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RJR CONFIDENTIAL

RJR

January 27, 1987

TO: Dr. G. Robert Di Marco

RE: Weekly Highlights - Biochemical/Biobehavioral
Week Ending January 16, 1987

ITEMS FOR WEEKLY BRIEF

- Additives - Scientists and regulatory agencies have expressed concern about ozone depletion in the earth's atmosphere. One group of agents which has been postulated to contribute to this ozone depletion is chlorofluorocarbons (Freon). Regulatory agencies are considering a production ceiling or production ban on Freon 11, 12 and 113. The more likely regulation is an immediate production ceiling and a gradual decrease in usage over several years. Freon 11 is used in our tobacco puffing operation (G-13). A review of potential Freon replacements has shown Freon 123 to be as non-toxic as Freon 11 but three times costlier. The ozone depletion potential of Freon 123 has been rated at less than 0.05 while Freon 11's potential has been rated at 1.0 by DuPont scientists. Freon 134a is not thought to cause ozone depletion but additional biological research must be conducted to ensure its lack of toxicity. Freon 141b has been reported to be a mutagen, Freon 133a is reported to be embryotoxic and Freon 31 is reported to be a mutagen and animal carcinogen. The physical characteristics make Freon 123 most compatible with our current G-13 process. The Engineers are currently evaluating Freon 123 as a potential replacement if Freon 11 production is reduced or banned.

  Nicotine levulinate was approved as an additive up to 5.0% in WINSTON Ultra low tar cigarettes if the test panelist smoked one cigarette. The nicotine delivery of this product does not exceed the nicotine levels in conventional cigarettes. Levulinic acid is relatively non-toxic and had been approved earlier for limited evaluation by panelists.

- Pesticides - Results of selected pesticide residues on 71 tobacco samples have been received from Del Monte. Nine samples from Greece, seven samples from China, one sample from

Canada and one sample from an unlabelled area contained residue levels which were above our guideline levels. The remaining samples from China, Thailand, Panama, Malaysia, Yugoslavia and Bulgaria were acceptable for purchase.

- Industrial Hygiene - Symptoms of eye irritation and skin reddening occurred in two employees following exposure to ultraviolet lights at the Tobaccoville Flavoring and Adhesive Storage area. Ultraviolet light is used to kill bacteria which may contaminate our flavorings and adhesives. It has been recommended that all facilities install limit switches and caution signs to prevent accidental exposure to ultraviolet light.

  Confined space entry work was proposed at the Bailey Power Plant coal silo. Our recommendation to Mechanical Contract Services stated that the contractor was not qualified to conduct such work. The work has been postponed until later in 1987 when a qualified contractor is located.

- Basic Sensory Research - Twenty White Carneaux pigeons were received from the Bowman Gray School of Medicine. The pigeons will be housed in the new pigeon facility in Building 611-9. The Standard Operating Procedure (SOP) for Laboratory Pigeon Care was revised and distributed to the internal Animal Care and Use Committee for review.

ITEMS OF GENERAL INTEREST TO R&D

Biobehavioral Research

- Support to Brand R&D - The analyses of plasma samples, obtained during the puffing/breathing/blood chemistry study completed in August, 1986, for nicotine and cotinine have been completed. Data are now being tabulated.

  Replications on the human mimic smoking machine resumed with smokings of NOW's from the DFC project. These replications will require two to three weeks to complete.

- Smoke Component Dose - Drs. John Robinson and Brad Ingebrethsen (Fundamental R&D) met to discuss the design of a smoke collection device that would allow the capture of exhaled smoke as well as smoke generated by a puff but allowed to "leak" out of the smoker's mouth before being inhaled. This device will be useful in studies designed to determine the dose of a particular smoke component actually absorbed by a smoker. Another meeting is planned this week to begin development of this system.

- Psychophysiology of Smoking - A total of seven subjects have now been run by Dr. Wally Pritchard and Ms. Sandra Ingram on the Bio-Logic Brain Atlas III Plus according to the Bio-Logic normative data base protocols. Very preliminary analysis of the data indicate that an inverse relation between neuroticism (assessed by the Eysenck Personality Questionnaire) and latency of the P300 wave of the evoked brain potential,

previously reported by German psychophysiologists, is replicated in the RJRT data.

