
**Combating anaemia and micronutrient deficiencies among young children
in rural Cambodia through in-home fortification and nutrition education**

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Contents

List of Abbreviations	iv
Protocol Summary	v
1 Key Roles.....	1
2 Background Information and Scientific Rationale	3
2.1 Background Information	3
2.2 Rationale.....	5
2.3 Potential Risks and Benefits	5
2.3.1 Potential Risks.....	5
2.3.2 Known Potential Benefits	5
3 Objectives	6
4 Study Design.....	7
5 Study Population.....	11
5.1 Subject Inclusion Criteria	12
5.2 Subject Exclusion Criteria	12
6 Enrollment/ Randomization/Masking Procedures	13
7 Study Procedures/Evaluations.....	14
7.1 Clinical Evaluations	14
7.2 Concomitant Medications/Treatments	14
7.3 Laboratory Evaluations	14
7.3.1 Clinical Laboratory Evaluations.....	14
7.3.2 Specimen Preparation, Handling and Shipping	15
8 Study Schedule.....	17
8.1 Screening	17
8.2 Enrollment/Baseline	18
8.3 Follow-up	18
8.4 Final Study Visit	19
8.5 Early Termination Visit	19
9 Study Intervention/Investigational Product.....	20
9.1 Study Product Acquisition	20
9.1.1 Formulation, Packaging and Labeling.....	20
9.2 Product Storage and Stability	20
9.3 Preparation, Administration and Dosage of Study Intervention/Investigational Product.....	21
9.4 Accountability Procedures for the Study Intervention/Investigational Product(s).....	21
9.5 Assessment of Subject Adherence to Study Intervention/Investigational Product.....	22
10 Assessment of Scientific Objectives (e.g., Safety or Immunogenicity or Efficacy)	23
10.1 Specification of the Appropriate Outcome Measures.....	23
10.1.1 Primary Outcome Measures.....	23
10.1.2 Secondary Outcome Measures.....	23
10.2 Methods and Timing for Assessing, Recording, and Analyzing Appropriate Outcome Measures	23

10.3	Modification and Discontinuation of Study Intervention/Investigational Product for a Participant	24
10.3.1	Dose/Schedule Modifications for a Subject.....	24
10.3.2	Criteria for Discontinuation of Study Intervention/Product for Withdrawal of a Subject (or a Cohort)	24
11	Assessment of Safety	25
11.1	Specification of Safety Parameters	25
11.2	Methods and Timing for Assessing, Recording, and Analyzing Safety Parameters.....	25
11.2.1	Adverse Events, Serious Adverse Events	25
11.3	Reporting Procedures	26
11.3.1	Serious Adverse Event Detection and Reporting	26
11.3.2	Procedures to be Followed in the Event of Abnormal Laboratory Test Values or Abnormal Clinical Findings.....	26
11.3.3	Type and Duration of the Follow-up of Subjects After Adverse Events.....	27
11.4	Halting Rules.....	27
12	Statistical Considerations.....	28
12.1	Overview and Study Objectives	28
12.2	Study Population	28
12.3	Study Design.....	28
12.4	Study Outcome Measures	28
12.5	Study Hypotheses	29
12.6	Sample Size Considerations	29
12.7	Participant Enrollment and Follow-Up.....	30
12.8	Planned Interim Analyses	30
12.8.1	Safety Review	30
12.9	Final Analysis Plan.....	30
13	source documents and Access to Source Data/Documents.....	32
14	Quality Control and Quality Assurance	33
15	Ethics/Protection of Human Subjects.....	35
15.1	Declaration of Helsinki	35
15.2	Institutional Review Board	35
15.3	Informed Consent Process	35
15.4	Subject Confidentiality	36
15.5	Study Discontinuation	36
16	Data Handling and Record Keeping	37
16.1	Data Management Responsibilities	37
16.2	Data Capture Methods	37
16.3	Types of Data.....	37
16.4	Timing/Reports.....	38
16.5	Study Records Retention	38
16.6	Protocol Deviations	38
17	Publication Policy.....	39

18	Literature References	40
	18.1.1 Appendix A	42
	18.1.2 Appendix B	44
	Middle Upper Arm Circumference.....	48
19	Protocol Amendment (July 2009).....	50

Abbreviations

AGP	Alpha-1 Glycoprotein
BASICS	Basic Support for Institutionalizing Child Survival (USAID)
CBC	Complete Blood Count
CDHS	Cambodia Demographic Health Survey
CESVI	Cooperazione e Sviluppo
CIPS	Cambodia Intercensal Population Survey
CRP	C-Reactive Protein
FNSPSP	Food Security and Nutrition Policy Support Project
GTZ	German Technical Cooperation
HKI	Helen Keller International
IMCI	Integrated Management of Childhood Illness
IYCF	Infant and Young Child Feeding
M & E	Monitoring & Evaluation
MOH	Ministry of Health
MPA	Minimum Package of Activities (to be implemented at Health Centre level)
MPA 10	Minimum Package of Activities Module 10 (Nutrition Module)
MTWG	Micronutrients Technical Working Group
NFP	Nutrition Focal Point (Person)
NIPH	National Institute of Public Health
NIPHL	National Institute of Public Health Laboratory
NNP	National Nutrition Program
OD	Operational District (Health)
PHD	Provincial Health Department
RBP	Retinol Binding Protein
StFr	Serum Transferrin Receptor
TASK	Tratrong ning Akphiwat Sokhaphiep neak Kre Kraw (Supporting the Development & Health of the Poor)
UNICEF	United Nation Children's Fund
VHSG	Village Health Support Group (Ministry of Health sanctioned Community Volunteer: 1 woman and 1 man from each village)
WHO	World Health Organization

Protocol Summary

Limit to 1-2 pages

Title: Cluster randomized study: Combating anaemia and micronutrient deficiencies among young children in rural Cambodia through in-home fortification and nutrition education

Population: Total sample size 3600 infants aged 6 months (sub-sample of 1200 infants for haematology and biochemical analysis of blood, anthropometry and other data) from Svay Rieng Operational Health District, Svay Rieng Province, Cambodia.

Number of Sites: 1 Operational Health District, 20 Health Centres

Study Duration: March 2008 – August 2009

Description of Agent or Intervention:

Infant & Young Child Feeding Education over 6 months for mothers of children 6-11 months of age.

Sprinkles: Multi-micronutrients in a powder form to be sprinkled onto and mixed into food for 6 months from 6-11 months of age

Overall Goal:

To inform policy on effective and feasible interventions to combat anaemia and other micronutrient deficiencies and promote growth in children in the first 18 months of life.

Objectives:

Primary Objective

To assess the effectiveness of 6 months of in-home fortification with Sprinkles starting at six months of age accompanied with IYCF education compared to IYCF education alone to reduce anaemia, iron, zinc, and vitamin A deficiencies and improve growth at 12 months and to sustain these changes at 18 months of age.

Hypotheses

1. Daily in-home fortification of infant's diets from age 6 through 11 months, delivered with IYCF education will reduce the prevalence of anaemia by at least 25 percentage points, while also reducing deficiencies of iron, zinc, vitamin A, and stunting and wasting at 12 months of age compared to infants whose mothers receive IYCF education alone.
2. Daily in-home fortification of infant's diets from age 6 through 11 months, delivered with IYCF education will produce a sustained reduction of anaemia prevalence by at least 25 percentage points, while also reducing deficiencies of iron, zinc, vitamin A, and stunting and wasting at 18 months of age compared to infants whose mothers receive IYCF education alone.

Protocol Summary - *continued*

Secondary Objective

To test the feasibility of the distribution of Sprinkles and IYCF education through the government health system down to community level.

Primary Outcome: The study will determine the extent to which:

- Anaemia, iron deficiency, zinc deficiency, vitamin A deficiency, stunting and wasting are decreased at age 12 months and reductions are sustained at 18 months compared to IYCF education alone.

Secondary Outcomes:

The study will assess the implementation feasibility through measure of the following indicators

- Commodity distribution of in-home fortificant Sprinkles
- Promotion and instruction on use of Sprinkles
- Use and adherence of Sprinkles in targeted children
- IYCF behaviour change communication activities

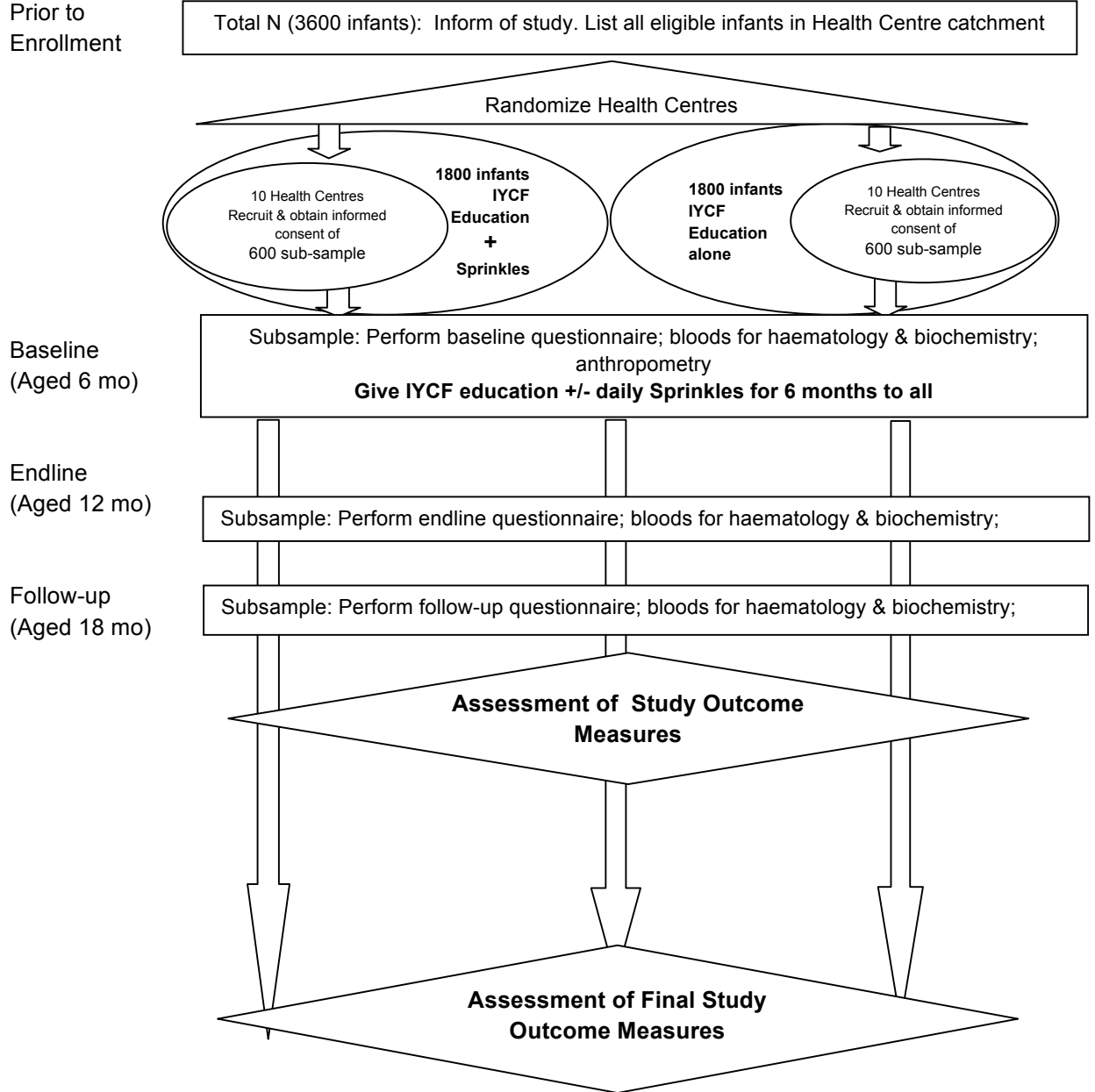
Other related study activities:

