149 SEASONAL UV DOSE AND DNA REPAIR CAPACITY PREDICT NON-MELANOMA SKIN CANCER RISK.

J. L. Matta[1], A. Ruiz[1], R. A. Armstrong[2], Y. Detres[2] and J. M. Ramos[1].
[1]Pharmacology and Toxicology, Ponce School of Medicine, Ponce, Puerto Rico and [2]Marine Sciences, University of Puerto Rico, Mayaguez, Puerto Rico.

Non-melanoma skin cancer (NMSC) is the most common cancer worldwide. UV radiation is an important risk factor for NMSC. UVA (320-400 nm) and UVB (280-320 nm) can cause DNA damage and mutations. Cancer risk is partly determined by the capacity of the body to repair DNA damage. DNA repair capacity (DRC) has been reported as an independent risk factor for the development of NMSC. A 3-year clinical study was performed in Puerto Rico to develop a model of how environmental factors (e.g. UV exposure and sunblock) and genetics (e.g. DRC and skin type) influence the risk of NMSC. UVA and UVB measurements were obtained over a 5-year continuous period using a Biospherical Instruments GUV-511 radiometer. All participants (n=550) completed an Informed Consent and a questionnaire that elicited information on risk factors. DRC was measured in peripheral blood lymphocytes using a host cell reactivation assay with luciferase reporter gene. NMSC risk factors were determined by logistic regression analysis. Monthly means of daily UV radiation fluxes ranged from 569 to 1504 kJ m-2 d-1. UV fluxes were 24% higher from March-September when compared with the October-February period. Persons with NMSC had a statistical significant lower DRC (42%) compared with controls. Significant risk factors ($p<0.005$) included a minimal of 3-hour weekly cumulative UV dose (OR=2.0, 95% CI=1.2-3.3), skin type I and II (OR=8.5, 95% CI=5.2-13.9), and the use of sunblock (OR=0.16, 95% CI=0.10-0.26). A high DRC (highest 10%) reduced NMSC risk 7-fold and a low DRC (lowest 33%) increased risk by 3.4-fold. Overall, for every 1% decrease in DRC, the risk of developing NMSC increased 20%. The model presented illustrates how genes-environment-disease interact to influence NMSC risk. Genetic factors are more important than environmental factors for the development of NMSC in the population studied. These findings may be useful for NMSC prevention programs. Supported by NIH-NCRR grant 2G12RR03050-19.

150 ALTERATION OF CHEMOTHERAPEUTIC-INDUCED DNA DAMAGE BY A COMMON HEALTH FOOD SUPPLEMENT.

W. Trinachartvanit[1], B. M. Francis[2] and A. Rayburn[3]. *[1]Animal Biology, University of Illinois, Urbana, IL, [2]Entomology, University of Illinois, Urbana, IL and [3]Crop Sciences, University of Illinois, Urbana, IL.*

In the US, use of health food supplements has increased rapidly in the last decade, especially in cancer patients. However, recent concerns have been raised as to whether specific health food supplements taken during chemotherapy interact with chemotherapeutic agents. If interactions occur, they could enhance the action of chemotherapy or interfere with it by decreasing the conventional medication efficacies. Our aim was to investigate any potential interactions between an over-the-counter health food supplement, Fruit of LifeTM (which is advertised to consist of highly active antioxidants) and a chemotherapeutic agent, cytosine arabinoside (ara-C). Outbred CD1 mice were fed a semi-synthetic diet, supplemented with 0% (control), 0.2% (low dose) or 1.0% (high dose) of Fruit of LifeTM, for 4 weeks. Ara-C at 8 mg/kg was administered ip to half of the control and treated mice, 72 hours prior to the end of the feeding period. Prior studies had demonstrated that this dose of ara-C damages DNA in bone marrow without causing acute illness in the mice. Bone marrow was collected from all treatment groups and flow cytometry was used to determine DNA damage to the bone marrow cells. There were no significant differences in DNA damage between negative control mice and mice fed with 0.2% and 1.0% Fruit of LifeTM. Combined treatments of ara-C with either 0.2% or 1.0% Fruit of LifeTM significantly decreased the DNA damage level compared to ara-C treated mice on the unsupplemented diet. These results suggest that the health food supplement of interest (Fruit of LifeTM) interacts with the chemotherapeutic agent (ara-C) to decrease DNA damage caused by ara-C to normal bone marrow cells. Future studies are needed to determine how Fruit of LifeTM may alter the effectiveness of ara-C during chemotherapy. *This work was supported by a grant from the Office of the Attorney General of the State of Illinois.*