Dr. Wally Pritchard completed writing programs for computer administration and scoring of the Eysenck Personality Questionnaire, the Anger Expression Scale and the State Trait Personality Inventory.

- Nicotine Neurophysiology - Collaborative studies with Dr. Larry Daniel (Bowman Gray School of Medicine) to determine the effects of nicotine on neuronal signal transduction mechanisms have shown promising results. The evidence suggests that cortical neurons are able to rapidly incorporate a number of markers, including phosphorus 32, 3H-inositol, 3H-lyso-platelet activating factor, and 3H-choline into cellular lipids. There is extensive incorporation into the phosphatidylcholine (PC), phosphatidylethanolamine (PE) and phosphatidylinositol (PI) fractions. In addition, the cells can be stimulated to produce diglyceride from PC. Diglyceride is the putative activator of an enzyme closely linked to modulation of neuronal growth and/or plasticity. Production of diglyceride as a possible second messenger from PC is a novel pathway identified previously by Dr. Daniel in other cell types. The ability of nicotine to stimulate this pathway through activation of nicotinic receptors is presently being investigated.

- Nicotine Receptor Pharmacology - Characterization of the pharmacological specificity of nicotine receptors in cultured cortical neurons is continuing. All compounds tested thus far have shown characteristics in cultured cells essentially identical to those observed in whole brain membrane preparations. Hexamethonium, decamethonium, tetramethyl-ammonium chloride, and dimethylphenylpiperazinium are presently being tested to complete the profile.

  Ms. Kay Fernandes visited the laboratory of Dr. Ken McCarthy (University of North Carolina at Chapel Hill) to discuss improvements to immunocytochemical and autoradiographic methodologies presently being implemented in-house. Application of these methods to identify specific cell types and nicotine receptor locations has begun. Antibodies to neuron-specific filament proteins and to non-neuronal (i.e. glial) markers are being used to distinguish cell types in cultures.

  Monoclonal antibodies to rat brain nicotine receptors are kindly being made available to us by Dr. Jon Lindstrom (Salk Institute). It is hoped that these radiolabeled antibodies can be used to autoradiographically identify putative receptors.

  Dr. John Langone (Baylor College) has forwarded antibodies and antiidiotypic antibodies to nicotine to be used as potential probes for nicotine receptor location and function. The efficacy of these probes is presently being evaluated.

- Miscellaneous - Dr. Jim Walker and Ms. Cheryl Miller were accepted as members by the Society for Neuroscience.

  Dr. Pat Lippiello and others from the Biochemical/ Biobehavioral Group are developing a proposal for a nicotine analogue research program.

Toxicology Research

- Special Toxicology

  Ms. Lisa Bates set up a new method for the HPLC analysis of nicotine, cotinine, and nicotine-1'-N-oxide in plasma. Since it has been demonstrated that these are the principal metabolites in plasma of rats, this method, which takes only 10 min per run, is much faster than the HPLC method that separates 12 metabolites. Data were obtained on a mixture of C14 nicotine and 14C-cotinine demonstrating baseline separation. The recovery of the 14C from the HPLC system was 99% and the efficiency for 14C detection was approximately 90%. The HPLC method will be set up along with the Ramona LS-4 14C detector so that the plasma samples from the intravenous group in the nicotine bioavailability study can be run unattended.

  Dr. Don deBethizy met with the computer group to discuss the disposition software system. The steps necessary to complete the validation of the system were discussed, as well as those items that are on the agenda for completion during phase 2.

  Dr. Dave Eaker (Fundamental R&D) completed sample collection of mainstream and sidestream cigarette smoke condensates. The samples will be sent to Pharmakon Laboratories for comparative genotoxicity assays.

  According to Dr. Chortyk, (USDA Agricultural Research Service, Athens, Georgia) the smoke condensate of Kentucky reference cigarettes 1R4F fortified with selenium (Na2Se03) induced 50% less revertants in the Ames assay than the control. Brian Smeeton of Fundamental Research obtained the selenium-treated cigarettes (10, 50 and 100 ug Se per cigarette) and we tested the condensates along with the control. In the S9 activated assay system, neither TA100 nor TA98 showed any statistically significant difference in the revertant number from that of the control. The same CSC will be tested in SCE/CHO assay.

