Final Report of

The Investigation of Unusual Illnesses Allegedly Produced by Endosulfan Exposure in Padre Village of Kasargod District (N.Kerala)

Submitted to the
Honourable
National Human Rights Commision

National Institute of Occupational Health
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Reports of unusual diseases in certain villages of Kasargod district of Northern Kerala allegedly caused by spray of a pesticide, endosulfan, over the cashew plantations were published in Down to Earth (February 28th, 2001), The Hindu (July 22nd 2001), India Today (July 23rd, 2001) and several other magazines, local news papers and TV channels.

The National Human Rights Commission initiated suo moto action on the report entitled "Spray of Misery" published in India Today (July, 23 2001) and asked a number of agencies including ICMR to submit a report within four weeks. On the directives of the Director General ICMR, a three-member team from NIOH visited the affected areas and collected first hand information and a preliminary report was submitted to the NHRC. (Annexure 1). In the report it was mentioned that NIOH will carry out an environmental epidemiological study and submit the report within six months of which the first part of the report will be submitted by the end of three months and informed the Commission that the first results of the study will be available by the end of December 2001.

The review of the published reports showed following features:

1. In Kasaragod district, apart from the private owned plantations under cashew cultivation, there are three plantations owned by the Plantation Corporation of Kerala.

    a. Kasaragod Estate: The cashew plantations of Periye, Muliyar, Adhur and Perla come under this with a total area of 2,209 ha.
    b. Rajapuram Estate: The plantations in Painikara, Kanady and Panathur with a total area of 1,526 ha.
    c. Cheemeni Estate: With a total area of 980 ha.

2. Around 1963-64, the agriculture department began planting cashew trees on the hills around Padre village in Kasargod district. In 1978, Plantation Corporation of Kerala (PCK) took over the plantations. The aerial spray of Endosulfan

is undertaken by PCK to control tea mosquito on cashew nut plantations for over 20 years.

3. Several villages in the valley below the hill experience severe exposure to Endosulf during the spray. The water bodies also get contaminated during the spray. There are unconfirmed reports of the disappearance of fish, frogs and snakes from the area following the aerial spray.

4. Cases of illnesses such as disorders of the central nervous system - cerebral palsy, retardation of mental and/or physical growth, epilepsy among the children - and congenital anomalies like stag horn limbs have been reported. There are also reports of cancer of the liver and blood; infertility and undescended testis among males; miscarriages and hormonal irregularities among women; skin disorders; and asthma, psychiatric problems and suicidal tendencies the villages nearby cashew plantations.

5. Several villages near the cashew plantation are said to be affected by the diseases enumerated above. Amongst all these, 6th and 7th wards situated along the Kodenkiri stream in Enmakaje Gram Panchayat (village council) are reported to be the worst hit.

6. Team of researchers from Thanal, a Thiruvananthapuram based non-government organization carried out a house-to-house health survey in Periya, Cashew Plantation area of Kasargod district from October 1999 to December 1999. The researchers observed cases of anemia and generalized weakness in women and children, poor physical growth, frequent attacks of fever, numerous cases of infertility among men, miscarriages and menstrual disorders in women, kidney problems and swelling and discolouration of the skin of the limbs.

7. Dr. Mohana Kumar Y S, a physician practicing in the area since 1982, has been keeping record of the cases coming to his clinic for treatment for the last ten years. His record as published in Down to Earth Magazine (28th February 2001) is follows:

List of confirmed cases of various diseases
reported by Dr. Y. S. Mohankumar.

Disease	No. of Cases
Cancer	49
Mental retardation	23
Congenital anomalies	9
Psychiatric cases	43
Epilepsy	23
Suicide	9
Total*	156
Total (by January 26)**	197
NOTE: * - cases counted by January 5, 2001 **- break-up not available	

7. Centre for Science and Environment (CSE) New Delhi analysed biological and environmental samples for Endosulfan residues on 17th February 2001, about one and a half month after the last aerial spray of Endosulfan carried out on 26th December 2000. The results published in the magazine "Down to Earth" showed that the concentration of Endosulfan in three water samples were 7 to 51 times higher than the maximum residue limit (MRL). Very high levels of Endosulfan were reported in samples of human blood, human milk, vegetables, spices, cow's milk, animal tissues, cashew, cashew leaves and soil. In one of the soil sample the levels of Endosulfan were 391 higher than MRL.

8. Fredrick Institute of Plant Protection and Toxicology (FIPPAT) at the request of PCK carried out evaluation of Endosulfan residues in 106 samples of human blood, one cow milk sample, one fish sample, 30 water samples, 29 soil samples and 28 cashew leave samples collected from 18.3.2001 to 02.05.2001 from Padre village. Their results show that there are no residues of Endosulfan in any of the blood samples, cow milk and water samples. However, some residue of Endosulfan was detected in soil and leaf samples.

The above reports have following weaknesses from scientific point of view:

1. **In the report of Dr. Mohan kumar** only the number of persons suffering from diseases such as congenital malformations, cancers, psychiatric illnesses etc. without referring to the denominator (i.e. out of how many) was reported. These diseases also occur with varying frequency in general population. Hence their occurrence does not indicate exposure to unusual causative factor unless, the excessive incidence/prevalence compared to other similar population is proved. Therefore to establish the higher prevalence/incidence of any disease one must include control (referent) population in the study and then compare prevalence/incidence of various diseases.

2. **CSE Study:** Quantitative estimation of Endosulfan levels in biological and environmental samples was done by a very sensitive and sophisticated technique called gas chromatography equipped with the ECD detector. This technique although very sophisticated for quantitative estimation, cannot identify an unknown substance, which needs to be confirmed by using standard tests. This was particularly essential because the investigators reported the levels of Endosulfan varying between 108 and 196 ppm in the blood of all subjects with varying degrees of illnesses. These levels are much higher than the reported blood levels of 4-8 ppm in three fatal Endosulfan poisoning cases (Coutselinis A., Kentarchou E and Boukis D. 1978 Concentration level of endosulfan in biological material (Report of three cases) Forensic Sc. 11:75) and 2.9 ppm in another case of fatal Endosulfan poisoning (Blanco-Coronado JL Repetto M Geinestal RJ et al, 1992. Acute intoxication by endosulfan J Clin Toxicol 30:575-583.). No attempt was made by the investigator to confirm the presence of Endosulfan.

3. **The FIPPAT study,** though started one month later than CSE, shows just the opposite results, i.e. complete absence of endosulfan residues in blood, cow milk and water samples. The study reported Endosulfan residues in the range of 0.001to 0.012 ppm in soil samples and 0.04 to 2.863 ppm in cashew leave samples. FIPPAT did not include the confirmatory tests of the compound. The report mentions only about the validation of the method, which involves recovery experiments on extraction, and clean up procedures.

4. A well-planned environmental epidemiological study was therefore necessary to confirm the high incidence of diseases in the villages near the cashew Plantation in

Kasargod district and evaluate exposure to endosulfan and to find out its relationship with Endosulfan if any. NIOH carried out the actual field study from 24th Sept. 2001 to 7th October 2001 with the following objectives.

