crystals in the nephrons. In previous experiments we evaluated the dose-response for dietary co-exposure to MEL and CYA for 7-days in F344 rats. We report here the dose-response obtained after a period of 28-days in F344 rats using comparable experimental conditions. Rats were provided feed fortified with 0 (control), 30, 60, 120, 180, 239, or 359 ppm of melamine and cyanuric acid, comparable to approximately 2.5, 5, 10, 15, 20 and 33 mg/kg bw. As assessed by histopathology, the lowest dose that produced alterations was 120 ppm, versus 229 ppm in the 7-day study. Wet-mount analysis of kidney sections, however, demonstrated the formation of melamine cyanurate spherulites in 1 of 24 rats at the 30 ppm dose and in 3 of 23 rats at the 60 ppm dose. Clinical chemistry and body weight were less sensitive endpoints. While the determination of a specific NOAEL or TDI from this study is a function of the endpoints considered, these data demonstrate that the length of exposure plays a role in the definition of a threshold of toxicity for these compounds, a fact that should be taken into consideration when conducting future risk assessments. Funded in part by NTP IAG 224-07-0007 between the FDA/NCTR and the NIEHS/NTP.

PS 2421 A 90-DAY DIETARY TOXICITY STUDY OF R, R-MONATIN SALT, A NATURAL HIGH POTENCY SWEETENER, IN RATS.

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The root bark of Schlerochitin ilicifolius, a plant native to South Africa, contains an intensely sweet substance identified as R,R-monatin. While S. ilicifolius has been consumed by indigenous populations historically, the toxicity profile of R,R-monatin is generally unknown. To contribute data relevant to evaluation of the safety of this natural sweetener for use in food, the subchronic toxicity of sodium/potassium (2R, 4R)-2-amino-4-carboxy-4-hydroxy-5-(3-indolyl) pentanoate, otherwise R,R-monatin salt, was assessed in Sprague-Dawley rats. Male and female rats (20 animals/sex/dose) were exposed to concentrations of 0, 5,000, 10,000, 20,000 or 35,000 ppm (0, 0.5, 1.0, 2.0 or 3.5% w/w) R,R-monatin salt in the diet for 91 consecutive days. Functional observational battery and motor activity assessments revealed no test article-related alterations. There were no toxicologically relevant clinical, macroscopic, or histopathological findings in any of the test article-treated groups. However, mean body weights in the 35,000 ppm group males and females were 7% and 12% lower (statistically significant), respectively, than the control group at study week 13. In the absence of other observations associated with systemic toxicity and lower food consumption, the magnitude of the body weight difference in the 35,000 ppm group females relative to the control group exceeded 10%, which is generally considered to be an adverse finding. Therefore, based on the results of this study, the no observed adverse effect level (NOAEL) for oral (dietary) administration of R,R monatin salt to female rats for a minimum of 91 consecutive days was 20,000 ppm (equivalent to an exposure level of 1,544 mg/kg/day). In the absence of evidence of systemic toxicity following dietary administration of R,R monatin salt to male rats for a minimum of 91 consecutive days, the NOAEL was 35,000 ppm (equivalent to an exposure level of 2,368 mg/kg/day), the highest dietary concentration used in this study.

PS 2422 EVALUATION OF THE HYPERSENSITIVITY POTENTIAL OF ALTERNATIVE BUTTER FLAVORINGS: ARE THEY SAFE SUBSTITUTES FOR DIACETYL?

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Concern has been raised over the potential association of diacetyl, a chemical used in the production of butter flavorings, with lung disease clinically resembling bronchiolitis obliterans in food manufacturing workers. This has resulted in the need for identification of alternative chemicals to be used in the manufacturing process. We have previously shown diacetyl to be a dermal irritant and sensitizer (EC3 1.9) when tested in a combined murine local lymph node assay (LLNA). Structurally similar chemicals, 2,3-pentanedione 2,3-hexanedione 3,4-hexanedione and 2,3-heptanedione, used as constituents of synthetic flavoring agents have been suggested as potential alternatives for diacetyl. Immunotoxicity data on these chemicals are limited. The present study evaluated the dermal irritation and sensitization potential of diacetyl alternatives using a murine model. None of the chemicals were identified as dermal irritants when tested at concentrations up to 50%. Similar to diacetyl, concentration-dependent increases in lymphocyte proliferation were observed following exposure to all four chemicals, with calculated EC3 values of 15.4% (2,3-pentanedione), 18.2% (2,3-hexanedione), 15.5% (3,4-hexanedione) and 14.1% (2,3-heptanedione). No biologically significant elevations in total serum IgE were identified, however significant elevations in local IgE (IgE+B220+ lymphocytes), IL-4, and INF-gamma production by stimulated draining lymphocytes were observed after exposure to 25-50% concentrations of these chemicals. These results demonstrate the potential for development of hypersensitivity responses to these proposed alternatives and raise concern about their use as a replacement for diacetyl as flavoring agents.

