistance has developed in nearly 300 insect species, and this problem has been countered by higher and more frequent rates of application. In turn, this has led to an increase of pest resistance and to higher levels of pesticide residues, particularly when the more persistent chemicals have been used. At present, resistance in some species has developed to such an extent that chemical pesticides no longer give economic and safe control, especially in those situations in which more persistent but less costly and less toxic pesticides (e.g., DDT) can no longer be used.

It is considered unlikely that insect and mite viruses could entirely replace chemical insecticides in all situations in which vectors and pests are susceptible to viruses. They promise to be most useful within the framework of an integrated pest or vector control programme. To be effective, integrated control requires the development and use of the best features of each component involved. However, the use of insect viruses in some ecosystems may be all that is necessary to keep pest populations at an acceptable medical or economic level.

2.1 Agriculture and forestry

Insect viruses, like most other pathogens of arthropods, are an integral part of the ecosystems in which they occur and as such can play an important part in regulating the numbers of their hosts.

Viruses have been isolated from more than 400 species of insects and mites ; the majority of isolations have been from species in the order Lepidoptera, a considerable number from species in the order Hymenoptera, a few from species in the orders Orthoptera, Coleoptera, and Diptera, and some from certain species of phytophagous mites. Insect and mite viruses infect some of the most important agricultural and forest pests in the world, including grasshoppers, the codling moth, many species of cutworm, armyworm, and other noctuids such as *Heliothis* spp., several species of sawfly and defoliating caterpillars attacking forest trees, and the citrus red mite.

In recent years several insect viruses have been used to combat agricultural and forest pests (Table 2). In the USA, at least 5 viruses have

TABLE 2. INSECT AND MITE VIRUSES USED TO CONTROL PESTS IN AGRICULTURE AND FORESTRY *

Host	Crops [a]	Importance of host [b]	Areas concerned [c]	Virus group [d]	Remarks
Insecta					
Coleoptera					
Scarabaeidae					
Oryctes rhinoceros (Coconut palm rhinoceros beetle)	TF	++++	PAC	NV	Current FAO project: impact of virus on beetle survival and plant damage under study on several islands.
Hymenoptera					
Diprionidae					
Gilpinia hercyniae (European spruce sawfly)	F	++++	EUR, NA	NPV	Reduces insect populations below economic damage and serious defoliation levels.
Neodiprion lecontei (Red-headed pine sawfly)	F	+++	NA	NPV	Epizootics following field applications of virus have devastated sawfly populations.
Neodiprion pratti banksianae (Jack pine sawfly)	F	++	NA	NPV	Control potential uncertain.
Neodiprion pratti pratti (Virginia pine sawfly)	F	++	NA	NPV	Tests show that pest can be controlled by dissemination of low concentrations of virus into outbreak populations.
Neodiprion sertifer (European pine sawfly)	F	+++	EUR, NA	NPV	Reduces insect populations below economic damage and serious defoliation levels.
Neodiprion swainei (Swaine's jack pine sawfly)	F	++	NA	NPV	Tests indicate that the virus can be utilized successfully for large-scale control.

TABLE 2. INSECT AND MITE VIRUSES USED TO CONTROL PESTS IN AGRICULTURE AND FORESTRY (*continued*)

Host	Crops [a]	Importance of host [b]	Areas concerned [c]	Virus group [d]	Remarks
Lepidoptera					
Gelechiidae					
Phthorimaea operculella (Potato tuberworm)	VC	+++	PAC	GV	One application of virus is at least equal to 8–10 applications of chemical pesticides during a season.
Lasiocampidae					
Dendrolimus spectabilis (Pine caterpillar)	F	U	AS	CPV	Best effects obtained when virus disseminated at rate of 125 000 million virus-inclusion bodies per ha in June, when larvae are in instars VII and VIII.
Malacosoma disstria (Forest tent caterpillar)	F	+++	NA	NPV	Studies showed that NPV can be used to reduce populations when used at 2.5×10^{11} polyhedra per ha, but it is essential to spray as soon as larvae hatch and begin feeding.
Malacosoma fragile (Great basin tent caterpillar)	F	++	NA	NPV	Control potential uncertain.
Lymantriidae					
Hemerocampa pseudotsugata (Douglas-fir tussock moth)	F	++++	NA	NPV	Excellent control has been obtained with a single application of the NPV, and it appears that, in many situations, applications may not be necessary more than once every 5 years.
Lymantria fumida	F	+++	AS	NPV, CPV	Applications of the viruses prevented excessive damage and resulted in a collapse of the pest population.
Porthetria dispar (Gypsy moth)	F	++++	EUR, NA	NPV	Excessive defoliation by this species in Europe or North America can be prevented by applying aqueous suspensions of 10^9–10^{10} polyhedra/ml at the rate of 37 litres/ha.
Noctuidae					
Agrotis segetum (Cotton cutworm)	FC	U	EUR	GV	Used on cotton in Russia.
Autographa californica (Alfalfa looper)	FC, VC	+++	NA	NPV	Cross-infective to larvae of *Pectinophora gossypiella*, *Bucculatrix thurberiella*, *Heliothis virescens*, *Trichoplusia ni*, *Spodoptera exigua*, *Estigmene acrea*, and *Plutella xylostella*.
Autographa sp.	VC	+++	EUR	NPV	Used on cabbage in Russia.
Hadena sordida (Cereal noctuid)	FC	U	EUR	GV	Used on wheat in Russia.
Heliothis armigera	FC, VC	++++ *RC*	EUR, AF, AS	NPV	Natural outbreaks reported
Heliothis virescens (Tobacco budworm)	FC, VC	++++ *RC*	NA, CA, SA	NPV	See *Heliothis zea*.

TABLE 2. INSECT AND MITE VIRUSES USED TO CONTROL PESTS IN AGRICULTURE AND FORESTRY (*continued*)

Host	Crops [a]	Importance of host [b]	Areas concerned [c]	Virus group [d]	Remarks
Heliothis zea (Bollworm, corn earworm, tomato fruitworm)	FC, VC	++++ *RC*	NA, CA, SA	NPV	Virus applied to extensive acreage in USA with good results. Commercial preparations (Viron/H, Biotrol VHZ) given temporary registration permits restricting their use to cotton. Much attention paid to the virus since 1964. Development impaired owing to competition from inexpensive chemical pesticides, lack of protocol for clearance and registration, and insufficient background research on manipulation and use.
Heliothis sp.	FC	++++	EUR	NPV	Used on cotton in Russia.
Prodenia praefica (Western yellow-striped armyworm)	FC, VC	+++	NA	NPV	Used on alfalfa in California, USA.
Spodoptera exempta (Nutgrass armyworm)	FC, VC	++++	AF	NPV	Control potential uncertain.
Spodoptera exigua (Beet armyworm)	FC, VC	++++ *RC*	NA, CA, SA	NPV	Used successfully in California, USA.
Spodoptera litura (Cotton leafworm)	VC, FC	++++ *RC*	AF, ME	NPV	Used on cotton, sweet potatoes, clover, and corn.
Trichoplusia ni (Cabbage looper)	FC, VC	+++ *RC*	NA, CA, SA	NPV	Used successfully on several crops in California, Arizona, Florida, and North Carolina in the USA, as well as in Canada and Colombia.
Trichoplusia sp.	VC	+++	EUR	GV	Used on cabbage in Russia.
Notodontidae					
Thaumetopoea pityocampa (Pine processionary caterpillar)	F	+++	EUR	CPV	Dust preparation applied to some 500 ha controlled pest without harming parasites and predators.
Olethreutidae					
Laspeyresia pomonella (Codling moth)	TF	++++ *RC*	COS	GV	Six years of field work showed the virus to be effective in reducing insect populations. Cross-infective to *Grapholitha molesta* (oriental fruit moth).
Pieridae					
Colias eurytheme (Alfalfa caterpillar)	FC	+++	NA	NPV	Reduces insect populations below economic damage level.
Pieris brassicae (Large white butterfly)	VC	+++	EUR	GV	Successfully used on kale crops.
Pieris rapae (Imported cabbageworm)	VC	+++	NA, EUR	GV	Several studies have shown that the virus may be used for the control and regulation of pest populations.
Pieris sp.	VC	+++	EUR	GV	Used on cabbage in Russia.
Psychidae					
Kotochalia junodi (Wattle bagworm)	F	+++	AF	NPV	Used to suppress damaging pest populations when applied early in the larval cycle at rates of 2.5×10^{10} polyhedra per ha and higher.

TABLE 2. INSECT AND MITE VIRUSES USED TO CONTROL PESTS IN AGRICULTURE AND FORESTRY (*continued*)

Host	Crops [a]	Importance of host [b]	Areas concerned [c]	Virus group [d]	Remarks
Tortricidae					
Argyrotaenia velutinana (Red-banded leaf roller)	TF	++++ *RC*	NA	GV	Results indicate control potential.
Choristoneura fumiferana (Spruce budworm)	F	++++ *RC*	NA	NPV, GV, CPV	Control potential uncertain.
Yponomeutidae					
Yponomeuta padella (Ermine moth)	TF	U	EUR	NPV	Used on apples in Russia.
Arachnida					
Acarina					
Tetranychidae					
Panonychus citri (Citrus red mite)	TF	++++ *RC*	NA	NV	Used on limited scale in California, USA, by establishing infection in population foci.

* The sources of information for this table and for Table 4 were:

Bergold, G. H. (1963) *The nature of nuclear-polyhydrosis viruses.* In: Steinhaus, E. A., ed., *Insect pathology, an advanced treatise*, New York & London, Academic Press, vol. 1, pp. 413–456

Burges, H. D. & Hussey, N. W. (1971) *Microbial control of insects and mites*, New York & London, Academic Press

Heimpel, A. M. (1972) *Insect control by microbial agents.* In: Agricultural Board, Division of Biology and Agriculture, National Research Council, *Pest control strategies for the future*, Washington, D.C., National Academy of Sciences, pp. 298–316

Huger, A. (1963) *Granuloses of insects.* In: Steinhaus, E. A., ed., *Insect pathology, an advanced treatise*, New York & London, Academic Press, vol. 1, pp. 531–575

Journal of Invertebrate Pathology, 1964–72, vols 6–20

Laan, P. A. van der, ed. (1967) *Insect pathology and microbial control. Proceedings of the International Colloquium on Insect Pathology and Microbial Control, Wageningen, The Netherlands, 5–10 September 1966*, Amsterdam, North-Holland Publishing Company

Martignoni, M. E. & Longston, R. L. (1960) Supplement to an annotated list and bibliography of insects reported to have virus diseases, *Hilgardia*, **30**, 1–40

Smith, K. M. (1963) The cytoplasmic virus diseases. In: Steinhaus, E. A., ed., *Insect pathology, an advanced treatise*, New York & London, Academic Press, vol. 1, pp. 457–497

Smith, K. M. (1967) *Insect virology*, New York & London, Academic Press.