Objectives:

1. To confirm the reported disease pattern in the exposed populations and evaluate the magnitude of the problem by comparison with reference populations through a well designed epidemiological study.
2. To search for etiological factors if the exposed populations show abnormal disease patterns and generate a hypothesis.
3. To confirm the presence of Endosulfan residues in environmental and biological samples and estimate their levels.

Methodology and Plan of Work:

Study design:

a) For designing the study, discussions were held with senior members of the Scientific Advisory Committee of NIGH and its scientists and it was decided to start the first phase of the study in school children of Padre village, which is to be taken as the exposed area. The school children were selected for the following reasons.

b) Majority of the illnesses have been reported among the children.

c) It is physically very difficult to conduct a house-to-house survey involving specialized medical examination and laboratory investigations, as houses in Padre village are very scattered due to the topography of the area.

d) To estimate prevalence of disease, it is important that all the individuals selected for the study through statistical process, participate in the study. Non-participation of the selected population could result in selection bias, which will make the validity and reliability of the estimates doubtful. It was expected that many of the villagers may not cooperate due to repeated visits of different committees and NGOs and only affected families (motivated groups) may come forward thus causing a bias in the selection.

e) Due to high literacy standards in Kerala, most of the children attend school and through them it would be easy to call the parents to the school and record details of any diseases encountered in the family members.

f) Co-operation among school children could be ensured due to goodwill of the state education department and the district collector.

g) Study parameters related to growth and development in children need information on accurate age. The school records provide date of birth for the purpose.

1.2. At our request, the district authorities of the state education department sent a circular to all schools in the district to cooperate with the NIOH team.

1.3. Special proforma were designed separately for school children and families. Prof U. V. Shenoy, Head, Dept. of Pediatrics, K. M C. Mangalore extended full cooperation of his department for examination of school children. He also helped in design of proforma for school children (see annexure 2,3).

1.4. Selection of control groups. For any epidemiological study it is necessary to select a control group, which should be comparable with the exposed group in all respects except for the exposure, which in the present case was exposure to aerial spray of Endosulfan. The study group consisted of 619 children studying in Govt. Higher Secondary School, P.O. Vaninagar in Padre village. For comparison, 416 school children from two schools in Miyapavadu, Meenja Panchayath viz. Sri Vidya Vardanaka High School and Vani Vilas aided L. P. School were selected. The selection of controls was finalized after ensuring that these children had a similar socioeconomic background as the exposed group, and the control schools were also Kannada medium schools like the school in Padre. Meenja Panchayath is about 25 km. North of Padre and also has cashew nut crops but it was confirmed that this crop has never had any aerial spray of endosulfan. Three small rivers separate Meenja from Padre area thereby excluding any possibility of cross contamination of water sources.

1.5 The Regional Remote Sensing Service Centre (RRSSC) at Bangalore, an organization belonging to Indian Space Research Organization (ISRO) was requested to provide physiography of the exposed and control areas through satellite imaging.(Annexure 4)

1.6 Teams from following three organizations participated in the study. (Annexure 5)

1. National Institute of Occupational Health (NIOH), Ahmedabad.

2. Regional Occupational Health Centre (ROHC) (S), Bangalore.

3. Department of Pediatrics, Kasturba Medical college Mangalore.

1.7 All staff were explained the objectives of the study and trained for different aspects. During briefing/training the importance of unbiased recording of abnormalities (using same technique and criteria for exposed and control subjects) was emphasized.

1.8 The laboratory techniques and methodologies were standardized at NIOH and the necessary glassware, chemicals, reagents and equipments were carried from NIOH/ROHC to the study site. A field laboratory was set up for lymphocyte cultures and separation of serum samples. The serum and environmental samples were coded to avoid bias.

1.9 Same teams performed examination of children belonging to exposed and control groups, using same instruments and similar techniques and same persons recorded the family details.

2. Conduct of the Study:

2.1 Examination of School children:

After all above mentioned preparations and two preliminary visits, the actual study was started from 24th September and continued till 7th of October 2001. Every day about 50-70 children were asked to bring their parents with them on the next day. The parents were explained the objectives of the study and consent form in the local language was read out to them. Only after taking written consent of one of the parents, the children were examined and only in willing cases blood samples were collected and the sexual maturity rating (SMR) examination was performed. The SMR examination of the boys and girls was carried out by male and female pediatricians respectively observing necessary privacy required for this delicate examination. Every case whether from exposed or control group, which showed any major abnormality, was referred to a senior pediatrician and only after reconfirming the findings, the proformas were filled up.

2.2: Recording of ethnicity and major illnesses in the family:

The parents who accompanied their wards were interviewed by trained staff, who could communicate well in the local languages (Kannada, Malayalam, Tulu or Konkani), on the same day. Details about all family members living in the household were recorded with

special reference to the important diseases reported in the area. Information was also collected regarding the ethnicity, occupations of men and women, land ownership, crops etc. from the parents accompanying children.

Deaths in the family and their causes were also recorded and the respondents were asked to bring related case papers if available.

Checking of the proformas and data entry: During the study, the proforma were checked daily by one of the senior investigators for any anomalies which were rectified; on the next day. Before data analysis, the investigators and the statistician rechecked the proforma and in doubtful cases the examining pediatricians were consulted.

2.3 Investigations:

2.3.1 **Satellite based assessment of physiographic disposition of villages in the cashew plantation area of Kasargod district:** The Regional Remote Sensing Service Centre (RRSSC), Bangalore, of Indian Space Research Organization (ISRO) was requested to assess and provide information on environmental data such as topographic location of the villages, water sources, land use, crop and type of plantation in the region. Dr. P. P. Nageswara Rao, Head, RRSSC, Bangalore and his team analysed the satellite data and visited the villages from 2nd to 4th November, 2001 .

2.3.2 **Analysis of Serum Samples:** A total of 248 (164 exposed + 84 control) school children agreed for the blood examination. After separation, the serum samples were carried by air under dry ice (-80°C) to NIOH. Lymphocyte culture were carried out in the field laboratory.

The blood samples were collected for the following investigations:

1. Endosulfan residues.

2. Hormonal Analysis.:

i. Thyroid Hormones: T3, T4 and TSH

ii. Sex Hormones: Testoterone, Oestradiol, Progestrone, FSH, LH, Prolactin and growth hormone.

2.3.3 METHODOLOGY FOR HORMONE ANALYSIS:

All the hormones were measured using specific radioimmunoassay (RIA) technique utilizing serum varying from 10 to 100 Ml. This method of hormone assay is very sensitive.

Serial dilution of serum along with curves parallel to standard curve were obtained and processed in one assay to rule out interassay variations. A linearity study was performed to assess the sensitivity of all the hormones by serial dilution of a high level with the zero standards. For each hormones all or minimum 10 samples in duplicates were analyzed. The radio immuno assay kits for all hormones were procured from either Immunotech or Medicorp, USA. Sex hormone assay was limited to children 10 years and above.

2.3.4 Cytogenetic Studies - Study of chromosomal aberrations and sister chromatid exchange in peripheral leucocyte culture.

