IMMUNOCYTOHISTOCHEMICAL LOCALIZATION OF CYTOCHROME P-450 ISOCYANASE IN HUMAN EPITHELIAL CELLS

P.J.A.M. van Petegem,1,2 Y.J.M. Olde Meerink1 and P.J.J.M. Weterings1

Cultured keratinocytes obtained from human hair follicles might be a useful tool to study mutagenicity in human epithelial cells. Human hair follicles possess a cytochrome P-450 dependent enzyme system which is capable to metabolize xenobiotics. The preservation of this enzyme in vitro is important for the application of hair follicle cell cultures in genotoxicity studies especially for promutagens and procarcinogens.

We studied the immunolocalization of cytochrome P-450 by using monoclonal antibodies (K03 and K07) raised against two isoforms. The antigens were present in freshly plucked hair follicles, fibroblasts and the cell line SVK14. In the cultured keratinocytes no staining was observed by the antibodies. Since the cell line SVK14 shows a medium degree of response on the antibodies, the absence of cytochrome P-450 in the hair follicle keratinocytes is ascribed to the culture conditions. Further studies on the relation between culture media and maintenance of cytochrome P-450 is required.

A.M.Wetzer, H. Weeinkt, E.C.R. Kretfeldt and F. Seutter-Berlage

Organic isocyanates are highly reactive chemicals characterised by the general formula R(NCO)x. The diisocyanates are widely used for the industrial production of polyurethanes. Exposure to isocyanates is known to cause pulmonary and skin irritation as well as immune necitization of the respiratory tract. In contrast to these well studied toxic effects, little is known about the mutagenic and possible carcinogenic effects of the isocyanates.

We present a study of the mutagenic action to Salmonella typhimurium of three isocyanates extensively used in polyurethane industry: toluene diisocyanate (TDI), 4,4' - diisocyanatodiphenylmethane (MDI) and hexamethylene diisocyanate (HDI). In addition, the closely related tolyliso
cyanate (TDA, MDA, HDA and toluidine) were incorporated in the Ames-tests. The mutagenicity testing was carried out with the plate incorporation assay as described by Ames et al. (1). The tests were performed with S. typh. strains TA 100, TA 1535, TA 98 and TA 1538 both with and without metabolic activation (S9-mix containing rat liver homogenate (9000 g)).

The isocyanates, particularly HDI, showed a large toxic effect on the Salmonella bacteria. Mutagenicity was observed with TDI, TDA, MDI and MDA in TA 100 and TA 98 with S9-mix. In both cases the amine was more mutagenic than the analogous isocyanate. This finding suggests that the mutagenic effect of isocyanates can be attributed to reactive metabolites of the amines formed during hydrolysis of the isocyanates.

1. B.N. Ames, J. McCann and E. Yamasaki, Mutation Research, 31 (1975) 347

Department of Pharmacology, University of Nijmegen, Geert Grooteplein N21, 6525 EZ Nijmegen, The Netherlands.