Steinhaus, E. A. (1957) New records of insect-virus diseases, *Hilgardia*, **26**, 417–430

[a] FC = field crops. VC = vegetable crops. TF = tree fruits. F = forests.

[b] U = importance unknown. ++ = of some importance. +++ = of considerable importance. ++++ = highly important. *RC* = resistant to control by chemical insecticides.

[c] NA = North America. CA = Central America. SA = South America. EUR = Europe. AF = Africa. ME = Middle East. AS = Asia. PAC = Pacific. COS = cosmopolitan.

[d] NPV = nuclear polyhedrosis virus. CPV = cytoplasmic polyhedrosis virus. GV = granulosis virus. NV = noninclusion virus.

TABLE 3. INSECT VIRUS PRODUCTS DEVELOPED BY INDUSTRY IN THE USA FOR USE ON A COMMERCIAL OR EXPERIMENTAL BASIS *

Host	Name of product	Source
Heliothis	Biotrol VHZ [a]	Nutrilite Products, Inc. (NPI)
	Viron/H [a]	International Minerals and Chemical Corp. (IMC)
	Vitrex	Hays-Sammons
Neodiprion	Polyvirocide	Indiana Farm Bureau Co-op. Assoc.
Prodenia	Biotrol VPO	NPI
	Viron/P	IMC
Spodoptera	Viron/S	IMC
	Biotrol VSE	NPI
Trichoplusia	*Trichoplusia* virus	Rudd Associates
	Trichoplusia virus	Biological Control Supplies
	Viron/T	IMC
	Biotrol VTN	NPI

* All viruses mentioned are nuclear polyhedrosis viruses.

[a] The only insect virus products for which complete safety and efficacy data have been submitted to the US Environmental Protection Agency and which have been granted temporary use permits in the USA.

been produced on a commercial or experimental scale for field use (Table 3). A large number of viruses have been discovered in insects and mites of agricultural importance (Table 4), and many of them could have a potential for biological control. An attempt should be made to find additional viruses in the nuclear-polyhedrosis and granulosis groups that might be

TABLE 4. A PARTIAL LIST OF INSECTS AND MITES OF AGRICULTURAL IMPORTANCE FROM WHICH VIRUSES OF UNDETERMINED CONTROL POTENTIAL HAVE BEEN REPORTED *

Host	Virus group [a]
Insecta	
Coleoptera	
Lucanidae	
Figulus sp.	PXV
Scarabaeidae	
Aphodius tasmaniae	PXV
Costelytra zealandica	IV
Melolontha melolontha	PXV
Othnonius batesi	PXV
Sericesthis pruinosa	IV
Diptera	
Tipulidae	
Tipula paludosa	NPV, IV
Hymenoptera	
Argidae	
Arge pectoralis	NPV

TABLE 4. A PARTIAL LIST OF INSECTS AND MITES OF AGRICULTURAL IMPORTANCE FROM WHICH VIRUSES OF UNDETERMINED CONTROL POTENTIAL HAVE BEEN REPORTED (*continued*)

Host	Virus group [a]
Diprionidae	
Diprion pallida	NPV
Diprion pini	NPV
Gilpina hercyniae	NPV
Neodiprion abietis	NPV
Neodiprion dyari	NPV
Neodiprion exitans	NPV
Neodiprion nanulus	NPV
Neodiprion taedae linearis	NPV
Neodiprion virginiana	NPV
Tenthredinidae	
Anoplonyx destructor	CPV
Nematus olfaciens	NPV
Trichocampus viminalis	NPV
Lepidoptera	
Anthelidae	
Anthelia hyperborea	NPV
Anthelia varia	NPV
Pterolocera amplicornis	NPV
Arctiidae	
Amsacta moorei	PXV
Arctia caja	NPV, CPV
Arctia villica	CPV
Ardices glatignyi	NPV
Cycnia mendica	NPV, CPV
Diacrisia purpurata	CPV
Dionycopus amasis	GV
Ecpantheria sp.	NPV
Estigmene acrea	CPV, GV
Euplagia quadripunctaria	CPV
Halisidota argentata	NPV
Hyphantria cunea	NPV, CPV, GV
Hypocrita jacobaeae	NPV, CPV
Panaxia dominula	NPV
Parasemia plantaginis	CPV
Phragmatobia fuliginosa	CPV
Rhyparia purpurata	CPV
Spilosoma lubricipeda	CPV
Spilosoma lutea	CPV
Cossidae	
Cossus cossus	PXV
Crambidae	
Chilo suppressalis	GV, IV
Dioptidae	
Phryganidia californica	NPV
Drepanidae	
Drepana lacertinaria	CPV
Epipaschiidae	
Tetralopha scortealis	NPV
Galleriidae	
Galleria mellonella	NPV, CPV, NV
Gelechiidae	
Pectinophora gossypiella	CPV
Phthorimaea operculella	GV
Recurvaria milleri	GV

TABLE 4. A PARTIAL LIST OF INSECTS AND MITES OF AGRICULTURAL IMPORTANCE FROM WHICH VIRUSES OF UNDETERMINED CONTROL POTENTIAL HAVE BEEN REPORTED (*continued*)

Host	Virus group [a]
Geometridae	
Abraxas grossulariata	NPV, CPV
Alsophila pometaria	CPV
Anaitis plagiata	CPV
Biston betularia	CPV
Bupalus piniarius	CPV
Caripeta divisata	NPV
Cingilia catenaria	CPV
Crocallis elinguaria	CPV
Ectropis crepuscularia	NPV
Ellopia somniaria	NPV
Ennomos quercinaria	NPV
Enypia venata	NPV
Erannis tiliaria	NPV, CPV
Erannis vancouverensis	NPV
Eulype hastata	GV
Eupithecia longipalpata	NPV
Hibernia defoliaria	NPV
Idaea seriata	NPV
Lambdina fiscellaria	NPV
Melanolophia imitata	NPV
Nepytia phantasmaria	NPV
Oporinia autumnata	NPV
Operophtera burceata	NPV
Operophtera brumata	NPV, CPV, PXV
Operophtera fagata	CPV
Ourapteryx sambucaria	CPV
Paleacrita vernata	CPV
Pero behrensarius	NPV
Ptychopoda seriata	NPV
Sabulodes caberata	NPV, GV
Selenia lunaria	CPV
Selidosema suavis	NPV
Semiothisa liturata	CPV
Synaxis pallulata	NPV
Hepialidae	
Porina sp.	NPV
Wiseana cervinata	NPV, IV
Lasiocampidae	
Dendrolimus sibiricus	GV
Dendrolimus spectabilis	NPV
Dendrolimus superans	CPV
Dendrolimus undans	NPV
Eriogaster lanestris	CPV
Gastropacha quercifolia	CPV
Gonometa podocarpi	NV
Kunugia yamadai	NPV
Lasiocampa quercus	CPV
Malacosoma alpicola	NPV
Malacosoma americanum	NPV, CPV
Malacosoma californicum	NPV, CPV
Malacosoma constrictum	NPV
Malacosoma neustria	NPV, CPV
Malacosoma pluviale	NPV
Limacodidae	
Darna trima	GV
Natada nararia	GV
Sibine stimulea	NPV
Lycaenidae	
Lycaena phlaeas	CPV

TABLE 4. A PARTIAL LIST OF INSECTS AND MITES OF AGRICULTURAL IMPORTANCE FROM WHICH VIRUSES OF UNDETERMINED CONTROL POTENTIAL HAVE BEEN REPORTED (*continued*)

Host	Virus group [a]
Lymantriidae	
Dasychira plagiata	NPV
Dasychira pudibunda	CPV
Euproctis chrysorrhoea	NPV, CPV
Euproctis flava	NPV
Euproctis phaeorrhoea	NPV
Euproctis pseudoconspersa	NPV
Hemerocampa leucostigma	NPV, CPV
Hemerocampa pseudotsugata	CPV
Hemerocampa vetusta	NPV
Ivela auripes	NPV
Lymantria incerta	NPV
Lymantria monacha	NPV, CPV
Stilpnotia salicis	NPV, CPV
Megalopygidae	
Megalopyge sp.	NPV, GV
Noctuidae	
Achaea janata	GV
Agrochola lychnidis	CPV
Agrotis segetum	NPV, CPV
Agrotis subterranea	GV
Amathes glareosa	CPV
Anchosceli helvola	CPV
Anticarsia gemmatilis	NPV
Antitype xantomista	CPV
Autographa biloba	NPV
Autographa brassicae	NPV
Autographa californica	GV
Calophasia lunula	CPV
Catabena esula	NPV
Ceramica picta	NPV, CPV
Chorizagrotis auxiliaris	NPV, CPV, GV, PXV
Diataraxia oleracea	CPV
Euxoa ochrogaster	GV
Hada nana	CPV
Hadena serena	CPV
Heliphobus albicolon	CPV
Heliothis armigera	CPV, GV
Heliothis obtectus	NPV
Heliothis peltigera	NPV
Heliothis phloxiphaga	NPV
Heliothis zea	CPV, GV
Lampra fimbriata	CPV
Lithoplane leautieri	CPV
Mamestra brassicae	NPV, CPV
Mamestra illoba	NPV
Mania maura	CPV
Melanchra persicariae	GV
Mythrimna (Aletia) oxygala luteopallens	NPV
Nephelodes emmedonius	NPV, GV
Orthosia brumata	NPV
Orthosia hibisci	NPV
Panthela portlandica	NPV
Peridroma saucia	NPV, GV
Phlogophora meticulosa	NPV, CPV
Plusia gamma	NPV, CPV
Prodenia androgea	GV
Prodenia eridania	NPV
Prodenia ornithogalli	NPV
Prodenia terricola	NPV
Pseudaletia unipuncta	NPV, CPV, GV, NV
Pseudaletia sp.	NV
Pseudoplusia includens	NPV
Rachiplusia ou	NPV
Scoliopteryx libatrix	CPV
Sesamia cretica	GV
Spodoptera exigua	GV

TABLE 4. A PARTIAL LIST OF INSECTS AND MITES OF AGRICULTURAL IMPORTANCE FROM WHICH VIRUSES OF UNDETERMINED CONTROL POTENTIAL HAVE BEEN REPORTED (*continued*)

