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VETERANS ADMINISTRATION
RESEARCH AND DEVELOPMENT INFORMATION SYSTEM
AGENT ORANGEURINARY 6-HYDROXYCORTISOL - PHYSIOLOGIC AND PHARMACOLOGIC STUDIES
(INCLUDING AGENT ORANGE)

ERTEL, NORMAN H., M.D.

561 EAST ORANGE, NJ

PROJECT 7

LAST REPORT, PROGRESS 09/83

ASSOCIATES: Kiernan, TW Luisada-Oppar, A Mittler, JC
Peterson, RE Pogach, L Samojlik, E Schneider, G
Yang, CS

6-BETA-HYDROXYCORTISOL, MICROSOMAL ENZYME INDUCTION, EUHYDROXY CUSHING'S SYNDROME

Objectives: To further characterize the normal and abnormal physiology of 6-hydroxycortisol, to investigate dogs and humans exposed to carcinogens, such as those in cigarette smoke, and to investigate the effects of TCDD(dioxin) on hepatic microsomal induction and testosterone metabolism in rat testis.

Research Plan: We measured the daily excretion of 6-hydroxycortisol and cortisol by radioimmunoassay in patients with pituitary dependent Cushing's Syndrome. We examined the in vitro response of hepatic microsomal oxygenase enzymes to inducing agents with a comparison of 6-hydroxylase to other P-450 oxidases. A study was performed in dogs who were taught to smoke by machine, measuring testicular and hepatic hydroxylases.

Methodology: Our previously published method for RIA of urinary 6-hydroxycortisol and cortisol was used in these studies. Cortisol, pentobarbital and aniline hydroxylases were assayed in rat liver microsomes. Purebred beagles were tracheostomized and smoked cigarettes held in a machine designed to duplicate a standard puff profile. The 6-beta, 7-alpha, and 16-alpha hydroxylases active on androgens were measured in both liver and testes, following long-term cigarette smoking.

Clinical Relationships: We developed the first RIA for urinary 6-hydroxycortisol in man, facilitating the study of 6-hydroxycortisol metabolism and widening its usefulness as a tool in studying drug metabolism and microsomal enzyme induction.

Results to Date: Urinary 6-hydroxycortisol is a sensitive index of increased cortisol secretion in Cushing's Disease, and more clearly indicates this disease than do urinary hydroxysteroids or free cortisol. Studies with aminoglutethimide and ACTH indicate that altered peripheral metabolism of cortisol may explain these findings. A heterogeneity of responses of microsomal enzymes was noted in studies in rats. Chronic cigarette smoking increased hepatic metabolism of testosterone in dogs, and was associated with decreased 7-alpha-hydroxylase activity. Testicular 6-beta and 16-alpha hydroxylases were not altered. Hepatic androgen 6-beta-hydroxylase activity in control animals was 6 times the testis levels and was stimulated markedly by smoking. Serum testosterone levels and prostate size decreased and LH levels increased. Studies with TCDD(dioxin) were not conducted in this project because of safety considerations in a hospital environment(see/MITTLER/008). Safety regulations are under study.

SEP 26, 1984

VETERANS ADMINISTRATION
RESEARCH AND DEVELOPMENT INFORMATION SYSTEM
AGENT ORANGE

EFFECT OF TCDD ON LIPID METABOLISM (DIOXINS)

DUNN, G. DEWEY, M.D.

626 NASHVILLE, TN

PROJECT 3

LAST REPORT, PROGRESS 01/84

HYPERCHOLESTEROLEMIA, ATHEROSCLEROSIS, LIPOPROTEIN METABOLISM, AGENT ORANGE, SERUM TRIGLYCERIDES, TCDD, TESTICULAR LIPIDS, BIRTH DEFECTS, IMPOTENCE

Human exposure to the toxin TCDD as a contaminant of herbicides (Agent Orange used in Vietnam) has concerned the Veterans Administration. I monitor the Agent Orange examinations and am a reference person for the hospital. Four veterans are being followed with chloracne, hypercholesterolemia, and abnormal liver tests.

