Human Subjects Committee ~~killed~~
Wayne State U
Killed Proposal
Never Funded or Performed
- Spathelf

CENTRAL MICHIGAN UNIVERSITY
PROVOST AND VICE PRESIDENT FOR ACADEMIC AFFAIRS

June 20, 1985

Dr. Joseph Miceli, Associate Professor
Pediatrics and Pharmacology, Children's
Hospital of Michigan
Wayne State University School of Medicine
3901 Beaubien Boulevard
Detroit, Michigan 48201

SUBJECT: PBB/PCB Detoxification Study

RE: Grant Proposal to the Michigan Department of Public Health

Dear Dr. Miceli:

On behalf of the administration of Central Michigan University, I am tentatively approving our collaboration with Wayne State University on your proposed "PBB/PCB Detoxification Study," to the Michigan Department of Public Health.

CMU's participation will be contingent upon approval by the University's Board of Trustees, and of course by WSU's School of Medicine, Human Subject Review Committee.

Dr. Douglas Friedrich, Dean of Graduate Studies/Associate Vice Provost for Research, who has been working with you to coordinate activities, will remain as the administrative liaison on the study for the University.

The Office of the Provost has approved space and remodeling for the research should funding become available for the project from external sources. The indirect cost rate for CMU on this project will be 15% of total direct costs.

Should this proposal be funded, we look forward to a most beneficial research endeavor.

Sincerely,

John Cantelon

John Cantelon
Provost

dms

MOUNT PLEASANT, MICHIGAN 48859

Detoxification Study - Request for pilot project proposals for elucidating and developing new mechanisms to safely reduce or remove polybrominated biphenyls and polychlorinated biphenyls from the human body.

This proposal remains valid until notification of its rejection by the contracting agency.

CENTRAL MICHIGAN UNIVERSITY
PROVOST AND VICE PRESIDENT FOR ACADEMIC AFFAIRS

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MOUNT PLEASANT, MICHIGAN 48859

Shirley A. Walkowski, Associate Director
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Wayne State University
Detroit, Michigan 48202 (313) 577-2283

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Individual to sign for the University:
Daniel J. Graf, Director
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Wayne State University
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Principal Investigator: Joseph N. Miceli
Associate Professor of Pediatrics and Pharmacology

Administrative and analysis facility: Wayne State University/Children's Hospital of Michigan

Subcontractor:
1. Central Michigan University
Mt. Pleasant, Michigan 48859

Contact Person:
Dr. Douglas Friedrich
Dean of Graduate Studies
Associate Vice-Provost for Research

The treatment protocol will be performed at Central Michigan University (facility site) and the expenses associated with this are to be sub-contracted to Central Michigan University. The work to be sub-contracted includes instillation of the sauna, renovation of the treatment facility site, hydrostatic weighing procedures, obtaining of EKG and chest x-rays.

III - B. STATEMENT OF THE PROBLEM

Michigan citizens have been exposed to PCBs and PBBs. Today, ten years after exposure to PBB, PBB still remains in body tissue and circulating blood in those individuals so exposed. Reduction of body burden of both xenobiotics without creating an increased risk from these compounds would be desirable. Additionally, evaluation of the physiological processes involved in storage and release of PBB/PCB from body tissues, identification of points in physiological functions at which reduction or removal of PBB/PCB may be enhanced and identification of selected intervention measures for body burden and blood level reduction need to be investigated. The purpose of this submitted study is to evaluate a proposed method to safely reduce the human PBB and PCB body burden.

Central Michigan University
(Treatment Facility Site)

The site at which the detoxification procedure will be conducted is Central Michigan University (CMU), located in Mount Pleasant, Michigan. The selection and use of this facility is based on the following factors: (1) It is centrally located in the heart of the "high" PBB exposure area of the state, (2) there exists indoor and outdoor field track facilities, (3) an under-water weighing tank is available for fat-mass measurements, (4) a modern, conveniently located student health facility is available for use, (5) space is available for remodeling to include the sauna, shower room and examining room facilities, and (6) Mount Pleasant Community Hospital, located about 1.5 miles away from the University has a fully operational emergency room and will be available if required.

The projected duration of use of the CMU site is approximately 3 months. This includes the time required for renovation of the facility site. Since the indoor and outdoor field tracks and under-water weighing facilities are already in place, no additional funding or requirements for these facilities are required.

III - C. MANAGEMENT SUMMARY

1. Narrative

ABSTRACT

This purpose of this proposal is to evaluate a proposed treatment protocol for the removal of polybrominated biphenyl (PBB) and polychlorinated biphenyl (PCB) from humans. The treatment protocol consists of dietary supplementation with vitamins, minerals and polyunsaturated oil; exercise and exposure to dry heat. The treatment protocol lasts for 21 consecutive days. During this time period, participants gradually receive increasing doses of the supplements and increase the daily time exposed to dry heat (sauna). A preliminary report (1) indicated an average 21% reduction in PBB body burden after 3 weeks and an average 42% reduction 4 months after the completion of this treatment protocol. Twenty individuals (10 males and 10 females) will take part in the present study.

All subjects will have 3 separate simultaneous blood and fat PBB and PCB measurements to assess the over-all body burden reductions of these compounds. These samples will be obtained immediately before, upon completion and 6 months after completion of the treatment protocol. This will provide baseline data (pre-treatment) for comparison to post treatment body burdens. Additionally, on about day 15 of the treatment protocol, several blood samples will be obtained from each individual so that any transient increases in blood PBB and PCB levels that may occur due to the treatment can be assessed. Evaluation of the data will determine the extent of blood PBB and PCB increases during treatment, the amount of PBB and PCB eliminated in the feces before and after the treatment protocol and the extent of body burden reduction by determining fat PBB and PCB concentrations before and after the treatment protocol. In order to ensure that any documented declines in body burdens are not due to fat-weight reduction, hydrostatic weighing will be performed each time a fat biopsy sample is obtained and results reported

will be normalized to fat mass for that individual. Each participant will serve as his own control according to the experimental design of the protocol. PBB and PCB will be analyzed by standard, published methods using capillary column electron capture gas chromatography. The isomeric PBB and PCB composition will be determined before and after participation in the treatment protocol to document reduction of individual isomers.

Central Michigan University (CMU) will provide support (see attached letter) as a subcontractor to this proposal by providing a centrally located facility site in which to conduct the treatment protocol aspects of this proposal.

Central Michigan University
Dr. Douglas Friedrich
Dean of Graduate Studies
Associate Vice-Provost for Research
Mt. Pleasant, Michigan 48859
(517) 774-3093

2. Technical Work Plans

a. Specific Aims

1. To evaluate the effectiveness of a detoxification protocol by documentation of human body burden reductions of PBB and PCB. Documentation will be accomplished primarily by analysis of adipose tissue PBB and PCB concentrations before and after treatment protocol.

2. To determine if there is a transient increase in PBB and PCB blood levels during mobilization under the conditions of the proposed treatment. Existing unpublished data suggest that xenobiotic blood levels do not increase significantly during body burden clearing.

3. To determine the amount of PBB and PCB eliminated via the feces. The stool has long been considered the major route of lipophilic xenobiotic elimination. This study will determine the amount excreted before and after completion of the protocol.