- Strengthening the capacity of the National Nutrition Program, the National Institute of Public Health (NIPH) including the laboratory, the Provincial Health Department (PHD), Operational District (OD), health centre staff and Village Health Support Groups (VHSGs) to implement and monitor effective interventions.

Protocol Summary - continued

Schematic of Study Design:

Flow diagram:



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2 BACKGROUND INFORMATION AND SCIENTIFIC RATIONALE

2.1 Background Information

Indicators of child survival are improving in Cambodia. However, 44% of children under five are stunted (<-2 SD) and 62% are anaemic (CDHS 2005) indicating that both micro and macronutrient malnutrition are widespread. Rates of anaemia in preschool children in Cambodia are among the highest in the Asia-Pacific region and critically high among infants aged 6 to 11 months (Table 1). The prevalence of other micronutrient deficiencies is also high. The prevalence of vitamin A deficiency was found to be 22% (Cambodia Micronutrient Survey, 2000). Assessment of zinc deficiency from a peri-urban sample of stunted children showed a prevalence of 75%.

Table 1: Prevalence of micronutrient deficiencies in Cambodian preschoolers

Study	Study Area	Population and Age	Number of children	Prevalence
Anaemia (Hb <110 g/L)				
CDHS 2005	National	6-59m	3,158	61.9%
CDHS 2005	National	6-8m	163	80.5%
		9-11m	170	87.1%
		12-17m	370	85.8%
Cambodia Micronutrient Survey 2000 HKI/MOH	Sample of 7 Rural Provinces	6-11m	~50	~92%
Kandal Study 2002	Kandal province	8-72m	159	66.7%
GTZ Preschool baseline 2003	Kampot province	6-24m	250	74.4%
CESVI baseline 2003	Tuk Phos district, Kampong Chhnang Province	6m	204	78.0%
Servants/TASK baseline 2003	Meanchey district Phnom Penh	Stunted children 12-35m	286	73%
Vitamin A deficiency (serum retinol <0.70 µmol/L)				
Cambodia Micronutrient Survey 2000 HKI/MOH	Sample of 7 Rural Provinces	0-59m	344	22.3%
Servants/TASK baseline	Meanchey district	Stunted	286	28.4%

2003	Phnom Penh (urban)	children 12-35m		
Zinc Deficiency (serum Zn <10.7 µmol /L)				
Servants/TASK baseline 2003	Meanchey district Phnom Penh (urban)	Stunted children 12-35m	286	75%

Anaemia and micronutrient deficiencies in children are recognized as a serious public health issues and have devastating effects on child health and development causing poor resistance to illness and disease, delayed cognitive development, decreased future learning ability, reduced future earning capacity and increased mortality (Gibson, 2004). The causes of anaemia are multi-factorial including iron deficiency, other micronutrient deficiencies, infection and haemoglobinopathies. Recent studies in Cambodia have shown prevalence rates of haemoglobinopathies to be between 30-70% (Giovanni et al, 2006; GTZ, unpublished).

Deficiencies of micronutrients other than iron can cause or hinder efforts to address anaemia (Fishman et al, 2000). Recent Cambodian (Giovanni et al, 2006; GTZ, unpublished) and international (Smuts et al, 2004; Zlotkin et al 2005) efficacy studies have shown that anaemia in infants and young children can be effectively treated with daily micronutrient supplementation through in-home fortification (adding micronutrient Sprinkles or crushed tablets to complementary foods). Other studies tested the effectiveness (or program efficacy) of Sprinkles distribution along with distribution of donated food commodities (Menon et al, 2006) and daily 2 month administration versus flexible 3 and 4 month administration of Sprinkles (Haseen et al, 2005). There are issues of negative interactions between iron and zinc when the two nutrients are given together in a supplement form (Lonnerdal, 2004), but some evidence shows that when the nutrients are given together with food this interaction is decreased.

The Cambodian population is still 86% rural (CIPS, 2004). Breastfeeding of infants is almost universal (97%, CDHS 2005) but 60% of mothers exclusively breastfeed until 6 months of age and only 49% are fed with continued breastfeeding, adequate frequency and variety (CDHS 2005). Research shows timely initiation, quality, quantity, consistency, frequency of feeds, hygiene and active feeding practices can be greatly improved (GTZ-FSNPSP, 2004; HKI, 1997). A recent review of child nutrition programs in Cambodia concluded that few organizations adequately address complementary feeding (HKI & BASICS II, 2004). Nutrient poor foods such as watery rice porridge (borbor) are commonly given to children of all ages often starting too early or too late. This replaces nutrient rich breastmilk and exposes infants to pathogens leading to the dangerous interaction of infection and malnutrition. New studies have shown that interventions encouraging optimal breastfeeding and complementary feeding practices increase calorie consumption, improve nutrient intake, and reduce stunting (Penny et al, 2005; Hotz & Gibson, 2005). Based on the information above, Cambodia is ready to move towards development of effective policy to combat anaemia and other micronutrient deficiencies in infants and young children.

2.2 Rationale

We know that under research conditions, Sprinkles given to Cambodian infants from 6 months of age for 12 months are effective at reducing anaemia (41.5% point decrease); we now want to see if this same effect can be replicated by delivering Sprinkles and infant and young child feeding education through existing government health services. From around 4-6 months of age the prevalence of under-weight and stunting and anaemia increases considerably, plateauing at a high level around 23 months of age (CDHS 2005), so we want to see if education and Sprinkles given during this critical growth period can reverse the trends and help these children grow normally with reduced anaemia. The project is a cluster randomized controlled community-based study to evaluate the effectiveness of providing normal infants 6-11 months of age with daily micronutrient powders (called “Sprinkles”), in addition to nutrition education targeted to caregivers to improve infant and young child feeding (IYCF) practices on anaemia, vitamin A and zinc deficiencies, and growth.

Hypotheses

In this cluster-randomized study we will test two hypotheses:

- Daily in-home fortification of infant’s diets from age 6 through 11 months, delivered with IYCF education will reduce the prevalence of anaemia by at least 26 percentage points, while also reducing deficiencies of iron, zinc, vitamin A, and stunting and wasting at 12 months of age compared to infants whose mothers receive IYCF education alone.
- Daily in-home fortification of infant’s diets from age 6 through 11 months, delivered with IYCF education from 6-18 months of age will produce a sustained reduction of anaemia prevalence by at least 26 percentage points, while also reducing deficiencies of iron, zinc, vitamin A, and stunting and wasting at 18 months of age compared to infants whose mothers receive IYCF education alone.

2.3 Potential Risks and Benefits

2.3.1 Potential Risks

Sprinkles – possibly dark coloured stools; constipation or diarrhoea

Pain during and bruising after blood tests.

2.3.2 Known Potential Benefits

Decrease anaemia and iron, zinc and Vitamin A deficiencies. Improve growth.

3 OBJECTIVES

Overall Goal:

To inform policy on effective and feasible interventions to combat anaemia and other micronutrient deficiencies and promote growth in children in the first 18 months of life. The testing of our hypotheses will allow Cambodian policy makers to determine the value of adding micronutrient powders to existing IYCF education interventions, in terms of reducing in anaemia, deficiencies of iron, zinc and vitamin A and improving growth.

Primary Objective

To assess the effectiveness of 6 months of in-home fortification with Sprinkles starting at six months of age accompanied with IYCF education compared to IYCF education alone to reduce anaemia, iron, zinc, and vitamin A deficiencies and improve growth at 12 months and to sustain these changes at 18 months of age.

Secondary Objective

To test the feasibility of distributing Sprinkles and IYCF education through the government health system down to community level.

Outcomes

Primary Outcomes

The study will determine the extent to which:

- Anaemia, iron deficiency, zinc deficiency, vitamin A deficiency, stunting and wasting are decreased at age 12 months and reductions are sustained at 18 months compared to IYCF education alone.

Secondary Outcomes

The study will assess the implementation feasibility through measure of the following indicators:

- Commodity distribution of in-home fortificant Sprinkles
- Promotion and instruction on use of Sprinkles
- Use and adherence of Sprinkles in targeted children
- IYCF behaviour change communication activities

Other related study activities are:

- Strengthening the capacity of the National Nutrition Program, the National Institute of Public Health (NIPH) including the laboratory, the Provincial Health Department (PHD), Operational District (OD), health centre staff and Village Health Support Groups (VHSGs) to implement and monitor effective interventions.

