(a mouse and rat liver tumor promoter) and dieldrin (a mouse only liver promoter) increased ROS and oh8dG in a dose and species specific manner. The P450 activity of the microsomes was key to the ROS and oh8dG formation. These results showed a direct correlation between the induction of oxidative stress and the species specific tumorigenic effects of these compounds.

1075 AFLATOXIN B_1-INDUCED OXIDATIVE DAMAGES IN THE LIVER OF F344 RATS

C Y Shi, H M Shen, B L Lee, C N Ong. *National University of Singapore Faculty of Medicine, Singapore, Republic of Singapore.* Sponsor: P K Chan

Aflatoxin B_1 (AFB_1) has been shown to be a potent hepatocarcinogen in laboratory animals. The mechanism of AFB_1 genotoxicity and hepatocarcinogenesis, however, is not fully understood. The present study examined the induction of lipid peroxidation as well as DNA oxidative damages by AFB_1 in the liver of F344 rats. Two lipid peroxidation products, malonaldehyde (MDA) and conjugated dienes, were measured in liver homogenate and sub-cellular fractions. 8-Hydroxydeoxyguanosine (8-OHdG), a form of DNA oxidative lesions, were determined in isolated hepatic DNA. The result showed time- and dose-dependent increases of all three indices following a single *i.p.* injection of AFB_1 (100 μg/100 g body wt.), indicating that AFB_1 was capable of inducing oxidative damages in the liver. Lipid peroxidation was localized in certain cellular organelles such as microsomes, nuclei and mitochondria, but not in the cytosol. Hepatic DNA was also extensively modified by AFB_1-induced oxidative damages (as high as 45 8-OHdG adducts per 10^6 dG). Pretreatment of rats with the antioxidant selenium and the ion-chelator deferoxamine significantly inhibited lipid peroxidation as well as 8-OHdG formation, suggesting that hydroxyl free radicals might be involved as the reactive oxygen species. These results provide evidence that AFB_1 causes oxidative DNA damages in rat liver, which may constitute an important pathway in AFB_1 hepatocarcinogenesis.

1076 HEPATITIS VIRUS SURFACE ANTIGEN INDUCES NITRIC OXIDE SYNTHESIS IN HEPATOCYTES

R H Liu, J R Jacob, B C Tennant, J H Hotchkiss. *Inst. of Comparative and Environmental Toxicology, Stocking Hall [RHL, JHH]; and College of Veterinary Medicine [JRJ, BCT], Cornell University, Ithaca, NY*

Nitric oxide (NO•) can react with DNA to exert untoward effects including deamination and/or indirect alkylation leading to mutagenesis. Endogenous production of NO• and hepatocarcinogenic N-nitroso compound are increased in woodchucks with chronic woodchuck hepatitis virus (WHV) infection. We now report WHV surface antigen (WHsAg) stimulates hepatocytes to express NO• synthase to produce NO• in culture. NO• synthesis by hepatocytes was positively correlated to WHsAg concentration, and was inhibited by N^G-monomethyl-L-arginine and anti-WHsAg antibody. These data, when considered in light of the known genotoxicity of NO•, raise the possibility that viral hepatitis increases the risk of liver cancer by increasing the production of NO•. This provides a new mechanism by which hepatitis B virus infection increases the risk of liver cancer.

1077 THE TIME/DOSE RESPONSE OF OVARIAN ATROPHY AND TUBULAR ADENOMAS IN MICE FOLLOWING ORAL ADMINISTRATION OF MGK® REPELLENT R.11

K L Gabriel [1], F J Preiss [2], C Wood [3], W H Butler [4]. [1] *Biosearch Inc, Philadelphia, PA;* [2] *McLaughlin Gormley King Co, Minneapolis, MN;* [3] *Toxicol. Laboratories, Ledbury, UK; BIBRA International, Carshalton, Surrey, UK*

