

Appendix E

Neutral Red Dye Experiments

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APPENDIX E

Neutral Red Dye Experiments

Appendix E-1: Assessment of Protocol Variables in the NICEATM/ECVAM Evaluation of Cytotoxicity Assays

IIVS performed experiments using the 3T3 cells and the NRU test methods before the NICEATM/ECVAM validation study was initiated. The laboratory examined: optimal solvents (DMSO vs. ETOH), cell seeding densities, doubling times, and exposure duration of a test chemical (24, 48, and 72-hour exposures). Data are presented in the appendix.

Appendix E-2: Neutral Red (NR) Dye Experiments – 3T3 Cells

IIVS performed three sets of experiments to compare the optical density (OD) readings obtained in an NRU assay using various concentrations of NR dye and different incubation periods.

- Experiment 1: NR Stain Time Course in 3T3 Cells; NRU incubation times: 0.25, 0.50, 1.0, 2.0, and 3.0 hour.
- Experiment 2: Neutral Red Stain Prepared in DMEM/5%NCS; Test of NR Preparation 1 Day Prior to Use; Tested in 90-100% Confluent 3T3 Cultures
- Experiment 3: Neutral Red Stain Prepared in DMEM/5%NCS; Filtered Immediately before Use; Tested in 90-100% Confluent 3T3 Cultures

Appendix E-3: Neutral Red (NR) Dye Experiments – NHK Cells

IIVS performed three sets of experiments to compare the optical density (OD) readings obtained in an NRU assay using various concentrations of NR dye and different incubation periods.

- Experiment 1: NR Stain Time Course in NHK Cells; NRU incubation times: 0.25, 0.50, 1.0, 2.0, and 3.0 hour.
- Experiment 2: Neutral Red Stain Prepared in KGM; Test of NR Preparation 1 Day Prior to Use; Tested in 90-100% Confluent NHK Cultures
- Experiment 3: Neutral Red Stain Prepared in KGM; Filtered Immediately before Use; Tested in 90-100% Confluent NHK Cultures

Appendix E-4: Neutral Red (NR) Dye Experiments – Concentration vs Time – 3T3 Cells

ECBC performed experiments using the 3T3 cells and the NRU test methods.

- *in vitro* cytotoxicity NRU tests (3T3 cells) using SLS (range = 100 µg/mL to 6.7 µg/mL)

- NR dye mixed with DMEM culture medium with 10% NCS; final concentrations = 25 µg/mL and 50 µg/mL
- Tests performed with two NRU incubation times: 1 hour and 3 hours

| µg NR dye/mL | NRU Incubation Time (hours) | Mean Vehicle Control OD ₅₄₀ Value |
|--------------|-----------------------------|--|
| 25 | 1 | 0.255 |
| 25 | 3 | 0.508 |
| 50 | 1 | 0.330 |
| 50 | 3 | 0.457 |

Appendix E1

Assessment of Protocol Variables in the NICEATM/ECVAM Evaluation of Cytotoxicity Assays

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ASSESSMENT OF PROTOCOL VARIABLES IN THE NTP EVALUATION OF CYTOTOTXICITY ASSAYS

Balb/C 3T3 Cells

I. What is the acceptable solvent concentration?

Two solvents, DMSO and EtOH, were assayed in the 3T3 assay to determine acceptable concentrations. Multiple exposure times were assessed since the final assay exposure time was not yet established. Various cell seeding concentrations were tested since these experiments were run concurrently with others which used to determine optimal seeding density.

Table 1.

| EtOH | | | | | | | | | |
|------|---------|-----|------|-------|------------------------------|------|------|--------|------------------------------|
| | Date | 2% | 1% | 0.50% | Seeding Density | | | | |
| 48h | 2/26/02 | 58% | 72% | 100% | 9X10 ³ cells/ml | | | | |
| | 2/26/02 | 49% | 73% | 102% | 4.5X10 ³ cells/ml | | | | |
| 72h | 2/26/02 | 67% | 75% | 105% | 9X10 ³ cells/ml | | | | |
| | 2/26/02 | 68% | 82% | 108% | 4.5X10 ³ cells/ml | | | | |
| DMSO | | | | | | | | | |
| | Date | 2% | 1% | 0.5% | 0.4% | 0.3% | 0.2% | 0.1% | Seeding Density |
| 24h | 3/19/02 | | 76% | 91% | 92% | 99% | 100% | 101.6% | 2X10 ⁴ cells/ml |
| 48h | 2/26/02 | 25% | 54% | 83% | | | | | 9X10 ³ cells/ml |
| | 2/26/02 | 27% | 56% | 78% | | | | | 4.5X10 ³ cells/ml |
| | 3/19/02 | | 116% | 123% | 122% | 120% | 117% | 108.8% | 1X10 ⁴ cells/ml |
| 72h | 2/26/02 | 20% | 52% | 86% | | | | | 9X10 ³ cells/ml |
| | 2/26/02 | 19% | 56% | 93% | | | | | 4.5X10 ³ cells/ml |
| | 3/19/02 | | 58% | 89% | 102% | 102% | 112% | 110.1% | 5X10 ³ cells/ml |

We concluded from these experiments that 0.5% EtOH was the optimal EtOH concentration (little to no toxicity), and that 0.5% was probably acceptable for DMSO as a trade-off between slight toxicity and ability to test chemicals to higher does levels.

From about the middle of March on, we used 0.5% in all of our experiments where DMSO was called for as a solvent. This gave us a number of

opportunities to further determine the toxicity of DMSO by comparing the solvent control wells with the media control wells in the same experiment.

Table 2.

| DMSO | | | |
|----------------------|-----------------------|------------------|-----------------------|
| Date & Exposure Time | OD Assay Medium Wells | OD Solvent Wells | % Survival in Solvent |
| 24h 3/19/02 | 0.502 | 0.474 | 94.5% |
| | 0.441 | 0.394 | 89.4% |
| 48h 3/19/02 | 0.587 | 0.536 | 91.4% |
| | 0.582 | 0.545 | 93.6% |
| 72h 3/19/02 | 0.687 | 0.601 | 87.6% |
| | 0.666 | 0.588 | 88.3% |

The average survival in 0.5% DMSO from Table 2 was 90.8%.

II. Doubling Time Experiments

We ran a series of experiments designed primarily to determine the appropriate original seeding density for 24, 48, and 72 h exposure times. We judged our results on visual observations of the cells at the conclusion of the experiment (control cells should be just confluent at 24, 48, or 72h), and on the shape of the growth curve.

Figure 1.

3T3 Density Growth Curves, seeded 2/17/2002?

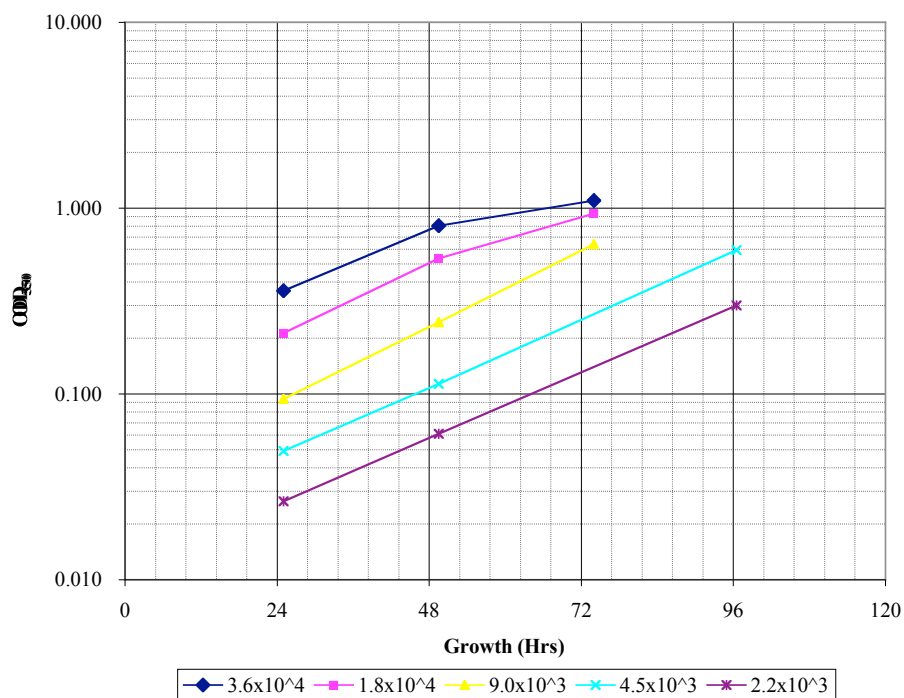
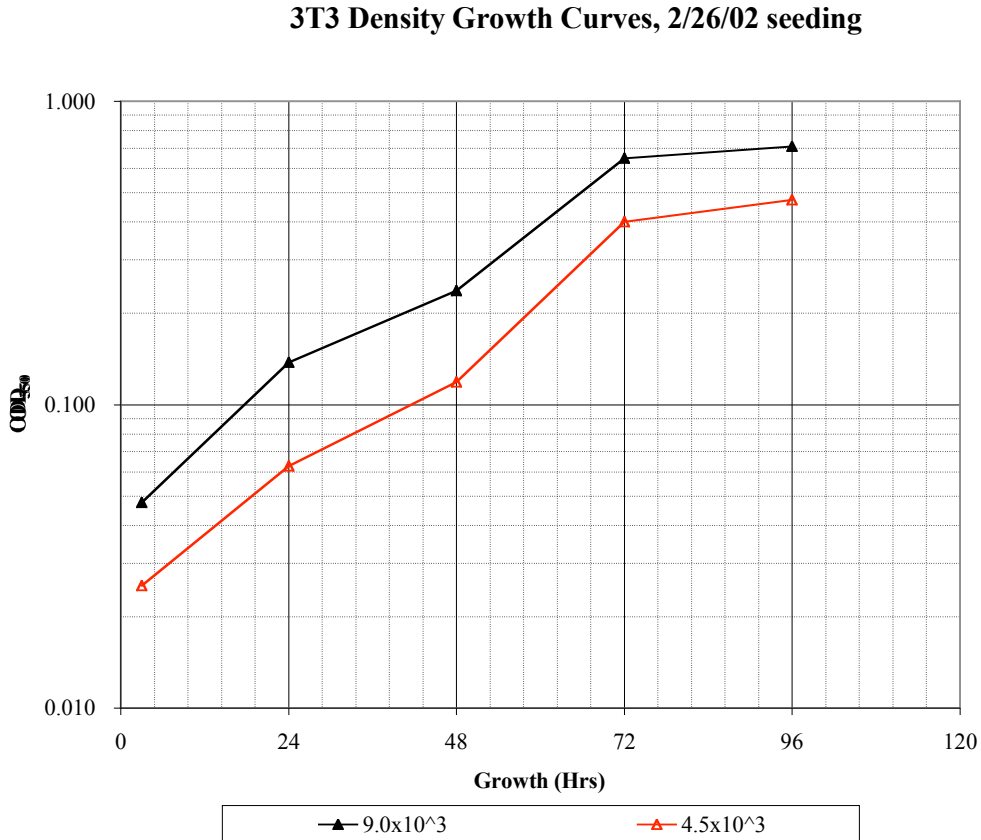


Figure 2.

We have concluded from these growth curves that our 3T3 cells have a doubling time of about 19 h and that cell concentration of: 1X10⁴ cells/ml (24h); 5X10³ cells/ml (48h); and 2.5X10³ (72h) are acceptable.

