

MEMORANDUM DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration
Center for Biologics Evaluation and Research
Office of Vaccines Research and Review
Division of Viral Products

Date: October 12, 2012

To: The File STN 125408

From: Haruhiko Murata, DVP

Through: Keith Peden, DVP
Jerry Weir, DVP
Anissa Cheung, DVP

Sponsor: Novartis Vaccines and Diagnostics, Inc.

Subject: Cell Substrate Review for STN125408;
Human Influenza Virus Type A (H1N1; H3N2) and B
Hemagglutinin Vaccine, Purified, Inactivated (Madin Darby
Canine Kidney Cells)

My descriptions/comments are in Arial font, while the texts provided by the Sponsor through the BLA are in Times New Roman font.

The following acronyms/abbreviations are used:

BPL: beta-propiolactone
CTAB: cetyltrimethylammonium bromide
EoP: end of production
FCC: influenza cell culture
MCB: master cell bank
MDCK: Madin-Darby canine kidney (cell)
TSE: transmissible spongiform encephalopathies
VRBPAC: Vaccines and Related Biological Products Advisory Committee
WCB: working cell bank

Source, History, and Generation of the Cell Substrate

The MDCK cell line was initially established from the kidney of an apparently normal, adult, male cocker spaniel in 1958 by Madin and Darby at the University of California, Berkeley, and this cell line was submitted to the ATCC at some time between 1958 and 1967. Cells originating from Madin and Darby (Berkeley, California, USA) were transferred to a colleague, -----(b)(4)----- within the -----(b)(4)----- for further study. These cells were passaged an undetermined number of times by ---(b)(4)-- before transfer to -----(b)(4)----- cloned the cell line in 1966 at passage (b)(4). Derivatives of the cloned cell line were provided to -----(b)(4)-----.

(b)(4) received passage number ---(b)(4)-- of the cells cloned by ---(b)(4)----- designated the (b)(4) passage as MDCK ---(b)(4)- and then passaged it (b)(4) times (MDCK ---(b)(4)--- to establish a Master Cell Stock for use in manufacture of veterinary vaccines. The Master Cell Stock (Bank) was passaged(b)(4) times, which represented the last passage to be used in production (MDCK --(b)(4)-. Both the Master Cell Bank (MDCK ---(b)(4)--- and end-of-production cells --(b)(4)-- were tested in accordance with 9 CFR 113 (United States Code of Federal Regulations) requirements for cell substrates intended for veterinary vaccine use. Approval for use of the MDCK cell line as a production substrate was granted by USDA-APHIS on 24 August 1972 (USDA license No. 263). US veterinary license No. 272 was subsequently granted in 1992 for production of an MDCK-based -----(b)(4)----- vaccine. Cellular material derived from the licensed MCB (MDCK --(b)(4)---- was provided to Novartis (formerly Behringwerke) in 1985.

Novartis (formerly Behringwerke) adapted the MDCK ---(b)(4)---- cell line to suspension growth and ----(b)(4)----- over the course of (b)(4) passages between 1988 and 1992. Novartis's suspension culture-adapted MDCK cell subline was designated MDCK (b)(4)-. The MDCK --(b)(4)-- subline was further adapted to ---(b)(4)--- conditions in 1995 (passages ---(b)(4)-----). A major research cell bank was established at passage (b)(4) in 1995. MCB ---(b)(4)----- was prepared from the suspension culture, ---(b)(4)---- adapted MDCK (b)(4) subline. In 1996, Novartis transferred the MDCK (b)(4) cell line, at -----(b)(4)----- for final adaptation to ---(b)(4)----- growth. (b)(4) completed the adaptation in 1996 and froze the cells. These cells were designated MDCK ----(b)(4)----- In 1998 (b)(4) passaged the MDCK --(b)(4)-- cell line (b)(4) more before returning them to Novartis for preparation of a new master cell bank ---(b)(4)-----.