Objective: TCDD given to guinea pigs and rats causes increased lipids. Our research is a study of TCDD on serum lipoproteins in rats.

Plan: To characterize lipoproteins, study synthesis, and catabolism. We will study fatty acid mobilization from adipose tissue and conduct chronic studies of TCDD administration to see effects of drug given in sublethal amounts. We also want to study testicular lipids because veterans are concerned about birth defects and impotence.

Methodology: Analysis of lipids, fatty acid composition of esterified lipids, electron microscopy of particles, electrophoresis of lipoproteins, lipoprotein separation by ultracentrifugation, studies of lipoprotein synthesis and catabolism using labeled lipoproteins and characterization of apolipoproteins.

Findings: Lipid abnormalities are produced in the rat by 1 microgram/kilogram TCDD with increased triglyceride levels. In chronic experiment dosages of 2 and 4 microgram/kilogram/day TCDD give consistent increases in cholesterol and triglycerides. Work at present is defining the lipoproteins increased in the rat and fatty acid composition. Rats are being injected with 10 microgram/kilogram and studies 5 days after TCDD injection. Cholesterol is elevated but triglyceride is the same as pair fed controls, but when Triton WR 1339 is used to block lipoprotein catabolism, studies demonstrates increased triglyceride synthesis after TCDD.

OCT 23, 1984

VETERANS ADMINISTRATION
RESEARCH AND DEVELOPMENT INFORMATION SYSTEM
AGENT ORANGE

The Chronic Effects of Herbicide Exposure on Testicular Function in Vietnam Veterans

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542 COATESVILLE, PA

PROJECT 2

LAST REPORT, INITIAL 12/83

ASSOCIATES: Snyder, PJ

Steinmann, WC

AGENT ORANGE, DIOXIN, TESTICULAR FUNCTION, VIETNAM VETERANS, INFERTILITY, PHENOXYACIDS, TESTICULAR TOXINS

OBJECTIVE: The purpose of this study is to determine whether or not certain levels of exposure to phenoxyacids and dioxin had a chronic adverse effect on the reproductive system of veterans in Vietnam and to correlate severity with exposure levels.

RESEARCH PLAN: To evaluate differences in sperm count and levels of follicle stimulating hormone (FSH), leutenizing hormone (LH) and testosterone in Vietnam veterans with high and low exposure to Agent Orange and compare to Vietnam era veterans who did not serve in Vietnam.

METHODOLOGY: The military service and possible exposure of individual veterans who have registered with the Agent Orange Registry in the Philadelphia/Coatesville VA Medical Centers will be determined. Using the military unit numbers, an evaluation of exposure will be determined using the resources of Army Agent Orange Task Force (Director: Richard Christian), who have available possible exposure to each veteran based on his area of service. Once exposure has been determined, 100 with highest and 100 with lowest exposure will be selected along with a cohort of 100 Vietnam era veterans. Sperm counts, LH, FSH and testosterone will be determined on three occasions after co-existing diseases have been excluded by history and physical exam. Analysis of variance and discriminant function analysis will be used to find how much variance in each symptom and the sperm count is accounted for by actual exposure and demographic variables.

FINDINGS: None to date.

OCT 23, 1984

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AGENT ORANGE

Epidermal Differentiation and Hyperplasia as Markers of TCDD Toxicity
MILSTONE, LEONARD M., M.D. 689 WEST HAVEN, CT