151 MODE OF ACTION FOR THE *IN VITRO* MUTAGENICITY OF BIOBAN CS-1246 AND IMPLICATIONS FOR ITS *IN VIVO* MUTAGENIC POTENTIAL.

B. B. Gollapudi[1], G. Charles[1], M. R. Schisler[1], M. Cifone[2], R. A. Budinsky[1] and P. J. Spencer[1]. *[1]The Dow Chemical Company, Midland, MI and [2]Covance Labs, Vienna, VA.*

The biocidal agent, 7-ethyl bicyclooxazolidine (Bioban CS-1246, CAS# 7747-35-5) was concluded to be non-mutagenic in a bacterial reverse mutation assay using Salmonella typhimurium strains TA98, TA100, TA1535, TA1537 and TA1538, and in an *in vitro* cytogenetics assay in Chinese hamster ovary cells both in the presence and absence of Aroclor-induced rat liver S-9. However, Bioban CS-1246 induced a mutagenic response in the mouse lymphoma (L5178Y TK+/-) forward mutation assay (MLA) both with and without S-9. Significantly, the mutagenicity was completely abrogated when the cultures were supplemented with formaldehyde (FA) dehydrogenase/NAD+ suggesting that the positive MLA response was attributable to the generation of FA in situ. The MLA findings are unlikely to be of relevance to an intact animal because of the efficient detoxification of low doses of FA *in vivo*. Evidence of this was obtained by the non-genotoxicity of Bioban CS-1246 in 2 *in vivo* assays. Firstly, in a mouse (male CD-1) bone marrow micronucleus test (MNT), in which there were no significant increases in micronucleated polychromatic erythrocytes following treatment with 0.5, 1 and 2 g/kg/day for 2 days and sacrificed 24 h later. Also, in an *in vivo*/*in vitro* unscheduled DNA synthesis (UDS) study, male F344 rats were given a single oral gavage at 0, 1 and 2 g/kg of CS-1246. Livers were perfused at 2 time points (2-4 and 14-16 h) and the extent of UDS was quantified in hepatocyte cultures after exposure to 3H-thymidine and autoradiography. CS-1246 did not elicit an UDS response indicating the lack of DNA reactivity *in vivo*. Based upon the weight of evidence from an extensive array of genotoxicity tests and the mode of action data, it is concluded that CS-1246 is not an *in vivo* mutagen. These results clearly demonstrate that *in vitro* genotoxicity of CS-1246 is associated with FA and unlikely to have relevance in animals or humans because of the occurrence of efficient detoxification mechanisms.

152 GENOTOXICITY EVALUATION OF THIODIGLYCOLND.

G. Reddy, M. A. Major and G. J. Leach. *Directorate of Toxicology, US Army Center for Health Promotion and Preventive Medicine, Aberdeen Proving Ground, MD.*

Sulfur mustard (HD) undergoes hydrolysis to form thiodiglycol (TG) and small amounts of compounds in biological and environmental systems. The US Army has proposed to neutralize HD through a hydrolysis process with subsequent biodegradation of reaction products. TG has been detected in soil and water at certain Army installations. The toxicity data on TG are limited to only a few reports. Genotoxicity data on TG are not available to complete health and environmental risk assessments. Therefore, we developed genotoxicity data under extramural contract. These tests were conducted in accordance with Environmental Protection Agency Health Effects Testing Guide Lines in compliance with Good Laboratory Practice. The summary of test results of completed work will be reported here. Thiodiglycol did not produce mutagenic effects at any dose up to 5000 mg/plate in Salmonella typhimurium (TA98, TA100, TA1535 and TA1537) and Escherichia coli (WP2uvrA) tester strains with and without a metabolic activation system. TG did not increase mutantion frequency at levels up to 5000 mg/ml in mouse lymphoma L5178ytk+/- cells with and without metabolic activation. The effects of TG on *in vitro* chromosomal aberrations in Chinese Hamster Ovary (CHO) cells showed chromosomal aberrations at 5 mg/ml (without metabolic activation) and 4 mg/ml with metabolic activation. Thiodiglycol (up to 2000 mg/kg) when tested *in vivo* in the mouse micronucleus assay was not mutagenic in mouse bone marrow. These studies revealed that TG is not mutagenic in three assays (Ames test, mouse lymphoma and mouse micronucleus assay) but it positive *in vitro* in CHO cells chromosomes. On the basis of these studies we believe that TG does not appear to pose a genetic hazard to humans (Abstract does not reflect US Army Policy)

