United States Department of Agriculture
Agricultural Research Service
Radiological Safety Staff
Beltsville, Maryland 20705

030-04530

November 9, 1987

19-00915-03

U. S. Nuclear Regulatory Commission
Region 1
631 Park Avenue
King of Prussia, PA 19406

Gentlemen:

This is letter is to inform you that the Radiological Safety Staff of the U.S. Department of Agriculture has approved modifications to Dr. R. J. Blank's approval to conduct an additional study involving human subjects at the USDA Human Nutrition Research Center, Grand Forks, North Dakota. The additional study is to determine the amount of intrasubject variation in absorption of zinc and copper from foods.

Enclosed are copies of correspondence received from Dr. R. J. Blank relative to this study.

If additional information is required, it will be provided upon request.

ROBERT D. JARRETT
USDA Radiological Safety Officer

Enclosures

FEE EXEMPT

"OFFICIAL RECORD COPY"

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12 NOV 1987

United States Department of Agriculture
Agricultural Research Service
Northern States Area
Grand Forks Human Nutrition Research Center
2420 2nd Avenue, N
P.O. Box 7166
University Station
Grand Forks, North Dakota
58202-7166

SUBJECT: Approval of Proposed Human Use Study

TO: Members, Human Use Subcommittee
USDA Radiological Safety Committee

FROM: USDA, ARS, Grand Forks Human Nutrition Research Center

1. Principal Investigator
Richard J. Blank, M.D.

1A. Telephone No.
701 780-6150

2. Associate Investigators
Phyllis E. Johnson, Ph.D. 701 795-8416 or FTS 783-0416
Glenn I. Lykken, Ph.D. 701 795-8418 or FTS 783-0418

3. Agency 4. Organization 5. Location and Address
USDA, ARS
Grand Forks Human Nutrition Research Center
PO Box 7166, University Station
Grand Forks, ND 58202-7166

6. Radioactive Drug Research Committee (RDRC)
University of North Dakota RDRC 0119

6A. Study Approval Dates
UND RDRC 0119 - Sept. 26, 1986
UND IRB - Nov. 21, 1986
USDA HSC - Jan. 9, 1987

7. Study Duration 12 weeks

8. Study Title Variability of Zn and Cu Absorption from Day to Day and Week to Week

9. Research Subjects (List: Sex, Number, Age)

Males and nonpregnant females, all over 18 years of age, total of 15 subjects.

10. Radioisotope(s) and Dose Administered

Zn-69m, 50 μCi; Zn-65, 0.10-0.15 μCi; Cu-67, 14 μCi.

11. Total Body Absorbed Dose Per Administration

6.6 mrad for Zn-69m @ 40% absorption
1.1-1.7 mrad for Zn-65 @ 40% absorption
5.2 mrad for Cu-67 @ 32% absorption

12. Total Body Absorbed Dose Per Study

14 mrad from all 3 isotopes

13. Critical Organ(s) and Absorbed Dose

Liver: 50 mrad for Zn-69 m
3-5 mrad for Zn-65 115 mrad total
60 mrad for Cu-67

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Approve_____ Disapprove_____ Signature_____ Date_____

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☐ EXPEDITED REVIEW REQUESTED UNDER ITEM ___ (NUMBER) OF HHS REGULATIONS

UNIVERSITY OF NORTH DAKOTA
HUMAN SUBJECTS REVIEW FORM
FOR NEW PROJECTS
OR
PROCEDURAL REVISIONS TO APPROVED PROJECTS
INVOLVING HUMAN SUBJECTS

780-6150
795-8418

PROJECT DIRECTOR: R.J. Blank, P.E. Johnson and G.I. Lykken TELEPHONE: 795-8418

DEPARTMENT: Grand Forks Human Nutr. Res. Center DATE: August 20, 19

CO-PDL:

PROPOSED PROJECT DATES: Oct. 1986 to Dec. 1989

PROJECT TITLE: Variability of Zn and Cu Absorption from Day to Day and Week to Week

FUNDING AGENCIES (IF APPLICABLE):

TYPE OF PROJECT:

☒ NEW PROJECT ☐ CONTINUATION ☐ RENEWAL ☐ DISSERTATION OR THESIS RESEARCH ☐ STUDENT RESEARCH PROJECT

☐ CHANGE IN PROCEDURE FOR A PREVIOUSLY APPROVED PROJECT

☐ PROJECT TO BE UNDERTAKEN AS AN ACTIVITY UNDER A PREVIOUSLY APPROVED TRAINING OR CENTER GRANT ENTITLED:

GRANT PROJECT DIRECTOR, THESIS ADVISER, OR STUDENT ADVISER:

PROPOSED PROJECT:

☐ INVOLVES NEW DRUGS (IND) ☐ INVOLVING NON APPROVED USE OF DRUG ☐ INVOLVES A COOPERATING INSTITUTION

(IF EITHER BOX IS CHECKED SECTIONS 5 OR 6 OF HHS FORM 596 MUST BE COMPLETED AND SUBMITTED IF YOU ARE SEEKING OUTSIDE FUNDING.)

☐ IS CLAIMED TO BE EXEMPT FROM HHS REGULATIONS BASED ON NUMBER(S) _____ OF HHS EXEMPTION LIST. (SEE INSTRUCTIONS.)

HUMAN SUBJECTS WOULD BE INVOLVED IN THE PROPOSED ACTIVITY AS EITHER:

☒ NONE OF THE FOLLOWING, OR INCLUDING:

☐ MINORS (<18 YEARS) ☐ PREGNANT WOMEN ☐ MENTALLY DISABLED

☐ FETUSES ☐ PRISONERS ☐ UND STUDENTS

☐ ABORTUSES ☐ MENTALLY RETARDED ☐ OTHER (PLEASE EXPLAIN)

ABSTRACT: (LIMIT TO 200 WORDS OR LESS AND INCLUDE JUSTIFICATION OR NECESSITY FOR USING HUMAN SUBJECTS.)

This study will be done in order to determine the amount of intrasubject variation in the absorption of zinc and copper from food. Subjects will be admitted to the Metabolic Unit at the Grand Forks Human Nutrition Research Center, where they will consume a diet composed of conventional foods and containing 10-12 mg Zn/d and about 1.5 mg Cu/d. Other nutrients will meet or exceed the average daily intake for the US population. Breakfasts labeled with stable and radioactive isotopes will be fed: Week 1, Day 1 - Zn69m, Day 2-Zn67, Day 3-Zn70; Week 2, Day 1 - Cu67, Day 2 - Cu65; Week 4, Day 1 - Zn65, Day 2 - Zn67, Day 3 - Zn70; Week 5, Day 2 - Cu655; Week 7, Day 2 - Zn67; Week 8, Day 2 - Cu65. Absorption of radioisotopes (Zn69m, Zn65, Cu67) will be determined by whole body counting. Absorption of stable isotopes will be determined by fecal monitoring and mass spectrometric analysis. Doses will be approximately 0.10-0.15 μCi Zn65, 50 μCi Zn69m, and 14 μCi Cu67. Stable isotope doses will be 2 mg Cu65 and 4 mg Zn67 and Zn70.