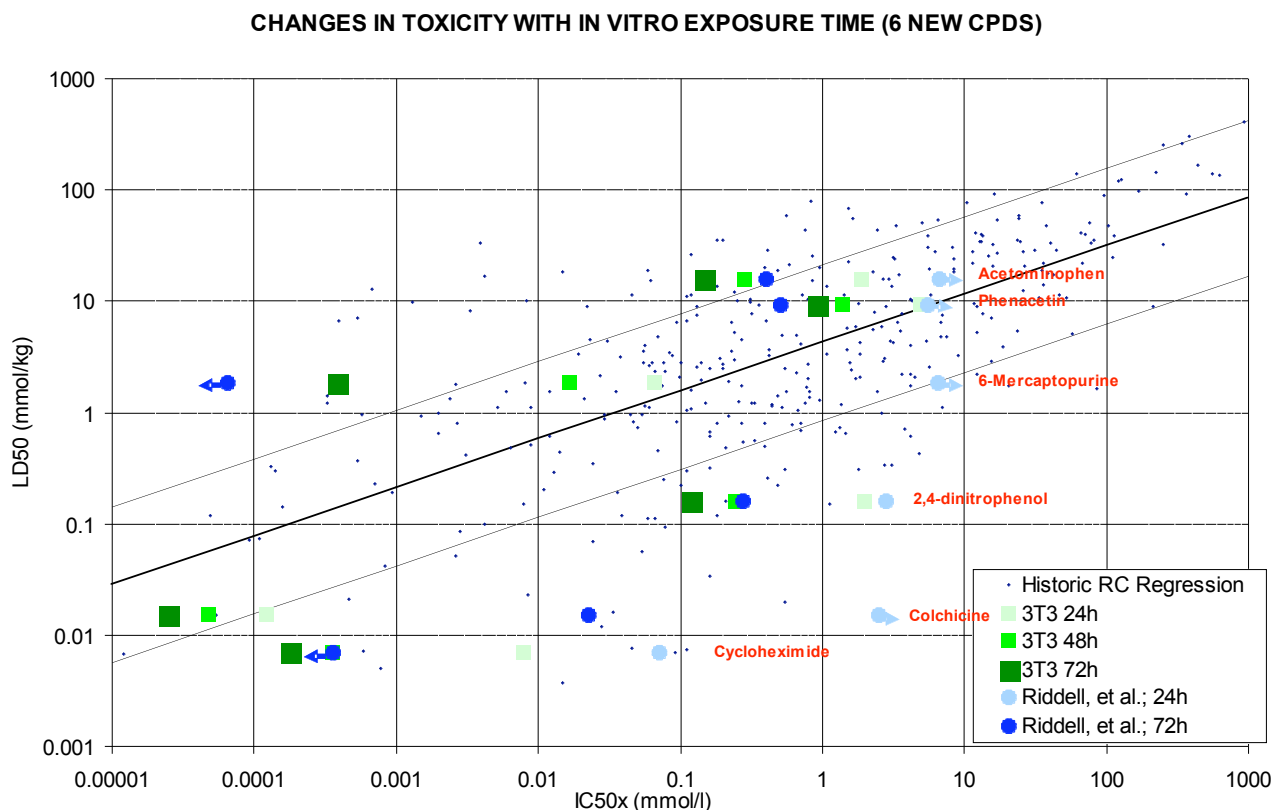
III. Exposure Duration

The exposure question was first raised by Richard Clothier who indicated that a paper by Riddell, et al. showed a number of chemicals whose toxicity changed greatly between a 24 h and a 72 h exposure (for 25/50 materials there was little change and for 25/50 materials there was a change). We examined the paper and chose to investigate six chemicals that showed some of the largest differences between 24h and 72h.

Our initial studies gave similar results to those of Riddell et al. However we felt that the cell number for the longer exposures was not optimal, and we conducted additional studies to determine a standard seeding density for each exposure period. Using this methodology we looked at the 6 materials in a standardized fashion at 24, 48 and 72h.

Our results are shown in Fig. 3.

Figure 3.



In this figure the historic Halle, et al. data are shown as small blue dots and the regression line as a dark black line. To add perspective we have included the Riddell, et al. data as a light blue diamond (24h) or a dark blue diamond (72h). Arrows emerging from certain points indicate that the value is less than or greater than that point. Our values are graphed in increasing shades of green from light (24h) to dark (72h). All green values are averages of at least two separate experiments. It appears that our data are somewhat different than Riddell, et al., i.e. most differences are not as great as originally seen. Nonetheless the values, as expected, do become more toxic with increased exposure time. We feel that 48 hrs is probably the optimal time for these data if the Halle regression is considered some type of a standard.

Next we asked whether a 48 h exposure time would affect our earlier results with the 11 chemicals presented in the Guidance Document. If these numbers were changed significantly, this might cause us to make significant modification to our guidance.

To assess the effect of increasing exposure time on the 11 chemicals, we tested them with exposure times of 24h, 48h and 72h as shown in Fig. 4.

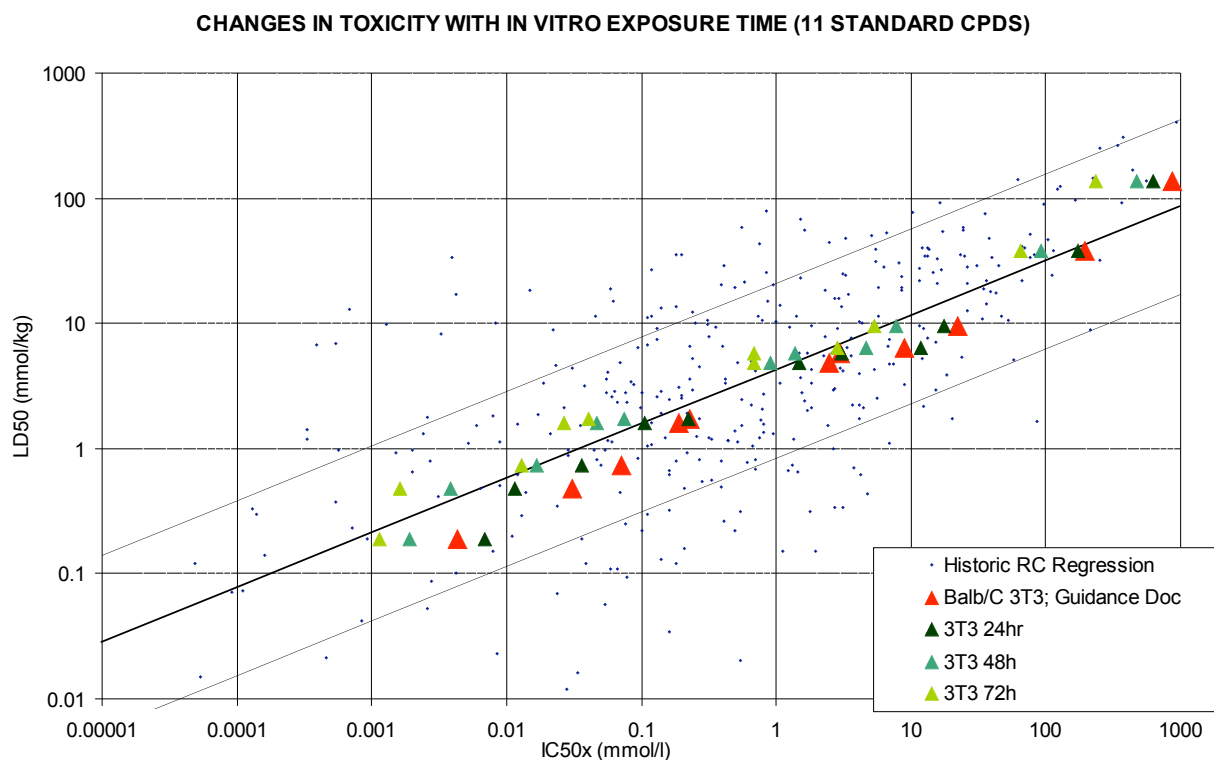
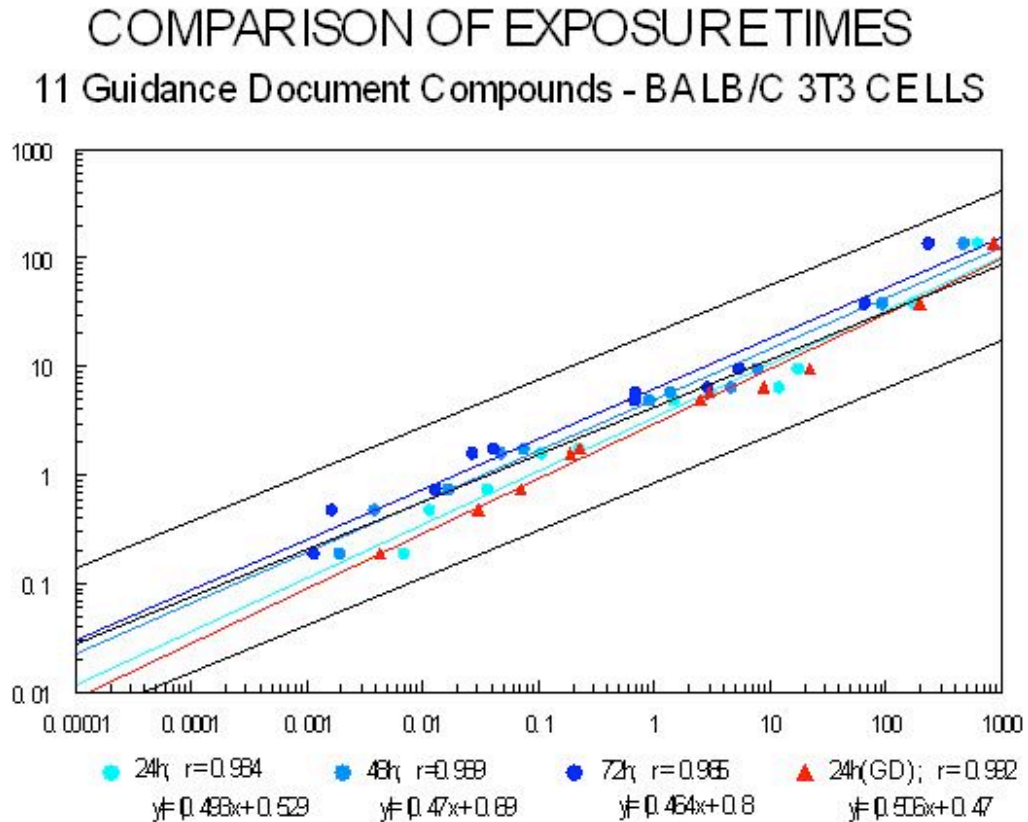


Figure 4.

The data shown on the graph are averages of duplicate experiments. It can be seen that although each of the chemicals becomes more toxic with increased exposure, all points are still within the 0.5 log range of the regression line. It again appears that 48 h exposure fits the regression more closely, however we regraphed the data in Fig. 5 to show the regression line and statistics for each of the new sets of data.

Figure 5.



In this figure it can be seen that all the regression lines for the 3 new time points plus the Guidance Document data (red triangles) fall within the regression boundaries. It again appears that the 48 hour values best fit the original regression line.

We now feel that for the 3T3 cells an extended exposure period (>24h) should be used, and that 48h seems to help identify the more toxic compounds while not over estimating the less toxic ones.