Overview of Cell Banks

A (b)(4)-tiered cell bank system represented by --(b)(4)----, master, working, -----(b)(4)----- cell banks was established for the FCC (Influenza Cell Culture) vaccine manufacturing process. The first cell bank was generated after the MDCK cell line had been adapted to suspension culture and --(b)(4)-- conditions. Preparation and testing of this early master cell bank ---(b)(4)---- is briefly described as the data establish absence

of detectable adventitious agents at this stage of derivation. The current set of cell banks --- (b)(4) ---, master, working, and ---- (b)(4) ----- was established after --- (b)(4) ----- adaptation of MDCK --- (b)(4) -. Working cell banks (----- (b)(4) ----- derived from -- (b)(4) --- MDCK --- (b)(4) ---- master cell bank ----- (b)(4) ----- will be used for commercial production of FCC monovalent bulk and influenza working seed virus. Qualification of the cell banks (MCB, WCB, --- (b)(4) -- as free from adventitious agents included assessment of microbiological purity, mycoplasma, standard *in vivo* and *in vitro* adventitious viral agent (AVA) testing, and additional testing for ----- (b)(4) -----
-----.

Reviewer's Comments:

A diagram depicting the derivation of the cell banks is provided in Appendix Fig. 1.

17 Pages Determined to be Not Releasable: (b)(4)

Reviewer's Comments:

- The testing program is consistent with 9 CFR regulations as well as the Guidance Document for cell substrate characterization and qualification issued by CBER in 2010.
- In addition to the standard *in vivo* and *in vitro* tests, (b)(4) tests were performed for specific adventitious agents, encompassing an extensive list of -----(b)(4)----- . These tests were demonstrated to have the appropriate level of sensitivity. Many of these tests were performed due to the passage history of the Sponsor's MDCK cells and their past exposure to biological material derived from various species (for example, sera from -----(b)(4)----- sources).
- The Sponsor's cell banks (MCB, WCB, ---(b)(4)---- appear to be free of adventitious agents.

Genetic Stability

To ensure consistent vaccine production, it was important to demonstrate genetic and phenotypic stability of the cell line over the period of production use. The maximum limit of use is specified in the batch records as ----(b)(4)----- from the (b)(4), which represents ----(b)(4)----- from the (b)(4). The heterogeneity of the karyotype previously shown for the (b)(4) was reflected in the subpopulations and -----(b)(4)-----, indicating genetic stability of the cell line over the culture period used during vaccine production. The stemlines for the Master, Working, --- (b)(4)--- cell banks ranged from --- (b)(4)--- with similar results in the interim research cell banks. These results differed significantly from that observed with ATCC --- (b)(4)----- cell lines.

[(b)(4)]

Reviewer's Comments:

The Sponsor's MDCK cells are genetically stable at the gross level as assessed by karyotype analysis. The data above are included in this review in order to emphasize the possibility that the Sponsor's MDCK cells may have properties distinct from "typical" MDCK cells (*i.e.*, as obtained directly from ATCC). -----

----- (b)(4) -----

----- (b)(4) -----
----- . The properties empirically demonstrated for the Sponsor's MDCK cells (for example, permissiveness for the replication of viruses other than influenza viruses as discussed in a later section on adventitious agents) might not be generalizable to "typical" MDCK cells.

Tumorigenic and Oncogenic Potential of MDCK Cell Substrate

Tumorigenicity and oncogenicity studies performed with either -----
----- (b)(4) ----- prepared from the MDCK cell
line used to produce Novartis's inactivated, trivalent influenza vaccine. The study designs
reflect ICH, CBER, and WHO guidance concerning cell characterization, with the
exception that the study duration was increased from ---- (b)(4) ----- . Studies were
initiated at the request of, and in consultation with, the Center for Biologics Evaluation
and Research (CBER) of the USFDA. The primary series of tumorigenicity and
oncogenicity studies were conducted with material from the --- (b)(4) --- cell bank. (b)(4)
studies were performed by ----- (b)(4) -----
----- . For all studies, the route of administration was by subcutaneous injection into
the flank of the hind limb to allow for ease of palpation/measurement of nodules.