PROJECT 2
LAST REPORT, INITIAL 08/84

ASSOCIATES: LaVigne, JF

KERATINOCYTES, CULTURES, TCDD, HYPERPLASIA, GENETICS, MECHANISMS,
RECEPTORS, CHLORACNE, TUMOR-PROMOTION

The objectives of the proposed research program are to determine the mechanisms by which tetrachlorodibenzo-p-dioxin (TCDD) induces epidermal hyperplasia and to ascertain whether the in vitro hyperplastic response to TCDD can be used to predict individual susceptibility to TCDD-induced tumor promotion and chloracne production. TCDD is the prototype for a group of chemicals that are toxic to man and animals. One well-studied manifestation of toxicity is enzyme induction. TCDD-induced epithelial hyperplasia occurs in only a limited number of species and tissues and may indicate susceptibility to other biological effects of TCDD which may not be discernable by the usual enzyme-induction assays. We have recently described the first in vitro assay for studying TCDD-induced epithelial hyperplasia. We propose to exploit this tissue culture methodology 1) to further characterize the effects of TCDD on growth and differentiation of human keratinocytes, 2) to examine the effect of TCDD on stimulation of biochemical markers for proliferating and terminally differentiating keratinocytes, 3) to determine whether the in vitro hyperplastic response to TCDD can be correlated with species or tissue susceptibility to TCDD-induced changes such as chloracne and tumor promotion and 4) to determine whether the in vitro assay can be used to identify human population at risk for TCDD-induced skin disease.

SEP 26, 1984

VETERANS ADMINISTRATION
RESEARCH AND DEVELOPMENT INFORMATION SYSTEM
AGENT ORANGE

Metabolism of the Herbicides Present in Agent Orange and Agent White
VESSEY, DONALD A., Ph.D. 662 SAN FRANCISCO, CA

PROJECT 1
LAST REPORT, PROGRESS 12/83

HERBICIDES, 2,4-D, 2,4,5-T, AGENT ORANGE, DIOXIN

The herbicides 2,4,5-T (2,4,5-trichlorophenoxy acetic acid) and 2,4-D (2,4-dichlorophenoxy acetic acid) are components of Agent Orange and other defoliant. It is known that 2,4-D and 2,4,5-T are metabolized by humans and other animals although identification of the metabolites has generally not been done. We will identify the metabolites of 2,4-D and 2,4,5-T using the rat as a model. We will determine what enzymes are involved in the metabolism of these herbicides and at what sites. We will characterize the regulatory properties of these enzymes in order to understand the factors affecting the rate of metabolism of 2,4-D and 2,4,5-T. We will determine whether the tremendous intra-individual variations in the extent of the metabolism of their herbicides by humans has toxicological significance, i. e. do the metabolites have a reduced (or enhanced) toxicity and are they eliminated at different rates? These metabolic studies will include investigations at high doses of 2,4-D and 2,4,5-T where there is increased metabolite formation and the possibility of long term effects. The potential for organ damage and carcinogenesis resulting from the formation of highly reactive metabolites will be assessed by looking for evidence of covalent binding of metabolites to cellular macromolecules. The extent to which dioxin (TCDD) potentiates this toxicity of 2,4-D and 2,4,5-T by induction of drug metabolizing activity will be determined in order to understand the toxicity of Agent Orange preparations that were contaminated with TCDD. It will also be determined to what extent 2,4-D and 2,4,5-T interfere with the metabolism of other toxins resulting in synergistic effects. The metabolism of these herbicides in neonatal rat liver will also be investigated.

SEP 26, 1984

VETERANS ADMINISTRATION
RESEARCH AND DEVELOPMENT INFORMATION SYSTEM
AGENT ORANGE

Effects of Agent Orange on Sleep
SIEGEL, JEROME M., Ph.D.

665 SEPULVEDA, CA

PROJECT 3
LAST REPORT, PROGRESS 05/84

AGENT ORANGE, SLEEP

Sleep problems are one of the most common and consistent complaints of veterans claiming exposure to Agent Orange. Humans with confirmed exposure and animals experimentally exposed have also been observed to have insomnia and waking lethargy. However no study of sleep changes resulting from exposure to Agent Orange or any of its constituents has been performed. We propose to determine the nature of the sleep disorder resulting from Agent Orange exposure. Specificity, using cats as a model, we will determine:

- 1) What dose of Agent Orange is required to disturb sleep.
- 2) Which constituents of Agent Orange are responsible for its effects.
- 3) What is the duration of the sleep disorder after administration.
- 4) Which stages of REM or nonREM sleep are disturbed.
- 5) What changes in the cyclicity of sleep stages and number of arousals within sleep occur.
- 6) What changes result in the power spectra of the sleep and waking electroencephalogram of affected cats.