2.3.5 Estimation of Endosulfan residues in environmental samples. The environmental samples for endosulfan residues were collected on two occasions: (1) At the time of medical survey during Sept./Oct. 2001 and (2) Mid June 2002.

The topography of area (Study - Padre; Reference - Meenja, Kasargod, Kerala) is typical hilly terrain comprising of mainly cashew plantation and also areca-nut, coconut, pineapple and banana etc. While the plantation exists on hill and hilltops, the residences are located at the valleys. The approximate distance between the hill tops and the residences range from 1-3 km. Each house has a pond which serves as water source for agricultural purposes. The water gets pooled in the ponds by the runoff water from hill tops. The open well and/or surangas (the water streams flowing in space between the rocks and tapped through a long tube for serving as drinking water source and for cooking purpose).

The insecticide (endosulfan) aerially sprayed could settle on soil and the runoff water can translocate the compound from hill tops to the ponds and other water bodies situated at valleys. Since the endosulfan has been reported to be strongly adsorbed in soil particles, the specimen of sediment of ponds, soil samples on hill terrain where the cashew plantation existed and the water samples from ponds and open-wells were collected to estimate the residue levels.

Procedure of Sediment sample collection:

Ponds of the residences in study and control area were the sources for sediment collection. The depth of water in ponds selected for sediment ranged from 1- 5 meters. The sediment samples were collected by diving in and reaching to the bottom of the

ponds. The soil-sediment collected from the bottom was brought to surface and transferred into the pre-numbered poly bags. In each of the pond selected for sediment sample, the pond bottom soil sediment was collected at 3 to 5 different spots and these were pooled as a composite sediment sample from that pond.

The slurry of sediment so collected was subjected for filtration using suction filtration apparatus (using buchner funnel, Whatman filter paper and a filtration suction pump). The sediment sample was compacted, labelled and preserved for transferring to the laboratory.

Procedure of Soil sample collection:

The hill terrain comprising of cashew plantation where previous aerial endosulfan spray was conducted in December 2000 was identified and selected for soil sample collection in the study area (Periyar area, approx. 5 km away from Periyar, Kasargod). The height of this terrain was approx. 2 km from the base. A kacha road-pavement existed to reach on the top of the hill from base and the cashew plantation was present on either side of the pavement.

The first sampling site was identified after walking up-hill from the base to a distance of about 750 meters. The sampling site measuring approx. 25 X 25 feet, square plot was chosen for soil sample collection. In each site, the soil sample was collected from 5 sub-spots (4 spots from the 4 corners of the square plot and one spot at the centre). The soil sample from each site was pooled into one pre-labelled poly bag which represented the identified soil sampling site.

The ground surface at each spot was cleaned using a cleaned and hexane rinsed iron hand spade. The soil samples were collected by digging the earth using a cleaned, sharp and pointed iron plate. At each spot identified, three soil samples were collected at three different depths or strata (2-3 inches deep - top soil, 5-7 inches down and 9-12 inches deep). The respective strata samples in the 5 spots of each soil sampling site were pooled up and labelled as soil composite sample of the particular site. Clean polybags which can hold about 3 kg of the soil were used for collection of soil samples. Thus, in each sampling site three soil samples, namely top-soil, mid-soil and lower soil corresponding to the three different strata. Each sampling site selected was about 150 to 200 meters away in the periyar hill area collecting in all 4 soil composite soil samples. Similarly, two soil composite samples were collected at Jintadka area which also had the same

topography but about 4 kms. away from Periyar, making a total of 6 soil composite samples at three different depths. (6 x 3 = 18 samples) representing the exposed area.

Water samples:

Sediment filtrate-water samples:

The water samples were collected from the ponds where the sediment samples were collected. The pond water collected in a polyethylene bucket. This water was subjected to filtration and the filtrate transferred to the well cleaned, l- liter, amber-coloured and hexane rinsed bottles. The bottles were labelled and preserved in the laboratory for further shipment to analytical laboratory.

Drinking Water Samples:

The drinking water sources were either open wells or Surangas in the residences. For collecting the drinking water samples the water was drawn from the wells and Surangas using poly bucket and then the water was subjected for filtration and the filtrate so obtained transferred into cleaned water collecting glass bottles. They were numbered and preserved for shipment to the laboratory.

Control (Reference) Area:

The control area chosen was Miyapadavu Gram Panchayat of Meenja village, about 25 km away from the exposed area of Padre area. Both the study and control areas were in the Kasargod district. The procedure of collection of soil, pond sediment-soil, sediment filtrate and drinking water samples in the control area was similar. Also equal number and type of samples were collected in the control area. However, it was ensured, by repeated enquiries that at the areas of sample collection there was neither previous aerial spray nor manual spray of endosulfan pesticide on the crops or plantation that existed in the area.

RESULTS:

1. Endosulfan Residue Levels:

Table 1 shows the levels of endosulfan in various water bodies collected in Sept./Oct, 2001. Most of these water bodies form the source of drinking and irrigation water for the villagers. These levels of endosulfan are much below the US Environmental Protection Agency (EPA) recommended maximum amount of endosulfan in lakes, rivers, and streams of 74 parts endosulfan per billion parts of water (74 ppb). But the detection of even very small endosulfan residues signifies continuous exposure of the population since the spray began more than 20 years back. It should however, be appreciated that the levels of endosulfan reported by us do not represent the real levels of exposure and probably represents the lowest levels for the year for following reasons:

1. Water samples were collected in September - October 2001, almost nine to ten months after the last spray of endosulfan and just two months before the next round was due had there been no ban on the aerial spray.

Table l. Levels of Endosulfan Residues in water Samples (collected in Sept/Oct 2001)

Sample Source	α- Endosulfan (ppb)	β- Endosulfan (ppb)	Endosulfan sulfate (ppb)	Total Endosulfan (ppb of α,β and Endosulfan sulfate)
Well	0.0086	0.0088	0.0035	0.0209
Well	0.0062	0.0023	N.D.	0.0085
Suranga	0.0065	0.0022	N.D.	0.0087
Well	0.0046	0.0032	N.D.	0.0078
Stream	0.0081	0.0123	N.D.	0.0204
Pond	0.0138	0.0416	0.0113	0.0667

N.D. = Not detected.

2. The district has an average annual rainfall of 3500 mm (140 inches) and the rainy season extends from end of May to October. Heavy rain would have washed out most of the endosulfan present in the water and soil.

Table 1-A . Mean Residues of Endosulfan in soil samples collected in Sept/Oct. 2001

Sample source	Area	Sample Number	α Endosulfan (ppb)	β Endosulfan (ppb)	Endosulfan sulfate (ppb)	Total Endo-sulfan (ppb)
Soil	Reference	2	0.338	Nd	0.033	0.372
	Study	8	0.890	0.0147	0.083	0.988

Table-2 shows endosulfan residues in different sources of drinking water in control and exposed areas. These samples were collected in mid-June 2002. There were no endosulfan residues isolated from any drinking water samples in control area. However, endosulfan residues were isolated from all drinking water samples from the study area and the levels were very low.