4. To determine if the protocol is effective in removing all congeners at a comparable rate. There is evidence that certain PBB and PCB congeners are more toxic than others; therefore, it would be important to document elimination of these compounds along with the major components.

b. Significance

In Michigan in 1973, Firemaster PB-6, a commercially prepared fire retardant composed primarily of a mixture of polybrominated biphenyls (PBBs) was accidentally substituted for Nutrimaster, an animal feed supplement. This subsequently caused contamination in cows, pigs, chickens and ultimately humans via the food chain. In 1978, 97% of individuals residing in Michigan had measurable levels of PBB in their adipose tissue (2). In the ensuing years, animal studies have suggested that PBB may be hepatotoxic, neurotoxic, immunotoxic and carcinogenic (3-7). Halogenated aromatic hydrocarbons, such as PCBs and PBBs, administered to experimental animals, have been shown to cause wasting syndromes, immunotoxicity, skin disorders, endocrine and reproductive deficits, porphyria, hepatotoxic, neurotoxic and genotoxic effects (8). Similar effects have been recorded in humans for these compounds, as well as subjective complaints for PBB exposed individuals including headache, joint pain, loss of memory, nervousness, etc (8).

PBBs persist in humans and contaminated Michigan residents will probably bear this xenobiotic burden throughout their lives (9-13). PBBs are transmitted transplacentally to human fetuses and into maternal breast milk (14-16). These observations have also been made for polychlorinated biphenyls (PCBs) in humans (15, 17-20) and for both compounds in animals (21-23). More recently, Miceli, et al (13) have demonstrated the persistence of PBB in human post-mortem tissue and its distribution into 15 different tissues 10 years following exposure. If these lipophilic substances were sequestered only in lipid deposits (evidence indicates that is not the case [13]) and did not mobilize from such tissues, the threat to human health presumably would be low. However, mobilization of lipids within the body occurs throughout the day, can be induced by several mechanisms and involves both the active and inactive fraction of the adipose tissue. The active fraction constitutes only 5% of total adipose tissue and does not appear to

contain many of the xenobiotics found in the inactive fraction (24). Mobilization of the inactive fraction does occur with some regularity. Starvation (fasting), including the fast between the evening meal and breakfast, results in significant turnover in adipose tissue stores and consequent mobilization of xenobiotic burdens (25). Exercise has also been shown to mobilize lipids with some indication that the rate of mobilization is dependent upon the rate of blood flow through the adipose tissue (26-28). Stress has been found to cause lipid mobilization apparently through sympathetic nerve stimulation. This increases lipolysis as a result of diffusion of norepinephrine from nerve terminals on blood vessels to nearby adipose cells (29). The fractional turnover of lipophilic xenobiotics has been found to be approximately equivalent to the fractional turnover of the lipid pool under fully fed conditions as well as during periods of induced depletion of lipid reserves (30). Such a turnover would not be expected to favor one homolog of PBB or PCB over another and the potential for mobilization of each in the same proportion as they are found in adipose tissue would be possible. This, however, has not been formally tested. The analytical procedure proposed (see methods) will permit examination of this question.

It is known that the toxicities of PBB and PCB are dependent on the various congeners for each substance (31-35) and that different PBB congeners disappear at different rates from rat serum (36). The ratio of blood to adipose tissue concentration of PBB and PCB homologs are not consistent (14) and varies from individual to individual. We propose to investigate and determine the levels of PBB and PCB homologs in human serum and homolog composition in adipose tissue. Previously published work by Schnare, et al (1) suggests that the congeners will be equally removed from the body. Scu

Mobilization of PBBs and PCBs will be required to reduce the body burden of these xenobiotics. A variety of means to enhance lipid mobilization have been reported, including heat stress and use of nicotinic acid (37-39). These

tend to increase blood flow through tissue, including adipose tissue. The degree to which their administration affects mobilization of xenobiotics has not been studied in depth. Mobilization of PBBs and PCBs will not lead to body burden reduction without concomitant excretion and/or metabolism to less toxic substances (40). Since PBBs and PCBs are not appreciably metabolized, the proposed means of elimination of these compounds are via biliary, intestinal and sebaceous routes. Studies have documented the fecal route for both PBB and PCB elimination (14, 41-42). However, there may be significant re-absorption of these substances via enterohepatic recirculation (43).

Various methods have been suggested to overcome enterohepatic recirculation. Cholestyramine, high fiber diets, vegetable diets, sucrose polyester and paraffin have all been used (43-47). Unfortunately, each has side effects which tends to stress the liver due to fat deposition. Additionally, none of these methods has proven effective in long term body burden reduction.

A less stressing approach to overcome enterohepatic recirculation is the use of dietary polyunsaturated oil. Total fecal steroid excretion has been increased by 45% through the use of corn oil as the source of dietary fats (48). This type of supplement has been found to produce a decrease in plasma cholesterol which appears to be indicative of increased fecal excretion (49-50). Use of polyunsaturated oil has increased fecal excretion of persistant xenobiotics in rhesus monkeys, with both the intestinal and biliary routes contributing to the load (42, 51). The intestinal route appeared to predominate. Determination of the predominate human excretion route has not been done, nor has the potential for preferential excretion of some homologs of PBB over others been investigated. The analysis of homologs in feces will indicate the amount of the various congeners excreted via this pathway.

In order to accurately determine total adipose tissue burdens of PBB and PCB, the total amount of adipose tissue (fat mass) must be known. Hydrostatic weighing (under-water weighing) with simultaneous measurement of residual volume (52) will be used to determine fat mass.

Evaluation of this protocol will determine the feasibility of reducing PBB and PCB body burdens in large scale populations, such as those residents of Michigan contaminated with PBBs.

C. EXPERIMENTAL DESIGN AND METHODS

1. Body Burden Reduction Procedure

Overview:

The treatment regimen, as described in this protocol, will be conducted under highly supervised conditions. The program entails exercise, dry heat (low temperature sauna exposure) and dietary supplementation (consisting chiefly of vitamins, minerals and polyunsaturated oil administration). This program can be strenuous; therefore, only "healthy" individuals will be allowed to participate in this study. However, individuals who have subjective complaints (headache, nervousness, etc.) attributed to PBB but who do not have abnormal clinical chemistries or physical impairment which would otherwise preclude them from participating in the study, can be included.

A. Preliminary Health Screening:

In the first month prior to the start of the treatment protocol, individuals selected for inclusion in the study will report to the facility site for collection of blood for determination of baseline clinical chemistries, lipid profile, complete blood count and urinanalysis.

Patients with abnormal results which indicate they may be at an increased risk to undergo the protocol will not be allowed to participate and replacements will be made from the reserve group (See Human Subjects). Those individuals selected for inclusion will be required to return the following week for a complete medical history and a physical examination performed by a physician. A chest x-ray and an EKG will be included in this examination. Copies of the forms to be used for this part of the study are in APPENDIX 1.

The purpose of the examination will be to evaluate the fitness of the patient to undergo the treatment protocol. The major criteria for elimination will be a history of cardiovascular and/or respiratory difficulties or other chronic disease, pregnancy, breast feeding by females and a history of unstable family or employment situation which may compromise full participation by the patient.