PS 2423 BEAUVERICIN-INDUCED OXIDATIVE STRESS IN CHO-K1 CELLS IS PREVENTED BY VITAMINS AND FLAVONOIDS.

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Mycotoxins affect cellular processes including inhibition of cell proliferation, oxidative stress and lipid peroxidation. The hypothesis that vitamin C, vitamin E, quercetin and pterostibene could prevent the mycotoxins-induced oxidative stress in the Chinese Hamster Ovary (CHO-K1) cells was investigated. The CHO-K1 cells exposed to the emergent beauvericin (BEA) mycotoxin (0.5 and 1.0μM for 24h) were simultaneously treated with vitamins C and E, quercetin and pterostilbene (2.5-50μM). MTT was used as indicator of cell viability, and determination of malonedialdehyde (MDA)– thiobarbituric acid (TBA) adduct was signed to determine lipid peroxidation. BEA cytotoxic effect was quantified as the concentration required for inhibiting cell growth by 50%(IC50). The IC50 value of BEA in CHO-K1 cells incubated for 24h was 12.08±1.10μM. Moreover, BEA increased significantly the production of MDA after 24h of treatment with 0.5 and 1μM (≈45%). However, when BEA and antioxidant are present simultaneously in cell culture, this will boost antioxidant activity. Vitamins were effective on decreasing MDA production at the lower concentration tested but not at the higher. On the other hand, pterostilbene shows protective effect only at lowest concentration tested, while quercetin showed it at all the concentrations. These results could be due to the ultimate balance between potentiating and protective effects may depend on the subtle redox equilibrium within the cells and the balance within the complete cellular antioxidant network. The results suggest that BEA exposure on CHO-K1 cells resulted in cytotoxicity and free-radical or other reactive species-mediated cell damage in a dose-dependent way. Oxidative stress was prevented by simultaneous treatment with vitamins and flavonoids. Quercetin was the most effective during BEA exposure. Supported by Conselleria de Sanitat (EVES2010-028) and Science and Innovation Spanish Ministry (AGL2010-17024/ALI)

PS 2424 SIMULTANEOUS DETERMINATION OF TYPE B TRICHOTHECENE MYCOTOXINS AND THEIR DERIVATIVES IN WHEAT BY LC/MS/MS.