4 STUDY DESIGN

Type of study:

Cluster randomized controlled unblinded implementation study in one Operational Health District with 20 Health Centres. Each health centre catchment area = one cluster. This is an implementation study to also test the feasibility of delivering infant and young child feeding education and Sprinkles through the existing Government health system

Size of study

The intervention arm consists of 10 clusters (health centre catchment areas) and the active control also of 10 clusters. There will be approximately 3600 children in the main sample of the study. Six hundred healthy children a month will be enrolled over six months. The sub-sample size for blood, anthropometry and in-depth data collection plus 20% extra for drop-outs makes a total sub-sample size of 1200. There are about 600 children reaching 6 months of age every month in the population. The sub-sample sizes were calculated to determine a significant difference between intervention and non-intervention for the outcomes of the prevalence of anaemia and vitamin A deficiency, the concentration of haemoglobin, serum zinc, serum ferritin and z-scores of stunting and wasting (Kirkwood and Sterne, 1999).

Intervention and sampling

Those infants in the intervention areas will receive sprinkles and nutrition education and those in the non-intervention will receive only nutrition education. Blood samples and other data will be collected a sample of children at age 6, 12, and 18 months.

Expected Duration of subject participation:

Six months for intervention for all subjects. 12 months for intervention and follow-up for the sub-sample population.

Randomisation (including rationale)

In order to assess the effectiveness of the interventions conducted through health centres, randomization will be done at the health centre level. The health centres within one operational district will be randomized, half to the intervention (IYCF education + in-home micronutrient fortification) and half to non-intervention (IYCF education alone). Randomization is done at cluster level as the tested intervention is delivered through the health centres. Randomizing by health centre will also decrease contamination or group crossover. Health centres were also stratified according to those Health centre catchment areas that had active Mother Support Groups (Baby Friendly Community Initiative) for assisting new mothers in infant and young child feeding education and support. All eligible infants will be enrolled (approximately 3600 infants), but only a sub-sample whose caregivers have given consent, will have blood testing, anthropometry and in-depth data collection.

The sub-sample will be randomly selected from each monthly cohort of eligible infants. The study will not collect in-depth information on all enrolled children. To select this sub-sample, first a listing of all children under six months of age in the OD will be completed by trained village health support group members. This listing will occur in the month prior to launch of the study. From this listing, the infants will be sorted by age in months and a random sub-sample of about 200 children per age month group will be selected. When the study begins, the first group of infants reaching the age of 6 months will join the study, the following month the second group of children reaching the age of 6 months will join. This process will continue until the sixth month of study implementation when all of the target population will have been included.

All subjects will participate in the study for 6 months from aged 6 months to 12 months. The sub-sample subjects will then be followed up after the completion of the intervention at aged 18 months.

Methods for data collection:

- Questionnaires for basic socio-economic data, pregnancy and birth history, feeding of infant history (breastfeeding and complementary feeding), immunization history, literacy level of mother;
- Blood tests including Complete Blood Count (CBC) including blood film examination, serum Zinc concentration, serum Ferritin concentration, Retinol Binding Protein (RBP) concentration (a measure of Vitamin A status), Serum Transferrin Receptor (StFr) concentration (measure of iron status), C-Reactive Protein (CRP) concentration (a measure of acute infection), and alpha-1 glycoprotein (AGP) concentration (a measure of chronic infection).
- Anthropometry including weight, length, mid upper arm circumference (MUAC) and head circumference.
- Spot checks to mothers to see if they have received and are putting into practice IYCF education; use of Sprinkles
- Collection of full and empty Sprinkles packaging to check for adherence
- Regular reported monitoring by the study team and provincial and operational health district staff

Centralisation of data management and laboratory analysis

All data from the questionnaire and anthropometry will be managed by Helen Keller International, Cambodia staff with double entry and data quality checks performed each month after the one week of data collection each month by trained staff.

The entered data will be stored on CD roms and stored in a locked cabinet in 2 locations: A2Z office and the WHO office.

The complete blood counts will be analysed at the National Institute of Public Health Laboratory, Cambodia by trained laboratory staff on a daily basis during the one week per month of data & blood collection. The following week, the results and blood films will be reviewed by a consultant Laboratory Scientist who will enter the data into Excel spread sheets. The data will be stored on CD roms and kept in a locked cabinet at WHO.

The thalassemia screening will be analysed at the National Paediatric Hospital, Cambodia by trained laboratory staff during Round 3 of data collection (follow-up when children are aged 18 months). The results will be reviewed by a consultant Laboratory Scientist who will enter the data into Excel spread sheets. The data will be stored on CD roms and kept in a locked cabinet at WHO.

Serum samples for biochemical analysis and zinc analysis will be stored in micro-containers, frozen at -70 Celcius ready for transportation to Germany and New Zealand respectively at the end of Round 3 data (August 2009) and blood collection and at the end of Round 4 data and blood collection (February 2010).

Primary Outcomes

The study will determine the extent to which:

- Anaemia, iron deficiency, zinc deficiency, vitamin A deficiency, stunting and wasting are decreased at age 12 months and reductions are sustained at 18 months compared to IYCF education alone.

Secondary Outcomes

The study will assess the implementation feasibility through measure of the following indicators:

- Commodity distribution of in-home fortificant Sprinkles
- Promotion and instruction on use of Sprinkles
- Use and adherence of Sprinkles in targeted children
- IYCF behaviour change communication activities

Interim analysis plans:

- Baseline results for both control and intervention groups, children aged 6 months (sub-sample)
- Round 2 (after 6 months of intervention/active control, children aged 12 months) results comparing control and intervention groups
- Round 3 (follow up 6 months after Round 2, children aged 18 months) results comparing control and intervention groups

Using the following parameters: Blood tests: anaemia; iron deficiency and iron deficiency anaemia (taking into account acute and chronic infection); zinc deficiency; vitamin A deficiency; selenium deficiency; haemoglobinopathies

Anthropometry: weight, length, mid upper arm circumference; head circumference; Body Mass Index (BMI)

Structure for oversight:

Study Steering Committee consisting of all collaborating institutions and agencies. Monthly reporting to A2Z, USAID Washington (main donor). Supervision of lead investigator by Otago University.

Test agent and specifics of administration

Sprinkles stored and packed into named bags at the Helen Keller International office which are delivered to the Operational Health District by HKI and NNP staff every month at the regular monthly meeting with Health Centre chiefs. Health Centre chiefs take the named Sprinkles bags back to their Health Centre for delivery to each mother/infant at their home by their Village Health Workers the following day. Empty and un-used Sprinkles sachets are collected at this time by the VHW's and returned to the HC for documenting adherence. Adherence reports are collected by the data collection team each month during the data collection week (first week of each month) and sent to HKI for computer entry.

5 STUDY POPULATION

The study population consists of all infants turning 6 months old during the baseline study period March – August 2008 living in Svay Rieng Operational Health District. All eligible children were listed before the study commences according to information from the Health Centres (Immunization records, birth records), Village Chiefs and Village Health Support Groups. This study has a rolling monthly recruitment for 6 months with each new cohort being enrolled during the first week of each month. Therefore a total of six cohorts will be enrolled to achieve the expected sample size. A sub-sample will be selected randomly from each cohort for detailed data collection (questionnaire; anthropometry and blood testing)

Sample Size Details

There will be 3600 children in the main sample of the study. Six hundred children a month will be enrolled over six months. The sub-sample size for blood, anthropometry and in-depth data collection plus 20% extra for drop-outs makes a total sub-sample size of 1200. Ministry of Planning population projections (2007) state in Svay Rieng operational district there is a total population of 292,000. From this population, there are about 600 children reaching 6 months of age every month. Only one third of eligible children need to participate in the study to fulfil the planned sub-sample size.

The sub-sample size of 1200 was calculated as a cluster randomized study accounting for the clustering of outcomes within the health centre operation areas with the intraclass correlation coefficient and increasing the estimated sample size by the variance inflation factor (Ukoumunne et al, 1999). The sub-sample sizes were calculated to determine a significant difference between intervention and non-intervention for the outcomes of the prevalence of anaemia and vitamin A deficiency, the concentration of haemoglobin, serum zinc, serum ferritin and z-scores of stunting and wasting with a power of 90% and alpha of 0.05 (Kirkwood and Sterne, 1999).

Strategies for subject recruitment and retention

All children who will turn 6 months of age during the study rolling recruitment period living in the 352 villages of Svay Rieng Operational Health District will be listed using Health Centre immunization records, Village Chief and Village Health Support Group information from each village. All these children will be enrolled in the study.

The sub-sample will receive an identification card with a study number. Transport costs will be paid to enable to mother/child to attend the Health Centre on the day of data collection. In addition, during the 2nd round (children aged 12 mo) mother's will receive a sarong.

The list of children will be generated for each cohort and given to the Health Centre chief each month, who will give it to his Village Health Support Group for each village in the HC catchment area. VHSG's will then go to each mother/child to invite them to participate. VHSG's will return

to these same mothers/children for the 2nd (children aged 12mo) and 3rd round (children aged 18 mo) to invite them to continue their participation with detailed questionnaire, anthropometry and blood testing.

OD and HC staff and VHSG's will have training before the study commences and refresher training 2 times during the study. Monthly meetings will be held between study staff, OD and HC chiefs who will in turn have monthly meetings with VHSG's.

5.1 Subject Inclusion Criteria

- Informed consent obtained and signed;
- aged 6-7 months at time of enrolment;
- absence of severe anaemia (<7g/dL) or severe acute malnutrition (<-3 Z-scores WHZ) or severe disability;
- normally living in Svay Rieng Operational Health District;
- willing to comply with the study procedures for the length of the study

5.2 Subject Exclusion Criteria

- Children aged 6 months of age who are eligible for the study but have severe anaemia (<7 g/dL) or severe acute malnutrition (<-3 Z-score WHZ) or are severely disabled.

6 ENROLLMENT/ RANDOMIZATION/MASKING PROCEDURES

The health centres within one operational district will be randomized, half to the intervention (IYCF education + in-home micronutrient fortification) and half to active control (IYCF education alone). According to sub-sample size calculations only about 1200 children are needed to test the hypotheses. To select this sub-sample, first a listing of all children under six months of age in the OD will be completed by trained village health support group members. This listing will occur in the month prior to launch of the study. From this listing, the infants will be sorted by age in months and a random sub-sample of about 200 children per age month group will be selected. When the study begins, the first group of infants reaching the age of 6 months will join the study, the following month the second group of children reaching the age of 6 months will join. This process will continue until the sixth month of study implementation when all target population will have been included.

