594 COMPARATIVE DEVELOPMENTAL TOXICITY OF URETHANE (URE), 2-METHOXYETHANOL (2ME) AND METHOTREXATE (MTX) IN *DROSOPHILA MELANOGASTER* OBTAINED FROM TWO SOURCES. D W Lynch. ETB, NIOSH, Cincinnati, OH.

The *Drosophila* Bioassay developed at NIOSH has been proposed as a screening test to help establish priorities for assessment of developmental toxicity *in vivo* (Lynch et al., *Teratogenesis Carcinog. Mutagen.* 11: 147-173, 1991). An independent laboratory used Oregon-R wild-type fruit flies obtained from Carolina Biological Supply Co. (CBSC) in this published effort. In order to further characterize the bioassay, an in-house study was performed 1) to determine if flies obtained from different sources respond both qualitatively and quantitatively in the same way to selected known developmental toxicants and 2) to compare the reproducibility of bioassay data obtained at different times in different labs using the same chemicals. Concurrent bioassays using flies obtained from both CBSC and the Mid-American Drosophila Stock Center were conducted at 25°C. Three developmental toxicants URE (5 and 7.5 mg/vial), 2ME (10 and 15 mg/vial) and MTX (100 and 200 μg/vial) were evaluated in separate experiments. Both URE and 2ME, previously documented to increase the incidence of bent bristles in developing *Drosophila*, statistically increased the incidence of this defect at both concentrations and in both strains compared to concurrent controls. MTX, reported to increase the incidence of wing notches, statistically increased the incidence of this wing blade defect in both strains and at both concentrations. These data indicate that the *Drosophila* Bioassay is not dependent on fly source and that bioassay results (at least with these three known developmental toxicants) can be replicated in independent labs. These results support further utilization of this bioassay as a screening test in developmental toxicology studies.

595 ULTRAMICROFIBEROPTIC pH SENSORS FOR MEASUREMENT OF REAL TIME RESPONSES TO CHEMICAL INSULT IN INTACT, ORGANOGENESIS-STAGE RAT CONCEPTUSES. C Harris, W. Tan, B A Thorsrud and R Kopelman. Departments of Environmental and Industrial Health and Chemistry, University of Michigan, Ann Arbor, MI.

A microfiberoptic sensor has been developed to provide dynamic, real time determinations of pH in very small organisms or single cells with millisecond response times. This probe has been used to examine changes in the pH of extraembryonic fluid (EEF) in viable, cultured rat conceptuses as a function of advancing gestational age and as a result of chemical exposure. The results of this analysis confirm the report of Collins et al. (*Am. J. Physiol.* 257: R542-549, 1989) showing an acidification of EEF from gestational day (GD) 10 through GD 12. Mean pH values of EEF decreased from 7.53 ± 0.07 on GD 10 (10-16 somites) to 7.37 ± 0.07 (22-26 somites) and 7.26 ± 0.06 (32-34 somites) on GDs 11 and 12, respectively. An embryotoxic dose of the thiol oxidant diamide (500 μM) produced an additional rapid acidification of EEF in the GD 12 conceptus. Values decreased 0.18 pH units within 30 sec of exposure and continued to decline to a final value of 6.87 over the subsequent 3 min. A slight alkalinization was seen at 3-5 min after exposure but pH values failed to recover to original GD 12 levels over 20 subsequent min of monitoring. This sensor demonstrates the ability to monitor dynamic, real time alterations of pH in intact, viable rat conceptuses *in vitro*. It will be useful in investigating the role of changing pH in developmental regulation and in studies of mechanisms of developmental toxicity. (Supported by DOE grant DE-FG02-90-ER 61085, NIH grants ES05235 and ES07062 and an OVPR grant from the Univ. of Michigan).

596 EFFECT OF CHRONIC DOXORUBICIN TREATMENT ON CARDIAC MITOCHONDRIAL CALCIUM HOMEOSTASIS. L E Solem and K B Wallace. Toxicology Graduate Program, Dept. of Pharmacol., University of Minnesota, Duluth, MN.