No medications, either prescription or non-prescription with the exception of oral contraceptives, will be allowed during participation in the study protocol, unless the physician deems it necessary for the welfare of the patient. This, however, is grounds for removal from the program. Each patient will be instructed regarding the signs and symptoms of heat stress and over-exertion as part of his preliminary introduction to the program. These signs and symptoms will be prominently displayed in writing in the facility site, particularly in the sauna area.

B. Mobilization (Body Burden Reaction) Procedure:

Mobilization of xenobiotics will be attempted during treatment via three interrelated processes: exercise, dietary supplementation and sauna-based dry heat exposure. All patients are required to undergo the treatment protocol on a daily basis until completion. The daily time required for participation in the protocol is approximately 5 hours.

1. Exercise: The exercise involved in the treatment protocol consists of a timed (usually 15-20 minutes) running or jogging program. This is performed shortly after arrival at the facility site and after consumption of the water soluble dietary supplements (see below). Exercise is performed only one time per day and is followed by sauna exposure.

2. Sauna: After running, the patient will spend the remaining time, intermittently in a sauna. Initially, this will consist of 10-15 minute exposure periods for a total daily time of 150 minutes. This is gradually increased to 15-20 minutes exposure periods for a total daily time of 240 minutes (APPENDIX 2). A dry sauna will be used and maintained at a temperature ranging between 140-180 degrees F. This mildly hot environment is used to enhance mobilization much in the same manner as does exercise by increasing peripheral blood flow. Usually a patient will start the program at the lower temperature and work up to the higher temperature as he progresses through the protocol. When a patient feels too warm he must leave the sauna and cool off. This can be accomplished by sitting or standing outside the sauna and/or taking a shower. After the cool down, they are permitted to return to the sauna again. The goal is to increase the daily sauna total time as shown in the daily treatment protocol (APPENDIX 2). Sauna time, replacement of fluid and electrolytes and body weight are carefully monitored by the staff.

It is possible that over-heating and salt depletion may occur. The signs of over-heating and/or salt (sodium and/or potassium) depletion include clammy skin, tiredness, weakness, headache, sometimes cramps, nausea, dizziness, vomiting and fainting. These signs will be explained to each participant and will be posted in the sauna area for constant reminder. If sweating ceases while in the sauna and the skin becomes hot and dry, this is an indication of impending heat stroke. The American National Red Cross has published the "Standard First Aid Personal Safety Booklet" which describes the symptoms and emergency management of heat stroke (APPENDIX 3).

The patient must be immediately removed from the sauna and cooled off via lukewarm shower or sponging - gradually decreasing the temperature of the water to cool. Water, salt and potassium should be administered during treatment. The emergency physician should be notified as soon as possible to determine if additional treatment is required. In conjunction with the foregoing, no person will be allowed to fall asleep in the sauna as a potentially dangerous situation may develop and no person shall be permitted in the sauna alone.

Salt repletion is not mandatory for each patient. It is necessary if the symptoms of salt depletion (heat exhaustion) occur. Since potassium is also lost during sweating, if salt (sodium chloride) does not correct the symptoms then potassium gluconate tablets or salt substitute should be administered. Sodium and potassium will be available for use if required. It is important that each participant replenish body water lost during the treatment protocol by drinking appropriate amounts of water.

3. Dietary Supplementation: The dietary supplements used in the protocol are niacin, vitamins A, B1, B complex, C, D, E, minerals and polyunsaturated oil. Details of the vitamin dosing regimen is provided in APPENDIX 4.

Niacin, nicotinic acid, is used to mobilize lipids via two separate mechanisms, the first of which is the brief but marked increase in peripheral blood flow. This is usually referred to as the niacin "flush." This flush typically lasts only a few minutes and may cause some transitory discomfort in certain individuals.

The second mechanism of action is the ability of niacin to produce a short term interruption of lipolysis. This causes a decrease in circulating blood lipids for a brief time period which is compensated later by a pronounced increase in circulating free fatty acid levels (38, 39). This brief period of lipid suppression has been found to have no effect on exercise performance (53). The daily niacin dose is gradually increased as the treatment progresses so that longer periods of increased peripheral blood flow are maintained. Doses are increased according to individual response to the niacin.

The above procedures are intended as a means to mobilize xenobiotics (PBB and PCB) from tissue stores. In order to reduce the body burden of these compounds, excretion must also be enhanced. Probable means of excretion are via sebum, bile and feces. Heat exposure increases sebaceous excretions by 10% for each 4 degree C rise in skin temperature (54). Sebum is a known reservoir for xenobiotics and due to the nature of its regular and continuous formation, constitutes a pathway from the blood to the skin and out of the body (54, 55).

The fecal and bile pathways are probably much more significant for elimination of PBB and PCB. Fecal elimination is probably due to intestinal transfer of xenobiotics from the blood into the gut and from bile concentration in the stool. Fecal and bile elimination of xenobiotics can be enhanced by ingestion of polyunsaturated oils (42, 56, 57). Since xenobiotics (in this case PBB and PCB) are expected to be concentrated in bile, the net effect is a reduction of these compounds from body tissues. The treatment

protocol requires the daily ingestion of from 2 to 8 tablespoons of blended polyunsaturated oil to promote elimination of the moblized PBB and PCB. Since the program is not designed as a means for losing weight, the doses are designed to maintain the initial body weight.

Animal studies have shown that significant dcreases of vitamin A in the liver and serum occur during PCB administration, leading to an increased requirement for this vitamin (58-60). The treatment protocol provides for large dose vitamin A dietary supplementation. Another vitamin potentially required during PCB mobilization is ascorbic acid. PCB contamination depresses the activity of L-gluconolactone oxidase and dehydroascorbatase and promotes urinary excretion of L-ascorbic acid. Dietary supplementation with L-ascorbic acid can provide protection from these deleterious effects (61,62). The treatment protocol also includes supplementation with this vitamin.

Because the treatment protocol is more vigorous than most people's normal routine, adequate sleep and a balanced diet is required. No change in normal dietary habits are usually required unless the usual diet is abnormally incomplete.

2. COLLECTION OF HUMAN SAMPLES

Overview

We propose to study a population of Michigan residents who experienced an unusually large acute exposure to PBB in 1973. The design of the protocol is such that each patient will serve as his own control. The effectiveness of the detoxification procedure will be evaluated by determining the PBB and PCB body burden in terms of fat and blood concentration of PBB and PCB congeners before and after the treatment protocol as described in this section.

To document that body burden reductions are not an artifact of redistribution to other tissue after mobilization and to document residual long term body reduction, a final body burden measurement will be performed at 6 months post treatment. Lean body mass measurements will be made conincidentally with adipose tissue sampling. Total body burdens will be estimated based on the concentration of PBB and PCB in the adipose tissue corrected for the mass of fat in the body. The body fat mass will be determined by underwater body weighing (hydrostatic weighing).

Since the proposed treatment protocol must enhance mobilization to effectuate PBB and PCB removal from the body, there is the potential that an increased risk to the patient may develop if the rate of excretion does not keep pace with the rate of mobilization. Preliminary data suggest that if there are increased blood levels during mobilization, they are low and transitory (1). In order to determine the extent of increased blood levels, the following indepth study will be performed: Approximately 15 days after the start of the treatment protocol, a Jelco catheter will be placed in either a forearm or antecubital vein. Patency will be maintained by a teflon stylet placed with a leur-lock connection. This will enable the patients to participate in the study with minimum impairment of physical activity as well as eliminate the need for a heparin lock for sample

collection. Five ml of blood will be obtained at the start of that day's treatment protocol and hourly thereafter until completion of that day's treatment. This will result in the collection of an addition 5 or 6 blood samples and evaluate the effect of the treatment protocol to cause an immediate transitory increase in serum PBB and PCB levels.

