

Attachment 1.
(FACE SHEET)

Date July 10, 1991

ETHICAL REVIEW COMMITTEE, ICDDR,B.

Principal Investigator Dr. D. Mahalanabis Trainee Investigator (if any) _____
Application No. 91-0068 Supporting Agency (if Non-ICDDR,B) _____
Title of Study Role of micronutrient mixture...in children: a randomized double-blind community intervention trial. Project status:
() New Study
() Continuation with change
() No change (do not fill out rest of form)

Circle the appropriate answer to each of the following (If Not Applicable write NA).

1. Source of Population:
 - (a) Ill subjects ☒ Yes ☐ No
 - (b) Non-ill subjects ☒ Yes ☐ No
 - (c) Minors or persons under guardianship ☒ Yes ☐ No
 2. Does the study involve:
 - (a) Physical risks to the subjects ☐ Yes ☒ No
 - (b) Social Risks ☐ Yes ☒ No
 - (c) Psychological risks to subjects ☐ Yes ☒ No
 - (d) Discomfort to subjects ☐ Yes ☒ No
 - (e) Invasion of privacy ☐ Yes ☒ No
 - (f) Disclosure of information damaging to subject or others ☐ Yes ☒ No
 3. Does the study involve:
 - (a) Use of records, (hospital, medical, death, birth or other) ☐ Yes ☒ No
 - (b) Use of fetal tissue or abortus ☐ Yes ☒ No
 - (c) Use of organs or body fluids ☐ Yes ☒ No
 4. Are subjects clearly informed about:
 - (a) Nature and purposes of study ☒ Yes ☐ No
 - (b) Procedures to be followed including alternatives used ☒ Yes ☐ No
 - (c) Physical risks ☐ Yes ☒ No
 - (d) Sensitive questions ☐ Yes ☒ No
 - (e) Benefits to be derived ☒ Yes ☐ No
 - (f) Right to refuse to participate or to withdraw from study ☒ Yes ☐ No
 - (g) Confidential handling of data ☒ Yes ☐ No
 - (h) Compensation &/or treatment where there are risks or privacy is involved in any particular procedure ☐ Yes ☒ No
 5. Will signed consent form be required:
 - (a) From subjects ☐ Yes ☒ No
 - (b) From parent or guardian (if subjects are minors) ☒ Yes ☐ No
 6. Will precautions be taken to protect anonymity of subjects ☒ Yes ☐ No
 7. Check documents being submitted herewith to Committee:
 - ☐ Umbrella proposal - Initially submit an overview (all other requirements will be submitted with individual studies).
 - ☒ Protocol (Required)
 - ☒ Abstract Summary (Required)
 - ☒ Statement given or read to subjects on nature of study, risks, types of questions to be asked, and right to refuse to participate or withdraw (Required)
 - ☒ Informed consent form for subjects
 - ☒ Informed consent form for parent or guardian
 - ☒ Procedure for maintaining confidentiality
 - ☒ Questionnaire or interview schedule *
- * If the final instrument is not completed prior to review, the following information should be included in the abstract summary:
1. A description of the areas to be covered in the questionnaire or interview which could be considered either sensitive or which would constitute an invasion of privacy.
 2. Examples of the type of specific questions to be asked in the sensitive areas.
 3. An indication as to when the questionnaire will be presented to the Cttee. for review.

I agree to obtain approval of the Ethical Review Committee for any changes involving the rights and welfare of subjects before making such change.

Dr. Mahalanabis
Principal Investigator

Trainee

RECEIVED 31 MAY 2005

SECTION I - RESEARCH PROTOCOL

Title : Role of micronutrient mixture containing zinc, selenium, iron, copper, and folate in reducing the incidence and severity of acute diarrhoea and acute respiratory infections, and in improving nutrition in children: a randomised double-blind community intervention trial

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Scientific Programme Head : 
Dr. Dilip Mahalanabis

ABSTRACT SUMMARY

The role of zinc, copper, selenium, iron, and folate in controlling and modulating immunologic responses has recently begun to attract attention in improving health and nutritional status. Sufficient biomedical and experimental evidence has accumulated over recent years to justify a community trial of an appropriate mixture of micronutrients to determine their public health impact. We propose to study the role of zinc, selenium, iron, copper, and folate in reducing morbidity (i.e. number and duration of diarrhoea and ARI episodes per child per year) and improving nutritional outcome compared to a single vitamin preparation. The randomised controlled design will be followed so that any observed difference in any outcome variables between groups can be confidently attributed to the real effect of the intervention. Comparable communities will be randomly allocated to different intervention groups. Of the target children in 24 communities, children in 6 communities will get a micronutrient mixture comprising of zinc, selenium, iron, copper, and folate (A), another 6 communities will get a mixture of iron, copper, and

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folate (B), children in 6 communities will receive a micronutrient mixture consisting of zinc and selenium (C), and the remaining 6 communities will receive a multivitamin preparation (D, control) which will also be present in the micronutrient mixture of the other three groups. Impact of all three micronutrient mixtures as a whole will be evaluated by comparing group A with group D. The combined effect of zinc and selenium will be evaluated by comparing group C and D. Combined effect of iron, copper and folate will be evaluated by comparing group B and D. The interaction between zinc + selenium as a group and iron + copper + folate as a group will be evaluated by using all 4 groups in an analysis of variance for factorial design:

zinc,selenium	+	zinc & selenium	-
iron,copper & folate	+	iron,copper & folate	+
(Group A)		(Group B)	
zinc & selenium	+	zinc, selenium	-
iron & copper & folate	-	iron, copper & folate	-
(Group C)		(Group D)	

Trained field volunteers will distribute nutrient mixtures and collect basic data. They will be supervised at the field level by male field workers. Field workers will also summarise data on monthly basis. Female interviewers will collect data and take anthropometric measurements. Performance of all staff will be monitored by regular observation at the field level. Whenever mothers feel necessary, sick children will be referred to the appropriate health facilities. The result of the study will help to formulate a cost-effective public health intervention and will have far reaching implications for child survival efforts.

Reviews: .

Research review committee : _____
(approved/not approved)

Ethical review committee : _____
(approved/not approved)

Director's signature : _____
(approved/not approved)

A. INTRODUCTION

Objectives:

The proposed randomised controlled community based study will evaluate:

1. Role of zinc, copper, selenium, iron, and folate in:
 - (a) reducing number of ARI (acute respiratory infections) and diarrhoeal episodes in each child
 - (b) reducing average duration of each ARI and diarrhoeal episode
 - (c) reducing total number of days of ARI and diarrhoea suffered by each child
2. Role of zinc, copper, selenium, iron, and folate in improving nutrition in infants and young children.

Background:

Depressed cell mediated immunity and malnutrition are important risk factors for diarrhoea and its severity in developing countries (1,2). In Bangladesh and Peru, anergy as judged by delayed type of hypersensitivity reaction to several antigens was associated with an increased incidence of diarrhoea even after controlling for any possible effect of nutritional status. The Bangladesh study further reported that among children aged more than 36 months, anergic children were 2.5 times more likely than non anergic children to have a diarrhoeal episode lasting 7 days or longer and about 23 times more likely to have an episode lasting 14 days or more. This effect persisted even after adjustment for the effects of age and initial nutritional status. The anergic children subsequently experienced majority of the prolonged respiratory infections (1,2). Recent study in rural Bangladesh observed that cell mediated immune deficiency independent of nutritional status is an important risk factor for persistent diarrhoea (3,4). The risk of persistent diarrhoea increased 2 fold in anergic children. The degree of association did not change even controlling for age or nutritional status or both. The role of zinc, copper, selenium, iron, and folate in controlling and modulating immunologic responsiveness has recently begun to attract attention. This interest may prove to be clinically important in improving health and nutritional status, thereby, influencing morbidity and mortality of infants and young children. Sufficient biomedical and experimental evidence has accumulated over recent years (as summarised below) to justify a community trial of an appropriate mixture of micronutrients to evaluate its impact on morbidity and nutritional status in children.

Role of zinc, copper, selenium, iron, and folate in immune system function:

Experimental zinc deficiency in baby pig was observed in 1968 to cause atrophy of the thymus gland and spleen in addition to lymphopenia, hypergammaglobulinemia, parakeratosis, and growth failure (5). Oral zinc therapy allowed correction of immune function. Children with protein energy malnutrition and hypozincemia showed an increase in thymic size after zinc supplementation (6).

Depressed cell mediated immunity has been reported in a 17-year-old decerebrate male on total parenteral nutrition lacking zinc supplements. However, Zinc therapy demonstrated positive delayed skin reaction and a normal lymphocyte response within three weeks (7). Zinc therapy resulted in dramatic resolution of the clinical manifestations of acrodermatitis enteropathica in a child with intractable diarrhoea with parenteral alimentation for five months. Cell mediated immune function was also restored to normal, suggesting an important role of zinc and other trace elements in cellular immune response (8). Severe depression of serum Ig G has been reported in patients with zinc deficiency and coexisting PEM (9). Zinc supplementation in deficient marasmic infants was followed by significant increase in serum Ig A only in zinc-supplemented infants (10).