Host	Virus group [a]
Noctuidae (*continued*)	
Spodoptera frugiperda	NPV, GV
Spodoptera litura	CPV, GV
Spodoptera mauritia	NPV
Synographa selecta	NPV
Trichoplusia ni	CPV, GV
Triphaena pronuba	CPV
Xylomyges conspicillaris	CPV
Notodontidae	
Cerura hermelina	NPV
Cerura vinula	CPV
Lophopteryx capucina	CPV
Phalera bucephala	NPV, CPV
Pygaera anastomosis	NPV, GV
Schizura concinna	CPV
Nymphalidae	
Aglais urticae	NPV, CPV
Argynnis dia	CPV
Junonia coenia	NPV, GV
Nymphalis antiopa	NPV, CPV
Nymphalis io	NPV, CPV
Polygonia c-album	CPV
Polygonia satyrus	NPV
Vanessa cardui	NPV, CPV
Olethreutidae	
Spilonota ocellana	NPV
Papilionidae	
Papilio machaon	CPV
Phycitidae	
Cadra cautella	NPV, CPV, GV
Pieridae	
Aporia crataegi	NPV
Colias electo	NPV
Colias eurytheme	CPV
Colias lesbia	NPV
Euchloe cardamines	CPV
Gonepteryx rhamni	CPV
Neophasia menapia	NPV
Pieris brassicae	NPV, CPV
Pieris napi	GV
Pieris rapae	NPV, CPV
Plutellidae	
Plutella xylostella	GV
Pyralidae	
Blepharomastix acutangualis	NPV
Lamprosema (Nacoleia) diemenalis	GV
Nacoleia octosema	NPV
Plodia interpunctella	GV
Witlesia sp.	IV
Saturniidae	
Actias selene	CPV
Antheraea eucalypti	CPV, NV
Antheraea mylitta	CPV
Antheraea paphia mylitta	CPV
Antheraea pernyi	NPV, CPV
Antheraea polyphemus	NPV, CPV
Antheraea yamamai	NPV
Automeris memusae	CPV
Coloradia pandora	NPV
Dictyoploca japonica	NPV
Hemileuca tricolor	NPV
Hyalophora cecropia	CPV, IV

TABLE 4. A PARTIAL LIST OF INSECTS AND MITES OF AGRICULTURAL IMPORTANCE FROM WHICH VIRUSES OF UNDETERMINED CONTROL POTENTIAL HAVE BEEN REPORTED (*continued*)

Host	Virus group [a]
Saturniidae (*continued*)	
Hylesia nigricans	NPV
Nudaurelia cytherea capensis	NV
Samia cynthia	NPV, CPV
Samia ricini	NPV
Saturnia pyri	NPV
Satyridae	
Dira megera	CPV
Pararge aegeria	CPV
Sphingidae	
Celerio euphorbiae	NPV, CPV
Erinnyis alope	GV
Hyloicus pinastri	CPV
Laothoe populi	NPV, CPV
Manduca sexta	GV
Mimas (Dilena) tiliae	CPV
Smerinthus ocellatus	CPV
Sphinx lingustri	NPV, CPV
Thaumetopoeidae	
Thaumetopoea pityocampa	NPV
Thaumetopoea processionea	NPV
Tineidae	
Tinea pellionella	NPV, CPV
Tineola bisselliella	NPV, CPV
Tortricidae	
Acleris variana	NPV
Adoxophyes orana	NPV, CPV
Amelia pallorana	GV
Choristoneura biennis	PXV
Choristoneura murinana	NPV, GV
Choristoneura rosaceana	NPV
Epiphyas postvittana	NPV
Pandemis lamprosana	NPV
Sparganothis pettitana	NPV
Tortrix viridana	NPV
Zeiraphera diniana	GV
Zeiraphera griseana	GV
Zygaenidae	
Harrisina brillians	GV
Orthoptera	
Acrididae	
Melanoplus bivittatus	NV
Melanoplus sanguinipes	NPV
Gryllidae	
Gryllus bimaculatus	NV
Teleogryllus oceanicus	NV
Teleogryllus commodus	NV
Arachnida	
Acarina	
Tetranychidae	
Panonychus ulmi	NV

* Prepared by L. A. Falcon and L. K. Etzel, Department of Entomological Sciences, University of California, Berkeley, Calif., USA. For sources of information, see footnote * to Table 2.

[a] NPV = nuclear polyhedrosis virus. CPV = cytoplasmic polyhedrosis virus. GV = granulosis virus. PXV = insect poxvirus. NV = noninclusion virus. IV = iridescent virus.

used for the control of agricultural and forest pests. Priority should be given to viruses of the desert locust, of rice and maize stem borers, and of coleopterous and lepidopterous pests of stored grain, for the following reasons : current methods of locust control depend primarily upon the use of dieldrin ; insect pests of stored grain are becoming resistant to malathion, the only pesticide that has been widely accepted for the long-term protection of commodities of this type ; and stem borers are particularly difficult to control by conventional methods.

2.2 Human and veterinary public health

Only a small number of viruses pathogenic to arthropods of medical and veterinary public health importance have been isolated to date. Several viruses showing nuclear and cytoplasmic polyhedrosis inclusion bodies have been isolated from mosquitos (*Aedes*, *Culex*, and *Anopheles*). An entomopoxvirus has been isolated from *Anopheles*, and iridescent viruses have been isolated from *Aedes* and *Simulium* spp. Experimental infections have been successful and particular properties have been defined for certain of these viruses. Current studies are concerned with structure, specificity, and epidemiology and limited to some viruses with nuclear and cytoplasmic polyhedrosis inclusions and to iridescent viruses. No viruses pathogenic to arthropods in other groups of medical and veterinary importance have been isolated, apparently because insufficient attention has been paid to the problem. It is probable that systematic research would find additional viruses that might be used for the control of insects in these groups and of other arthropods such as ticks, fleas, and triatomid bugs.

2.3 Production and application of insect viruses

The living target host is generally used to propagate insect viruses and will probably continue for some time to be the main substrate for the production of those intended to control arthropods of agricultural and medical importance. Production techniques for a few viruses of agricultural pests have advanced to the point at which the viruses can be produced commercially. This stage of development has not yet been reached for the virus of any medically important invertebrate pest.

The present industrial method for *Heliothis* NPV [1, 2] permits the continuous systematic production of the virus with minimal risk for the production personnel and reduces contamination of the product by extraneous microorganisms to an acceptable level. Each batch of commercially produced virus is characterized as to identity, activity, mammalian toxicity

---

[1] Ignoffo, C. M. (1966) In : Smith, C. N. ed., *Insect colonization and mass production*, New York & London, Academic Press, pp. 501–530.

[2] Ignoffo, C. M. (1965) *Entomophaga*, **10**, 29–40.

and pathogenicity, and content of extraneous microorganisms. Other recently described techniques for the propagation of insect viruses, e.g., tissue culture, cannot at present be utilized for their commercial production.

Heliothis NPV is currently formulated as a powder containing the inclusion bodies. This is suspended in water and sprayed on the crop in the same manner as chemical insecticides, by means of knapsack sprayers, hydraulic sprayers, mist blowers, or aircraft. The droplet size ranges from 300 µm with the usual methods of spraying to 100 µm or less for most low-volume applications of concentrated suspensions.

Heliothis NPV has suppressed natural populations of *Heliothis* spp. on cotton and other crops. The virus is highly specific and does not directly destroy parasites and predators of the target pest. Cadavers of the *Heliothis* caterpillars killed constitute a further, continuing source of new virus. This bonus effect is characteristic of infective agents and has been known to lead to the control of *Heliothis* pest populations over entire areas.

The *Heliothis* virus has shown no change in specificity over the past 12 years. Attempts to select *H. zea* resistant to the NPV have failed. The occluded virions of *Heliothis* NPV appear to be inactivated by sunlight, so that their half-life in the open environment is less than 24 hours; improved formulations applied on cotton have, however, increased their half-life to about 5 days. The current cost of the commercial preparation is intermediate between that of the most and the least expensive chemical insecticides.

The NPV affecting *Heliothis* spp. has been extensively tested for safety in vertebrates.[1] The tests were conducted on man, monkey, dog, rabbit, guineapig, rat, mouse, chicken egg, quail, sparrow, mallard, 7 species of fish, and many invertebrates. The virus was administered in a variety of ways: by ingestion, by inhalation, by topical application, and by intradermal, intracerebral, intravenous, intramuscular, and intraperitoneal inoculation. Tests were made for cutaneous or respiratory sensitivity, toxicity, pathogenicity, teratogenicity, carcinogenicity, and growth in primary primate cells, and all proved negative. Heimpel & Buchanan[2] reported no adverse effects on man in feeding experiments with this virus.

In 1970, the US Department of Agriculture and the US Food and Drug Administration, after considering the results of these tests, issued a temporary permit allowing the use of *Heliothis* NPV on a large acreage of cotton, together with a temporary exemption from the tolerance[3] requirement, and approval will probably be extended to its use on other crops. The NPV of the alfalfa looper also will probably shortly satisfy the requirements of the

---

[1] Greer, F., Ignoffo, C. M. & Anderson, R. F. (1971) *Chem. Techn.*, June, 342–347.

[2] Heimpel, A. M. & Buchanan, L. K. (1967) *J. invertebr. Path.*, **9**, 55–57.

[3] The word "tolerance" is applied to the permissible level of insecticidal material on a crop.

US Environmental Protection Agency and be released for use on cotton and other crops.

Insect virus preparations have been formulated as dusts or wettable powders or for use with baits. These preparations are applied, either alone or in combination with compatible chemical pesticides, by means of the equipment used for applying pesticides; alternatively, they may be used before or after applications of such pesticides. However, it is likely that even more suitable formulations, application methods, and equipment will be developed. Insect viruses can be used in conjunction with parasites, predators, and other insect pathogens. They could be included in sterile-male control programmes to aid in reducing the target population or to control other pests in the treated area. Insect viruses can also be disseminated by the release of treated laboratory-reared insects. In addition wild insects could be attracted to a source such as a light trap, contaminated with an insect virus, and released.

2.4 Training of personnel

Progress in the discovery, characterization, and evaluation of viruses of invertebrates as potential control agents is going to depend largely on the availability of scientists trained in the virology and pathology of invertebrates. There is a great shortage of such scientists at present. Attention is drawn to the worldwide scarcity of suitable educational programmes in this field.

