141 **COUMARIN HYDROXYLASE ACTIVITY IS INDUCED BY THE METACESTODE FORMS OF TAENIA TAENIFORMIS IN RAT LIVER.** Montero R[1], Serrano L[1], Plancarte A[2]. [1]Instituto de Investigaciones Biomédicas, UNAM. Apdo. Postal 70228, CP 04510, México D.F. [2]Facultad de Medicina, UNAM.

Infection caused by trematode parasites in liver has been reported to induce the activity of a specific CYP450 enzyme, coumarin-hydroxylase (COH) in mice infected with *Fasciola hepatica*, as well as in hamsters infected with *Opistorchis viverrini*. This enzyme participates in the metabolism of certain xenobiotics that have been identified as mutagenic and/or carcinogenic, such as the nitrosamines NDEA, NDMA, NNN, NNK, as well as AFB_1 in different species such as mouse, human, hamster and rat.We are investigating whether this effect caused by trematodes would occur every time that the liver is infected, as has been demonstrated with the hepatitis-B virus, and whether other CYP450 enzymes relevant to the metabolism of xenobiotics would be affected, thus altering the susceptibility of the infected organisms to environmental agents. We infected rats with *Taenia taeniformis* eggs, allowed 30 to 60 days for the larval forms to develop and then measured the activity of COH by injecting the animals with coumarin (0.5 mg/10 g, b.w.) and collecting the urine during 4 h. We measured the presence of the fluorescent metabolite hydroxy-coumarin in the urine and found a 6 to a 100 fold increase in the urine of infected rats with respect to controls which almost do not produce this metabolite. This result supports our hypothesis that any liver infection would cause the induction of COH. Other tests such as immunoblots to determine an alteration of other CYP450 isoforms, as well as in vitro genotoxicity studies are under way to determine the specific activity of other CYP450 isozymes.

142 **REDUCTION OF SPONTANEOUS MUTATION IN MISMATCH REPAIR DEFICIENT HUMAN COLON CANCER CELL LINES BY DIETARY ANTIOXIDANTS.** Mure K[1], Rossman T[1]. [1]New York University School of Medicine, Tuxedo, NY10987.

Spontaneous mutagenesis probably plays an important role in spontaneous carcinogenesis. Previously, this laboratory found that antioxidants could greatly reduce spontaneous mutagenesis in a model Chinese hamster cell line (G12) suggesting that oxidative damage to DNA is a major cause. It is possible that mismatch repair plays a role in repair of oxidative DNA lesions. The mismatch repair deficient human colon cancer cell line HCT116 (lacking MLH1), was used to test this hypothesis. For measurement of spontaneous mutagenesis, mutation rates at the *hprt* locus were measured by scoring resistance to 6-thioguanine. Prior to spontaneous mutation tests, toxicity of antioxidants was tested and non-toxic/non-growth-inhibiting doses were selected. Because serum contains antioxidants, two serum concentrations (1% or 10%) were chosen. The HCT116 cell line had a 22-fold higher spontaneous mutation rate compared with the HCT116/ch3 cell line, in which normal human chromosome 3 has been added to restore mismatch repair. We found that 5 μM lycopene reduced spontaneous mutagenesis in HCT116 cells by 75%. Ascorbate (284 μM), α-tocopherol (50 μM) and (-)-epigallocatechin gallate (10 μM) gave nearly 50% reductions. HCT116 cells cultured in 1% serum had a higher spontaneous mutation rate, which was more dramatically reduced by antioxidants, compared with cells cultured in 10% serum. The finding that such a large proportion of spontaneous mutagenesis can be blocked by antioxidants in mismatch repair-deficient cells supports the hypothesis that a major cause of spontaneous mutagenesis is endogenous oxidative damage to DNA, and suggests that mismatch repair may also repair endogenous oxidative lesions.

143 **A MICROSUSPENSION SCREENING VERSION OF THE AMES TEST TO PREDICT THE OUTCOME OF GLP ASSAYS FOR GENE MUTATIONS.** Muster W[1], Albertini S[1], Chètelat AA[1], Kirchner S[1], Gocke E[1]. [1]Pharma Research Nonclinical-Development - Safety PRNS, F. Hoffmann La Roche Ltd. CH-4070 Basel, Switzerland.

In order to predict/recognize genotoxic liabilities at a very early stage in drug development we employ a) structure activity prediction (using the DEREK system), b) a miniaturized version of the Ames test (microsuspension assay) and c) an in vitro MNT test with mouse lymphoma cells. Here we report our initial validation efforts and current testing experience with the microsuspension Ames test. The method is based on a modified preincubation version described by Kado et al (Mutat. Res. 1983, 121, 25-32). We use all five strains employed in the standard test (TA1535, TA97, TA98, TA100, TA102). The preincubation period is extended to 1 h, the preincubation volume is decreased to 210 ul (100 ul of 5 times concentrated overnight culture, 100 ul of S9 mix (2% S9), and 10 ul test compound solution). Three plates per dose point are prepared. The predominant aim of these modifications is to reduce the amount of test compound, rather than to reduce the workload per assay. For a number of positive controls the microsuspension assay is about 10 to 20 fold more sensitive than the standard assay. Thus, a reduction of test material to about 30 mg per test can be achieved. For other compounds (e.g. Na azide) the preincubation phase does not sensitize the test system or, alternatively, the cytotoxic action is increased more strongly than the genotoxic action, leading to a 'false negative' prediction. Several examples (established mutagens or development candidates) are shown and likely explanations for the discrepancies between microsupension vs 'standard' preincubation vs plate incorporation assays are listed. The value of the microsupension assay as a component of early screening strategies is discussed.