Sub-sample for blood testing, anthropometry & survey questionnaire. Listing of all eligible infants will be made at village level. Children will be randomly selected from a list by a statistician not involved in the day to day running of the study, by month blocks. The random allocation of Health Centres will be done by a permuted block design. This was to ensure 10 health centres in each arm. The randomization was also stratified to account for Health Centre catchment villages where the Baby Friendly Community Initiative (infant and young child feeding counseling to mothers) was already implemented or being planned for implementation during the intervention phase.

An expected 200 children per cohort will be invited to participate in the sub-sample data collection, however, if not enough children are being recruited, up to 250 children per cohort may be invited using the same random selection process.

Interim analysis will occur after the data and blood collection of Round 3 (follow-up at aged 18 months) for all parameters. This is not a blinded study, however the data have been entered in a blinded manner and will not be analyzed according to intervention and control groups until the time specified.

Full analysis will occur at the end of Round 3.

7 STUDY PROCEDURES/EVALUATIONS

7.1 Clinical Evaluations

Sub-sample will undergo data collection by questionnaire, anthropometric measurements and blood collection at baseline/Round 1 (aged 6 months); Round 2 (aged 12 months), Round 3 (aged 18 months)

At baseline data will be collected on:

Medical history: the pregnancy and birth history of the child; basic socio-economic information on the family including literacy of the mother; breastfeeding and infant feeding practices; immunizations

Medications history: the mother will be asked if she took iron/folate tablets during and after the pregnancy with the study child

Physical exam: no clinical physical exam will occur apart from anthropometric measurements including: height; weight; head circumference and mid upper arm circumference.

For Rounds 2, 3 and 4 data will be collected on:

Breastfeeding and infant and young child feeding practices and immunizations

Counseling procedures: all mothers will receive counseling on recommended infant and young child feeding practices by HC staff and VHSG's either in group counseling sessions or individual counseling sessions at home. Mothers of children receiving Sprinkles will also receive counseling on use of Sprinkles.

7.2 Concomitant Medications/Treatments

Vitamin A and mebendazole given according to national protocols and recorded on the child's Health Card through existing health systems.

Children who are found to be severely anaemic will be withdrawn from the study and treated with iron and multiple micronutrients. They will be followed up and referred for further treatment if indicated.

7.3 Laboratory Evaluations

7.3.1 Clinical Laboratory Evaluations

- Haematology: haemoglobin, haemocrit, WBC with differential, platelet count and a blood film on each sample (1mL EDTA anticoagulated blood)

-
- Biochemistry: Serum Ferritin concentration, Retinol Binding Protein (RBP) concentration (a measure of Vitamin A status), Serum Transferrin Receptor (StFr) concentration (measure of iron status), C-Reactive Protein (CRP) concentration (a measure of acute infection), and alpha-1 glycoprotein (AGP) concentration (a measure of chronic infection) and serum Zinc concentration (2mL in trace element free tube)
 - Haemoglobinopathy screening for alpha and beta thalasseмииs .

The blood tests will be conducted by trained experienced phlebotomists of the National Institute of Public Health Laboratories (NIPHL). They will follow standard safety protocols and deposit all bio-hazard waste into bio-waste containers for incineration at the Svay Rieng hospital or the NIPHL. The NIPHL will perform the complete blood counts and the National Paediatric Hospital will perform the screening tests for haemoglobinopathies. Dr J. Erhardt's laboratory (Germany) was selected for the five tests done together on a serum micro-sample for low cost and high quality. The zinc analysis will be run at the University of Otago, Department of Human Nutrition Laboratory at cost as this test is not yet available in Cambodia.

7.3.2 Specimen Preparation, Handling and Shipping

7.3.2.1 Instructions for Specimen Preparation, Handling and Storage

The blood will be collected into two properly labelled tubes, one with EDTA and the other trace element free and deposited immediately into a stand inside a cool box. At the end of the half day of blood collection, the bloods will be taken to the Svay Rieng Hospital lab where the trace element free tube will be spun and the serum pipetted into two labelled trace element free tubes. All blood samples will be delivered on the same day as collection to the NIPHL. The complete blood count will be run on the same day as collection. The trace element free tubes will be stored at -20 Celsius at the NIPHL until all samples are collected. One set of frozen samples will be sent to University of Otago for analysis of serum zinc concentration. The other set will be sent to Dr J. Erhardt's Laboratory in Germany for analysis of concentrations of serum ferritin, retinol binding protein, serum Transferrin Receptor (StFr) concentration, C-reactive protein and alpha-glycoprotein. Permission has only been granted by the Cambodia National Ethics Committee for Health Research to perform the tests indicated, therefore the samples will be disposed of by the respective laboratories after analysis is complete.

7.3.2.2 Specimen Shipment

The 5 tests for analysis of concentrations of serum ferritin, retinol binding protein, serum transferrin receptor sites, C-reactive protein and alpha-glycoprotein will be sent to DBS-Tech Laboratory, Kastanienweg 5, 77731 Willstaett, Germany (Dr J Erhardt) at the completion of the 3 data collection rounds after August 2009. Specimens are labelled

with special alcohol resistant labels and will be sent frozen at -70 C packed in dry ice by air transport via World Courier (or similar) shipping company.

The zinc and selenium samples, also labelled with alcohol resistant labels and frozen at -70 C will be sent on dry ice to Otago University, Dept of Human Nutrition Laboratory, Dunedin, New Zealand by air transport via World Courier shipping company.

8 STUDY SCHEDULE

- Phase 1 (9 months) Start-up (June 2007 – February 2008)
A detailed activities plan will be developed, the micronutrient packaging created and field-tested, stocks acquired, training and promotional materials developed in line with the NNP strategy, monitoring tools designed, trainings will be prepared and conducted for the staff of the Operational District (OD), health centres and community health volunteers. A listing of all infants under 6 months in the OD will be completed. Systems for study implementation will be put in place.
- Phase II (18 months) Implementation & Follow-up (March 2008 – February 2010)
Study sample will be identified and invited to participate, consent will be obtained and baseline data collected. All infants becoming 6 months of age will join each month for 6 months. This rolling recruitment will take 6 months with a new cohort entered each month. Due to the rolling recruitment and rolling intervention, data collection will occur monthly for a period of 18 months. Routine monitoring of trainings will occur. Routine monitoring of delivery of Sprinkles and nutrition education will occur monthly by study staff and OD/HC staff to VHSG's. Refresher training for OD/HC and VHSG's will occur 2 times in this period. Qualitative assessments of the distribution process will be conducted. Questions on the feasibility of the IYCF messages will be asked of selected caregivers of all children and on the feasibility of Sprinkles of caregivers who received them
- Phase III (3 months) Evaluation and Information Dissemination (September 2009 – November 2009)

Following the period of in-home fortification and 6 months of follow-up (when children are aged 18 months) interim analysis will be performed, written-up and disseminated to all stake-holders through workshops.

Then a full analysis of all results will be performed, written up and disseminated. . All evidence will be reviewed in a national workshop to produce policy recommendations.

8.1 Screening

Screening of listed children who are randomly selected as the sub-sample for data collection will occur at the enrolment time after consent has been obtained and will include: checking age at enrolment against Immunization Health Card or other birth registration details; checking for severely acute malnutrition by measuring weight and height and computing weight for length Z-scores and those children <-3 Z-score weight for length will be excluded from the study and referred or transported to an appropriate hospital for appropriate management. Checking for

severe anaemia (Hb <7 g/dL) with such children detected will be referred to an appropriate health facility for treatment and excluded from the study.

Consent will be obtained by study staff by reading out the information sheet, giving a copy of the information sheet to each mother and obtaining thumb-prints of all those consenting.

8.2 Enrollment/Baseline

All children in the intervention group will listed by the VHSGs and assigned to one village VHSG member in their village. This will facilitate distribution and monitoring of use of the in-home fortificants. All 1200 children selected for in-depth data collection will be registered and given a study ID card. Data entry systems and forms will be developed in conjunction with data processing team (HKI) and NIPHL for blood test data. Data will be collected from caregivers who refuse involvement in the study in order to determine if non-participants vary from participants. Methods will be developed to collect information on children who die or drop-out during the course of the study to ensure study safety and representability

Parents of eligible children will be invited to give verbal consent at the time of listing. Screening of listed children who are randomly selected as the sub-sample for data collection will occur at the enrolment time after written (thumb-print) consent has been obtained and will include: checking age at enrolment against Immunization Health Card or other birth registration details; checking for severely acute malnutrition by measuring weight and height and computing weight for length Z-scores and those children <-3 Z-score weight for length will be excluded from the study and referred or transported to an appropriate hospital for appropriate management. Checking for severe anaemia (Hb <7 g/dL) with such children detected will be referred to an appropriate health facility for treatment and excluded from the study.

8.3 Follow-up

Children found to be severely anaemic will be notified to the Health Centre and treated according to national protocols (iron and multiple micronutrients as indicated). Those children not responding will be invited to be screened for haemoglobinopathies at the National Paediatric Hospital and further follow up arranged at the Svay Rieng Provincial Referral Hospital. Severely acutely malnourished children will be referred to Svay Rieng Provincial Referral Hospital who are trained in the management of severe acute malnutrition for inpatient management according to WHO protocols

Any deaths of children aged between 6mo and 12mo, and 12mo and 18 mo will be reported by the Health Centres to the study staff. These families will be followed up and a verbal autopsy performed to try to ascertain the cause of death.

Any adverse events reported will be followed up by a home visit to the family concerned by the Health Centre, Operational District and/or study staff. Detailed questioning to ascertain the

nature of the adverse event and likelihood or otherwise of it being related to Sprinkles will occur. The mother/caregivers will be counseled and referred to the Health Centre or Referral Hospital if needed. Any serious adverse event will be reported immediately to the principal investigators and follow up made as necessary.

8.4 Final Study Visit

Final study visit will occur 18 months after each cohort was enrolled (Sept 2009 to February 2010) as each cohort completes the follow-up period 12 months after the intervention was completed.