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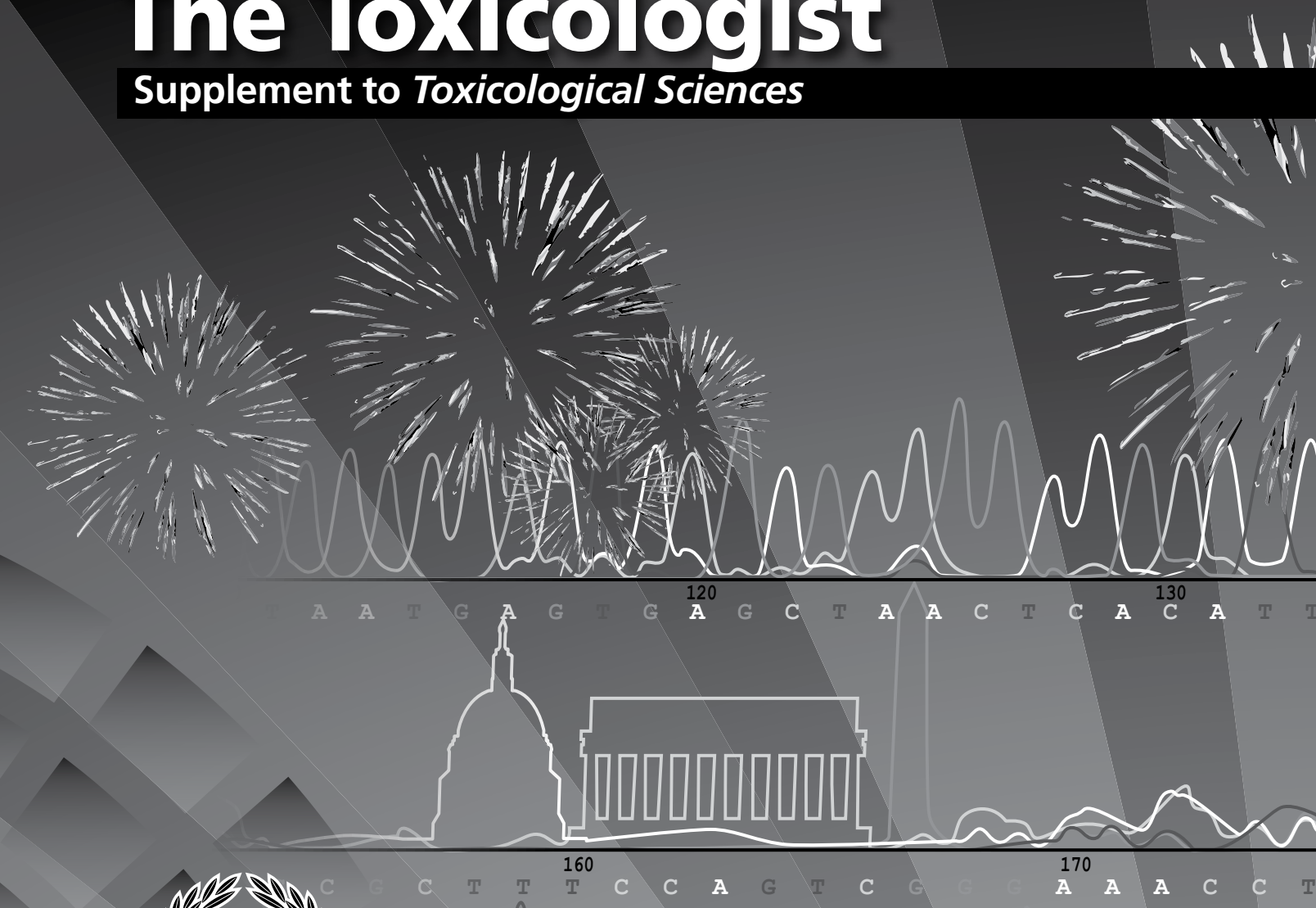
Type B trichothecene mycotoxins are toxic metabolites produced by Fusarium fungi that contaminate agricultural crops. Trichothecenes are a large family of sesquiterpene mycotoxins and many derivatives are present in the biosynthetic pathway. There are concerns that these derivatives have toxicity and reside at high concentrations in agricultural products. In this study, we developed a LC/MS/MS method for the determination of type B trichothecene mycotoxins and their derivatives in wheat. Derivatives of type B trichothecene, isotrichodermin, calonectrin (CAL), 3-deacetyl-CAL, 15-deacetyl-CAL, 3,15-diacetyl-NIV, 4,15-diacetyl-NIV, 3,15-diacetyl-DON, and 3,4,15-triacetyl-NIV were purified from trichothecene blocked mutants. DON, NIV, 3-acetyl-DON, 15-acetyl-DON, fusarenon-X and DON-3-glucoside standards were prepared from the commercial products. Wheat samples were extracted with acetonitrile/water/acetic acid (85:14:1) and the extract was treated with multifunctional column. The identification and determination of each trichothecene were achieved by LC/MS/MS system. Chromatographic separation of each toxin was performed by using a C18 column with a linear gradient of methanol and water contained 10 mM ammonium acetate. Trichothecene mycotoxins were purified with multifunctional column cleanup procedure, and good recoveries ranging 84.3–110% and RSDs (0.4–7.2%) were obtained in recovery tests. The limit of detection and quantification in wheat were 0.03–1.4 and 0.1–4.7 ng/g, respectively. The contaminated wheat samples inoculated by fungi in the field were measured for the screening of trichothecenes and their derivatives. The results revealed that derivatives and metabolite (DON-3-glucoside) were detected along with DON and NIV. Accordingly, LC/MS/MS method developed in this study is a practical method for the determination of type B trichothecene mycotoxins and their derivatives.

The Toxicologist

Supplement to *Toxicological Sciences*



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An Official Journal of the Society of Toxicology

OXFORD UNIVERSITY PRESS

ISSN 1096-6080
Volume 120, Supplement 2
March 2011

www.toxsci.oxfordjournals.org

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Preface

This issue of *The Toxicologist* is devoted to the abstracts of the presentations for the Continuing Education courses and scientific sessions of the 50th Annual Meeting of the Society of Toxicology, held at the Walter E. Washington Convention Center, March 6–10, 2011.

An alphabetical Author Index, cross referencing the corresponding abstract number(s), begins on page 578.

The issue also contains a Key Word Index (by subject or chemical) of all the presentations, beginning on page 606.

The abstracts are reproduced as accepted by the Scientific Program Committee of the Society of Toxicology and appear in numerical sequence.

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