[(b)(4)]

----- (b)(4) -----

(b)(4)

- The Sponsor's MDCK cells are tumorigenic (*i.e.*, the cells are capable of forming tumors when inoculated into immunosuppressed animals). This is notable, as Flucelvax will represent the first licensed vaccine in the United States to be manufactured in tumorigenic cells.
- Oncogenicity is a property of biological agents (*e.g.*, viruses) or materials (*e.g.*, nucleic acids) that are capable of immortalizing cells and endowing them with the capacity to form tumors. Oncogenicity is distinct from tumorigenicity. Lysates and DNA derived from the Sponsor's MDCK cells are non-oncogenic.
- In general, the theoretical risks perceived to be associated with tumorigenic cells can be addressed by (1) comprehensive testing of the cell substrate for adventitious agents, and (2) clearance of residual cell-substrate DNA from the final product. Both of these subjects are discussed in relevant sections of this review.
- The use of tumorigenic cells (in particular, the Sponsor's MDCK cells) in vaccine manufacture was discussed during the Vaccines and Related Biological Products Advisory Committee (VRBPAC) Meeting in November 2005. At that time, Committee members were in general agreement that

tumorigenic MDCK cells could be used for the manufacture of inactivated influenza vaccine.

- More recently, a VRBPAC Meeting held in September 2012 discussed the use of human tumor-derived cell lines (such as HeLa cells) for the manufacture of vaccines. The Committee came to a general consensus that potential safety issues (primarily adventitious agents and DNA oncogenicity) can be adequately addressed. No issues were identified that *a priori* precluded the use of such cells.

10 Pages Determined to be Not Releasable: (b)(4)

Adventitious Agents Safety Evaluation

The adventitious agents risk evaluation can be organized into the following categories:

- Cell substrate-related safety data
- Seed virus-related safety data
- Process-related safety data

Cell Substrate-Related Safety Data

Non-Viral Adventitious Agents

The final product is a sterile solution containing ---(b)(4)-- of endotoxin. Entry of microbial organisms into the process is limited by use of a validated facility with established cleaning and sterilization methods. The bulk manufacturing process has routine bioburden testing at ---(b)(4)----- to monitor control along with ---(b)(4)----- steps with capability to reduce potential bioburden.

In accordance with the European Council Directive 2001/83/EC amended by Directive 2003/63/EC, the components within the FCC vaccine manufacturing process have been evaluated with consideration of the current “Note for guidance on minimizing the risk of transmitting animal spongiform encephalopathy agents via human and veterinary medicinal products” (EMA/410/01). Separate TSE assessments have been conducted for the FCC manufacturing process and the MDCK --(b)(4)-- master cell bank (MCB). The FCC manufacturing process from the MDCK working cell bank (WCB) through to the final product was evaluated. It was determined that no potential TSE risk materials have been or are used in the virus seed materials, nor in the routine production of FCC subunit vaccine. The use of ruminant-derived materials complies with the requirements of the current TSE note for guidance (EMA/410/01). In addition, a study/data based TSE risk assessment on the MDCK cell line was performed in collaboration with Dr. A. Aguzzi (Zurich, Switzerland) and was published in a peer reviewed journal (Vaccine 26:2601, 2008). -----

-(b)(4)

Viral Adventitious Agents

The MDCK cells used to manufacture the FCC vaccine have been banked, and the master and working cell banks have been characterized for growth, identity, donor history, purity from adventitious agents, and tumorigenicity. Qualification of the cell banks (MCB, WCB, ---(b)(4)--- as free from adventitious agents included microbial and fungal sterility, mycoplasma, standard *in vivo* and *in vitro* adventitious viral agent (AVA) testing, and assessment of -----(b)(4)----- . Virus specific and degenerate (b)(4) assays were performed on cells from the MCB, WCB, ---(b)(4)- samples.

Reviewer's Comment:

Cell bank-related adventitious agent testing was described in previous sections of this review.