PLEASE NOTE: ONLY INFORMATION PERTINENT TO YOUR REQUEST TO UTILIZE HUMAN SUBJECTS IN YOUR PROJECT OR ACTIVITY SHOULD BE INCLUDED ON THIS FORM. WHERE APPROPRIATE ATTACH SECTIONS FROM YOUR PROPOSAL, BUT DO NOT REFER TO PAGE NUMBERS IN THE PROPOSAL SINCE NOT ALL BOARD MEMBERS WILL HAVE A COPY OF YOUR PROPOSAL FOR REFERENCE PURPOSES.

2. PROTOCOL: (DESCRIBE PROCEDURES TO WHICH HUMANS WILL BE SUBJECTED. USE ADDITIONAL PAGES IF NECESSARY.)

Healthy adult males and nonpregnant females will be invited to participate in this study. They will live in the metabolic unit of the Grand Forks Human Nutrition Research Center for twelve weeks (2 weeks of equilibration and 10 weeks for absorption measurements). A mixed diet composed of conventional foods served on a three-day rotating menu cycle will be used. The diet will contain 10-12 mg Zn/d, approximately 1.5 mg Cu/d and other nutrients in amounts that meet or exceed the average daily intake of the US population.

Radioisotopes and stable isotopes of zinc and copper will be used to measure absorption on a number of occasions in order to determine the amount of intrasubject variation in absorption of Zn and Cu. Doses of 0.10-0.15 μCi Zn-65, 50 μCi Zn-69m, 14 μCi Cu-67, 2 mg Cu-65, 4 mg Zn-67, and 4 mg Zn-70 will be used. They will be administered with breakfast according to the following schedule:

Schedule of Isotope Feeding

Week	Day 1	Day 2	Day 3
1	Zn^{69m}	Zn^{67}	Zn^{70}
2	Cu^{67}	Cu^{65}	
3			
4	Zn^{65}	Zn^{67}	Zn^{70}
5		Cu^{65}	
6			
7		Zn^{67}	Zn^{70} (optional)
8		Cu^{65}	(optional)
9			
10			

Designation of weeks is weeks after equilibration period.

Absorption of radioisotopes will be determined by whole body counting. Absorption of stable isotopes will be determined by fecal monitoring and mass spectrometric analysis. Zinc and copper balance will also be determined.

Breakfasts labeled with isotopic tracers will be fed after an overnight fast. Before each meal, a fasting blood sample (5 ml) will be obtained for measurement of plasma Zn and Cu and ceruloplasmin. At the beginning and end of the study, blood (70 ml) will be drawn for a general nutritional assessment.

Measures of body composition to be made during the study include body impedance, deuterium oxide dilution (measurement of total body water), exercise and fitness testing, underwater weighing, anthropometry and skinfold thickness measurements.

Psychological testing will include electroencephalography (EEG), general psychological assessment, and sleep behavior inventory.

3. BENEFITS: (DESCRIBE THE BENEFITS TO THE INDIVIDUAL OR MANKIND)

There is no direct benefit to the subjects from participating in this study. The knowledge gained from it will benefit humanity by advancing our knowledge of human requirements for zinc and copper.

4. RISKS: (DESCRIBE THE RISKS TO THE SUBJECT AND PRECAUTIONS THAT WILL BE TAKEN TO MINIMIZE THEM. THE CONCEPT OF RISK GOES BEYOND PHYSICAL RISK AND INCLUDES RISKS TO THE SUBJECT'S DIGNITY AND SELF RESPECT, AS WELL AS PSYCHOLOGICAL, EMOTIONAL OR BEHAVIORAL RISK. IF DATA ARE COLLECTED WHICH COULD PROVE HARMFUL OR EMBARRASING TO THE SUBJECT IF ASSOCIATED WITH HIM OR HER, THEN DESCRIBE THE METHODS TO BE USED TO INSURE THE CONFIDENTIALITY OF DATA OBTAINED, INCLUDING PLANS FOR FINAL DISPOSITION OR DESTRUCTION, DEBRIEFING PROCEDURES, ETC.

Radioisotopes: The radiation exposure from each of the radioisotope labeled meals (three total) and the sodium-22 is less than that received from a single chest x-ray. It is approximately equivalent to that received from cosmic rays while flying for seventeen hours in an airplane at 30,000 feet above sea level. A person receives about 10 times as much exposure each year just by living in Grand Forks.

Venipuncture: There may be some discomfort as the needle enters the skin, but this lasts only a few seconds. About 10% of the time a small bruise may develop at the needle site, but this resolves in two weeks or less. A maximum of 70 ml (5 tablespoons) of blood will be taken at any one time. No more than 250 ml (17 tablespoons) of blood will be taken each month. This is within blood bank donation limits.

Stable isotopes: There is no known risk associated with the ingestion of stable isotopes of Zn and Cu. Deuterium has previously been fed to adults, pregnant women, and infants with no ill effects.

Exercise stress testing: The progressive exercise tests described in this protocol are not without some risk for cardiovascular complications, including sudden death. Subjects will be examined by a physician and an EKG performed prior to any testing. During all testing, the subject's EKG will be monitored continuously and a physician, emergency medications, and equipment will be present in the laboratory.

Anthropometry and skinfold thickness measurements: There are no known risks involved in the anthropometric and skinfold thickness measurements. However, some individuals may experience transient, minor discomfort during the measurement of skinfold thickness.

Records: Information published in scientific journals as a result of this study will be in a form not identifiable with the subjects. Subjects may request copies of the results of any and all tests. Records will be kept at the Human Nutrition Research Center for times in accordance with government regulations.

5. CONSENT FORM: A COPY OF THE CONSENT FORM TO BE SIGNED BY THE SUBJECT (IF APPLICABLE) AND/OR ANY STATEMENT TO BE READ TO THE SUBJECT SHOULD BE ATTACHED TO THIS FORM. IF NO CONSENT FORM IS TO BE USED, DOCUMENT THE PROCEDURES TO BE USED TO ASSURE THAT INFRINGEMENT UPON THE SUBJECT'S RIGHTS WILL NOT OCCUR.
DESCRIBE WHERE SIGNED CONSENT FORMS WILL BE KEPT AND FOR WHAT PERIOD OF TIME.

