the case of malformation, the use of 20 eggs per replicate resulted in a marked reduction in CI size. Additional known and unknown teratogens (6-aminonicotinamide, ethanol, valproic acid and diclofenac) were tested in order to confirm the preliminary findings with caffeine. In comparison to the FETAX assay [ASTM E 1439-91], an established embryotoxicity and teratogenesis assay using South African clawed frog (*Xenopus laevis*) embryos, DRETA appeared to have a higher experimental robustness in terms of animal maintenance, handling, egg production, egg quality, low spontaneous mortality and malformation and high reproducibility of results.

1280 OPTIMIZING THE EXPERIMENTAL DESIGN OF THE FETAX ASSAY.

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The experimental design recommendations of the ASTM E 1439-91 Standard Guide for Conducting the Frog Embryo Teratogenesis Assay-*Xenopus* (FETAX) were evaluated using statistical power analysis. FETAX is an 96 hour *in vivo*, whole embryo, developmental toxicity test using the South African clawed frog, *Xenopus laevis*, and is currently employed as an alternative assay for the routine detection of teratogenic compounds. Power analysis of the current protocol indicated that the recommended minimum of 3 concentrations for the definitive test can lead to questionable results. There is less than a 60% chance that 3 concentrations could produce an 0.80 correlation coefficient with $p < 0.05$. Present results indicated that a minimum of 5 concentrations (2 replicates) were required for an 80% chance of obtaining a correlation coefficient of 0.80 with $p < 0.05$. In addition to the number of concentrations needed, the spacing is crucial in obtaining narrow confidence intervals for LC50 or EC50 estimates. Five substances (6-aminonicotinamide, caffeine, diclofenac, ethanol and valproic acid) were used to demonstrate the effectiveness of a new strategy for selecting the appropriate range and spacing of concentrations. This "percent effect" based strategy provides an 80% chance of consistently obtaining reliable LC50 or EC50 estimates.

1281 TOXICITY OF THREE NITRO-MUSKS IN EARLY LIFESTAGES OF *XENOPUS LAEVIS*.

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Among the natural musk substitutes, musk xylene (MX; 1-tert-butyl-3,5-dimethyl-2,4,6-trinitrobenzene), musk ketone (MK; 4-tert-butyl-3,5-dinitro-2,6-dimethylacetophenone) and musk moskene (MM; 1,1,3,3,5-pentamethyl-4,6-dinitroindane)) are three of the most important nitro-containing synthetic fragrances. As benzene derivatives with various nitro side groups, these compounds exhibit very low bio-degradation rate, high lipophilicity and relatively high BCFs. Consequently, these compounds, especially MX and MK, have been detected ubiquitously in global surface water including the ocean, and have been also detected in various aquatic organisms including fish, shellfish and shrimps. To date little data is available as to the toxicity of these nitromusks to early-life-stages of fish and amphibians. To investigate the potential developmental toxicity posted by these three compounds to amphibians, *Xenopus laevis* embryos were exposed to nominal concentrations of 4, 40 and 400 µg/l of MK and MM, respectively, while exposure to MX was carried out only at 400 µg/l. Exposures started at 4 hours after fertilization and continued up to 11 days (static renewal every 24h). Feeding of the embryos started after 5 days exposure. A significantly increased mortality rate was observed for all three compounds, however only at the highest dose (400 µg/l) tested. However, the mortality was only increased two-fold over the control. No differences were observed between the 3 compounds tested. No treatment related developmental effects (malformation) or growth inhibition were observed with any compound.