In order to document that the mobilized PBB and PCB is excreted, collection of feces will be performed at three specified times: at the start of treatment, at the completion of treatment and at the 6 month follow up time.

A. Preliminary Blood Profile

Prior to the start of the treatment protocol, a fasting blood specimen (at least 12 hours) will be obtained from each participant. The blood samples will be analyzed (SMA-12 biochemical profile) at Roche Biomedical Laboratories (RBL) for the following: BUN, uric acid, calcium, phosphate, alkaline phosphatase, total bilirubin, SGOT, LDH, cholesterol, glucose, total protein and albumin. RBL will also perform: lipid profiles (cholesterol, triglyceride, LDL, HDL, VLDL fractions) CBC, including differential and a urinalysis for each patient. Four ml of whole (unclotted) blood is required for the SMA-12 profile, 7 ml of anticoagulated blood (EDTA) for the CBC and differential, 6 ml of whole blood for the lipid profile and 10 ml of urine for the urinalysis. The lipid profiles will be repeated on day 15 of the protocol, at the end of the protocol and at the 6 month follow up.

Each patient will be assigned a time to report to the facility site so that blood can be obtained via venipuncture. This will be performed by a nurse or a physician. Each patient will be instructed to remain fasting from 8:00 p.m. of the day prior to the sample taking until the blood is

obtained; after which they are free to resume their normal daily activities. These initial blood chemistries and lipid profile will serve as baseline values for each individual. These tests will also serve to identify patients with abnormal values. Those persons judged unable to undergo the rigors of the program will not be allowed to participate in the study and acceptable replacements will be obtained from the reserve group (see Human Subjects).

B. Serum Concentration Measurements

At the start of treatment, at completion of treatment and at the 6 month follow up time, 5 ml of whole blood will be obtained from each patient for PBB and PCB blood concentration determination. The first sample represents the baseline (pretreatment) value. The second sample will document the extent of serum reduction at the conclusion of the treatment period and the third sample will document the 6 month follow up serum reduction. These samples will be collected via venipuncture in a standard manner (5 ml vacutainer red top tube or 5 ml syringe collection and subsequent expulsion into a glass tube) by a nurse or physician at the facility site.

C. Adipose Tissue Concentration and Fat Mass Measurement

All adipose tissue samples will be taken by a physician at the facility site. The method and the procedure for fat needle biopsy has been previously described (63) and widely used for collection of adipose tissue samples. Approximately 150-500 mg of lipid can be collected using this method.

Samples of adipose tissue will be obtained on three separate occasions, corresponding to the serum collection times (see above). The first sample will provide an indication of initial PBB and PCB body burden and will serve as the immediate pre-treatment body burden measurement. The second

sample will be taken upon conclusion of the treatment and will indicate immediate post-treatment body burden reduction and the last sample will be obtained 6 months following completion of the protocol and will determine the extent of continued body burden reduction.

Fat mass measurements will be performed at the facility site (CMU), which has an under-water weighing tank facility and is available for use in this study. Measurements will be conducted by trained personnel under the supervision of the CMU staff. Corrections will be made for residual lung air. Fat mass measurements will be performed each time a fat sample is obtained. Fat mass will be calculated using the equation of Brozek et al, (64). A description of the technique is described here. Each patient will be individually seated on a platform in shoulder depth water with neither the patient nor the platform touching the pool sides or bottom. The platform will be suspended from a spring dial scale previously calibrated to account for the weight of any ballast weights required for patient stability in the water. The patient will wear a nose clip and breath room air. When ready, the patient will make a maximal exhalation and bend forward until completely submerged. During maintenance of expiration (2-3 seconds) the underwater weight is manually recorded. The patient then lifts his head above the water and resumes normal breathing. An estimation of residual air in the lungs will be made by use of standard tables based on age, sex, body size and weight (64).

D. Bile and Fecal Collections

In order to document that the mobilized PBB and PCB are excreted and not recirculated, feces will be collected to coincide with serum and fat collection. This sampling timetable will determine the amount of PBB and

PCB excreted prior to treatment, amount excreted immediately after treatment and determine if there is continued excretion after cessation of the protocol. A 24-hour fecal collection will be obtained from these patients using standard disposable collection containers. All feces collected during the 24-hour interval will be placed in a deordorized pre-weighted metal storage container. These containers will be stored in a freezer until analyzed (see Analysis of Fecal Samples) for PBB and PCB content. Weight of the stool sample will be determined by subtracting the weight of the can from the weight of the can plus stool. Analysis of these samples will document the total amount excreted via the feces. Although it would be of importance to continue the work of Eyster, et al (14), and determine the extent of PBB and PCB excretion in bile, budgetary constraints preclude the inclusion of this important aspect of determining the excretory process of these compounds.

E. Lipid Determinations

The lipid profile tests are those described above and are for the determination of total cholesterol, triglyceride, VLDL, LDL and HDL fractions in fasting blood. Samples for these tests will be obtained as part of the screening profile prior to inclusion into the study, at the completion of the treatment and at the 6 month follow up examination. Analysis will be performed by Roach Biomedical Laboratories using their usual and established procedures for these tests.

F. Group Identification

In order to evaluate the protocol on the 20 patients, 2 groups of patients will be formed. The groups will participate in a staggered schedule so that one group a week for 2 consecutive weeks will be started. Both groups are required to return for a 6 month follow up visit for final blood, fat, fat mass and lipid profile measurements.

3. ANALYSIS OF SAMPLES FOR PBB AND PCB CONTENT

Overview

All samples will be analyzed using capillary column electron capture gas chromatography. This technology is routinely used for the quantitation of halogenated hydrocarbons such as PBB and PCB. The extraction procedure for each sample will be presented prior to the sections pertaining to the gas chromatography methodology. All samples (except feces) will be placed in PBB- and PCB-free containers for transportation and storage until analysis. Determined values of PBB and PCB will be normalized relative to the amount of lipid present in each sample.

A. Adipose Tissue and Blood Analysis

Procedures for the extraction of PBB have been used by Miceli (12, 13) and are based on other techniques (65, 66). These extraction procedures will be used for the simultaneous extraction of PBB and PCB. Approximately 100 mg of adipose tissue and 2 ml of serum are required for extraction. The concentrate will then be chromatographed (see below) so that PCB and PBB congeners will be simultaneously determined.

B. Fecal Samples

Extraction of PBB and PCB from fecal specimens will be performed according to the procedure described by McCoy (67) and listed here.

