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January 16, 2006

Dr. William S. Stokes, Director
Director of NICEATM
NIEHS
79 West Alexander Drive
P.O. Box 122233
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Research Triangle, NC 27709

Re: Submission of the MCF-7 Estrogenic Activity Cell Proliferation Assay for cross-laboratory validation studies by ICCVAM and NICEATM

Dear Dr. Stokes:

On behalf of CertiChem, Inc (CCi), we are submitting for consideration for cross-laboratory validation studies our MCF-7 Cell Proliferation Assay as a robotic *in vitro* screen for estrogenic activity (EA). We submit our test method for validation by NICEATM and ICCVAM because our assay is sensitive and reliable in manual or robotic format, as determined by comparing data generated using this assay against previously published *in vitro* data, such as those listed in Table 4 of ICCVAM (2003). The rationale for recommending this assay for cross-laboratory validation studies is as follows:

Need for a valid EA assay, especially in robotic format

- Recent scientific investigations have shown that many chemicals used to manufacture plastics, pharmaceuticals, pesticides, cosmetics, foodstuffs, etc., are endocrine disruptors (EDs) that interfere in various ways with the actions of estrogen, androgen or thyroid hormones (EDSTAC, 1998; NRC, 1999; EPA, 2000; ICCVAM, 2002a-c, 2003).
- The most common ED activity is mediated through the estrogen receptor, and which can have significant deleterious effects on many physiological processes. Most importantly, adverse EA effects sometimes occur at very low (picomolar to nanomolar) concentrations, especially in fetal or developing mammals, including humans (EDSTAC, 1998; NRC, 1999; NTP, 2001; ICCVAM, 2002a-c, 2003; Palanza, 2002).
- Since a large number of chemicals should be screened for possible EA, the U.S. Environmental Protection Agency (EPA) and ICCVAM have set a high priority on the



development of high throughput *in vitro* robotic screening assays for EA (EDSTAC, 1998; ICCVAM 2002a-c, 2003).

- No methods to test for EA have yet been approved by the EPA or validated by ICCVAM and NICEATM

Reasons why CCI's MCF-7 cell proliferation assay for EA should be considered by ICCVAM and NICEATM for cross-laboratory validation studies:

- Our *in vitro* assay can reduce animal use and does not use radioactive materials, in compliance with ICCVAM recommendations for such test methods.
- Our assay is highly sensitive, i.e. capable of detecting chemicals with high EA (e.g. beta-estradiol, diethylstilbesterol) at less than picomolar concentrations and chemicals with low EA (octylphenol, estriol) at less than micromolar concentrations.
- Our assay can measure the EA of single chemicals, as well as of complex mixtures of known and/or unknown chemicals.
- Our assay requires only a small amount of a suspect chemical or chemical mixture.
- The cost of our assay is relatively low.
- The time it takes to run the assay is short (days).
- Our manual and robotic protocols meet or exceed all published ICCVAM recommendations (ICCVAM, 2002a-c, 2003).
- The data obtained using this assay in manual or robotic format are reliable, i.e. reproducible within and between laboratories.
- The results obtained using this assay are accurate, i.e. the data contain very few, if any, false negatives or false positives when compared with data compiled and published by ICCVAM (2000a-c, 2003).
- Our assay is applicable to the needs of various federal agencies (FDA, DOD, DHS, DOA) and commercial firms, especially since we have developed procedures to screen animal feeds, human foodstuffs, aqueous extracts from plastics, etc. for EA.

For these and other reasons, CCI submits its MCF-7 cell proliferation assay in its manual and robotic formats for consideration for cross-laboratory validation studies. The supporting documentation for this nomination is provided in the accompanying CDs sent by express mail.



CCi is requesting that ICCVAM and NICEATM initiate the cross-laboratory studies needed to formally validate this MCF-7 cell proliferation assay. CCi proposes that it be one of the laboratories chosen for formal validation studies and that CCi act as the laboratory to provide training and technical support to other laboratories chosen to validate this assay.

Sincerely,

George D. Bittner, Ph.D. CEO, CertiChem

Cathy Yang, MD, PhD COO, CertiChem



LITERATURE CITED

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