Carcinogenicity studies in CD_1 mice resulted in an increase of tubular adenomas of the ovary. Tubular adenomas are secondary to ovarian atrophy and pituitary stimulation. The time course and dose response have been studied to demonstrate the correlation of atrophy and adenomas and establish a no effect level. Mice received 5, 30, 60, 90 and 500 mg/kg/day and groups were killed at 30, 60, 90 days and 26, 55 and 80 weeks. Mice receiving 500 mg/kg/day at 26 weeks showed ovarian atrophy, 6/10 and at 55 weeks all had ovarian atrophy and 7/10 mice tubular. At 80 weeks atrophy was present in 31/37 mice from the 500 mg/kg/day group and 8/40 from the 90 mg/kg/day group. Tubular hyperplasia was only observed in the high dose group. Tubular adenomas, 9/37, granuloma cell tumour, 3/37, and luteoma, 4/37, were observed in the high dose group. No tubular adenomas were seen in the 90 mg/kg/day group. The findings show a clear dose and time related occurrence of atrophy, hyperplasia and adenomas with a no observable effect level of 60 mg/kg/day.

1078 A COMPARISON OF URINARY CHEMISTRY CHANGES IN MALE AND FEMALE RATS AND MICE TREATED WITH SODIUM SACCHARIN

L L Arnold, T Anderson, M Cano, M St. John, B Mattson, Jan Wehner, S M Cohen. *Department of Pathology and Microbiology and Eppley Institute, University of Nebraska Medical Center, Omaha, NE*

Male rats fed high doses (≥3%) of sodium saccharin (NaSac) develop urothelial cell proliferation and increased bladder tumor formation. Calcium phosphate precipitate forms in the urine of these rats and may be part of the mechanism by which the bladder changes occur. These changes occur to a lesser extent in the female rat and not at all in mice. We treated F344 male and female rats and Swiss male and female mice with 7.5% NaSac for twelve weeks. Urinary chemistry parameters evaluated showed similarities between the males and females in each species in pH level, protein and NaSac concentration, and various minerals. Calcium levels were higher in control rats compared to control mice. Treatment further increased levels in male rats but not in the mice or female rats. This increased level of calcium may play a role in the mechanism by which NaSac selectively causes bladder changes in the male rat. Supported by NIH grant #CA32513 and a grant from Intl. Life Sci. Inst.

1079 CELL KINETIC STUDY OF SQUAMOUS CARCINOMA DEVELOPMENT DURING BLADDER TRANSITIONAL CELL CARCINOGENESIS IN RATS TREATED WITH BUTYL-(4-HYDROXYBUTYL)-NITROSAMINE

M Mutai [1], T Masui [2], K Kobayashi [1], R Hasegawa [3], M Tatematsu [2]. *Toxicol. Lab., Yokohama Research Center, Yokohama, Japan;* [1] *Mitsubishi Chemical Corp., Yokohama, Japan;* [2] *Lab. Pathol., Aichi Cancer Center Research Institute, Nagoya, Japan;* [3] *1st Dept. Pathol., Nagoya City Univ. Med. Sch., Nagoya, Japan.* Sponsor: T Suga

To clarify the origin and development of the squamous cell carcinoma (SCC) of rat bladder, cell kinetics of the squamous and transitional neoplastic lesions induced by butyl-(4-hydroxybutyl)-nitrosamine (BBN) was investigated. A total of 220 male F344 rats, aged 6 wks, were given 0.05% BBN in the drinking water fort 12 wks and then given normal tap water. Rats were sequentially sacrificed at 8, 12, 18, 30 and 60 wk. Bromodeoxyuridine (BrdU) was applied as a single injection or by contineous labeling using osmotic mini pumps for 4 or 7 days before sacrifice. The bladder were fixed and processed for routine staining with hematoxylin-eosin, and for immunohistochemical demonstraion of BrdU. Small neoplastic squamous lesions (NSL) were firstly observed in transitional cell papillomas and carcinomas (TCP/TCC) at 18 wk. And then the number and area of NSL in the TCP/TCC was increased during the course of the study. Flash and continuous BrdU labeling revealed NSL to consist of cells showing twofold high mitotic activity compared with surrounding TCP/TCC cells. These results suggest that NSL cells might derive from TCP/TCC cells, and this highly labeled NSL replace the TCP/TCC and develop to bladder SCC.