4 Pages Determined to be Not Releasable: (b)(4)

Seed Virus-Related Safety Data

Influenza vaccine seed viruses are made from "reference virus strains" that are distributed by custodian laboratories (e.g. the Center of Disease Control, Atlanta and NIBSC, London) on behalf of the WHO Collaborating Centers for Reference and Research in Influenza. The strains have been isolated in WHO-associated laboratories or National Influenza Reference Centers by inoculation of human clinical samples, such as throat swabs, into embryonated hen's eggs and by monitoring these for growth of haemagglutinating agents as an indicator for influenza viruses. Recovered isolates are then typed and selected by haemagglutination inhibition tests and by analyzing their genetic phenotype. By the time recommended vaccine strains or reassortants are eventually distributed to the vaccine manufacturers, they have usually undergone 3-7 passages in embryonated eggs. Influenza virus isolation and early passage was usually conducted in normal embryonated eggs and did not follow specific procedures to exclude the presence of adventitious agents, e.g., use of eggs from pathogen-free chicken flocks or inclusion of viral screening tests to identify and exclude potential contaminants. Until recently passage at the custodian laboratories (e.g., the NIBSC in London) was done in normal embryonated eggs. According to information by the NIBSC, a change to the use of embryonated eggs from controlled, specific pathogen-free herds has been implemented at the CDC and the NIBSC in 2007, while the Australian Center has used such eggs already for a longer time. However, repeated, rapid passaging followed by assay of influenza-related haemagglutinin (HA) activity and typing of the recovered isolates via influenza-specific HA-inhibiting antibodies and genetic characterization are effective means to exclude most adventitious agents. Many influenza A virus strains used for vaccine production are reassortants. The selection process used after the reassortment are essentially high dilution viral cloning steps. The high dilution cloning would also be expected to effectively eliminate contaminants.

-(b)(4).

14 Pages Determined to be Not Releasable: (b)(4)

[(b)(4)]

Risk Assessment

A simple method was established to rate the risk of adventitious agents if different cell substrates (embryonated eggs, MDCK cells, ---(b)(4)-- or combinations thereof) were used for influenza virus isolation and subsequently for virus propagation during vaccine manufacturing. Data regarding the replication of various viruses and microbial agents were gathered from published literature or by internal laboratory studies and translated into semi-quantitative scores. Other characteristics of the viruses relevant to vaccine manufacturing (e.g., enveloped or naked, resistance to environmental and processing conditions, stability against chemical inactivation) were also scored. Based on these scores, an algorithm was defined and used to calculate summary scores, which rate the relative risk of occurrence of an adventitious agent in the final influenza vaccine. The methodology and results of this approach were published in a peer-reviewed journal (Vaccine 26:3297, 2008). The main purpose of this scoring system was to rate the risks associated with the introduction of MDCK cells as a new cell substrate to produce influenza vaccines. According to this analysis, MDCK cells were found to reduce contamination risks associated current influenza vaccines. Detailed observations include:

- Avian non-enveloped viruses introduced via embryonated eggs appear to be the most likely virus contaminants to be expected. Low to negligible risks were found for mycoplasma and chlamydia and for almost all enveloped viruses. Of the enveloped viruses only avian retrovirus and herpes simplex virus had risk scores above background.
- Of the mammalian agents, mainly reovirus should be considered a potential source of viral contamination.
- Compared with existing egg-derived vaccines, the MDCK cells used to produce vaccine virus from influenza virus strains isolated in eggs do not introduce new or higher risk, as the host spectrum of MDCK cells is similar to that of embryonated eggs. For all of the viruses scored, MDCK cells used to produce vaccines from egg-derived isolates gave either the same or lower risk scores than embryonated eggs used for isolation and production.