A copy of the consent form is attached. Signed consent forms will be kept at the Human Nutrition Research Center for times prescribed by govenment regulations.

IF NO EXEMPTION IS CLAIMED (SEE PAGE 1).

6. FORWARD A SIGNED ORIGINAL AND 8 COPIES OF THIS COMPLETED FORM, AND WHERE APPLICABLE, 9 COPIES OF THE PROPOSED CONSENT FORM, QUESTIONNAIRES, ETC. AND ANY SUPPORTING DOCUMENTATION TO:

OFFICE OF RESEARCH AND PROGRAM DEVELOPMENT
UNIVERSITY OF NORTH DAKOTA
BOX 8158, UNIVERSITY STATION
ROOM 101, TWAMLEY HALL
GRAND FORKS, NORTH DAKOTA 58202

IF AN EXEMPTION IS CLAIMED, ONLY THE ORIGINAL AND, WHERE APPLICABLE, ONE COPY OF THE PROPOSAL ALONG WITH CONSENT FORM, QUESTIONNAIRE, ETC. SHOULD BE FORWARDED TO ORPD. IF THE PROPOSED ACTIVITY INVOLVES A PROPOSAL TO A POTENTIAL FUNDING AGENCY, ONE COPY OF THE PROPOSAL ALSO SHOULD BE INCLUDED.

FOR EXPEDITED REVIEW FOLLOW THE ABOVE FOR EXEMPTION IS CLAIMED.

THE POLICIES AND PROCEDURES ON USE OF HUMAN SUBJECTS OF THE UNIVERSITY OF NORTH DAKOTA APPLY TO ALL ACTIVITIES INVOLVING USE OF HUMAN SUBJECTS PERFORMED BY PERSONNEL CONDUCTING SUCH ACTIVITIES UNDER THE AUSPICIES OF THE UNIVERSITY. NO ACTIVITIES ARE TO BE INITIATED WITHOUT PRIOR REVIEW AND APPROVAL AS PRESCRIBED BY THE UNIVERSITY'S POLICIES AND PROCEDURES GOVERNING THE USE OF HUMAN SUBJECTS.

SIGNATURES:

9-18-86
DATE

R. J. Blank, M.D.
PROJECT DIRECTOR

8-25-86
DATE

Phyllis Johnson

9-18-86
DATE

TRAINING OR CENTER GRANT DIRECTOR, OR STUDENT ADVISER (WHERE APPLICABLE)

12/86

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VARIABILITY OF ZN AND CU ABSORPTION FROM DAY TO DAY AND WEEK TO WEEK

Study No. 051-123

Assessment of bioavailability of Zn and Cu from the diet is an essential component of the determination of dietary requirements for these minerals. Measurement of their bioavailability in humans is often done by feeding foods labeled intrinsically or extrinsically with radio or stable isotopes of Zn or Cu and measuring the absorption of the isotopic tracer. Interpretation of results is sometimes limited by the lack of information concerning intrasubject variation in Zn and Cu absorption (1). Flanagan et al. (2) used a dual radioisotope stool counting technique and found that intrasubject, as well as intersubject, variation in Zn absorption from a turkey meal was about 20% (2). There are no data showing the intrasubject variability in Zn or Cu absorption for the techniques used in our laboratory to measure absorption, whole-body gamma counting and fecal excretion of stable isotopes (4). The findings of Flanagan (2) may not be applicable to the techniques we use because he administered a laxative after each isotope dose as a routine part of his protocol.

Because we have at our disposal the facilities and expertise to measure Zn and Cu absorption with both radioactive and stable isotopes, we can assess variability in the absorption of these elements on three consecutive days by using different isotopes to prevent individual absorption tests interfering with each other. We propose to measure the

day-to-day and week-to-week variability in Zn absorption using radioactive Zn-65 and Zn-69m and stable Zn-70 and Zn-67. Variability in Cu absorption will be measured with radioactive Cu-67 and Stable Cu-65.

General Procedure

This study will involve the oral administration of a dose of each radioisotope, Zn-65, Zn-69m and Cu-67 to humans in amounts as low as practicable. Doses will be approximately 0.10-0.15 µCi Zn-65, 50 µCi of Zn-69m, and 14 µCi of Cu-67. The half-lives of these isotopes are shown in Table I. Doses of 50 µCi Zn-69m have been used by other investigators (5,6). Studies using Cu-67 in humans have not been done previously. Administration of radioisotopes to humans as part of a general project entitled "Bioavailability of Zinc, Copper, and Iron for Absorption in Humans" has been approved by the following committees on the dates listed:

University of North Dakota Radioactive Drug Research Committee 0119 (Committee approved by HEW-DHS-FDA October 12, 1979), March 18, 1980.

University of North Dakota Human Subjects Committee, March 1, 1980.

University of North Dakota Radiation Safety Committee through North Dakota State Department of Health License Number 33:12827-01, Amendment No. 02, January 8, 1981.

Adult male and nonpregnant females will participate in the study. Urinary HCG screening tests will be performed on all premenopausal women before their admittance into the study and prior to the feeding of each radiolabeled meal. Subjects will be required to withdraw from the study if they become pregnant. No subject known by the investigators to be pregnant will be given subsequent doses of radioisotopes.

Subjects will be admitted to the metabolic unit of the Human Nutrition Research Center. They will be fed a controlled diet composed of conventional foods on a three-day rotating menu cycle. The diet will contain approximately 10-12 mg Zn/d and 1.5 mg Cu/d. Other nutrients will meet or exceed the average daily intake for the U.S. population. Identical breakfasts will be fed on the days that isotope labels are added to the breakfast. Two weeks will be allowed for equilibration to the diet before the isotopically labeled meals are fed. The schedule for administration of isotopically labeled meals is given in Table II. All volunteers will have a whole body count before the first isotope dose to determine individual background and correction factors. This will involve a one-minute exposure to 1.3 μCi Na-22 placed under the mattress of the counter. The stable isotope doses in weeks 7 and 8 will be done if time available on the metabolic unit allows; otherwise the subjects will be discharged at the end of week 7.

Stools will be collected for 14 days following each of the stable isotope labeled meals. Absorption of the stable isotopes will be determined by calculating the difference in the amount of isotope fed and that recovered in the stools. Doses of 4 mg Zn-67 and Zn-70, and 2 mg Cu-65 will be used. These doses are similar to those we have used in the past, and well within the range of normal dietary intake. The use of stable isotopes of Zn, Cu, and Fe has been previously approved by the University of North Dakota Human Subjects Committee, April 11, 1979 and the USDA Human Subjects Review Committee.

