**Materials and Methods**

**ATP Assay Procedures**

- After treatment termination, cultures were transferred into 24-well plates containing 0.5 mL of a 1:10 mg/mL MTT stock solution for 4 hours at 37°C in a standard tissue culture incubator.

- The MTT reduction in tissue samples can be calculated as:

\[
\% \text{ of Control} = \frac{\text{corrected test formulation RLU} - \text{mean blank RLU}}{\text{mean control RLU} - \text{mean blank RLU}} \times 100
\]

**ATP Assay Procedures**

- Output of the entire set of ATP assay procedures and results are reported in the MTT vs. ATP graph below.

- The MTT reduction in tissue assay is plotted as the % of Control vs. formulation exposure time.

**Results**

- Test formulation residues on tissue killed tissues frequently showed <150 net OD values using the MTT endpoint (not shown in figure).

- All MTT results from the identical test batch of formulation were identical with one exception: 0.03% α-t, 120 min exposure time response in formulation without α-t was similar to 0.03% α-t, 240 min exposure time response in formulation with α-t, indicating a more conservative irritancy prediction.

- Test formulation residues on tissue killed tissues observed no net nil signals using the ATP assay.

- Exposure time response curves of treatments with α-t were identical to the exposure time response of formulation without α-t.

- Both MTT and ATP endpoints predicted progressive shorter exposure time responses in formulations with decreasing concentrations of α-t.

- Relative viability values using the ATP endpoint were lower than those from the MTT endpoint. Relative viability by the ATP endpoint was less than 100% of control for the baseline formulations in both tissue models.

**Conclusions**

- Freeze killed control tissues demonstrated that test chemical (selenium) or residues on treated tissue could directly induce MTT in addition to the MTT reduction from viable cells.

- Relative viability values of the MTT endpoint can be calculated for the amount of MTT reduced directly by test chemical residues. The correction for direct MTT reducer reaction increases the sensitivity for assessing relative viability. The relative viability values from the ATP endpoint may not be directly comparable for these from the MTT endpoint.

- The absence of reference material for the MTT endpoint is a potential barrier to using this endpoint for routine irritancy testing.

**References**


