The European Agency for the Evaluation of Medicinal Products
Evaluation of Medicines for Human Use

London, 25 April 2002
CPMP/BWP/1412/02

Testing for SV40 in poliovirus vaccines

1. Background

SV40 is a polyoma virus that naturally infects rhesus macaques. Infected animals are generally asymptomatic (1). Prior to discovery of the virus in 1960 (2) SV40 was present as an unrecognised contaminant of the macaque kidney cell cultures used to prepare inactivated poliovirus vaccines, experimental lots of oral poliovirus vaccines and adenovaccines. Consequently, large numbers of people were exposed to SV40 between 1955 and early 1963. Once the presence of SV40 in vaccines was discovered, regulatory measures were introduced to detect and exclude it.

2. SV40 and human tumours

SV40 is known to cause specific types of tumours in rodents and from 1992, viral specific sequences have been reported in the homologous tumours in humans, most recently in two reports of SV40 sequences in 42% of non-Hodgkin's lymphomas (3,4). The origin of the viral sequences detected is not known but studies in the USA and in Europe are continuing with a view to identifying the source of the SV40 sequences in human tumours in which the sequences have been detected. The outcome of these studies will be kept under review.

There is no epidemiological link between the use of contaminated poliovaccine and the relevant tumours, and there is no evidence that the SV40 from the old vaccines causes cancer in humans (1). For example, large epidemiological studies in Germany, Sweden and the United States found no increase in people who received poliovaccine contaminated with SV40 30 years ago compared to people who did not receive such vaccine.

3. Vaccine issues

WHO and Ph Eur regulations require that several approaches are used to detect and exclude SV40 contamination from oral poliovirus vaccines produced in primary cell cultures (7,8) or from inactivated poliovirus vaccines produced in primary cell cultures (9,10). These are:

1. That animals from closed colonies are used where possible

2. That animals must be shown to be free of antibodies to SV40 prior to use

3. That the virus seed, control cells and each single harvest of poliovirus vaccine must be tested and shown to be free of SV40 in at least one sensitive cell culture. Tests shall be maintained for at least 4 weeks

4. That in addition to screening for cytopathic effects of SV40, fluorescent antibody tests may be used to detect SV40 in cell culture test

5. That the virus seed is free of SV40 sequences

Most requirements were implemented soon after the discovery of the virus. Over the last few years the effectiveness of the measures taken to exclude SV40 from poliovaccines has been assessed using state of the art techniques. Three separate laboratories (5,6,11) have tested bulks of poliovaccines and failed

7 Westferry Circus, Canary Wharf, London, E14 4HB, UK
Tel. (44-20) 74 18 84 00 Fax (44-20) 74 18 8545
E-mail: mail@emea.eu.int http://www.emea.eu.int

to detect SV40 sequences by sensitive PCR assays. Seeds for vaccine production which have been tested are negative for SV40 sequences.

It has been noted that different strains of SV40 may not be readily identified by *in vitro* culture methods. However, as implemented by manufacturers, the assays have been shown to be highly sensitive and able to detect a variety of SV40 virus strains whose growth properties were predicted to differ (12). Furthermore, animals infected with different virus strains produced antibodies, which can be detected by standard laboratory strains (13).

Collectively, these studies provide clear evidence that the requirements introduced after the discovery of SV40 are effective and that polio vaccines produced on primary cell cultures are free of contamination by the virus.

REFERENCES

1. Butel JS. Simian virus 40, poliovirus vaccines, and human cancer: research progress versus media and public interests. Bulletin of the World Health Organisation, 2000, 78, 195-198.

2. Sweet, Hilleman MR. The vaculating virus, SV40. Proceedings of the Society for Experimental Biology and Medicine, 1960, 185 713-718.

3. Shivapurkar, Harada, Reddy, Scheuermann, Xu, McKenna, Milchgrub, Kroft, Feng, Gazdar. Presence of simian virus 40 DNA sequences in human lymphmas. Lancet, 2002, 359, 851-852.

4. Vilchez RA, Madden CR, Kozinetz CA, Halvorson SJ, White, Jorgensen, Finch CJ, Butel JS. Association between simian virus 40 and non-Hodgkin Lymphoma. Lancet, 2002, 359, 817-823.

5. Sangar D et al. Examination of poliovirus vaccine preparations for SV40 sequences. Biologicals, 1999, 27, 1-10.

6. Sierra-Hongimann and Krause P. Live oral poliovirus vaccines do not contain detectable simian virus 40 (SV40) DNA. Biologicals, 28, in press.

7. World Health Organisation. Recommendations for oral poliomyelitis vaccine (revised 1999), in press.

8. European Pharmacopoeia. Poliomyelitis vaccine (oral). 1997.

9. World Health Organisation. Requirements for inactivated poliomyelitis vaccine. Technical Report Series, 673, (1982).

10. European Pharmacopoeia. Poliomyelitis vaccine (inactivated). 2000.

11. Rizzo P et al. Unique strains of SV40 in commercial poliovaccines from 1955 not readily identifiable with current testing for SV40 infection. Cancer Research, 1999, 59, 6103-6108.

12. Lednicky JA et al. Natural isolates of simian virus 40 from immunocompromised monkeys display extensive genetic heterogeneity: new implications for polyoma disease. Journal of Virology, 1998, 72, 3980-3990.

13. P D Minor, G Dunn and PA Pipkin. Detection and growth of SV40 preparations with different enhancer element copy number. Vaccine in press.