We have completed pilot dose-response studies and have been running experimental subjects. A low and high dose group has been formed and 4 cats dosed. Each cat has been run for at least 500 hours. Sleep disturbances were seen in the high dose condition during the first week after administration. Preliminary indication are that more normal sleep patterns were seen in subsequent weeks. Long term repeated monitoring is being used to detect any delayed or persistent effects. Subsequent work will employ Agent Orange with higher levels of dioxin contamination.

SEP 26, 1984

VETERANS ADMINISTRATION
RESEARCH AND DEVELOPMENT INFORMATION SYSTEM
AGENT ORANGE

MECHANISMS OF DIOXIN INDUCED TOXICITY USING THE CHLORACNE MODEL
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PROJECT 3
LAST REPORT, INITIAL 01/81

ASSOCIATES: Puhvel, SM

Because of the apparent selective sensitivity of follicular epithelial cells to polyhalogenated aromatic hydrocarbons, elucidating the mechanisms of toxicity for such cells may provide a better understanding of the mechanisms of such toxicity in general.

In human beings, development of chloracne is the most sensitive indicator of exposure to toxic polyhalogenated aromatic hydrocarbons. The mechanisms of pathogenesis of this disease are not known. It appears that epithelial cells lining the ducts of human sebaceous follicles are primary target organs and are transformed into an abnormal pattern of differentiation upon exposure to such chemicals. This results in formation of retention hyperkeratosis, comedones and chloracne. To understand the mechanisms involved in this transformation, we propose to use experimental animals for in vivo, and human skin biopsies for in vitro studies to investigate the biochemical and metabolic parameters which may be affected upon exposure to dioxin into polychlorinated biphenyl. Specifically, we will monitor certain enzymatic markers during experimentally induced chloracne in hairless mice and in rabbit ears. We will also examine the effect of dioxin and polychlorinated biphenyl exposure on sebaceous follicle lipid biosynthesis and androgen metabolism in in vitro assays and cellular kinetics of follicular epithelial cells in tissue culture. Finally, we will attempt to identify the localization of these aromatic hydrocarbons in the skin by using radiolabeled tracers.

OCT 23, 1984

VETERANS ADMINISTRATION
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AGENT ORANGE

Mechanism of TCDD Absorptions and Toxicity on Lipid & Lipoprotein Metabolism
LAKSHMANAN, RAJ M., Ph.D. 688 WASHINGTON, DC

PROJECT 2
LAST REPORT, PROGRESS 03/84

AGENT ORANGE, TCDD, CHYLOMICRONS

The main objectives of the project in the 1st year were (1) to investigate the absorption and distribution of TCDD and (2) to evaluate the half-life of TCDD in the plasma compartment. Since the Bag in/Bag out HEPA Filter system has not yet been installed in our Agent Orange laboratory safety hood, we couldn't carry out extensive studies as originally planned. However, limited studies using [(supra 3)H] TCDD (trace quantities) have been conducted with promising results.

It was found that about 31% of the intraduodenally administered [(supra 3)H] TCDD was recovered in the lymph chylomicron fraction, whereas about 58% was excreted in the feces. Thus it is clear that lymph chylomicrons are the major carriers of newly absorbed TCDD. Administration of [(supra 3)H] TCDD labeled chylomicrons intravenously & analysis of [(supra 3)H] radioactivity in the arterial blood samples at various intervals revealed that the half life [(supra 3)H] TCDD in the plasma compartment was around 17 min.

