CHAPTER III
MATERIALS AND METHODS

In this chapter, we shall describe the materials and methods adopted in the present study. These materials and methods are related to those adopted for collecting, analyzing and interpreting the data in the present study.

Study Area and Population

The present study was conducted in Domiasiat area of the West Khasi Hills district of Meghalaya during the months of January-June 2005 and January-June 2006. It is located between 25° 47' and 25° 57' north latitude and between 91° 10' and 91° 57' east longitude. It is situated to the south-western part of the West Khasi Hills district about 60 km from Nongstoin. The Domiasiat area as well as the whole West Khasi Hills district is dominated by the Khynriam Khasis, one of the Khasi sub-groups, who speak the Mon-Khmer language of the Austro-Asiatic group and have been following the matrilineal system of society. Thus in the present study, the term “Khasis” refers to the “Khynriam Khasis” inhabiting mainly in the West Khasi Hills district of Meghalaya, particularly those living in Domiasiat area.

The sampling design for this study was based on the geographical distribution of villages, which are located within 10 Km radius from the uranium mining site. Therefore, in this thesis, the term “Domiasiat area” refers to the area within 10 km radius from the uranium mining site. According to our survey, there were altogether 10 villages with total households of 443 and about 2826 souls. These villages include Phlangdiloin (106
households), Umjarain (63 households), Domiasiat (8 households), Mawkhlaitngap (33 households), Wahkajee (76 households), Nongjynrin (10 households), Nongtynger (56 households), Nongtynniaw (75 households), Mawthabah (10 households) and Mawlaikhap (6 households). We made complete enumeration of households for demographic information. For other measurements, only those individuals who were willing to co-operate to this study were included in our samples.

As already mentioned, data were also collected from 239 households of Rangblang village, which is located about 25 km from Domiasiat. Rangblang is the biggest village in the northern part of Domiasiat area. It is considered to be more advanced than Domiasiat area in terms of socioeconomic conditions. The basic purpose is to make a comparative analysis of data between the two areas of study for better understanding of the health status of the people in Domiasiat area, i.e., even if Rangblang sample is not necessarily considered as a control group.

**NATURE OF DEMOGRAPHIC DATA**

The nature of demographic data collected for the present study was based on those parameters suggested by the World Health Organization Working Group (WHO, 1964, 1968; Mahadevan, 1986). These may be briefly described as follows:

**Individual records:** These include name of informant, age, sex, marital status, relationship to head of the household, date and place at which record was taken, clan, tribe, religion, community affiliation, total number of family members, place of birth, place of residence, etc.

**Fertility records:** They include pregnancy history of each married woman or mother,
present age of mother, approximate age at each conception, total number of live-births, birth order; age, sex and marital status of each offspring.

*Mortality records:* These include total number of conception, number of dead children, sex, date of birth, age at death, causes of death, if any, number of reproductive wastage (abortions and still-births), etc.

*Socio-economic variables:* These include occupation, education, monthly and annual income of the household, monthly expenditure of the household, age at marriage, and religion.

The entire demographic data were collected through schedules from all the 682 households in the eleven villages, viz., Wahkajee, Nongjynrin, Mawthabah, Umjarain, Mawlaikhap, Nongtynger, Phlangdiloin, Mawkhlaitngap, Domiasiat, Nongtynniaw and Rangblang. Information on age, sex, marital status, tribe, religion, occupation, income, education, community affiliation, place of birth, place of residence, etc. was collected from the head of the household or elder member who was capable of furnishing all the relevant information as per household schedule.

The fertility schedule was completed by filling-in the information on the number of conceptions, number of live births, number of reproductive wastages (abortion and still births), sex, present age, age at death, birth order, etc. from all the ever married women. Sometimes, information given by the mothers was cross-checked from their respective husbands. It may be mentioned that great difficulties were experienced in the assessment of age, particularly that of the elderly women because many of them were not aware of
their real age. Consequently in such cases, the age was estimated with the help of other persons in the household/village. So, there could be some mistakes, in some cases, in the estimation of age.