Improvement of cell mediated immunity dependent on T cell, including thymic hormonal inductive factors with selenium supplementation has been reported (11). Depressed humoral immune response has also been reported in selenium deficient chicks (12).

Studies suggest that folic acid deficiency is responsible for the observed depression of cell mediated and humoral immunity in animal and human populations (13,14). It has been observed that impairment of cell mediated immunity due to folate deficiency in mice causes more severe rotaviral diarrhoea (15).

In copper-deficient animals the function of the reticuloendothelial system is depressed and the microbicidal activity of granulocytes is reduced. Impairment of antibody response and low levels of thymic hormone activity in copper-deficient animals have been observed when compared with pair-fed controls (16).

Certain abnormalities have been demonstrated in humoral immunity in iron deficient children. Effects of iron deficiency in children not only decrease the white cell phagocytic function but also produce or potentiate immunodeficient state, thereby, results in worsening of malnutrition and infection (17). Iron deficiency and impaired cellular immunity are common findings in patients with chronic mucocutaneous candidiasis (18). Lymphopenia and depressed lymphocyte transformation response in iron-deficient subjects returned to normal after correction of their iron status. Chandra (19) reported return of polymorph function in children within one week of starting of iron therapy. Such change occurred even prior to an increase in haemoglobin concentration.

Role of zinc, copper, selenium, iron, and folate in growth:

Zinc is an essential trace element required for RNA, DNA, and protein synthesis. Zinc is known to influence cell division (20), growth, and development in animals and human beings (21-23). It is increasingly apparent that zinc deficiency is commonly prevalent in malnourished children of developing countries (24,25). Infants and young children during their growing age are at risk of becoming deficient in zinc. Since the size of the total zinc store is extremely limited, therefore, recommended dietary allowance for zinc is relatively high and unlikely to be met on an ordinary diet mainly based on cereal proteins in Bangladesh and other developing countries (26,27). Zinc requirements are increased during catch-up growth (23). Zinc

supplementation promotes better weight gain and enhances the rate of clinical recovery from severe PEM (28,29). In Bangladesh 73 per cent more weight gain was observed in severely malnourished but zinc supplemented group than the healthy controls (30). A double-blind, randomised controlled trial with a zinc acetate supplement to young children with acute diarrhoea reported a 25 per cent increase in linear growth in both lighter and shorter children as compared to unsupplemented group. In Bangladesh, suboptimum linear growth in young children when compounded by diarrhoea results in further deterioration of zinc status, thus leads to further stunting and increased diarrhoeal risk (31).

The first evidence of possible human need for selenium was demonstrated in malnourished Jordanian infants who exhibited weight gain in response to selenium supplementation (32). Selenium supplementation to patients and deficient animals resulted in increased enzyme activity in both cells and plasma (33).

Folate has a special role in renewal of epithelial cells of the small bowel mucosa as well as cellular DNA synthesis (35). Folate deficiency caused inhibition of weight gain during recovery from rotavirus diarrhoea in mice (15).

In 1964 Cordano et al. (36) reported occurrence of copper deficiency in severely malnourished infants probably as a result of long standing intestinal losses due to chronic diarrhoea and intestinal malabsorption as well as grossly inadequate intakes. However, a prompt response to copper therapy was observed.

A study of 68 babies aged 6-24 months in Guatemala reported substantial developmental deficits in iron deficient anaemic infants as compared to their normal peers (37). Another study reported slower development of young children (aged around 20 months) due to iron deficiency anaemia (38). Aukett et al. (39) reported faster weight velocity and proportionately more success in expected increase in psychomotor skills in anaemic children who received treatment with iron for two months when compared with a control group of anaemic children without iron therapy.

Role of zinc, copper, selenium, iron, and folate in resistance to infection:

In recent past, diminished serum (40-45), hair (42), and rectal mucosa (45,46) zinc levels have been documented in hospitalised children with acute (40,41,43,44,45,46) and chronic (40,42,45) diarrhoea. The zinc deficiency led to reduced absorption of water and electrolytes, loss of intercellular tight junction, appearance of large lateral space, and limited regeneration of gut epithelium (46). Decreased plasma zinc levels and negative correlation between zinc level and duration of acute diarrhoea in infants has been reported (40). Diarrhoea may influence zinc status by reduction of dietary intake, impaired intestinal absorption or increased faecal loss of endogenous stores in acute and chronic diarrhoea (43-45). Zinc depletion occurs more in chronic diarrhoea, the depletion progresses with prolongation of duration or recurrent illnesses (41,45,47). Significant reduction in water and electrolytes absorption has been demonstrated in zinc deficient animals (48,49). Zinc repletion restores transport of water and electrolytes to normal (49). A

recent clinic based study reported that zinc supplementation results in significant rise in serum levels of zinc and alkaline phosphatase in acute and persistent diarrhoea. Increase in mucosal permeability as well as reduction in intestinal fluid loss has been observed. Moreover, decrease in diarrhoeal duration in lighter children and duration of both diarrhoea and respiratory tract illnesses in shorter children has also been reported (31,50).

There are reports that selenium deficient animals are more susceptible to infectious diseases than those who have adequate intake of selenium. Such recognition of the importance of selenium in animal population has led to a federal regulation that allowed selenium fortification of chick and turkey rations (51). In selenium-deficient swine with experimentally induced dysentery led to increase in gut lesions as well as frequency of stool and severity of the disease (33).

Several studies have documented folate deficiency in coeliac disease (52,53), tropical sprue (54,55), ulcerative colitis (56,57), and crohn's disease (58,59). Nelson et al. (60) observed that infant guineapigs fed a folate deficient diet for 2 weeks were highly susceptible to infection with *Shigella flexneri* when challenged. Of them, 89 per cent died as compared to no deaths in similar animals fed a normal diet. The same was true for *Salmonella typhimurium* (61). Administration of oral folate during the acute phase of infantile diarrhoea has been found to significantly shorten the duration of diarrhoea. Therefore, it is thought that folic acid accelerates the normal regeneration of the damaged epithelial cells in the intestinal brush border.

In 1968 Newberne et al. (62) reported increased mortality in copper-deficient rats exposed to *Salmonella typhimurium*. Copper deficiency in mice is accompanied by bacterial infections (*Escherichia coli*, *Staphylococcus aureus*), diarrhoea, and bronchopneumonia (63).

Iron deficiency is the most prominent nutrient deficiencies in the world (64). Preschool children are particularly at risk of acquiring iron deficits (65). Low birth weight with consequent reduction in iron stores, frequent infections, and low intake of iron particularly in socioeconomically poor groups are significant risk factors for iron under-nutrition (66). An expert group of the World Health Organisation reported that individuals with nutritional anaemia tend to have more frequent infections (67). Significant impairment of intracellular bacterial killing capacity by polymorphonuclear leukocytes in children with iron deficiency anaemia has been reported (19). Hussein et al. (68) studied the effect of iron supplementation on the number of diarrhoeal episodes and the course of illness. The study observed significant reduction in the mean number of episodes per child (2-6 years old) per month after iron treatment as compared to those who received placebos. Also, there was a non-significant decrease in the average duration. Mackay (69) observed a modest decrease in the number of episodes of bronchitis and gastroenteritis in iron-supplemented infants from low-income families in London. Andelman et al. (70) observed significantly less respiratory infections in infants who received iron-fortified milk formula. High prevalence of diarrhoea and respiratory illnesses have been reported in iron-deficient children in Alaska (71).

Micronutrient profile of Bangladeshi children:

In a urban clinic 60 severely malnourished children aged between 5 and 60 months were screened for their plasma zinc concentration on admission (29). Zinc concentration was significantly lower in malnourished groups compared with healthy controls (Mean 8.23, SD 0.7 or Mean 7.9, SD 0.7 vs. Mean 11.60, SD 2.4).

Serum zinc and serum copper levels were determined in 128 young children admitted into Children's Nutrition Unit (CNU), Dhaka with different degrees of malnutrition on admission. The results obtained were compared with the serum zinc levels of 48 apparently healthy Bangladeshi children. The results are shown in the following (72):

Nutritional status	# cases	Age (months) Mean (SD)	Zinc (micromol/L) Mean (SD)	Copper (micromol/L) Mean (SD)
Control	48	37.75 (11.27)	11.60 (2.40)	19.14 (1.75)
marasmus	30	35.43 (13.53)	6.83 (1.63)	14.19 (2.59)
Marasmic-kwashiorkor	47	31.55 (12.19)	7.33 (2.0)	14.39 (4.02)
Kwashiorkor	51	33.64 (10.73)	5.47 (1.72)	12.86 (3.43)

Following is the haematocrit value and total serum protein findings of the same children (72):

Nutritional status	# cases	Age (months) Mean (SD)	HCT (per cent) Mean (SD)	Total protein (g/dL) Mean (SD)
Control	48	37.75 (11.27)	36.78 (4.03)	7.8 (0.8)
Marasmas	30	35.43 (13.53)	33.85 (2.5)	4.9 (0.88)
Marasmic-kwashiorkor	47	31.55 (12.19)	34.04 (5.51)	5.0 (0.92)
Kwashiorkor	51	33.64 (10.73)	31.92 (3.10)	4.1 (0.68)

In another study 60 rural children aged between 1 and 5 years were studied for their biochemical parameters (73). Blood haematocrit values were found to be lower than normal in majority of the children ((Mean (SD) 34.8 (6.3)). Sixty children aged 10 to 40 months who were exclusively fed with cows milk were examined in a urban clinic (74). All children suffered from moderate to severe anaemia ((haemoglobin concentration (Mean (SD) 46.5 (8.6) per cent)). Moreover, 18.3 per cent children suffered from iron and folate deficiency as evidenced by blood film.