3. CHARACTERISTICS AND SPECIFICITY OF INSECT VIRUSES

It is important to determine whether infection by insect viruses used as control agents will be limited to one insect species or extended to others or possibly to vertebrates. The specificity of a virus for a particular invertebrate can be used to characterize and identify it. Full characterization of specificity by *in vivo* and *in vitro* studies will determine whether there is a possibility of any interaction between insect viruses and vertebrates.

3.1 Identification and characterization

If studies on specificity are to be meaningful, it is essential to obtain more detailed information on the physical, chemical, and biological properties of insect viruses. The following characteristics, among others, should be determined:

(1) *Virion*

(*a*) Nature of the nucleic acid—single- or double-stranded, molecular weight;

(*b*) Symmetry of the capsid ;
(*c*) Nucleocapsid enveloped or naked ;
(*d*) Dimensions of the nucleocapsid ;
(*e*) Number of capsomers ;
(*f*) Whether embedded or not in crystalline protein body, characters of the crystals ;
(*g*) Antigens ;
(*h*) Sensitivity to temperature ;
(*i*) Stability.

(2) *Replication of the virus*
(*a*) Nature of the target cells and nature of cellular lesions ;
(*b*) Site of viral replication—cytoplasm or nucleus ;
(*c*) Upper and lower temperature limits for development.

(3) *Pathogenicity*
(*a*) Symptomatology and diagnosis of the disease ;
(*b*) Host specificity.

3.2 In vivo specificity for insects

3.2.1 *Infection by ingestion*

It is probable that viruses enter the insect host mainly through the mouth parts. Tests of host specificity are made by feeding the insects doses of virus particles, or polyhedra containing virus particles, in natural food (e.g., leaves) or artificial diets. The criterion of infection is the mortality of the test insects, usually expressed in LD_{50} values. This means that it is difficult to take sublethal infections, which may be common in nature, into account. The available methods for assaying the level and extent of these

TABLE 5. TRANSMISSION OF INSECT VIRUSES ACROSS SPECIES BARRIERS *

Group [a]	Total number of attempted transmissions [b]	Percentage of all attempts successful [b]	Attempted transmissions to other Families (% of attempts successful)	Attempted transmissions to other Orders (% of attempts successful)
GV	56	11	3	—
NPV	187	32	22	33
CPV	45	64	46	29
NV	39	90	85	95

* Adapted from : Ignoffo, C. M. (1968) *Bull. ent. Soc. Amer.*, **14**, 265–276.

[a] GV = granulosis virus. NPV = nuclear polyhedrosis virus. CPV = cytoplasmic polyhedrosis virus. NV = noninclusion virus.

[b] Including within-Family transmissions.

possible sublethal infections are both tedious and time-consuming. There is thus an urgent need to develop easier and more sensitive methods for assaying and monitoring all stages of an infection and determining the precise identity of the virus causing it. In some situations the dose levels of virus to which insects are exposed in the field are lower than those applied in host range experiments in the laboratory. Table 5, which is based on laboratory experiments on the cross-transmission of insect viruses, shows that the groups can be ranked in order of their specificity, granulosis viruses being the most specific and non-inclusion viruses the least.

3.2.2 *Infection by intrahaemocoelic injection*

The dosages required to infect and cause death are invariably much higher when the virus is introduced *per os* than when it is introduced by intrahaemocoelic inoculation. The phenomenon responsible for this is usually referred to as the gut barrier. Little is known about the type and importance of the defence mechanisms involved in the response of invertebrates to infection with viruses, since they do not have the system of circulating immunoglobulins found in vertebrates. The gut barrier may play a role in the defensive reactions of invertebrates, but the responses of the invertebrate host to virus infection need more intensive study. With most pathogenic viruses of insects, invasion of the haemocoele and associated tissues is a prerequisite for fatal disease. For this reason, any infection limited to the gut tissues would be undetected by existing methods. It is therefore clear that reliance on mortality data may give an imprecise picture of the prevalence of infection and result in an inaccurate assessment of the invertebrate host range of any particular insect virus.

Intrahaemocoelic infection may occur in nature when, for example, parasitic wasps inject virus into host larvae together with their eggs.[1]

There is evidence that very little virus is needed to cause fatal disease when it is injected directly into the haemocoele. Thus a much wider host range for a particular virus can be determined by this method than by the ingestion method. For example, the iridescent virus of the crane fly (*Tipula paludosa*) has been shown, by intrahaemocoelic injection in the class Insecta, to have an extremely wide host range infecting species in the orders Diptera, Lepidoptera, and Coleoptera,[2] although it is very difficult to infect even the original host by ingestion.

3.3 In vitro specificity for tissue cultures

Virulence patterns of viruses may change when mutations permit them to adapt to a different host. Insect viruses tend to be less specific *in vitro*

---

[1] Kurstak, E. & Vago, C. (1967) *Rev. canad. Biol*, **26**, 311–316.

[2] Smith, K. M. (1967) *Insect virology*, New York & London, Academic Press.

than *in vivo*. The fact that a virus is capable of infecting cultured cells from a particular host does not necessarily imply that it is a pathogen for that host. The pathogenicity of a virus for an insect host can only be determined by *in vivo* experiments.

The infection of cells in culture does not always lead to destructive changes but may result in transformations that could indicate carcinogenesis. For example the vertebrate virus SV40, which causes no apparent disease in monkeys, induces tumours when injected into newborn hamsters.

3.3.1 *Cell cultures derived from invertebrates*

Tissue cultures have been derived from Lepidoptera, Coleoptera, Hymenoptera, Diptera, Homoptera, Dictyoptera, and Orthoptera—mainly from ovarian sheaths, heart, blood cells, and gut epithelium. Both primary cultures and cell lines may be available from several laboratories engaged in research on viruses of invertebrates.[1] All groups of insect viruses have been grown experimentally in primary insect cell cultures. Nuclear polyhedrosis, granulosis, and cytoplasmic polyhedrosis viruses infect lepidopteran ovarian and cardiac cell cultures.

Whatever the group of virus, replication has been demonstrated mainly in cells of the species in which it originated. However, the nuclear polyhedrosis, cytoplasmic polyhedrosis, and granulosis viruses will replicate in cell cultures of Lepidoptera other than the original Lepidopteran host species. Densonucleosis virus can infect only the wax moth (*Galleria mellonella*) but will replicate in cultured cells of the silk worm (*Bombyx mori*). Iridescent viruses can be grown routinely in cell cultures from Diptera, Lepidoptera, or Dictyoptera.

Insect tissue cultures are essential for the investigation of viral replication and may help to indicate the range of host susceptibility. Systematic studies should be made of the effects of insect viruses on cell cultures derived from various invertebrates. However, any cell line used must be characterized so as to identify its nature and origin clearly.

3.3.2 *Cell cultures derived from vertebrates*

There is so far little or no evidence that insect viruses can replicate in cell cultures derived from vertebrates. Himeno et al.[2] have reported that viral DNA extracted from the NPV of *Bombyx mori* infected human amnion (FL) cells and produced infectious virions as well as polyhedra. The virus produced was morphologically and serologically similar to virus produced in insects, was infectious for insects, and caused typical disease. These results,

---

[1] Burges, H. D. & Hussey, N. W. (1971) *Microbial control of insects and mites*, New York & London, Academic Press.

[2] Himeno, M. et al. (1967) *Virology*, **33**, 507–512.

however, await confirmation. Experiments in several cell lines from vertebrates, using NPVs of *Heliothis* and *Hemerocampa*, a cytoplasmic polyhedrosis virus (CPV) of *Bombyx*, an entomopoxvirus of *Amsacta*, and a noninclusion virus (NV) of *Tipula*, provided no evidence of viral replication.

Densonucleosis virus (DNV) provides an interesting example of the relationship between an insect virus and cell cultures derived from vertebrates.[1, 2] The purified virus succeeded in adapting to mouse L cells and to rat embryo cells, which then produced viral antigens localized in the nucleus, as shown by the immunofluorescent technique. Some mouse L cells produced DNV virions, although in smaller quantities than can be obtained from insects. These virions caused a disease typical of DNV when injected into *G. mellonella* larvae. However, the virus did not replicate or cause disease in mice.

3.4 Possible interactions between insect viruses and vertebrates

The properties of a virus isolated from the moth *Gonometa podocarpi* clearly indicated that it belonged to the picornavirus group of vertebrate viruses. In the United Kingdom, this virus has been shown to react with antibodies in sera from cattle, horses, sheep, pigs, dogs, and deer (Longworth — unpublished data). These were antibodies of the IgM type which are usually a response to recent infection. It is unlikely that the animals were exposed to the *Gonometa* virus, as it is exotic to the United Kingdom. It is more probable that they had been infected with some other virus or microorganism that shared a common antigen with the *Gonometa* virus.

Tests on sera from laboratory staff working with insect viruses revealed that 8 out of 24 individuals had antibodies to at least one of the following: a GV polyhedral protein, an iridescent virus, and a picornavirus of invertebrates. These findings could be interpreted as the result either of exposure to these viruses suspended in the air of the laboratory or—which is much less likely—of previous infections with other microorganisms of related antigenic composition. There was no associated medical evidence of overt disease in the individuals concerned. In other situations, no antibody responses were detected in persons occupationally exposed to *Heliothis* NPV or *Bombyx* NPV.[3] While these findings indicate that careful attention should continue to be paid to the possibility that some insect viruses may be capable of infecting vertebrates, the reports considered above are difficult to interpret.

---

[1] Kurstak, E. (1971) *Ann. Parasit. hum. comp.*, **46**, No. 3 bis, 277–288.

[2] Kurstak, E. (1972) *Advanc. Virus Res.*, **17**, 207–241.

[3] Aizawa, K. (1954) *Nature (Lond.)*, **174**, 748–749.

4. SAFETY CONSIDERATIONS AND POSSIBLE HAZARDS IN THE USE OF INSECT VIRUSES

The hazards that the use of insect viruses may involve for man, other vertebrates, non-target invertebrates, and the environment must be considered.

4.1 Present evidence of safety

Insect viruses have long been present in the environment, and vertebrates, especially insectivorous species, have certainly been exposed to them on countless occasions. During epizootics of virus disease in insect populations, insectivorous birds and animals ingest high concentrations of virus; virus-infected insects may even be eaten by preference, since infections can alter their colour and behaviour and make them more vulnerable to predators.[1] Wild animals are also exposed to aerosols containing naturally occurring virus particles, and vegetables are often heavily contaminated with naturally occurring insect viruses.