**Measures of Fertility and Mortality**

**Age Specific Fertility Rate:** The total marital fertility rate is estimated by generalized Poisson regression using SPSS. The dependent variable is the number of births over the three years preceding the survey; we include five-year age groups (as dummy variables) on the right hand side of the model and control for the length of exposure (three years for each woman) using a term called the offset. Fertility rates are obtained as exponents of the regression coefficients for each of the five age groups (Schoumaker, 2004), and the total fertility rate is equal to the sum of the rates multiplied by five.

**Mortality:** For analysing data on mortality, three parameters are taken into consideration. These are: infant mortality (i.e., those who died before one year of life); child mortality (i.e., death between 1-4 years); juvenile mortality (deaths between 5-14 years of age) and reproductive wastage (abortions and still-births). In the present study, abortion refers to any foetus or embryo which is expelled from its mother’s womb on or before the twenty eight weeks of pregnancy because of its failure to develop or otherwise. It refers to both spontaneous and induced abortions. Still-birth is defined as “any child which has been issued forth from its mother after the twenty eight weeks of pregnancy and which did not at any time after being expelled from its mother, breaths or shows any other signs of life” (Marry Cross and Mackintosh, 1954).
**Completed Family Size**

It is a fertility measure showing the number of live-births per woman who married only once and lived continuously in wedlock till the age of 45 years and above. It is obtained with the help of the following formula:

$$\text{CFR} = \frac{B}{M} \times K,$$

Where,

- $\text{CFR} =$ Completed family size,
- $B =$ Total number of live-births to married women of completed family size,
- $M =$ Total number of women who married only once and lived continuously in wedlock till the age of 45 + years,
- $K =$ Constant (in the present study, we have taken as 1)

**ANTHROPOMETRY**

Following are the anthropometric measurements taken on adults aged 18-60 years and children aged 2-6 years of age, following standard techniques (Weiner and Lourie, 1981, Heyward and Wagner, 2004):

- Weight (Kg)
- Height (cm)
- Sitting Height (cm)
- Mid Upper Arm Circumference (MUAC)
- Hip Circumference (cm)
- Waist Circumference (cm)
Besides the above measurements, following indices and ratio were computed for both adult males and females for correlating with body composition:

1. Body mass index or BMI = weight (kg)/height (m)^2
2. Fat free mass index or FFMI = FFM (kg)/height (m)^2
3. Body fat mass index or BFMI = BFM (kg)/height (m)^2
4. Cormic index or relative-sitting height = sitting height (cm)/height (cm)
5. Conicity index or CI = waist circumference (m)/0.109x\(\sqrt{\text{weight (kg)/height (m)}}\)
6. Waist-to-Hip ratio or WHR = waist circumference (cm)/hip circumference (cm)

Anthropometric measurements were used to estimate the body composition (FM and FFM), using the prediction equations of Durnin and Womersley (1974) and Siri (1961) based on age, weight, height, and skinfold thickness. Body density was calculated according to Durnin and Womersley formula, which was in turn used to estimate the percent body fat (PBF) or percent BFM by using Siri’s equation:

\[ \%\text{BFM} = (4.95/\text{density}-4.50) \times 100 \]

BFM was calculated as body weight multiplied by percent FM and then divided by 100:

\[ \text{BFM} = \text{weight (kg)} \times \%\text{BFM}/100 \]

FFM was then calculated as body weight minus BFM:

\[ \text{FFM} = \text{weight (kg)} - \text{BFM (kg)} \]

The BMI (body weight in kg divided by the square of height in meters) was separated into two components: body fat mass index (BFMI = BFM in kg divided by the square of height in meters) and fat-free mass index (FFMI = FFM in kg divided by the square of height in meters) to test the relationship between body composition in terms of BFMI and FFMI with morbidity and other parameters.
Methods of Taking Measurements

Weight: The body weight was taken with a spring weighing machine, asking the subject to stand on it with an erect posture and light apparel. The weighing machine was checked from time to time with a known standard weight. No deduction was made for the weight of light apparel while taking the final reading.

