1376 EFFECTS OF 5-AZA-2-DEOXYCYTIDINE (D-AZA) ON REPRODUCTIVE CAPACITY AND POST-NATAL DEVELOPMENT OF CD-1 MICE.

F. J. Cisneros and S. Branch. *North Carolina State University, Department of Toxicology, Raleigh, NC.*

It has been reported that d-AZA induces DNA hypomethylation and can therefore alter gene expression. The embryonic developmental toxicity of d-AZA may be mediated by this mechanism. In addition, body weight gain and post-natal development is gene dependant. It has been shown that here is a correlation between reproductive ability and body weight. The objective of this study was to evaluate the effects of 5-aza-2'-deoxycytidine (d-AZA) on reproductive capacity and post-natal development of CD-1 mice. Pregnant CD-1 mice were administered 1.0 mg/kg of d-AZA at gestation day (GD) 10. Controls were left untreated. The mice were allowed to give birth. The body weights of the resulting F1 control and treated pups were recorded at three different times points (3, 5 and 6 months of age). To evaluate the reproductive capacity, 5 month old treated F1 males and females were mated with normal females and males (Groups 1 and 2, respectively). The control group consisted of mated control males and females (Group 3). Mating pairs were housed separately for four days and the presence of plugs were recorded (plug day designated as GD 0). At gestational day 17, pregnant females were killed. Dam, uterine, and litter weights were recorded. Fecundity and teratological data were also recorded. Our data suggest that body weight differences between treated and control parents are statistically significant and were more pronounced with increased age ($p>0.01$). There was a significant difference ($p>0.01$) in number of plugs and pregnancy rates between each group compared to controls. Ten percent of group 1 females had plugs, but no pregnancies were observed. 100% of group 2 females had plugs resulting in a 75% of pregnancy rate. Forty-five percent of group 3 females presented plugs resulting in a 18.18% pregnancy rate. The reproductive data obtained suggests that reproductive capacity of d-AZA treated males is more adversely affected than that of treated females. Detailed reproductive analyses are ongoing to better characterize the male reproductive toxicity of d-AZA.

1377 TOMUDEX-INDUCED MAMMALIAN DEVELOPMENTAL TOXICITY IS PREVENTED BY CO-TREATMENT WITH FOLINIC ACID.

J. E. Andrews, J. M. Rogers, H. P. Nichols, C. S. Lau and J. E. Schmidt. *US Environmental Protection Agency, Research Triangle Park, NC.*

The thymidylate synthase (TS) inhibitor, Tomudex (TM), is a structural analogue of folate. TS inhibition has been shown to result in impaired embryonic development. We evaluated the developmental toxicity of TM in a rodent whole embryo culture (WEC) system and tested the ability of folinic acid to rescue embryos from TM toxicity. Gestational day (GD) 9.5 rat embryos were cultured for 48 hours in the presence of 0, 50, 100, 150, 250, 350 or 500 ng TM/4ml culture medium(CM). Following culture, embryos were evaluated for growth and development. Embryos exposed to ≥150 ng TM had a concentration dependent increase in incidence of open neural tubes, rotational defects, eye defects and abnormal caudal appendages, and a concentration dependent decrease in developmental score. Significant embryo lethality was observed at concentrations ≥ 350 ng TM /4ml CM. Additional embryos were cultured with 0-1100 ng TM/4ml CM and co-treated with 10 µg folinic acid/4ml CM. At the end of 48 hours, embryos were evaluated as above. All TM exposed embryos co-treated with folinic acid developed similarly to control embryos. Folinic acid co-treatment completely prevented the occurrence of malformations resulting from exposure to TM. Folinic acid alone (10 µg folinic acid /4ml CM) had no impact on embryonic development. Preliminary studies have been conducted on GD 9.5 embryos cultured with 0-950 ng TM/4ml CM for 2 hours and then transferred to CM with no TM. At the end of 48 hours of culture, TM exposed embryos were evaluated and found to be indistinguishable from control embryos suggesting that embryos can recover from short term exposure to as much as 950 ng TM/4ml CM. These data suggest that TM is developmentally toxic to the midgestational embryo and that folinic acid can competitively inhibit TM toxicity.