Appendix E2

Neutral Red (NR) Dye Experiments – 3T3 Cells – IIVS

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Test Facility : IIVS
 Chemical Code : N/A
 2nd Chem. Code* : NRU

Study Number: R&D - NR Stain Time Course in 3T3
 96-Well Plate ID : 1
 Experiment ID : RD96023T

96-WELL PLATE MAP

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|-------|-------|-------|-------|-------|-------|-------|--------|--------|--------|--------|-------|
| A | Blank | Blank | Blank | Blank | Blank | Blank | Blank | Blank | Blank | Blank | Blank | Blank |
| B | Blank | 3 hr | 3 hr | 2 hr | 2 hr | 1 hr | 1 hr | 30 min | 30 min | 15 min | 15 min | Blank |
| C | Blank | | | | | | | | | | | |
| D | Blank | | | | | | | | | | | |
| E | Blank | | | | | | | | | | | |
| F | Blank | | | | | | | | | | | |
| G | Blank | | | | | | | | | | | |
| H | Blank | Blank | Blank | Blank | Blank | Blank | Blank | Blank | Blank | Blank | Blank | |

RAW ABSORBANCE DATA (OD₅₅₀)

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| A | 0.048 | 0.046 | 0.045 | 0.047 | 0.047 | 0.046 | 0.046 | 0.044 | 0.044 | 0.043 | 0.044 | 0.038 |
| B | 0.048 | 0.753 | 0.794 | 0.595 | 0.607 | 0.415 | 0.396 | 0.267 | 0.282 | 0.219 | 0.213 | 0.039 |
| C | 0.047 | 0.866 | 0.766 | 0.668 | 0.668 | 0.406 | 0.391 | 0.257 | 0.256 | 0.227 | 0.220 | 0.038 |
| D | 0.046 | 0.844 | 0.794 | 0.607 | 0.622 | 0.393 | 0.387 | 0.228 | 0.262 | 0.213 | 0.217 | 0.038 |
| E | 0.046 | 0.717 | 0.805 | 0.627 | 0.610 | 0.384 | 0.375 | 0.239 | 0.266 | 0.210 | 0.206 | 0.038 |
| F | 0.044 | 0.776 | 0.769 | 0.618 | 0.665 | 0.378 | 0.398 | 0.277 | 0.301 | 0.186 | 0.202 | 0.038 |
| G | 0.043 | 0.717 | 0.807 | 0.639 | 0.616 | 0.385 | 0.349 | 0.265 | 0.269 | 0.211 | 0.195 | 0.036 |
| H | 0.044 | 0.044 | 0.045 | 0.044 | 0.045 | 0.045 | 0.043 | 0.043 | 0.045 | 0.045 | 0.041 | 0.036 |

CORRECTED ABSORBANCE (Sample OD₅₅₀ - Mean Blank OD₅₅₀)

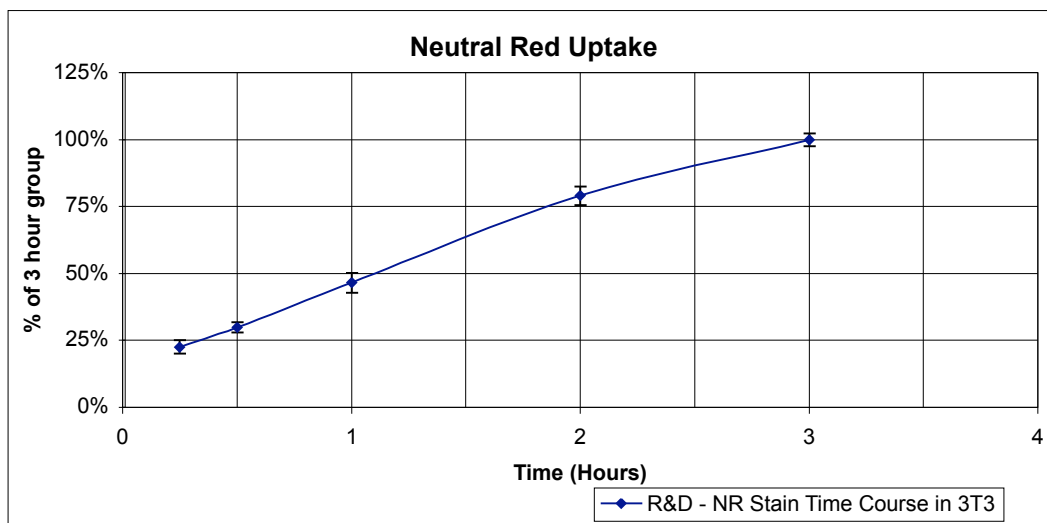
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|--------|--------|
| A | 0.005 | 0.003 | 0.002 | 0.004 | 0.004 | 0.003 | 0.003 | 0.001 | 0.001 | 0.000 | 0.001 | -0.005 |
| B | 0.005 | 0.710 | 0.751 | 0.552 | 0.564 | 0.372 | 0.353 | 0.224 | 0.239 | 0.176 | 0.170 | -0.004 |
| C | 0.004 | 0.823 | 0.723 | 0.625 | 0.625 | 0.363 | 0.348 | 0.214 | 0.213 | 0.184 | 0.177 | -0.005 |
| D | 0.003 | 0.801 | 0.751 | 0.564 | 0.579 | 0.350 | 0.344 | 0.185 | 0.219 | 0.170 | 0.174 | -0.005 |
| E | 0.003 | 0.674 | 0.762 | 0.584 | 0.567 | 0.341 | 0.332 | 0.196 | 0.223 | 0.167 | 0.163 | -0.005 |
| F | 0.001 | 0.733 | 0.726 | 0.575 | 0.622 | 0.335 | 0.355 | 0.234 | 0.258 | 0.143 | 0.159 | -0.005 |
| G | 0.000 | 0.674 | 0.764 | 0.596 | 0.573 | 0.342 | 0.306 | 0.222 | 0.226 | 0.168 | 0.152 | -0.007 |
| H | 0.001 | 0.000 | 0.002 | 0.001 | 0.002 | 0.002 | 0.000 | 0.000 | 0.002 | 0.002 | -0.002 | -0.007 |

Mean Blank = 0.043

RELATIVE VIABILITY (% OF VEHICLE CONTROL)

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|--------|--------|-------|-------|-------|-------|-------|-------|-------|-------|----|
| A | | | | | | | | | | | | |
| B | | 95.8% | 101.4% | 74.5% | 76.1% | 50.2% | 47.6% | 30.2% | 32.2% | 23.7% | 22.9% | |
| C | | 111.1% | 97.6% | 84.3% | 84.3% | 49.0% | 46.9% | 28.9% | 28.7% | 24.8% | 23.9% | |
| D | | 108.1% | 101.4% | 76.1% | 78.1% | 47.2% | 46.4% | 24.9% | 29.5% | 22.9% | 23.5% | |
| E | | 91.0% | 102.8% | 78.8% | 76.5% | 46.0% | 44.8% | 26.4% | 30.1% | 22.5% | 22.0% | |
| F | | 98.9% | 98.0% | 77.6% | 83.9% | 45.2% | 47.9% | 31.6% | 34.8% | 19.3% | 21.4% | |
| G | | 91.0% | 103.1% | 80.4% | 77.3% | 46.1% | 41.3% | 29.9% | 30.5% | 22.6% | 20.5% | |
| H | | | | | | | | | | | | |

| | 3 hr | 3 hr | 2 hr | 2 hr | 1 hr | 1 hr | 30 min | 30 min | 15 min | 15 min |
|----------------|-------|--------|--------|-------|-------|-------|--------|--------|--------|--------|
| Conc. (µg/mL): | | | | | | | | | | |
| Mean Corr. OD: | 0.736 | 0.746 | 0.582 | 0.588 | 0.350 | 0.339 | 0.212 | 0.229 | 0.168 | 0.166 |
| SD: | 0.064 | 0.018 | 0.026 | 0.028 | 0.014 | 0.018 | 0.019 | 0.016 | 0.014 | 0.010 |
| Mean 3 hour: | 0.741 | | | | | | | | | |
| Mean Blank: | 0.043 | | | | | | | | | |
| % of 3 hour: | 99.3% | 100.7% | 78.6% | 79.4% | 47.3% | 45.8% | 28.6% | 31.0% | 22.6% | 22.3% |
| SD: | 8.6% | 2.4% | 3.5% | 3.7% | 1.9% | 2.5% | 2.5% | 2.2% | 1.9% | 1.3% |
| % CV: | 8.63% | 2.37% | 4.42% | 4.72% | 4.08% | 5.42% | 8.73% | 7.14% | 8.22% | 5.76% |
| hours | | | 3 | 2 | 1 | 0.50 | 0.25 | | | |
| % of 3 hour: | | | 100.0% | 79.0% | 46.5% | 29.8% | 22.5% | | | |



Neutral Red Stain Prepared in DMEM5%NCS - TEST OF NR PREP 1 DAY PRIOR TO USE
 Tested in 90-100% Confluent 3T3 Cultures

96-WELL PLATE MAP

| | | | | | | | | | | | | |
|---|-------|-----------------------|-------|-------|---------------------|-------|-------|-------|---------------------|-------|-------|-------|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| A | Blank | Blank | Blank | Blank | Blank | Blank | Blank | Blank | Blank | Blank | Blank | Blank |
| B | Blank | 50 ug/ml | | | 50 ug/ml | | | | 33 ug/ml | | | Blank |
| C | Blank | Prepared and filtered | | | Filtered before use | | | | Filtered before use | | | Blank |
| D | Blank | in evening before use | | | | | | | | | | Blank |
| E | Blank | Filtered before use | | | | | | | | | | Blank |
| F | Blank | | | | | | | | | | | Blank |
| G | Blank | | | | | | | | | | | Blank |
| H | Blank | Blank | Blank | Blank | Blank | Blank | Blank | Blank | Blank | Blank | Blank | Blank |

RAW ABSORBANCE DATA (OD₅₅₀)

| | | | | | | | | | | | | |
|---|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| A | 0.045 | 0.045 | 0.045 | 0.044 | 0.056 | 0.056 | 0.056 | 0.057 | 0.053 | 0.051 | 0.051 | 0.052 |
| B | 0.043 | 0.383 | 0.459 | 0.417 | 0.541 | 0.631 | 0.639 | 0.635 | 0.637 | 0.686 | 0.656 | 0.052 |
| C | 0.045 | 0.389 | 0.397 | 0.379 | 0.557 | 0.536 | 0.621 | 0.559 | 0.590 | 0.618 | 0.612 | 0.051 |
| D | 0.043 | 0.383 | 0.429 | 0.350 | 0.539 | 0.575 | 0.545 | 0.629 | 0.613 | 0.658 | 0.652 | 0.053 |
| E | 0.042 | 0.361 | 0.345 | 0.334 | 0.579 | 0.585 | 0.577 | 0.573 | 0.626 | 0.635 | 0.599 | 0.051 |
| F | 0.044 | 0.368 | 0.412 | 0.374 | 0.582 | 0.588 | 0.578 | 0.572 | 0.687 | 0.647 | 0.641 | 0.050 |
| G | 0.042 | 0.415 | 0.451 | 0.422 | 0.600 | 0.620 | 0.616 | 0.632 | 0.572 | 0.744 | 0.637 | 0.050 |
| H | 0.044 | 0.042 | 0.043 | 0.043 | 0.057 | 0.059 | 0.055 | 0.057 | 0.050 | 0.057 | 0.050 | 0.054 |

CORRECTED ABSORBANCE (Sample OD₅₅₀ - Mean Blank OD₅₅₀)

| | | | | | | | | | | | | |
|---|--------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| A | 0.002 | 0.002 | 0.002 | 0.001 | 0.013 | 0.013 | 0.013 | 0.014 | 0.010 | 0.008 | 0.008 | 0.009 |
| B | 0.000 | 0.340 | 0.416 | 0.374 | 0.498 | 0.588 | 0.596 | 0.592 | 0.594 | 0.643 | 0.613 | 0.009 |
| C | 0.002 | 0.346 | 0.354 | 0.336 | 0.514 | 0.493 | 0.578 | 0.516 | 0.547 | 0.575 | 0.569 | 0.008 |
| D | 0.000 | 0.340 | 0.386 | 0.307 | 0.496 | 0.532 | 0.502 | 0.586 | 0.570 | 0.615 | 0.609 | 0.010 |
| E | -0.001 | 0.318 | 0.302 | 0.291 | 0.536 | 0.542 | 0.534 | 0.530 | 0.583 | 0.592 | 0.556 | 0.008 |
| F | 0.001 | 0.325 | 0.369 | 0.331 | 0.539 | 0.545 | 0.535 | 0.529 | 0.644 | 0.604 | 0.598 | 0.007 |
| G | -0.001 | 0.372 | 0.408 | 0.379 | 0.557 | 0.577 | 0.573 | 0.589 | 0.529 | 0.701 | 0.594 | 0.007 |
| H | 0.001 | 0.000 | 0.000 | 0.000 | 0.014 | 0.016 | 0.012 | 0.014 | 0.007 | 0.014 | 0.007 | 0.011 |

Mean Blank = 0.052 (Only the 14 wells from the 33 ug/ml group)

| | | Neutral Red Stain Concentration | | | | | | | | | | |
|---------------------|--|---------------------------------|-------|-------|-------|-------|-------|-------|-------|-------|-------|--|
| Conc. (µg/mL): | | 50.0 | | | | 50.0 | | | | 33.0 | | |
| Mean Corr. OD: | | 0.340 | 0.372 | 0.336 | 0.523 | 0.546 | 0.553 | 0.557 | 0.578 | 0.621 | 0.590 | |
| SD: | | 0.019 | 0.042 | 0.035 | 0.025 | 0.034 | 0.035 | 0.035 | 0.040 | 0.045 | 0.023 | |
| Group mean corr OD: | | 0.349 | | | | 0.545 | | | | 0.596 | | |

Note: Significant crystal formation was observed in the DMEM5%NCS/NR prepared 1 day prior, and the color was essentially medium-colored. Much NR stain stripped out of solution. No ppt or crystallization observed in the wells during the NR loading of cells.