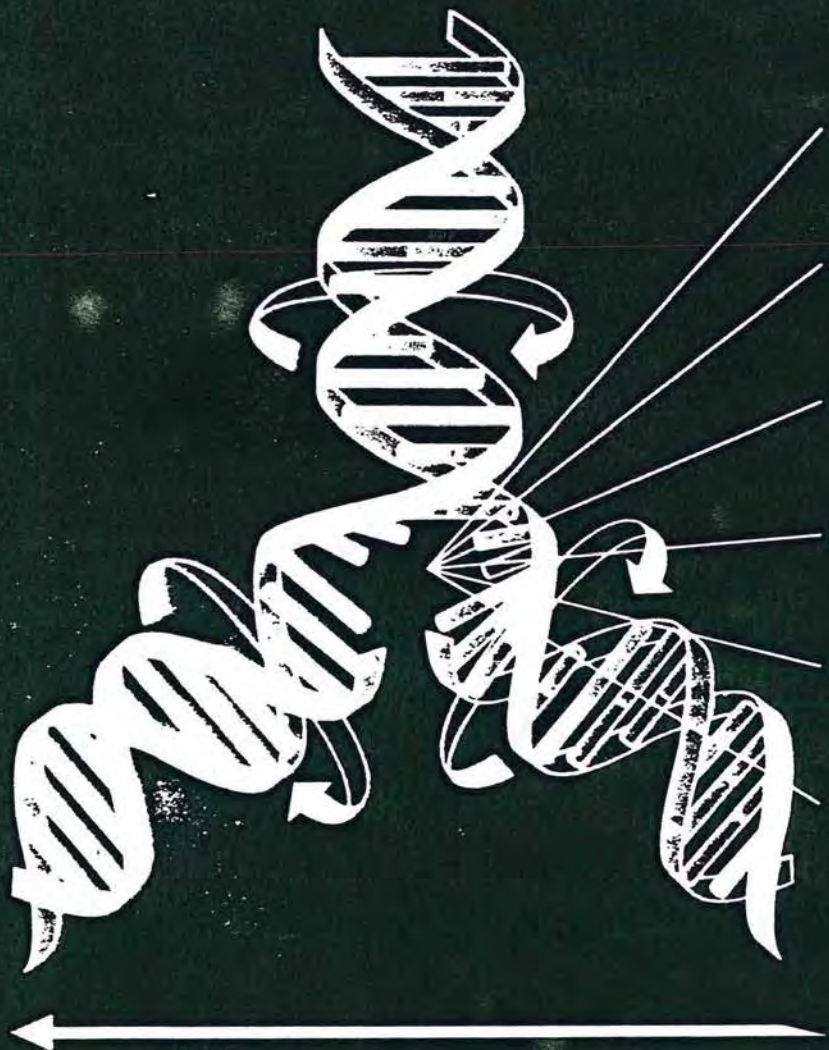
144 **ANALYSIS OF K-*RAS* AND *P53* MUTATIONS IN MESOTHELIOMAS FROM HUMANS AND RATS EXPOSED TO ASBESTOS.** Ni Z[1,2], Liu YQ[2], Keshava N[1], Zhou G[1], Ong T[1]. [1]Toxicology and Molecular Biology Branch, National Institute for Occupational Safety and Health, Morgantown, WV 26505. [2]Department of Toxicology, West China University of Medical Sciences, Chengdu, China 610041.

Malignant mesothelioma is known to be associated with asbestos exposure. However, the mechanism of mesothelial carcinogenesis in relation to activation of proto-oncogenes or inactivation of tumor suppressor genes remains unclear. In this study, the PCR-Primer Introduced Restriction Site (PCR-PIRS) assay was employed to examine mutations in the K-*ras* proto-oncogene of mesothelioma tissues from workers exposed to asbestos and from rats treated with asbestos. Direct DNA sequence analysis was also performed to determine the mutations in exons 1 and 2 of the K-*ras* gene and exons 5 to 8 of the *p53* tumor suppressor gene. Results of the PCR-PIRS analysis revealed no mutations in codons 12, 13 or 61 of K-*ras* gene in all 17 human and 22 rat mesothelioma tissue samples. The sequence was confirmed by the direct DNA sequence analysis. No mutation was found in exons 5 to 8 of the *p53* gene in all the mesothelioma tissue samples analyzed. These results further indicate that the K-*ras* proto-oncogene and the *p53* tumor suppressor gene may not play a critical role in the induction of mesothelioma by asbestos either in humans or in rats.

An International Journal Specializing in Environmental Mutagenesis

Volume 35
Supplement 31
2000

Environmental and Molecular Mutagenesis



WILEY-LISS
ISSN: 0893-6692