Plasma zinc and copper, ceruloplasmin, and red cell superoxide dismutase will be determined, after an overnight fast, before each labeled meal. These data will be examined for any possible correlations with Zn and Cu absorption.

Other Procedures

Measures of body composition to be made during the study include body impedance, deuterium oxide dilution (measurement of total body water), exercise and fitness testing, underwater weighing, anthropometry, and skinfold thickness measurements.

In order to maintain the body weight of the subjects at ± 2%, we will adjust calories as necessary, but they will also be given tri-weekly exercise on a treadmill or stationary bicycle. Each subject's peak work capacity, 90% age-adjusted heart rate (220-age) will be determined. From this, the tri-weekly, prescription exercise will be calculated (50% of peak). Subjects will be retested at weekly intervals to determine if fitness (oxygen consumption and carbon dioxide production) has changed. Blood will be drawn before and after exercise on those days.

A dose of approximately 10 g of deuterium oxide will be used for total body water determinations.

Physiological testing will include electroencephalography (EEG), general psychological assessment, and sleep behavior inventory.

Risks

The total radiation exposure from all 3 of the radioisotope labeled meals (3 total) and the sodium-22 is less than (about one eighth of) that received from a single chest x-ray. It is approximately equivalent to

that received from cosmic rays while flying for 40 hours in an airplane at 30,000 feet above sea level. A person receives about 10 times as much exposure each year just by living in Grand Forks. The radiation exposure from the Zn-69m will be about 5% of that from one chest x-ray; exposure from the Zn-65 will be about equal to 0.1% of a chest x-ray and exposure from Cu-67 will be approximately equal to 4% of that from a chest x-ray. The radiation dose from a one-minute exposure to sodium-22 is equivalent to that received from the body's potassium-40 in 30 minutes. This is less than 0.002% of the exposure from a chest x-ray.

Venipuncture:

There may be some discomfort as the needle enters the skin, but this lasts only a few seconds. About 10% of the time, a small bruise may develop at the needle site, but this resolves in two weeks or less. About 0.01% of the time, infection may develop, but this is easily treated with antibiotics. A maximum of 70 ml of blood will be taken at any one time. No more than 250 ml of blood will be taken each month. This is within blood bank donation limits.

The progressive exercise tests described in this protocol are not without some risk for cardiovascular complications, including sudden death. Subjects will be examined by a physician and an EKG performed before any testing. During progressive exercise testing, the subject's EKG will be monitored continuously and a physician, emergency medications and equipment will be present in the laboratory.

There are no known risks involved in the anthropometric and skinfold measurements. However, some individuals may experience transient, minor, discomfort during the measurement of skinfold thickness.

Information published in scientific journals as a result of this study will be in a form not identifiable with the subjects. Subjects may request copies of the results of any and all tests at the end of the study. Records will be kept at the Human Nutrition Research Center for times in accordance with government regulations.

There is no direct benefit to the subjects from participating in this study. The knowledge gained from it will benefit humanity by advancing our knowledge of human requirements for zinc and copper in the diet.

References

1. Johnson, P.E. and Lykken, G.I. Unpublished data.
2. Flanagan, P.R., Cluett, J., Chamberlain, M.J., Valberg, L.S. Dual isotope method for determination of human zinc absorption: the use of a test meal of turkey meat. J. Nutr. 115, 111-122 (1985).
3. Lykken, G.I. A whole body counting technique using ultralow doses of ^{59}Fe and ^{65}Zn in absorption and retention studies in humans. Am. J. Clin. Nutr. 37, 652-662 (1983).
4. Johnson, P.E. A mass spectrometric method for use of stable isotopes as tracers in studies of iron, zinc, and copper absorption in human subjects. J. Nutr. 112, 1414-1424 (1982).
5. Aamodt, R.L., Rumble, W.F., Johnston, G.S., Foster, D., Henkin, R.I. Zinc metabolism in humans after oral and intravenous administration of Zn-69m. Am. J. Clin. Nutr. 32, 559-569 (1979).
6. Molokhia, M., Sturniolo, G., Shields, R., Turnberg, L.A. A simple method for measuring zinc absorption in man using a short-lived isotope (69Znm). Am. J. Clin. Nutr. 33, 881-886 (1980).

Table I

Isotope	Half-life
Zn-69m	13.9 hr
Zn-65	243.6 d
Cu-67	61.9 hr

Table II

Schedule of Isotope Feeding

Week	Day 1	Day 2	Day 3
3	Zn-69m	Zn-67	Zn-70
4	Cu-67	Cu 65	
5			
6	Zn-65	Zn-67	Zn-70
7		Cu-65	
8			
9		Zn-67	Zn-70 (optional)
10		Cu-65	(optional)
11			
12			

Appendix II

Chapter 3

DOSIMETRY CONSIDERATIONS FOR ORALLY ADMINISTERED ^{67}Cu

Copper is absorbed from the gastronintestinal tract, particularly from the upper small intestine.(1)(2) After oral ingestion of radiocopper there is a rapid increase in plasma radioactivity followed by a similarly rapid fall.(3) Intravenously administered radiocopper disappears rapidly from the blood with 66 - 95% appearing in the liver in 2 - 8 hours.(4) With whole-body counting following intravenous injection of radiocopper,(5) a significant concentration of activity has been found in the liver and, to a less degree, in the kidneys. Subsequent observations determined that only 0.6% of the dose was excreted in the urine.

Cartwright and Wintrobe(6) have estimated that of the 2-5 mg.of Cu ingested daily by man, 32% is absorbed, 26% is excreted in the bile, 6% passes directly through the bile and 1.2% appears in the urine. King et al(7) using the stable tracer ^{65}Cu, found an average overall copper absorption of 56% in 22 women 19-25 years of age. The absorption was determined from the difference between intake and fecal output of the stable isotope ^{65}Cu as determined by neutron activation analysis. This high amount of absorption was possible due to naturally occurring ^{65}Cu or to a higher absorption level due to a relatively low (2 mg Cu per day) pre-study diet.