1282 PROTECTIVE ROLE OF FOLINIC ACID ON THE DEVELOPMENTAL TOXICITY OF METHOTREXATE IN INTACT *DROSOPHILA*.

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Methotrexate (MTX), a structural analogue of folic acid and a known developmental toxicant, is used clinically as a chemotherapeutic agent and as a treatment for psoriasis and rheumatoid arthritis. MTX inhibits dihydrofolate reductase, preventing the formation of folinic acid (FA; tetrahydrofolate) and stopping one carbon metabolism needed for the synthesis of DNA in rapidly dividing cells. To determine if exogenous FA could mitigate the developmental toxicity of MTX in developing fruit flies, the effect of co-administration of FA on the incidence of wing notches induced by MTX was evaluated. *Drosophila* were exposed throughout development (egg through third instar larva) in culture vials to medium containing 10 µg/vial MTX with or without FA. 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 (Carolina Biological Supply Co.) was added to each culture vial and allowed to oviposit for 20 hours, then removed. Emerging offspring were collected over 10 days, and examined microscopically (25×) for wing blade notches, a morphological defect shown to occur with an increased incidence in flies exposed to developmental toxicants. The incidence of wing notches was statistically increased (64/137; $p<0.001$) by MTX alone and by MTX plus 10 µg/vial FA (103/208; $p<0.001$). However, no wing notches were found in flies treated with MTX plus 50 µg/vial FA (0/271) nor in the concurrent control (0/218). Mortality was significantly decreased ($p<0.001$) in both groups of flies treated with MTX plus FA compared to the MTX alone group. These results parallel the protective effects of folinic acid analogues on the developmental toxicity of MTX reported in mammals. Furthermore, they demonstrate an addition capability of this *Drosophila*-based assay to investigate mechanisms of developmental toxicity in addition to its proposed role as a prescreen.

1283 ACUTE TOXICITY OF BENZENE ON LARVAE OF JAPANESE MEDAKA (ORYZIAS LATIPES).

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Environmental pollutants are known to elicit adverse effects in man, animals and aquatic organisms. Benzene is a known human carcinogen. With its widespread use, there is a high potential for large amounts of benzene to be released to the environment. Since eggs and larvae are generally the stages in life most sensitive to environmental stress, experiments were conducted to study the effects of benzene on larvae of Japanese medaka. Fertilized eggs obtained from Gulf Coast Research Laboratory, Ocean Springs, MS were reared in our laboratory in a rearing medium at 25°C. Fry hatched on day 12 were collected and 24 hours post-hatch larvae were used for the experiments in a static closed system without renewal. The exposure chamber consisted of a 125-ml Teflon capped bottle. 100 ml of the medium, with various test concentrations (0, 2.5, 5.0, 7.5, 10.0, 12.5 and 15 mg/L) of benzene, were filled in the bottle and 10 fry were placed in each exposure chamber. Samples withdrawn at the beginning and the end of 168 hours were analyzed on a gas chromatograph to verify the benzene concentration in the test chamber. The result showed that all the 10 fry survived at 0 and 2.5 mg/L, 8 fry survived at 5 mg/L, 4 survived at 7.5 mg/L and all of them died at 10, 12.5 and 15 mg/L. Based on the survival of the fry LC_{50} was calculated to be approximately 7 mg/L. Supported by MHPF/ATSDR grant # U50/ATU 398948.

1284 PATHOGENESIS OF HALOACETIC ACID-INDUCED EMBRYOTOXICITY IN MOUSE WHOLE EMBRYO CULTURE.

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Haloacetic acids (HAs) are embryotoxic contaminants commonly found in chlorinated drinking water. Several HAs inhibit protein kinase C (PKC) activity in the neurulation-stage mouse embryo; this inhibition may be in part responsible for their embryotoxic effects. This study was conducted to evaluate the pathogenesis of HA-induced malformations during mouse neurulation, and to compare these data with those from a known PKC inhibitor (Bis I). Dichloro-, dibromo-, and bromochloroacetate and Bis I were administered to CD-1 mouse embryos (3–6 somite staged) in culture for varying lengths of time. Cell cycle analysis was performed by flow cytometry following nuclear staining with propidium iodide; apoptosis was examined by staining embryos with Lysotracker (LT) and visualizing with fluorescence and/or confocal microscopy. At concentrations producing 100% embryotoxicity with no consequent embryolethality, neither Bis I nor the HAs perturbed the cell cycle. However, flow cytometry revealed significant accumulation of sub-G1 particles (an indicator of cell death) across time with HA, but not Bis I, treatment. Sub-G1 particles were particularly prominent in the head region, and remained at control levels in the heart. LT staining confirmed a similar pattern of apoptosis in the intact embryo. The HAs produced intense