Any adverse events after this period will be notified by the Health Centres to the Operational Health District chief to the study staff and appropriate action taken.

8.5 Early Termination Visit

Children and mothers of children from the sub-sample for detailed data collection who refuse blood tests will still be encouraged to participate in the questionnaire and anthropometry if they are willing. Children and mothers who refused blood or data collection at one round will still be invited and encouraged to participate in the following round.

Subjects may withdraw voluntarily from participation in the study at any time. Subjects may also withdraw voluntarily from receiving the study intervention for any reason. Due to the design of this study this will only be known at the time of data and blood collection so no further evaluations will take place although every effort will be made to encourage the subjects to remain in the study as above. In addition, at the time and day scheduled for data and blood collection, if any subjects do not attend, they will be followed up by the VHSG or Health Centre staff contacting them by telephone or in person to encourage them to attend.

Usual care at the Health Centre will continue.

9 STUDY INTERVENTION/INVESTIGATIONAL PRODUCT

9.1 Study Product Acquisition

9.1.1 Formulation, Packaging and Labeling

The project will use Sprinkles, a micronutrient powder-like formulation as the in-home fortificant. Sprinkles are microencapsulated micronutrients in powder form that can be sprinkled onto complementary food without changing the colour or taste of the food. Sprinkles are packaged in single-dose sachets to ensure that the correct amounts of micronutrients are given.

Sprinkles formulation:

• Iron (microencapsulated ferrous fumarate)	12.5mg
• Zinc (Zinc gluconate)	10mg
• Vitamin A (retinol acetate)	300µg
• Iodine	90µg
• Vitamin B 1	0.5mg
• Vitamin B 2	0.5mg
• Vitamin B 6	0.5mg
• Vitamin B 12	0.9µg
• Niacin	6mg
• Folate (folic acid)	160µg
• Vitamin C (ascorbic acid)	30mg
• Copper	0.3mg
• Vitamin D	5µg
• Vitamin E	6 IU

Sprinkles will be packaged in single dose sachets with a metalized polyethylene film (MET-PET) that provides adequate water and oxygen protection.

9.2 Product Storage and Stability

Sprinkles should be stored in dry cool conditions. Testing for stability in hot humid climates has shown a shelf life of up to 24 months (unclear if this was field conditions – need to check). The Sprinkles will be given monthly to the mothers in a plastic zip-lock bag and the mothers instructed to keep the bags tightly zip-locked and stored in the coolest, driest area of their house.

9.3 Preparation, Administration and Dosage of Study Intervention/Investigational Product

Use of Sprinkles:

- a. Wash hands and utensils with soap
- b. Put appropriate amount of complementary food in the bowl
- c. Tear to open the top of the sprinkles packet/sachet
- d. Pour the contents of sprinkles into the bowl of complementary food. Mix the powder well with complementary food. You can do this with any food that young child (aged 6-11 months) eats. The food should NOT be hot when you add the sprinkles to it. It should be cool enough to mix the sprinkles for the child to eat. Each child should receive only one package per day. Do not give more than one package per day but you may divide the dose to give over two or more meals.
- e. After mixing it well, mixed food is ready to feed child
- f. All of mixed food should be eaten by child
- g. Don't share mixed food with any member of family

9.4 Accountability Procedures for the Study Intervention/Investigational Product(s)

This project will use the existing public health system to deliver the in-home fortificants for free to caregivers of children starting at 6m of age in the intervention area.

The VHSGs in each village will receive training from the HC staff on micronutrients and optimal IYCF practices. The Sprinkles will be stored at the Helen Keller International office in Phnom Penh. Every month the named packs of Sprinkles sachets will be delivered to the Operational Health District at the time of the OD/Health Centre chief's monthly meeting. The HC chiefs will take all the Sprinkles for children in their catchment area to their HC for the next days monthly meeting with the Village Health Support Groups. HC staff will be responsible for distributing the Sprinkles to the VHSGs at their usual VHSG monthly support meeting. The VHSGs will then distribute the Sprinkles to the infants with each care giver being given a monthly supply of micronutrient supplement with clear instructions on how and when to administer them. Nutrition, in particular, IYCF education will be given by HC staff and VHSGs and Mother's Groups to caregivers as per their usual practice to reinforce and support breastfeeding and good complementary feeding.

If needed, further supplies of Sprinkles will be taken from the central storage place (HKI office) monthly and stored at Health Centres.

Overall 600,000 sachets were procured. This was well over what was required for this study but was the minimum shipment possible. The study steering committee along with World Health Organization (who purchased the Sprinkles) will decide what happens to the left over Sprinkles. They will not be used during the study period for any other children in the study location or near the study location. Other NGO's may be able to use the Sprinkles if they are implementing similar programmes.

9.5 Assessment of Subject Adherence to Study Intervention/Investigational Product

Spot checks to mothers individually or as small groups will occur regularly by study staff and OD/Health Centre staff to check on compliance to IYCF practices and consumption of Sprinkles. In addition, all children receiving Sprinkles will be advised to keep all empty and full sachets. These will be collected monthly by Health Centre staff and the numbers of consumed and remaining sachets documented per child. This data will be collated monthly in order to give ongoing feedback to Health Centre staff and VHSG's by study staff in order to improve or maintain compliance as the study goes along.

10 ASSESSMENT OF SCIENTIFIC OBJECTIVES (E.G., SAFETY OR IMMUNOGENICITY OR EFFICACY)

10.1 Specification of the Appropriate Outcome Measures

10.1.1 Primary Outcome Measures

Anaemia: Haemoglobin concentration

10.1.2 Secondary Outcome Measures

Iron deficiency: Iron (Serum ferritin and Serum Transferrin Receptor (StFr) concentration)

Vitamin A deficiency: Serum Retinol Binding Protein concentration

Zinc deficiency: Serum Zinc concentration

Anthropometry: weight; length; head circumference; mid upper arm circumference

Haemoglobinopathy screen: testing for alpha and beta thalassemia traits

10.2 Methods and Timing for Assessing, Recording, and Analyzing Appropriate Outcome Measures

Blood tests will all be taken under the following conditions: non fasting, morning, children sitting, no tourniquet used or if used then for less than 1 minute.

Anthropometry will be performed using standard procedures (see Appendix B; Standard Operating Procedures)

10.3 Modification and Discontinuation of Study Intervention/Investigational Product for a Participant

10.3.1 Dose/Schedule Modifications for a Subject

For all children the daily dose of Sprinkles (1 sachet) may be divided and given 2-3 times with food during the day as long as the whole sachet is consumed in one day if the child does not tolerate for any reason the sachet being given at one time.

10.3.2 Criteria for Discontinuation of Study Intervention/Product for Withdrawal of a Subject (or a Cohort)

A study subject will be discontinued from further investigational product if:

- Any clinical adverse event, laboratory abnormality, intercurrent illness, other medical condition or situation occurs such that continued participation in the study would not be in the best interest of the subject.
- Development of any exclusion criteria may be cause for discontinuation.

Note that subject will continue to be followed with subject's permission if study intervention/product is discontinued.

11 ASSESSMENT OF SAFETY

There are no safety issues expected. Adverse events will be reported and followed up by personal interview with the mother/caregiver.

11.1 Specification of Safety Parameters

N/A.

11.2 Methods and Timing for Assessing, Recording, and Analyzing Safety Parameters

11.2.1 Adverse Events, Serious Adverse Events

- *Any adverse events will be reported by the VHSG, Health Centre or study staff. A visit to the mother/child will occur for elicit full details and to determine if the episode is indeed an adverse event related to Sprinkles or a concomitant event. Verbal and written reports will be given to the principal investigators and discussed at the monthly steering committee meeting.*
- *Unsolicited events thought to be related to Sprinkles will be captured in the same way as described above.*
- *Any adverse event will be reported and checked as soon as the study staff are alerted to a problem. If the study staff are not available for further investigation, the Health Centre or Operational District staff will check the child.*

11.2.1.1 Definition of Adverse Event

An AE is any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and that does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not related to the medicinal (investigational) product. Include time period of collection.

11.2.1.2 Serious Adverse Event

A Serious Adverse Event (SAE) is any adverse event/experience occurring at any study drug dose that results in any of the following outcomes:

- Death

-
- Life-threatening (subject at immediate risk of death)
 - Requires in-patient hospitalization or prolongation of existing hospitalization
 - Results in congenital anomaly/birth defect
 - Results in a persistent or significant disability or incapacity
 - Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse event/experience when, based upon appropriate medical judgment, they may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition

11.3 Reporting Procedures

Any adverse event should be reported by the VHSG to the HC chief who in turn will notify the OD chief and study staff who will notify the lead investigators and study steering committee. Follow up will be organized by the OD chief in conjunction with the HC chief and study staff.

Adverse events include:

Serious Adverse Events: none expected

Other adverse events: None expected although minor symptoms of diarrhoea or constipation may occur.

11.3.1 Serious Adverse Event Detection and Reporting

For those events meeting the previously described definition of Serious Adverse Events, the completion of a Serious Adverse Event report form is required. This will be sent to the principal investigators and discussed at the monthly steering committee meeting.

11.3.2 Procedures to be Followed in the Event of Abnormal Laboratory Test Values or Abnormal Clinical Findings

At baseline (aged 6 months) and endline (12 months), and follow up at 18 months and 24 months any children with severe anaemia and severe malnutrition will notified to the principal investigators.

Children with severe anaemia (Hb <7g/dL) will be excluded from the study; given iron/folate supplementation according to national standard treatment guidelines and followed up. Children not responding to iron/folate supplementation will be invited to be screened for haemoglobinopathies at the National Paediatric Hospital in Phnom Penh with any treatment and follow up managed by the hospital staff.

Children with severe acute malnutrition (weight for length < 3 Z-score) will be excluded from the study and will be invited to be admitted to Svay Rieng Provincial Referral Hospital for further management and follow up.

11.3.3 Type and Duration of the Follow-up of Subjects After Adverse Events

Any child with an adverse event will be followed up by their VHSG and Health Centre staff as needed.

11.4 Halting Rules

There are no halting rules.

