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Cytotoxicity of Methacrylonitrile

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A variety of compounds are suspected to be mutagenic and carcinogenic. These include polycyclic and poly halogenated aromatic hydrocarbons as well as nitroaromatic compounds, most of which have not been fully investigated in terms of toxicological properties (Frazier 1990, 1992). Methacrylonitrile is a widely used industrial chemical. It is used as an intermediate in the preparation of common laboratory chemicals (Windholz 1983). It is also used as a replacement for acrylonitrile in the manufacture of carbonated beverage containers (Cosidine 1974). MeAN has been identified as a component of main stream smoke of unfiltered cigarettes (Baker *et al,* 1986). Reports of occupational exposure to MeAN are available in the literature (ACGH 1986 ; Amoore and Hautala 1983).

Liver is an organ of particular importance in toxicological research and it is the most frequently used tissue in carcinogenesis bioassay. The use of an *in vitro* hepatic system for toxicity studies has received increasing attention in recent years. Freshly isolated suspensions and primary cultures of hepatocytes represent the most popular models, because primary hepatocytes maintain most of their xenobiotic metabolizing capabilities for several days in culture (Blaaboer *et al,* 1985).

Mammalian cell culture systems have also been used in risk evaluation, both for investigating the mechanism of chemical carcinogenesis and as bio assay systems, for monitoring environmental genotoxins (Russell *et al,* 1984 ; Purchan *et al,* 1976). Difficulties associated with the preparation and maintenance of primary hepatocyte culture limit their widespread use. The human hepatoma cell line Hep G2 provides an alternative as it is a more convenient and less labor - intensive hepatocyte system. HepG2 cells remain highly differentiated in culture and retain most of the normal functions of the human hepatocyte. They have been used in the examination of cyto and genotoxicity of chemicals.

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MATERIALS AND METHODS

HepG2 cells were obtained from the National Facility for Animal tissue and Cell Culture (NFATCC), Pune, India. Cell culture media were obtained from HiMedia (Bombay). [^{3}H] Thymidine was obtained from

Bhabha Atomic Research Centre (BARC), Bombay. All the other reagents were obtained from Sigma Chemicals Co., USA.

HepG2 monolayer cell culture were maintained in a humidified 5% CO_2 atmosphere at 37°C in a minimum essential medium with Earhli'salts, NEAA, phenolred (without L. glutamine, Antibiotic and Sodium bicarbonate), supplemented with 20µl/lit pencillin and streptomycin; 20µl/lit fungizone and 3% glutamine.

Genotoxicity in HepG2 cell line was investigated using the LSC based assay for unscheduled DNA synthesis (UDS) (Furat Naji *et al*, 1994). HepG2 cells were grown to confluency in 35 mm petridishes and were maintained at this stage in 0.5% (v/v) Fetal Bovine Serum (FBS) for 4 days to minimize scheduled DNA synthesis. The cells were then incubated for 1 hr with 10 mmol/L hydroxyurea to inhibit residual scheduled DNA synthesis. Different concentrations of test compound (10nm, 20nm and 40nm of solution of MeAN) in acetone were added to the cells (to 1% v/v final solvent concentration) together with 5 mCi/L of [^{3}H] thymidine, and incubated for 24 hr. The cells were then collected onto 0.6 pm Sartorius filters, washed in succession with 3 ml PBS, 10 ml of 5% (w/v) TCA, and 5 ml of 95% (v/v) ethanol. the filters were air dried, combined with 10 ml scintillation cocktail (0.5% w/v PPO, 0.015% w/v POPOP in 2:1 toluene: Triton X-100), and then counted in a LKB Pharmacia liquid scintillation counter. In this assay, an increase in the incorporation of [^{3}H] thymidine indicated the induction of UDS and presented graphically.

Quantitative estimations were made with at least six plates for each concentration of MeAN. Statistical analysis was done by students 't' test and 'p' value was arrived at to assess the statistical significance of the changes observed. 'p' value less than 0.05 was considered significant. The experimental values were compared with the control values where no MeAN was added.

RESULTS AND DISCUSSION

HepG2 cells grown in complete medium were used to develop a cytotoxicity assay and UDS assay. Cytotoxicity assay developed was based on the measurement of the level of UDS in HepG2 cells. The LSC-based method measures the level of [^{3}H] thymidine uptake by HepG2 cells.