1080 *IN VITRO* EXPOSURE OF HUMAN UROEPITHELIAL CELLS TO OCCUPATIONAL CHEMICAL RESULTS IN ALTERATIONS IN PROTEIN EXPRESSION AS DETERMINED BY HIGH RESOLUTION TWO DIMENSIONAL POLYACRYLAMIDE GEL ELECTROPHORESIS

M H Kanitz, R E Savage, Jr., K Athota. *National Institute of Occupational Safety and Health, Cincinnati, OH, C Reznikoff, S Swaminathan, and S Frederickson, University of Wisconsin, Madison, WI*

A study has been initiated to investigate the carcinogenicity of 4,4'-methylene bis(2-chloroaniline) (MOCA) and 2-phenyl-1,4-benzoquinone (PBQ) and to identify biomarkers of effects of exposure to these workplace-associated suspect carcinogens. High resolution two dimensional polyacrylamide gel electrophoresis (2D PAGE) was used to examine changes in protein expression in chemically-treated human uroepithelial cells (HUC). Previously, HUC has been shown to be sensitive to malignant transformation by 4-aminobiphenyl (4-ABP) and its metabolites (Bookland, et. al., *Cancer Research*, 52, 1606–1614, 1992). HUC was incubated for 24 hr with the controls, dimethyl sulfoxide (DMSO) or N-OH-AABP, as well as N-OH-MOCA (2.5 and 10 uM), or PBQ (1.5 and 3.5 uM) which resulted in 25% and 75% cell kill, respectively. A 2D PAGE protein map of treated HUC was developed using the ISODALT method of Anderson. Electrophoretic pattern comparison across treatment groups by scanning densitometer and computer analysis revealed quantitative changes in a specific subset of proteins in the 10 uM MOCA-treated HUC and N-OH-AABP-treated HUC but not the DMSO-treated HUC. Preliminary studies of tumor incidence in athymic mice suggest that neo-plastic

transformation occurred only in the 10 uM MOCA-treated HUC. These results show that occupational chemical exposure of HUC results in 2D PAGE protein alterations which may be useful for the identification of biomarkers associated with bladder tumorigenesis.

1081 TWO-YEAR DERMAL STUDY OF TRIETHANOLAMINE IN F344 RATS AND B6C3F$_1$ MICE

M R Hejtmancik, J D Toft, R L Persing, R L Melnick [1], P J Kurtz. *Battelle, Columbus, OH; [1] NIEHS/NTP, Research Triangle Park, NC*

Human dermal exposure to triethanolamine (TEA, CAS No. 102-71-6) occurs during the normal use of many cosmetic preparations. F344 rats received TEA in acetone by topical application at doses of 0, 32 (males only), 63, 125, and 250 (females) mg/kg. Male B6C3F$_1$ mice received TEA at doses of 0, 200, 630 and 2000 mg/kg and female mice received doses of 0, 100, 300, and 1000 mg/kg. Animals were treated for 5 days/week for up to 103 weeks. An interim evaluation conducted during Week 66 included 10 animals/dose group of each sex. After two years, dosing was suspended and survivors terminated two weeks later, followed by complete necropsy and histopathology. Treatment was not associated with an increased incidence of spontaneous deaths or moribund terminations. The growth rate of treated animals was similar to that of their respective control groups. Irritation at the site of application increased with dose level (rats and male mice). Microscopic examination of interim and two year rats (particularly females) revealed dose-related acanthosis, chronic-active inflammation, and epidermal ulcerations. There were no treatment-related lesions at the application site in mice, although acanthosis apppeared to be a treatment-related change at the 66-week interim termination. In mice, there was a treatment-related increase in the number of liver tumors at necropsy which were classified predominately as hepatocellular adenomas upon microscopic examination. (Supported by NIEHS Contract No. NO1-ES-85226).