Having accumulated data on the virus replication in the cell substrate used, on the inactivation or process removal of various viruses, and with consideration of applicable detection limits for virus-exclusion tests, a process-specific risk assessment was made using quantitative data relative to infectious doses. When necessary, realistic worst-case assumptions were made to adequately cover variables, such as potential virus titers, virus passage numbers, dilutions, or inactivation results with different types of the same virus family. The assessment starts at the earliest relevant step, which is the isolation of an influenza virus strain from a human throat sample, considers egg passages, MDCK cell passages to prepare viral seeds, (b)(4) detection limits for tested materials, and extends through -----(b)(4)-----

----- . In contrast to risk analysis described above (which rates the probability of occurrence of a process contaminant), this risk assessment invariably assumed a real contamination. For example contamination by human viruses was assumed in the primary isolate and – because of human manipulation – also in the seed virus. Avian virus contaminants were assumed to occur during egg-passage. Two extreme model cases were also included: a porcine circovirus that survived the double inactivation within the trypsin preparation and a parvovirus introduced from the environment. The analysis was published in a peer reviewed journal (Vaccine 26:3332, 2008). The assessment encompasses 24 different virus types or groups and also include Chlamydia and mycoplasma. The results for all viruses and agents clearly show that any of these modeled contaminants would be reduced to levels that are unable to cause infection. For viruses that are infectious to humans, the results would mean that more than 100,000 doses would need to be administered to one individual to accumulate a single infectious unit. Even if the calculated worst-case contaminant level is considered, the final vaccine product would still be unable to transmit infectious viruses.

The Sponsor's strategy for mitigation of adventitious agents risk consists of complementary and orthogonal approaches: (1) qualification of cell banks and seed viruses, (2) assessment of the permissiveness of the MDCK cell substrate to replicate potential adventitious agents, and (3) validation of clearance of model adventitious viruses through key manufacturing steps.

The qualification of the cell banks with respect to adventitious agents was discussed in an earlier section of this review. The testing program appears to be comprehensive, and results support the conclusion that the Sponsor's cell substrate is free of adventitious agents. The use of ---(b)(4)--- medium for cell propagation as well as mitigation steps involving key components of animal origin (for example, the ------(b)(4)-----) reduce the likelihood of introducing adventitious agents during routine manufacturing. The Sponsor's cell banks (MCB, WCB, --(b)(4)-- were extensively tested by (b)(4) for -----(b)(4)----- viruses; all tests were negative.

Following licensure, Flucelvax will be the first vaccine in the United States to be manufactured using a tumorigenic cell substrate. It is notable that the cell bank qualification encompassed (b)(4) testing for virus families known to harbor oncogenes capable of transforming cells (for example, -----(b)(4)-----).

An extensive list of viruses was empirically tested for the ability to replicate in the Sponsor's MDCK cells. A systematic literature search was also performed to identify viruses capable of growth in MDCK cells. The results of these studies corroborate each other and suggest that the Sponsor's MDCK cells are permissive to a narrow spectrum of viruses (comparable with eggs). Relevant viruses that appear to grow in MDCK cells to a significant extent are parainfluenza virus, herpes simplex virus, and reovirus; these three viruses are appropriately prioritized for specific testing --(b)(4)- involving the (b)(4) viruses and -----(b)(4)-----.

Empirical testing of virus replication, as described above, usually involves the use of laboratory-adapted virus strains, thereby potentially limiting the generalizability of findings. -----

In general, the two processing steps evaluated for inactivation of viruses (BPL inactivation and CTAB splitting/--(b)(4)----- demonstrated acceptable robustness. The analysis included viruses considered to be highly resistant to inactivation (such as ----(b)(4)-----. Viruses with relatively

Information Requests

Responses to information requests pertinent to the cell substrate are discussed below.

Amendment 10 (125408/0.9; received by CBER on April 26, 2012)

This amendment contains responses to the following two queries:

Please provide the following documents related to the assessment of risk associated with residual DNA: (1) DRA-Application of Defined Risk Assessment to Use of Cell Bank, (2) Chiron pre-read 5-4188B1_18, (3) Report 228847, (4) Report 235243, (5) Report 229373, (6) Report 250384, and (7) Report 280908.