Zinc requirements of a child is relatively higher than adult. Subsistence on a diet consisting of cereals, and green leafy vegetables with very little animal protein is responsible for low serum zinc level in young children of rural Bangladesh. Unrefined grains are generally good sources of zinc. Pulses and legumes are excellent sources of zinc, highest being in lentil (3.67 mg zinc per 100 gm). Such zinc concentration is comparable to meat. However, high level of fibre and phytate associated with unrefined grain, pulses, and legumes severely limit the availability of zinc. Both leafy and non-leafy vegetables are not good sources of zinc. Limited bioavailability of zinc from some green leafy vegetables has also been reported. Moreover, low dietary protein content results in high urinary excretion of zinc. Furthermore, concomitant diarrhoea may impair absorption of zinc. Presence of associated infection or stress also increases requirement and excretion of zinc. Copper is widely distributed in food stuffs, particularly in cereals. When cereals are consumed in inadequate amounts thereby fail to meet the normal demand. This results in deficiency of copper. Moreover, deficiency of appropriate- α -2 globulin due to decreased intake of protein in the diet is also the cause of low serum copper. Copper deficiency is further aggravated by concomitant diarrhoea. In the developing countries it has been observed that iron deficiency anaemia is due to low availability of dietary iron. Repeated episodes of diarrhoea may also be one of the major contributors of anaemia in rural Bangladeshi children. Although folate is present in many natural foods, However, 50 to 95 per cent of the folate content of foods may be destroyed by cooking or other processing and a restrictive diet pattern of infants and young children would limit folate intake. Folic acid content in breast milk also parallels closely the seasonal variations in vitamin intake.

Interaction among micronutrients:

Addition of a trace element to the diet may alter the absorption and/or metabolism of other elements. In some cases the interaction between two elements is complimentary; for instance, the dietary level of iron needed to maintain a given concentration of haemoglobin is dependent upon the level of copper in the diet (75). In others the interaction may be antagonistic. For instance, high levels of zinc in the diet may lead to copper deficiency. A suitable ratio of zinc:copper in the diet prevents copper deficiency; a ratio of 10:1 to 5:1 for zinc/copper appears to be adequate to prevent adverse interaction (76).

The other important interaction is between zinc and iron. Zinc and iron partially share absorptive sites in the intestine. In animal experiments a high iron/zinc ratio inhibits zinc absorption; on the other hand a very high zinc/iron ratio (e.g. 8:1) was necessary to inhibit iron absorption. Zinc is therefore a less effective inhibitor of iron absorption than iron is for zinc absorption (77). Human studies suggest that inorganic iron added to test solutions of zinc salts in iron/zinc ratio of 2 or more significantly lowers zinc absorption. In physiological ratios of iron/zinc e.g. 1:1 or less, absorption of zinc is not affected (78,79,80). However, when large iron supplements are ingested in the absence of food, it is possible that iron could adversely affect zinc absorption. Physiological ratio of zinc and iron will be maintained in the mineral mixture to be used in this study.

In the micronutrient mixture to be used in this study, we propose to use the recommended dietary allowances of children 1-3 yr. of age for copper, selenium and iron and twice the recommended dose for zinc and folate. In this combination, zinc/iron and zinc/copper interaction for intestinal absorption should be favourable.

Recommended dietary allowances (RDA) of zinc, copper, selenium, iron, and folate:

Age		Zinc(mg)	Copper(mg)	Selenium(micro gm)	Iron(mg)	Folate(micro gm)
0-5	Mo	5	0.4-0.6	10	6	25
5-12	Mo	5	0.6-0.7	15	10	35
1-3	Yr.	10	0.7-1.0	20	10	50
4-6	Yr.	10	1.0-1.5	20	10	75

Rationale:

1. Micronutrients promote growth, reduce morbidity and severity of illness. In many ways several micronutrients behave similarly.
2. In Bangladesh widespread nutrient deficiency may be an important factor for suboptimum growth, increased morbidity, and its duration and severity.
3. It is now established that deficiencies of even a single micronutrient may impair immune responses. The need for improved health and nutrition is an impetus for research of control and modulation of immune status by micronutrients. It is clear that maintenance of nutrition and immunocompetence by micronutrients will alter morbidity and severity of illnesses.
4. There are only few studies in rural Bangladesh that have looked concurrently at the impact of single micronutrient on the morbidity and severity of illnesses in young children. Since the individual effect of single micronutrient is known it has become imperative to assess the additive effect of different micronutrients. A polymicronutrient mixture may have practical appeal from public health point of view. Role of polymicronutrient mixture is yet to be studied in Bangladesh.
5. There is sufficient biomedical evidence to suggest that a suitable combination of micronutrients should be evaluated for their public health impact in a community based trial. Deficiency of one micronutrient is likely to be associated with deficiency of another and it is easier and cheaper to produce a mixture of trace metals than to produce one trace metal in pure form. The best studied micronutrients are zinc, iron, copper, selenium, and folate.

6. A randomised controlled trial of a polymicronutrient mixture if found beneficial may be a cost-effective public health intervention for better child survival.

B. SPECIFIC AIMS

Does daily supplementation with a polymicronutrient mixture in infants and young children in rural Bangladesh reduce morbidity particularly due to diarrhoea and ARI, their duration, total number of days suffered, and improve growth as compared to a control intervention?

C. EXPERIMENTAL DESIGN

The experimental intervention and control:

Twenty four communities (clusters of 40-50 households with at least one under four child in each household), geographically separated from each other will be selected in the project area. To avoid bias comparable communities will be assigned randomly to receive either intervention polymicronutrient mixture or control vitamin mixture.

- A - Children in 6 communities will get a micronutrient mixture comprising of all 5 micronutrients i.e. zinc, selenium, iron, copper and folate.
- B - Children in 6 communities will get a mixture of iron, copper, and folate.
- C. - Children in 6 communities will receive a micronutrient mixture consisting of zinc and selenium.
- D - Children in 6 communities will receive a multivitamin preparation (control) which will also be present in the micronutrient mixture of the other three groups.

Impact of all four micronutrient mixtures as a whole will be evaluated by comparing group A with group D. The combined effect of zinc and selenium will be evaluated by comparing group C and D. Combined effect of iron, copper and folate will be evaluated by comparing group B and D. The interaction between zinc + selenium as a group and iron + copper + folate as a group will be evaluated by using all 4 groups in an analysis of variance for factorial design:

zinc,selenium	+	zinc & selenium	-
iron,copper & folate	+	iron,copper & folate +	
(Group A)		(Group B)	
zinc & selenium	+	zinc, selenium	-
iron & copper & folate	-	iron, copper & folate -	
(Group C)		(Group D)	

Through discussions with the local community leaders and researchers of a nearly completed study on growth monitoring (supported by National Nutritional Council) we gathered some preliminary information as the location and distribution of the villages, existing health problems and we made an assessment of the feasibility of the randomised controlled community trial design.

The need for randomised control design:

Ideally like clinical trial each individual is to be randomised in intervention trial as gold standard. However, randomisation at the individual level is not possible for large scale community trials for logistical problems any observed difference in outcome variables between the intervention and control groups can be confidently explained due to the real effect of the intervention and not attributed to any difference in the baseline composition in the groups. Therefore, it is crucial at the design stage that no severe imbalance in composition of the groups occurs. It has been suggested that even in extreme situations at least four communities are needed in each group to demonstrate a statistically significant difference (81). Closely similar communities will be randomly assigned to receive either the intervention or to act as control intervention. Thus any difference in observed outcome between interventions and controls is due to the intervention and not due to chance. Moreover, randomisation will:

1. ensure that initial composition of the intervention and control groups are similar
2. enable to infer a causal relationship of observed difference between intervention and control groups and among groups
3. ensure that conclusions drawn out of the study are generalizable to other areas.

Setting

The project area is situated about 15 miles away from ICDDR,B in easternly direction of the capital city, and stands on the bank of the river Shitalakhaya under Bandar Upa-zila (sub-district) in Narayanganj district (appendix 1).

In order to maximise the likely impact of intervention the selection of the study site was based on the following criteria:

1. the area is known for diarrhoeal disease and ARI endemicity with high morbidity thus warranting an intervention to minimise the problems.
2. lack of adequate static health facilities, and poor outreach coverage for diarrhoea and ARI treatment is likely to influence the achievement of desired outcomes when more effective and convenient intervention is introduced in the study population.

3. Given the existing health and socio-economic problems, the proposed intervention is feasible by utilising available resources.