1082 NINETY-DAY TOXICITY STUDIES OF 2-METHYL-IMIDAZOLE IN FISCHER RATS AND B6C3F1 MICE

P C Chan, M L Wenk. *NIEHS, RTP, NC and Microbiological Associates, Inc., Bethesda, MD.* Sponsor: J R Bucher

2-Methylimidazole (2MI) is used as chemical intermediate in the manufacture of pharmaceuticals, photographic and photothermographic chemicals, dyes and pigments, agricultural chemicals and rubber. 2MI is also used as polymerization crosslinking accelerator and hardener for epoxy resin systems for semiconductor potting compounds and soldering masks. 2MI has been identified in ammoniated hay forage for livestocks and in caramel coloring, soy sauce, Worchestershire sauce, wine, ammoniated molasses, caramel-colored syrups, and cigarette smoke. Toxicity studies of 2 MI was conducted because of its widespread use, potential for widespread exposure in food to humans, lack of toxicity data, and suspicion of carcinogenicity. Groups of 10 male and 10 female Fischer rats and B6C3F1 mice were dosed fed 2MI at 0, 625, 1250, 2500, 5000 or 10000 ppm for 90 days. All animals survived. No signs of neurotoxicity were observed. Mean body weights were significantly reduced in male rats at ≥2500 ppm and in female rats at 10000 ppm. Food consumption was reduced in male and female rats at ≥5000 ppm. Relative liver and kidney weights were increased in male and female rats. Serum T3 and T4 levels were decreased and TSH levels increased significantly in male and female rats at ≥2500 ppm. Dose-related thyroid follicular cell hyperplasia occurred in male and female rats; in 2 of 10 male rats at 10000 ppm the lesions were large. Uterine atrophy and adrenal cortical necrosis were also observed in the 2 highest dose groups of female rats. Mean body weights were significantly reduced in male and female mice at ≥5000 ppm. Food consumptions were similar among the male and female mouse groups. Relative kidney and liver weights were increased in dosed male and female mice. Thyroid follicular cell hyperplasia and splenic hematopoietic cell proliferation were found in male and female mice at ≥2500 ppm. Hemosiderin was observed in kidney tubules in male mice at ≥1250 ppm and in female mice at ≥2500 ppm. Serum TSH levels were increased and T3 and T4 levels were decreased in male and female mice. Induction of thyroid follicular cell hyperplasia in rats and mice by 2MI may be due to enhanced metabolism of T3 and T4 by increased hepatic UDPglucuronosyltransferase activities. This indirectly stimulated TSH secretion and in turn thyroid gland hyperplasia. Prolonged exposure to 2MI in rats and mice would probably lead to thyroid follicular cell adenoma formation.

1083 MODIFYING EFFECTS OF ADI DOSE MIXTURES OF 20 OR 40 PESTICIDES ON THE MEDIUM-TERM CARCINOGENESIS MODELS IN RATS

M Futakuchi, R Hasegawa, M Kawabe, A Hagiwara, K Imaida, N Ito, T Shirai. *First Department of Pathology, Nagoya City University Medical School, Nagoya, Japan*

The modifying effects of acceptable daily intake(ADI) dose mixtures of 20 or 40 pesticides of interest because of large quantity manufactured or suspectable carcinogenicity in rats, were investigated using our medium-term liver and multi-organ bioassays for rapid detection of carcinogens. No adverse effects in terms of body weight, organ weights, and tumor incidences were observed in rats exposed to the mixtures of 40 or 20 pesticides. In contrast, elevated quantitative values for immuno-histochemically demonstrated GST-P positive hepatocyte foci, as well as increased incidences of preneoplastic renal lesions, were found in rats given captofol used as a positive control. The safety factor used for ADI by the Japanese Ministry of Health and Welfare and the FAO/WHO is usually 100. There are a number of potential problems associated with the safety factor approach, including the fact that the observation of no treatment-related effects may depend on the number of animals exposed and dose levels used, and biological justification for general use may therefore be lacking. However, the present results indicate that the regulation of pesticides based on ADI levels is appropriate with regard to carcinogenicity.