Neutral Red Stain Prepared in DMEM5%NCS/Filtered immediately before use
 Tested in 90-100% Confluent 3T3 Cultures

96-WELL PLATE MAP

| | | | | | | | | | | | | |
|---|-------|----------|----------|----------|----------|----------|----------|---------|---------|---------|---------|-------|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| A | Blank | Blank | Blank | Blank | Blank | Blank | Blank | Blank | Blank | Blank | Blank | Empty |
| B | Blank | 50 ug/ml | 50 ug/ml | 28 ug/ml | 28 ug/ml | 16 ug/ml | 16 ug/ml | 9 ug/ml | 9 ug/ml | 5 ug/ml | 5 ug/ml | |
| C | Blank | | | | | | | | | | | |
| D | Blank | | | | | | | | | | | |
| E | Blank | | | | | | | | | | | |
| F | Blank | | | | | | | | | | | |
| G | Blank | | | | | | | | | | | |
| H | Blank | Blank | Blank | Blank | Blank | Blank | Blank | Blank | Blank | Blank | Blank | |

RAW ABSORBANCE DATA (OD₅₅₀)

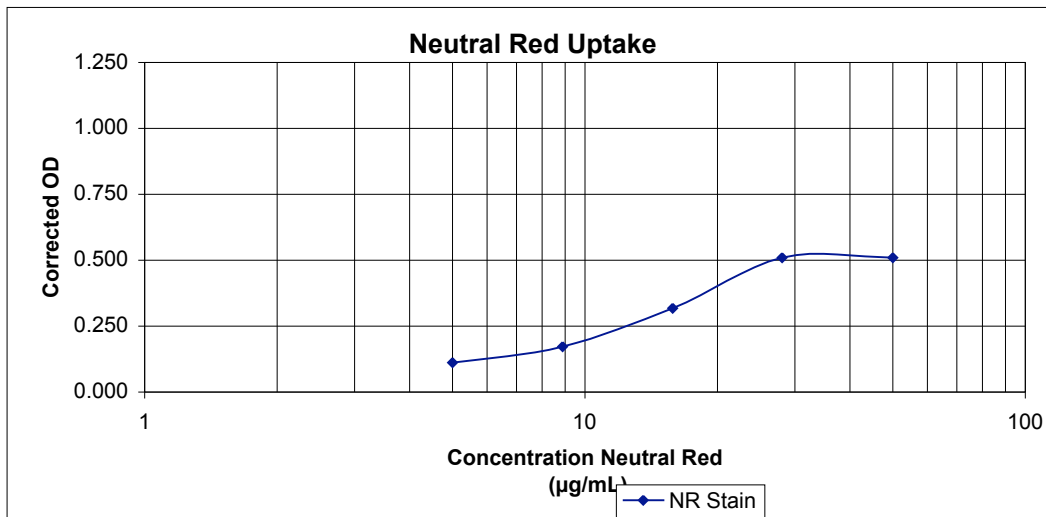
| | | | | | | | | | | | | |
|---|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| A | 0.076 | 0.051 | 0.05 | 0.045 | 0.044 | 0.041 | 0.041 | 0.041 | 0.039 | 0.038 | 0.037 | 0.037 |
| B | 0.058 | 0.553 | 0.535 | 0.58 | 0.587 | 0.421 | 0.353 | 0.225 | 0.221 | 0.149 | 0.145 | 0.037 |
| C | 0.053 | 0.561 | 0.503 | 0.517 | 0.549 | 0.338 | 0.345 | 0.213 | 0.203 | 0.144 | 0.155 | 0.035 |
| D | 0.048 | 0.493 | 0.527 | 0.489 | 0.495 | 0.351 | 0.331 | 0.196 | 0.196 | 0.143 | 0.161 | 0.038 |
| E | 0.047 | 0.491 | 0.497 | 0.528 | 0.571 | 0.312 | 0.321 | 0.188 | 0.195 | 0.132 | 0.172 | 0.038 |
| F | 0.073 | 0.606 | 0.697 | 0.53 | 0.6 | 0.36 | 0.373 | 0.239 | 0.218 | 0.143 | 0.163 | 0.036 |
| G | 0.072 | 0.63 | 0.497 | 0.563 | 0.592 | 0.399 | 0.39 | 0.235 | 0.21 | 0.145 | 0.157 | 0.037 |
| H | 0.056 | 0.089 | 0.055 | 0.043 | 0.045 | 0.041 | 0.04 | 0.039 | 0.039 | 0.042 | 0.04 | 0.036 |

CORRECTED ABSORBANCE (Sample OD₅₅₀ - Mean Blank OD₅₅₀)

| | | | | | | | | | | | | |
|---|-------|-------|-------|-------|-------|--------|--------|--------|--------|--------|--------|--------|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| A | 0.033 | 0.008 | 0.007 | 0.002 | 0.001 | -0.002 | -0.002 | -0.002 | -0.004 | -0.005 | -0.006 | -0.006 |
| B | 0.015 | 0.510 | 0.492 | 0.537 | 0.544 | 0.378 | 0.310 | 0.182 | 0.178 | 0.106 | 0.102 | -0.006 |
| C | 0.010 | 0.518 | 0.460 | 0.474 | 0.506 | 0.295 | 0.302 | 0.170 | 0.160 | 0.101 | 0.112 | -0.008 |
| D | 0.005 | 0.450 | 0.484 | 0.446 | 0.452 | 0.308 | 0.288 | 0.153 | 0.153 | 0.100 | 0.118 | -0.005 |
| E | 0.004 | 0.448 | 0.454 | 0.485 | 0.528 | 0.269 | 0.278 | 0.145 | 0.152 | 0.089 | 0.129 | -0.005 |
| F | 0.030 | 0.563 | 0.654 | 0.487 | 0.557 | 0.317 | 0.330 | 0.196 | 0.175 | 0.100 | 0.120 | -0.007 |
| G | 0.029 | 0.587 | 0.454 | 0.520 | 0.549 | 0.356 | 0.347 | 0.192 | 0.167 | 0.102 | 0.114 | -0.006 |
| H | 0.013 | 0.000 | 0.012 | 0.000 | 0.002 | -0.002 | -0.003 | -0.004 | -0.004 | -0.001 | -0.003 | -0.007 |

Mean Blank = 0.039 (Only the 4 wells from the 5.0 ug/ml group)

| | | Neutral Red Stain Concentration | | | | | | | | | | |
|---------------------|-------|---------------------------------|-------|-------|-------|-------|-------|-------|-------|-------|-------|--|
| Conc. (µg/mL): | | 50.0 | 50.0 | 28.0 | 28.0 | 15.8 | 15.8 | 8.9 | 8.9 | 5.0 | 5.0 | |
| Mean Corr. OD: | | 0.512 | 0.499 | 0.491 | 0.522 | 0.320 | 0.309 | 0.173 | 0.164 | 0.099 | 0.116 | |
| SD: | | 0.057 | 0.077 | 0.033 | 0.039 | 0.040 | 0.026 | 0.021 | 0.011 | 0.006 | 0.009 | |
| Group mean corr OD: | | 0.506 | | 0.507 | | 0.315 | | 0.168 | | 0.107 | | |
| | | | | | | | | | | | | |
| | graph | x | 50.0 | 28.0 | 15.8 | 8.9 | 5.0 | | | | | |
| | | y | 0.506 | 0.507 | 0.315 | 0.168 | 0.107 | | | | | |



Appendix E3

Neutral Red (NR) Dye Experiments – NHK Cells – IIVS

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Test Facility : IIVS
 Chemical Code : N/A
 2nd Chem. Code*: NRU

Study Number.: R&D - NR Stain Time Course in NHK
 96-Well Plate ID : 1
 Experiment ID : RD9602NK

96-WELL PLATE MAP

| | | | | | | | | | | | | |
|---|-------|-------|-------|-------|-------|-------|-------|--------|--------|--------|--------|-------|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| A | Blank | Blank | Blank | Blank | Blank | Blank | Blank | Blank | Blank | Blank | Blank | Blank |
| B | Blank | | | | | | | | | | | Blank |
| C | Blank | | | | | | | | | | | Blank |
| D | Blank | 3 hr | 3 hr | 2 hr | 2 hr | 1 hr | 1 hr | 30 min | 30 min | 15 min | 15 min | Blank |
| E | Blank | | | | | | | | | | | Blank |
| F | Blank | | | | | | | | | | | Blank |
| G | Blank | | | | | | | | | | | Blank |
| H | Blank | Blank | Blank | Blank | Blank | Blank | Blank | Blank | Blank | Blank | Blank | Blank |

RAW ABSORBANCE DATA (OD₅₅₀)

| | | | | | | | | | | | | |
|---|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| A | 0.057 | 0.053 | 0.059 | 0.058 | 0.054 | 0.055 | 0.053 | 0.052 | 0.051 | 0.048 | 0.049 | 0.035 |
| B | 0.068 | 1.501 | 1.564 | 1.311 | 1.327 | 0.998 | 1.052 | 0.671 | 0.649 | 0.438 | 0.474 | 0.037 |
| C | 0.057 | 1.549 | 1.482 | 1.376 | 1.372 | 1.082 | 1.076 | 0.714 | 0.697 | 0.494 | 0.474 | 0.034 |
| D | 0.058 | 1.540 | 1.503 | 1.415 | 1.422 | 1.026 | 0.995 | 0.724 | 0.698 | 0.482 | 0.474 | 0.036 |
| E | 0.057 | 1.553 | 1.532 | 1.388 | 1.453 | 1.060 | 1.010 | 0.675 | 0.634 | 0.459 | 0.462 | 0.034 |
| F | 0.057 | 1.632 | 1.600 | 1.396 | 1.380 | 1.066 | 1.074 | 0.656 | 0.628 | 0.470 | 0.429 | 0.033 |
| G | 0.054 | 1.462 | 1.514 | 1.357 | 1.439 | 1.069 | 1.010 | 0.708 | 0.606 | 0.474 | 0.437 | 0.035 |
| H | 0.057 | 0.054 | 0.053 | 0.052 | 0.051 | 0.055 | 0.051 | 0.049 | 0.047 | 0.050 | 0.046 | 0.034 |

CORRECTED ABSORBANCE (Sample OD₅₅₀ - Mean Blank OD₅₅₀)

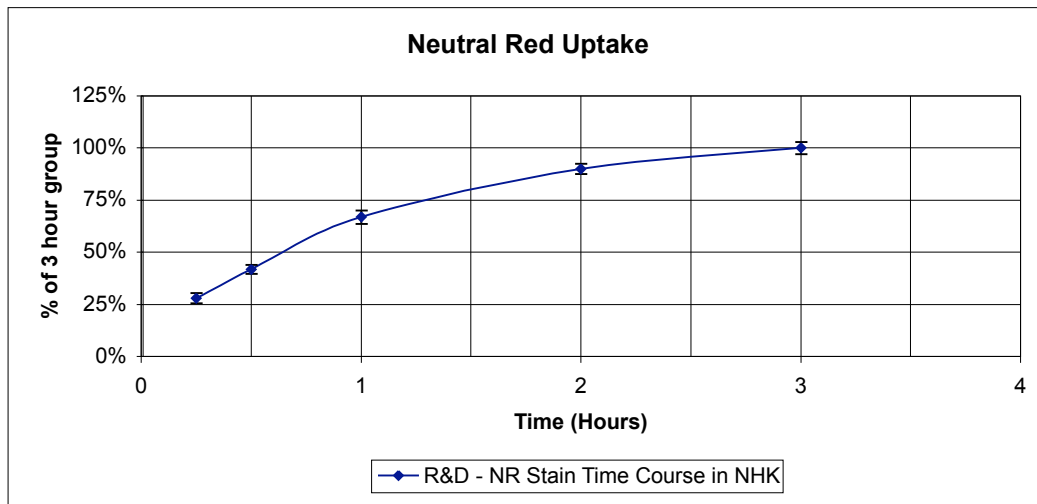
| | | | | | | | | | | | | |
|---|-------|-------|-------|-------|-------|-------|-------|--------|--------|--------|--------|--------|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| A | 0.007 | 0.003 | 0.009 | 0.008 | 0.004 | 0.005 | 0.003 | 0.002 | 0.001 | -0.002 | -0.001 | -0.015 |
| B | 0.018 | 1.451 | 1.514 | 1.261 | 1.277 | 0.948 | 1.002 | 0.621 | 0.599 | 0.388 | 0.424 | -0.013 |
| C | 0.007 | 1.499 | 1.432 | 1.326 | 1.322 | 1.032 | 1.026 | 0.664 | 0.647 | 0.444 | 0.424 | -0.016 |
| D | 0.008 | 1.490 | 1.453 | 1.365 | 1.372 | 0.976 | 0.945 | 0.674 | 0.648 | 0.432 | 0.424 | -0.014 |
| E | 0.007 | 1.503 | 1.482 | 1.338 | 1.403 | 1.010 | 0.960 | 0.625 | 0.584 | 0.409 | 0.412 | -0.016 |
| F | 0.007 | 1.582 | 1.550 | 1.346 | 1.430 | 1.016 | 1.024 | 0.606 | 0.578 | 0.420 | 0.379 | -0.017 |
| G | 0.004 | 1.412 | 1.464 | 1.307 | 1.389 | 1.019 | 0.960 | 0.658 | 0.556 | 0.424 | 0.387 | -0.015 |
| H | 0.007 | 0.000 | 0.003 | 0.002 | 0.001 | 0.005 | 0.001 | -0.001 | -0.003 | 0.000 | -0.004 | -0.016 |