The *Heliothis* NPV (section 2.3) has frequently been used for the field control of pest insects, but only after extensive testing. In recent years, however, a cytoplasmic polyhedrosis virus, an entomopoxvirus, and a picornavirus, none of which had undergone adequate testing beforehand for hazards to vertebrates, have been sprayed out-of-doors. A virus from the coconut beetle (*Oryctes rhinoceros*) has also been spread by the release of infected insects—a less hazardous procedure. To date, no deleterious effects have been reported in connexion with these operations.

At present there is no direct evidence that any virus of invertebrates presents a hazard to man or other vertebrates, although there is recent evidence of the widespread occurrence of what appears to be antibody reacting with a non-occluded virus of invertebrates in several vertebrate species. Antibodies to certain insect viruses have also been reported in several workers in a laboratory (section 3.4).

4.2 Possible hazards

Whenever insect viruses are used for the control of pests and disease vectors, man and other non-target animals will inevitably be exposed; the numbers of species involved and the degree of exposure will depend on the nature and scale of the operation, the method used, and the geograph-

[1] Hostetter, D. L. & Biever, K. D. (1970) *J. invertebr. Path.*, **15**, 173–176.

ical area. The risks to animals exposed within the treated area will depend basically on the specificity of the virus.

Risks to non-target animals outside the treated area will depend on the extent to which the virus spreads beyond the area, which in turn will depend on the method of application, the possible dissemination of the virus through the faeces of predators that have eaten diseased insects, the movement of diseased insects and their parasites, and dissemination by wind and rain. The rates at which introduced viruses spread may vary in different localities. For instance, when the NPV of the European spruce sawfly (*Gilpinia hercyniae*) was applied to a small area in Newfoundland, it spread rapidly over considerable distances ; in the United Kingdom, however, its spread has been slower.

In testing vertebrates for susceptibility to infection with insect viruses, considerations such as their body temperatures, the virus dose, the species and age of the animal, the duration of the experiment (whether in the laboratory or the field), and the passage level of the virus should be taken into account. Such risks for vertebrates as oncogenicity, teratogenicity, and genetic recombination between viruses would arise only if the insect virus were capable of infecting cells of vertebrates.

The passage of a virus in any host by unnatural means or the infection of an unfamiliar host may result in the selection of a mutant virus with different properties ; this phenomenon is well known in vertebrate virology. During the development and production of insect viruses, mutations may occur that might cause a change in virulence or host range. If an insect virus capable of infecting cells of vertebrates were disseminated in the field, there would be a remote possibility of recombination between this virus and a vertebrate virus genome. If poxviruses of insects and of vertebrates could be induced to replicate in the same cell system, for example, an opportunity for experiments to test this possibility might be provided, but suitable safety precautions would have to be maintained.

4.2.1 *Man and other vertebrates*

In any large-scale use of insect viruses, there is a considerable risk that vertebrates will be exposed to contamination. The routes to be considered are the respiratory and gastrointestinal tracts and the skin and eyes. The possible effects may be infective, allergic, or toxic.

Possible risks to man arise during manufacture, formulation, and packaging, during field preparation, during application, and for a period after application that varies according to the stability of the virus used. Before application, the risks are probably confined mainly to the production workers, but after application it must be assumed that every animal in the treated area and substantial numbers outside it have been exposed to some extent.

Routes involved in exposure

(i) *Respiratory tract*

Respiratory risks arise for domestic and wild animals from the inhalation of small droplets resulting from the application of insect virus preparations to pastures or to neighbouring crops. Although wild animals might have been exposed to natural aerosols containing virus particles, they will not have been exposed to droplets as small as those produced by spraying machines.

When material is sprayed as an aqueous slurry or suspension or disseminated as a powder, the nature and degree of the respiratory risk depend on the dose inhaled and on the proportional distribution of the different sizes of particle produced. Most inhaled particles with diameters over 10 μm will lodge in the upper respiratory tract while those with diameters smaller than 5 μm can penetrate to the bronchioles. Intermediate-sized particles will penetrate the respiratory tract in inverse proportion to their size. The smaller the particle, the longer it will remain in suspension. Large particles sediment rapidly from the atmosphere, but particles of diameters below 5 μm may remain suspended in the air for a prolonged period and can travel substantial distances, their concentration being relatively unaffected by rain or other forms of precipitation. Their movement is governed by the prevailing meteorological conditions, especially wind speed and direction, and their concentration largely by the amount dispersed and the height of any temperature inversion, which acts as a barrier.

On being sprayed, suspensions of material in water dry out in the atmosphere in a matter of seconds, the actual time depending on the relative humidity and the resultant particle load and distribution on the amount of dried solids in the preparation sprayed. The stability of the virus particles will be more or less strongly influenced by sunlight, relative humidity, and other atmospheric factors, depending on the organism used. When nonaqueous carriers, such as cottonseed oil, are employed in control programmes, evaporation is not an important factor.

It is clear that all animals downwind of an aerosol source will inhale material suspended in the air (man at a rate of about 10 litres/min and other animal species roughly in proportion to their size). An aerosol dispersion of a slurry of *Bacillus subtilis*, even under conditions in which only 5% of the material was expected to remain airborne, was found to persist at least 37 km downwind at levels that could have been dangerous for man had a pathogen been involved. When about 490 litres were sprayed from a ship along a distance of 3.2 km, an area of about 260 km^2 was contaminated.[1]

[1] Fothergill, L. D. (1961) *J. Amer. diet. Ass.*, **38**, 249–252.

(ii) *Gastrointestinal tract*

Although many insect viruses are likely to be destroyed in the stomach, they could certainly have the opportunity, when ingested, of infecting the nasopharyngeal region. Viruses occluded in protein crystals may retain their infectivity in the guts of birds,[1] but not in the guts of mammals.[2,3] It is not known, however, whether non-occluded virus particles remain infective in the same circumstances. Insects infected with occluded viruses may be expected to synthesize non-occluded particles, which will be ingested by their vertebrate predators. Hence, to be comprehensive, tests should cover the effects of free virions. Ingestion risks, like respiratory risks, obviously arise at all stages of the preparation of the virus before application. Afterwards they arise when treated crops are used for human or animal food. In addition, viruses of prolonged viability in soil, notably nuclear polyhedrosis viruses,[4] will be washed into streams and other bodies of water for some time after application. Consequently the possibility that any hazard to fish or other components of aquatic ecosystems will be prolonged, perhaps for several years, will have to be considered.

(iii) *Skin and eyes*

Possible risks from the exposure of eyes and skin are likely to arise only for the workers involved in the application or production of viral agents or for animals or persons within the treated area at the time of application.

Possible effects of exposure

(i) *Infective*

As regards infection, the hazards presented by exposure to insect viruses can only be assessed by very extensive testing, but if there is evidence that such a virus can initiate any sort of infection in any species of vertebrate, a potentially serious risk must be presumed to exist, even if no overt disease occurs, because of the possibility of the adaptation and spread of the virus to other species of vertebrates in which disease may develop.

The problem of infectivity is a difficult one to elucidate and has extremely serious implications since infections can persist and spread and, if established in wildlife, may be impossible to eradicate. Infection might occur by any route, but ingestion and inhalation are certainly the most important. The latter is probably paramount because, for an animal the

[1] Hostetter, D. L. & Biever, K. D. (1970) *J. invertebr. Path.*, **15**, 173–176.

[2] Ignoffo, C. M. et al. (1971) *Fate of* Heliothis *nucleopolyhedrosis virus following oral administration to rats*. In : *Proceedings of the IV International Colloquium on Insect Pathology and Microbial Control, College Park, Maryland*, Society for Invertebrate Pathology, pp. 357–352.

[3] Smirnoff, W. A. & MacLeod, C. G. (1964) *J. Insect Path.*, **6**, 537–538.

[4] Jacques, R. P. (1964) *J. Insect Path.*, **6**, 251–254.

size of a man, a very low virus concentration in air could be infective—for example, a mean concentration of only one infective dose in 300 litres of air can suffice if 10 litres of air are inhaled per minute and the aerosol cloud is present for 30 minutes (a not unlikely period). The risks presented by contaminant microorganisms must once again be stressed.

All infective hazards are enhanced by immunodepression which can be caused by congenital defects, malnutrition, diseases such as leukaemia, infections such as malaria, and immunodepressive drugs. In many parts of the world, malnutrition and immunodepressive infections are very common in man, and increasing numbers of people are being treated with immunodepressive drugs. As a result, the potential susceptibility of man and other vertebrates will be different in different areas.

(ii) *Allergic*

Allergic manifestations are also possible, whatever the type of exposure involved. These could be caused by the insect virus itself, the insect host material (e.g., urticating hairs), contaminant microorganisms, or any potentially allergenic material added to the formulation. Effects on the skin and eyes would present a particular hazard for workers involved in production and application and for animals present in the area during application, but respiratory effects could be much more widespread. Allergic effects are more likely to follow repeated exposure, but it is by no means improbable that some individuals may react on what is apparently the first exposure, because of unrecognized previous exposure or because of previous exposure to antigenically related allergens. Particular attention must be drawn to the potentially serious risk of immune-complex pulmonary disease, leading to progressive and disabling pulmonary fibrosis, which may follow chronic exposure to allergens by inhalation (such as occurs in farmer's lung — aspergillosis—and in budgerigar-fancier's lung) ; again this is most probably a special hazard for the workers involved in production or application. However, respiratory exposure downwind may be sufficient to cause asthma in allergized individuals, and efforts should be made to ensure that insect virus preparations proposed for field use do not contain substantial amounts of the allergens commonly incriminated in this disease.

(iii) *Toxic*

Whatever the route involved, toxic effects are more likely to arise from heavy contamination than as the cumulative result of frequently repeated exposure to small doses of the control agent. Such effects could be caused not only by the virus material but also by any material added to the formulation or by contaminant materials, including other microorganisms—a consideration that applies particularly to the uncontrolled production and use of insect viruses. Hence the need for high microbiological standards in the manufacture of virus material. Testing for acute and chronic toxicity should be required before the field use of the material, and suitable precautions will be necessary to protect workers involved in the various stages of manufacture and use.

4.2.2 *The environment and insect ecosystems*

Infection with an insect virus may be used for the control of insect populations by making a sufficient number of applications to ensure that the disease becomes self-perpetuating during the crop-growing season; by supplementing its natural incidence (where this is inadequate) early in the season; or by making repeated applications, as with chemical pesticides.

In addition to the factors discussed above, the degree of hazard to ecosystems will depend on whether the control measure involves the supplementation of a virus that is already present or the artificial introduction of a new virus; the hazard is of course much greater in the latter instance.