Table-3 shows endosulfan residue levels in pond sediments and pond filtrate in study and reference villages. All the samples in study village showed varying amounts of endosulfan residues. However the residue concentrations were higher in pond sediments as compared to filtrate. In pond sediment only a-endosulfan was found; whereas in two samples of pond filtrate, all the residues were found.

These findings of higher endosulfan residue in sediment support the published reports about binding of endosulfan to soil particles carried by the runoff water. Since this water is used for irrigation purpose there is a likelihood of exposure through food.

Table 2 Levels of Endosulfan residues in drinking water samples (collected in mid-June 2002).

Type of sample	Area	Sample Number	α Endosulfan	β Endosulfan	Endosulfan sulfate	Total Endo-sulfan
Drinking Water	Meenja N=5	1 (suranga)	Nd	Nd	Nd	Nd
		2 (suranga)	Nd	Nd	Nd	Nd
		3 (well)	Nd	Nd	Nd	Nd
		4 (well)	Nd	Nd	Nd	Nd
		5 (well)	Nd	Nd	Nd	Nd
	Padre N=5	1(suranga)	0.003	Nd	Nd	0.003
		2 (well)	0.0005	Nd	Nd	0.0005
		3 (tank)	0.003	Nd	Nd	0.003
		4 (well)	0.014	0.010	0.006	0.030
		5 (tank)	0.0004	Nd	Nd	0.0004

Table-4 shows endosulfan residue (Meas±S.D) levels at different depths of soil in study and reference areas. The levels were higher in study area as compared to reference area and a-endosulfan was the major residue in both areas.

Table-5 shows the results of endosulfan residues in serum samples according to sex. Endosulfan residues were found in 85% and 78% of female and male subjects, respectively of study area; whereas they were found in 34% and 29% of female and male subjects in the reference group. These differences were statistically highly significant ($p<0.01$). The frequency of positive serum samples was highest for α-endosulfan followed by β-endosulfan and endosulfan-sulphate.

Table 3 Levels of endosulfan in pond water and filterate in Padre and Meenja villages

Type of sample	Area	Sample Number	α Endosulfan (ppb)	β Endosulfan (ppb)	Endosulfan sulfate (ppb)	Total Endo-sulfan (ppb)
Pond sediment	Reference N=4	1	N.D.	N.D.	N.D.	N.D.
		2	N.D.	N.D.	N.D.	N.D.
		3	N.D.	N.D.	N.D.	N.D.
		4	N.D.	N.D.	N.D.	N.D.
	Study N=4	1	0.282	N.D.	N.D.	0.282
		2	0.307	N.D.	N.D.	0.307
		3	0.179	N.D.	N.D.	0.179
		4	0.155	N.D.	N.D.	0.155
Pond filtrate	Reference	1	N.D.	N.D.	N.D.	N.D.
		2	N.D.	N.D.	N.D.	N.D.
		3	N.D.	N.D.	N.D.	N.D.
		4	N.D.	N.D.	N.D.	N.D.
	Study	1	0.004	N.D.	N.D.	0.004
		2	0.019	N.D.	0.23	0.042
		3	0.011	0.006	0.002	0.019
		4	0.021	0.023	0.009	0.053

N.D. = Not detected.

Table 4 Residues of Endosulfan (Mean±SD) in soil samples collected in June 2002

Type of sample	Area	No. of Samples	α Endosulfan (ppb)	β Endosulfan	Endosulfan sulfate (ppb)	Total Endo-sulfan (ppb)
Top soil (1"-3")	Reference	6	0.153±0.067	0.002±0.004	0.007±0.012	0.162±0.08
	Study	6	0.274±0.161	0.0018±0.004	0.025±0.03	0.030±0.18
Mid soil (5"-7")	Reference	6	0.089±0.096	N.D.	0.007±0.012	0.096±0.091
	Study	6	0.183±0.076	0.005±0.001	0.008±0.018	0.191±0.08
Lower soil (10"-12")	Reference	6	0.0623±0.06	N.D.	0.0005±0.001	0.062±0.059
	Study	6	0.128±0.076	N.D.	0.012±0.028	0.106±0.085

Table 5 Positive cases of endosulfan residues in serum of the subjects according to sex and exposure status

Endosulfan	Study		Reference	
	Female	Male	Female	Male
α Endosulfan	11/35(31.43)	14/48(29.17)	54/67(80.60)**	71/97(73.20)**
β Endosulfan	5/35(14.29)	7/48(14.58)	46/67(68.66)**	51/97(52.58)**
Endosulfan Sulfate	3/35(8.57)	2/48(4.17)	34/67(50.75)**	44.97(45.36)**
Total Endosulfan	12/35(34.29)	14/48(29.17)	57/67(85.07)**	76/97(78.35)**

Figures in bracket indicate percentage. ** $p < 0.01$

Table-6 shows Mean ± SEM of the serum endosulfan residues in subjects according to sex. The levels of α-endosulfan, β-endosulfan and endosulfan-sulfate were significantly higher in subjects belonging to study group as compared to reference group. Higher levels were that of α-endosulfan.

The higher levels of endosulfan found in the environment and serum samples in study areas as compared to reference population indicate continued exposure of the subjects in study area. There was significant fall in endosulfan levels in drinking water in study area over a period of 8-9 months (Sept-Oct 2001 to June 2002).

Table 6 Mean ± SEM of the endosulfan residues in serum of the subjects according to sex and exposure status

Endosulfan	Reference Group		Study Group	
	Female (35	Male (48)	Female (67)	Male (97)
α Endosulfan	0.60 ± 0.18	0.89 ± 0.22	5.26 ± 0.65**	4.17 ± 0.66**
β Endosulfan	0.25 ± 0.12	0.40 ± 0.16	3.44 ± 0.48**	2.48 ± 0.52**
Endosulfan Sulfate	0.11 ± 0.06	0.09 ± 0.07	2.73 ± 0.47**	2.06 ± 0.41**
Total Endosulfan	0.95 ± 0.27	1.38 ± 0.38	11.43 ± 1.34**	8.71 ± 1.38**

Figures in parenthesis indicate number of subjects in the group. ** $p < 0.01$

Medical Findings in Children:

Comparison of Anthropometric data:

Table 7 shows the comparison of mean age, height and weight of the study and reference group of study population. The sex wise distribution is comparable in study and reference groups. The mean age of the study group is higher as compared to reference population. This is because the school taken for study population at Padre has classes up to XII standard, whereas the schools in control village has classes up to X standard.

