Report for ASEA: Data from an in vitro study relating to product safety

Study Performed by Pacific Northwest National Laboratory (PNNL)*

Purpose and Disclaimer:

This data is taken from an in-vitro scientific study done to determine the bioactivity of ASEA on eukaryotic cells in a controlled environment. This report offers a summary of the results related to safety data for ASEA on a cellular level. This data answers the question: "Is there any evidence of a toxic response if ASEA comes into contact with eukaryotic cells?"

Explanation of transcription-factor-activation science:

When a cell is stressed by a toxin, the cell responds by sending a certain set of transcription factors into the nucleus. Once inside the nucleus, these transcription factors activate the genes responsible for cellular defense and protection against toxins (such as the inflammatory response). The translocation of certain transcription factors into the nucleus can be seen under a fluorescent microscope when the cells stained by specific indicator dyes.

If the cell undergoes a toxic response, the fluorescent dye is pulled into the nucleus along with the transcription factor. In this experiment two transcription factors, the p65 subunit of NF-kappaB and P-Jun, were monitored. These two transcription factors are known to be activated in all toxic responses. In the photographs from the fluorescent microscopic images of the cells, a toxic response is registered if the green dye is seen to move into the nucleus.

Procedure:

The target eukaryotic cells were cultured in dishes and exposed respectively to (1) Phosphate Buffered Saline (PBS)—the negative control (no toxic response expected), (2) 5% ASEA—Equivalent to replacing 5% of the nutrient solution (blood plasma) with ASEA, (3) 20% ASEA—Equivalent to replacing 20% of the nutrient solution with ASEA, and (4) A known toxin—the positive control (toxic response expected).

The response of the transcription factors, the p65 subunit of NF-kappaB and P-Jun, were photographed under a microscope after exposure to the four solutions listed above. A DAPI stain was also applied to the nuclei in order to help computer software to find the nucleus in the pictures. The software automatically tallied the amount of dye in the nuclei. In the case of P-Jun, measurements of over one hundreds cells were made in order to compile the summary chart.

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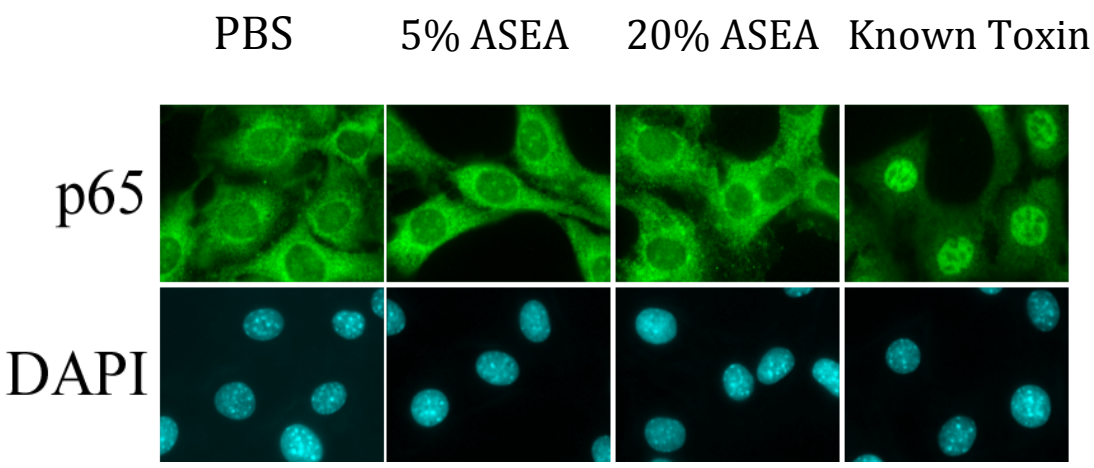
Results:

P65/NF-kappaB

The images cells with stained p65 subunit of NF-kappaB are shown below. It is visually evident that no toxic response is registered for exposure of the cells to ASEA compared to the clear positive response is seen in the rightmost positive control. The p65 subunit remains on the outside of the nuclei in the leftmost 3 pictures, indicating that no toxic response is registered by the cells.



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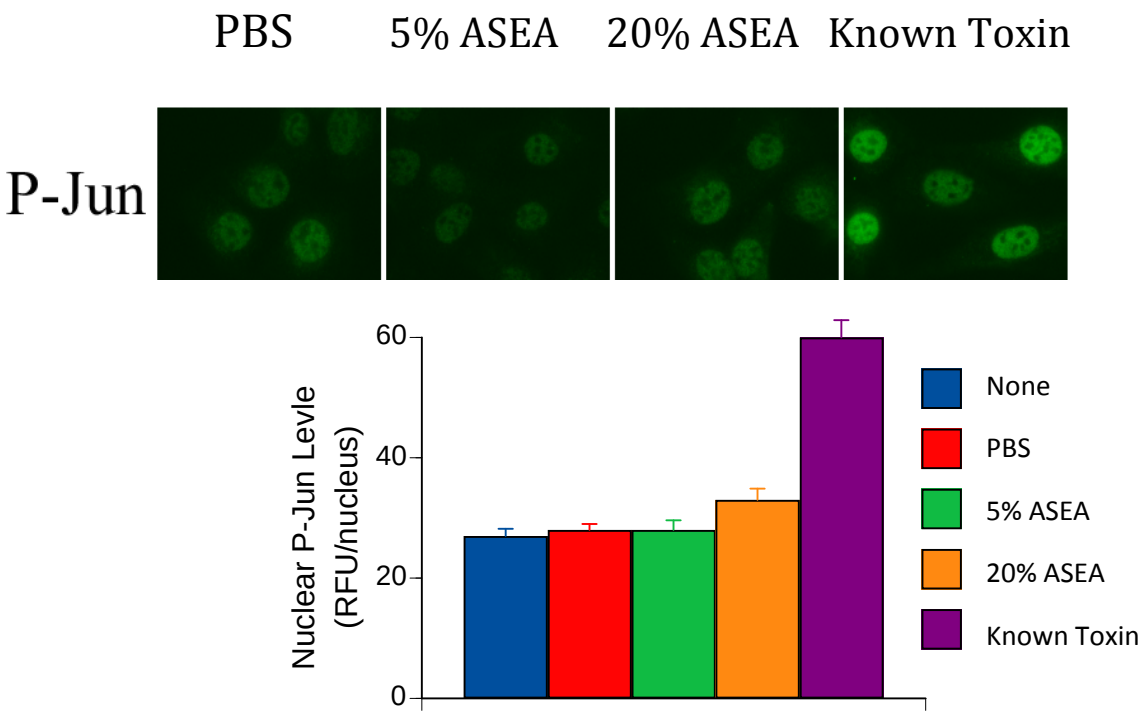
P-Jun

As reinforcement of the NF-kappaB results, the P-Jun data also shows visual evidence that no major toxic response registers. It was necessary for the P-Jun data to be averaged over more than 100 cells in order to get statistically significant numerical results. The resulting chart clearly shows that no toxicity exists for the 5% ASEA. Blood concentration for oral doses, however, will never get anywhere near even 1%.



Conclusions

Direct exposure of cells to relatively high concentrations of ASEA does not register a significant toxic response as measured by NF-kappaB and P-Jun nuclear translocation. Based on these results, ASEA, orally administered, should not manifest a toxic response or inflammation to any of the exposed tissues.

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