Response variables:

The major response variables are:

1. Number of diarrhoeal episodes : i.e. number of episodes of diarrhoea experienced by each child each year.
2. Number of ARI episodes : i.e. number of episodes of ARI experienced by each child each year.
3. Duration of diarrhoea : i.e. time in hours from initiation of the illness until diarrhoea stops as ascertained by the mothers.
4. Duration of ARI : i.e. time in hours from initiation of the illness until the ARI stops as ascertained by the mothers.
5. Total number of days of diarrhoea : i.e. cumulative number of days of diarrhoea suffered by each child each year.
6. Total number of days of ARI : i.e. cumulative number of days of ARI suffered by each child each year.
7. Weight gain (or loss) : i.e. weight gain (or loss) in g/month compared with base line weight in long follow-up period; for age independent comparison Z-scores of weight for age and weight for height will be used.
8. Height increment : i.e. height increment in cm/month compared with base line height in long follow-up period; for age independent comparison Z-scores of weight for height and height for age will be used.

Secondary response variables are:

1. Proportion without diarrhoea in a year
2. proportion without ARI in a year

Eligibility criteria for study subjects are:

1. Children aged 3-47 months
2. Children of both sexes
3. Children not critically ill or without congenital malformations or without metabolic disorders
4. Children irrespective of feeding habit
5. Resident in the community

Sample size estimate:

Comparison of outcome variables between the groups will be performed on all the study children independent of the compliance. It is assumed that the compliance may not be hundred per cent at the community level. Therefore, the expected change is relatively less than that of a clinical trial.

(a) To determine impact on number of diarrhoeal episodes:

Hussein et al. (68) reported a drop in the mean number of episodes in two-to six-year-old children per month, from 0.38 before iron supplementation to 0.25 after supplementation (SD=0.37). Based on this information the sample size (80 per cent power, alpha=0.05) to detect a difference of 0.09 (75 per cent of observed difference) is:

$$N = \frac{2 \times 0.37 \times 0.37}{0.09 \times 0.09} \times 8$$

$$= 270 \text{ in each group}$$

While calculating the sample size an adjustment for outmigration (10 per cent) from the area of residence is critical. Therefore, the sample size is 297 in each group.

(B) To determine impact on diarrhoeal duration:

Roy (50) reported a significant difference in the duration of young childhood acute diarrhoea between the placebo and zinc supplemented group. A reduction of duration from 6.1 days (SD=2.9 days) in control group to 4.7 days in intervention group was observed. Based on this data the sample size (80 per cent power, alpha=0.05) to detect a difference of 1.05 day (75 per cent of reported difference) is:

$$N = \frac{2 \times 2.9 \times 2.9}{1.05 \times 1.05} \times 8$$

$$= 122 \text{ in each group}$$

(c) To determine impact on number of ARI episodes and its duration:

In rural Bangladesh under 5 children were observed to suffer from more diarrheal episodes than respiratory illnesses. However the duration of respiratory episodes was much longer than diarrhoeal illnesses (1). Therefore, we estimate that the sample size should be smaller than the that calculated for diarrhoea.

(d) To determine impact on weight change:

We base this calculation from data of Walravens et al. (29) who reported weight increment in a randomised trial of zinc supplementation in children. In control group the mean weight increment was 3867 gram (SD=721 g) where as supplemented group had 4282 gram. The sample size (80 per cent power, alpha=0.05) to detect a difference of 311 gram (75 per cent of observed difference) is :

$$N = \frac{2 \times 721 \times 721}{311 \times 311} \times 8$$

$$= 86 \text{ in each group}$$

(e) To determine impact on height change:

Hambidge et al. (82) reported mean height increment of 6.66 cm per year in zinc supplemented group as compared to an increment of 5.92 cm per year (SD=0.69 cm) in non supplemented group of children aged 2-6 years. Based on this information the sample size (80 per cent power, alpha=0.05) to detect a difference of 0.55 cm (75 per cent of reported difference) is :

$$N = \frac{2 \times 0.69 \times 0.69}{0.55 \times 0.55} \times 8$$

$$= 25 \text{ in each group}$$

After review of the sample sizes for different outcomes and to enable us to carry out subgroup analysis we propose to enrol 300 in each group.

Enrolment of subjects:

Informed consent : Informed consent will be obtained from parents (preferably mothers) of all children before their participation in the study. Mothers will be fully informed about the study and freely- given consent for the subject to participate will be obtained, preferably in writing. The consent form in simple and understandable words will indicate:

- the general purpose of the study
- the benefits and known risk (if any) to the mother
- length of the follow-up

The consent form will also clearly indicate the mothers right to withdraw her child from the study at any time and the fact that the child will continue to receive health care along with ORS packets for the diarrhoeal episodes. Depot holders will obtain the informed consent from mothers.

Baseline examination:

Female interviewers will identify family units and children. They will collect baseline information with particular attention to socio-economic and environmental status (appendix 2). Households will be mapped according to their distribution in the community. Baseline anthropometric measurements of young children prior to regular distribution of polymicronutrient mixtures will also be conducted by them.

Randomisation:

Steps will be taken to ensure that equal numbers of communities are assigned to each of the three different intervention groups and one control group. Since the most important variable that may confound the outcome is the mother's literacy, relevant information will be collected. These community clusters will then be sorted and arranged in descending order according to proportion of the mothers with no education. Permuted block randomisation technique will be used to assign an equal number of communities into four groups, three intervention groups and one control group from a set of four closely comparable communities from top. Therefore, children in 6 intervention communities will receive zinc, selenium, iron, copper, and folate mixture, 6 intervention communities will receive iron, copper, and folate mixture, another 6 communities will receive zinc and selenium mixture and the remaining 6 communities will receive a multivitamin preparation (control). In fact, a stratified randomisation will be implemented to achieve comparability in base line characteristics; however, for the purpose of analysis the strata will be ignored.

Standard case management and the experimental treatment:

We have decided to use RDA of children 1-3 yr. of age for copper, selenium, and iron and twice the recommended dose for zinc and folate. Therefore, the daily doses for elemental copper, selenium, iron will be 1 mg, 20 microgram, 10 mg respectively. The daily doses for zinc and folate will be 20 mg and 100 microgram respectively. The composition of the mixtures will be developed in such a way so that doses for all children can be administered conveniently in one teaspoonful (5 ml.). Each child will be given the nutrient/vitamin mixture daily by the community depot holders (field volunteers). The study will also be double blind. Distribution of micronutrient mixtures and observation of these children for morbidity and nutritional status will continue daily and once a month, respectively, for 12 consecutive months.

Standard case management

Both mothers and depot holders will receive educational messages to indicate our definition of diarrhoea: three or more loose or liquid or watery stool without any visible blood in a 24-hour period (watery diarrhoea) or any number of stools with visible blood (dysenteric diarrhoea). Since diarrhoea is difficult to define objectively, particularly in children, we will also follow mothers 'qualitative' definition (i.e. whenever they feel their children have

diarrhoea). An episode of ARI will be defined as a group of signs and symptoms that include: cough, blocked or runny nose, ear discharge, hoarseness, rapid breathing, breathing difficulty, visible sucking in of ribs, very rapid breathing, and inability to drink. However, measles as well as coughs and/or runny nose lasting longer than two weeks will not be considered as ARI. Depot holders will provide ORS packets whenever they detect diarrhoea children during their daily disease surveillance rounds. The intervention micronutrient mixture and placebo multivitamin preparation will be identical in appearance (i.e. for flavour and colour) and will be packaged in identical bottles. Community depot holders will serve as the depot holders of the micronutrient mixtures. These field volunteers will be advised to administer the stated dose once daily to the assigned study children during their disease surveillance rounds. Repeat dose will only be given if there is vomiting immediately after the intake. Moreover, mothers will be advised to collect ORS packets for their diarrhoea children from the community depot holders. Depot holders will educate and demonstrate the preparation and administration of ORS to the mothers. A standard measure (water mug) will be used to measure the required volume of water (half a litre). Breast-fed children will be encouraged to receive milk while being treated. These children will also be followed at the households by the depot holders. The mothers will be further advised on the continuation of unrestricted breast-feeding, diet, and plain water from time to time in small volumes. Pretested forms will be used for recording relevant information. Field volunteers will be instructed to refer the children to the nearest appropriate health facility whenever the mothers feel the need.