12 STATISTICAL CONSIDERATIONS

12.1 Overview and Study Objectives

Overall Goal:

To inform policy on effective and feasible interventions to combat anaemia and other micronutrient deficiencies and promote growth in children in the first 18 months of life.

Primary Objective

To assess the effectiveness of 6 months of in-home fortification with Sprinkles starting at six months of age accompanied with IYCF education compared to IYCF education alone to reduce anaemia, iron, zinc, and vitamin A deficiencies and improve growth at 12 months and to sustain these changes at 18 months of age.

Secondary Objective

To test the feasibility of distributing Sprinkles and IYCF education through the government health system down to community level.

12.2 Study Population

Intervention (IYCF education + Sprinkles) and active control (IYCF education alone) groups will be children living in Svay Rieng Operational Health District who turn 6 months of age during the rolling recruitment period of the study (March 2008 to August 2008).

Rationale for active control for ethical reasons of wanting all children to receive adequate infant and young child feeding during this critical growth period.

12.3 Study Design

Cluster randomized non-blinded implementation study. One operational health district with 20 Health Centres, where each health centre is a cluster. 10 clusters receiving the intervention and 10 clusters as active controls. Rolling recruitment of 6 month old infants over a period of 6 months to reflect real field programmatic conditions for distribution of IYCF education and Sprinkles at 6 months of age after the completion of 6 months of exclusive breastfeeding.

12.4 Study Outcome Measures

Primary Outcomes

The study will determine the extent to which:

- Anaemia is decreased at age 12 months and reductions are sustained at 18 months compared to IYCF education alone
- Also; Iron deficiency, zinc deficiency, vitamin A deficiency, stunting and wasting are decreased at age 12 months and reductions are sustained at 18 months compared to IYCF education alone

Secondary Outcomes

The study will assess the implementation feasibility through measure of the following indicators:

- Commodity distribution of in-home fortificant Sprinkles
- Promotion and instruction on use of Sprinkles
- Use and adherence of Sprinkles in targeted children
- IYCF behaviour change communication activities

12.5 Study Hypotheses

Hypotheses

1. Daily in-home fortification of infant's diets from age 6 through 11 months, delivered with IYCF education will reduce the prevalence of anaemia by at least 26 percentage points, while also reducing deficiencies of iron, zinc, vitamin A, and stunting and wasting at 12 months of age compared to infants whose mothers receive IYCF education alone.
2. Daily in-home fortification of infant's diets from age 6 through 11 months, delivered with IYCF education from 6-18 months of age will produce a sustained reduction of anaemia prevalence by at least 26 percentage points, while also reducing deficiencies of iron, zinc, vitamin A, and stunting and wasting at 18 months of age compared to infants whose mothers receive IYCF education alone.

12.6 Sample Size Considerations

There will be 3600 children in the main sample of the study. Six hundred children a month will be enrolled over six months. The sub-sample size for blood, anthropometry and in-depth data collection plus 20% extra for drop-outs makes a total sub-sample size of 1200. Ministry of Planning population projections (2007) state in Svay Rieng operational district there is a total population of 292,000. From this population, there are about 600 children reaching 6 months of age every month. Only one third of eligible children need to participate in the study to fulfil the planned sub-sample size. The rolling enrolment of subjects is designed to reflect the real program conditions where new children are enrolled each month.

The sub-sample size was calculated as a cluster randomized study accounting for the clustering of outcomes within the health centre operation areas with the intraclass correlation coefficient and increasing the estimated sample size by the variance inflation factor (Ukoumunne et al, 1999). The sub-sample sizes were calculated to determine a significant difference between intervention and non-intervention for the outcomes of the prevalence of anaemia and vitamin A deficiency, the concentration of haemoglobin, serum zinc, serum ferritin and z-scores of stunting and wasting with a power of 90% and alpha of 0.05 (Kirkwood and Sterne, 1999).

The sub-sample size of 1200 children (1000 plus 20% for drop-outs) divided into the intervention and non-intervention reference group is sufficient to show a statistically significant difference of 26 percentage points or more for anaemia prevalence (Hb concentration of less than 110 g/L). For vitamin A deficiency, the sub-sample will identify a 19 percentage point decrease or more (vitamin A deficiency is considered as serum retinol <0.7 µmol/L). Concerning micronutrient concentrations, the sub-sample size will identify a statistically significant difference of 7 g/L for Hb (0.6 SD), 1.15 µg/L of serum ferritin (0.4 SD) and 0.9 µg/L of serum zinc (0.4 SD). As children grow, nutrition stores decline and demands for growth increase and stunting and wasting increase. The intervention intends to prevent malnutrition. The sub-sample size will have the power to show a significant difference of 0.4 SD of Height for Age and Weight for Height Z scores.

12.7 Participant Enrollment and Follow-Up

Rolling recruitment into the study over 6 months with an estimated 600 children per month. 200-250 of these will be invited to participate as the sub-sample for detailed data collection including questionnaire, anthropometry and blood testing. 20 Health Centre catchment areas with an expected 30 children per Health Centre per month enrolled and 10 per Health Centre per month for sub-sample data collection

Expected total of approximately 3600 children with 1200 in sub-sample by the end of the rolling recruitment period and for the remainder of the study intervention (6 months) and follow-up (6 months after completion of the intervention). 20 Health Centre catchment areas with an expected 180 children per Health Centre enrolled and 60 in the sub-sample.

Expected overall 20% drop out rate of sub-sample leaving 960 by the end of the study.

12.8 Planned Interim Analyses

Planned interim analyses will occur after Round 3 of data collection.

12.8.1 Safety Review

It is not anticipated to have any reason to halt this study.

12.9 Final Analysis Plan

Full analyses will be performed at the end of the study after the final round of data and blood collection. The analyses will take into account the effects of randomization by cluster. Children excluded from the study will be excluded from the final analysis.

With Round 2 data (children at 12 months of age) comparisons will be made between blood data, anthropometry and IYCF data between intervention and non-intervention groups to determine if the project had a significant effect on anaemia, micronutrient deficiencies, body mass index, stunting and wasting Z-scores (calculated according to the new WHO child growth standards) and IYCF knowledge and practices.

With follow-up data (children at 18 months of age) comparisons will be made using the blood data, anthropometry and IYCF data between intervention and non-intervention groups to determine if the addition of Sprinkles made a significant lasting effect to prevent on anaemia and micronutrient deficiencies during the most vulnerable period of growth in childhood. Body mass index, stunting and wasting Z-scores will also be analyzed to determine if the project intervention prevented stunting and averted wasting during this critical time period. IYCF knowledge and practices will be analyzed to determine if there were any lasting differences between groups

13 SOURCE DOCUMENTS AND ACCESS TO SOURCE DATA/DOCUMENTS

Source documents will be held in 3 locations: World Health Organization, Cambodia office; A2Z Cambodia office and Helen Keller International, Cambodia office.

The Principle Investigators will have access to the records, although no records will be analyzed until the Round 3 data collection is completed. Helen Keller International staff involved in data entry and monitoring will have access to questionnaire data and adherence data but no analysis will be performed until Round 3 data collection is completed. Robyn Devenish (Laboratory Scientist) will have access to complete blood count and blood film records but no analysis will be done until the Round 3 data collection is completed.

14 QUALITY CONTROL AND QUALITY ASSURANCE

This study will be conducted at a single site with 20 clusters (Health Centre catchment areas).

Standard Operating Procedures were developed for

- data collection: questionnaire/interviews; anthropometry; blood collection
- Blood specimen management: labelling; centrifuging; separating serum & transferring to micro-containers; freezing of serum samples; transport of blood specimens to National Institute of Public Health Laboratory.
- Sprinkles storage, distribution channels and adherence monitoring
- Monitoring and spot checks of Sprinkles and IYCF education at community and household level

Training in the study protocol & systems, Infant and Young Child Feeding Education and Sprinkles of Provincial Health Department and Operational Health District staff will be conducted by the study team led by and with National Nutrition Program staff. NNP staff and study staff along with PHD and OD staff will train the Health Centre staff who will then train the Village Health Support Groups (VHSG's). Refresher training will be held at least twice during the 12 months of intervention.

Data collectors will be trained by study and National Nutrition Programme staff in questionnaire technique and anthropometry with continuous quality checks by spot checks and computer generated quality checks on questionnaire data and anthropometric measurements. Feedback will be given monthly by the PI. Refresher training will be held at least once during the 18 months of data collection. Any new staff will have individual on the job training and close supervision by study staff.

Blood collectors will be trained by experienced National Institute of Public Health Laboratory staff and the PI and quality checks maintained by spot checks on technique by PI and study staff and feedback on labeling errors given monthly.

Following written standard operating procedures, the monitors will verify that the clinical trial is conducted and data are generated, documented (recorded), and reported in compliance with the protocol, and the applicable regulatory requirements. Reports will be submitted to the study steering committee on monitoring activities.

The investigational site will provide direct access to all trial related sites, source data/documents, and reports for the purpose of monitoring and auditing by the sponsor, and inspection by local and regulatory authorities.

The study steering committee will implement quality control procedures beginning with the data entry system and generate data quality control checks that will be run on the database. Any missing data or data anomalies will be communicated to the site(s) for clarification/resolution.

15 ETHICS/PROTECTION OF HUMAN SUBJECTS

15.1 Declaration of Helsinki

"The investigator will ensure that this study is conducted in full conformity with the current revision of the Declaration of Helsinki, or with the International Conference for Harmonisation Good Clinical Practice (ICH-GCP) regulations and guidelines, whichever affords the greater protection to the subject."

15.2 Institutional Review Board

The study is approved by the National Ethics Committee for Health Research, Ministry of Health, Cambodia and the Human Ethics Committee, Academic Committees, University of Otago, Dunedin, New Zealand.