Materials and Reagents

Petroleum ether, distilled in glass, Burdick & Jackson Laboratories

Ethyl ether, anhydrous, reagent grade, Mallinckrodt

Benzene, distilled in glass, Burdick & Jackson Laboratories

Florosil, 60/200 mesh, Applied Science Laboratories

Sodium Sulfate, anhydrous, granular analytical reagent, Mallinckrodt

Hexabrominated biphenyl, technical material, Food and Drug Administration, 1050 74-555

Soxhlet extraction apparatus, Pyrex

Extraction thimbles, 33 mm x 80 mm, Whatman

Glass columns, Pyrex No. 8552, 7/16 in. ID x 12 in. long, equipped with teflon stopcocks

Procedure

1. A representative fecal sample of between 50-500 mg is placed in a 50 ml beaker and mixed with sufficient sodium sulfate to completely cover the sample. The sample is allowed to air dry at room temperature for at least 24 hours.

2. The sample and sodium sulfate are transferred to an extraction thimble and extracted on a soxhlet extractor for 8 hours using 200 ml of 50:50 petroleum ether:ethyl ether.

3. The resulting extract is evaporated to dryness using a warm water bath and air stream. The residue is volumetrically reconstituted to 10 ml with petroleum ether.

4. Columns are prepared by first placing a glass wool plug above the stopcock. Florosil is then packed in the column to give a height of 3 inches and then sodium sulfate is added to give an additional 2 inches in height.

5. Each column is washed with approximately 50 ml petroleum ether which is discarded.

6. 2 ml of the reconstituted sample prepared in step 3 is placed in a tared 10 x 75 disposable test tube. The solvent is evaporated in a hot water bath with the aid of an air stream. The tube is then heated at 50 degrees in an oven for 2 hours and after cooling to room temperature, the tube is placed in a dessicator

for several hours. The tube is then weighed again. This weighed amount of fat extracted from the fecal sample is used as a basis for calculations later.

7. Six ml of the reconstituted sample prepared in step 3 is placed on the prepared column and allowed to drain into the packing materials. The column is then eluted with 50 ml of petroleum ether which is collected in 20 x 150 mm glass tubes.

8. The eluate is evaporated in a warm water (50°C) bath and in the final stages is transferred to a 12 x 75 mm glass disposable tube.

9. This dried residue is reconstituted with 500 microliters of benzene and mixed on a vortex for 30 seconds. One microliter is injected into the gas chromatograph. If the sample has an unusually high PBB content it may be necessary to reconstitute the sample in a suitable larger volume.

10. The concentration of PBB in the sample is determined by reference to a standard curve prepared using PBB standards of 9, 10, 30 and 50 ng/ml in benzene.

11. A reagent blank is run through the above steps for each analytical run to check for contamination.

Calculation

Specimen weight -------- gm

Tube with lipid -------- gm

Tared tube -------- gm

Lipid -------- gm x 3 = _____ gm lipid on column

PBB = _____ ng/sample

On extracted lipid basis PBB = _____ ng gm

C. Gas Chromatography

Analysis of PBB and PCB will be performed using electron-capture gas chromatography. In order to determine the different PCB congeners present, the procedure recently described by Safe and Mullin (68) will be used. These authors state (personal communication) that the PBB congeners can also be measured simultaneously with this procedure by merely extending the length of the chromatographic run prior to resetting to the initial conditions. Both Drs. Safe and Mullin are consultants for this aspect of the study.

D. Quality Control

Dr. Stephen Safe has agreed to participate in this aspect of the study. His laboratory will prepare a pooled serum standard containing a known amount of PBB and PCB congeners. The amount and choice of PBB and PCB will be his. His laboratory will analyze an aliquot of this standard and ship (frozen) the remainder to the laboratories at Wayne State University. Upon receipt, an aliquot will be analyzed for PBB and PCB content. If the determined results are in agreement with the targeted results, analysis of patient samples will be permitted. If the results are not to Dr. Safe's satisfaction, there will be a repeat analysis until we are given the "go ahead" to proceed. The pooled serum will be aliquoted into individual 2 ml samples and stored frozen as such. These "quality control" samples will be analyzed after every 10 patient samples to insure the integrity of the assay. If the results of analysis of this standard indicate an inconsistency with time, Dr. Safe will prepare a fresh standard for a new quality control standard. The procedure to be followed in this case will be the same as the initial one.

4. Data Analysis

The final report will contain the raw data points for all participants in the study, including complete PBB and PCB congener listing and quantitation for all aspects of the study. Specifically, the data will be analyzed to determine if the treatment protocol successfully removed PBB and PCB from the study population and to what extent removal occurred. This will be accomplished by determining the amount of PBB and PCB congeners present in the blood and fat prior to the start of the protocol and comparing these values to subsequent values obtained at the completion of the protocol and 6 months after treatment is completed. The data will be analyzed to determine the overall decreases in body burden and to determine if the various congeners are removed from the body at the same rate.

PBB and PCB congener amount in the feces prior to treatment will be compared to those values obtained after the treatment. These values will then be correlated with the amount in the blood and fat. To determine if there is a transient increase in circulating PBB and PCB during mobilization, the amount of PBB and PCB congeners determined during the acute blood mobilization phase (hourly samples) will be compared to the amount present in the blood at the start, completion and 6 months after the completion of the protocol.

The mean value for each type of sample (blood, fat, feces) will be calculated and tabulated for each collection time so that "mean" body burden reductions can be estimated. Additionally, the amount of PBB and PCB body burden reduction will be determined for each individual.

The estimated number of samples for PBB and PCB analysis is:

Tissue	Numbers
Fat	60
Blood	160
Feces	60
Quality control	30
Standards	50
Total:	360

This figure does not include duplicate analysis or other unforeseen events requiring analysis of additional samples. Since PCB and PBB consist of large numbers of congeners (8, 68) it is reasonable to assume that each sample analyzed will contain at least 150 identifiable congeners, as gas chromatographic peaks (68).

Standard statistical tests, such as the paired t-test, linear regression calculations to determine correlation coefficients and multiple analysis of variance (ANOVA) of individual data will be used to determine the significance of any observed changes in body burden. These methods will be used for calculation of each PBB and PCB homolog concentration in blood, adipose tissue, feces and bile as well as for analysis of changes in the circulating blood lipids during the treatment protocol. Data forms for this protocol are provided in APPENDIX 5.

5. HUMAN SUBJECTS:

Human subjects will be used to evaluate the proposed treatment protocol. A copy of this application has been submitted to the Wayne State University Human Experimentation Committee for review. Approval is pending. Risks associated with this proposed treatment are discussed in this section along with other pertinant information regarding human subjects. This information is presented in a uniform document rather than segregation in section a, b, c, etc., as outlined in the RFP. All samples (materials) and/or data will be obtained specifically for the purpose of this contract. Previous information from patients selected for inclusion will be used to document changes in body burden (see below). Informed consent (APPENDIX 6) will be obtained by either Dr. Cuillo or Dr. Miceli at the Central Michigan facility site prior to inclusion in the study. Each patient will also be given a "patient information sheet" (APPENDIX 7). As mentioned in the significance section, currently there appears to be no alternate, viable procedure for long-term elimination and body burden reduction of PBB and PCB. Emergency medical intervention is available through Mount Pleasant Community Hospital, a Hospital with a fully operational emergency room and located approximately 1.5 miles from the Central Michigan University facility site. The Hospital has been informed of this study and will respond if necessary. The risks to the patient, as described below, are minimal considering that the potential benefit may be to reduce the body burden of PBB and PCB in these individuals.