153 DNA DAMAGE IN HUMAN LEUKOCYTES INDUCED *IN VITRO* BY 1- OR 2- BROMOPROPANE.

M. Toraason[1], N. P. Singh[2] and D. W. Lynch[1]. *[1]NIOSH, Cincinnati, OH and [2]University of Washington, Seattle, WA.*

1-Bromopropane (1-BP; n-propyl bromide) (CAS No. 106-94-5) is an alternative to ozone-depleting chlorofluorocarbons that has a variety of potential applications as a cleaning agent for metals and electronics, and as a solvent vehicle for spray adhesives. Its analogue, 2-brompropane (2-BP; isopropyl bromide) (CAS No. 75-26-3) impairs antioxidant cellular defenses, enhances lipid peroxidation, and causes DNA damage *in vitro*. In the present study, DNA damage was assessed in human leukocytes exposed *in vitro* to 1-BP or 2-BP at 0, 0.01, 0.1, or 1 mM for 8 hr; or at 1 mM for 1, 2, 4 or 8 hr. Exposures to 1-BP or 2-BP were performed in triplicate samples of fresh heparinized venous blood from an adult male volunteer. For estimation of DNA damage, comets were produced by alkaline microgel-electrophoresis. In each sample, DNA damage was estimated in a minimum of 100 leukocytes using VisComet image analysis software. Apoptosis was assessed in leukocytes exposed to 1-BP or 2-BP using the DNA diffusion assay. 1-BP or 2-BP induced a significant increase in comet tail moment at 1 mM but not at 0.01 or 0.1 mM. DNA strand break number was significantly increased at 0.1 and 1 mM of 1-BP or 2-BP. In temporal studies, a significant increase in DNA damage was evident 1, 2, 4, or 8 hrs after exposure to 2-BP, whereas increased DNA damage was observed only after 4 or 8 hrs of exposure to 1-BP. Significant increases in apoptosis were evident after

exposure to 0.1 or 1 mM 2-BP, but only after exposure to 1 mM 1-BP. Results demonstrate the potential for 1-BP and 2-BP to induce DNA damage *in vitro* in human leukocytes.

154 GENOTOXICITY EVALUATION OF THE CHLOROHYDRIN HYDROLYSIS PRODUCT OF BISPHENOL A DIGLYCIDYL ETHER (BADGE-2HCL).

J. M. Waechter, V. A. Linscombe, M. R. Schisler, K. M. Jackson and P. J. Spencer. *Toxicology & Environmental Research and Consulting, The Dow Chemical Co., Midland, MI.*