15.3 Informed Consent Process

Informed consent is a process that is initiated prior to the individual's agreeing to participate in the study and continuing throughout the individual's study participation. Discussion of risks and possible benefits of this therapy will be provided to the subjects and their families. Consent forms describing in detail the study interventions/products, study procedures and risks are given to the subject and written documentation of informed consent is required prior to starting intervention/administering study product. Consent forms will be ethics board approved and the subject's caregiver will be asked to read, or listen to if unable to read, and review the document. Upon reviewing the document, the investigator will explain the research study to the subject's caregiver and answer any questions that may arise. The subjects' caregivers will thumb print the informed consent document prior to any procedures being done specifically for the study. The subjects' caregivers should have the opportunity to discuss the study with their surrogates or think about it prior to agreeing to participate. The subjects' caregivers may withdraw consent at any time throughout the course of the trial. A copy of the informed consent document will be given to the subjects for their records if requested. The rights and welfare of the subjects will be protected by emphasizing to them that the quality of their medical care will not be adversely affected if they decline to participate in this study

15.4 Subject Confidentiality

Subject confidentiality is strictly held in trust by the participating investigators, their staff, and the sponsor(s) and their agents. This confidentiality is extended to cover testing of biological samples and genetic tests in addition to the clinical information relating to participating subjects.

The study protocol, documentation, data and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party, without prior written approval of the study steering committee.

The study monitor or other authorized representatives of the sponsor may inspect all documents and records required to be maintained by the Investigator, including but not limited to, medical records (office, clinic or hospital) and pharmacy records for the subjects in this study. The clinical study site will permit access to such records.

15.5 Study Discontinuation

In the event that the study is discontinued:

- Subjects who were found to be severely anaemic or severely acutely malnourished will be managed and followed up following national protocols (malnutrition) or advice (haemoglobinopathies) from National Paediatric Hospital or Svay Rieng Provincial Referral Hospital staff as appropriate.

16 DATA HANDLING AND RECORD KEEPING

Data will be collected as per SOP's, double entered and cleaned as per standard data entry procedures. Data quality checks will be run monthly on entered data of all questionnaires and anthropometric measurements to check for accuracy, consistency, completeness and reliability. These will include quality checks by data collector allowing for monthly individual feedback as needed. Every month updated copies of all data will be burnt onto CD roms and held at the WHO Cambodia office, A2Z Cambodia office and Helen Keller International Cambodia office.

Confidentiality will be maintained at all stages.

Hard copies of all questionnaires and anthropometric measurements will be stored in labeled boxes at the Helen Keller International Cambodia office.

16.1 Data Management Responsibilities

Data collection and management for questionnaires, anthropometry and Sprinkles adherence will be the responsibility of Helen Keller International Cambodia office staff under the supervision and direction of the study steering committee and PI's.

Blood data management will be under the responsibility of National Institute of Public Health Laboratory staff and Robyn Devenish (Laboratory Scientist) under the supervision and direction of the study steering committee and PI's.

Training materials, schedules, monitoring and supervision of study implementation records including spot check reports will be under the responsibility of National Nutrition Programme staff under the supervision and direction of the study steering committee and PI's.

The PI's will be responsible for overall monitoring of the study, ensuring data collection occurs, ensuring quality is maintained and correct record keeping occurs. The PI will write monthly progress reports incorporating data collection reports from HKI and Robyn Devenish.

16.2 Data Capture Methods

Data will be captured by paper and transferred to electronic means. Data will be entered monthly.

16.3 Types of Data

Questionnaire; Anthropometric; Laboratory; Adherence; Study reports; Safety.

16.4 Timing/Reports

Monthly study progress reports on: data collection; trainings; refresher trainings; any other issues

Data analysis of questionnaires, anthropometry and haemoglobin levels will occur as soon as Round 3 data collection is completed (August 2009). No interim analysis will occur before then. Other biochemistry analysis will occur once blood has been sent to respective laboratories in Germany and New Zealand and analyzed and results sent to the PI's.

It is expected preliminary results of the study will be available by the end of 2009. In depth full analysis will occur after the final round of data and blood collection is completed (February 2010) over the following 2 years.

16.5 Study Records Retention

Study records will be kept for at least 5 years following the publication of the results of the study.

16.6 Protocol Deviations

Protocol deviations will not be allowed unless agreed upon by the study steering committee. The study steering committee, sponsors and advisors may detect, review and report on deviations. An investigator will not implement any deviation from, or changes of, the protocol without agreement by the steering group and prior review and documented approval opinion from the IRB of an amendment, except where necessary to eliminate an immediate hazard to trial subjects, or when the change(s) involves only logistical or administrative aspects of the trial (e.g., change of monitor(s), change of telephone number(s)).

The steering committee may approve deviations if it is deemed in the best interest of policy formation for the control of anaemia in children in Cambodia. Further ethical approval will be sought for any protocol deviation.

17 PUBLICATION POLICY

Any publications will be authored by the study investigators and under the PI's and Study Steering Committee supervision and approval.

Any sub-studies will be authored by the study investigators same supervision and approval

No data may be used for publication without the full approval of the PI's and Study Steering Committee.

18 LITERATURE REFERENCES

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SUPPLEMENTS/APPENDICES

18.1.1 Appendix A

Blood Tests

1. Complete Blood Count (CBC) (including blood film examination)
2. Zinc
3. Ferritin
4. Retinol Binding Protein (RBP) (Measure of Vitamin A status)
5. Serum Transferrin receptor (Stfr) (Measure of Iron status)
6. C-Reactive Protein (CRP) (Measure of acute infection)
7. α -1-glycoprotein (AGP) (Measure of chronic infection)

Testing Laboratories

1. National Institute of Public Health Laboratory, Cambodia (NIPHL)
2. SEAMEO-TROPMED RCCN, University of Indonesia or German Laboratory (Dr J. Erhardt)
3. Otago University, Department of Human Nutrition Laboratory. New Zealand (NZ)

Sample Requirements

1. CBC

Tube: EDTA (paediatric tube) **Volume:** 0.5 ml whole blood

Stability: Should be analyzed within 12 hrs of collection.

Analysis: At NIPHL

 Sysmex KX21 haematology analyzer

 May-Grunwald Geimsa blood film stain

 Analysis will include red cell distribution width.

2. Serum ZINC

Tube: Trace-element free tube **Volume:** 0.5ml serum

Stability: Clotted sample must be spun down within 30 – 60 minutes and serum separated into trace-element vials, using trace-element free transfer pipettes.

 Serum can be stored for 1 year at -20°C at NIPHL

Analysis: At University of Otago, Dept of Human Nutrition Lab

 Atomic Absorption Analysis

3. Ferritin, RBP, Stfr, CRP, AGP

Tube: Trace-element free tube **Volume:** 0.1 ml serum for all 5 analytes

Stability: Clotted sample should be spun down within 30 – 60 mins and serum separated into small plastic vials. Serum can be stored for 1 year at -20°C at NIPHL

Analysis: At Dr J. Erhardt Lab

 Sandwich ELISA technique

Third round of blood testing for screening for Haemoglobinopathies

Haemoglobinopathies begin to become evident around one year of age. If, after 6 months of MMN supplements and 6 months of follow up (children at 18 months of age), the CBC shows a MCV below 80 fl and/or abnormal red cell morphology, a haemoglobinopathy may be present. The following screening tests for alpha, beta thalassaemia and Hb E will be done at NIPHL on the EDTA sample used for the CBC to diagnose these conditions. If funding is available all children in the sub-sample will be tested for haemoglobinopathies.

Osmotic Fragility Test:

Principle: The red cells of thalassaemia (alpha and beta) carriers will resist haemolysis under lower osmotic concentrations than normal red cells.

DCIP Test: (Test for Hb E)

Principle: Reactive sulphhydryl groups are oxidized by a dichlorophenolindophenol (DCIP) dye which results in the selective precipitation of the haemoglobin E molecules. Positive screening tests could be followed up with Hb electrophoresis for confirmation if required. (Note: Hb electrophoresis cannot be used to confirm α thalassaemia)

Hb H inclusions: (Test for α thalassaemia)

Principle: Excess β Hb chains are precipitated in the red cells as Hb H inclusions. These inclusions can be seen on a blood film made after incubating red cells with Brilliant Cresyl Blue stain.

18.1.2 Appendix B

Training Plan

Provincial Health Department and Operational District Staff

- Introduction to PHD and OD staff to study (1 session by study staff)
- Training of Health Centre Staff (4 staff x 2 sessions [44 participants total (2 from each HC's and 2 from Referral Hosp)])
- Field Supervision (2 monthly for 12 months (4 visits to Health Centres, 1 day, ~20 Health Centres), 2 staff: 1 OD and 1 PHD).

Health Centre Staff

- Training of Community Health Volunteers (6 sessions (20 participants per session, total 120 VHSGs) 2 sessions per day = 3 days; 2 HC staff per session plus study staff x 4 training sessions (1. Introduce study 2. Enrolment strategy, register. 3. MMN 4. IYCF; 3 monthly refresher training (MMN and IYCF).
- Delivery of MMN supplements - MMN held at the HC and distributed on a monthly basis by HC staff to the VHSGs
- Supervision of VHSGs – monthly basis when VHSGs come to receive the supplements.

Appendix A: Schedule of Procedures/Evaluations

	2008											2009											
	Mar	April	May	June	July	Aug	Sept	Oct	Nov	Dec	Jan	Feb	Mar	April	May	June	July	Aug	Sept	Oct	Nov	Dec	
Baseline Data Collection Age 6 mo with Intervention for 6 mo																							
Age 6 mo-12 mo																							
Cohort 1																							
Cohort 2																							
Cohort 3																							
Cohort 4																							
Cohort 5																							
Cohort 6																							
Endline Data Collection Age 12 mo (Round 2)																							
Cohort 1																							
Cohort 2																							
Cohort 3																							
Cohort 4																							
Cohort 5																							
Cohort 6																							
Follow up Data Collection Age 18 mo (Round 3)																							
Cohort 1																							
Cohort 2																							
Cohort 3																							
Cohort 4																							
Cohort 5																							
Cohort 6																							
Blood samples sent to Germany & NZ for analysis																							
Preliminary analysis of data from baseline, endline and first follow up																							
Dissemination of preliminary results in Cambodia																							

Anthropometry: (Put in appendix as SOP)

Tared weighing

- Turn on the scale. When the number 0.0 appears, the scale is ready.
- The mother will remove her shoes and step on the scale to be weighed first alone. Have someone else hold the undressed baby wrapped in a blanket.
- Ask the mother to stand in the middle of the scale, feet slightly apart (on the footprints, if marked), and to remain still. The mother's clothing must not cover the display.
- Ask the mother to step off the scales then step back on immediately as this will tare the scales.
- Hand the undressed baby to the mother and ask her to remain still.
- The baby's weight will appear on the display (shown to the nearest 0.1 kg). Record this weight.
- Repeat this procedure to weigh again and record. If the 2 weights are >0.5kg different, you will need to repeat this procedure a third time and record the weight.