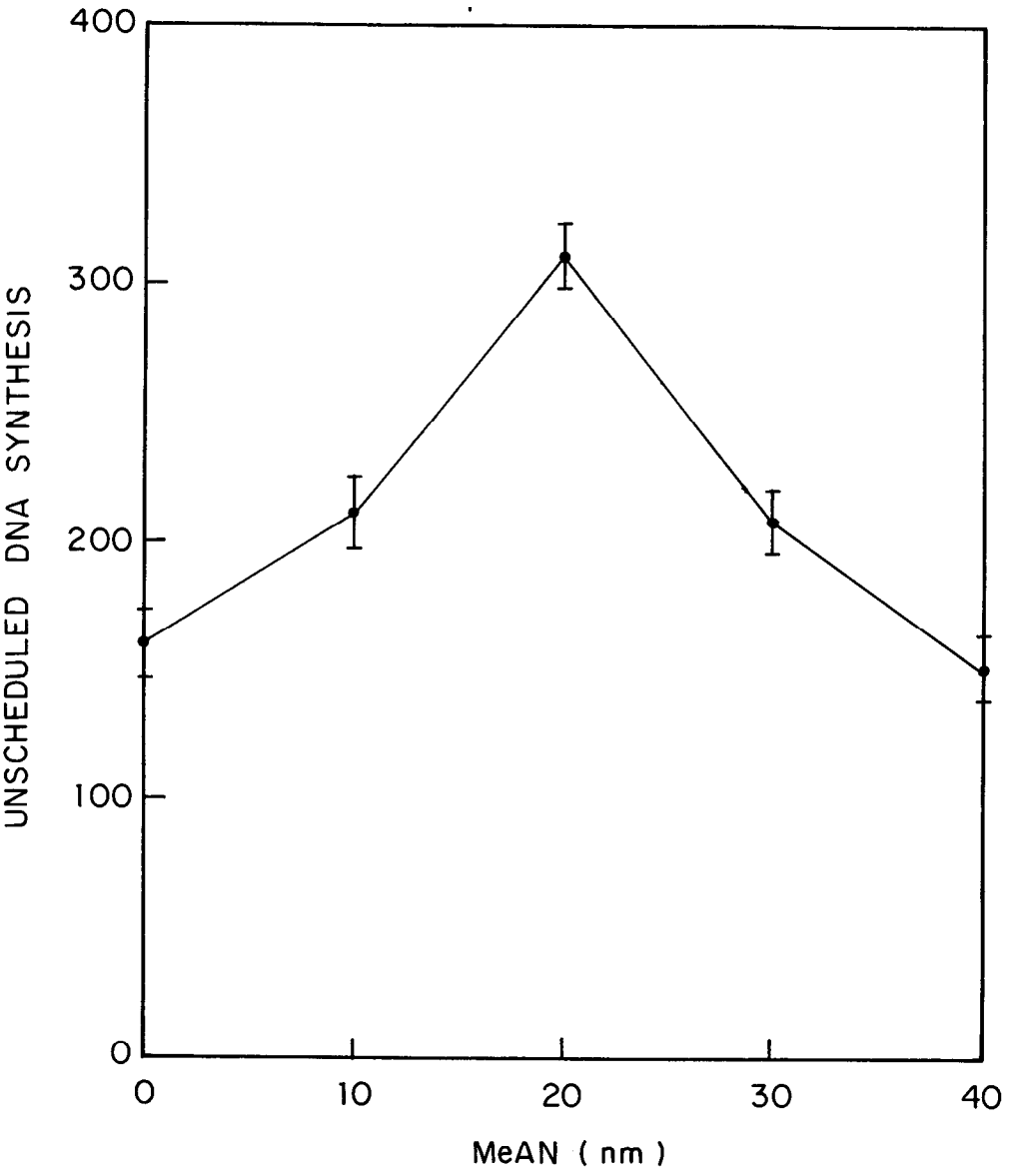

Figure. l Unscheduled DNA systhesis (UDS) in HepG2 cells exposed to MeAN at various concentrations. Values are Mean ± S.D. for 6 plates.

Dose response relationship for MeAN in the LSC-based assay is shown in Figure 1. Maximal UDS activity was observed at a concentration of 20nm/plate. The highest concentration tested, namely 40nm/plate, yielded a modest depression of the UDS response. The plates 1,2,3 and 4 show untreated HepG2 cells; 10nm of MeAN and HepG2 cells, 20nm of MeAN + HepG2 cells and 40nm of MeAN + HepG2 respectively. Plates 2,3 and 4 show dose dependent cell proliferation. The relationship did not hold when the xenobiotic concentration reached a level that was cytotoxic to the cells.

Acrylonitrile (AN) a homologue of MeAN, the most investigated chemical in the class of nitriles, is mutagenic (Lambotte *et al, 1984),* a known animal carcinogen (Maltoni *et al,* 1977) and suspected human carcinogen (Koerrelman and Vander (1984). MeAN has been recommended as a chemical which, probably, is carcinogenic in humans (NIOSH 1988). For acrylonitrile; the epoxide is considered the ultimate carcinogenic species (Roberts *et al,* 1991). The formation of epoxide intermediate in MeAN metabolism (Farooqui *et al.* 1990) may be the cause for the increased uptake of [^{3}H] thymidine by HepG2 cells at the concentration of 20nm/plate. But at the highest concentration tested (40nm/plate) modest uptake of [^{3}H] thymidine was observed which may be due to the cytotoxicity of MeAN. In the United States, under the Resource Conservation and Recovery Act, MeAN has been designated as a hazardous waste (waste no. 10152) in Appendix VIII, a listing of chemicals that have been shown to have toxic, carcinogenic, mutagenic or teratogenic effects on humans or other life forms (USEPA 1986).

It can be concluded that MeAN may be a mutagen/carcinogen at lower doses, but at higher doses, it has cytotoxic effect.

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