For the purpose of absorbed dose estimation it is assumed here that the orally administered dose passes through the GI tract remaining 3 hours in the stomach and 23 hours in each of the small intestine (SI), upper large intestine (ULI), and the lower large intestine (LLI). A 32 % absorption in the small intestine(1)(6)(7)

is assumed with 70% of the absorbed radiocopper[1] going to the liver, 3% to the kidneys and the remaining 27% going to the rest of the body (total body). Since the half-life of ^{67}Cu (61.7h) is short relative to typical biological half-lives, the effective half-life may be taken to be 61.7h. Table 1 shows the average absorbed doses (per microcurie administered dose) expected for these assumptions for ^{67}Cu and Table 2 shows the estimated doses for ^{64}Cu under similar assumptions. The absorbed doses were obtained from tables in the NM/MIRD Pamphlet No. 11.[8]

TABLE 1 ABSORBED DOSE PER MICROCURIE DOSE OF ^{67}Cu(m rad/μCi_D)

TARGET ORGANS	SOURCE ORGANS							
	Stomach (3h)	SI (23h)	ULI (23h)	LLI (23h)	Kidneys	Liver	Total Body	Total
Adrenals	6.8 E-3	2.2 E-2	1.1 E-2	5.0 E-3	7.9 E-3	8.4 E-2	4.9 E-4	0.14
Bladder Wall	6.5 E-4	4.7 E-2	2.6 E-2	8.3 E-2	2.2 E-4	2.8 E-3	4.8 E-4	0.16
Bone (Total)	2.2 E-3	2.0 E-2	1.3 E-2	1.8 E-2	1.0 E-3	1.8 E-2	4.9 E-4	0.073
GI (STOM Wall)	2.1 E 0	6.7 E-2	4.5 E-2	2.2 E-2	2.6 E-3	3.4 E-2	4.8 E-4	2.3
GI (SI)	7.1 E-3	9.1 E 0	2.1 E-1	1.1 E-1	2.2 E-3	3.0 E-2	5.0 E-4	9.5
GI (ULI Wall)	9.4 E-3	4.2 E-1	1.1 E+1	5.1 E-2	2.2 E-3	4.4 E-2	4.9 E-4	12
GI (LLI Wall)	3.2 E-3	1.3 E-1	3.7 E-2	1.8 E+1	5.9 E-4	4.5 E-3	4.8 E-4	18
Kidneys	9.1 E-3	5.7 E-2	3.4 E-2	1.1 E-2	9.4 E-1	7.0 E-2	4.8 E-4	1.1
Liver	5.3 E-3	3.2 E-2	3.2 E-2	3.2 E-3	2.9 E-3	4.2 E 0	4.8 E-4	4.3
Lungs	4.4 E-3	4.3 E-3	3.2 E-3	9.9 E-4	6.4 E-4	4.4 E-2	4.6 E-4	0.058
Marrow (Red)	3.8 E-3	6.9 E-2	4.2 E-2	5.6 E-2	2.6 E-3	2.6 E-2	5.2 E-4	0.20
OTH TISS (MUSC)	3.5 E-3	2.8 E-2	1.8 E-2	2.1 E-2	1.0 E-3	1.9 E-2	4.6 E-4	0.091
Ovaries	1.2 E-3	1.9 E-1	1.4 E-1	2.3 E-1	8.5 E-4	8.2 E-3	4.9 E-4	0.57
Pancreas	4.7 E-2	3.6 E-2	2.9 E-2	8.7 E-3	5.0 E-3	7.2 E-2	5.1 E-4	0.20
Skin	1.2 E-3	7.7 E-3	5.2 E-3	6.2 E-3	4.2 E-4	9.0 E-3	4.2 E-4	0.030
Spleen	2.7 E-2	2.8 E-2	1.8 E-2	9.6 E-3	6.7 E-3	1.6 E-2	4.8 E-4	0.11
Testes	1.5 E-4	5.9 E-3	3.6 E-3	2.3 E-2	7.6 E-5	1.3 E-3	4.5 E-4	0.034
Thyroid	2.7 E-4	3.2 E-4	2.5 E-4	8.5 E-5	4.3 E-5	3.2 E-3	4.5 E-4	0.0046
Uterus (NONGRVD)	2.1 E-3	1.7 E-1	6.3 E-2	8.7 E-2	7.5 E-4	6.8 E-3	5.2 E-4	0.33
Total Body	8.9 E-3	1.1 E-1	5.5 E-2	6.3 E-2	5.4 E-3	1.3 E-1	4.6 E-4	0.37

TABLE 2 ABSORBED DOSE PER MICROCURIE DOSE OF ^{64}Cu(mrad/μCi_D)

TARGET ORGANS	SOURCE ORGANS							
	Stomach (3h)	SI (23h)	ULI (23h)	LLI (23h)	Kidneys	Liver	Total Body	Total
Adrenals	8.9 E-3	2.5 E-2	1.3 E-2	6.6 E-3	2.2 E-3	3.0 E-2	1.2 E-2	0.098
Bladder Wall	1.9 E-3	6.7 E-2	2.5 E-2	7.5 E-2	6.9 E-5	2.1 E-3	1.1 E-2	0.18
Bone (Total)	2.5 E-3	1.6 E-2	9.8 E-3	1.4 E-2	1.8 E-4	4.5 E-3	9.9 E-3	0.057
GI (STOM Wall)	1.7 E 0	6.7 E-2	4.8 E-2	2.4 E-2	5.9 E-4	1.2 E-2	1.1 E-2	1.9
GI (SI)	1.1 E-2	5.3 E 0	2.1 E-1	1.2 E-1	4.8 E-4	9.9 E-3	1.1 E-2	5.7
GI (ULI Wall)	1.4 E-2	4.6 E-1	6.3 E 0	5.6 E-2	5.0 E-4	1.6 E-2	1.1 E-2	6.9
GI (LLI Wall)	5.3 E-3	1.3 E-1	4.0 E-2	9.8 E 0	1.5 E-4	2.0 E-3	1.1 E-2	10
Kidneys	1.4 E-2	6.2 E-2	3.7 E-2	1.1 E-2	1.2 E-1	2.3 E-2	1.1 E-2	0.28
Liver	8.6 E-3	3.6 E-2	3.3 E-2	4.7 E-3	7.0 E-4	7.8 E-1	1.1 E-2	0.87
Lungs	7.8 E-3	6.7 E-3	4.6 E-3	1.4 E-3	1.6 E-4	1.5 E-2	1.0 E-2	0.046
Marrow (Red)	4.4 E-3	4.9 E-2	2.9 E-2	4.2 E-2	4.3 E-4	6.6 E-3	1.0 E-2	0.14
OTH TISS (MUSC)	5.8 E-3	3.0 E-2	2.0 E-2	2.2 E-2	2.6 E-4	7.0 E-3	9.9 E-3	0.095
Ovaries	2.2 E-3	1.7 E-1	1.7 E-1	2.1 E-1	2.1 E-4	1.4 E-3	1.1 E-2	0.56
Pancreas	7.2 E-2	4.1 E-2	2.4 E-2	9.8 E-3	1.1 E-3	3.0 E-2	1.1 E-2	0.19
Skin	2.5 E-3	1.1 E-2	7.5 E-3	8.0 E-3	1.2 E-4	3.8 E-3	8.6 E-3	0.043
Spleen	3.9 E-2	3.2 E-2	1.7 E-2	1.3 E-2	1.4 E-3	6.2 E-3	1.1 E-2	0.12
Testes	1.4 E-4	7.4 E-3	6.9 E-3	2.8 E-2	2.7 E-5	7.4 E-4	1.1 E-2	0.054
Thyroid	6.4 E-4	8.2 E-4	5.9 E-4	2.5 E-4	1.8 E-5	1.2 E-3	9.9 E-3	0.017
Uterus (NONGRVD)	3.9 E-3	1.7 E-1	6.0 E-2	7.4 E-2	1.5 E-4	2.8 E-3	1.1 E-2	0.32
Total Body	1.1 E-2	6.0 E-2	4.3 E-2	4.6 E-2	7.6 E-4	2.7 E-2	9.9 E-3	0.22