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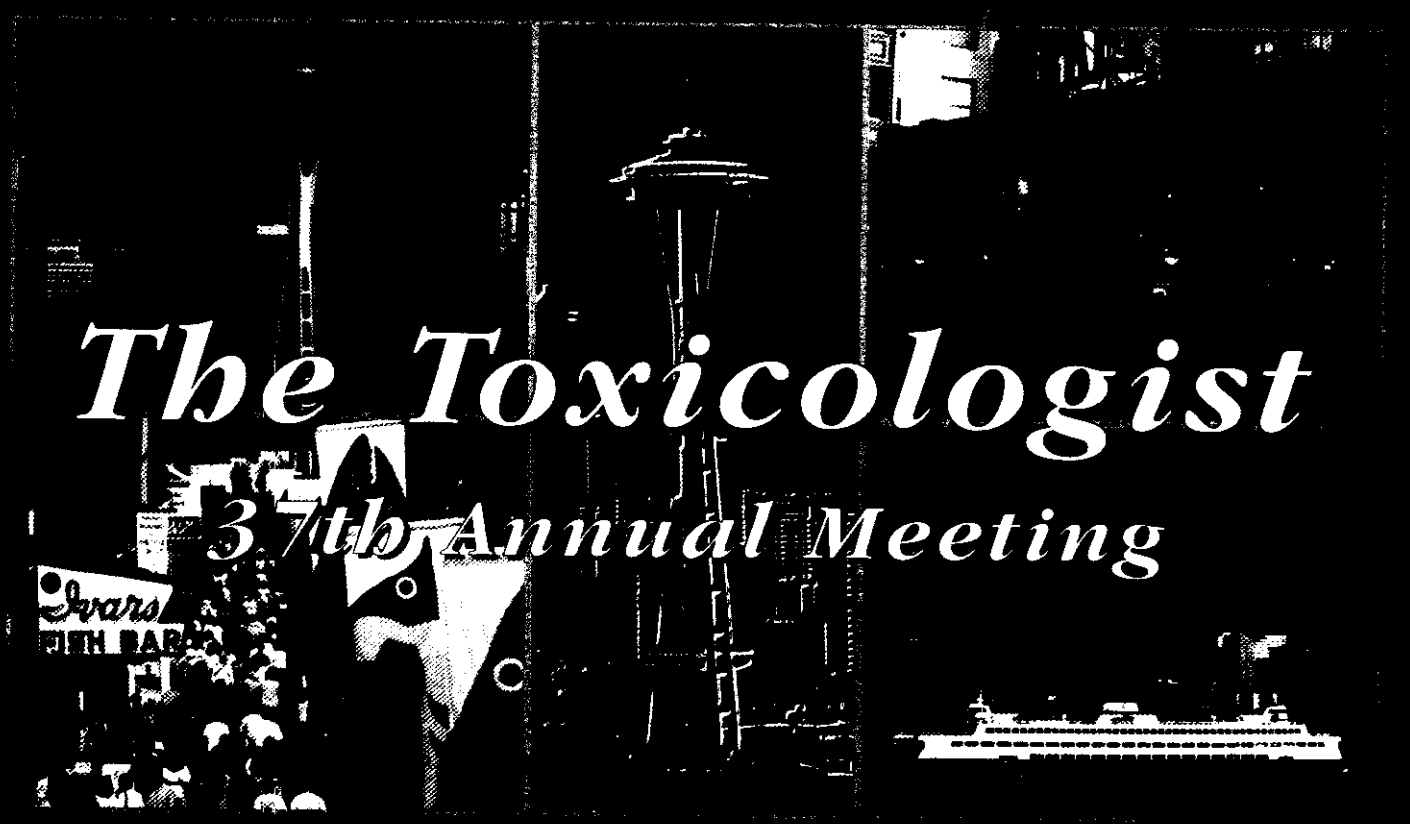

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Preface

This issue of *The Toxicologist* is devoted to the abstracts of the presentations for the symposium, platform, poster / discussion, workshop, roundtable, and poster sessions of the 37th Annual Meeting of the Society of Toxicology, held at the Washington State Convention Center, Seattle, Washington, March 1-5, 1998.

An alphabetical Author Index, cross referencing the corresponding abstract number(s), begins on page 407.

The issue also contains a Keyword Index (by subject or chemical) of all the presentations, beginning on page 433.

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