SEP 26, 1984

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AGENT ORANGE

TCDD-Exposed Rhesus Monkeys: Effects on Behavior and Stress Hormones
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PROJECT 4
- LAST REPORT, PROGRESS 01/84

ASSOCIATES: Bowman, RE Gross, ML

AGENT ORANGE, TCDD, 2,3,7,8-TETRACHLORO -DIBENZO-P-DIOXIN, RHESUS MONKEYS,
ACTH, HPA AXIS, CORTISOL, STRESS, LEARNING, MOTOR CONTROL

A. Project Objectives: This project is designed to investigate the possible neuroendocrine and behavioral effects of 2,3,7,8-tetrachloro-dibenzo-p-dioxin (TCDD) upon perinatally exposed rhesus monkeys. TCDD is a major contaminant of the defoliant-Agent Orange.

B. Plan: We have rhesus monkeys which were perinatally exposed to TCDD and age-matched controls. We plan to test motor function, cognitive performance, and endocrine and behavioral responses to stress. We will also study the hypothalamic-pituitary-adrenal (HPA) axis diurnally and using both the dexamethasone suppression test (DST) and adrenocorticotrophic hormone (ACTH) infusion. TCDD concentrations in omnia fat samples will be measured and correlated with behavioral and endocrine measures.

C. Method: We will use radioimmunoassay (RIA) to measure ACTH and cortisol concentrations in plasma samples. TCDD will be measured using GC-mass spectrometry. Wisconsin General Testing Apparatus (WGTA) will be employed for cognitive performance. Operant chambers will be used to measure discriminative motor control.

D. Results: We have studied locomotor activity and found the initial hyperactivity seen in our TCDD group, at 6 mos. has subsequently normalized. The HPA axis function as tested by ACTH infusion, DST, diurnal rhythm of ACTH and cortisol, and endocrine response to stress is undifferentiated between the groups. A wide range of TCDD concentrations was detected in omnia fat samples taken at 5 mos. of age from the TCDD exposed animals. We were unable to detect any TCDD in omnia fat samples taken from our age-matched controls.

E. Clinical Relationship: Clinical reports suggest psychological and neurological effects from TCDD exposure, but because of other factors, it is difficult to assess the role of TCDD in humans. The rhesus monkey is a good model of the human for behavioral neuroendocrine responses to stress, as well as its toxic response to TCDD and similar compounds. We are studying TCDD's effects in a controlled experiment to assess its role in mediating human symptoms.

SEP 26, 1984

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AGENT ORANGE

Effects of 2,3,7,8-tetrachlorodibenzodioxin on hepatobiliary function in animals

FUJIMOTO, JAMES M., Ph.D.

695 WOOD, WI

PROJECT 4

LAST REPORT, PROGRESS 04/84

ASSOCIATES: Calvanico, N

Dawson, C

2,3,7,8-TETRACHLORODIBENZODIOXIN, HEPATOTOXICITY

TCDD is known to cause liver damage in rats. Several different aspects have been undertaken in our studies.

PROJECT OBJECTIVES: 1. Does TCDD affect the liver damage produced by another hepatotoxin, CCl(sub-4).
2. Does TCDD affect the biliary excretion of epidermal growth factor (EGF).

PLAN AND METHODS: Rats are given 10 mug/kg of TCDD in oil orally.

- (1). Three days later they are challenged with a hepatotoxic dose of CCl(sub-4) and 2 hours later their liver function was tested by ability excrete (supra 3)H ouabain (given i.e.) in bile.
- (2). On day 4, the rats are given a load of EGF, followed in 4 hours by another load of EGF. Total excretion of EGF in bile is measured.

RESULTS TO DATE: It appears that TCDD pretreatment decreases the hepatotoxicity of CCl(sub-4). CCl(sub-4) by itself profoundly decreases biliary excretion of (supra 3)H ouabain. TCDD pretreatment appears to protect against this effect. It may be that TCDD inhibits the microsomal cytochrome P-450 system responsible for activating CCl(sub-4) to its free radical form (CCl(sub-3)). A preliminary experiment seems to indicate that TCDD treatment decreases biliary excretion of intravenously administered EGF. The measurement of EGF in human urine has not yet started.

PUBLICATIONS: None.