Mean Blank = 0.050

RELATIVE VIABILITY (% OF VEHICLE CONTROL)

| | | | | | | | | | | | | |
|---|---|--------|--------|-------|-------|-------|-------|-------|-------|-------|-------|----|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| A | | | | | | | | | | | | |
| B | | 97.6% | 101.9% | 84.9% | 85.9% | 63.8% | 67.4% | 41.8% | 40.3% | 26.1% | 28.6% | |
| C | | 100.9% | 96.4% | 89.2% | 89.0% | 69.5% | 69.1% | 44.7% | 43.6% | 29.9% | 28.6% | |
| D | | 100.3% | 97.8% | 91.9% | 92.3% | 65.7% | 63.6% | 45.4% | 43.6% | 29.1% | 28.6% | |
| E | | 101.1% | 99.7% | 90.0% | 94.4% | 68.0% | 64.6% | 42.1% | 39.3% | 27.5% | 27.7% | |
| F | | 106.5% | 104.3% | 90.6% | 89.5% | 68.4% | 68.9% | 40.8% | 38.9% | 28.3% | 25.5% | |
| G | | 95.0% | 98.5% | 88.0% | 93.5% | 68.6% | 64.6% | 44.3% | 37.4% | 28.6% | 26.1% | |
| H | | | | | | | | | | | | |

| | | | | | | | | | | |
|-----------------|--------|-------|--------|-------|-------|-------|--------|--------|--------|--------|
| | 3 hr | 3 hr | 2 hr | 2 hr | 1 hr | 1 hr | 30 min | 30 min | 15 min | 15 min |
| Conc. (µg/mL) : | | | | | | | | | | |
| Mean Corr. OD : | 1.490 | 1.483 | 1.324 | 1.349 | 1.001 | 0.987 | 0.642 | 0.602 | 0.420 | 0.409 |
| SD : | 0.057 | 0.043 | 0.036 | 0.048 | 0.032 | 0.036 | 0.028 | 0.038 | 0.019 | 0.020 |
| Mean 3 hour : | 1.486 | | | | | | | | | |
| Mean Blank : | 0.050 | | | | | | | | | |
| % of 3 hour : | 100.2% | 99.8% | 89.1% | 90.8% | 67.3% | 66.4% | 43.2% | 40.5% | 28.3% | 27.5% |
| SD : | 3.8% | 2.9% | 2.4% | 3.2% | 2.1% | 2.4% | 1.9% | 2.5% | 1.3% | 1.4% |
| % CV : | 3.83% | 2.91% | 2.75% | 3.53% | 3.17% | 3.61% | 4.29% | 6.28% | 4.62% | 4.97% |
| hours | | | 3 | 2 | 1 | 0.50 | 0.25 | | | |
| % of 3 hour : | | | 100.0% | 89.9% | 66.8% | 41.9% | 27.9% | | | |



Neutral Red Stain Prepared in KGM - TEST OF NR PREP 1 DAY PRIOR TO USE
 Tested in 90-100% Confluent NHK Cultures

96-WELL PLATE MAP

| | | | | | | | | | | | |
|---|-------|--|-------|-------|---------------------------------|-------|-------|-------|---------------------------------|-------|-------|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 |
| A | Blank | Blank | Blank | Blank | Blank | Blank | Blank | Blank | Blank | Blank | Blank |
| B | Blank | 50 ug/ml Prepared and filtered in evening before use | | | 50 ug/ml Filtered before use | | | | 33 ug/ml Filtered before use | | |
| C | Blank | Prepared and filtered in evening before use | | | Filtered before use | | | | Filtered before use | | |
| D | Blank | Filtered before use | | | | | | | | | |
| E | Blank | | | | | | | | | | |
| F | Blank | | | | | | | | | | |
| G | Blank | | | | | | | | | | |
| H | Blank | Blank | Blank | Blank | Blank | Blank | Blank | Blank | Blank | Blank | Blank |

RAW ABSORBANCE DATA (OD₅₅₀)

| | | | | | | | | | | | |
|---|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 |
| A | 0.062 | 0.061 | 0.063 | 0.064 | 0.063 | 0.062 | 0.060 | 0.060 | 0.052 | 0.053 | 0.051 |
| B | 0.055 | 1.306 | 1.545 | 1.530 | 1.514 | 1.403 | 1.421 | 1.297 | 1.249 | 1.136 | 1.134 |
| C | 0.060 | 1.530 | 1.520 | 1.554 | 1.471 | 1.536 | 1.416 | 1.415 | 1.308 | 1.160 | 1.189 |
| D | 0.062 | 1.454 | 1.527 | 1.513 | 1.511 | 1.472 | 1.491 | 1.438 | 1.217 | 1.192 | 1.173 |
| E | 0.067 | 1.423 | 1.433 | 1.505 | 1.577 | 1.469 | 1.448 | 1.474 | 1.199 | 1.249 | 1.158 |
| F | 0.057 | 1.423 | 1.591 | 1.577 | 1.577 | 1.403 | 1.431 | 1.347 | 1.250 | 1.235 | 1.102 |
| G | 0.065 | 1.430 | 1.468 | 1.393 | 1.319 | 1.432 | 1.304 | 1.416 | 1.243 | 1.117 | 1.110 |
| H | 0.064 | 0.059 | 0.060 | 0.064 | 0.064 | 0.065 | 0.061 | 0.064 | 0.060 | 0.055 | 0.060 |

CORRECTED ABSORBANCE (Sample OD₅₅₀ - Mean Blank OD₅₅₀)

| | | | | | | | | | | | |
|---|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 |
| A | 0.012 | 0.011 | 0.013 | 0.014 | 0.013 | 0.012 | 0.010 | 0.010 | 0.002 | 0.003 | 0.001 |
| B | 0.005 | 1.256 | 1.495 | 1.480 | 1.464 | 1.353 | 1.371 | 1.247 | 1.199 | 1.086 | 1.084 |
| C | 0.010 | 1.480 | 1.470 | 1.504 | 1.421 | 1.486 | 1.366 | 1.365 | 1.258 | 1.110 | 1.139 |
| D | 0.012 | 1.404 | 1.477 | 1.463 | 1.461 | 1.422 | 1.441 | 1.388 | 1.167 | 1.142 | 1.123 |
| E | 0.017 | 1.373 | 1.383 | 1.455 | 1.527 | 1.419 | 1.398 | 1.424 | 1.149 | 1.199 | 1.108 |
| F | 0.007 | 1.373 | 1.541 | 1.527 | 1.527 | 1.353 | 1.381 | 1.297 | 1.200 | 1.185 | 1.052 |
| G | 0.015 | 1.380 | 1.418 | 1.343 | 1.269 | 1.382 | 1.254 | 1.366 | 1.193 | 1.067 | 1.060 |
| H | 0.014 | 0.000 | 0.010 | 0.014 | 0.014 | 0.015 | 0.011 | 0.014 | 0.010 | 0.005 | 0.010 |

Mean Blank = 0.055 (Only the 14 wells from the 33 ug/ml group)

| | | Neutral Red Stain Concentration | | | | | | | | | |
|---------------------|--|---------------------------------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| Conc. (ug/mL) : | | 50.0 | | | 50.0 | | | | 33.0 | | |
| Mean Corr. OD : | | 1.378 | 1.464 | 1.462 | 1.445 | 1.403 | 1.369 | 1.348 | 1.195 | 1.132 | 1.095 |
| SD : | | 0.072 | 0.056 | 0.064 | 0.096 | 0.051 | 0.062 | 0.064 | 0.037 | 0.053 | 0.035 |
| Group mean corr OD: | | 1.435 | | | 1.391 | | | | 1.141 | | |

Note: No crystal formation was observed in the KGM/NR prepared 1 day prior.
 No ppt or crystallization observed in the wells during the NR loading of cells.

Neutral Red Stain Prepared in KGM/Filtered immediately before use
 Tested in 90-100% Confluent NHK Cultures

96-WELL PLATE MAP

| | | | | | | | | | | | | |
|---|-------|----------|----------|----------|----------|----------|----------|---------|---------|---------|---------|-------|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| A | Blank | Blank | Blank | Blank | Blank | Blank | Blank | Blank | Blank | Blank | Blank | empty |
| B | Blank | 50 ug/ml | 50 ug/ml | 28 ug/ml | 28 ug/ml | 16 ug/ml | 16 ug/ml | 9 ug/ml | 9 ug/ml | 5 ug/ml | 5 ug/ml | |
| C | Blank | | | | | | | | | | | |
| D | Blank | | | | | | | | | | | |
| E | Blank | | | | | | | | | | | |
| F | Blank | | | | | | | | | | | |
| G | Blank | | | | | | | | | | | |
| H | Blank | Blank | Blank | Blank | Blank | Blank | Blank | Blank | Blank | Blank | Blank | |

RAW ABSORBANCE DATA (OD₅₅₀)

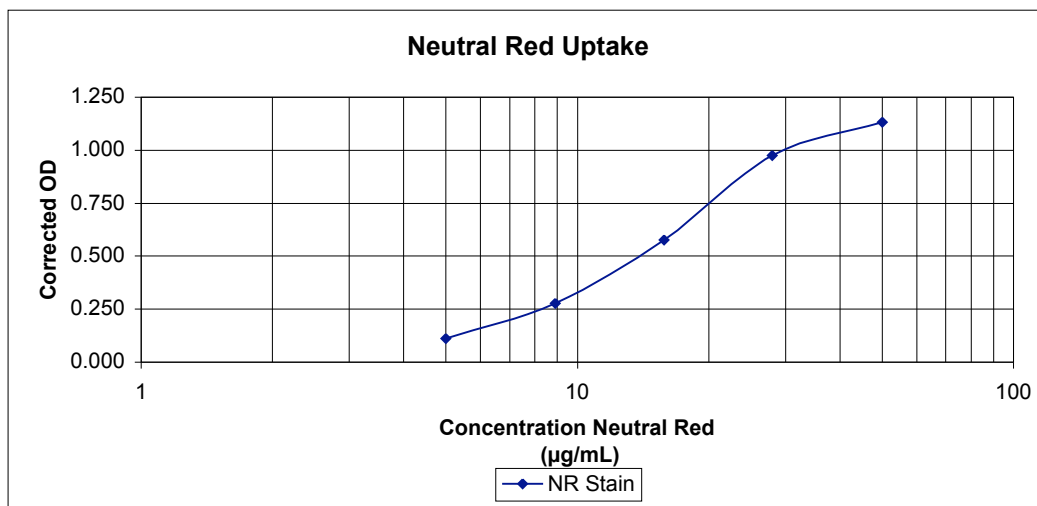
| | | | | | | | | | | | | |
|---|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| A | 0.067 | 0.064 | 0.066 | 0.049 | 0.049 | 0.040 | 0.040 | 0.038 | 0.038 | 0.036 | 0.037 | 0.035 |
| B | 0.048 | 1.255 | 1.119 | 1.103 | 1.054 | 0.623 | 0.605 | 0.325 | 0.334 | 0.156 | 0.150 | 0.034 |
| C | 0.050 | 1.035 | 1.004 | 1.020 | 0.956 | 0.624 | 0.601 | 0.345 | 0.312 | 0.151 | 0.154 | 0.034 |
| D | 0.047 | 1.131 | 1.352 | 1.094 | 1.078 | 0.643 | 0.635 | 0.331 | 0.314 | 0.157 | 0.147 | 0.035 |
| E | 0.047 | 1.117 | 1.227 | 0.923 | 0.893 | 0.595 | 0.618 | 0.323 | 0.302 | 0.155 | 0.150 | 0.035 |
| F | 0.046 | 1.245 | 1.129 | 0.976 | 0.988 | 0.607 | 0.617 | 0.308 | 0.313 | 0.156 | 0.156 | 0.035 |
| G | 0.047 | 1.136 | 1.282 | 1.061 | 0.995 | 0.624 | 0.582 | 0.283 | 0.282 | 0.131 | 0.127 | 0.037 |
| H | 0.063 | 0.056 | 0.060 | 0.061 | 0.048 | 0.042 | 0.042 | 0.038 | 0.039 | 0.040 | 0.038 | 0.036 |