Reviewer's Comments:

These documents were requested because the Sponsor's risk analysis strategy had undergone a significant change since the 2005 VRBPAC meeting (from an analysis based on DNA size distribution to one based on ---(b)(4)----. The derivation of the estimated ratio of oncogenes to (b)(4) elements ---(b)(4)-- oncogenes/(b)(4) is explained more completely in Report 280908. The evolution of the Sponsor's DNA risk analysis during the course of product development is described more completely in the requested documents.

Please provide the validation report for the host-cell protein (b)(4) (Report 403410).

Reviewer's Comments:

The validation of host-cell protein removal during ---(b)(4)----- processing was described in 3.2.S.3.2.5-1 (study period 04/2004-10/2005); this document references the requested assay validation report 403410 for the host-cell protein (b)(4). The information contained in 403410 is acceptable and corroborates a later supplemental assay validation report included in the original submission (Attachment 3.2.R.3-257228; carried out in 2007/2008).

The validation of host-cell protein removal during -----(b)(4)--- processing (described in 3.2.S.3.2.5-1) was performed with process 1.0 samples. In discussion with other CMC reviewers (Zhiping Ye, Xianghong Jing, and Anissa Cheung), this was found to be acceptable because other analytical data (assessment of -----(b)(4)----- corroborate the removal of host-cell proteins, and process 1.1 includes an -----(b)(4)----- step --(b)(4)-. It is notable that the -----(b)(4)----- step assessed for process 1.0 -----(b)(4)----- step) contributed the most to host-cell protein removal ---(b)(4)--

The Sponsor is proposing to assess total protein rather than host-cell protein (by --(b)(4)- for release testing; in discussion with other CMC reviewers (Zhiping Ye and Xianghong Jing), this was deemed acceptable.

Amendment 12 (125408/0.12; received by CBER on June 20, 2012)

This amendment contains responses to the following two queries:

On p. 46 of 3.2.A.2 (Adventitious Agents Safety Evaluation), Attachment 3.2.A.2.5.2.2-1, a summary of BPL inactivation studies for model viruses is provided. However, this Attachment only describes inactivation by BPL of --- (b)(4)----. Please submit the correct referenced general summary of the BPL inactivation studies.

Table 3.2.A.2.5.2.2-1 and Table 3.2.A.2.5.2.2-2 of 3.2.A.2 (Adventitious Agents Safety Evaluation) provide an overview of the BPL inactivation results. Please provide the study reports for viruses capable of replicating in MDCK cells: --- (b)(4)----- (Study No. AS 02/00-18), -----(b)(4)----- (Study No. AS 03/02-52 and Study No. AS 10/02-58), -----(b)(4)----- (Study No. 02/02-50).

Reviewer's Comments:

As described in the section on Adventitious Agents Safety Evaluation, the Sponsor completed (b)(4) sets of studies on BPL inactivation of model viruses: ---

----- (b)(4)-----

-----, only tabulated summaries were provided in the original submission (Table 3.2.A.2.5.2.2-1 and Table 3.2.A.2.5.2.2-2 of 3.2.A.2).

However, the log-reduction values from these studies are incorporated in an analysis of overall clearance by BPL and CTAB

splitting/----- (b)(4)----- (Table 3.2.A.2.6-1 and Table 3.2.A.2.6-2).

As these studies provide critical data to assure safety in terms of adventitious viruses, additional details were sought. Due to the extensive testing performed the Sponsor, only study reports for viruses capable of replicating in MDCK cells were requested (i.e., ----- (b)(4)-----

--; these viruses represent potential vulnerabilities and are the focus of specific testing at the --- (b)(4)---- stage. The studies, as described by the reports submitted in Amedment 12 (AS 02/00-18, AS 03/02-52, AS 10/02-58, and AS 02/02-50), were performed in an acceptable manner.

Amendment 34 (125408/0.34; received by CBER on September 21, 2012)

This amendment contains the response to the following query:

Please clarify at which stage the "Absence of extraneous viruses" testing will be performed----- (b)(4)-----
----- If these tests will be performed ----- (b)(4)-----, please comment on whether these processes would affect the test results.