Height: It measures the vertical distance from the floor to the vertex. The subject was made to stand as erect as possible with his/her arms hanging at the sides with thumbs forward, heels holding together and eyes directing towards the horizon (Hooton, 1946). The anthropometer was placed at the back and between the heels of the subject, taking care that it is kept absolutely vertical. The sliding sleeve of the anthropometer was then lowered down towards the middle of the head (Sagital line) so that it would touch the vertex lightly. Reading is in centimeter and its fractions was recorded.

Sitting height: It measures the vertical distance from the vertex to the sitting surface of the subject. The subject was made to sit on the stool, or a flat wooden chair, or at the end of wooden bench. Then he/she was positioned in an erect sitting posture, with ankles crossed, knees spread about 20 cm apart and hands rested on the thighs. The anthropometer was placed at the back and between the two buttocks, taking care that the lumbar curve of the subject was not flattened, but concave from behind. The sliding sleeve was then lowered down to touch the vertex lightly.

Mid upper arm circumference: The measurement was taken with a steel tape at the middle (midway between acromion and elbow) part of the left upper arm on the naked skin (Sen, 1994), while the arms were hanging at the sides of the body.
**Waist Circumference:** Waist circumference was measured midway between the lower rib margin and the superior anterior iliac spine. This measurement was taken with a steel tape at the right angle to the axis of the body when the subject exhaled normally.

**Hip Circumference:** Hip circumference was taken at the widest point over the greater trochanters. This measurement was taken with a steel tape at right angle to the axis of the body when the subject exhaled normally.

**Biceps:** The skinfold was picked up between the thumb and forefinger and the caliper jaws was applied at exactly the level marked. The measurement was read after the full pressure of the caliper jaws was applied to the skinfold. Harpenden Skinfold Caliper was used for taking the skinfold thickness. The skinfold was picked up on the front of the upper arm directly above the centre of the cubital fossa and the level marked on the skin for the arm circumference.

**Triceps:** The skinfold was picked up at the back of the upper arm about 1 cm above the level marked on the skin for the arm circumference and directly in line with the olecranon process.

**Sub-scapular:** The skinfold was picked up under the inferior angle of the left scapula. According to the natural cleavage of the skin, the fold was measured either vertical or slightly inclined downward and laterally.

**Anthropometric Analyses**

Anthropometric measurements were used to evaluate the growth and nutritional status of participants. Human growth is generally measured in terms of anthropometric measurements like height and weight at different age intervals. The velocity or rate of change in these measurements over time is indicative of the growth of an individual. As
already mentioned, the comparison of these measurements with growth references or standards relative to age and sex is known as growth assessment. The quantities of such comparison are often expressed in terms of anthropometric indices, which are used as indicators of nutritional status.

In the present study, three anthropometric indices, such as weight-for-age, height-for-age and weight-for-height were used as indicators of the nutritional status of children (WHO, 1995). These indices are expressed as a Z-score of a child’s measurement to the median weight of the WHO reference population (WHO, 2006a), using the Lambda-mu-sigma (LMS) method (Cole, 1990). The formula for obtaining the Z-score for a given measurement relative to the WHO growth reference is given as:

\[ Z = \frac{(X_M^L - 1)}{L \times S} \]

where, X is the anthropometric measurement (weight or height) and L (power in the Box-Cox transformation), M (median) and S (standard deviation) are the reference values corresponding to a given sex and age in months. For example, according to the WHO growth reference 2006, the L, M and S values for the height of boys aged 24.0 months are 1.0, 87.1161 and 0.0351, respectively. If the height of 24.0 months old boy in our study population is 82.25 cm, the height-for-age Z-score of the boy will be as follows:

\[ Z = \frac{(82.25^{1.0} - 1)}{1 \times 0.0351} = -1.59 \]
The Z-score of -2 was used as the cut-off point for screening the children who were likely to be undernourished. Children with the Z-score below -3 were arbitrarily categorized as having moderate to severe undernutrition.