CORRECTED ABSORBANCE (Sample OD₅₅₀ - Mean Blank OD₅₅₀)

| | | | | | | | | | | | | |
|---|--------|-------|-------|--------|--------|--------|--------|--------|--------|--------|--------|--------|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| A | 0.017 | 0.014 | 0.016 | -0.001 | -0.001 | -0.010 | -0.010 | -0.012 | -0.012 | -0.014 | -0.013 | -0.015 |
| B | -0.002 | 1.205 | 1.069 | 1.053 | 1.004 | 0.573 | 0.555 | 0.275 | 0.284 | 0.106 | 0.100 | -0.016 |
| C | 0.000 | 0.985 | 0.954 | 0.970 | 0.906 | 0.574 | 0.551 | 0.295 | 0.262 | 0.101 | 0.104 | -0.016 |
| D | -0.003 | 1.081 | 1.302 | 1.044 | 1.028 | 0.593 | 0.585 | 0.281 | 0.264 | 0.107 | 0.097 | -0.015 |
| E | -0.003 | 1.067 | 1.177 | 0.873 | 0.843 | 0.545 | 0.568 | 0.273 | 0.252 | 0.105 | 0.100 | -0.015 |
| F | -0.004 | 1.195 | 1.079 | 0.926 | 0.938 | 0.557 | 0.567 | 0.258 | 0.263 | 0.106 | 0.106 | -0.015 |
| G | -0.003 | 1.086 | 1.232 | 1.011 | 0.945 | 0.574 | 0.532 | 0.233 | 0.232 | 0.081 | 0.077 | -0.013 |
| H | 0.013 | 0.000 | 0.010 | 0.011 | -0.002 | -0.008 | -0.008 | -0.012 | -0.011 | -0.010 | -0.012 | -0.014 |

Mean Blank = 0.038 (Only the 4 wells from the 5.0 ug/ml group)

| Neutral Red Stain Concentration | | | | | | | | | | |
|---------------------------------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| Conc. (µg/mL): | 50.0 | 50.0 | 28.0 | 28.0 | 15.8 | 15.8 | 8.9 | 8.9 | 5.0 | 5.0 |
| Mean Corr. OD: | 1.104 | 1.136 | 0.980 | 0.944 | 0.570 | 0.560 | 0.270 | 0.260 | 0.101 | 0.098 |
| SD: | 0.083 | 0.126 | 0.070 | 0.067 | 0.017 | 0.018 | 0.021 | 0.017 | 0.010 | 0.010 |
| Group mean corr OD: | 1.120 | | 0.962 | | 0.565 | | 0.265 | | 0.100 | |
| graph | x | 50.0 | 28.0 | 15.8 | 8.9 | 5.0 | | | | |
| | y | 1.120 | 0.962 | 0.565 | 0.265 | 0.100 | | | | |



Appendix E4

Neutral Red (NR) Dye Experiments – 3T3 Cells – ECBC

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Test Facility : ECBC
 Chemical Code : SLS
 2nd Chem. Code*: none

Study Number.: ECBC-3T3 Ia 0#
 96-Well Plate ID : 090602-1
 Experiment ID : SLS-B(25ug NR/ml 1hr)

96-WELL PLATE MAP

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| A | Blank | Blank | Blank | Blank | Blank | Blank | Blank | Blank | Blank | Blank | Blank | Blank |
| B | Blank | VC1 | C1 | C2 | C3 | C4 | C5 | C6 | C7 | C8 | VC2 | Blank |
| C | Blank | VC1 | C1 | C2 | C3 | C4 | C5 | C6 | C7 | C8 | VC2 | Blank |
| D | Blank | VC1 | C1 | C2 | C3 | C4 | C5 | C6 | C7 | C8 | VC2 | Blank |
| E | Blank | VC1 | C1 | C2 | C3 | C4 | C5 | C6 | C7 | C8 | VC2 | Blank |
| F | Blank | VC1 | C1 | C2 | C3 | C4 | C5 | C6 | C7 | C8 | VC2 | Blank |
| G | Blank | VC1 | C1 | C2 | C3 | C4 | C5 | C6 | C7 | C8 | VC2 | Blank |
| H | Blank | Blank | Blank | Blank | Blank | Blank | Blank | Blank | Blank | Blank | Blank | Blank |

RAW ABSORBANCE DATA (OD₅₄₀)

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| A | 0.049 | 0.051 | 0.048 | 0.052 | 0.048 | 0.050 | 0.050 | 0.046 | 0.044 | 0.045 | 0.046 | 0.047 |
| B | 0.050 | 0.262 | 0.050 | 0.046 | 0.130 | 0.274 | 0.254 | 0.322 | 0.315 | 0.329 | 0.333 | 0.046 |
| C | 0.052 | 0.283 | 0.053 | 0.051 | 0.145 | 0.231 | 0.252 | 0.276 | 0.283 | 0.293 | 0.321 | 0.050 |
| D | 0.050 | 0.307 | 0.055 | 0.053 | 0.135 | 0.242 | 0.252 | 0.291 | 0.280 | 0.302 | 0.314 | 0.049 |
| E | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| F | 0.000 | 0.000 | 0.100 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| G | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| H | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |

CORRECTED ABSORBANCE (Sample OD₅₄₀ - Mean Blank OD₅₄₀)

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|
| A | 0.000 | 0.002 | -0.001 | 0.003 | -0.001 | 0.001 | 0.001 | -0.003 | -0.005 | -0.004 | -0.003 | -0.002 |
| B | 0.001 | 0.214 | 0.001 | -0.003 | 0.082 | 0.226 | 0.206 | 0.274 | 0.267 | 0.281 | 0.285 | -0.003 |
| C | 0.003 | 0.235 | 0.004 | 0.002 | 0.097 | 0.183 | 0.204 | 0.228 | 0.235 | 0.245 | 0.273 | 0.001 |
| D | 0.001 | 0.259 | 0.006 | 0.004 | 0.087 | 0.194 | 0.204 | 0.243 | 0.232 | 0.254 | 0.266 | 0.000 |
| E | -0.049 | -0.049 | -0.049 | -0.049 | -0.049 | -0.049 | -0.049 | -0.049 | -0.049 | -0.049 | -0.049 | -0.049 |
| F | -0.049 | -0.049 | 0.052 | -0.049 | -0.049 | -0.049 | -0.049 | -0.049 | -0.049 | -0.049 | -0.049 | -0.049 |
| G | -0.049 | -0.049 | -0.049 | -0.049 | -0.049 | -0.049 | -0.049 | -0.049 | -0.049 | -0.049 | -0.049 | -0.049 |
| H | -0.049 | 0.000 | -0.049 | -0.049 | -0.049 | -0.049 | -0.049 | -0.049 | -0.049 | -0.049 | -0.049 | -0.049 |

Mean Blank = 0.049

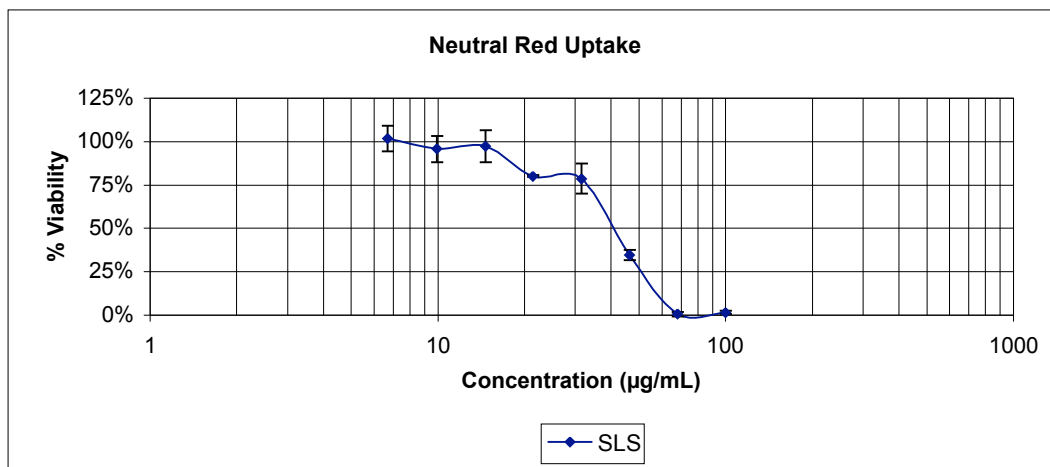
RELATIVE VIABILITY (% OF VEHICLE CONTROL)

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|----|
| A | | | | | | | | | | | | |
| B | | 83.8% | 0.6% | -1.0% | 32.0% | 88.5% | 80.6% | 107.3% | 104.6% | 110.1% | 111.6% | |
| C | | 92.0% | 1.8% | 1.0% | 37.9% | 71.6% | 79.9% | 89.3% | 92.0% | 95.9% | 106.9% | |
| D | | 101.4% | 2.6% | 1.8% | 33.9% | 75.9% | 79.9% | 95.2% | 90.8% | 99.5% | 104.2% | |
| E | | -19.0% | -19.0% | -19.0% | -19.0% | -19.0% | -19.0% | -19.0% | -19.0% | -19.0% | -19.0% | |
| F | | -19.0% | 20.2% | -19.0% | -19.0% | -19.0% | -19.0% | -19.0% | -19.0% | -19.0% | -19.0% | |
| G | | -19.0% | -19.0% | -19.0% | -19.0% | -19.0% | -19.0% | -19.0% | -19.0% | -19.0% | -19.0% | |
| H | | | | | | | | | | | | |

Test Facility : ECBC
 Chemical Code : SLS
 2nd Chem. Code*: none

Study Number.: ECBC-3T3 Ia 0#
 96-Well Plate ID : 090602-1
 Experiment ID : SLS-B(25ug NR/ml 1hr)

| | VC1 | C1 | C2 | C3 | C4 | C5 | C6 | C7 | C8 | VC2 |
|------------------------|--------|--------|---------|-------|--------|-------|-------|-------|--------|--------|
| Conc. (µg/mL) : | 0.0 | 100.0 | 68.0 | 46.3 | 31.5 | 21.4 | 14.6 | 9.9 | 6.7 | 0.0 |
| Mean Corr. OD : | 0.236 | 0.004 | 0.001 | 0.088 | 0.201 | 0.204 | 0.248 | 0.244 | 0.260 | 0.274 |
| SD : | 0.023 | 0.003 | 0.004 | 0.008 | 0.022 | 0.001 | 0.023 | 0.019 | 0.019 | 0.010 |
| Mean Vehicle Control : | 0.255 | | | | | | | | | |
| Mean Blank : | 0.049 | | | | | | | | | |
| % of Vehicle Control : | 92.4% | 1.6% | 0.6% | 34.6% | 78.7% | 80.1% | 97.3% | 95.8% | 101.8% | 107.6% |
| SD : | 8.8% | 1.0% | 1.4% | 3.0% | 8.8% | 0.5% | 9.2% | 7.6% | 7.4% | 3.8% |
| % CV : | 9.56% | 60.40% | 240.37% | 8.66% | 11.14% | 0.57% | 9.47% | 7.95% | 7.22% | 3.50% |
| Mean VC - VC1 (%) : | 7.59% | | | | | | | | | |
| Mean VC - VC2 (%) : | -7.59% | | | | | | | | | |
| Mean Absolute OD : | 0.303 | | | | | | | | | |