Bisphenol A diglycidyl ether is the monomer for epoxy resins used for the production of lacquer coatings for food and beverage cans. Therefore, human exposure to BADGE and/or its hydrolysis products *via* the migration of the residual monomer from cured can coatings into foodstuffs may occur. Previous work has shown that one of the hydrolysis products of BADGE in the presence of aqueous gastric fluid simulants is its *bis*-chlorohydrin (2, 2-*bis*[4-(3-chloro-2-hydroxypropoxy)phenyl]propane) (BADGE-2HCl) reported to increase micronuclei in cultured human lymphocytes treated *in vitro* (Suarez et al., Mutation Res. 470: 221-228, 2000). The genotoxicity potential of BADGE-2HCl was studied in three *in vitro* assays with or without the presence of S9 activation. In a Salmonella-Escherichia coli reverse mutation assay, six concentrations of the test article were tested ranging from 1 to 1000 micrograms/plate and 10 to 5000 micrograms/plate, respectively. In a chromosomal aberration assay, rat lymphocytes were treated with concentrations ranging from 6.25 to 37.5 micrograms BADGE-2HCl/ml of media for determining the incidence of chromosomal abberations. In a mouse lymphoma forward mutation assay, concentrations of BADGE-2HCl ranged from 1.25 to 80 micrograms/ml without S9 and from 1.25 to 100 micrograms/ml with S9. BADGE-2HCl did not induce a mutagenic or clastogenic response in these assays. The *in vivo* genotoxic potential of BADGE-2HCl was studied using the mouse bone marrow micronucleus (MN) test using oral doses of 0, 250, 500, or 1000 mg/kg/day administered on two consecutive days. Groups of six CD1 mice per dose were sacrificed at 24 hours after the second treatment for the evaluation of polychromatic erythrocytes for MN (2000 cell/animal) which were not significantly increased at any dose relative to the negative controls. In summary, these data indicate that BADGE-2HCl does not represent a genotoxic hazard. (Sponsored by the Epoxy Resins Committee of the Association of Plastics Manufacturers in Europe)

155 INDUCTION OF DNA DAMAGE (COMET ASSAY) BY BISPHENOL A IN CHINESE HAMSTER OVARY (CHO) CELLS.

K. Rao. *Toxicology, MicaGenix, Greenfield, IN.*

Induction of DNA Damage (Comet Assay) by Bisphenol A in Chinese Hamster Ovay (CHO) Cells. Yong Xu, Ellen Shaw, K.S. Rao, and J.W. Parton, MicaGenix Inc., Greenfield, IN Bisphenol A (4, 4-isopropylidene-2-diphenol, BPA) is a key industrial monomer used for the synthesis of polycarbonate plastics and epoxy resins. BPA produces a weak response in uterotropic assays and there has recently been renewed attention to estrogenic potential of BPA due to widespread contamination of laboratory experiments by BPA through leaching from autoclaved polycarbonate cages (Koehler et. Al. Lab. Animal 32:24, 2002). Epoxy resin-based sealers were reported to induce DNA damage with Comet assay (Huang et al. J Endodontics 27:744, 2001), which might be related to recent findings of genotoxic activity of BPA. This study investigated the potential activity of BPA on DNA damage using the Comet assay in CHO cells in the presence or absence of S9 activation. A trypan blue exclusion assay was performed to determine the cytotoxic activity of BPA. A 30-50% cytotoxicity was observed at concentration levels of 39.1 ug/ml with S9 and 78.1 ug/ml without S9. It was concluded that the microsomal fraction S9 enhanced cytotoxicity of BPA. A Comet assay was performed by treating CHO cells with BPA for 2 hours with and without S9. A dose-related increase of DNA damage, represented by Comet moment, Tail length and % tail DNA, was observed. The effects were again higher in the cells with S9 activation than without S9. The results indicate a DNA damage effect of BPA and suggest this effect may be related to the metabolism of BPA.

156 PHOTOMUTAGENICITY OF BERGAMOTTIN AND ISOPIMPINELLIN.

J. Cocchiara and A. Api. *Research Institute for Fragrance Materials, Inc., Woodcliff Lake, NJ.*

The European Commission's Scientific Committee on Cosmetics and Non-Food Products (SCCNFP) recently released an opinion to limit all "furocoumarin-like" substances in cosmetic products to 1 ppm based on potential photocarcinogenic and photomutagenic effects. Since it is not proven that all furocoumarins are photocarcinogens, the photomutagenicity of two furocoumarins found in citrus oils used in perfumery, bergamottin and isopimpinellin, was investigated in *Salmonella typhimurium* tester strains TA98, TA100, TA1535 and TA1537 and *Escherichia coli* strain WP2. Treatments were performed in all strains at concentrations up to precipitating dose levels. Specifically the bacteria were plated with bergamottin at doses of 15.8-5000 µg/plate, and isopimpinellin at doses of 3.16-1000 µg/plate in both the presence and absence of UV light. Irradiations of each strain used appropriate UVA light exposures, the higher exposure being the mutagenicity limit for each strain, having previously been demonstrated to induce approximately a doubling of revertant numbers or a 50% decrease in cell survival, and the lower exposure being a non-mutagenic and non-toxic dose. For bergamottin, none of the treatments resulted in any increases in revertant numbers indicative of any photomutagenic activity, and this material was considered negative under the conditions of this study. For isopimpinellin, there were no notable increases in revertant numbers in *Salmonella typhimurium* strains TA98, TA100, TA1535 or TA1537. However, statistically significant increases in revertant numbers in strain TA102 were observed following irradiation (both with the low and high UV irradiation levels), and were dose-related up to the precipitating dose levels. A confirmatory test in this strain was conducted. The increases were reproducible and these data are therefore considered indicative of photomutagenic activity of isopimpinellin in strain TA102 in this assay system. Additional photomutagenicity testing on other furocoumarins identified in essential oils is planned.

