Appendix B

List of Test Protocols for Basal Cytotoxicity

European Centre for the Validation of Alternative Methods (ECVAM)

Scientific Information System (SIS) http://www.ivtip.org/protocols.html#basalcyto

THE FRAME MODIFIED NEUTRAL RED UPTAKE CYTOTOXICITY TEST
The cytotoxic effect of chemicals upon cells in culture is measured by cell viability (neutral red uptake) method. Topics: Basal Cytotoxicity. Contact: Dr. Richard H. Clothier, Queen's Medical Centre, UK Last update: September 1990. Protocol no: 3.

HUMAN LYMPHOCYTE CYTOTOXICITY ASSAY
This method measures the leakage of DNA and lactate dehydrogenase (LDH, EC. 1.1.1 27) from lymphocytes into the surrounding medium as an indicator of cytotoxicity. This method also includes an assay of intracellular (mitochondrial) diaphorase as a measure of cellular activity (MTT assay). Topics: Basal Cytotoxicity. Contact: Prof. Jorgen Clausen, Roskilde University, DK. Last update: May 1991. Protocol no: 6.

THE USE OF MEMBRANE PERMEABILITY AS A MEASURE OF CYTOTOXICITY IN PERFUSED CELL CULTURES
Membrane permeability of perfused cell cultures, as determined by the afflux of [3H]-2-deoxy-D-glucose-6-phosphate, is used as an indicator of the cytotoxic effect of chemicals. Topics: Basal Cytotoxicity. Contact: Dr. E. Walum, Bioscience Centre, SEK. Last update: June 1989. Protocol no: 9.

HEL-30 CYTOTOXICITY TEST
The ability of cultured cells to synthesize protein is used to assess the effect of a test compound on cellular anabolic competence. Topics: Basal Cytotoxicity Contact: Dr. Marina Marinovich, Universita di Milano, I. Last update: April 1990. Protocol no: 14.

THE FRAME CYTOTOXICITY TEST (KENACID BLUE)
The cytotoxic effect of chemicals upon cells in culture is measured by the change in total cell protein arising from the inhibition of cell proliferation (Kenacid Blue R dye binding method). Topics: Basal Cytotoxicity. Contact: Dr. Richard H. Clothier, Queen's Medical Centre, UK. Last update: July 1992. Protocol no: 15.

CYTOTOXICITY AND GENOTOXICITY IN PRIMARY CULTURES OF HUMAN HEPATOCYTES
This test determines the cytotoxic and genotoxic effect of test compounds on primary cultures of human hepatocytes, by measuring cell viability, DNA damage, and unscheduled DNA synthesis. Topics: Basal Cytotoxicity, Mutagenicity. Contact: Prof. Giovanni Brambilla, University of Genoa, I. Last update: May 1992. Protocol no: 16.

MTT ASSAY
This method outlines a simple assay to determine the viability/number of cells in culture, through the formation of a colored product (in a mitochondria-dependent reaction) to which the cell membrane is

impermeable. Topics: Basal Cytotoxicity. Contact: Dr. Rosanna Supine, Istituto Nadonale Tumori, I. Last update: April 1990. Protocol no:17.

CYTOSKELETAL ALTERATIONS AS A PARAMETER FOR ASSESSMENT OF TOXICITY
Changes in the balance of cytoskeletal proteins after exposure to test compounds can be detected by indirect immunofluorescence microscopy and quantitative biochemical methods. Topics: Basal Cytotoxicity. Contact: ECVAM SIS. Last update: July 1991. Protocol no: 24.

YEAST GROWTH RATE CYTOTOXICITY TEST
The cytotoxic effect of chemicals upon yeast (Saccharomyces cerevisiae) cells in culture is determined by inhibition of cell proliferation, as measured by cell density. Topics: Basal Cytotoxicity. Contact: Dr. Ingolf Cascorbi, Institute of Clinical Pharmacology, D. Last update: January 1994. Protocol no: 33.

YEAST PLASMA MEMBRANE H+-ATPASE TOXICITY TEST
The effect of chemicals on the activity of the plasma membrane-bound H+-ATPase, isolated from yeast (Saccharomyces cerevisiae) cells, is used as a measure of their toxicity. Topics: Basal Cytotoxicity. Contact: Dr. Ingolf Cascorbi, Humboldt-University, D. Last update: January 1994. Protocol no: 34.

CHINESE HAMSTER OVARY CELL NA+/K+-ATPASE TEST
The effect of chemicals on the activity of the plasma membrane-bound Na+/K+ -ATPase isolated from Chinese Hamster Ovary (CHO) cells is used as a measure of their toxicity. Topics: Basal Cytotoxicity Contact: Dr. Ingolf Cascorbi, Humboldt-University, D. Last update: January 1994. Protocol no: 35.

CHINESE HAMSTER OVARY (CHO) CELL PROLIFERATION TEST
The inhibition of CHO cell proliferation provides an overall assessment of the toxicity of the test substance. Topics: Basal Cytotoxicity Contact: Dr. Ingolf Cascorbi, Humboldt-University, D. Last update: January 1994. Protocol no: 36.