Dose and composition of the micronutrient/vitamin mixtures:

SYRUP A :	Quantity/5 ml
1. Zinc acetate	: 20 mg
2. Sodium selenite	: 20 micro gm
3. Ferrous sulphate	: 75 mg
4. Copper sulphate	: 1 mg
5. Folic acid	: 200 micro gm
6. Vitamin A (acetate)	: 500 micro gm (1500 I.U.)
7. Ascorbic acid	: 30 mg
8. Vitamin D 3	: 200 I.U.
9. Vitamin E	: 10 mg
10. Glycerine U.S.P.	: 1.2 ml
11. Propylene glycol U.S.P.	: 0.75 ml
12. Sorbitol 70% B.P.	: 2.0 ml
13. Methyl-paraben U.S.F.N.	: 3.5 mg
14. Polysorbate-80 (Tween-80) B.P.C.	: 50 mg
15. Lemon oil Ph. Grade	: .0125 ml
16. Caramel Brown colour powder Ph Grade	: 1 mg
17. Purified water	: .75 ml

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SYRUP B

Quantity/5 ml

1. Ferrous sulphate	:	75	mg
2. Copper sulphate	:	1	mg
3. Folic acid	:	200	micro gm
4. Vitamin A (acetate)	:	500	micro gm (1500 I.U.)
5. Ascorbic acid	:	30	mg
6. Vitamin D 3	:	200	I.U.
7. Vitamin E	:	10	mg
8. Glycerine U.S.P.	:	1.2	ml
9. Propylene glycol U.S.P.:	:	0.75	ml
10. Sorbitol 70% B.P.	:	2.0	ml
11. Methyl-paraben U.S.F.N.:	:	3.5	mg
12. Polysorbate-80 (Tween-80) B.P.C.	:	50	mg
13. Lemon oil Ph. Grade	:	.0125	ml
14. Caramel Brown colour powder Ph Grade	:	1	mg
15. Purified water	:	.75	ml

SYRUP C

Quantity/5 ml

1. Zinc acetate	:	20	mg
2. Sodium selenite	:	20	micro gm
3. Vitamin A (acetate)	:	500	micro gm (1500 I.U.)
4. Ascorbic acid	:	30	mg
5. Vitamin D 3	:	200	I.U.
6. Vitamin E	:	10	mg
7. Glycerine U.S.P.	:	1.2	ml
8. Propylene glycol U.S.P.:	:	0.75	ml
9. Sorbitol 70% B.P.	:	2.0	ml
10. Methyl-paraben U.S.F.N.:	:	3.5	mg
11. Polysorbate-80 (Tween-80) B.P.C.	:	50	mg
12. Lemon oil Ph. Grade	:	.0125	ml
13. Caramel Brown colour powder Ph Grade	:	1	mg
14. Purified water	:	.75	ml

SYRUP D

Quantity/5 ml

1. Vitamin A (acetate)	:	500	micro gm (1500 I.U.)
2. Ascorbic acid	:	30	mg
3. Vitamin D 3	:	200	I.U.
4. Vitamin E	:	10	mg
5. Glycerine U.S.P.	:	1.2	ml
6. Propylene glycol U.S.P.:	:	0.75	ml
7. Sorbitol 70% B.P.	:	2.0	ml
8. Methyl-paraben U.S.F.N.:	:	3.5	mg
9. Polysorbate-80 (Tween-80) B.P.C.	:	50	mg
10. Lemon oil Ph. Grade	:	.0125	ml
11. Caramel Brown colour powder Ph Grade	:	1	mg
12. Purified water	:	.75	ml

Organisation of the study:

Curriculum and form development

The curriculum for training of the depot holders includes lectures, demonstrations, and participatory practice on the identification of the ARI cases, assessment of dehydration, and treatment of diarrhoea by prepackaged ORS. In addition, tools for measurement of outcome variables will also be developed and pretested. A questionnaire will be developed for recording relevant information on ORS intake, frequency of stool and vomiting, and feeding practices along with information on ARI. However, prior to its use, the questionnaire will be field tested for validity, comprehension, and acceptability in 10-20 mothers. A picture calendar will be developed to use as an instrument to record the duration of ARI and diarrhoea along with ORS use. Each mother will be demonstrated how to use the picture calendar. Each calendar will have hand drawn pictures of: (a) a sick child passing diarrhoea stool (b) coughing and (c) a child with diarrhoea is being given ORS by tea spoon out of the feeding cup. Next to these pictures there will be hand-drawn blank boxes in series. Of them, four boxes will be earmarked for recording events occurring in four six-hourly periods of each day. On the top of each box, pictures of the sun and moon will indicate morning, noon, evening, and night in sequence. Mothers will be expected to give check marks with pencils, one mark for each event i.e. presence of diarrhoea or ARI during that 6 hourly period. Moreover, one mark for ORS indicating that ORS has been given during that 6 hourly period. Such a simple and easily understandable picture calendar will facilitate recording of ARI and diarrhoea duration and provision of rehydration therapy with a precision of plus or minus 6 hours. However, this calendar will be field tested in 10-20 mothers before finalising (appendix 3).

Selection and training of depot holders

Community leaders will be explained the objectives and expected benefit out of the proposed study and their active co-operation will be sought for successful implementation of the intervention. With their active assistance, 24 women, one from each community will be selected as depot holders. Women aged 20-40 years, with some education (years of schooling no less than 6 years), intelligent, willing to serve the community and has the permission of their legal guardians to serve as depot holders will be preferred. They will be trained on diarrhoea management at the household level. They will also be trained on face-to-face teaching and demonstration techniques. Depot holders will attend a 2-day lecture, demonstration and practical sessions in ICDDR,B's Dhaka hospital. The method of preparation of ORS by measuring the required volume of water using a standard measure, a half litre mug will also be shown. The other aspects of education are demonstration of administration of ORS to the child by the depot holder for a while. Moreover, mothers will be advised on continuation of breast-feeding, diet, and plain water from time to time. Once the depot holder is convinced that the mother can prepare and administer ORS up to her satisfaction, then the mother will be allowed to continue the management of the child.

Training of male field workers and female interviewers

Six male field workers will be trained to provide guided and supportive supervision to the field volunteers and two female interviewers will be trained to collect detailed household information, health-related data, and anthropometric measurements by using prescribed forms.

Enrolment of children

Distribution of polymicronutrient mixtures, disease and nutritional surveillance of children between 3 to 47 months will start in all communities at a time.

Data collection

Once intervention started, the monitoring of the impact will continue for 12 consecutive months. Distribution of micronutrient/multivitamin mixtures and surveillance for diarrhoea, ARI, and other common ailments in all study children will be conducted daily by depot holders using field tested forms. Mothers will be asked about the active cases or cases occurring since the last visit. Detected cases will be followed till recovery or adverse outcome by depot holders. Male field workers will visit communities, contact depot holders and based on their logs, will also visit all children with acute diarrhoea and ARI in each community to ensure collection of data with better accuracy. The end of an episode will be recorded when the child is found to have normal stools or no ARI related complaints according to the mother and that cessation of the episode continues for three consecutive days for diarrhoea and two consecutive weeks for ARI. This will also be confirmed by three subsequent daily visits. If the child is taken to any health care provider or health facility, the nature and type of health care provider and medicines received will be recorded. Anthropometric measurements will be repeated every month for twelve consecutive months by female interviewers. Investigators during their routine visits to the communities and one investigator, a paediatrician with good familiarity with the communities and population, will treat if necessary sick children and if needed will refer the children to the nearby Rotary Club operated MCH Clinic, Upa-zila Health Complex, and Narayanganj District Hospital. An appropriate referral linkage will be established with the concerned health facilities well ahead of starting the project. If required, ARI and diarrhoeal children will be referred straight to the ICDDR,B hospital. To facilitate smooth follow-up visits, a diary will be maintained by each field volunteer, field worker, and female interviewer. The diary will readily provide information on dates of follow-up visits for each child.

Quality control

To ensure quality control of data, staff will not be specifically informed of the hypothesis being tested and composition of the mixtures being used. Moreover, female interviewers will be trained on interviewing techniques and taking anthropometric measurements. The performance of depot holders and field workers will be monitored by regular observation at the field level. Regular checks on data will be carried out. In 10% cases data collection will be repeated by the supervisor and 5% cases by the investigators. Errors, detected if any will be corrected on the spot. Moreover, forms will be reviewed daily

for completeness by field workers. For the sake of standardisation any intra-observer and inter-observer variations will be monitored on weekly basis for controlling such variations. Monthly meetings will review the progress of the work. Identical forms, equipments, definitions, and methods will be used throughout the study period in both intervention and control communities.

Consideration of withdrawals and treatment failures during analysis:

The reasons for withdrawing a patient from the study are: non-compliance of the subject, either because the patient requires unscheduled intervention for a serious intercurrent illness or complications that prevented the planned intervention from being conducted, or migration from his place of residence. Results from all study children will be included in the analysis. Data from patients withdrawn because of any reason will be included in the analysis up to the time of withdrawal.

Summary of procedures:

1. Baseline information with particular attention to the socio-economic and environmental status will be collected. Households will be mapped.
2. Permuted block randomisation technique will be used to assign an equal number of communities into four groups, three intervention groups and one control intervention group from a set of four closely comparable communities.
3. Community leaders will be explained the objectives and expected benefit out of the proposed study and with their active assistance community depot holders will be selected.
4. Community depot holders will be trained on identification of ARI cases, assessment of dehydration, and diarrhoea management at the household level. Appropriate dietary advice will also be given. Each mother will be provided with a picture calendar and its use will be carefully explained to record the ARI and diarrhoea events and provision of rehydration therapy. Patients will also be home visited regularly..
5. Anthropometry of eligible children will be conducted prior to starting of distribution of polymicronutrient mixture and ORT packets.
6. Standardised procedures will be followed to ensure quality control.

Methods to measure the response variables:

- body weight : by weighing children undressed on a scale sensitive to 20 grams;
- duration : by counting number of six hourly periods on the picture calendar;

— anthropometry : by supine length board (to the nearest of 0.1 cm)
— (length, MUAC) by measuring tape (to the nearest of 0.1 cm).

Study schedule:

An outline schedule indicating the time to be required for the different phases of the project is given below:

Year 1:

August–September 1991 : Recruitment and training of study personnel;
Development of study materials;
Field test of study materials;
Procurement of supplies;
Establishment of methods.