Table 7 Mean (± SD) age, height and weight in control and study population

	Reference (n=416)		Study (n=619)	
	Female 183(44.0%)	Male (48) 233(56.0%)	Female (67) 258(41.7%)	Male (97) 361(58.3%)
Age (years)	10.5 ± 3.03	10.7 ± 3.11	12.0± 3.27	12.2 ± 3.17
Height (cms)	129 ± 15.6 (182)	130 ± 15.4 (231)	136 ± 16.4 (254)	138 ± 16.7 (359)
Weight (kg)	25.9 ± 9.37	25.7 ± 8.16	30.8 ± 11.1	30.2 ± 11.1

Table 8 Average (± SD) height (cms) and weight (kg) of the study and control groups according to sex

Age (completed years	Reference				Study			
	Female		Male		Female		Male	
	Height	Weight	Height	Weight	Height	Weight	Height	Weight
Below 10 (337)	116 ± 9.75	18.6 ± 3.50	116 ± 9.63	19.1 ± 3.78	115 ± 8.07	18.4 ± 4.27	118 ± 8.44	20.3 ± 5.82
10 (91)	131 ± 6.22	25.1 ± 4.07	130 ± 4.35	24.5 ± 2.73	128 ± 7.72	23.7 ± 3.24	128 ± 5.86	22.9 ± 2.22
11 (117)	138 ± 6.71	28.7 ± 4.80	135 ± 7.07	26.6 ± 3.91	134 ± 6.52	26.5 ± 3.88	134 ± 7.43	25.4 ± 4.47
12 (117)	140 ± 5.82	30.8 ± 5.54	138 ± 7.28	29.1 ± 6.03	144 ± 9.23	34.7 ± 7.59	138 ± 7.76	29.5 ± 6.36
13 (105)	143 ± 6.63	33.8 ± 9.14	141 ± 8.33	31.0 ± 6.63	147 ± 6.23	35.9 ± 5.38	142 ± 9.36	32.7 ± 7.30
14 (95)	147 ± 6.13	37.4 ± 6.20	149 ± 8.21	35.0 ± 4.81	148 ± 5.70	40.3 ± 6.58	151 ± 9.28	37.4 ± 12.9
15 (83)	151 ± 6.78	44.0 ± 5.15	153 ± 6.47	39.7 ± 5.07	154 ± 8.57	42.7 ± 7.96	155 ± 7.47	40.2 ± 6.89
16 (43)	146 ± 6.78	36.3 ± 4.35	154 ± 7.03	41.8 ± 9.24	147 ± 7.25	39.8 ± 6.69	159 ± 8.13	43.7 ± 7.00
17 (21)	144 ± 0.00	30.0 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	154 ± 5.38	49.0 ± 9.47	160 ± 6.10	45.8 ± 6.45
18 and above (17)	140 ± 0.00	34.0 ± 0.00	145 ± 2.83	35.5 ± 3.54	155 ± 8.96	41.0 ± 5.48	164 ± 7.85	50.8 ± 9.28
Figures in bracket indicate number of subjects whose data on height and weight is available								

Table 8 shows comparison of mean height and weight of the study and reference population according to age and sex. It may be noted that the data for height and weight were not available fond 9 subjects. It is seen that mean height and weight are in study and control subjects are comparable fond the same age and sex group. Nutrition and ethnic background are the two major factors, which determine the height and weight. The results thus signify that the nutritional status of the study and reference population of the two groups are comparable.

Neurobehavioural problems: These were investigated both subjectively as well as by objective tests.

Table 9. Prevalence of scholastic backwardness (learning disability) as reported by the class teacher and the results of annual examinations.

	Reference (416)	Study (619)	Significance	Relative Risk (95% Conf. Interval)
Learning disability	11 (2.60%)	66 (10.7%)	P<0.001	4.03 2.16 - 7.54)
Retained in the Same class	56 (13.50%)	126 (20.40%)	P=0.0055	1.51 1.13 - 2.02)

Table 9 shows the prevalence of scholastic backwardness (learning disability) as reported by the class teacher and results of annual examinations. It is seen that the prevalence of poor scholastic performance and the incidence of being detained into the same class was significantly higher in the study children as compared to the reference population.

Table 10 shows the performance of the two groups of children in a test called 'Draw A Man Test' which is a standard but preliminary test used to evaluate Intelligence Quotient (IQ) of children between 3-15 years of age. This is a simple screening test for IQ. The child is asked to draw a man as perceived by him and the scoring is done on the basis of standard laid down criteria. The test was administered to children up to 15 years of age in both groups and the results show that the proportion of children having low IQ was

significantly higher in the study group. The proportion of children having higher IQ was also lower in the study group. As an isolated test, it has very limited significance however, if the results of this test are seen in the light of the findings shown in previous table, it assumes greater significance.

Table 10. Results of IQ evaluation by Draw A Man Test in Study and reference population.

IQ range	Reference	Study	Significance
<84	155/239 (64.90%)	398/511 (77.90%)	P<0.001
84-115.99	68/239 (28.50%)	107/511 (20.90%)	
116+	16/239 (6.70%)	6/511 (1.20%)	

Table 11. Behaviour in the class as reported by the class teacher.

Behaviour	Reference (416)	Study (619)	Significance
Aggressive	0/416 (0.00%)	8/619 (1.30%)	P=00012
Arrogant	1/416 (0.20%)	11/619 (1.80%)	
Restless	0/416 (0.00%)	2/619 (0.30%)	
Normal	415/416 (99.80%)	598/619 (96.60%)	

Table 11 shows abnormal behaviour as reported by the teachers in two groups. The prevalence of arrogant and aggressive behaviour and restlessness were higher in the study group as compared to the reference population. The overall prevalence of these behavioural abnormalities was significantly higher in study group as compared to reference population. The findings reported in tables 5, 6 and 7 taken together signify that in the study

group the children are having a number of functional abnormalities of the nervous system which though not severe enough to prevent them from attending school, may interfere with their optimal intellectual development and total personality.

Table 12 Prevalence of seizure disorders in study and control subjects according to sex.

		Female	Male
Epilepsy/seizure disorders	Reference	1 /183 (0.55)	4/ 233 (1.72)
	Study	5 /258 (1.94)	4/ 361(1.10)
Febrile convulsion	Reference	3 /183 (1.64)	1/ 233(0.43)
	Study	4 /258 (1.55)	4/ 361(1.11)
Any of the above	Reference	4 /183 (2.19)	5/ 233(2.15)
	Study	9/258 (3.49)	9/ 361(2.49)

Figures In Parenthesis Are Percentages.

Table 12 shows the prevalence of seizure disorders in study and reference population. The diagnosis of epilepsy or seizure disorder was made by the examining pediatrician only in those cases where there was a clear-cut history suggesting the diagnosis. The history of convulsions regularly associated with fever has been classified as febrile convulsions. The prevalence of epilepsy was higher in study girls, however, the differences were statistically non-significant.

Congenital abnormalities: Table 13 shows the prevalence of various congenital abnormalities in study and control subjects. The overall prevalence of congenital abnormalities was significantly higher in the female in study group as compared to the control female. The prominent abnormalities were congenital heart diseases and skeletal abnormalities. The diagnosis of congenital heart disease was based on clinical findings and the examining pediatricians have suggested confirmation diagnosis by echocardiography and other investigations. The abnormalities of testes like Cryptorchidism and hydrocele were reported exclusively amongst the study subjects. The former abnormality is

suspected to be the result of exposure to the environmental oestrogens of the mother during the pregnancy.