1084 CARCINOGENICITY OF TRIFLURALIN — NOT TRIGGERED BY ITS IMPURITY N-NITROSODI-N-PROPYLAMINE (NDPA)

R Cabral, K Hakoi, T Hoshiya, R Hawegawa, N Ito. *Department of Pathology, NCU Medical School, Nagoya, Japan*

Trifluralin is a selective pre-emergence herbicide used worldwide on more than 28 crops. Several years ago, the US EPA raised concerns about trifluralin regarding its carcinogenic potential. There was concern because trifluralin products contained significant levels of a carcinogenic contaminant — NDPA. Trifluralin, with or without NDPA contamination, was studied in a medium-term bioassay based on the induction of preneoplastic lesions in the liver. Rats were administered diethylnitrosamine (DEN) i.p. at a dose of 200 mg/kg bw and two weeks later were treated with different samples of trifluralin at a dose of 5000 ppm in the diet for six weeks and then killed; all rats had a partial hepatectomy at week 3. Hepatocarcinogenic potential was assessed by comparing the number and area of glutathione-S-transferase (placental form)-positive foci in the liver with those of controls given DEN alone.

Positive results were noted with all samples of trifluralin with or without NDPA contamination. The present results would suggest that trifluralin itself (without contaminant) was hepatocarcinogenic in this bioassay.

1085 SUBCHRONIC TOXICITY EVALUATION OF TETRYL IN FISCHER 344 RATS

T V Reddy [1], F B Daniel [1], M Robinson [1], J Torsella [1], G R Olson [2], B Wiechman [2], G Reddy [3]. *[1] EMRD, EMSL, USEPA, Cincinnati, OH; [2] PAI, Westchester, OH; [3] USACHPPM, Fort Detrick, MD*

Subchronic toxic effects of the explosive chemical N-Methyl-N-2,4,6-tetranitroaniline (tetryl) in male and female Fischer 344 rats were evaluated by feeding Purina® rodent chow supplemented with varied concentrations of tetryl (0, 200, 1000 and 3000 mg/kg diet) for ninety days. Food intake was reduced in both sexes at all dose levels throughout the study and resulted in a significant decrease in terminal body weights in the 3000 and 1000 mg tetryl dose groups. The calculated average tetryl intake was 14, 69, and 199 mg/kg BW/day for females and 13, 62 and 180 mg/kg BW/day for males. An increase in the relative liver and kidney weights in both sexes in the 3000 and 1000 mg tetryl dose groups and an increase in the relative spleen weight in the 3000 mg dose group were noted. Hemoglobin content was decreased and methemoglobin levels were increased in both sexes in the 3000 and 1000 mg tetryl dose groups. Decreased red cell count and increased reticulocyte count from both sexes in the 3000 mg tetryl dose group were apparent. Histopathological examinations revealed pigment deposition and erythroid cell hyperplasia in spleen, and tubular degeneration and cytoplasmic droplets in kidney, from both high dose groups. From this study the no observed adverse effect level (NOAEL) of 13 mg/kg/day was established. (This abstract does not necessarily reflect U.S. EPA/U.S. Army policy.)

SOCIETY OF TOXICOLOGY

34th Annual Meeting

THE TOXICOLOGIST

Volume 15, No. 1, March 95

FUNDAMENTAL AND APPLIED TOXICOLOGY