October–December 1991 : Carry out follow-up;
Quality control;
Data checking.

Year 2:

January–September 1992 : Carry out follow-up;
Quality control;
Data checking;
Data entry.

October–January 1993 : Data analysis.

February–April 1993 : Report writing.

Data management and analysis:

Information will be recorded on the field tested forms. Field workers will be rotated from one group of communities to another every three months to avoid intra and inter-observer variations. Moreover, communities assigned to female interviewers for data collection and anthropometric measurements will also be changed periodically. Data forms will be checked by field workers (if information is collected by depot holders), and senior health assistant (if information is collected by female interviewers and if forms are checked by female interviewers). Then all the forms will be checked by the investigators. Data will be cleaned as they are collected and entered into the computer. Apart from visual checks, logical and range checks of the data will be conducted. System files will then be created using SPSS PC+ software for analysis.

During this study two types of data will be collected:

(a) Pre-intervention data:

- Age (months)
- Base line weight (Kg)
- Base line height (Cm)
- Base line weight for height (%)
- Base line MUAC (Cm)

(b) Response data:

- Weight change g/month
- Height change cm/month
- MUAC change cm/month
- Change in weight for height and height for age over time
- Number of diarrhoeal episodes per child per year
- Number of ARI episodes per child per year
- Duration of diarrhoea from start to recovery (6 hourly units)
- Cumulative diarrhoea days per child per year
- Duration of ARI from start to recovery (6 hourly units)
- Cumulative ARI days per child per year

Although the unit of intervention is a community cluster, the confounders (initial nutritional status of the child, child's age and sex, breastfeeding status, maternal education, socio-economic condition of the parents etc.) will operate at individual child level. We, therefore, plan to analyse the outcome variables taking the individual child as the unit of analysis. Moreover, it is very likely that in this longitudinal study data may not be complete in case of all children due to dropouts or temporary absence because of social visits. Therefore, simple and straight forward analysis for differing lengths of follow-up on an individual child basis is crucial.

Data will be summarised, and different nutrient mixture groups will be compared. To assess the impact on morbidity and nutrition, groups will be compared with regard to mean and median of age (in months), and duration of diarrhoea and ARI (in hours), and base line weight, height, and mid upper arm circumference. Similarly outcome responses will also be summarised and compared. Data on number of ARI and diarrhoea episodes, weight gain (g/month), height increment (cm/month) and duration of ARI and diarrhoea (hours) will be used.

Statistical analysis will include descriptive as well as analytic methods, arithmetic mean and standard deviation if the distribution is normal, and median and ranges if the distribution is not normal will be computed. If the outcome is expressed as a categorical variable, the significance of differences will be evaluated by the chi-square tests. If the outcome is expressed as a continuous variable, the statistical significance of group mean comparisons will be determined by analysis of variance and student's t test. If necessary equivalent nonparametric tests will be computed because asymmetric distribution of measurements may occur.

To estimate the impact on nutrition, the primary objective of the analysis will be to see changes over time. The monthly measurements will be compared with those at base line to reveal whether the effect of intervention was progressive. Control communities will also be followed in the same fashion. Moreover, monthly changes between intervention and control communities will be compared. The Chi-square for trends will be performed in order to evaluate the significance of differences.

For continuous outcome variables, multiple regression analysis will be used to adjust for several independent variables. In addition, for dichotomous outcome variables logistic regression will be computed where the variable of interest will be regressed on independent variables to estimate relative risk while adjusted simultaneously for various potentially confounding variables. Mantel-Haenszel procedure for stratified analysis will also be performed to check on the validity of logistic regression models.

Weight for height, weight for age, and height for age expressed as percentage of the National Centre for Health Statistics (NCHS) median as well as Z scores will be used as the indicators of nutritional status of the children. For all tests a p value less than 0.05 will be considered to be significant.

To ascertain the changes in nutritional status for those who will be present throughout the intervention and to guard against bias from differential dropouts or absence, we will perform separate analysis according to differing lengths of follow-up in addition to the subgroup of children present throughout the full year of analysis.

Personnel requirements:

A. Professional scientific staff	#	% time	# months	
			Yr. 1	Yr. 2
1. Dr. Dilip Mahalanabis		20	12	12
2. Dr. ASG Faruque		20	12	12
3. Dr. Dilip Kumar Das		100	12	12
4. Dr. Samiul Hoque		10	12	12
5. Mr. MA Wahed		5	8	8
6. Mr. Mujibur Rahman		5	8	8
B. Technical staff				
1. Senior Health Assistant	1	100	12	8
2. Female Interviewer	2	100	12	8

3. Data entry technician	1	100	12	12
4. Field Worker	6	100	12	8
5. Community Depot Holder	24	100	12	8

SUMMARY BUDGET

		Yr. 1	Yr. 2
1. Personnel	: US \$	47,320	41,300
2. Local travel	: US \$	5,500	5,000
3. Supplies	: US \$	8,500	4,000
4. Interdepartmental Services	: US \$	3,200	2,800
5. Total	: US \$	64,520	53,100

ABSTRACT SUMMARY FOR ETHICAL REVIEW COMMITTEE

1. This study aims to evaluate the beneficial effects of supplementation of micronutrients like; zinc, copper, selenium, iron, and folate on morbidity and growth of infants and young children in a rural community. ICDDR,B is carrying out research for improving health and nutritional status of Bangladeshi children since its inception. These micronutrients have been found to be effective in decreasing duration of diarrhoea and ARI, and improving growth of infants and young children. Polymicron nutrient mixtures will be given to the children every day. Infants and children (3-47 months) will be included in this study because they are the children vulnerable to high risk of diarrhoea, ARI and malnutrition. These children will be given mixture and followed for consecutive 12 months.

2. No potential risk to infants and young children is involved.

3. The children will be under constant observation of field volunteers, field workers, female volunteers, and physicians.

4. During data analysis only the identification numbers of children will be used and confidentiality will be maintained.

5. Informed consent in a consent form will be obtained from the authorised legal guardian or parents of the children before being included in the study. Mothers will be fully informed about the study and freely given consent for the subject to participate will be obtained, preferably in writing.

6. Interview of mothers will be conducted at their households by female interviewers and field volunteers to know baseline information with particular attention to socio-economic and environmental variables. Information on morbidity, and nutritional status will also be collected at regular intervals. Each interview shall not take more than half an hour.

7. Children with diarrhoea will be provided with ORS and followed by depot holders, field workers, female interviewers, and physicians if necessary at households. They will also be referred to the appropriate health facilities if required. The results of the study will help to formulate future health policy in ARI and diarrhoeal disease control and management, and will have far reaching implications for child survival efforts.

8. The study will not use records, organs, tissues, body fluids, fetus or the abortus.

CONSENT FORM

(Will be read and explained clearly before consent is obtained)

The International Centre for Diarrhoeal Disease Research, Bangladesh is planning to undertake a study in your community to know the role of micronutrients in reducing occurrence of diarrhoea and ARI episodes, their duration, and in improving weight gain in children 3-47 months.

Your child will receive micronutrient mixture daily based on random allocation. We will collect information related to health problem, take weight and measure length. Your child will be followed for at least 12 months.

We believe better knowledge of micronutrient mixtures will bring benefit to the children. The results may help in designing future action programme for control of diarrhoeal disease, acute respiratory infections, and betterment of nutritional status.

Your child will be home visited daily. Anthropometric measurements (such as measuring of weight, length, and mid upper arm circumference) will also be conducted once a month.

The study involves no risk. We will maintain confidentiality of the information given to us. If you do not agree, your child will still get ORS for diarrhoeal episodes. At any stage of the study, you may withdraw your child, but his care by us will not be hampered.

If you agree to participate in this study then please sign below.