1378 DEVELOPMENTAL TOXICITY OF AMINOPTERIN IN *DROSOPHILA*.

D. W. Lynch. *NIOSH, Experimental Toxicology Branch, Cincinnati, OH.*

To further characterize the *Drosophila*-based prescreen to detect developmental toxicants, aminopterin (4-aminofolic acid [APN]; CAS No. 54-62-6), was studied. APN, a documented developmental toxicant and teratogen, was formerly used clinically as an antineoplastic agent. APN inhibits dihydrofolate reductase preventing the formation of folinic acid and stopping one carbon metabolism needed for the synthesis of DNA in rapidly dividing cells. Initially, 7 APN concentrations ranging from 4 - 80 µg/vial were evaluated. In a second experiment, 6 concentrations ranging from 0.01 - 2 µg/vial were utilized to determine if a threshold of developmental toxicity could be observed. Each experiment included a concurrent control. *Drosophila* were exposed throughout development (egg through third instar larva) in culture vials to medium containing APN. 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 vial and allowed to oviposit for 20 hours, 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 each experiment, the incidence of the two defects at each concentration was compared to the controls using chi-square. The incidence of wing blade notches was significantly increased (all $p< 0.001$) at 2 µg/vial, 17/138; 4 µg/vial, 20/170; 10 µg/vial, 25/96; 14.1 µg/vial, 9/81; 20 µg/vial 13/82; 28.3 µg/vial 9/43; 40 µg/vial 18/61; and at 80 µg/vial, 10/25. One wing blade notch was observed among 414 control flies. Bristle defects were not increased by APN. Mortality of the offspring was increased and the first day of emergence was delayed at the two highest APN concentrations. The results with APN 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.

1379 AMPHETAMINE-STIMULATED DOPAMINE (DA) RELEASE IN F_1 AND F_2 MALE RATS EXPOSED TO THE ESTROGENIC COMPOUND, GENISTEIN.

B. J. Gough[1], S. A. Ferguson[1], K. M. Flynn[1], K. B. Delclos[1] and R. R. Newbold[2]. *[1]National Center for Toxicological Research/FDA, Jefferson, AR and [2]NIEHS, Research Triangle Park, NC.*

Developmental steroid hormone treatment causes adverse effects; however, the potential effects of developmental exposure to endocrine disruptors such as the phytoestrogen genistein are relatively unknown. Because estradiol affects striatal DA release, it is plausible that genistein might have similar effects. Here, Sprague-Dawley rats (F_0 generation) were exposed to 0, 100, or 500 ppm genistein in a soy-free diet. Genistein treatment continued throughout the F_1 and F_2 generations. Between postnatal days (PND) 100-175, striatal microdialysis samples (DA, DOPAC, HVA, and 5-HIAA) were collected from awake males of the F_1 (n=10 for 0 ppm; n=9 for 500 ppm) and F_2 (n=7 for 0 ppm; n=4 for 100 ppm; n=7 for 500 ppm) generations for 2 hr at 20 min intervals. After the baseline period, 2 mg/kg d-amphetamine was injected ip and sample collection continued for the subsequent 3.7 hr. There were no statistically significant effects of genistein treatment on any measure. Results indicated that all rats responded similarly to amphetamine with increased DA levels, decreased DOPAC and HVA levels and only minor changes in 5-HIAA levels. These results would indicate that developmental/chronic genistein exposure has few effects on striatal DA release. (Supported by Interagency Agreement #224-93-001 between the FDA and the NIEHS.)

1380 TERATOGENIC EFFECTS OF VALPROIC ACID IN THE FETAX SYSTEM: SIMILARITIES TO EFFECTS OBSERVED IN HUMANS.

E. O'Brien, A. Prietz and D. R. Dietrich. *Environmental Toxicology, University of Konstanz, Konstanz, Germany.*

The vast number of new chemicals and pharmaceuticals which requiring safety evaluation and teratogenicity testing has lead to renewed interest from regulatory and industrial communities in the development and licensing of alternative *in vitro* teratogenesis assays. The FETAX assay has a number of advantages to offer in this respect. It is a high-throughput, rapid assay system (96h), allowing large numbers of samples to be screened for morphological, histological and biochemical effects. The assay in-corporates all the major stages of embryonic development. Since development occurs ex utero very specific interventions are possible. The relevance of the assay with regard to the detection of compounds teratogenic to mammals, specifically humans, may, however, be questioned due to species differences. Therefore, further investigation of this testing system requires using several known mammalian teratogens and non-teratogens in addition to the comparison of the results with published human epidemio-logical studies. One good candidate for such an investig

The Toxicologist

An Official Publication of the Society of Toxicology

and

Abstract Issue of

TOXICOLOGICAL SCIENCES

An Official Journal of the Society of Toxicology

Published by Oxford University Press, Inc.

Abstracts of the
39th Annual Meeting
Volume 54, Number 1
March 2000