SEP 26, 1984

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AGENT ORANGE

Neuromuscular Toxicity of Agent Orange

JOHNSON, KENNETH P., M.D.

512 BALTIMORE, MD

PROJECT 3

LAST REPORT, INITIAL 12/82

PROJECT OBJECTIVE: To investigate the effects of 2,4-dichlorophenoxyacetic acid (2,4-D) and 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in the neuromuscular system in rats.

PLAN: To assess neuromuscular function in a broadbased, multidisciplinary approach involving behavior, neurochemistry, neurophysiology, and neuropathology. Specifically, we propose the following:

- 1) To determine whether short or long term exposure to 2,4-D or TCDD causes peripheral neuropathy in rats.
- 2) To determine whether 2,4-D or TCDD causes pathological alteration in the neuromuscular function in rats.
- 3) To determine whether 2,4-D or TCDD causes muscular atrophy, altered fiber type distribution, myopathy, or muscle degeneration and regeneration.
- 4) To determine whether 2,4-D or TCDD alters the ability of the neuromuscular system to repair itself following injury or to carry on normal turnover processes.
- 5) To determine whether 2,4-D or TCDD causes altered responses of muscle to the anabolic effects of androgens or to the catabolic effects of glucocorticoids.

It is anticipated that the results of this research will answer the question whether delayed neuromuscular toxicity results from exposure to the components of agent orange.

SEP 26, 1984

VETERANS ADMINISTRATION
RESEARCH AND DEVELOPMENT INFORMATION SYSTEM
AGENT ORANGE

Behavioral Toxicity of an Agent Orange Component 2,4-D
DOUGHERTY, JOHN A., Ph.D. 596 LEXINGTON, KY

PROJECT 4
LAST REPORT, PROGRESS 01/84

2,4,-DICHLOROPHENOXY ACETIC ACID N-BUTYL ESTER, 2,4-D, AGENT ORANGE,
HERBICIDE, BEHAVIORAL TOXICITY, OPERANT BEHAVIOR, AMPHETAMINE, METABOLISM

Objectives: To determine if the herbicide 2,4-D n-butyl ester exerts behaviorally-toxic effects in albino rats by altering food-reinforced performance, memory, and motor function after acute and chronic administration.

Plan: Determine 2,4-D dose-response and time-response functions on lever pressing and compare with that of similarly administered amphetamine. After pharmacodynamic parameters have been explored, the effects of 2,4-D on more diverse behaviors will be determined.

Methodology: 2,4-D will be injected subcutaneously in polyoxyethylated castor oil at various times and doses before the following behavioral tests: responding reinforced by sucrose pellets on a variable-interval, time-out schedule; discretetrial spatial alternation between two levers; photocell activity; and ability to maintain balance on a rotating drum.

Results to Date: Suppression of responding occurred within 60 minutes of 2,4-D injection, reaching a peak at 4-6 hours. Pre-injection baselines were recovered within 24-48 hours. The suppression of responding was completely reversible and no cumulation occurred when injections were spaced 48 hours apart. Doses of 10-300 milligrams per kilogram (mg/kg) were effective; motor incoordination was evident at the highest dose. Castor oil vehicle injections in a control group had no systematic effect. In comparison, d-amphetamine produced biphasic effects at 0.1 - 1.0 mg/kg. Metabolism studies showed that 95 percent of a 100 mg/kg dose of 2,4-D was excreted in urine within 48 hours, 97 percent as 2,4-D acid.

Clinical Relationship: These results are relevant to the possible effects of Agent Orange in exposed Vietnam-era veterans.