The Z-score, as calculated above, is a measure of the distance between the child's measurement and the median of the growth reference. It depends to a great extent on the growth reference. The choice of appropriate growth reference is therefore very crucial for getting comparable and reliable information on growth and nutritional status. It has become an academic issue that has received considerable attention during the last three decades (Khongsdier, 2009). The justification for the use of international growth reference is that the effect of ethnic/genetic differences in growth of young children, especially below 5 years of age, is not large enough when compared with the effects of environmental factors including poverty and inadequate nutrition (Habitch et al., 1974). In India, several studies have supported such a contention (Gopalan, 1992; Bhandari et al., 2002). In other words, growth retardation among under-five children is generally considered an indicator of poor nutritional status, or a failure in the expression of the "genetic potential" for growth.

It may also be noted that the clinical and public health implications of the use of growth reference are more important than the choice of reference. It is, therefore, necessary to distinguish between reference and standard. A reference is a statistical tool used to classify and analyse data, whereas standard "embodies the concept of a norm or target, and thus involves a value judgment" (WHO Working Group, 1986; WHO, 1995). Accordingly, the WHO growth reference was used in the present study as a means for screening and monitoring to detect children at greater risk of health and nutritional
problems, but not as a fixed target or diagnostic tool which is the main characteristic of the standard. In addition, the WHO growth reference was used with a view to making our data comparable at the national and international levels.

**Weight-for-age index:** It is a composite measure of body weight or body mass relative to age. It is used an indicator of **underweight** when the children are below $-2$ Z score of the median of the WHO reference population. Such a decrease in body weight is associated with a reduction in body fat mass (body fat stores) and lean tissues (fat-free mass), besides a disproportionate loss of skeletal tissues and a tendency to over-hydration of the body, depending upon the time and levels of undernutrition. Weight-for-age index is, however, a crude measure of underweight, because it is not independent of height.

**Weight-for-height index:** It is widely used as a practical measure of fatness or body fat stores in terms of body weight relative to height among children. Low weight-for-height ($-2$ Z score of the reference population) is an indicator of **thinness** or **wasting** characterized by a deficit in body fat mass and other tissues. Wasting is considered to be a short-term response to nutritional and socioeconomic deprivation. It can develop rapidly under poor environmental conditions, but it can also be restored rapidly under favourable conditions. Along with weight-for-age index, weight-for-height index is very important for assessing the current health and nutritional status of an individual, or a population.

**Height-for-age index:** It is a composite measure of height relative to age among children. Low height-for-age ($-2$ Z score of the reference population) is an indicator of growth retardation, or **stunting** (short stature) relative to a given age. Stunting is
considered to be the result of slow skeletal growth, which is generally a long-term response to nutritional and socioeconomic deprivation.

**Body mass index (BMI):** It is also known as Quetelet’s index which is obtained as weight in kg divided by the square of height in meter (m). It is widely used as a measure of fatness, or the nutritional status of adult individuals. For example, a BMI of $<18.5$ kg/m$^2$ is widely used as an indicator of chronic energy deficiency (CED), i.e., a ‘steady’ underweight in which an individual is in energy balance irrespective of a loss in body weight or body energy stores (Khongsdier, 2005). Such a ‘steady’ underweight is likely to be associated with morbidity or other physiological and functional impairments.