Table 12 Prevalence of seizure disorders in study and control subjects according to sex.

	Group	Female	Male
Congenital hydrocele	Reference	0 /183 (0.00)	0/ 233 (0.00)
	Study	0 /258 (0.00)	4/ 361 (1.11)
Undescended Testes (Cryptorchidism)	Reference	0 /183 (0.00)	0/ 233 (0.00)
	Study	0 /258 (0.00)	2/ 361(1.55)
Congenital Inguinal Hernia	Reference	0 /183 (0.00)	1/ 233(0.43)
	Study	0/258 (0.00)	1/ 361(0.28)
Macrocephaly	Reference	0 /183 (0.00)	1/ 233(0.43)
	Study	0/258 (0.39)	0/ 361(0.00)
Minor malformation	Reference	0 /183 (0.00)	1/ 233 (0.43)
	Study	0/258 (0.00)	0/ 361(0.00)
Congenital heart disease	Reference	1 /183 (0.55)	4/ 233 (1.72)
	Study	9/258 (3.49)	2/ 361 (0.55)
Cerebral Palsy	Reference	0 /183 (0.00)	0/ 233 (0.00)
	Study	0/258 (0.00)	1/ 361 (0.28)
Congenital skeletal disorder	Reference	2 /183 (1.09)	1/ 233 (0.43)
	Study	5/258 (1.94)	3/ 361 (0.83)
Congenital Cataract	Reference	0 /183 (0.00)	1/ 233 (0.43)
	Study	1/258 (0.39)	2/ 361(0.55)
Congenital retinopathy	Reference	0 /183 (0.00)	0/ 233 (0.00)
	Study	1/258 (0.39)	1/ 361(0.28)
Any Congenital abnormality	Reference	2 /183 (1.09)	8/ 233 (3.43)
	Study	15/258 (5.8)	14/ 361 (3.88)
Relative Risk (95% C.L.)		5.32 (1.23–22.98) ($p<0.05$)	1.13(0.48–2.65) NS

Figures in parenthesis apre percentages

*One male and one female in study group and one female in reference population showed two congenital abnormalities.

Reproductive Development:

Female Subjects:

In most of the girls the definite time of menarche could not be elicited. Therefore comparison is made between distribution of menstruating girls according to age. It is seen from table 14 that the proportion grils who had attained menarche was higher in study groups 11,12,13,14 and 15 years. Overall these differences are statistically significant. It may be noted that 5 girls above 16 years in the study group and one girl in control group who had not attained menarche need to be investigated

Table 14 comparison of age wise distribution of menstruating girls.

Age Group (completed years)	Reference	Study
Below 10	0 / 89 (0.00)	0 / 71 (0.00)
10	0 / 11 (0.00)	0 / 18 (0.00)
11	0 / 16 (0.00)	3 / 29 (10.3)
12	1 / 21 (4.8)	8 / 35 (22.9)
13	4 / 16 (25.0)	13 / 25 (52.0)
14	11 / 17 (64.7)	16 / 26 (61.5)
15	5 / 8 (62.5)	21 / 23 (91.3)
16	4 / 4 (100.0)	17 / 21 (81.0)
17 and above	0 / 1 (0.00)	9 / 10 (90.0)

Odds Ratio: 1.56

95% Confidence Interval 1.10 – 2.21

$p<0.05$

Table 15 Prevalence of Menstrual Cycle Disorders

	Reference 25	Study 87	Significance
Menstrual period more than 4 days	5 (20.00%)	52 (59.77%)	P = 0.001
Excessive flow	1 (40.00%)	11 (12.60%)	NS
Irregular cycle	0 (0.00%)	11 (12.60%)	NS
Excessive flow or Irregular cycle	1 (4.00%)	19 (21.80%)	P=0.041

Table 15 shows that the prevalence of menstrual disorders was significantly higher in the study group.

Table 16. Female subjects showing SMR grade 2 or more for breast development and or public hair

Age Group (completed years)	Reference	Study
5 – 9	0 / 67 (0.00)	0 / 71 (0.00)
10	0 / 2 (0.00)	2 / 14 (14.29)
11	0 / 1 (–)	3 / 5 (60.00)
12	2 / 3 (66.67)	6 / 8 (75.00)
13	0 / 1 (0.00)	2 / 3 (66.7)
14	0 / 1 (0.00)	2 / 2 (100.0)
15	1 / 1 (100.0)	1 / 1 (100.0)
16	0 / 0 (–)	4 / 4 (100.0)

Table 16 shows the number of female subjects showing SMR grade 2 or more for breast development and/or public hair. It may noted that limited number of girls consented for SMR evaluation. Although the number of girls attaining puberty earlier is higher in study group, definite conclusions can not be drawn because of small number in each group.

Table 17 . Age-wise distribution of skin fold thickness in control and study groups

	Female						
	Reference			Study			
Age Group (completed years)	Mean	S.d.	No	Mean	S.d.	No	Significance
5 – 9	9.0227	2.2282	88	8.3429	2.5981	70	NS
10	10.0000	2.0000	10	7.8235	2.7440	17	P=0.039
11	8.6250	3.7749	16	8.7586	1.9938	29	NS
12	7.9500	2.5021	20	9.7647	3.1340	34	P=0.032
13	8.3750	4.0641	16	10.6800	2.7647	25	p=0.037
14	8.2353	2.7733	17	13.6923	3.9575	26	p=0.001
15	9.3750	3.0208	8	11.3333	3.1038	21	NS
16	8.2500	1.5000	4	14.0000	5.4290	20	P=0.001
17 and above	10.0000		1	15.1250	2.9665	8	
	Male						
	Reference			Study			
Age Group (completed years)	Mean	S.d.	No	Mean	S.d.	No	Significance
<5				11.0000		1	
5-9	8.8947	4.7164	95	7.7108	2.2821	83	P=0.039
10	8.1250	2.1328	24	6.7179	1.7464	39	P=0.006
11	8.1143	3.0270	35	7.6757	2.1089	37	NS
12	7.8235	2.4808	17	7.8537	2.1396	41	NS
13	7.6910	2.3974	21	8.3864	2.9900	44	NS
14	6.6111	1.19140	18	7.6667	2.2032	33	NS
15	6.9231	1.4412	13	7.2927	2.2276	41	NS
16	7.0000	2.0000	6	7.5385	3.0988	13	NS
17				7.8667	1.9223	15	
18				7.6667	2.0656	6	
19	6.0000	.0000	2	9.7500	6.2915	4	NS

Table 17 shows the average skin fold thickness in study and reference population according to age and sex. In the female subjects the average skin fold thickness is higher in the age groups 12,13, 14, 15 and 16. These differences are statistically significant in the age groups 12, 13, 14, and 16. This age groups belong to the pre-pubertal and pubertal period. In male subjects, the average thickness of the skin fold was significantly lower in the age groups Below 10 and 10 years. In addition to nutrition, the skin fold thickness particularly in female subjects is affected by the levels of sex hormones.