The possible effects of the accumulation of virus particles in the soil following repeated applications must be considered. In Canada, it was found that the NPV of *Trichloplusia ni* persisted in soil for at least five years.[1] There is, however, no evidence at present that such accumulation could lead to any risks similar to those associated with the organochlorine pesticides.

Changes in susceptibility of target insects

Any natural population of insects must contain individuals differing in their susceptibility to virus. Thus the selection pressure resulting from the frequently repeated application of a virus or from an artificially induced enzootic might result in the emergence of a less susceptible insect population. However, long-term efforts to obtain strains of *Bombyx mori* resistant to the grasserie virus have been unsuccessful and selection over many generations has failed to produce a strain of *Heliothis zea* resistant to its NPV.[2] The induction of reduced susceptibility to a CPV has been reported in *Bombyx*,[3] and the existence of strains of *Pieris* with consistently reduced susceptibility to a GV has been recognized.[4] In the field, the sawfly *Gilpinia hercyniae* has remained susceptible to its NPV for more than 20 years.[5]

The possibility of mutations in an insect virus that might evoke an apparent change in the susceptibility of the host is discussed in section 4.2.

Non-target invertebrates

The wider the host range of a virus, the greater the dangers to non-target species. The more specific a viral agent is in its action, the more precisely it can be used in pest management. The method of application (especially the amount of drift outside the target crop) and the natural rate

---

[1] Jacques, R. P. (1964) *J. Insect Path.*, **6**, 251–254.
[2] Ignoffo, C. M. & Allen, G. E. (1972) *J. invertebr. Path.*, **20**, 187–192.
[3] Watanabe, H. (1967) *J. invertebr. Path.* **9**, 474–479.
[4] David, W. A. L. & Gardiner, B. O. C. (1965) *J. invertebr. Path.*, **7**, 285–290.
[5] Bird, F. T. & Burke, J. M. (1961) *Canad. Ent.*, **93**, 228–238.

of transmission and spread of the virus are variables with an important bearing on the degree of risk for non-target insect species.

Parasites and predators

The extent to which a viral control agent will have a direct effect on parasites and predators will depend on its specificity, but the destruction of a large part of the prey population will undoubtedly cause a major reduction in the numbers of their parasites and predators (the effect on those with a wide range of prey or hosts will be less marked). If this occurs in a semi-permanent ecosystem (such as an orchard or forest), a cessation of viral control could be followed by a resurgence of the pest. An example of this has been reported in the case of the codling moth following a period of control by a GV, during which the natural parasites became scarce.[1]

As NPVs and GVs do not normally act directly on parasitic and predatory insects, their use would not lead to the rise of secondary pests as a result of the decimation of the latter's natural enemies. Theoretically, secondary pests could increase through being released from interspecific competition with the primary pest.

5. STAGES OF DEVELOPMENT AND REGULATION OF AN INSECT VIRUS AS A CONTROL AGENT

The experience and skills required for the development of an insect virus as a control agent are complex. Specialists and suitable equipment are needed in order to progress from the initial isolation of a virus to the distribution of a tested and safe product. In any country contemplating the use of viruses to control pests, a national authority should be set up to regulate safety testing and application. In the USA and in Canada, for example, guidelines are being developed to ensure the safe use of viruses for pest control. Proposed guidelines will shortly be published by the US Environmental Protection Agency in the Code of Federal Regulations (see Annex 1).[2]

5.1 Discovery and characterization of a candidate virus

The discovery of an insect virus is usually the result of the observations and efforts of an individual scientist. Viruses, particularly the NPVs and GVs, are often detected as causing massive epizootics in entire insect populations, while many have been isolated from single insect specimens.

---

[1] Falcon, L. A., Kane, W. R. & Bethell, R. S. (1968), *J. econ. Ent.*, **61**, 1208–1213.

[2] A limited number of copies of these guidelines in draft form are available from: Office of Pesticide Programs, US Environmental Protection Agency, Washington, D.C. 20460, USA.

Stability is one of the desirable features of an insect virus if it is to be effective as a control agent. The inclusion body viruses are particularly suitable, because they are occluded in inert protein crystals that protect them from destructive elements in the environment. It is these viruses that have been most extensively developed as control agents in the past three decades. The non-occlusion viruses are less stable and thus more difficult to store for extended periods of time.

After isolation, a candidate virus has to be purified and fully characterized. It should then be propagated in its original host in sufficient quantity to provide material for subsequent tests. Propagation in disease-free colonies of insects reared on clean fodder will eliminate much of the tedious process of purifying the virus before testing. Methods of rearing the insects on an artifical diet should be developed as soon as possible.

Pathogenicity tests in the original host should then be conducted; virulence can best be measured as an LD_{50} with confidence limits. If field-collected insects have to be used, large numbers of test and control insects will be required. If none of the required information is available at the outset, it could take 2 years to carry out the various procedures indicated.

Infection tests (including determination of the LD_{50}) should be performed on other species of insects in order to determine the specificity of the virus. These tests can be carried out either by the investigator or by experts elsewhere.

It should be emphasized that during this development period, aseptic microbial techniques must be strictly applied in order to protect laboratory personnel and to prevent contamination of the candidate virus and of test insects.

5.2 Safety tests and limited field trials

At this stage, the national authority should require the performance of basic safety tests. The design and interpretation of these tests must be primarily microbiological rather than toxicological. For example, the research organizations in agriculture and forestry of the US Department of Agriculture have decided that all experimental materials selected for field-testing must be submitted to 7 short-term tests proposed by the US Environmental Protection Agency (Annex 1, Section A). Any virus belonging to a group other than the NPV and GV groups might require the complete range of safety tests listed in Annex 1, Sections A-E. These tests cover most of the serious risks discussed earlier in this report. Details of the safety testing procedures followed by the Insect Pathology Laboratory of the US Department of Agriculture are given in Annex 2.

If a virus should prove innocuous in all the tests cited in Annex 1, Section A, limited field trials under expert supervision could be initiated with the approval of the national authority. These could gradually be expanded, if this appears justified by the efficacy and safety of the virus. Judging from past experience, this procedure would probably require up to 2 years, so that it would take about 4 years in all to develop the virus to this stage.

If, on the other hand, a serious reaction were to be detected during these tests, safety testing would be extended after consultation with the national regulatory agencies, probably in accordance with Section B of Annex 1, until sufficient data had been acquired to permit a decision on whether to discard the idea of further development or to continue testing.

5.3 Large-scale testing

If, at this point, the efficacy of the virus against the target insect or insects warranted its further development as a control agent, and if there was no indication of deleterious effects in other organisms in the environment, the virus could be admitted for mass production and large-scale testing might be authorized. From this point on, the development of the virus into a product authorized for general use by the appropriate regulatory authority will require a specially trained team of scientists. The end result should be a standardized product that will remain efficacious after long storage and shipment under any conditions and can be manufactured in sufficient quantities for the treatment of large areas. At this point safety testing on fish, wildlife, and plants should be initiated in accordance with Section E of Annex 1.

It should then be possible to proceed with mass production, storage, formulation, further safety testing, and, finally, careful countrywide testing of the virus against target insects.

If this development process has continued without any impediment or sign of harm to the environment as a whole, permission might be given to carry out very large-scale tests for efficacy. To reach this stage may take up to 5 or 6 years.

6. CONCLUSIONS

Of the insect viruses, the nuclear polyhedrosis (NPV) and granulosis (GV) viruses offer the greatest potential for the control of arthropods of importance in human and veterinary medicine and in agriculture and forestry. They are particularly suitable for this purpose because of their probable efficacy and safety in use, good storage properties, relative ease of production, and wide distribution among insects. The use of other groups of viruses might involve greater difficulties particularly from the standpoint of specificity.

Adequate safety testing and precautions are essential not only to safeguard vertebrates and other invertebrates, but also to minimize the risk of untoward incidents that might prejudice the future development and use of insect viruses as control agents.

This form of insect control will probably be most effective when it is employed in conjunction with other measures within the context of an integrated control project.

Detailed information on the physical, chemical, and biological properties of insect viruses is required in order to determine the pattern of specificity with accuracy. In this connexion, the characterization and pathogenicity tests for known insect viruses are of particular importance.

There is an urgent need for easier and more sensitive methods for assaying all stages of virus infection in invertebrates and for monitoring and precisely identifying the virus or viruses responsible.

More research is required on the defensive responses of invertebrates to infection.

In safety testing, the most important question is whether a candidate insect virus can infect cells of vertebrates. If it is unable to infect such cells, only the allergic and toxic risks need be considered.

The considerable difficulties associated with testing for possible carcinogenicity and teratogenicity will also be largely avoided if it can be shown that: (*a*) the virus cannot multiply in cells at the body temperature of man or in cells of other important species; (*b*) the viral nucleic acid cannot infect the cells of man and other important species.

Selective changes may occur in the susceptibility of the target insect and in the virulence of the viral agent, and careful checks on mutability are needed.

Respiratory risks could affect a much wider range of vertebrates, but their importance will depend on the mode of dissemination and on prevailing weather conditions. Such risks appear to be low with NPVs and GVs.

The enhanced susceptibility of humans and domestic animals whose immunity is depressed must be taken into account. Workers can be protected by suitable respirators.

Workers associated with the production and application of viral agents may suffer allergic reactions due to eye and skin exposure. However, by careful attention to the necessary precautions, these risks can be greatly reduced.

Possible risks from ingested viral particles arise mainly in connexion with food consumed by herbivores and man, but with NPVs and GVs these risks appear to be small.

The potential risks for non-target invertebrates will increase with the host range of the viral agent used and the extent to which it spreads outside the target area.

The wider the host range of a virus, the greater the dangers to non-target species ; the more specific the viral agent, the greater the safety and precision with which it can be used in pest management.

Where the viruses used affect only a narrow host range, resurgences of the primary pest and the emergence of secondary pests are less probable and, if they occur, likely to be less pronounced than those associated with the use of chemical pesticides.

A careful choice of methods of application minimizes the possible effects of the virus material beyond the target pest or disease vector. When a new virus is tested in the field, the potential risks will be greater if it is applied in aerosols with very small droplets or in such a way that there is drift outside the control area. Restrictions may need to be placed on the methods and timing used in field-testing new viruses.

The accumulation of virus particles in the soil does not seem to pose any special risks.

Attention is drawn to the world-wide paucity of educational programmes for the training of specialists in the virology and pathology of invertebrates.