A. Population Selection

The population for inclusion in the study will be obtained from the area surrounding CMU, encompassing three Michigan counties: Isabella, Newwaygo and Mecosta. These areas had an high exposure to PBB 10 years ago (2). In order to be included in the study, each individual must have a history of residency in the state (preferably in the above

counties) at the time of the contamination. The population to be studied will include families residing on farms quarantined by the state, workers employed by the manufacturer of PBB, residents of farms which had lower levels of PBB contamination and were not quarantined, farm product consumers and children, who will now be attending high school or college.

The total population reasonably considered available for this study is estimated at about 20,000 people. Volunteers for inclusion in the study will be obtained from a list of about 1,000 individuals who are part of the Michigan PBB cohort (to be supplied by the Michigan Dept. of Public Health) or the population will be obtained by news media solicitation.

The initial contact will consist of an information/qualifying questionnaire (APPENDIX 8). This questionnaire will be used to identify the population (20 males and 20 females, aged 18 to 40 years). Individuals with previously determined PBB measurements will be given preference for inclusion in the study. Screening blood chemistries will be obtained from this study population.

At the time of selection, a medical release for information concerning previously determined fat and blood PBB concentrations will be obtained and directed to the appropriate source. This baseline data will be included in the patient's chart and document the extent, if any, of spontaneous PBB tissue decline during the past decade in these individuals. From this group, the final participants will be selected and alternates identified. The 20 selected will then proceed to have the physical evaluation and ultimately inclusion or exclusion from the study. Criteria for disqualification will include a history of drug

abuse, unstable family situation, unwillingness to cooperate, pregnancy, cardiovascular, chronic pulmonary, or renal disease, breast feeding in females, diabetes, and/or any other physical or medical hinderance to participation in the program.

Informed consent will be obtained from each patient prior to participation in the study and a patient information sheet will be given to each patient prior to the start of treatment. In order to assure confidentiality, each patient will be assigned a code number. All samples from that patient will be placed in collection containers appropriately labeled for that individual. Only the project manager, the principal investigator and the attending physician will have access to the code. A daily progress report will be maintained for each patient and included in his chart.

B. Risks

This program involves exercise, sauna-induced dry heat, dietary supplementation, collection of blood, fat and feces. Each of these procedures has potential risks associated with it as detailed here. In order to minimize any risks associated with exercise, sauna or dietary supplementation, only "healthy" individuals will be selected for inclusion in the study. Criteria for noninclusion in the program include: a history of cardiovascular, renal or respiratory disease, pregnancy, breast feeding by females and a history of unstable family or employment situation. Subjective complaints attributed to PBB, such as headache, nervousness, etc., will not be grounds for exclusion from participating in the treatment protocol. History will be evaluated in conjunction with the screening blood tests (routine clinical chemistries and lipid profile), chest x-ray, EKG and physical examination. In addition, there

is careful daily monitoring of each participant's activities (weight, running time, sauna time) by the study staff. Therefore, any risks associated with this aspect of the protocol are exceedingly small.

Risks attributed to the dietary supplements, likewise, should also be small. Ingestion of large doses of niacin do produce the so-called niacin "flush." This is due to peripheral vasodilitation, is transient (duration a few minutes), does not occur in all individuals and although the flush may feel discomforting in some people, it is not dangerous or harmful. Although there may be some side effects of "high" dose vitamin therapy for certain vitamins, the dose and duration of treatment with the vitamins in this protocol should present little increased risk. Daily monitoring of the patients by the staff will help ensure that signs of toxicity are detected early. Likewise, the ingestion of cholesterol-free polyunsatruated oil should pose no increased health risk to the patient. The determination of the lipid profiles at 3 separate times will enable the objective determination of atherogenic risk to these patients.

Risks attributed to the collection of blood are also very minor. Potential risks include bruise formation and very rarely, infection. These risks will be minimized by using sterile collection techniques and by having the blood collected by a nurse or a physician. A greater potential risk exists for the indepth, acute mobilization study. To conduct that study, a Jelco catheter will be placed in a forearm or antecubital vein by the nurse or physician. Patency will be maintained by a teflon stylet placed with a leur-lock connection. This will eliminate the need for a heparin lock, providing greater comfort to each patient for the multiple blood sampling. The potential for the stylet to come loose in

the sauna or during the exercise period is small. The staff will carefully monitor this aspect of the study to prevent unwanted bleeding.

The potential risks associated with the needle fat biopsy technique is also low. This procedure will be performed by the physician and is usually well tolerated, although some individuals may experience some discomfort for a short time period. The procedure will be performed under local anesthesia so that any pain associated with the procedure will be minimized. It should be pointed out that most, if not all, of the people to be included in the study are familiar with this procedure from previously obtained fat samples. There should be no known health risks associated with the collection of the urine and fecal samples or with the hydrostatic weighing procedure.

6. TIMETABLE

Start date: September, 1985.

September:

1. Obtain from Michigan Dept. Public Health names of individuals in the PBB cohort who reside in the vicinity of Central Michigan University.
   a) Alternate procedure: news media solicitation.
2. Identify 20 males and 20 females, ages 18-40 years, for inclusion in the study.
3. Identify 10 males and 10 females for primary group; 10 males, 10 females for reserve group.
4. Place order for gas chromatograph.
5. Start renovation of Central Michigan facility site.

October:

1. Finish renovation of facility site.
2. Schedule screening tests and physical examinations for primary group.
3. Hire program supervisor.
4. Start treatment protocol.
5. Preparation of quality control by Dr. Safe.
6. Collection and storage of samples for analysis.

November:

1. Obtain and place gas chromatograph in operational order.
2. Conclude treatment protocol.
3. Collect all samples, store and transport to laboratory for analysis.
4. Establish analytical procedure in conjunction with Dr. Mullin.

December:

1. Validate analytical procedure.
2. Validate quality control samples.
3. Start analysis of patient samples.

1986:

January - April:

1. Analyze samples, perform quality control, tabulate results.

May:

1. Six month follow up sample collection.

May - June:

1. Analyze samples, quality control samples.

July - August:

1. Evaluate data, prepare report.
2. Project terminated.

3. Prior Experience

Principal Investigator: Dr. Miceli has a long standing interest in the mobilization of lipophilic toxins from human tissue. Initial studies in this area of toxicology concerned the body burden reduction of phencyclidine (PCP), a drug widely abused by a large segment of the drug subculture. PCP is a highly lipophilic substance which is sequestered in fatty tissue (69, 70). Prior to the development of the treatment protocol designed by Dr. Miceli and his colleagues, the only treatment for acutely intoxicated humans was supportive in nature.

In a preliminary report (71) and in subsequent reports (72-74), the pharmacokinetics of PCP in acutely intoxicated patients was described. Evaluation of the kinetics led to the development of the current treatment protocol which is currently used to manage PCP intoxicated patients (75-78).

Dr. Miceli's research with PBB has led to two published papers which extend our understanding of the disposition of these compounds. The first (12) involved a study using rats in which the test animals were forced fed PBB dissolved in corn oil. At 6, 12, 24 and 36 weeks, groups of 10 rats were sacrificed and the adrenal, brain, fat, gonad, heart, kidney, liver, lung, pituitary and spleen were removed for PBB analysis. The results indicated a slight drop of PBB tissue and serum concentrations accompanied by a concomitant early increase of fat PBB concentration, which subsequently declined and remained constant for the remainder of the study time. It was impossible to assess longer term tissue storage because funding was terminated. It was calculated, however, that the rat would never spontaneously eliminate its PBB body burden during its natural life time. Similar calculations have been made by others for PBB in rats (40) and for PCB in mouse, rat, dog and monkey (79).