Recumbant Length

- Measure the child's **length** lying down (recumbent) using a length board which should be placed on a flat, stable surface such as a table.
- Be prepared to measure length/height immediately after weighing, while the child's clothes are off.
- Remove the child's shoes and socks.
- Undo braids and remove hair ornaments if they will interfere with the measurement of length.
- Explain all procedures to the mother and enlist her help
- Cover the length board with a thin cloth or soft paper for hygiene and for the baby's comfort.
- Explain to the mother that she will need to place the baby on the length board herself and then help the assistant if needed to hold the baby's head in place while you take the measurement. Show her where to stand when placing the baby down, i.e. opposite you, on the side of the length board away from the tape.
- Allow the assistant to place the baby's head (against the fixed headboard) quickly and surely without distressing the baby.
- Ask her to lay the child on his back with his head against the fixed headboard, compressing the hair.
- Quickly allow the assistant to position the head so that an imaginary vertical line from the ear canal to the lower border of the eye socket is perpendicular to the board. (The child's eyes should be looking straight up.) The assistant should be behind the headboard and hold the head in this position.
- Speed is important but so is accuracy. Standing on the side of the length board where you can see the measuring tape and move the footboard:
- Check that the child lies straight along the board and does not change position. Shoulders should touch the board, and the spine should not be arched. Ask the assistant to inform you if the child arches the back or moves out of position.
- Hold down the child's legs with one hand and move the footboard with the other. Apply gentle pressure to the knees to straighten the legs as far as they can go without causing injury by applying minimum pressure.

- If a child is extremely agitated and both legs cannot be held in position, measure with one leg in position.
- While holding the knees, pull the footboard against the child's feet. The soles of the feet should be flat against the footboard, toes pointing upwards. If the child bends the toes and prevents the footboard from touching the soles, scratch the soles slightly and slide in the footboard quickly when the child straightens the toes.
- Read the measurement and record the child's length in centimetres to the last completed 0.1 cm. This is the last line that you can actually see. (0.1 cm = 1mm)
- Repeat this measurement and record the length. If the 2 measurements are more than 0.5cm different, you will need to do a third measurement and record the length.

Head Circumference

Head circumference is a measurement of the circumference of the child's head at its largest area (above the eyebrows and ears and around the back of the head).



Figure X. Measurement of Head Circumference

Equipment:

Use a flexible, nonstretchable measuring tape.

The procedure for measuring head circumference is as follows:

1. Position the child in a sitting position in the lap of the caregiver.
2. Place the lower edge of the measuring tape just above the child's eyebrows, above the ears and around the occipital prominence at the back of the head.
3. Pull the tape snugly to compress the hair. The objective is to measure the maximal head circumference.
5. Record the numerical value immediately on the questionnaire.

Middle Upper Arm Circumference

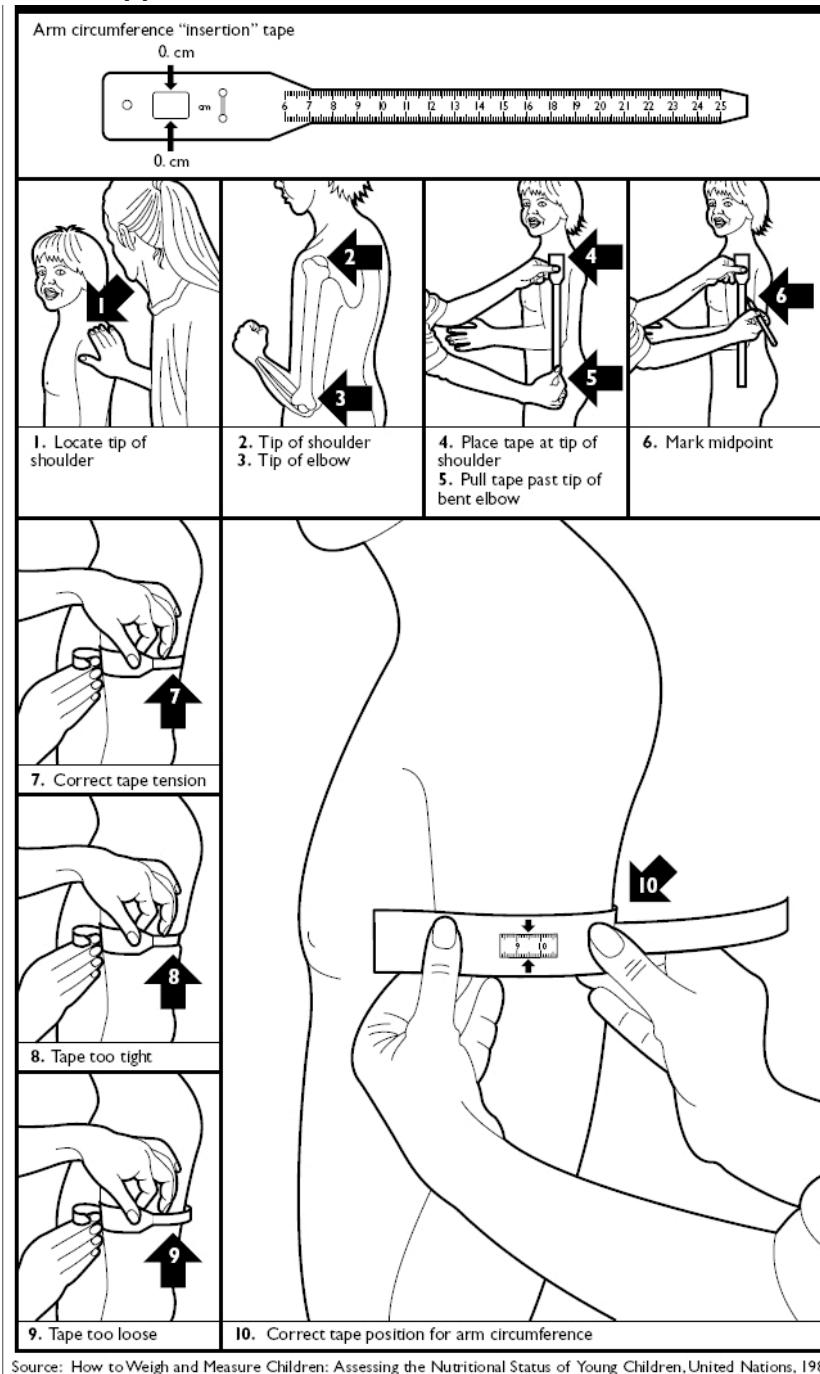


Figure 2. Child Mid-Upper Arm Circumference (MUAC) Procedure

The procedure for measuring recumbent length is as follows:

1. Measurer: Keep your work at eye level. Sit down when possible. Very young children can be held by their mother during this procedure. Ask the mother to remove clothing that may cover the child's left arm.

2. Measurer: Calculate the midpoint of the child's left upper arm by first locating the tip of the child's shoulder (Arrows 1 and 2) with your finger tips. Bend the child's elbow to make a right angle (Arrow 3). Place the tape at zero, which is indicated by two arrows, on the tip of the shoulder (Arrow 4) and pull the tape straight down past the tip of the elbow (Arrow 5). Read the number at the tip of the elbow to the nearest centimeter. Divide this number by two to estimate the midpoint. As an alternative, bend the tape up to the middle length to estimate the midpoint. A piece of string can also be used for this purpose. Either you or an assistant can mark the midpoint with a pen on the arm (Arrow 6).
3. Measurer: Straighten the child's arm and wrap the tape around the arm at midpoint. Make sure the numbers are right side up. Make sure the tape is flat around the skin (Arrow 7).
4. Measurer and assistant: Inspect the tension of the tape on the child's arm. Make sure the tape has the proper tension (Arrow 7) and is not too tight or too loose (Arrows 8-9). Repeat any steps as necessary.
5. Assistant: Have the questionnaire ready.
6. Measurer: When the tape is in the correct position on the arm with the correct tension, read and call out the measurement to the nearest 0.1cm (Arrow 10).
7. Assistant: Immediately record the measurement on the questionnaire and show it to the measurer.
8. Measurer: While the assistant records the measurement, loosen the tape on the child's arm.
9. Measurer: Check the recorded measurement on the questionnaire for accuracy and legibility. Instruct the assistant to erase and correct any errors.
10. Measurer Remove the tape from the child's arm.

19 PROTOCOL AMENDMENT (JULY 2009)

Funding was sought and secured through the Global Alliance for Improved Nutrition (GAIN, Geneva, Switzerland) for a further round of data collection for the study children at 24 months of age. This would allow the investigators to determine if any observed effects on anaemia, deficiencies in iron, vitamin A and zinc, and wasting, underweight and stunting were sustained through to age 24 months.

Ethical approval from National Ethics Committee for Health Research, Ministry of Health, Cambodia and the Human Ethics Committee, Academic Committees, University of Otago, Dunedin, New Zealand was sought and granted.

All data collection, data entry, analysis was to be performed as per the first 3 rounds of data collection. Round 4 data collection to be conducted from September 2009 to February 2010.

The amended schedule of Procedures/Evaluations is as follows:

	2008												2009												2010							
	Mar	April	May	June	July	Aug	Sept	Oct	Nov	Dec	Jan	Feb	Mar	April	May	June	July	Aug	Sept	Oct	Nov	Dec	Jan	Feb	Mar	April	May	June	July	Aug		
Baseline Data Collection Age 6 mo with Intervention for 6 mo Age 6 mo-12 mo																																
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Follow up Data Collection Age 18 mo (Round 3)																																
Cohort 1																																
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Preliminary analysis of data from baseline, endline and first follow up																																
Dissemination of preliminary results in Cambodia																																
Follow up Data Collection Age 24 mo (Round 4)																																
Cohort 1																																
Cohort 2																																
Cohort 3																																
Cohort 4																																
Cohort 5																																
Cohort 6																																
Final analysis including Round 4 data																																
Dissemination of final results in Cambodia																																