

SHORT COMMUNICATION

Nongenotoxic Effects of Polycyclic Aromatic Hydrocarbons and Their Ozonation By-Products on the Intercellular Communication of Rat Liver Epithelial Cells

Nongenotoxic Effects of Polycyclic Aromatic Hydrocarbons and Their Ozonation By-Products on the Intercellular Communication of Rat Liver Epithelial Cells. UPHAM, B. L., MASTEN, S. J., LOCKWOOD, B. R., AND TROSKO, J. E. (1994). *Fundam. Appl. Toxicol.* 23, 470-475.

Since polycyclic aromatic hydrocarbons (PAHs) are known to have epigenetic effects, we evaluated the effect of the parent chemical and the ozonated products on *in vitro* cell to cell communication bioassays which measures a nongenotoxic event. The scrape loading/dye transfer (SL/DT) technique was used to determine the effect of the following PAHs on gap-junction intercellular communication (GJIC): fluorene, 1-methyl-fluorene, fluoranthene, anthracene, 9-methyl-anthracene, phenanthrene, pyrene, benzo(*a*)pyrene, and benzo(*e*)pyrene. The methylated PAHs were more inhibitory to GJIC than the unmethylated counterparts. Fluoranthene, which has an additional ring added to fluorene, was more effective in inhibiting GJIC than fluorene. The three-ringed PAHs were also more inhibitory than the four- and five-ringed PAHs. A time-course study of fluoranthene and of pyrene resulted in maximal inhibition occurring within 30 min of incubation with the cells. The cells recovered from the inhibition within 1 hr after fluoranthene and pyrene were removed from the cell culture medium. Pyrene, benzo(*a*)pyrene, fluorene, and fluoranthene were ozonated until the parent compound was completely eliminated as determined by reverse-phase high-pressure liquid chromatography (RP-HPLC). An increased level of inhibition of GJIC was observed for the ozonated mixtures of by-products of pyrene, fluoranthene, and benzo(*a*)pyrene, but not for fluorene, as monitored with the SL/DT technique. The products of the ozonated pyrene mixture were fractionated and collected by RP-HPLC. Each fraction was found to be inhibitory to GJIC as monitored by fluorescence recovery after photobleaching. In conclusion, current treatment technologies, such as ozonation or biologically based oxidations and methylations, do not necessarily eliminate toxicity. Therefore, it is imperative that toxicological studies be used to complement traditional chemical detection techniques used to monitor the fate of a pollutant in environmental treatment systems.

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Environmental pollutants can effect the health of living systems through various mechanisms such as genotoxicity,

cytotoxicity, and epigenetic toxicity (Trosko *et al.*, 1990a). A primary health concern resulting from environmental pollutants is cancer and past research has focused primarily on the mutagenicity (genotoxicity) of environmental contaminants on carcinogenic events in animal systems. However, many known chemicals appear not to be genotoxic (Ashby and Tennant, 1991) but rather modify gene expression by their ability to alter homeostatic control of multicellular organisms (Trosko *et al.*, 1990b).

A central mechanism in maintaining the homeostatic health of multicellular organisms is intercellular communication. The intercellular communication resulting from the transfer of ions and molecules (≤ 1200 Da) occurs by way of a membrane structure called the gap junction (Loewenstein, 1979). Molecules known to diffuse through gap junctions are cAMP, amino acids, calcium, inositol, and triphosphates (Pitts and Finbow, 1986; Lawrence *et al.*, 1978; Saez *et al.*, 1989). A family of highly evolutionarily conserved genes codes for the gap-junction proteins (Willecke *et al.*, 1991; Fishman *et al.*, 1991). Gap junctions in different tissues are not identical but they do share a basic structure consisting of six protein subunits (connexin) forming a hexameric channel (connexon) traversing the plasma membrane which is anchored with the connexon of the opposing membrane to form a complete channel between two cells (Yamasaki, 1990). Intercellular communication through these gap junctions plays a crucial role in maintaining the homeostasis of multicellular organisms (Sheridan, 1987). The gap junction has been linked to many regulatory roles such as growth control, developmental and differentiation processes, synchronization, and metabolic regulation (Trosko *et al.*, 1993). Alteration of gap-junction communication by chronic exposure to toxicants has been implicated in tumor promotion and carcinogenesis (Trosko *et al.*, 1991), in teratogenesis (Trosko *et al.*, 1982), in reproductive dysfunction (Gilula *et al.*, 1976; Larsen *et al.*, 1986; Ye *et al.*, 1990), and the alteration of muscle contractions in the heart and uterus (Cole and Garfield, 1986; DeMello, 1982). Trosko *et al.* (1983) also noted that neurotoxicity could result from the inhibition of intercellular communication in the central nervous system. A recent report has im-