Reviewer's Comments:

(b)(4) testing for extraneous viruses is performed on the ---(b)(4)-----

---(b)(4)-----

Reviewer's Summary/Conclusions

The Sponsor's MDCK cell substrate is tumorigenic when inoculated into nude mice. A long-standing proscription of the use of tumorigenic cells for vaccine manufacture has been in place since a recommendation in 1954 by the US Armed Forces Epidemiology Board (Natl Cancer Inst Monogr 29:463, 1968) to use primary cells for the manufacture of adenovirus vaccines rather than HeLa cells (derived from human cervical cancer). The Sponsor's product (Flucelvax), upon licensure, will be the first vaccine marketed in the US that is produced in tumorigenic cells.

The use of the Sponsor's MDCK cells for manufacture of inactivated influenza vaccine was publicly discussed at the November 2005 VRBPAC meeting. In addition, a related discussion on human tumor-derived cell substrates took place at the September 2012 VRBPAC meeting. While some members of the VRBPAC expressed reservations regarding the use of tumorigenic cells, the Committee came to a general agreement that the Sponsor's approach to risk mitigation was acceptable (documented in the publicly available transcripts for the VRBPAC meetings in November 2005 and September 2012).

The theoretical risks associated with the use of tumorigenic cells (centering on the concern over the potential transfer of factors that might neoplastically transform cells of the vaccine recipient) can be mitigated by (1) comprehensive testing for adventitious agents and (2) reducing or inactivating residual cell-substrate DNA (Biologicals 37:190, 2009). On the basis of the extensive testing performed by the Sponsor, I agree with the conclusion that the Sponsor's MDCK cell banks are free of adventitious agents. The clearance validation studies of multiple model viruses (including viruses known to be resistant to inactivation by physical and chemical means, such as ----(b)(4)----- provide additional safety information. Furthermore, the probability of contamination by adventitious agents during vaccine manufacture is greatly reduced by (1) the testing strategies in place with respect to the vaccine seed and the vaccine bulk harvest, (2) the use of media free of animal-derived components, and (3) the inactivation protocol applied to --- (b)(4) ----- . Finally, the Sponsor provides compelling evidence that the Sponsor's MDCK cells are inherently permissive only to a narrow spectrum of viruses (comparable with chicken eggs), thus forming a basis for a natural obstacle to contamination by adventitious agents. Theoretical risks associated with residual cell-substrate DNA is effectively addressed by the Sponsor through (1) reduction of DNA quantity to a maximal level of 10 ng per dose and (2) reduction of --- (b)(4) --- through the manufacturing process (largely due to BPL). The negative results obtained by in vivo oncogenicity testing of the Sponsor's MDCK cell lysate/DNA provide supportive assurance of safety.

There are substantial benefits to using MDCK cells in the manufacture of influenza vaccine. Cells that are banked and qualified can supply the requisite biomass of vaccine substrate on short demand (as opposed to chicken eggs, which are the conventional substrate for currently licensed influenza vaccines),

thereby allowing a more rapid response in the event of an influenza pandemic. In addition, human influenza viruses are known to undergo adaptations in avian substrates that can impact antigenicity; thus, a vaccine manufactured entirely* in mammalian cells, such as MDCK cells, may have improved efficacy by providing a better antigenic match to circulating human viruses.

On the other hand, the risks associated with tumorigenic cell substrates are largely theoretical, and thus, exceedingly difficult to quantify; they may, in actuality, be non-existent, although such a conclusion can never be empirically established. However, engendering public confidence in vaccination programs necessitates a prudent course and the theoretical risks need to be adequately addressed. It is my conclusion that the Sponsor has done so through extensive characterization of the Sponsor's MDCK cell substrate. With respect to use of the Sponsor's MDCK cells, the known benefits appear to me to outweigh the unknown (theoretical) risks.

On the basis of information pertaining to the MDCK cell substrate, I recommend approval of the Sponsor's licensure application for Flucelvax.

* The Sponsor is presently seeking licensure for a process using egg-grown reassortant virus seed progenitors; -----

----- (b)(4) -----

3 Pages Determined to be Not Releasable: (b)(4)