Test Facility : ECBC
 Chemical Code : SLS
 2nd Chem. Code* : none

Study Number.: ECBC-3T3 Ia 0#
 96-Well Plate ID : 090602-2
 Experiment ID : SLS-B(50ug NR/ml 1hr)

96-WELL PLATE MAP

| | | | | | | | | | | | | |
|---|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| A | Blank | Blank | Blank | Blank | Blank | Blank | Blank | Blank | Blank | Blank | Blank | Blank |
| B | Blank | VC1 | C1 | C2 | C3 | C4 | C5 | C6 | C7 | C8 | VC2 | Blank |
| C | Blank | VC1 | C1 | C2 | C3 | C4 | C5 | C6 | C7 | C8 | VC2 | Blank |
| D | Blank | VC1 | C1 | C2 | C3 | C4 | C5 | C6 | C7 | C8 | VC2 | Blank |
| E | Blank | VC1 | C1 | C2 | C3 | C4 | C5 | C6 | C7 | C8 | VC2 | Blank |
| F | Blank | VC1 | C1 | C2 | C3 | C4 | C5 | C6 | C7 | C8 | VC2 | Blank |
| G | Blank | VC1 | C1 | C2 | C3 | C4 | C5 | C6 | C7 | C8 | VC2 | Blank |
| H | Blank | Blank | Blank | Blank | Blank | Blank | Blank | Blank | Blank | Blank | Blank | Blank |

RAW ABSORBANCE DATA (OD₅₄₀)

| | | | | | | | | | | | | |
|---|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| A | 0.056 | 0.061 | 0.063 | 0.055 | 0.052 | 0.051 | 0.058 | 0.050 | 0.050 | 0.052 | 0.050 | 0.051 |
| B | 0.088 | 0.377 | 0.057 | 0.053 | 0.192 | 0.315 | 0.325 | 0.364 | 0.402 | 0.403 | 0.396 | 0.053 |
| C | 0.058 | 0.378 | 0.062 | 0.058 | 0.158 | 0.277 | 0.337 | 0.379 | 0.400 | 0.391 | 0.386 | 0.051 |
| D | 0.061 | 0.373 | 0.054 | 0.051 | 0.182 | 0.308 | 0.343 | 0.367 | 0.425 | 0.420 | 0.409 | 0.050 |
| E | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| F | 0.000 | 0.000 | 0.100 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| G | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| H | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |

CORRECTED ABSORBANCE (Sample OD₅₄₀ - Mean Blank OD₅₄₀)

| | | | | | | | | | | | | |
|---|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| A | 0.007 | 0.013 | 0.015 | 0.006 | 0.003 | 0.002 | 0.009 | 0.001 | 0.001 | 0.003 | 0.001 | 0.002 |
| B | 0.040 | 0.329 | 0.008 | 0.004 | 0.144 | 0.267 | 0.277 | 0.316 | 0.354 | 0.355 | 0.348 | 0.004 |
| C | 0.009 | 0.330 | 0.014 | 0.009 | 0.110 | 0.229 | 0.289 | 0.331 | 0.352 | 0.343 | 0.338 | 0.002 |
| D | 0.013 | 0.325 | 0.005 | 0.002 | 0.134 | 0.260 | 0.295 | 0.319 | 0.377 | 0.372 | 0.361 | 0.001 |
| E | -0.049 | -0.049 | -0.049 | -0.049 | -0.049 | -0.049 | -0.049 | -0.049 | -0.049 | -0.049 | -0.049 | -0.049 |
| F | -0.049 | -0.049 | 0.052 | -0.049 | -0.049 | -0.049 | -0.049 | -0.049 | -0.049 | -0.049 | -0.049 | -0.049 |
| G | -0.049 | -0.049 | -0.049 | -0.049 | -0.049 | -0.049 | -0.049 | -0.049 | -0.049 | -0.049 | -0.049 | -0.049 |
| H | -0.049 | 0.000 | -0.049 | -0.049 | -0.049 | -0.049 | -0.049 | -0.049 | -0.049 | -0.049 | -0.049 | -0.049 |

Mean Blank = 0.056

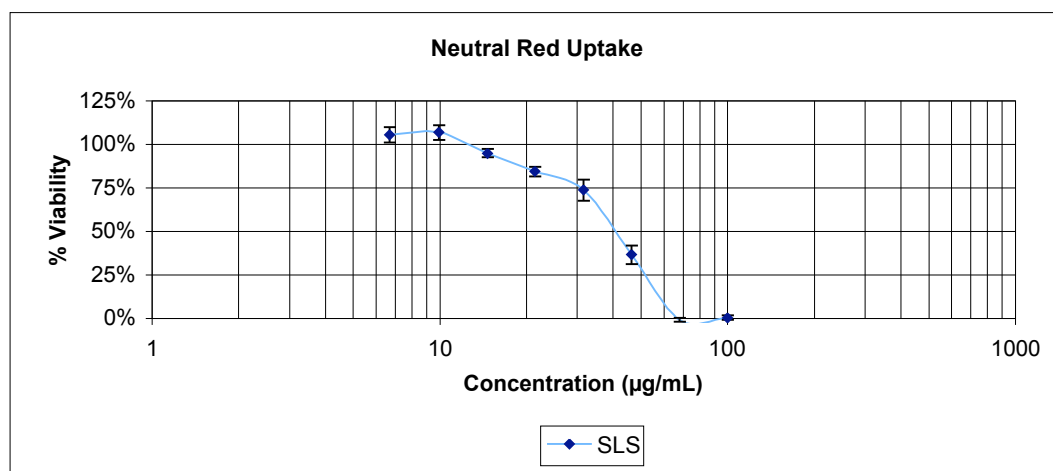
RELATIVE VIABILITY (% OF VEHICLE CONTROL)

| | | | | | | | | | | | | |
|---|---|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|----|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| A | | | | | | | | | | | | |
| B | | 128.9% | 3.3% | 1.8% | 56.3% | 104.6% | 108.5% | 123.8% | 138.7% | 139.1% | 136.4% | |
| C | | 129.3% | 5.3% | 3.7% | 43.0% | 89.7% | 113.2% | 129.7% | 137.9% | 134.4% | 132.4% | |
| D | | 127.3% | 2.2% | 1.0% | 52.4% | 101.8% | 115.6% | 125.0% | 147.7% | 145.8% | 141.5% | |
| E | | -19.0% | -19.0% | -19.0% | -19.0% | -19.0% | -19.0% | -19.0% | -19.0% | -19.0% | -19.0% | |
| F | | -19.0% | 20.2% | -19.0% | -19.0% | -19.0% | -19.0% | -19.0% | -19.0% | -19.0% | -19.0% | |
| G | | -19.0% | -19.0% | -19.0% | -19.0% | -19.0% | -19.0% | -19.0% | -19.0% | -19.0% | -19.0% | |
| H | | | | | | | | | | | | |

Test Facility : ECBC
 Chemical Code : SLS
 2nd Chem. Code* : none

Study Number.: ECBC-3T3 Ia 0#
 96-Well Plate ID : 090602-2
 Experiment ID : SLS-B(50ug NR/ml 1hr)

| | VC1 | C1 | C2 | C3 | C4 | C5 | C6 | C7 | C8 | VC2 |
|------------------------|--------|--------|--------|--------|-------|--------|--------|--------|--------|--------|
| Conc. (µg/mL) : | 0.0 | 100.0 | 68.0 | 46.3 | 31.5 | 21.4 | 14.6 | 9.9 | 6.7 | 0.0 |
| Mean Corr. OD : | 0.328 | 0.009 | 0.005 | 0.129 | 0.252 | 0.287 | 0.322 | 0.361 | 0.356 | 0.349 |
| SD : | 0.003 | 0.004 | 0.004 | 0.017 | 0.020 | 0.009 | 0.008 | 0.014 | 0.015 | 0.012 |
| Mean Vehicle Control : | 0.338 | | | | | | | | | |
| Mean Blank : | 0.056 | | | | | | | | | |
| % of Vehicle Control : | 128.5% | 3.6% | 2.2% | 50.6% | 98.7% | 112.4% | 126.2% | 141.5% | 139.8% | 136.8% |
| SD : | 1.0% | 1.6% | 1.4% | 6.9% | 7.9% | 3.6% | 3.1% | 5.5% | 5.7% | 4.5% |
| % CV : | 0.81% | 44.09% | 65.56% | 13.56% | 8.04% | 3.20% | 2.47% | 3.85% | 4.09% | 3.31% |
| Mean VC - VC1 (%) : | 3.11% | | | | | | | | | |
| Mean VC - VC2 (%) : | -3.11% | | | | | | | | | |
| Mean Absolute OD : | 0.387 | | | | | | | | | |



Test Facility : ECBC
 Chemical Code : SLS
 2nd Chem. Code*: none

Study Number.: ECBC-3T3 Ia 0#
 96-Well Plate ID : 090602-2
 Experiment ID : SLS-B(25ug NR/ml 3hr)

96-WELL PLATE MAP

| | | | | | | | | | | | | |
|---|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| A | Blank | Blank | Blank | Blank | Blank | Blank | Blank | Blank | Blank | Blank | Blank | Blank |
| B | Blank | VC1 | C1 | C2 | C3 | C4 | C5 | C6 | C7 | C8 | VC2 | Blank |
| C | Blank | VC1 | C1 | C2 | C3 | C4 | C5 | C6 | C7 | C8 | VC2 | Blank |
| D | Blank | VC1 | C1 | C2 | C3 | C4 | C5 | C6 | C7 | C8 | VC2 | Blank |
| E | Blank | VC1 | C1 | C2 | C3 | C4 | C5 | C6 | C7 | C8 | VC2 | Blank |
| F | Blank | VC1 | C1 | C2 | C3 | C4 | C5 | C6 | C7 | C8 | VC2 | Blank |
| G | Blank | VC1 | C1 | C2 | C3 | C4 | C5 | C6 | C7 | C8 | VC2 | Blank |
| H | Blank | Blank | Blank | Blank | Blank | Blank | Blank | Blank | Blank | Blank | Blank | Blank |

RAW ABSORBANCE DATA (OD₅₄₀)

| | | | | | | | | | | | | |
|---|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| A | 0.052 | 0.047 | 0.050 | 0.048 | 0.046 | 0.048 | 0.046 | 0.048 | 0.046 | 0.046 | 0.046 | 0.046 |
| B | 0.049 | 0.559 | 0.047 | 0.050 | 0.175 | 0.387 | 0.506 | 0.474 | 0.580 | 0.489 | 0.610 | 0.048 |
| C | 0.052 | 0.613 | 0.051 | 0.061 | 0.183 | 0.414 | 0.525 | 0.518 | 0.487 | 0.444 | 0.520 | 0.047 |
| D | 0.052 | 0.554 | 0.052 | 0.052 | 0.195 | 0.364 | 0.507 | 0.523 | 0.527 | 0.555 | 0.485 | 0.057 |
| E | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| F | 0.000 | 0.000 | 0.100 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| G | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| H | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |

CORRECTED ABSORBANCE (Sample OD₅₄₀ - Mean Blank OD₅₄₀)

| | | | | | | | | | | | | |
|---|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| A | 0.003 | -0.002 | 0.001 | -0.001 | -0.003 | -0.001 | -0.003 | -0.001 | -0.003 | -0.003 | -0.003 | -0.003 |
| B | 0.000 | 0.511 | -0.002 | 0.001 | 0.127 | 0.339 | 0.458 | 0.426 | 0.532 | 0.441 | 0.562 | -0.001 |
| C | 0.003 | 0.565 | 0.002 | 0.013 | 0.135 | 0.366 | 0.477 | 0.470 | 0.439 | 0.396 | 0.472 | -0.002 |
| D | 0.003 | 0.506 | 0.003 | 0.003 | 0.147 | 0.316 | 0.459 | 0.475 | 0.479 | 0.507 | 0.437 | 0.008 |
| E | -0.049 | -0.049 | -0.049 | -0.049 | -0.049 | -0.049 | -0.049 | -0.049 | -0.049 | -0.049 | -0.049 | -0.049 |
| F | -0.049 | -0.049 | 0.052 | -0.049 | -0.049 | -0.049 | -0.049 | -0.049 | -0.049 | -0.049 | -0.049 | -0.049 |
| G | -0.049 | -0.049 | -0.049 | -0.049 | -0.049 | -0.049 | -0.049 | -0.049 | -0.049 | -0.049 | -0.049 | -0.049 |
| H | -0.049 | 0.000 | -0.049 | -0.049 | -0.049 | -0.049 | -0.049 | -0.049 | -0.049 | -0.049 | -0.049 | -0.049 |