Male Subjects:

Table 18 shows number of male subjects showing SMR grade 2 or more for pubic hair, penis and testes. It is seen that in most of the age groups, the number of boys showing SMR changes were lower in study group as compared to reference population. The overall differences were statistically significant. Figures 1, 2 and 3 illustrate average S MR scores for pubic hair, penis and testes for each age group. The average SMR scores were lower in the study group as compared to reference population.

Table 18. Male subjects showing SMR grade 2 or more for public hair, penis and testes.

	Public Hair		Penis		Testes	
Age Group (completed years)	Reference	Study	Reference	Study	Reference	Study
5-9	1 / 86 (1.16)	0 / 81 (0.00)	1 / 86 (1.16)	2 / 81 (2.46)	1 / 86 (1.16)	1 / 81 (1.23)
10	3 / 17 (17.65)	2 / 27 (7.41)	7 / 17 (41.18)	9 / 27 (33.33)	5 / 17 (29.41)	9 / 27 (33.33)
11	4 / 17 (23.53)	2 / 21 (9.52)	6 / 17 (35.29)	5 / 21 (23.81)	5 / 17 (29.41)	6 / 21 (28.57)
12	9 / 12 (75.00)	1 / 19 (5.26)	9 / 12 (75.00)	5 / 21 (26.32)	5 / 17 (66.67)	6 / 21 (31.58)
13	11 / 13 (84.62)	7 / 17 (41.18)	11 / 13 (84.62)	11 / 17 (64.71)	11 / 13 (84.62)	11 / 17 (64.71)
14	14 / 15 (93.33)	10 / 12 (83.33)	14 / 15 (93.33)	9 / 12 (75.00)	14 / 15 (93.33)	10 / 12 (83.33)
15	8 / 9 (88.89)	7 / 11 (63.64)	9 / 9 (100.0)	9 / 11 (81.82)	8 / 9 (88.89)	9 / 11 (81.82)
16	4 / 5 (80.0)	2 / 3 (66.67)	5 / 5 (100.0)	1 / 3 (33.33)	5 / 5 (100.0)	1 / 3 (33.33)
Odds Ratio	2.66		1.61		1.43	
95% Confidence Interval	1.77 – 3.99		1.17 – 2.21		1.04 – 1.97	
Statistical Significance	P<0.01		P<0.01		P<0.05	

Figures in parenthesis indicate percentage.

Figure 1 Mean (SEM) of SMR Score for Pubic Hair

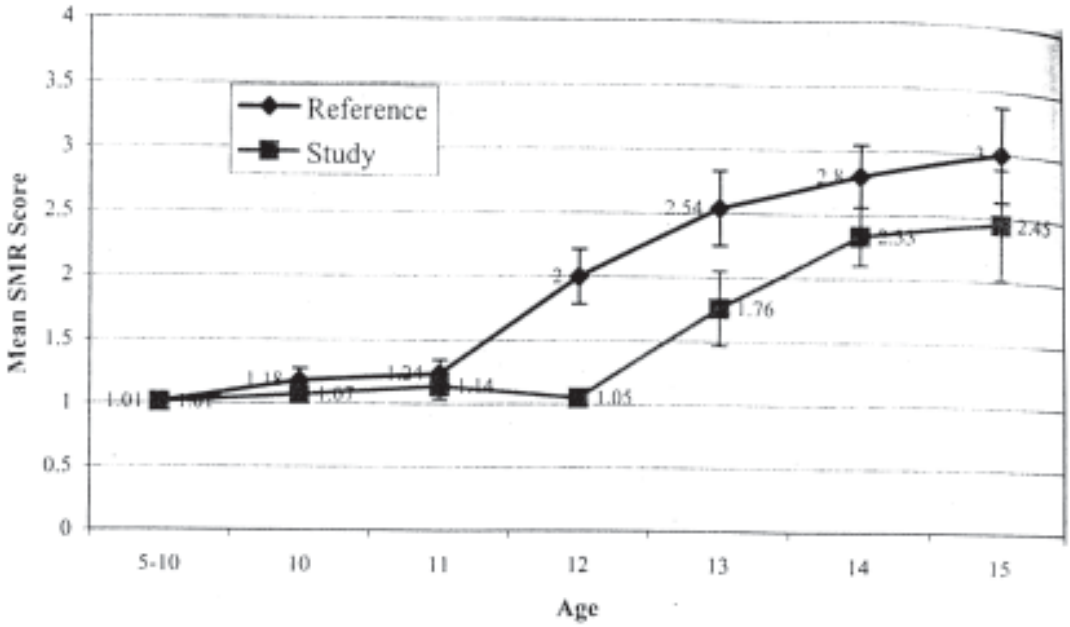


Figure 2 Mean SMR Score for Penis according to age

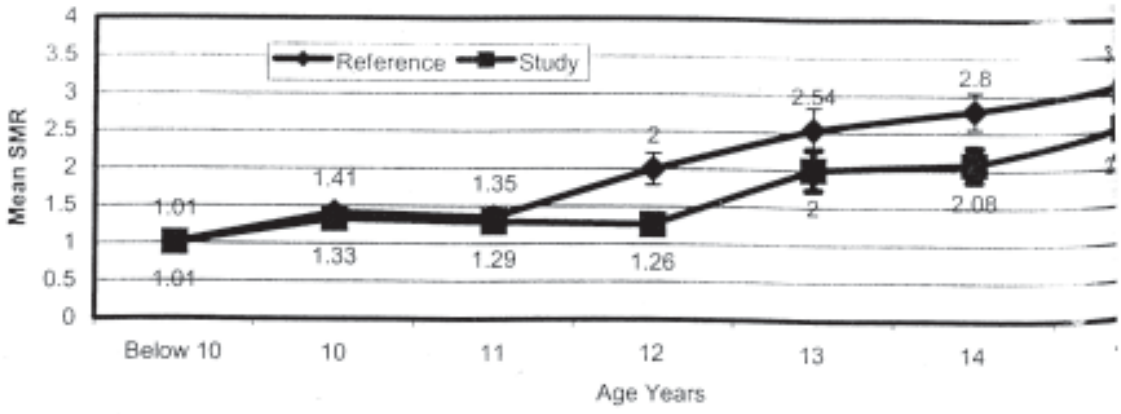
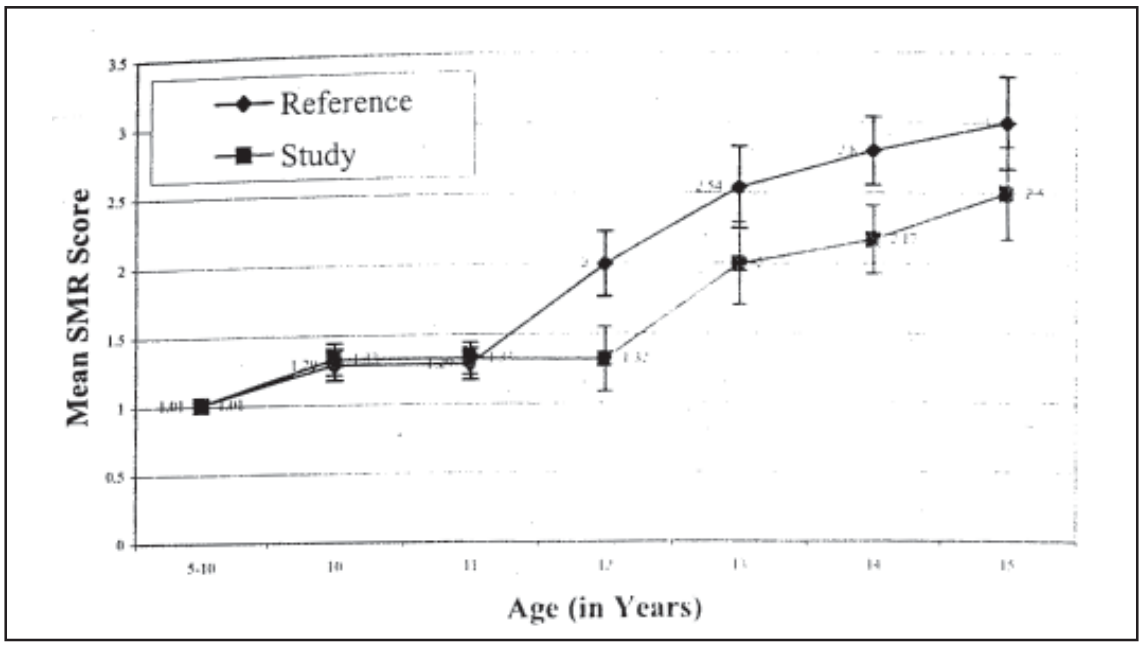