Doxorubicin (DXR) disrupts mitochondrial Ca^{2+} homeostasis in vitro by selectively activating the cyclosporine A (CyA)-sensitive Ca^{2+} release channel. The objective of this investigation was to determine whether a similar effect is manifested following chronic DXR treatment. Mitochondrial respiration was monitored using a Clark-type oxygen electrode, Ca^{2+} accumulation was determined with arsenazo III, and membrane potential ($\Delta\psi$) was assessed using rhodamine 123 in cardiac mitochondria isolated from rats treated with 2mg/kg/week (s.c.) DXR (DXR-mito) or 5-iminodaunorubicin (IDR-mito) for 13 weeks. DXR-mito exhibited a decreased RCR but no change in the ADP:O ratio compared to saline control. Compared to controls, DXR-mito accumulated less Ca^{2+} and released that Ca^{2+} which was accumulated back into the medium following a short latency period. These effects of DXR on mitochondrial Ca^{2+} accumulation and retention were reversed by CyA. Finally, whereas saline and IDR-mito were able to maintain mitochondrial $\Delta\psi$, DXR-mito demonstrated a Ca^{2+}-dependent depolarization of $\Delta\psi$, which was reversed by adding either CyA or ruthenium red. In all cases, IDR-mito were not different from saline controls. These data demonstrate that selective activation of the CyA-sensitive Ca^{2+} channel by DXR is manifested in cardiac mitochondria following chronic drug treatment. Furthermore, this event may sensitize the mitochondria to Ca^{2+}-dependent depolarization of $\Delta\psi$ and interference with mitochondrial respiration. (Supported by The American Heart Association, Minnesota Affiliate).

597 EPR EVIDENCE FOR THE GENERATION OF REACTIVE OXYGEN FROM THE COPPER-MEDIATED OXIDATION OF 1,4-HYDROQUINONE (HQ). Y Li, P Kuppusamy, J L Zweier, and M A Trush. The Johns Hopkins Medical Institutions, Baltimore, MD.

It has been previously demonstrated that oxidation of HQ by Cu(II) results in plasmid DNA cleavage. In this study, using EPR spectroscopy we have investigated whether this chemical-metal redox system can generate reactive oxygen species which induce DNA damage. In order to set the stage for the EPR experiments and the inhibitors to be used in these experiments, some preliminary O_2 consumption and plasmid DNA cleavage experiments were performed. Mixing 100 μM HQ with 10 μM Cu(II) in PBS resulted in a marked consumption of O_2 and the concomitant generation of H_2O_2, and extensive DNA degradation in øX-174 RFI DNA. The presence of SOD or mannitol did not affect either the O_2 consumption, H_2O_2 generation or DNA damage. In contrast, the Cu(I) chelators, bathocuproinedisulfonic acid (BCS) and GSH, extensively inhibited the HQ/Cu(II)-mediated O_2 consumption and DNA damage. The presence of catalase also prevented the DNA damage. Although the HQ/Cu(II)-mediated O_2 consumption increased in the presence of azide, azide markedly inhibited the HQ/Cu(II)-induced H_2O_2 formation and DNA degradation. POBN-spin trapping experiments showed that the interaction of HQ with Cu(II) produced POBN-CH_3 and 4-POBN-CH(OH)CH_3 adducts in the presence of DMSO and ethanol, respectively, demonstrating that hydroxyl radical and/or another oxidizing intermediate are generated from the HQ/Cu(II) system. The presence of catalase, BCS or GSH but not SOD completely prevented the formation of the POBN-CH_3 adduct from the HQ/Cu(II) plus POBN/DMSO system. Anaerobic conditions induced an 80% decrease in the POBN-CH_3 adduct. The formation of the POBN-CH_3 adduct was also significantly inhibited by azide but not by mannitol. The above results indicate that the copper-mediated oxidation of HQ generates reactive oxygen, which participates in DNA damage.