The above cut-off points were taken after taking into consideration the recent studies from many Asian countries. It may be noted that the WHO (1995) has recommended the cut-off points of 25.0 kg/m$^2$ and 30.0 kg/m$^2$ for defining overweight and obesity, respectively. However, there is considerable evidence that these cut-off values are not applicable across ethnic groups, especially among Asian populations. It has been reported that Asian Indians, for example, have higher percentage body fat, waist-to-hip ratio and abdominal fat at a lower level of BMI compared with the Caucasian populations (Ramachandran et al., 1997; Deurenberg-Yap et al., 2000; Deurenberg et al., 2002; Chakraborty et al., 2009). In Asian subjects, the risk of association with diabetes and heart disease occurs at lower levels of BMI compared with the Caucasians (McKeigue and Shah, 1991; Banerji et al., 1999; Chandalia et al., 1999). In this connection, the WHO Regional Office for Western Pacific Region, along with the International Association for the Study of Obesity and the International Obesity Task Force, has proposed new BMI cut-off points of $23.0$ kg/m$^2$ and $25.0$ kg/m$^2$ for defining
overweight and obesity, respectively, for Asian populations (WHO, 2000).

HAEMOGLOBIN ESTIMATION

Data on haemoglobin content of adults were collected using Sahli’s Haemometer by following standard techniques (WHO, 1980), which may be described as follows:

1. 3 to 4 ml or 3 g/dl of N/10 HCL was taken in the clean graduated tube or measuring tube.

2. The blood sample was taken directly from the subject after piercing his/her left middle finger tip. Sahli’s pipette with rubber tubing and mouthpiece was used for drawing or sucking the capillary blood up to 0.02 ml of the pipette. After drawing the capillary blood up to the desired mark, the outside of the pipette was wiped with absorbent or filter paper, making sure that the blood was still on 0.02 ml mark.

3. The blood was then blown from the pipette into the graduated tube containing N/10 HCL. The mixture was shaken thoroughly and allowed to stand for five minutes or so in the Sahli’s Haemometer.

4. After 5 minutes or so, two or three drops of distilled water were added to the mixture with the help of dropping pipette. Special care was taken that the blood was thoroughly diluted by tiring it with the glass rod.

5. Seeing that the mixture had changed its colour, care was taken by adding drop by drop of distilled water after stirring it thoroughly. Reading was recorded when the colour of the mixture matched with those of the two reference tubes in the Haemometer.
Precautions

1. All apparatus were cleaned thoroughly.

2. The first drop of the capillary blood was avoided for taking the measurement.

3. Care was taken not to allow air bubbles to enter Sahli’s pipette before sucking or drawing the blood from the middle finger tip of the subjects.

BLOOD PRESSURE

Mercury sphygmomanometer was used to measure blood pressure of the individuals included in the present study. All measurements were taken on left hand when subjects were being seated position. Each participant was asked to relax and take rest for 10 minutes before taking the measurement. Systolic blood pressure was recorded as the first Korotkov sound (phase 1). Distolic blood pressure was taken as the disappearance of the Korotkov sounds (Phase V). Measurements were recorded for three times, and the average of the three was taken as recorded measurement. Digital blood pressure monitor (M2 Model, Omron Health Care Co. Ltd., Japan) was also used to cross-check the measurement. However, mercury type of measurement was reported for the present study.

DATA ON MORBIDITY

Data on morbidity were based on "self-reported illness experience" of a subject as generally adopted in surveys, which did not involve a clinician (Strickland & Ulijaszek, 1993; Garcia & Kennedy, 1994; Strickland & Tuffrey, 1997). SRM is also more preferable from the point of view that a clinical diagnosis involves much time, cost and technical expertise, which are not always possible when carrying out community-based
studies in developing countries including India. Despite its limitations (Sadana, 2000), SRM might be considered to be the second alternative proxy for assessing the morbidity status of populations in developing countries. Nevertheless, the term “morbidity” in this study was defined simply in terms of the number of 'days ill' and/or 'days unable to work' in the last four weeks before the survey. Each subject included in the study was asked whether or not he had been ill at any time in the last four weeks? If the answer was yes, he was asked how many days had he been in bed or unable to work due to illness? A subject who reported at least two days ill was classified as being “ill.”

