127 CYTOTOXICITY AND MUTAGENICITY IN HUMAN TK6 LYMPHOBLASTOID CELLS EXPOSED *IN VITRO* TO METHYLPHENIDATE

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Methylphenidate(Ritalin, MPH) is a psycho-stimulant widely used for the treatment of attention deficit/hyperactivity disorder (ADHD); however, a recent report of cytogenetic effects in peripheral blood lymphocytes of children treated with MPH for 3 months (El-Zein et al., Cancer Letters, in press) has raised concerns that long-term exposure may pose a potential health risk. To investigate the mutagenic effects of MPH in human cells in culture, a cloning assay was used to measure cloning efficiencies (CEs) and mutant frequencies (MFs) in the hypoxanthine-guanine phosphoribosyltransferase (*HPRT*) and thymidine kinase (*TK*) genes in TK6 cells exposed to MPH with/without human hepatic S9. TK6 cells exposed to 500 MPH μg/ml + S9 (n=6) for 3 days impeded cell growth with <2 rounds of replication (compared with >3 in control cells) but was significantly mutagenic (MF=8.65±2.12/million cells; control MF=5.39±1.91/million cells; p=0.01). In a follow-up experiment, TK6 cells were exposed to 0, 250, or 500 μg MPH/ml, with/without S9, for 4 days to acquire at least 3 rounds of cell replication for treated cells. MPH treatments with or without S9 were cytotoxic relative to controls (CE in treated cells were 50-56% of control values). Dose-related increases in *HPRT* MFs were found in cells exposed to MPH in the presence of hepatic S9, but not in its absence. Drug-induced MFs were significantly elevated in the 500 μg MPH/ml + S9 group (n= 6, MF=13.86±7.12/million cells; p=0.007) but not the 250 μg MPH/ml + S9 group (n=5,MF=7.13±4.89/million cells) compared with controls (n=7, MF=3.79±3.62/million cells). For both experiments, there was no significant increase in *TK* MFs in any MPH treatment group, with/without S9. These findings demonstrate that human hepatic enzymes have the capacity to convert MPH to a mutagenic metabolite(s) that can induce mutations in exposed lymphocytes. Further investigations of drugs used to treat children with ADHD are needed to characterize the mode of action and potential risks of long-term exposure to therapeutic agents such as MPH and d-amphetamine (Adderall) in children.

128 HUMAN DIETARY ALKALOIDS INHIBIT HEDGEHOG SIGNALING

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The Hedgehog (Hh) signaling pathway plays an integral role in the patterning and development of diverse structures in the vertebrate embryo and aberrations in Hh signaling are associated with a range of developmental defects including cleft lip/palate and cyclopia; collectively termed holoprosencephaly (HPE). Postnatally, Hh signaling is postulated to play a pivotal role in healing and repair processes and inappropriate pathway activation has been implicated in several types of cancers. The Veratrum alkaloid cyclopamine is a potent inhibitor of Hh signaling and causes HPE-related defects in diverse species. Here we show that structurally related alkaloids in the human diet also inhibit Hh signaling. We determined the relative potency and maximum efficacy of cyclopamine, tomatidine (from tomatoes) and solandine (from potatoes) in inhibiting Hh target gene induction in Hh-ligand responsive mouse embryo fibroblasts (MEFs). We found that cyclopamine was 40 times more potent than tomatidine and 120 times more potent than solanidine and that each was capable of complete inhibition of Hh signaling. We then set out to establish an in vivo assay capable of detecting varying potencies of Hh signaling inhibition. Consistent with previous reports, we found that zebrafish embryos exposed to graded concentrations of waterborne cyclopamine displayed classic endpoints of Hh inhibition including U-shaped somites, lack of the horizontal myoseptum, and reduced interocular distance; the severity of each defect increased in a cyclopamine concentration-dependent manner. These results support use of the zebrafish embryo as a potentially useful model for identifying other compounds with a wide range of potencies for inhibiting Hh signaling. Thus, we determined that certain human dietary alkaloids inhibit Hh signaling in vitro and have established a zebrafish embryo model for assaying correlative in vivo effects. The identification of dietary compounds that abrogate Hh signaling may have substantial implications in a wide range of human health issues related to Hh signaling.

129 DEVELOPMENTAL TOXICITY OF PENTAERYTHRITOL ESTERS APPLIED DERMALLY IN RATS

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Esters of pentaerythritol (PE, or 1,1,1-tris(hydroxy-methyl)ethanol) and dipentaerythritol (DPE) are used as synthetic base oils in a variety of lubricants. Because of potential dermal exposure of people to these products, a developmental toxicity screening study was performed with a mixture of long-chain esters of PE and DPE (viscosity ~5 cSt at 100°C). The esters were administered on gestation days 0-19 to the uncovered skin of presumed-pregnant rats (15/group) at doses of 0, 800, and 2,000 mg/kg. Dams were sacrificed on GD 20. Maternal parameters of food consumption, body weight gain, and clinical signs were unaffected except for slight erythema and flaking of the skin during treatment. Clinical chemistry was unaffected in dams at sacrifice. No adverse effects were observed at cesarean section on reproductive parameters (number of implants, resorptions, or viable fetuses) or on fetal parameters (body weight or crown-rump length). Levocardia was noted in 3.2 and 10.1% of 800 and 2,000 mg/kg groups, respectively, but was considered to be an artifact of the methods for fixation and examination of the fetuses. Otherwise, no significant adverse effects were noted in fetuses during external, skeletal, or visceral examinations. The NOAEL for both maternal and fetal toxicity was 2,000 mg/kg/day.

130 TERATOLOGY STUDY OF DIBUTYLTIN IN CYNOMOLGUS MONKEYS GIVEN DURING ORGANOGENESIS

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We previously reported that dibutyltin dichloride (DBTCl) during organogenesis caused increased incidence of fetal malformations in rats. The present study was conducted to determine the teratogenic potential of DBTCl given to pregnant