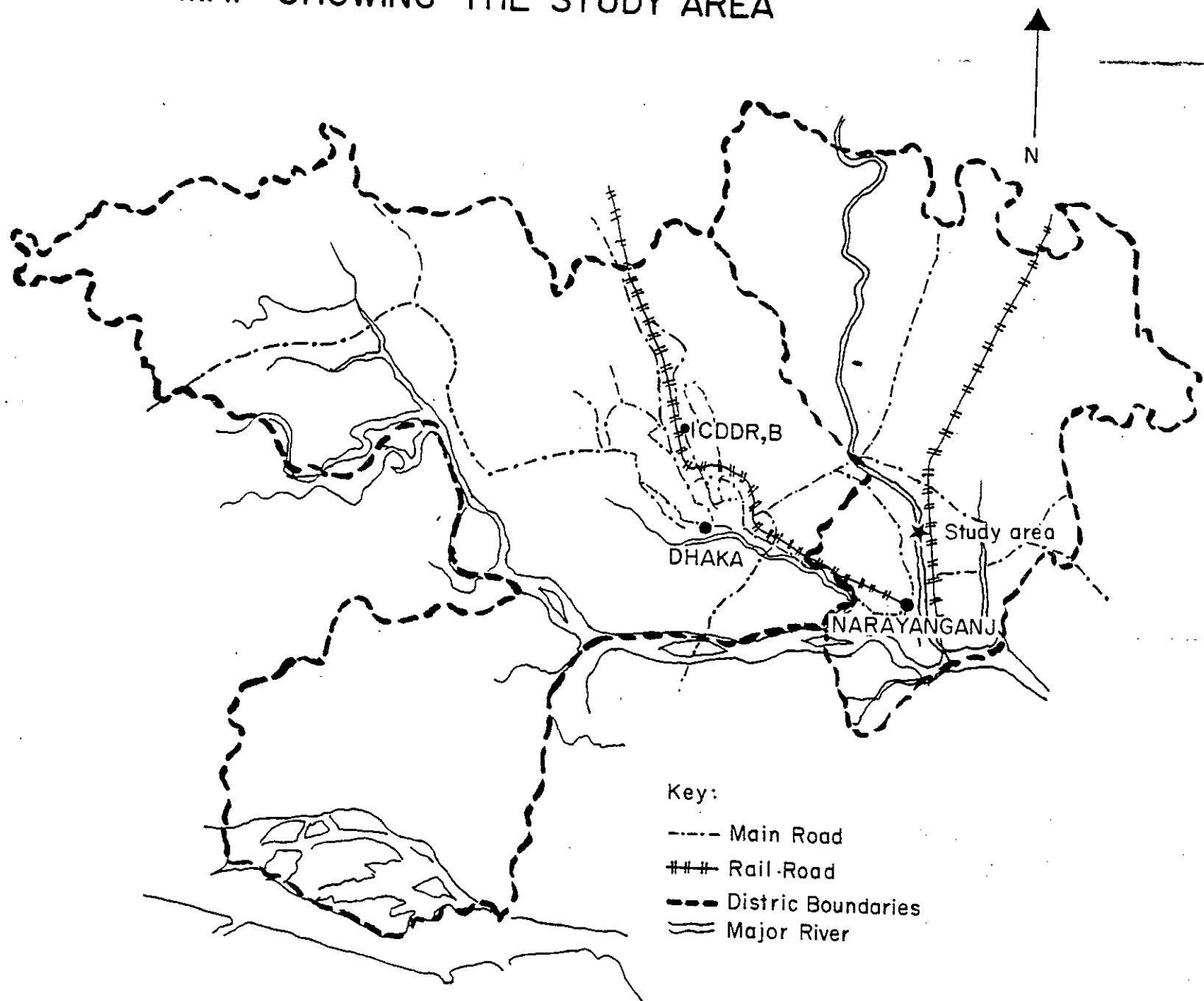
Signature of the
Principal Investigator

Date

Signature or left thumb
impression of the legal
guardian

Signature or left thumb
impression of the witness

MAP SHOWING THE STUDY AREA



Village					Bari			
Sl. No.	Name	Relation	Age	Sex	I.D. No.	Education	Occupation	Land holding
1								
2								
3								
4								
5								
6								
7								
8								
9								
10								
11								
12								

Source of water: Drinking : Tubewell ☐ Pond ☐ Canal ☐ River ☐ Well ☐ Other ☐

Cooking : Tubewell ☐ Pond ☐ Canal ☐ River ☐ Well ☐ Other ☐

Washing : Tubewell ☐ Pond ☐ Canal ☐ River ☐ Well ☐ Other ☐

Bed-room floor : Cemented ☐ Non-cemented ☐

Electricity : Yes ☐ No ☐

Animals : Cow : Yes ☐ No ☐ # ☐

Goat : Yes ☐ No ☐ # ☐

Chicken : Yes ☐ No ☐ # ☐

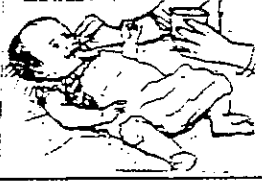
Duck : Yes ☐ No ☐ # ☐

Name of the worker-----

Date-----

ছবি পদ্ধতি

লোথায় : হাসপাতালে _____ বাড়ীতে _____

প্রতিবার পাঠ্যখানা হলে এক বার দাগ দিন	তারিখ : বার				তারিখ : বার				তারিখ : বার				তারিখ : বার			
																
	সকাল	দুপুর	সন্ধ্যা	রাত্রি	সকাল	দুপুর	সন্ধ্যা	রাত্রি	সকাল	দুপুর	সন্ধ্যা	রাত্রি	সকাল	দুপুর	সন্ধ্যা	রাত্রি
																
																

যদি এই সময়ের মধ্যে স্যানাইন খাওয়াছে থাকেন তবে প্রতি করে একবার দাগ দিন :

যদি এই সময়ের মধ্যে বাচ্চার ঠান্ডা, কফ, কাশি, শ্বাস রুখি থাকে তবে প্রতি করে একবার দাগ দিন :

সকাল : সকাল ৬টার পর থেকে দুপুর ১২টা

দুপুর : দুপুর ১২টার পর থেকে সন্ধ্যা ৬টা

সন্ধ্যা : সন্ধ্যা ৬টার পর থেকে রাত ১২টা

রাত্রি : রাত ১২টার পর থেকে সকাল ৬টা পর্যন্ত

রোগীর নাম : _____

গ্রাম : _____

বাড়ীর নম্বর : _____

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DAILY DISEASE SURVEILLANCE
(Long term follow-up)

Name of the worker: _____

Date of visit: _____

1. Child I.D. # ____/____/____/____/____/____/

2. Age (months) ____/____/

3. Sex (Male=1, Female=2) ____/

4. Any diarrhoea between last visit and this visit ____/
(Yes=1, No=2)

5. If yes date of onset: ____/____/____/____/____/____/
D D M M Y Y

6. Time of onset: ____/

6 AM - 12 Noon=1, 12 Noon - 6 PM=2,
6 PM - 12 MN=3, 12 MN - 6 AM=4

7. If yes character of stool: ____/
(Watery/loose=1, bloody=2, mucoid=3)

8. If yes, use of ORS: ____/
(Yes=1, No=2)

9. Use of any drug: ____/
(Allopathic=1, Homeopathic=2, Herbal=3)

10. Drug received from:

Drug store [Yes=1, No=2]	____/
Qualified physician [Yes=1, No=2]	____/
non qualified physician [Yes=1, No=2]	____/
Kabiraj [Yes=1, No=2]	____/
health complex [Yes=1, No=2]	____/
MCH clinic [Yes=1, No=2]	____/
health subcentre [Yes=1, No=2]	____/
government health worker [Yes=1, No=2]	____/
homeopath [Yes=1, No=2]	____/
district hospital [Yes=1, No=2]	____/
ICDDR,B hospital [Yes=1, No=2]	____/
other [Yes=1, No=2]	____/
not applicable [Yes=1, No=2]	____/

11. Outcome: ____/
(recovered=1, died=2,
still continuing=3)

D D M M Y Y

6 PM - 12 MN=3, 12 MN - 6 AM=4

✓

____/____/____/____/____/____
D D M M Y Y

/

6 PM - 12 MN=3. 12 MN - 6 AM=4





1

1



homeopath	[Yes=1, No=2]	<u> </u> /
district hospital	[Yes=1, No=2]	<u> </u> /
ICDDR,B hospital	[Yes=1, No=2]	<u> </u> /
other	[Yes=1, No=2]	<u> </u> /
not applicable	[Yes=1, No=2]	<u> </u> /

21. Outcome: /

(recovered=1, died=2,
still continuing=3)

22. If recovered, date of recovery / / / / / /

D D M M Y Y

23. If recovered, time of recovery: / /

6 AM - 12 Noon=1, 12 Noon - 6 PM=2,
6 PM - 12 MN=3, 12 MN - 6 AM=4

24. Any illness:

Measles	[Yes=1, No=2]	<u> </u> /
Scabies	[Yes=1, No=2]	<u> </u> /
Conjunctivitis	[Yes=1, No=2]	<u> </u> /
Night blindness	[Yes=1, No=2]	<u> </u> /
Stomatitis	[Yes=1, No=2]	<u> </u> /
Marasmus	[Yes=1, No=2]	<u> </u> /
Kwashiorkor	[Yes=1, No=2]	<u> </u> /
Impetigo	[Yes=1, No=2]	<u> </u> /
Sore throat	[Yes=1, No=2]	<u> </u> /
Mumps	[Yes=1, No=2]	<u> </u> /
TB	[Yes=1, No=2]	<u> </u> /
Other	[Yes=1, No=2]	<u> </u> /

MONTHLY SURVEILLANCE SUMMARY FORM
(Long term follow-up)

1. Name of the child: _____
2. Father's name: _____
3. Age(months) _____ 4. Sex: Male _____ Female _____
5. Child I.D. # _____
6. Name of the month _____ Year _____
7. Number of diarrhoeal episodes in this month: _____
8. If any diarrhoea:

Stool character for first episode:

watery/loose _____ bloody _____ mucoid _____

ORS use: Yes _____ No _____

Duration of diarrhoea(# 6 hourly completed period) _____

Use of any drug: Allopathic _____ Homeopathic _____ Herbal _____

Source of drug:

Drug store _____ Qualified physician _____

Non qualified physician _____ Kabiraj _____

health complex _____ MCH clinic _____

health subcentre _____ homeopath _____

district hospital _____ ICDDR,B hospital _____

other _____

Stool character for second episode:

watery/loose _____ bloody _____ mucoid _____

ORS use: Yes _____ No _____

Duration of diarrhoea(# 6 hourly completed period) _____

Use of any drug: Allopathic _____ Homeopathic _____ Herbal _____

Source of drug:

Drug store_____ Qualified physician _____
Non qualified physician_____ Kabiraj_____
health complex_____ MCH clinic_____
health subcentre_____ homeopath_____
district hospital_____ ICDDR,B hospital_____
other_____

Stool character for third episode:

watery/loose_____ bloody_____ mucoid_____
ORS use: Yes_____ No_____
Duration of diarrhoea(# 6 hourly completed period) _____
Use of any drug: Allopathic _____ Homeopathic_____ Herbal_____

Source of drug:

Drug store_____ Qualified physician _____
Non qualified physician_____ Kabiraj_____
health complex_____ MCH clinic_____
health subcentre_____ homeopath_____
district hospital_____ ICDDR,B_____
other_____

9. Outcome of diarrhoeal episode: recovered_____ Died_____ Unknown_____

10. Number of diarrhoeal episodes in this month: _____

11. If any ARI:

Clinical features for first ARI episode:

Fever_____ cough_____ runny or blocked nose_____
Hoarseness of voice_____ rapid breathing_____
breathing difficulty_____ visible sucking in of ribs_____

very rapid breathing_____ ear discharge or pain in the ear_____

Unable to drink_____

Duration of ARI (# 6 hourly completed period) _____

Use of any drug: Allopathic _____ Homeopathic_____ Herbal_____

Source of drug:

Drug store_____ Qualified physician _____

Non qualified physician_____ Kabiraj_____

health complex_____ MCH clinic_____

health subcentre_____ homeopath_____

district hospital_____ ICDDR,B hospital_____

other_____

Type of ARI: Mild_____ Moderate_____ Severe_____

Outcome of ARI: recovered_____ Died_____ unknown_____

Clinical features for second ARI episode:

Fever_____ cough_____ runny or blocked nose_____

Hoarseness of voice_____ rapid breathing_____

breathing difficulty_____ visible sucking in of ribs_____

very rapid breathing_____ ear discharge or pain in the ear_____

Unable to drink_____

Duration of ARI (# 6 hourly completed period) _____

Use of any drug: Allopathic _____ Homeopathic_____ Herbal_____

Source of drug:

Drug store_____ Qualified physician _____

Non qualified physician_____ Kabiraj_____

health complex_____ MCH clinic_____

health subcentre_____ homeopath_____

district hospital_____ ICDDR,B hospital_____
other_____

Type of ARI: Mild_____ Moderate_____ Severe_____

Outcome of ARI: recovered_____ Died_____ unknown_____

Clinical features for third ARI episode:

Fever_____ cough_____ runny or blocked nose_____

Hoarseness of voice_____ rapid breathing_____

breathing difficulty_____ visible sucking in of ribs_____

very rapid breathing_____ ear discharge or pain in the ear_____

Unable to drink_____

Duration of ARI (# 6 hourly completed period) _____

Use of any drug: Allopathic _____ Homeopathic_____ Herbal_____

Source of drug:

Drug store_____ Qualified physician _____

Non qualified physician_____ Kabiraj_____

health complex_____ MCH clinic_____

health subcentre_____ homeopath_____

district hospital_____ ICDDR,B hospital_____

other_____

Type of ARI: Mild_____ Moderate_____ Severe_____

Outcome of ARI: recovered_____ Died_____ unknown_____

12. Any other illness:

Measles_____ Scabies_____ Conjunctivitis_____

Night blindness_____ Stomatitis_____ Marasmus_____

Kwashiorkor_____ Impetigo_____ Sore throat_____

Mumps_____ TB_____

Other(_____)_____

13. Weight:_____ Kg

14. Length:_____ Cm

15. MUAC:_____ Cm

1

Page 1 (of 2)

Title: Role of micronutrient mixture containing zinc, selenium, iron
and folic acid in reducing the incidence a
randomised community intervention trial

Summary of Referee's Opinions: Please see the following table to
evaluate the various aspects of the proposal by checking the
appropriate boxes. Your detailed comments are sought on a
separate, attached page.

Rank Score

Quality of Project

Adequacy of Project Design

Suitability of Methodology

Feasibility within time period

Reasonableness of budget

Potential value of field of knowledge

High	Medium	Low
X		
X		
X		
X		
	X	
	X	

Comments:

Impact of the application:

a) without qualification

(X)

I do not support the application

Name of Referee: E FONTAINE

Position: Medical Officer

Institution: WHE/CMB

Signature: *E Fontaine*

Date: 13 June 1991

Handwritten scribbles and marks at the top of the page.

This is a well well - ad well designed project. I have no major comments and only one query. Is it possible to make the already decide build?

Respected: C. Ferreira

Topic: Role of manufacturing mixtures

(The following pages if necessary)

Please briefly provide your opinions of this proposal giving special attention to the originality and feasibility of the project. Its potential for providing new knowledge and the justification of financial support sought. Include suggestions for modifications (scientific or financial) where you feel they are justified.

Relevant comments

2

Title: Role of micronutrient mixture containing zinc, selenium, iron copper, and folate in reducing the incidence a randomised community intervention trial

Summary of Referee's Opinions: Please see the following table to evaluate the various aspects of the proposal by checking the appropriate boxes. Your detailed comments are sought on a separate, attached page.

Rank Score

	High	Medium	Low
Quality of Project		✓	
Adequacy of Project Design		✓	
Suitability of Methodology		✓	
Feasibility within time period		✓	
Appropriateness of budget			
Potential value of field of knowledge		✓	

CONCLUSIONS

I support the application:

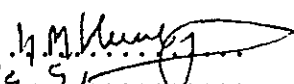
a) without qualification ☐

b) with qualification

- on technical grounds ☒

- on level of financial support ☐

I do not support the application ☐

Name of Referee: HAMBIDGE V.M. Signature: 

Date: 4.14.91

Position: Professor Pediatrics

Institution: Univ. Colorado Health Sciences Center

Detailed Comments

Please briefly provide your opinions of this proposal, giving special attention to the originality and feasibility of the project, its potential for providing new knowledge and the justification of financial support sought; include suggestions for modifications (scientific or financial) where you feel they are justified.

(Use additional pages if necessary)

Title:

PI:

Reviewer:

Strengths

- This proposal addresses questions of potential public health significance.
- A polymicronutrient mixture has practical appeal from a public health perspective.
- Good justification for the number subjects required.
- Randomized, placebo-controlled, blinded study.

Weaknesses

- No pilot data on micronutrient status of study population. ✓
- No rationale for choice of micronutrients allocated to each group. Potential problems with current choices do exist, e.g. zinc supplements without copper supplements could lead to copper deficiency if dietary copper is marginal.

Questions

- What is included in placebo vitamin preparation?
- What impact will this preparation have on total folate intake for folate-supplemented group?
- Does a 12 month observation period mean a 12 month supplementation period also?

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3



Department of Epidemiology and Population Sciences

Dr D Mahalanabis
Associate Director and Head, Clinical Sciences Division
International Centre for Diarrhoeal Disease Research
GPO Box 128
Dhaka 1000
BANGLADESH

19th April 1991

Dear Dilip

Thank you for sending me the proposal "Role of micronutrient mixture containing zinc, selenium, iron, copper, and folate in reducing the incidence and severity of acute diarrhoea and acute respiratory infections, and in improving nutrition in children: a randomised community intervention trial" for review. I apologize for any delay in responding; I have been in Burkina Faso for the past month and found the proposal waiting for me on my return. My comments on the proposal are as follows.

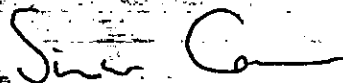
1. My main query concerning the proposed design is why the investigator has chosen to randomize the intervention at the community level? I realize that randomization at the individual level would introduce some complications. That each depot holder would have to deliver 4 different polymicronutrient mixtures to the children in her community. However, the depot holders will be women with at least 6 years of schooling who will be responsible for the completion of various forms. I would have thought that, with the aid of colour coding or some other simple device, it should be possible to ensure the delivery of the correct mixture to each child randomized on an individual basis. Randomization at the individual level would remove any doubts about the validity of analyses conducted at the individual level. I am not convinced that all confounders operate at the level of the individual child (p.19, para 1).

2. Is daily delivery of the micronutrient mixtures the most appropriate regime? On page 8 (Rationale 6) it is suggested that a polymicro-nutrient mixture might prove to be a cost-effective public health intervention. Is it realistic to imagine that in a programme setting these mixtures could be delivered on a daily basis? If not, would it be more realistic to adopt a regime which called for less frequent delivery? Is the main objective to evaluate the theoretical "efficacy" of these mixtures or to evaluate their practical effectiveness.

3. On a more mundane level, each day each depot holder will be expected to deliver the micronutrient mixture to about 50 children and record morbidity for those children. This sounds like quite a heavy workload to me. In addition, it is stated that all forms will be reviewed daily for completeness. By whom? The female interviewers? How will forms be collected from the 24 communities each day?

In summary, the proposal is a very interesting and important one. I would urge the investigator to consider the possibility of randomizing the intervention at the level of the individual child rather than the level of the community.

Yours sincerely



Simon Cousens

Ext. 2422