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LYNCH, D.W., NIOSH, DBBS, Experimental Toxicology Branch, Cincinnati, Ohio. Eye defects in *Drosophila* exposed to vinblastine sulfate during development.

To further characterize the *Drosophila* bioassay as a screen to detect developmental toxicants, vinblastine sulfate (VBS; CAS No. 143-67-9), a documented teratogen and developmental toxicant, was evaluated. VBS concentrations were investigated in two experiments using our published protocol (*Teratogenesis, Carcinogenesis, and Mutagenesis* 11:147-173, 1991). Each experiment utilized seven VBS concentrations plus a concurrent control. Initially, concentrations ranging from 40-200 µg/vial were evaluated. Since no flies were found to emerge at concentrations greater than 40 µg/vial, lower concentrations (3-30 µg/vial) were utilized in the second experiment to better characterize any developmental toxicity and to determine if a threshold could be observed. *Drosophila* were exposed throughout development (egg through third instar larva) in culture vials to medium containing VBS. Each vial contained 1g of powdered medium and 5ml of distilled deionized water or a solution of test chemical in water. A mated, untreated, Oregon-R wild-type female (Mid-American *Drosophila* Stock Center, Bowling Green State University, Ohio) was added to each culture vial, allowed to oviposit for 20 hours, and then removed. Emerging offspring were collected over 10 days and examined microscopically (25x) for bent humeral bristles and wing blade notches; morphological defects shown to occur with an increased incidence in flies exposed to developmental toxicants. In addition, offspring were also examined for the presence of eye abnormalities after an unusual eye defect was observed in a fly exposed to VBS in the 40 µg/vial group in the first experiment. In each experiment, the incidence of specific defects at each concentration was compared to the concurrent controls using chi-square. The incidence of bristle defects was significantly increased at 10 µg/vial, 15/128, $p<0.05$ and at 40 µg/vial, 1/5, $p<0.001$. The incidence of eye defects was significantly increased at 15 µg/vial, 2/78, $p<0.05$, 20 µg/vial, 2/48, $p<0.01$, 25 µg/vial, 5/44, $p<0.001$, and at 40 µg/vial, 1/5, $p<0.001$. No wing blade defects were observed at any VBS concentration. The results with VBS parallel the developmental toxicity reported in mammals, and replicate and expand published *Drosophila* data. These findings provide additional support for increased utilization of this assay as a prescreen for the detection of developmental toxicants.

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HAN, S.Y., D.H. LEE, C.K. KIM, J.H. SHIN, H.J. MOON, H.S. KIM, S.H. KIM, S.D. OH, R.D. LEE, T.S. KIM, E.H. WON and K.L. PARK, Department of Toxicology, National Institute of Toxicology Research, Korea Food & Drug Administration, Seoul, Korea. Embryotoxicity and teratogenicity studies of epoxidized soy bean oil (ESBO) using in vitro battery test system.

Epoxidized soy bean oil (ESBO) is used as a plasticizer in PVC products. The purpose of this study was to evaluate embryotoxicity and teratogenicity of ESBO using in vitro battery test system such as whole embryo, midbrain and limb bud culture system. Rat embryos at gestation day 9.5 were cultured for 48 h with 0, 250, 500 and 750 µg/Ml of ESBO dissolved in ethanol. For evaluation of embryo development and abnormality, morphological changes were observed and scored by the method of Van Maele-Fabry. Micromass cells from midbrain and limb bud of rat embryos at gestation day 12.5 were cultured for 4 days with 2×10-4~ 1×103 µg/Ml of ESBO. Cell proliferation was assessed by neutral red staining, and differentiation was determined by measuring of foci area after hematoxylin staining (midbrain cells) or alcian blue staining (limb bud cells). There was no effect of ESBO in whole embryo culture up to 750 µg/Ml as well as midbrain culture and limb bud culture system up to 1 mg/Ml. These results show that ESBO is not embryotoxic nor teratogenic in vitro battery test system.

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HANSEN[1], J.M., E.W. CARNEY[2] and C. HARRIS[1], [1]University of Michigan, Ann Arbor, Michigan and [2]The Dow Chemical Company, Midland, Michigan. Alteration in differentiation of rat and rabbit limb bud micromass cultures by glutathione modulating agents.

Glutathione (GSH) accounts for more than 90% of the reducing equivalents in cells, which establishes the redox potential that controls important developmental events like differentiation, proliferation and apoptosis. Using limb bud micromass cultures from two species, Sprague-Dawley rats and New Zealand White rabbits, we were able to quantitatively determine chondrogenic foci formation in response to known GSH modulating agents, L-buthionine-S,R-sulfoximine (BSO) and diethyl maleate (DEM). On GD 13 (rat) and GD 12 (rabbit), embryos were removed, limbs were dissected, cells dissociated and plated in micromass. Cultures were incubated for 5 days and treated with BSO (0-100 µM) or DEM (0-25 µM). Differentiation was confirmed by positive staining of chondrogenic foci with Alcian blue. GSH content was determined by HPLC. Although GSH was significantly depleted by 39% and 85% in the 50 and 100 µM BSO rat cultures, respectively, rat cultures showed no significant decrease in chondrogenic foci formation. Rabbit limb bud cultures treated with BSO showed complete inhibition of chondrogenic differentiation, coupled with a significant GSH decrease of 41% (50 µM) and 80% (100 µM). With 10, 20 and 25 µM DEM concentrations, rat cultures showed a significant decrease in differentiation by 36%, 47% and 59%, respectively. In these same cultures, GSH was significantly increased to contain at least 68% more than controls. Rabbit cultures treated with 10 µM DEM showed a 74% decrease in chondrogenic foci, but 20 and 25 µM DEM cultures showed no chondrogenesis. GSH was not depleted in either the 10 or 20 µM DEM rabbit cultures, but 25 µM DEM cultures were significantly depleted by 28%. These results show selective intrinsic depletion of GSH in rat vs. rabbit limb bud cells and indicate that GSH may also affect differentiation.

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ABSTRACTS