7. RECOMMENDATIONS

The following recommendations are intended to further national and international cooperation and efforts for the continuing development of insect viruses as control agents.

7.1 Priorities

(1) FAO and WHO should encourage the development and testing for safety and efficiency of those insect viruses already characterized that have an established potential for the control of important agricultural and forest pests or of vectors of important human or veterinary diseases.

(2) Research to evaluate and develop insect viruses for possible use in the control of arthropods important in human or veterinary medicine and in agriculture or forestry should be actively supported. In the field of agriculture, for example, attention should be given to providing and characterizing viruses potentially useful for the control of :

(*a*) the desert locust,

(*b*) the principal pests of stored products,

(*c*) stem borers of rice and maize.

In the field of veterinary and medical public health, the information available on pathogenic insect viruses is fragmentary. Particular attention could be given to those disease vectors in which resistance to insecticides is already a problem.

7.2 Conditions for safe use

(1) Countries contemplating the use of insect viruses as control agents should establish a control authority to regulate their development and use and should discourage any use of such agents other than in accordance with the general programme of development and safety testing laid down in section 5. Safety tests should take the enhanced susceptibility associated with immunodepression into account. Regulatory authorities should ensure that safety tests take account of other substances, such as insect tissues, in insect virus preparations.

(2) The general use of any insect virus product must be conditional on satisfactory evidence that it cannot cause infection or unacceptable harm to man, domestic and wild animals (including beneficial arthropods), and plants.

(3) Suitable precautions should be taken to protect workers involved in the various stages of manufacture and use of insect virus preparations.

(4) Studies should be encouraged on the possible relationships between insect viruses and other viruses, and experiments should be performed to investigate the mutability of insect viruses.

7.3 Technology of production and application

(1) Methods for the application of insect viruses as control agents should be developed so as to minimize the spread of an agent beyond the target area especially during the field-trial phase of development.

The development of improved formulations and application equipment should also be encouraged.

(2) Infection-free colonies of insects should be developed whenever they are required for experimentation, for pathogenicity tests, and especially for the production of insect virus preparations. Artificial diets for the mass production of all economically and medically important invertebrates should be developed.

(3) Insect cell lines, whether for experimentation, pathogenicity tests, or production, should be shown to be free of extraneous microorganisms and must be carefully characterized to identify clearly both their nature and origin.

7.4 Field monitoring

(1) The safety testing of a new insect virus or virus product should not be confined to the laboratory, but should form an integral part of a

staged programme of field trials based on sufficient prior evidence of safety. Field trials must include adequate surveillance to detect important effects on fauna and flora not only in the treated area but in the surrounding areas. An environment in which viruses have been used should be monitored and surveyed for a substantial period to detect harmful effects.

(2) It is recommended that, whenever the first use of a viral control agent is contemplated in a particular area, surveys should be made of all target pest species, of their parasites, predators, and pathogens, and of beneficial or aesthetically important invertebrate species present in the area. These surveys should continue throughout the trial period and for a substantial time afterwards. WHO and FAO should provide, on request, all possible help in setting up field studies of this kind.

(3) In areas where there is extensive and continuing application of an insect virus as a control agent, special efforts should be made to monitor the abundance and condition of non-target vertebrates and invertebrates and assess the overall impact on the environment.

7.5 Information

(1) FAO and WHO should use their resources to collect information about major problems pertaining to arthropod pests in agriculture and forestry and to vectors of medical and veterinary importance, with particular reference to those that might be amenable to control by insect viruses, and publish such information regularly.

(2) FAO and WHO should arrange for the compilation and progressive amendment of lists of insect viruses isolated from pests in agriculture and forestry and from vectors of medical and veterinary importance. These records should be classified according to the source, availability, state of characterization, specificity, and pathogenicity of the viruses.

(3) FAO and WHO should collect and disseminate information on the guidelines available for the evaluation and safety testing of insect viruses developed as control agents, and encourage further research in this field.

7.6 Special recommendations

(1) FAO and WHO should consider the feasibility of establishing laboratories responsible for reference collections of insect viruses and for the identification and characterization of newly isolated viruses.

(2) FAO and WHO should ensure continuous liaison, through their designated experts, in order to :

(i) review in depth the current situation as regards the development and use of insect viruses for pest and vector control ;

(ii) decide how they can further encourage the development as control agents of insect viruses that have been shown to be safe and effective for the purpose ;

(iii) review the use of approved virus agents in the control of pests and vectors within the context of integrated control programmes.

(3) FAO and WHO should facilitate the training of scientists in insect virology and pathology by awarding suitable fellowships, especially to candidates from countries where expertise in these fields is at present lacking.

Annex 1

GUIDELINES PROPOSED BY THE UNITED STATES ENVIRONMENTAL PROTECTION AGENCY FOR SAFETY EVALUATION OF MICROBIAL AGENTS IN THE USA *

A. Required in all cases :

Single-dose exposure

Studies in D, 1 and 2 are incorporated in A (see text)

1. Oral (two vertebrate species)
2. Primary tissue cultures of vertebrate and human origin
3. Dermal (rabbits)
4. Inhalation (rats) [1]
5. Eye irritation (rabbits)
6. Primary skin irritation (rabbits)[1]

Multiple-dose short-term exposure

1. Ninety-day feeding (rats and one other species)

B. These requirements depend on the results of short-term testing and the nature of the formulation.

2. Twenty-one day dermal (rabbits)
3. Fourteen-day inhalation (rats) [1]
4. Skin sensitization—Landsteiner allergenic test (guinea-pigs) [1]
5. Respiratory sensitization — allergenicity test (guinea-pigs) [1]
6. Reproductive and teratogenicity studies (rats)

C. *Long-term exposure studies* (same stipulation as in B)

1. Carcinogenicity studies (these will definitely be required for fungi)

D. *Other related studies* (required)

1. Persistence and replication of the biological agent in mammalian species
2. Antibody studies
3. Mutation studies
4. Chemical and biological controls to ensure uniformity of product

---

* See section 5.

[1] Since the present report repeatedly draws attention to the possible risks of pulmonary involvement and allergenic reactions, these guidelines will be changed to include multidose exposure experiments for the assessment of skin sensitization and of inhalation toxicity and respiratory sensitization. In addition, the inclusion of immunodepressed animals in some of the safety tests will be strongly recommended.

E. *Beneficial insect, wildlife, plant toxicology* (required)

1. Acute toxicity to honey-bees
2. Acute toxicity to fish (rainbow trout and bluegills)
3. Eight-day acute toxicity to birds (quail and mallards)
4. Subacute and/or chronic toxicity to birds (including reproduction)
5. Phytotoxicity

Annex 2

PROTOCOLS FOLLOWED BY THE INSECT PATHOLOGY LABORATORY, UNITED STATES DEPARTMENT OF AGRICULTURE, FOR SAFETY TESTING OF INSECT VIRUSES

1. Acute oral toxicity — rats

Duration. 21-day observation period.

Animals. One hundred and twenty weanling albino rats (60 males and 60 females) will be randomly assigned to the experimental groups listed below. The animals will weigh between 150 and 250 g at initiation of the test. All animals will be individually housed with food and water available *ad libitum*.

Group number	*Number of animals*		*Test material*
	Male	*Female*	
1	20	20	saline control
2	20	20	lactose carrier + saline
3	20	20	*Autographa californica* + lactose + saline

Pathogen administration. Acetone-precipitated lactose-virus polyhedra slurries, dried *in vacuo,* will be resuspended in 0.8% saline at the rate of 40×10^9 polyhedra per ml.

This dosage will be administered to each animal in Group No. 3 at the rate of 1.0 ml per rat by gastric intubation. The remaining animals will receive 1.0 ml of the appropriate control by gastric intubation.

Observations. Daily observations for appearance and mortality will be made. Body weights and food consumption determinations will be made weekly. Body temperature of each animal will be recorded twice daily for the duration of the experiment.

Clinical studies. Blood glucose and serum glutamic-pyruvic transaminase will be determined on five males and five females from each group at Days 0 and 21.

The following hematological parameters will be determined on five males and five females from each group at Days 0, 14, and 21 :

hemoglobin	erythrocyte count
microhematocrit	total leukocyte count
coagulation time	differential leukocyte count
prothrombin time	thrombocyte count

Interval sacrifice. Four animals from each group (two males and two females) will be sacrificed on the following days : Day 0 (immediately after dosing), Day 1, Day 3, Day 7, and Day 14. Six animals from each group (three males and three females) will be sacrificed on Day 21. One ml of serum from each animal will be frozen for pick-up by the sponsor. Additionally, the following tissues will be removed, stored in sterile containers, and reserved for the sponsor :

mesentery	mesenteric lymph node
liver	gastro-intestinal contents from various sections along the GI tract

Note. These tissues must be removed with sterile instruments. The same instrument must not be used to remove more than one of the above organs. Possible cross-contamination by the instruments must be prevented.

Termination. The study will be terminated at 21 days and necropsies will be performed. The following procedure will be followed for any animal which dies during the experiment and in all survivors at termination :

Organ weights (for each rat)

heart	adrenals
lung	thyroid
liver	prostate
kidneys	uterus
gonads	pituitary

Portions of these tissues plus portions of the following will be frozen and delivered to the sponsor :

duodenum	cervical lymph node
intercostal muscle	bronchial lymph node
pancreas	bone marrow
urinary bladder	stomach
mesenteric lymph node	brain

Additionally, eyes will be taken, grossly observed, preserved in Zenker's fixative, and delivered to the sponsor.

Histopathological examination. The following tissues will be examined from any animal that dies and from three males and three females from each group sacrificed at Day 21 :

fat	spleen	cervical lymph node
heart	lung	mesenteric lymph node

liver	salivary gland	bronchial lymph node
kidneys	small intestine	

All slides will be delivered to the sponsor at completion of the study.

Report. At termination a report will be submitted giving:

experimental design	hematology data
body weight data	clinical chemistry data
food consumption data	gross necropsy findings
mortality data	microscopic examination results

2. Acute dermal toxicity — guinea pigs

Duration. 14-day observation period

Animals. Thirty albino guinea pigs (15 males and 15 females) will be randomly assigned to the experimental groups listed below. The animals will be individually housed with feed and water freely available.

Group number	*Number of animals*		*Test material*
	Male	*Female*	
1	5	5	lactose control
2	5	5	*A. californica* virus polyhedra
3	5	5	*A. californica* ME virus (released rods)

Pathogen administration

Group No. 1. The vehicle control (2% lactose in 0.8% saline) will be administered in the manner described for the experimental groups.