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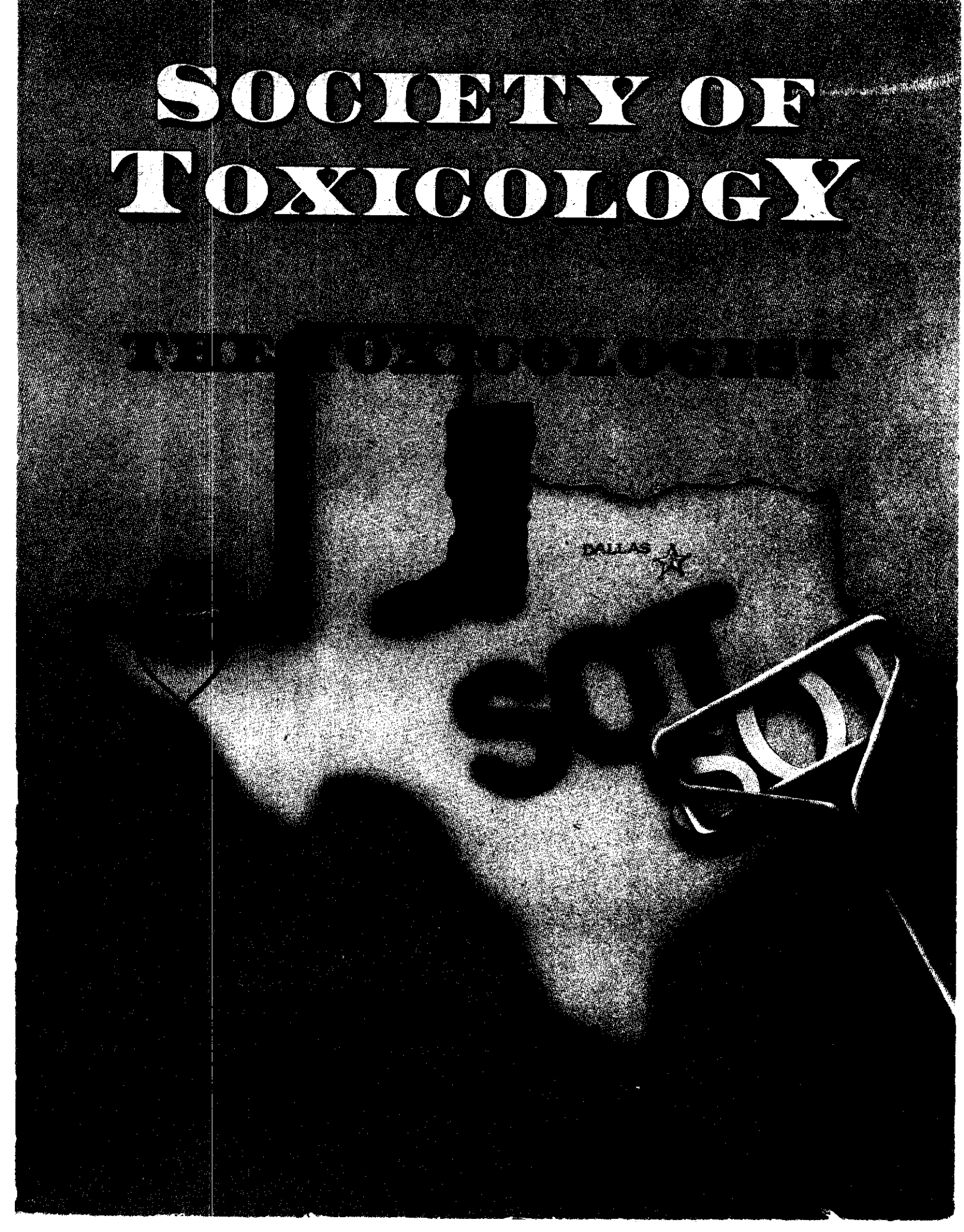

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Preface

This issue of *The Toxicologist* is devoted to the abstracts of the presentations for the symposium, platform, poster/discussion, and poster sessions of the 33rd Annual Meeting of the Society of Toxicology, held at the Loews Anatole Hotel, Dallas, Texas, March 13-17, 1994.

An alphabetical Author Index, cross-referencing the corresponding abstract number(s), begins on page 439.

The issue also contains a Keyword Index (by subject or chemical) to the titles of all the presentations, beginning on page 467.

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