GENETICALLY MODIFIED CHO CELLS EXPRESSING HUMAN NAT2 *N*-ACETYLTRANSFERASE OR BACTERIAL *O*-ACETYLTRANSFERASE FOR STUDYING THE GENOTOXICITY OF HETEROCYCLIC AMINES FROM COOKED FOODS.

R.W. Wu, J.D. Tucker, K.J. Sorensen, L.H. Thompson, & J.S. Felton. Biology and Biotechnology Research Program, Lawrence Livermore National Laboratory, Livermore CA, 94551.

We earlier developed the CHO UV5P3 cell line that expresses cytochrome P450IA2 and lacks nucleotide excision repair for studying heterocyclic amines produced in cooked food. The UV5P3 cells were considerably more sensitive to PhIP than IQ with respect to cytotoxicity and mutation induction. To test the hypothesis that the important missing activity in our CHO system for IQ genotoxicity was acetyltransferase, we transfected the UV5P3 cells with plasmids of either the human NAT2 *N*-acetyltransferase or a bacterial *O*-acetyltransferase gene used by Watanabe (kindly provided by Drs. T. Nohmi and T. Deguchi). After screening transformants with IQ in the differential cytotoxicity assay and subcloning them, two clones designated 5P3NAT2 and 5P3YG (expressing human and bacterial transferases, respectively) were studied further. The expression of these acetyltransferase genes was further confirmed by measuring the enzyme activity with isoniazid as substrate. The activity fold-increase over the parental line was 10.94 and 40.27 for 5P3YG and 5P3NAT2, respectively. Both cell lines showed killing even at the lowest dose of IQ tested (0.004 μg/ml). Based on the 50% killing dose (D_{50}), the acetyltransferase expressing cells showed approximately 1000-fold increase in sensitivity over the parental UV5P3 cell line. The same dramatic change in sensitivity was also seen with the mutation response at the *aprt* locus, and with chromosome aberrations and sister chromatid exchanges. In contrast, when these cells were exposed to PhIP, they showed cytotoxicity similar to that of the parental line UV5P3. This result suggests that PhIP does not require acetyltransferase for metabolic activation related to genotoxicity in these cells. The data also show that when *N*-acetyltransferase or the bacterial *O*-acetyltransferase was present, IQ was 10 fold more potent than PhIP for the equivalent cytotoxicity and mutational responses. This relative potency for PhIP and IQ in these specifically engineered CHO cells now resembles more closely the *Salmonella* response for mutations. These new cell lines provide a more sensitive system for evaluating the genotoxicity of compounds requiring metabolic activation by both P450IA2 and acetyltransferase as well as a system for better understanding of the mechanisms for mutagenesis. (This work was performed under the auspices of the U.S. DOE by LLNL under W-7405-Eng-48 and supported by NCI grant CA55861).