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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

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OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

MEMORANDUM

SUBJECT: Response to Requested Information about Human Cell Culture Studies to Support the Registration of Spod-X® and Cyd-X Viral Insecticides

TO: Linda Hollis (PM-18)
Insecticide-Rodenticide Branch
Registration Division (7505C)

FROM: John L. Kough, Ph.D., Biologist
Biological Pesticides Section
Science Analysis Branch
Health Effects Division (7509C)

THROUGH: Roy D. Sjoblad, Ph.D., Section Head
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DATA REVIEW RECORD

Active Ingredients:	Nuclear Polyhedrosis Viruses of *Cydia pomonella* and *Spodoptera exigua*
Product Name:	Spod-X & Cyd-X
ID No:	058788-00001 Spod-X
Submission No:	S475138
Chemical No:	129078
DP Barcode:	D209244

ACTION REQUESTED
To review the responses to the questions asked in the review of the cell culture assays for Spod-X and Cyd-X (memoranda from J. Kough to L. Hollis, June 10, 1994 & July 12, 1994).

BACKGROUND
The company has submitted product identity and toxicology packages to support the registration of these viral agents. The packages have been reviewed and been found substantially complete. The 2 human cell culture studies submitted were found to be supplementary. This rating was given because while there were no obvious signs of cytotoxicity or cytopathogenicity, the cultures were not monitored for effects on cell generation times which is one of the endpoints required for viral cell culture studies. In addition certain assumptions about the volume of viral suspension that could be imbibed by the target pest were made to calculate the

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number of LD_{90}'s added as test substance to the cell cultures. A literature citation or other reference was requested to justify these assumptions.

Cell Culture Generation Times

After discussion with the company, they decided it was possible to justify not determining cell generation time for these assays. The basis for this justification is that any toxicity or infectivity due to the presence of the test virus would be manifested in the endpoints already examined: cell confluence and general morphological assessment. The visual assessment of the growth rate of control and virus inoculated flasks of WI-38 and Detroit 551 cell lines indicated that both cultures in both virus tests reached confluency on day 5 post inoculation. No treatment differences were apparent. There was a difference in the time the cultures reached confluency due to different inherent doubling times. In the Cyd-X inoculated cell culture study, the WI-38 cells were 90% confluent on day 3 whereas the Detroit 551 cells were only 60% confluent. The cultures in the Spod-X inoculation study were not examined at this earlier time point but were both confluent by day 5.

The company adds an explanation that there is an inherent variability in doing cell counts that would mask any differences between treatments. The reasons for this variability are the incomplete recovery of trypsinized cells, cell clumping and errors associated with hemocytometer counting. The company claimed it would require a large number of flasks to detect significant differences between treatments using cell counts.

SAB COMMENTS

SAB recognizes there are problems associated with doing cell counts as stated. This is the reason that plating efficiency determinations are requested rather than simple cell counts. However, even simple cell counts that show no significant differences due to high intra-treatment variability are preferable to no data on general cell culture population dynamics.

Justification for Estimates of Larval Uptake Volumes

The company was unable to obtain references for their estimates of volume uptake by neonate larva of either *Spodoptera exigua* or *Cydia pomonella*. However, using literature references for similar sized or larger larva, the company was able to show that their volume estimates were reasonable for dose estimation. Hughes and Wood ("*In vivo* and *In vitro* Bioassay Methods for Baculoviruses" in The Biology of Baculoviruses, Vol. II, R.R. Granados & B.F. Federici, eds., 1986, CRC Press, Boca Raton, FL) report that mean feeding volumes are consistent and gives values for 3 species that are comparable to *S. exigua*: 14 nl for *Helicoverpa zea*, 13 nl for *Tricoplusia ni* and 6 nl for *S. frugiperda*. In another study the volume ingested by *T. ni* larvae was determined spectroscopically to be 7 nl. (N.A.M. van Beek & P.R. Hughes, 1986, "Determination by Fluorescence Spectroscopy of the Volume Ingested by Neonate Lepidopterous Larvae," J. Invert. Path. 48:249-251) For *Cydia*

pomonella the 2 nl estimate is comparable to the 2 nl value obtained for *Plutella xylostella*, a similarly sized larva. (M.S. Ridout, J.S. Fenlon and P.R. Hughes, 1993, "A Generalized One-Hit Model for Bioassays of Insect Viruses," Biometrics 49:1136-1141)

SAB RECOMMENDATIONS

The company has substantially answered the questions that required clarification for the human cell culture studies (for Spod-X MRID 430743-01 & 430743-02; for Cyd-X MRID 431905-01). While the responses for cell generation time were not adequate to specifically answer the question, the cells in the studies did not show any cytopathic or toxic responses and apparently reached confluence at similar rates. This should suffice for rating these studies as acceptable. In the future, if any cell culture studies are done by CGI, adequate provisions in experimental design should be made to provide cell generation times or plating efficiency results.

The responses for volumes imbibed by neonate larvae by comparison to other Lepidopteran species were adequate and insured that the cell cultures were exposed well in excess of the 7X the LD_{50} required in the guidelines.