  A flavor package was tested in the chromosome aberration assay with metabolic activation. The cells were treated for two hours and harvested after 10 and 14 hour recovery periods. The doses tested were 0, 100, 200, 400, 600, 800 and 1000 ug/ml. The results are negative in all the doses tested. The same sample, tested in a nonactivated assay, was also negative.

  Genetic toxicology staff went through the first week of training in in vivo cytogenetics experiments. They learned the basic technique of BrdU tablets implantation and bone

marrow preparation. The training will continue several more weeks.

Three samples for Ames testing with 2 strains and S9, and four samples for testing with 5 strains both with and without S9 were submitted by Dr. Bill Casey.

Mr. Jack White submitted two samples for Ames testing with TA98 and TA100 with S9. Preliminary testing on potential bacterial interference was performed.

Dr. Bill Rice mailed 15 samples (plus 1 reference) to Hazleton for Ames testing. The remaining samples of KC98, KC99, 1R4F, and BLANK were mailed to Dr. Bill Blazak of SRI.

Quality Assurance testing for Ms. Debbie Kay continued. Ten samples were tested for general bacteria count and yeasts and mold count.

- Applied Toxicology

Dr. Arnold Mosberg was co-author on a paper entitled "Pharmacokinetics and Metabolism of Inhaled Radiolabeled Tixocortol Pivalate" published in the "Proceedings of the 6th International Congress for Aerosols in Medicine." The paper described research done prior to his joining RJRT.

Sacrifices of animals from the reversibility groups of a 90-day inhalation animal study were completed, with the team from VERITAS performing the necropsies.

The move of Applied Toxicology equipment from the second floor 611-9 to the new facilities on the first floor began. This move and the re-calibration of instruments, etc., will take at least another 10 days.

Very preliminary work on a modified puffer device showed promise. The work is at the moment aimed at the replacement of mechanical pumps on the smoking machines. It is considered that the methods under test, if successful, could result in an application for a patent.

Scientific Affairs

- Manufacturing - Two potential product contaminations were reviewed. One potential contamination involved glass which is a physical hazard but not of toxicological significance. The second incident involved potential contamination of 1,776 cases of WINSTON 85 with plastic film. The plastic components were identified as polyethylene, polyethylene terephthalate and ethylene acetate copolymer. These components are not expected to generate or cause any biological effect.

It was recommended that a spray tank coating be applied during a weekend when there was no cigarette production. The coating contained methyl ethyl ketone, toluene, titanium dioxide, silica and xylene.

- Industrial Hygiene - The proposed North Carolina Air Toxics Regulations were reviewed and comments were furnished to Environmental Engineering. The theory supporting the regulations is a conservative interpretation often used by regulatory agencies. The theory uses "safety factors" which are multiplied times the published and generally accepted threshold limit values for chemical exposures in the workplace. If the safety factors are not used, it would cost state regulatory agencies a great deal of time and money to establish additional environmental concentrations of chemicals. It was suggested that an industry representative who is familiar with biological data be a member of the advisory committee.

  Mr. David Taylor attended a Radiation Safety Officer's course at the University of Texas. This course is necessary for proper licensing of a radiation safety officer.

Center of Excellence in Toxicology

- Dr. John Kirschman has been selected as a touring lecturer for the Institute of Food Technologist's National Speakers Bureau. The title of his lecture will be "Food Safety is a Risky Business."

- Tate and Lyle presented data to Nabisco Brands personnel and Center personnel on the biological effects of methoxyphenoxy-propionic acid, a sweetness inhibitor. A report on the biological data will be forthcoming.

New Product Support

- The QA team has moved to 611-13W/1 where they have established offices and an archival area. They have issued their first SOP concerning how to prepare SOP's. They will be converting all currently issued SOP's to this format.

- The QA team completed audit of the data transfer for the serum lipid analysis from a recently completed 90-day subchronic study. Jerry Avalos of the Applied Toxicology group will perform statistical analysis of this data.

A. Wallace Hayes

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