In the second study (13, APPENDIX 9), Dr. Miceli and colleagues determined the residual tissue distribution and concentration of PBB in 15 human post-mortem subjects who were exposed to PBB approximately 10 years earlier. He obtained autopsied samples of the following human tissues: adrenal, aorta, brain, heart, kidney, liver, lung, pancreas, fat, skeletal muscle, spleen, thymus and thyroid. Of 196 separate tissues analyzed for PBB, only 4 were at or below the limit of detection of the analytical technique. These results clearly indicate that PBB is distributed throughout the body and not merely confined to adipose tissue. They also indicate that PBBs are persisting in human tissue a decade after the initial exposure and that PBBs in humans, like the rat, will be expected to persist throughout the lifetimes of individuals contaminated with this xenobiotic. Rudimentary calculations gave an estimated mean half time of at least 7.8 years. This is in good agreement with an estimated half time calculated by Tuey and Matthews (40) based on rat data that was extrapolated to humans. Both of Dr. Miceli's PBB projects were funded, in part, by the Michigan Department of Public Health. Each project was successfully concluded as evidenced by the publication of results from each project.

Scn

Consultant: Dr. David Schnare has promulgated the treatment protocol, described in this document, as a means of enhancing body burden reduction of PBB and PCB (1). An initial pilot evaluation of the treatment protocol shows that this treatment is generally safe for use in humans (80).

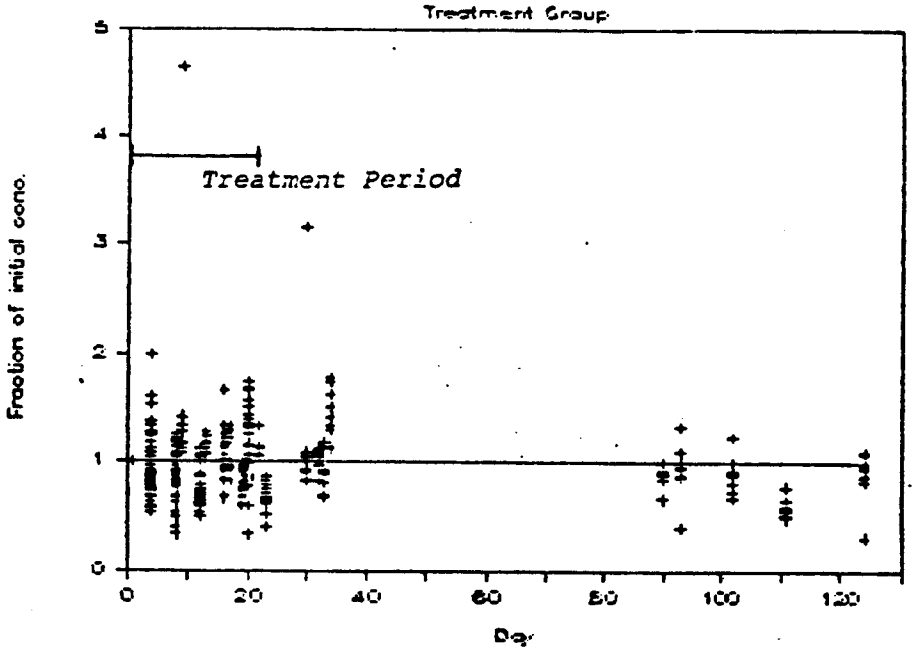
A pilot study of the efficacy of the treatment protocol for reduction of PBB body burden was carried out in 7 patients exposed to PBB ten years ago. The results of that study (1) indicated statistically significant reduction in 7 of the 16 organohalides that were examined.

Dr. Schnare has continued his studies evaluating the treatment protocol by examining the fate of PCB body burden reduction in 10 electrical workers occupationally exposed to this substance. Reduction of PCB adipose tissue burden was 15.5% at immediate post treatment and 14.7% at a 4 month follow up. There was a significant reduction after treatment in all 10 PCB congeners monitored. An additional element of the study was examination of blood PCB levels during treatment to determine if blood levels temporarily increased during mobilization. Figures 1A and 1B indicate excretion processes appear to keep pace with mobilization and as a result, no excessive or permanent increase was observed.

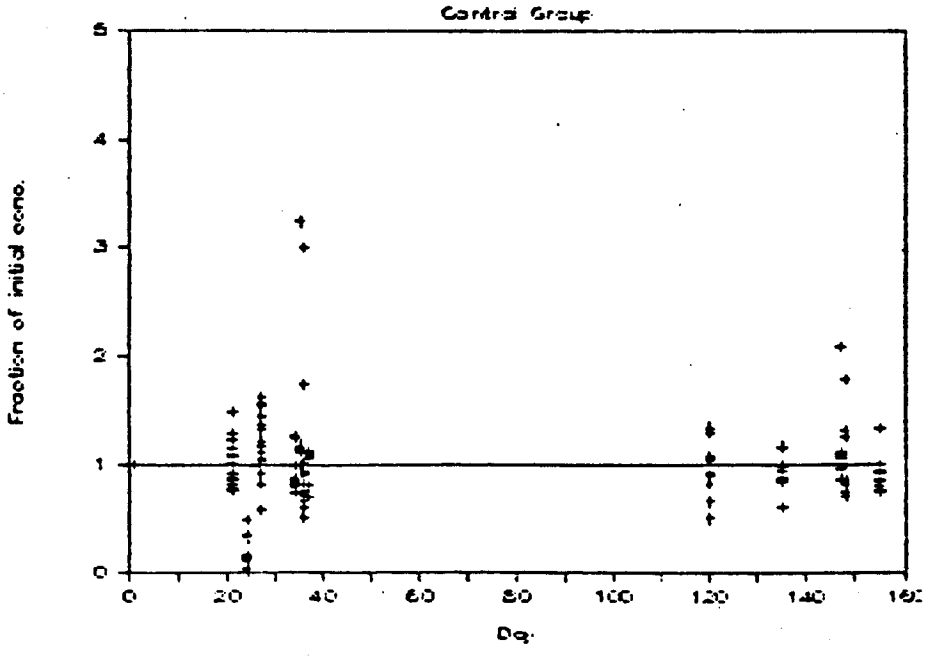
LEGENDS FOR FIGURES

Figure	Legend
1	PCB blood concentrations
1A	Standardized (normalized) PCB blood concentrations in the treated group. Workers had blood taken on the days indicated for PCB measurement. Total time was about 120 days. Concentration is plotted as fraction of the initial concentration.
1B	Standardized PCB blood concentrations in the control group. See 1A for explanation of coordinates. Total time was about 160 days.

Standardized PCB Blood Conc.



Standardized PCB Blood Conc.