Group No. 2. Acetone-precipitated lactose-virus polyhedral slurries, dried *in vacuo*, will be resuspended in 0.8% saline at the rate of 40×10^9 polyhedra per ml. This dosage will be applied to the shaved backs of 10 guinea pigs on two areas of intact skin and two areas of abraded skin on each animal at the rate of 0.10 ml per application (Draize's technique).

Group No. 3. Freed virus rods (by the carbonate technique), prepared by the sponsor, will be used. The free virus from 40×10^9 clean polyhedra (43.5 mg) will be suspended per 1.0 ml of saline and applied to the test animals in the same manner as the polyhedra (using Draize's technique).

Observations. Animals will be examined daily for the first week and at termination on the 14th day. Animals will be observed daily for mortality. Body weight and food consumption will be determined weekly. Body temperature will be recorded for each animal daily for the first seven days and on the 14th day.

Termination. The study will be terminated on the 14th day and gross necropsies performed. The following will be performed on all animals that die during the experiment and on all survivors at termination :

Organ weights (for each animal)

heart	adrenals
lung	thyroid
liver	prostate
kidneys	uterus
gonads	pituitary

Portions of these tissues plus portions of the following tissues will be frozen and shipped to the sponsor :

duodenum	cervical lymph node
intercostal muscle	bronchial lymph node
urinary bladder	bone marrow
pancreas	stomach
mesenteric lymph node	brain

In addition, eyes will be taken, grossly observed, and preserved in Zenker's fixative prior to delivery to the sponsor.

Histopathological examination. The following tissues will be examined from any animal which dies during the study and from three males and three females from each group sacrificed on Day 14 :

fat	lung	bronchial lymph node
heart	salivary gland	small intestine
liver	cervical lymph node	mesenteric lymph node
kidneys	spleen	

All slides will be delivered to the client at completion of the study.

Report. At completion of the study a report will be submitted giving :

experimental design	results of dermal exposure
physical appearance	mortality data
body weight data	gross necropsy observations
food consumption data	results of microscopic examination

3. Eye irritation test — rabbits

Duration. 14-day observation period

Animals. Twenty albino rabbits (10 males and 10 females) will be randomly assigned to the following groups. The animals will be individually housed with food and water freely available. Prior to initiation of the study all animals will be examined for corneal injury by instillation of 5% fluorescein solution and flushing the eye with distilled water 20 seconds after application. The animals will then be examined with a band slit lamp. Any animal with damaged cornea will be excluded from the study.

Group number	*Number of animals*		*Test material*
	Male	*Female*	
1	5	5	ME virus in saline suspension
2	5	5	freed virus in saline suspension

Pathogen administration

Group No. 1. Acetone-precipitated lactose-virus polyhedral slurries, dried *in vacuo*, will be resuspended in 0.8% saline at the rate of 40×10^9 polyhedra per animal. This dosage will be applied to the left eye of each animal at the rate of 0.1 ml per animal (4.35×10^6 polyhedra per eye). The right eye will serve as a control and will receive 0.1 ml of a 0.8% saline solution containing 2% lactose.

Group No. 2. Freed virus rods (by carbonate technique), prepared fresh by the sponsor, will be used. The free virus from 43.5 mg of clean polyhedra will be suspended in 1.0 ml of saline. The left eye of each rabbit will receive 0.1 ml of this suspension. The right eye will serve as the control and receive 0.1 ml of the 0.8% saline solution.

Observations. Examination for injury or irritation will be made at 24, 48, and 72 hours after treatment, and at seven and 14 days. Eye irritation will be graded and scored according to the method of J. H. Draize. Body temperature will be recorded for each animal daily for the first seven days and at weekly intervals thereafter.

Necropsy. Should damage be present at 14 days, the animal should be sacrificed and the eye and conjunctivae preserved in 10% neutral buffered formalin for histopathological examination. All slides should be sent to sponsor at completion of the study.

Report. At completion of the study, a report will be submitted giving :

experimental design
ocular findings
tabulation of eye scores
microscopic findings

4. Primary skin irritation — rabbits

Duration. 72-hour observation period.

Methods. Six albino rabbits, clipped free of hair, will be divided into two equal groups.

The exposure area of one group will be abraded and the skin of the remaining group will remain intact. Acetone-precipitated lactose-virus polyhedra (*A. californica*), dried *in vacuo*, will be resuspended in 0.8% saline at the rate of 40×10^9 polyhedra per animal; 0.5 ml of this material will be introduced under a $1'' \times 1''$ gauze patch which will be secured in place with adhesive tape.

The animals will be immobilized in stocks and the entire trunk of each animal will be wrapped in a non-absorbent binder for 24 hours.

Observations. After the 24-hour exposure, the patches will be removed and the skin reactions will be evaluated. A second evaluation will be made 48 hours later (72-hour observation). The reaction will be scored according to the method of J. H. Draize. Body temperature will be recorded for each animal daily.

Report. The report will include the following:

- details of experimental design
- rating of the irritancy according to the Federal Hazardous Substances Act
- tabulated scoring of skin reactions

5. Acute inhalation toxicity — rats

Objective. The purpose of this test is to determine the toxicity and pathogenicity of a single one-hour exposure of rats to a single concentration of *A. californica* ME virus.

Animals. Forty SPF albino rats obtained as weanlings (23-26 days of age) from Charles River Breeding Laboratories will be used after a seven-day acclimation period in an air-conditioned holding room.

Groups. Two groups, each consisting of 10 males and 10 females, will be formed by random selection from the original 40. Group No. 1 will be exposed to the experimental material at 40×10^9 polyhedra per animal and Group No. 2 will be exposed to 2% lactose powder as a control.

Exposure conditions. Both groups will be exposed to their respective materials in 100-liter glass and stainless steel inhalation chambers of cubical design with pyramidal tops and bottoms. The materials will be drawn into the chambers from the top by vacuum at the bottom at a rate of 10 liters per minute of airflow. The rats will be individually housed in stainless steel mesh cages in two layers during exposure. A Wright dust feeder situated at the top of each chamber will feed the material to be generated into filtered and dehumidified make-up air at 72°F to provide a constant concentration of material in air during the one-hour exposure after equilibration.

Materials needed. A quantity of pathogen (suitably mixed with the carrier) to provide the desired dosage/hour will be required from the sponsor. Assuring a delivery concentration in air of 10 mg/liter and a flow rate of 10 liters/min through the chamber, 12 g of material will be generated in one hour. Thus, approximately 15 g of material should be supplied. Also, 15 g of control material will be needed.

Post-exposure observations. The two groups will be housed in separate rooms in individual metal cages for 14 days after the termination of exposures. The animals will have continuous access to water. Each animal will be observed daily for signs of toxicity and any moribund animal will be sacrificed for necropsy immediately. Total food consumption will be determined by weight for individual animals weekly by preweighing and post-weighing the food remaining in the food bin.

Necropsies. All animals will be necropsied after 14 days (sacrificed with barbiturate overdose) or upon death. The following organs will be weighed :

heart	lung	liver
kidneys	gonads	adrenals
fat	thyroid	prostate or uterus
pituitary		

Portions of these organs plus portions of the following tissues will be grossly examined and preserved in 10% formalin :

duodenum	intercostal muscle
urinary bladder	pancreas
mesenteric lymph node	cervical lymph node
bronchial lymph node	bone marrow
stomach	brain
spinal cord	

In addition, eyes will be taken, grossly observed, and preserved in Zenker's fixative. All preserved tissues shall be sent to the Insect Pathology Laboratory, Entomology Building A, ARC, Beltsville, Maryland 20705.

Histopathology. The following tissues will be examined from any animal dying and from three males and three females from each group on Days 4 and 21 :

brain	cervical lymph node	kidneys
fat	bronchial lymph node	lung
liver	spinal cord	small intestine
spleen	heart	mesenteric lymph node
salivary gland		

All slides shall be sent to the Insect Pathology Laboratory, Beltsville, Maryland.

Report. A final report will be submitted, describing the methodology and results in detail, within 30 days after completion of the histopathological examination.

6. Subacute dietary administration — rats

Duration. 13-week observation period.

Animals. Forty healthy, young albino rats (Sprague-Dawley) will be selected and placed in the following experimental groups by stratified randomization :

Group number	*Number of animals* *Male*	*Female*	*Test material*
1	10	10	2% lactose
2	10	10	*A. californica* ME virus in 2% lactose

The animals will be individually housed in screen bottom cages with feed and water available *ad libitum*.

Pathogen administration. Acetone-precipitated lactose-virus polyhedral slurries, dried *in vacuo*, will be mixed into the diet at a level specified by the sponsor. The pathogen will be administered in the diet for the 13-week observation period. Fresh diets will be prepared weekly.

Observations. Body weights and food consumption will be recorded weekly. Daily observations for mortality will be made and weekly records will be maintained of appearance, behavior, and signs of toxic or pharmacologic effects. Body temperature will be recorded for each animal daily for the first seven days and at weekly intervals thereafter.

Clinical studies. The following observations will be made in five males and five females from the control and test groups :

Hematology (at 45 and 90 days)

erythrocyte counts	leukocyte counts
hemoglobin	

Urine analysis (pooled samples) (at 45 and 90 days)

specific gravity	glucose
pH	blood (presence or absence)
total protein	

Interval sacrifice. Four animals (two males and two females) from each group at 45 days and 90 days. One ml of serum from each animal will be frozen for pick-up by the sponsor. Additionally, the following tissues will be removed, stored in sterile containers, and reserved for the sponsor:

mesentery	mesenteric lymph node
liver	gastro-intestinal contents from various sections along the GI tract

Note. These tissues must be removed with sterile instruments. The same instrument must not be used to remove more than one of the above organs. Possible cross-contamination by the instruments must be prevented. These tissues will be bioassayed by incorporation of the blended tissues into the diet, and tested against susceptible insects.

Termination. The study will be terminated at 90 days.

Necropsy procedure. The following will be performed on all animals, when sacrificed:

Gross examination

Organ weights

liver	kidney
heart	gonads
spleen	

Histopathological examination. The following tissues should be processed:

brain	colon	kidney
thyroid	adrenal	heart
lung	pancreas	stomach
salivary gland	liver	spleen
duodenum	uterus	bone marrow
jejunum	prostate	ovaries
lymph nodes	testes with epididymis	

Report. The report will include the following:

experimental design	signs of toxic or pharmacologic effects
general appearance and behavior	clinical findings
effects on growth, body weights, food consumption, and survival	gross and microscopic necropsy findings