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Zinc-69m Dosimetry

Zinc-69m is an isomeric state[a] of Zn-69, it decays by emission of a 440 KeV photon and has a half-life of 13.9 h whereas Zn-69 decays by emission of a 925 KeV beta-minus and has a half-life of 58 min. In calculating absorbed doses therefore, one must consider both the 440 KeV photon and the 925 KeV beta-minus.

In this work it will be assumed that the ingested zinc remains in the stomach for a period of time and that the stomach emptying function may be described as (1)

$$C_s(t) = C_0 \exp(-\lambda_s t^2) \quad (1)$$

Where $C_s(t)$ is the content of the stomach at time t after ingestion of an amount of a substance C_0 and λ_s is related is related to the characteristic emptying time, assumed here to be 0.03 h^{-2}. The activity in the stomach will then be described as

$$A_s(t) = Q_p \exp(-\lambda_s t^2) \exp(-\lambda_I t) \quad (2)$$

Where Q_p is the amount of radiozinc ingested and λ_I is the natural decay constant of Zn-69m.

Aamodt et al (2) have studied Zn-69m metabolism and have shown that absorbed Zn-69m went rapidly to the liver (35% absorption at 6 h, 40% at 12 h) and liver activity could be described for the period 2 days (48 h) to 5 days (120 h) by the expression

$$A_{L3}(t) = 0.076 \exp(-.005t) + 0.194 \exp(-.00033t) \quad (3)$$

The liver activity for periods $0 \leq t \leq 12$ h and $12 \leq t \leq 48$ h may be expressed, when natural decay is considered, respectively as,

$$A_{L1}(t) = 0.0833(t - t^2/20) \exp(-.05t) \quad (4)$$

and

$$A_{L2}(t) = 0.456 \exp(-0.051t) \quad (5)$$

It will be conservatively assumed that the remaining unabsorbed activity remains in the small intestine for 23 h, in the upper large intestine for 23 h and in the lower large intestine for 23 h.

Absorbed doses due to photons and beta-minus particles can be calculated with the use of definitions found in MIRD/Pamphlet No. 1 (3) and absorbed fractions and coefficients of variation for organs within a nonhomogeneous phantom found in MIRD/Pamphlet No. 5 (4). For photons as well as for particulate radiations it is convenient to define an equilibrium absorbed dose constant Δ which gives the total energy in g-Sv/μCi-h emitted by that component (3). These may be written for the photons from Zn-69m as

$$\Delta_{\gamma i} = 2.13 \times 1 \times 0.44 \times 10^{-2} \text{ g-Sv}^b/\mu\text{Ci-h} \quad (6)$$

[a] Metastable state or relatively long lived state

[b] 1 Sv(Sievert) is equal to 10^2 rem

and for beta-minus particles from Zn-69 as

$$\bar{\Delta}_{\beta i} = 2.13 \times 1 \times 0.925 \times 10^{-2} \text{ g-Sv/}\mu\text{Ci-h} \quad (7)$$

For target and source in the same volume (which must be the case for nonpenetrating beta-minus particles) the absorbed dose may be written as (3)

$$\bar{D} = \bar{A}/m \sum \phi_{i_i} \Delta_i \text{ Sv} \quad (8)$$

where $\bar{A}$ is the cumulated activity defined as $^{a}_{1}$ Sv(Sievert) is equal to 10^2 rem.

$$\bar{A} = \int_{t_1}^{t_2} A(t)dt \ \mu\text{Ci-h} \quad (9)$$

where A(t) is defined above and t_1 to t_2 is the time interval that the activityy is in the volume which contains the mass m, ϕ is the ratio of the energy absorbed by the target to that emitted by the source, values of which are tabulated in Appendix A of reference 4 for selected photon energies; for simplicity, tne 440 KeV photon from Zn-69m is taken to be a 500 KeV photon.

For Zn-69m ingestion the critical organ is the liver but, the total body dose is also of interest; photon doses, beta-minus doses and total doses to both the liver and the total body for an ingested dose of 50 uCi Zn-69m are shown in Table 1.

Table 1

Photon doses, beta-minus doses and total doses to both the liver and the total body for an ingested dose of 50 μCi Zn-69m.

Organ	D_γ(m Sv)	D_β(m Sv)	D_{Total}(m Sv)	(mrem)*
Liver	0.25	0.25	0.50	50
Liver[a]	0.81	0.75	1.60	160
Total Body[b]	0.046	0.02	0.066	6.6

[a]Calculated under a worst possible case scenario where all Zn-69m goes rapidly to the liver and decays there.
[b]Calculated under the condition of a uniform Zn-69m source in the body with all Zn-69m decaying in the body.

Our calculations give slightly less liver and greater total body doses than do those of Molokhia et al. (5) by factors of 2.9 and 4.4 respectively.

*1 rem = 10^{-2} Sv

References

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University of North Dakota Radioactive Drug Research Committee 0119 (Committee approved by HEW-DHS-FDA October 12, 1979), March 18, 1980.

University of North Dakota Human Subjects Committee, March 31, 1980.

USDA Human Studies Review Committee, April 18, 1980.

University of North Dakota Radiation Safety Committee through North Dakota State Department of Health License Number 33-12827-01, Amendment No. 02, January 8, 1981.

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3. A schema for absorbed-dose calculations for biologically-distributed radionuclides-MIRD/Pamphlet No. 1. Society of Nuclear Medicine, 211 E 43 St, New York, NY 10017.