LS-L929 CYTOTOXICITY TEST
This simple cell culture-based cytotoxicity test (in which cell viability is determined by uptake of the dyes ethidium bromide and fluorescein acetate) has been developed as a general test for acute toxicity. Topics: Basal Cytotoxicity, Eye Irritation. Contact: Dr. R.B. Kemp, University College of Wales, UK. Last update: July 1992. Protocol no: 38.

V79 CYTOTOXICITY/ TEST FOR MEMBRANE DAMAGE
The cytotoxic effect of test chemicals in V79 cell culture can be determined by assessing damage to the plasma membrane as determined by a nucleic acid leakage assay. Topics: Basal Cytotoxicity. Contact: Prof. Vera Bianchi, University of Padova, I. Last update: June 1990. Protocol no: 39.

BALB/C 3T3 CYTOTOXICITY TEST
The cytotoxic effect of chemicals upon Balb/c 3T3 cells in culture is measured by cell viability (Neutral Red Uptake) and total cell protein (Kenacid Blue R dye binding method). Topics. Basal Cytotoxicity, Eye Irritation. Contact: Dr. med. Horst Spielmann, ZEBET BgVV, D. Last update: January 1992. Protocol no: 46, German EGA Validation Study Protocol.

QUANTITATIVE VIDEO MICROSCOPY OF INTRACELLULAR MOTION AND MITOCHONDRIA-SPECIFIC FLUORESCENCE
AVEC-DIC microscopy in combination with mitochondria-specific fluorescence allows a quantitative analysis of cell organelle dynamics and fine structure in cell cultures exposed to test compounds. Topics: Basal Cytotoxicity. Contact: Dr. Toni Lindl, Inst. f. Angewandte Zellkultur, D. Last update: April 1992. Protocol no: 52.

UV ABSORPTION AS AN APPROXIMATION FOR CELL NUMBER
The absorption of UV at 260nm in a fixed volume of solubilized cells is proportional to the cell number, and therefore can be used as a simple means of obtaining a cell count. Cell counts obtained in this way can be combined with measurements of the inhibition of DNA synthesis ([3H]-thymidine incorporation) by test compounds, to produce an index of cytotoxicity. Topics: Basal Cytotoxicity. Contact: Dr. Ming J.W. Chang, Chang Gung Medical College, Rep. of China. Last update: September 1992. Protocol no: 58.

***IN VITRO* PREDICTION OF THE MAXIMUM TOLERATED DOSE**
The results of cytotoxicity tests in primary cultures of rat hepatocytes and in MDCK and McCoy cells can be used to predict the *in vivo* 4-wk maximum tolerated dose in rats and dogs. A correlation between *in vitro* cytotoxicity, as measured in this system, and LD50 values in rats and mice has also been established. Topics: Basal Cytotoxicity, Acute Systemic Toxicity. Contact: Dr. R. Shrivastava, RL-CERM, F. Last update: February, 1992. Protocol no: 66.

TWO-COMPARTMENT HUMAN TISSUE CYTOTOXICITY TEST
The activating system (human liver microsomes) is separated by a semi-permeable membrane from the target cells (human mononuclear leukocytes or red cells) in order to identify cytotoxic metabolites that are capable of diffusing away from the site of production. Topics: Basal Cytotoxicity, Hepatotoxicity I Metabolism - Mediated Toxicity. Contact: Dr. M.D. Tingle, University of Liverpool, UK. Last update: January 1994. Protocol no: 73.

TETRAHYMENA ASSAY FOR MEMBRANE-STABILIZING ACTIVITY
The effect of a test compound on lipid structure and protein ion channels in biological membranes can be determined by using video image analysis to assess its effect on the swimming speed of the ciliated protozoan, Tetrahymena pyriformis. Topics: Basal Cytotoxicity, Ecotoxicity, Aqueous contamination. Contact: Dr. S.L. Cassidy, Dow Corning Corporation, USA. Last update: February 1994. Protocol no: 76.

CYP1A1-INDUCING POTENCY AND CYTOTOXICITY TEST IN THE HEPA-1 MOUSE HEPATOMA CELL LINE
This bioassay utilizes cultured Hepa-lclc7 (Hepa-l) mouse hepatoma cells to assess the CYP1A1-inducing potency or cytotoxicity of pure test chemicals or environmental samples. In the Hepa-l induction test, the CYP1A1-inducing potency of the test sample is detected as increased aryl hydrocarbon hydroxylase (AHH) and 7-ethoxyresorufin-O-deethylase (EROD) activities. In the Hepa-l cytotoxicity test, the effect of the sample on cell viability is measured. Topics: Basal Cytotoxicity, Ecotoxicity. Contact: Dr. Sirpa Kärenlampi, Dr. Riitta Torronen, Dr. Paivi Kopponen, University of Kuopio, FIN. Last update: October 1995. Protocol no: 112, MEIC Project Protocol.