The study was symptom-based in which the symptoms were grouped into three categories as suggested in many studies (Strickland and Ulijaszek, 1994; Strickland and Tuffery, 1997; Sadana, 2000). For the present analysis, self reported symptoms of morbidity are broadly classified into three groups: (i) Cold and respiratory include those symptoms such as cough, runny nose, fever, breathing problem, chest pain, sore throat, etc. (ii) Intestinal disorders include diarrhea, dysentery, worms, vomiting, and other self-reported problems of stomach pain. (iii) Self-reported symptoms of morbidity like headache, diabetes, hypertensions and other than the two categories above were included in the category of other health problems.

SOCIO-ECONOMIC CATEGORIES

In the present study, three important socio-economic variables were taken into consideration. These include age at marriage, monthly income of the households and educational level. These socio-economic variables were classified arbitrarily into different groups and/or categories with a view to understanding their influence on
demographic characteristics, growth, health and nutritional status of the study population.

Our classification may be briefly described as follows:

**Income groups:** Data on household income were collected directly from the heads of the households and they were cross-checked taking into consideration some aspects of socio-economic conditions like housing condition, types of occupation, land holding, and monthly expenditure. The per capita monthly income of the households was classified as follows:

- Above 75\(^{th}\) percentile (>Rs.667) = High income group (HIG)
- 50\(^{th}\) to 75\(^{th}\) percentile (Rs.400-667) = Middle income group (MIG)
- Below 50\(^{th}\) percentile (<Rs. 400) = Low income group (LIG)

**Educational Level:** Data on educational attainment of individuals in the present study were arbitrarily classified into four broad educational levels, namely, illiterate, primary, secondary and above secondary. In the present study, the number of illiterates (i.e., those individuals who were not able to read or write) was included in the category of illiterates. **Primary level** of education includes lower primary and upper primary, i.e., up to standard VIII. In the **secondary level** of education, we included those individuals who attended standard VIII to X. **Higher Secondary or above secondary level** included other individuals who attended standard XI and other higher levels of education. This educational classification is highly arbitrary. However, we assumed that if education is really important in regulating nutritional and health indicators like in the western countries, its effects can be observed even if the individuals were dichotomized only into two categories, say, lower and higher levels of education.
**Family Size:** The family size was classified into three categories. The individuals who lived in a household with less than 4 family members were considered as having a *Small Family Size*. The *Average/Medium Family Size* includes those individuals who lived in a household with 4-8 family members. The individuals who lived in a household with more than 8 family members were grouped in the category of *Large Family Size*.

**Statistical analyses**

The basic design of the study is to analyse and present comparative data between Domiasiat and Rangblang. In addition, the main focus of analysis was to understand how nutritional and health status is related to biosocial variables, such as age, sex, anthropometric variables, self-reported morbidity, blood pressure, household income, education and family size in both the areas of study.

All data were managed and analysed using SPSS/PC Software. The analysis was first carried out to present the basic descriptive statistics of demographic and anthropometric variables, blood pressure, hemoglobin, and morbidity prevalence in relation to socio-economic characteristics of the study samples for both Domiasiat and Rangblang. The differences between two means were tested, using t-student test, while the differences between more than two means were determined, using one-way analysis of variance (ANOVA). Analysis of covariance was also carried out for testing the differences among means, allowing for the effects of other covariates. The differences between proportions were tested, using chi-square test. Multiple regression analysis was also carried out for understanding the effects of socio-economic factors on demographic characteristics of the population. Logistic regression analysis was used for analyzing the effects of biosocial variables on dependent variables that are dichotomous. Receiver
operating characteristic (ROC) was adopted for determining the necessary cut-off points by using MEDCALC 12 Windows Software. Curve fitting and graphical presentations were carried out by using ORIGINLAB 8.5 Software for Windows.