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INFORMED CONSENT STATEMENT

PROJECT: Variability of Zn and Cu Absorption
from Day to Day and Week to Week
051-123

You are being asked to participate in this research project so that we can find out by how much people vary from day to day and week to week in their ability to absorb zinc and copper from the same diet. You are being asked to participate because you are over 18 years of age, not pregnant (or contemplating pregnancy in the next 6 months), and are in good general health.

All aspects of this project will be carried out at the Human Nutrition Research Center. A private room with radio, television, telephone, and semi private bath will be provided. Only food and drink provided by our kitchen may be eaten. You may leave the laboratory only when accompanied by a chaperone provided by us. A daily schedule of your research activities and tests will be provided before you agree to participate. During the equilibration period you will consume a normal mixed diet and become accustomed to living on the Clinical Research Unit. The diet will be controlled and will be the same for the equilibration period and the rest of the entire study (9-12 wk). You will be expected to consume all of the food and drink served to you and nothing else. You will be expected to save all stools and urine in the containers provided while you are living on the Human Studies Unit. Premenopausal female subjects will also be expected to save menstrual losses using the sanitary napkins or tampons provided. Your cooperation and attention to the rules and regulations governing subject conduct on the clinical research unit is expected. A nurse

is present on the unit 24 hours a day and a physician and psychologist are on call for any problems (medical emergencies or otherwise) which may arise. While you are a subject, health care will be provided for, except in case of a pre-existing condition, for example, dental care or new eyeglasses.

After the equilibration period of two weeks, you will be fed breakfasts labeled with various radioactive and stable isotopes of zinc and copper on different days. These isotopes will be incorporated into the breakfast you will be served on these days. You are not likely to notice any change in the quality of the food because of their presence. The schedule for the isotope doses is as follows:

Schedule of Isotope Feeding

Week	Day 1	Day 2	Day 3
3	Zn^{69m}	Zn^{67}	Zn^{70}
4	Cu^{67}	Cu^{65}	
5			
6	Zn^{65}	Zn^{67}	Zn^{70}
7		Cu^{65}	
8			
9		Zn^{67}	Zn^{70} (optional)*
10		Cu^{65}	(optional)*
11			
12			

*depending on time available in metabolic unit schedule

Before you consume the first radioisotope labeled meal, you will have a whole body count (WBC). During this WBC you will be exposed for approximately one minute to a small amount of gamma radiation from

sodium-22 (a radioactive form of sodium) contained in a sealed sheet under the mattress in the whole body counter. This will allow us to determine your self-absorption of radiation because of your particular body size and density.

After eating each radioisotope labeled meal, and at regular intervals afterwards, you will have a WBC. Before each WBC, you will shower and put on clean gown which we supply. A technician will direct you into the whole body counter steel room where you will be assisted in lying down on the WBC bed. During a WBC you will stay in the enclosed steel room, lying on a bed between two banks of 16 detectors each. You will be observed on a closed circuit TV system, will be able to contact the WBC technician by intercom, and will have a choice of listening to radio, or having silence. Each WBC will take 10-30 minutes or less, including data processing time.

Zinc-67, zinc-69m, and copper-67 are radioactive forms of these elements. The total radiation exposure from all 3 of the radioisotope labeled meals (three total) and the sodium-22 is about one-twelfth that received from a single chest x-ray. It is approximately equivalent to that received from cosmic rays while flying for 40 hours in an airplane at 30,000 feet above sea level. You receive about 10 times as much exposure each year just by living in Grand Forks. The radiation exposure from the Zn-69m will be about 5% of that from one chest x-ray; exposure from the Zn-65 will be about equal to 0.1% of a chest x-ray and exposure from Cu-67 will be approximately equal to 4% of that from a chest x-ray. The radiation dose from a one-minute exposure to sodium-22 is equivalent to that received from the body's potassium-40 in 30 minutes. This is less than 0.002% of the exposure from a chest x-ray. The effects of small doses of radiation on the developing human fetus are not known. Because of this we are not knowingly allowing pregnant women to participate in the

study. If you become pregnant during the study, you will be discharged.

Before you eat each labeled meal, after an overnight fast, your blood will be drawn so that we can test for factors which may help us predict how much zinc or copper you will absorb. Blood will also be drawn at the beginning and end of the study so that we can assess your general state of nutrition. The blood tests will be done b· a medical technician using a needle and syringe. You may experience some discomfort as the needle enters the skin, but this lasts only a few seconds.

Many measures of body composition and function will be made on a daily, weekly, or monthly basis. They include the following:

Whole Body Scintillation Counting. Your lean body mass (muscle and bone) contains potassium-40, a naturally occurring radioisotope of potassium, not found in fat. By counting the amount of potassium-40 in on a regular basis, we will be able to estimate your fat-free mass and see whether it changes over time. The whole body counter is located in the Grand Forks Human Nutrition Research Center, within a steel-lined room. You will lie on a mattress above and below from which are suspended a number of sodium iodide crystals used to count the energy emissions from the decaying potassium-40. This process takes about 30 minutes. At monthly intervals a sodium-22 (radioisotope) source will be placed under the mattress. You will be counted for 1 minute to determine your individual count correction factor which depends on body size, mass and geometry.

Bioelectrical Impedance. This process is used to estimate total body water. Electrodes will be pasted to the skin of your hands and feet. A low level alternating current will be passed through your body. The electrical resistance is a function of the salt ions present in your body

water. You will not be able to feel the current. This all will take less than ten minutes.

Exercise/Fitness Testing. In order to maintain your body weight at ± 2% we will adjust calories as necessary, but you will need to do tri-weekly exercise on a treadmill or stationary cycle. Your peak work capacity, 90% age-adjusted heart rate (220-age), will be determined, from which the tri-weekly, or prescription exercise is calculated (50% of peak). At weekly intervals you will be retested to determine whether your fitness (oxygen consumption and carbon dioxide output) is changing. Blood will be taken from a vein in your arm before and after exercise on these days. The peak exercise sessions will be supervised by physiologists with nurses and physicians present in the Grand Forks Human Nutrition Research Center.

Electroencephalography (EEG). The electrical activity of your brain will be recorded weekly while you sit in a sound-proof booth and perform a variety of mental tasks. Visual tasks are presented on a television screen and auditory tasks are presented using stereo speakers. A trained EEG technician will fit you with a cap resembling those worn by astronauts, which contains numerous electrodes designed to detect electrical activity on the scalp. A small amount of conductive gel will be applied to your scalp to facilitate the recording, and washes easily from the hair. The weekly EEG recording session will require approximately one hour.