157 BACTERIAL MUTAGENICITY OF CIGARETTE SMOKE GAS/VAPOR PHASE.

F. J. Tewes and T. J. Meisgen. *PHILIP MORRIS Research Laboratories GmbH, Cologne, Germany.* Sponsor: H. Haussmann.

The *in vitro* mutagenicity of cigarette smoke is generally determined from the particle phase. In this study the mutagenicity of the gas/vapor phase of cigarette smoke was investigated. Mainstream smoke from University of Kentucky standard reference cigarettes and a research cigarette, generated according to ISO standards, was passed through a glass fiber filter to trap the total particulate matter (TPM) and then bubbled through ice-cold phosphate-buffered saline to trap the 'watersoluble fraction of the gas/vapor phase' (GVP). Mutagenicity was determined in the microsuspension modification of the Ames Salmonella mutation assay according to Kado (1983). Results revealed that GVP is mutagenic; however, in contrast to TPM, GVP contains significant amounts of direct-acting mutagens, as indicated by the response in the absence of a metabolic activation system, and causes mainly base-pair substitutions, as indicated by the higher response in strain TA100 than in strain TA98. Validation of the microsuspension assay using the solvent as negative control and methyl methanesulfonate as positive control showed that the intra-day and inter-day variability of the assay, with a coffecient of variation of less than 20%, was well within the usual range of other *in vitro* genotoxicity assays. Comparison of the reference cigarettes with the research cigarette showed that these cigarettes could be discriminated on the basis of GVP mutagenicity (e.g., response at equivalent TPM doses was approximately four-fold higher for the research cigarette compared to the Reference Cigarette 2R4F). Screening for mutagenic constituents in GVP showed that acrolein and formaldehyde were active in the microsuspension assay and that acrolein was responsible for up to 40% of the GVP activity. The results of the study demonstrate that the gas/vapor phase of cigarette smoke is mutagenic and that the microsuspension assay may be a useful tool for further investigations.

158 GENOTOXICITIES OF SAMPLES FROM NICKEL REFINERIES: PREDICTIONS OF CARCINOGENIC POTENTIALS.

R. Verma and J. Landolph. *Cancer Research Laboratory, Depts. of Mol. Microbiol. and Immunol., Path., and Mol. Pharmacology/Toxicol., USC/Norris Cancer Center, Keck School of Medicine/School of Pharmacy, University of Southern California, Los Angeles, CA.*

Epidemiological studies and animal carcinogenicity data indicate that different nickel compounds have different carcinogenic potentials. This poses the problem of how to predict carcinogenic potentials of those nickel samples for which there are no valid animal inhalation studies and no, or inconclusive, epidemiological data. We have used short-term *in vitro* assays to determine the relative genotoxicities of several nickel samples, finding good agreement between *in vitro* and *in vivo* results. We have now extended this work to include four samples of nickel refinery dust: two samples from a sulfidic nickel ore refinery (MD, RD) and two samples from a lateritic nickel ore refinery (NiCOM, GNiO [<15µm]). The sample of refinery matte dust (MD), was significantly cytotoxic to cultured C3H/10T1/2 (10T1/2) mouse embryo fibroblastic cells, with an LC_{50} concentration of (1.5 ± 1.2) µg/ml. This sample induced morphological transformation in 10T1/2 cells although it was

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