III-C. MANAGEMENT SUMMARY

4. Manpower

Dr. Joseph N. Miceli

As principal investigator (PI), Dr. Miceli is responsible for the implementation, overall coordination and completion of the study. As PI, he assumes responsibility that the study will be performed in accordance with the proposal. In this regard, he will participate in population selection, supervise and assist in the treatment protocol, work with the research associate in development of analytical methodology (PBB, PCB), assist in the analysis of samples, assure that appropriate quality control is performed and maintained, be responsible for accurate recordkeeping, and supervise and maintain a working relationship with Central Michigan University. He will also be responsible for data analysis and interpretation in conjunction with the other members of the team. Dr. Miceli will assure confidentiality of records as well as protect patient welfare and rights under this protocol.

Dr. Ralph Kauffman

As Co-investigator, Dr. Kauffman will assist the PI with: population selection, evaluation of screening clinical information, give advice concerning the clinical management of patients and assist in data evaluation and analysis. He will also aid the PI in assuring that the patients' rights are not compromised under this protocol. Dr. Kauffman has also had input in the design of this proposal.

Dr. Jerome Ciullo

As Co-investigator, Dr. Ciullo will participate with Drs. Miceli and Kauffman in population selection, perform the physical examinations on the individuals selected for inclusion in the study and obtain the needle-fat biopsy and concurrent blood samples.

Paul Misiak

As research assistant, Paul Misiak will work with the PI to establish and implement the PBB/PCB analysis procedure, validate the methodology, perform quality control and patient sample analysis.

Project Administrator

The project administrator (PA), will have responsibility to oversee the day-to-day treatment aspects of the protocol. The project administrator will work closely with the PI in population selection, coordinate the daily routine, maintain appropriate health records, assure samples are collected in the correct manner and stored properly labeled until shipped to the laboratory. The PA will assist in scheduling of staff, be responsible for project personnel, coordinate supplies to coincide with the study time table, assist in supply purchases, be responsible for storage and inventory of supplies, assure the treatment site is adequately maintained and be available to answer patient inquiries. The PA will coordinate and implement the protocol, have a thorough familiarity with the treatment protocol and coordinate schedules with the various groups of patients. The PA should be a R.N. and have supervisory experience in the use of the proposed treatment protocol in humans.

Program Supervisor

The program supervisor (PS) oversees the daily patient routine and is trained in the treatment protocol. The PS should be trained in CPR, capable of monitoring blood pressure, be able to spot the signs and symptoms of salt depletion, heat exhaustion and anemia and be able to take the appropriate action. The PS is responsible for daily sauna maintenance, such as temperature control, ventilation and proper disinfectant procedures. The PS is responsible for the daily dispensing of the nutritional supplements. In this regard, he must assure that each patient receives his correct dose and on time in his presence. The PS

reports to the PI, PA or the physician in charge. The PS is responsible for daily communications with the patients. This is accomplished by review of the daily report forms and in informal conversations.

Secretary (part-time)

This part-time secretary (can be a student) will perform secretarial duties and computer data input at the Wayne State site (PI's office). In addition to routine secretarial duties, this individual will be required to enter generated data into the computer for storage and subsequent retrieval. This position is required throughout the length of the project.

CONSULTANTS

There are three consultants to this application: Dr. Michael Mullin of the U.S. E.P.A. Laboratory located in Grosse Ile, Michigan; Dr. Stephen Safe, located at Texas A&M University in College Station, Texas, and Dr. David Schnare, Scn of the U.S. E.P.A., located in Washington, D.C.

Drs. Mullin and Safe will provide analytical consulting services pertaining to validation of detection methodologies developed by these two researchs. In that regard, Dr. Mullin is available for telephone and site visit consultation. Dr. Safe will prepare the appropriate quality control samples and will be available for telephone consultation. Attached are copies of letters of intent from both of these individuals. These letters are for intent to participate with the principal investigator in a grant submission application (in preparation) on an expanded project to the NIH. Funding by the State for this project is not mutually exclusive with funding by the NIH. Although both of these scientists could not provide a letter specifically in response to this request (both are unavailable at the present time), I have spoken to each individually and they have agreed to participate as stipulated in the NIH letter. Additionally, I have spoken with Dr. Dan Jones of Dr. Safe's laboratory who also confirmed his intention to participate at the amount budgeted for this study. Dr. Schnare has also given his verbal approval to participate as consultant and for inclusion of a copy of his letter for the proposed NIH grant. Dr. Schnare is also unavailable at the present time to submit a letter specifically for this application. The time dead-line for this proposal does not permit the principal investigator to await the return of these individuals. The consultants' letters are in APPENDIX 10.

Consultants:

Dr. David Schnare Sen

Dr. Schnare will advise the study team concerning the treatment protocol, provide information concerning the nutritional supplementation and exercise parts of the protocol and assist in decision making concerning the conduct of the protocol, and participate with the other team members in the analysis of data. Dr. Schnare has had input into the design of this proposal and this study will evaluate in detail his earlier work (1).

Dr. Stephen Safe

Dr. Safe will serve as one of the analytical consultants. His laboratory will serve as a quality control center to validate the methodology, submit a serum sample prepared with PBB and PCB for use as a quality control and assist in obtaining appropriate analytical standards.

Dr. Michael Mullin

Dr. Mullin will serve as the other analytical consultant. He will provide assistance in the implementation of the gas chromatography techniques developed in his laboratory for the measurement of PBB and PCB from tissue extracts.

III-C. MANAGEMENT SUMMARY

5. Subcontractors

Central Michigan University
Mt. Pleasant, MI 48859

John Harper - Dean of Grad.
↓
Doug Spathelf - Current Research Ass

Contact Person:
Dr. Douglas Friedrich (Gone)
Dean of Graduate Studies
Associate Vice-Provost for Research
(517) 774-3093

Doug Spathelf

Central Michigan University is a corporation registered in the State of Michigan. The work to be sub-contracted includes the instillation of the sauna, renovation of the treatment facility site, hydrostatic weighing procedures, obtaining EKG and chest x-rays.

The site at which the detoxification procedure will be conducted is Central Michigan University (CMU), located in Mount Pleasant, Michigan. The selection and use of this facility is based on the following factors: (1) It is centrally located in the heart of the "high" PBB exposure area of the state, (2) there exists indoor and outdoor field track facilities, (3) an under-water weighing tank is available for fat-mass measurements, (4) a modern, conveniently located student health facility is available for use, (5) space is available for remodeling to include the saunas, shower room and examining room facilities, and (6) Mount Pleasant Community Hospital, located about 1.5 miles away from the University has a fully operational emergency room and will be available if required.

The projected duration of use of the CMU site is approximately 3 months. This includes the time required for renovation of the facility site. Since the indoor and outdoor field tracks and under-water weighing facilities are already in place, no additional funding or requirements for these facilities are required.

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APPENDIX

1. Medical History Form; Physical Examination Form; Daily Report Form.

2. Daily Treatment Protocol and Group Scheduling of Events.

3. Management of Salt Depletion.

4. Content of Dietary Supplements and Wind-down Procedure.

5. Data Collection Form.

6. Informed Consent (Pending).

7. Patient Information Sheet.

8. Information/Qualifying Questionnaire for Inclusion in the Study.

9. Miceli, J.N., Nolan, D.C., Marks, B.H., Hariharan, M.: Persistence of polybrominated biphenyls (PBB) in human post-mortem tissue. Environ. Hlth. Persp. 60, (in press).

10. Consultants Letters of Intent

11. Letter from the Foundation for the Advancements in Science and Education.

12. Curriculum Vitaes