Mean Blank = 0.049

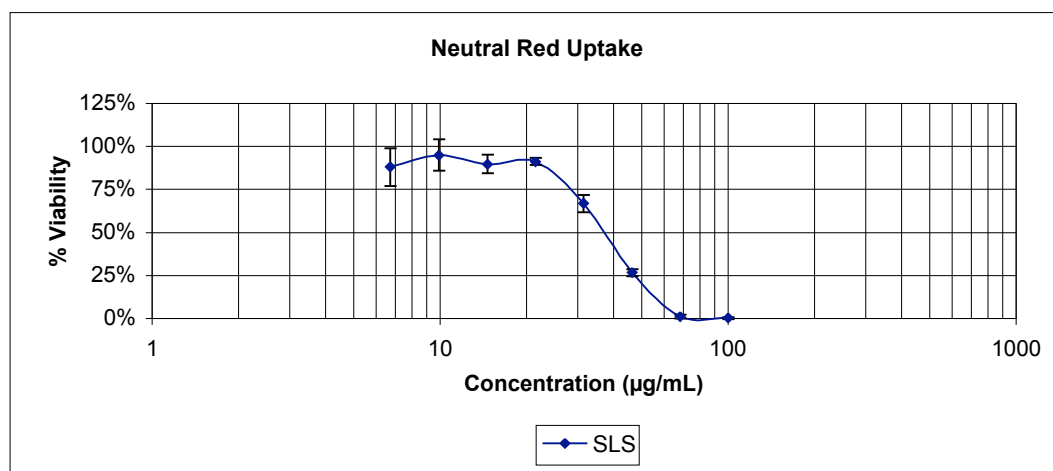
RELATIVE VIABILITY (% OF VEHICLE CONTROL)

| | | | | | | | | | | | | |
|---|---|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|----|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| A | | | | | | | | | | | | |
| B | | 200.3% | -0.6% | 0.6% | 49.6% | 132.8% | 179.5% | 167.0% | 208.6% | 172.9% | 220.3% | |
| C | | 221.5% | 1.0% | 4.9% | 52.8% | 143.4% | 187.0% | 184.2% | 172.1% | 155.2% | 185.0% | |
| D | | 198.4% | 1.4% | 1.4% | 57.5% | 123.8% | 179.9% | 186.2% | 187.8% | 198.8% | 171.3% | |
| E | | -19.0% | -19.0% | -19.0% | -19.0% | -19.0% | -19.0% | -19.0% | -19.0% | -19.0% | -19.0% | |
| F | | -19.0% | 20.2% | -19.0% | -19.0% | -19.0% | -19.0% | -19.0% | -19.0% | -19.0% | -19.0% | |
| G | | -19.0% | -19.0% | -19.0% | -19.0% | -19.0% | -19.0% | -19.0% | -19.0% | -19.0% | -19.0% | |
| H | | | | | | | | | | | | |

Test Facility : ECBC
 Chemical Code : SLS
 2nd Chem. Code*: none

Study Number.: ECBC-3T3 Ia 0#
 96-Well Plate ID : 090602-2
 Experiment ID : SLS-B(25ug NR/ml 3hr)

| | VC1 | C1 | C2 | C3 | C4 | C5 | C6 | C7 | C8 | VC2 |
|------------------------|--------|---------|---------|-------|--------|--------|--------|--------|--------|--------|
| Conc. (µg/mL) : | 0.0 | 100.0 | 68.0 | 46.3 | 31.5 | 21.4 | 14.6 | 9.9 | 6.7 | 0.0 |
| Mean Corr. OD : | 0.527 | 0.001 | 0.006 | 0.136 | 0.340 | 0.464 | 0.457 | 0.483 | 0.448 | 0.490 |
| SD : | 0.033 | 0.003 | 0.006 | 0.010 | 0.025 | 0.011 | 0.027 | 0.047 | 0.056 | 0.064 |
| Mean Vehicle Control : | 0.508 | | | | | | | | | |
| Mean Blank : | 0.049 | | | | | | | | | |
| % of Vehicle Control : | 206.7% | 0.6% | 2.3% | 53.3% | 133.4% | 182.1% | 179.1% | 189.5% | 175.6% | 192.2% |
| SD : | 12.8% | 1.0% | 2.3% | 4.0% | 9.8% | 4.2% | 10.6% | 18.3% | 21.9% | 25.3% |
| % CV : | 6.21% | 176.38% | 100.45% | 7.41% | 7.36% | 2.30% | 5.91% | 9.66% | 12.48% | 13.16% |
| Mean VC - VC1 (%) : | -3.64% | | | | | | | | | |
| Mean VC - VC2 (%) : | 3.64% | | | | | | | | | |
| Mean Absolute OD : | 0.557 | | | | | | | | | |



Test Facility : ECBC
 Chemical Code : SLS
 2nd Chem. Code*: none

Study Number.: ECBC-3T3 Ia 0#
 96-Well Plate ID : 090602-2
 Experiment ID : SLS-B(50ug NR/ml 3hr)

96-WELL PLATE MAP

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| A | Blank | Blank | Blank | Blank | Blank | Blank | Blank | Blank | Blank | Blank | Blank | Blank |
| B | Blank | VC1 | C1 | C2 | C3 | C4 | C5 | C6 | C7 | C8 | VC2 | Blank |
| C | Blank | VC1 | C1 | C2 | C3 | C4 | C5 | C6 | C7 | C8 | VC2 | Blank |
| D | Blank | VC1 | C1 | C2 | C3 | C4 | C5 | C6 | C7 | C8 | VC2 | Blank |
| E | Blank | VC1 | C1 | C2 | C3 | C4 | C5 | C6 | C7 | C8 | VC2 | Blank |
| F | Blank | VC1 | C1 | C2 | C3 | C4 | C5 | C6 | C7 | C8 | VC2 | Blank |
| G | Blank | VC1 | C1 | C2 | C3 | C4 | C5 | C6 | C7 | C8 | VC2 | Blank |
| H | Blank | Blank | Blank | Blank | Blank | Blank | Blank | Blank | Blank | Blank | Blank | Blank |

RAW ABSORBANCE DATA (OD₅₄₀)

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| A | 0.059 | 0.065 | 0.053 | 0.052 | 0.054 | 0.052 | 0.054 | 0.053 | 0.056 | 0.053 | 0.054 | 0.051 |
| B | 0.057 | 0.513 | 0.057 | 0.056 | 0.154 | 0.302 | 0.416 | 0.485 | 0.473 | 0.457 | 0.485 | 0.050 |
| C | 0.059 | 0.488 | 0.058 | 0.056 | 0.152 | 0.326 | 0.420 | 0.460 | 0.500 | 0.438 | 0.562 | 0.059 |
| D | 0.059 | 0.516 | 0.054 | 0.056 | 0.146 | 0.326 | 0.496 | 0.447 | 0.478 | 0.455 | 0.508 | 0.051 |
| E | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| F | 0.000 | 0.000 | 0.100 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| G | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| H | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |

CORRECTED ABSORBANCE (Sample OD₅₄₀ - Mean Blank OD₅₄₀)

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|
| A | 0.011 | 0.017 | 0.004 | 0.003 | 0.005 | 0.003 | 0.005 | 0.004 | 0.007 | 0.004 | 0.005 | 0.002 |
| B | 0.008 | 0.465 | 0.008 | 0.007 | 0.106 | 0.254 | 0.368 | 0.437 | 0.425 | 0.409 | 0.437 | 0.001 |
| C | 0.011 | 0.440 | 0.009 | 0.007 | 0.104 | 0.278 | 0.372 | 0.412 | 0.452 | 0.390 | 0.514 | 0.011 |
| D | 0.011 | 0.468 | 0.005 | 0.007 | 0.098 | 0.278 | 0.448 | 0.399 | 0.430 | 0.407 | 0.460 | 0.002 |
| E | -0.049 | -0.049 | -0.049 | -0.049 | -0.049 | -0.049 | -0.049 | -0.049 | -0.049 | -0.049 | -0.049 | -0.049 |
| F | -0.049 | -0.049 | 0.052 | -0.049 | -0.049 | -0.049 | -0.049 | -0.049 | -0.049 | -0.049 | -0.049 | -0.049 |
| G | -0.049 | -0.049 | -0.049 | -0.049 | -0.049 | -0.049 | -0.049 | -0.049 | -0.049 | -0.049 | -0.049 | -0.049 |
| H | -0.049 | 0.000 | -0.049 | -0.049 | -0.049 | -0.049 | -0.049 | -0.049 | -0.049 | -0.049 | -0.049 | -0.049 |

Mean Blank = 0.055

RELATIVE VIABILITY (% OF VEHICLE CONTROL)

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|----|
| A | | | | | | | | | | | | |
| B | | 182.3% | 3.3% | 2.9% | 41.4% | 99.5% | 144.2% | 171.3% | 166.6% | 160.3% | 171.3% | |
| C | | 172.5% | 3.7% | 2.9% | 40.6% | 108.9% | 145.8% | 161.5% | 177.2% | 152.8% | 201.5% | |
| D | | 183.5% | 2.2% | 2.9% | 38.3% | 108.9% | 175.6% | 156.4% | 168.5% | 159.5% | 180.3% | |
| E | | -19.0% | -19.0% | -19.0% | -19.0% | -19.0% | -19.0% | -19.0% | -19.0% | -19.0% | -19.0% | |
| F | | -19.0% | 20.2% | -19.0% | -19.0% | -19.0% | -19.0% | -19.0% | -19.0% | -19.0% | -19.0% | |
| G | | -19.0% | -19.0% | -19.0% | -19.0% | -19.0% | -19.0% | -19.0% | -19.0% | -19.0% | -19.0% | |
| H | | | | | | | | | | | | |

Test Facility : ECBC
 Chemical Code : SLS
 2nd Chem. Code*: none

Study Number.: ECBC-3T3 Ia 0#
 96-Well Plate ID : 090602-2
 Experiment ID : SLS-B(50ug NR/ml 3hr)

| | VC1 | C1 | C2 | C3 | C4 | C5 | C6 | C7 | C8 | VC2 |
|------------------------|--------|--------|-------|-------|--------|--------|--------|--------|--------|--------|
| Conc. (µg/mL) : | 0.0 | 100.0 | 68.0 | 46.3 | 31.5 | 21.4 | 14.6 | 9.9 | 6.7 | 0.0 |
| Mean Corr. OD : | 0.457 | 0.008 | 0.007 | 0.102 | 0.270 | 0.396 | 0.416 | 0.435 | 0.402 | 0.470 |
| SD : | 0.015 | 0.002 | 0.000 | 0.004 | 0.014 | 0.045 | 0.019 | 0.014 | 0.010 | 0.040 |
| Mean Vehicle Control : | 0.464 | | | | | | | | | |
| Mean Blank : | 0.055 | | | | | | | | | |
| % of Vehicle Control : | 179.4% | 3.1% | 2.9% | 40.1% | 105.8% | 155.2% | 163.0% | 170.8% | 157.6% | 184.4% |
| SD : | 6.0% | 0.8% | 0.0% | 1.6% | 5.4% | 17.7% | 7.6% | 5.6% | 4.1% | 15.5% |
| % CV : | 3.36% | 26.57% | 0.00% | 4.08% | 5.14% | 11.40% | 4.65% | 3.30% | 2.60% | 8.41% |
| Mean VC - VC1 (%) : | 1.37% | | | | | | | | | |
| Mean VC - VC2 (%) : | -1.37% | | | | | | | | | |
| Mean Absolute OD : | 0.512 | | | | | | | | | |