General Psychological Assessment (GPA). An assortment of behavioral and mood assessments will be made on a regular basis. Tests of sensory-motor performance, attention, perception, learning, memory, decision-making, and problem solving abilities will be presented on a microcomputer during a 90-minute session supervised by a trained examiner

from Psychology. Measurement of mood, transient personality states, and a variety of verbal, reading, and mathematical skills will be accomplished by our participation in paper-and-pencil testing requiring approximately 1-2 hours.

Sleep Behavior Inventory (SBI). Prior to weighing each morning you will fill out a card containing several questions regarding your sleep behavior the prior night. Information requested includes: what time you went to sleep and awoke, whether your woke up during the night, whether you remember dreaming (not the content), and a subjective assessment of the quality of your sleep. Completion of this card will require approximately two minutes.

Underwater weighing. Underwater weighing to determine body density (a measure of body fat) will be performed weekly during the first month, then monthly until the end of study. You will change into a bathing suit and kneel on a weighing platform in a tank of warm water. A mouthpiece and attached hose will be placed in your mouth. With guidance from the scientist you will duck under the water surface, exhale completely and hold still for seven seconds before resurfacing. You will not be restrained or held under so that you my surface at any time. The process will be repeated two or three times and lasts thirty minutes.

Anthropometry and Skinfold Thickness Measurements. Determination of circumferences of the upper arm, calf, and waist, and of linear measures at the elbow and the knee will be made using an anthropometric caliper. Assessments of skinfold thickness will be performed by pinching the skin, holding the pinched area, then measuring the size of the pinched skin and adipose tissue at the front and back of the upper arm, back, waist, and calf. Standing height and body weight will also be determined. These anthropometric and skinfold measurements will be performed weekly.

Deuterium oxide and bromide dilution. The amount of total water and extracellular fluid in your body will be measured using a procedure called deuterium oxide and bromide dilution. Deuterium oxide or "heavy water" is a nonradioactive, naturally occurring substance which looks and tastes like ordinary water. You will drink a small amount (4 tsp.) of deuterium oxide and sodium bromide on an empty stomach. It may taste slightly salty. Blood samples will be taken from a vein in your arm before, and two hours after, you drink the deuterium oxide and bromide. Deuterium oxide and sodium bromide have previously been fed to adults, pregnant women, and infants without ill effects.

Exercise Stress Testing. The progressive exercise tests described in this protocol are not without some risk for cardiovascular complications, including sudden death. You will be examined by a physician and an electrocardiogram (EKG) performed prior to any testing. During maximum exercise stress testing, your EKG will be monitored continuously and a physician, will be present.

There are no known risks involved in the anthropometric and skin fold thickness measurements. However, some individuals may experience transient, minor discomfort during the measurement of skinfold thickness.

Radioisotope exposure. The estimated total radioisotope exposure in this study is 12 mrem. It is about one twelfth of the dosage received from a chest x-ray. This radiation exposure is equivalent to spending 40 hours in an airplane at 30,000 feet.

Stable isotopes. Zinc-67, zinc-70, and copper-65 are naturally occurring substances which are found in many foods. There are no known ill effects associated with their ingestion.

Venipuncture. About 10% of the time a small bruise may develop at the needle site, but this resolves in two weeks or less. Occasionally (0.01%)

infection may develop, but this is easily treated with antibiotics. A maximum of 70 ml (5 tablespoons) of blood will be taken at any one time. No more than 250 ml (17 tablespoons) of blood will be taken each month. This is within blood bank donation limits.

Benefits. There is no direct benefit to you from participating in this study. The knowledge gained from the study will benefit humanity in that it will advance our knowledge of the human requirements for dietary minerals. You will be given a stipend of $30 per day at regular intervals to compensate you for the inconvenience of living in a restricted environment. You are free to leave at any time; however, if you leave before the end of the study you forfeit any unpaid stipend. We reserve the right to dismiss you at any time if, in our opinion you have disobeyed regulations, have disrupted research or have exhibited inappropriate behavior. If it is felt that continuing in the study might be harmful to you mentally or physically, or there is the development of an illness or condition that might affect research results, you will be dismissed. If we dismiss you for health reasons you will receive the stipend to the day of discharge. During the first weeks of the study your blood and urine will be tested for pregnancy if you are female. At monthly intervals thereafter, urine tests for pregnancy will be done. If you are pregnant at the beginning of the study or become pregnant during the study, you will be dismissed. In the unlikely event that you are injured or develop an illness as a direct result of your participation in this study, we will make arrangements for medical care. Compensation for any injury or damages may be yours by filing suit under the Federal Tort Claims Act.

All information (other than name, address, and Social Security number) about you is confidential. Any results from your participation in this project may be published in a scientific journal, but only in a form not

identifiable with you. You may request copies of any and all blood, exercise, etc., tests at the end of the study. These will be sent to a qualified professional for review with you. You are free to ask questions at any time during the study by calling Dr. Johnson, 795-8416 or Dr. Lykken, 777-3519 or 795-8418.

Your signature below signifies that you have read this form, have had the study explained, have had any questions answered to your satisfaction, and that you now understand what will be expected of you.

_____ _____

Subject Date

_____ _____

Witness Date

_____ _____

Investigator Date

8/86

Project: Variability of Zinc and copper absorption from day to day and week to week.

BETWEEN:

LICENSE FEE MANAGEMENT BRANCH, ARM
AND
REGIONAL LICENSING SECTIONS

(FOR LFMS USE)
INFORMATION FROM LMS

PROGRAM CODE: 03610
STATUS CODE: 2
FEE CATEGORY: 2X 3L
EXP. DATE: 19840331
FEE COMMENTS: _____

LICENSE FEE TRANSMITTAL

A. REGION

1. APPLICATION ATTACHED
APPLICANT/LICENSEE: AGRICULTURE, DEPARTMENT OF
RECEIVED DATE: 871112
DOCKET NO: 3004530
CONTROL NO.: 108094
LICENSE NO.: 19-00915-03
ACTION TYPE: AMENDMENT

2. FEE ATTACHED
AMOUNT: _____
CHECK NO.: _____

3. COMMENTS

SIGNED _____
DATE _____

B. LICENSE FEE MANAGEMENT BRANCH (CHECK WHEN MILESTONE 03 IS ENTERED /__/)

1. FEE CATEGORY AND AMOUNT: _____

2. CORRECT FEE PAID. APPLICATION MAY BE PROCESSED FOR:
AMENDMENT _____
RENEWAL _____
LICENSE _____

3. OTHER _____
_____

SIGNED _____
DATE _____