SEP 26, 1984

VETERANS ADMINISTRATION
RESEARCH AND DEVELOPMENT INFORMATION SYSTEM
AGENT ORANGE

Mechanism of Porphyria caused by TCDD and Related Chemicals
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PROJECT 5
-LAST REPORT, PROGRESS 10/83

ASSOCIATES: Sinclair, JF Sinclair, PR

PORPHYRIA, TETRACHLORODIBENZODIOXIN, LIVER, DELTA-AMINOLEVULINATE, CULTURE

Objectives: The purpose of this project is to determine the mechanism by which 2,3,7,8-tetrachlorobenzo-p-dioxin (TCDD) and related polyhalogenated aromatic hydrocarbons (PHAH's) such as polychlorinated and polybrominated biphenyls cause inhibition of hepatic uroporphyrinogen decarboxylase, an enzyme of the heme biosynthetic pathway. When this enzyme is inhibited, the liver becomes overloaded with uroporphyrin (URO).

Research Plan: This study uses tissue cultures of chick embryo liver cells in which URO accumulation occurs in hours after TCDD or PHAH treatment. The project with TCDD is to extend and complete studies already begun with PHAH's. Dose response, time courses and the roles of different isozymes of cytochrome P450 are to be investigated. Our collaborator, Prof. G. Elder of the Welsh National Medical School, is to assay uroporphyrinogen decarboxylase activities in the cells and to purify the enzyme so that antibodies can be prepared.

Methodology: The chick liver cell culture is used in our laboratory in investigations of the regulation of heme and cytochrome P450. Decrease in uroporphyrinogen decarboxylase is most readily assayed by the change in composition of porphyrins accumulated after addition of the heme precursor, delta-aminolevulinate. Control cells accumulate protoporphyrin. The compositions are readily determined fluorometrically and confirmed by HPLC.

Results to Date: No TCDD experiments have been carried out due to delays in completion of a safe laboratory (expected to be completed in early 1984). Decrease in the URO decarboxylase caused by PHAH's has been assayed in intact cells by the fluorometric assay and by the direct enzyme assay of homogenates of the same cells. The surprising result was that low concentrations of PHAH were required to cause the URO accumulation by intact cells, whereas higher concentrations were required to cause decrease in enzyme activity. Furthermore, the inhibition in intact cells could be reversed by homogenization of the cells. These results have been published (FEBS Lett 152:217-221, 1983) and suggest i) that the usual homogenate assay might underestimate the inhibition of the enzyme in the intact liver, and ii) that the mechanism of the enzyme inhibition does not involve covalent binding of PHAH's or their metabolites.

SEP 26, 1984

VETERANS ADMINISTRATION
RESEARCH AND DEVELOPMENT INFORMATION SYSTEM
AGENT ORANGE

Effects of Low Dose TCDD on Mammalian Chromosomes and Liver Cells
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PROJECT 2

- LAST REPORT, PROGRESS 01/84

DIOXIN, TCDD, CHROMOSOME DAMAGE, LIVER DAMAGE

Project Objectives: We propose to assess possible health hazards of sublethal exposure to TCDD by seeing if this agent causes chromosomal aberrations or liver damage.

Work accomplished in 1983: In the first year of the project we have accomplished the following project goals: 1) developed and implemented a safety plan for the safe handling of TCDD and TCDD-exposed materials; 2) formulated TCDD in corn oil and assayed content independently by two outside laboratories; 3) established a threshold (10 mug/Kg) of i,p. TCDD that does not cause liver damage to mice; 4) established that mice injected with high doses of TCDD (150 mug/Kg) that causes severe liver disease do not show any damage to bone marrow chromosomes as measured by the structural abberation, sister chromatid exchange, and micronucleus tests; 5) established a new method of preparing liver nuclei for cytophotometric assays of hepatotoxic effects of TCDD. The following publications are in preparation from this work:

1) Meyne JM, Allison DC, Smith J, Jordon S, Bose K: Hepatotoxic doses of Dioxin do not cause damage to mouse bone marrow chromosomes.

(in preparation)

2) Bose K, Allison DC: An improved method of preparing nuclei for absorption cytophotometric measurements. (in preparation)

Work Planned in 1984: In 1984 we plan to develop an assay to see if TCDD causes damage to mouse liver chromosomes. We will also study the capacity of low doses of TCDD to act synergistically in combination with low doses of other hepatatoxins.