Figure 3 Mean (SEM) Score for testicular development



Other Abnormalities:

Table 15 shows the prevalence of goitre in the study and control subjects. It may be noted that all cases of goire were classified as physiological goitre occurring at the onset of puberty. The significance of higher prevalence of this physiological variation in the study girls is not clear and may be investigated in further details.

Table 19 Prevalence of goitre

	Female	Male
Reference	2 / 183 (1.09)	1 / 233 (0.43)
Study	14 / 258 (5.41)* P=0.032978	0 / 361 (0.00)

Figures in parenthesis indicate percentage.

Table 20 Prevalence of hypersensitivity disorders

		Female	Male
Bronchial asthma	Reference	2 / 183 (10.93)	15 / 233 (6.44)
	Study	21 / 258 (8.14)	28 / 361 (7.76)
Allergic dermatitis	Reference	2 / 183 (1.09)	3 / 233 (1.29)
	Study	6 / 258 (2.33)	3 / 361 (0.83)
Eczema	Reference	1 / 183 (0.55)	2 / 233 (0.86)
	Study	1 / 258 (0.39)	1 / 361 (0.28)
Any of the above	Reference	23 / 183 (12.57)	20 / 233 (8.58)
	Study	27 / 258 (10.47)	32 / 361 (8.86)

Table 20 shows the prevalence of various hypersensitivty disorders such as bronchial asthma, allergic dermatitis and eczema in study and reference population. It is seen that the prevalence of these abnormalities are comparable in study and reference populations.

Table 21. Prevalence of past history of jaundice

	Female	Male
Reference	0 / 183 (0.0%)	3 / 233 (1.3%)
Study	10 / 258 (3.9%) P=0.0064	8 / 361 (2.22%)

Figures in parenthesis indicate percentage.

Table 21 shows the prevalence of past history of jaundice in study and reference population according to the sex. The prevalence higher in both male and female study population and the differences are statistically significant in female subjects.

Table 22. Incidence of Chromosomal aberration and sister chromatic exchange per cell in study and reference population (Chromosomal aberration per cell)

	Reference			Study		
	Mean	S.d.	No	Mean	S.d.	No
Female	0.0387	0.0201	3	0.0401	0.0085	11
Male	0.03200	0.0109	5	0.0393	0.0130	18
Total	0.0345	0.0140	8	0.0396	0.0113	29
Sister Chromatic Exchange						
	Reference			Study		
	Mean	S.d.	No	Mean	S.d.	No
Female	6.409	0.411	3	6.374	0.230	8
Male	6.051	0.572	5	6.395	0.215	13
Total	6.185	0.519	8	6.387	0.215	21

Table 22 shows incidence of Chromosomal aberrations and sister chromatic exchange per cell in study and reference population. The incidence of these abnormalities are comparable in study and control subjects. It may be noted that the chromosomal abnormalities like dicentric chromosome and chromatic exchange (figure 4) were observed in two each of the study subjects. These abnormalities are rarely observed in healthy children.

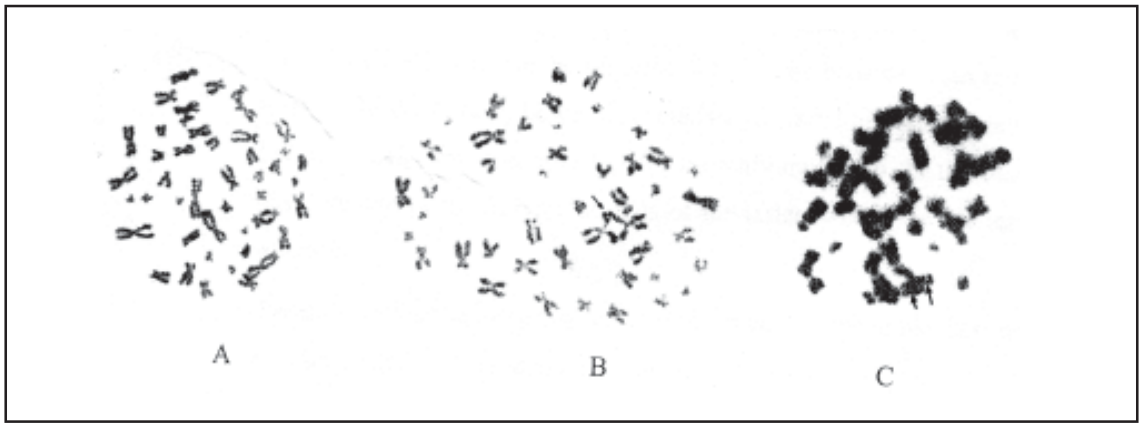


Figure 4. Chromosomal aberrations in phytohemagglutinin stimulated peripheral leucocytes A. Normal, B. Chromatic exchange C. Dicentric chromosome

Hormone Estimations:

Hormones play an important role in growth and development, therefore the hormonal profile study is essential in any investigation related to growth and developments. Following is the brief description of the hormones related to growth and development.

Thyroid Hormones: Triiodothyronine (T_3) and tetraiodothyronine (thyroxin or T_4) are secreted by the thyroid gland and controls basal metabolic rate, growth and development. Levels of T_3 and T_4 are regulated by the thyroid stimulating hormone (TSH) secreted by the pituitary gland through a feed back mechanism. Deficiency of these hormones lead to poor physical and mental development in children.

Growth Hormone: Growth hormone (GH), essential for normal musculoskeletal growth in children of both sexes is secreted by pituitary in short pulses.

Prolactin is a single-chain protein hormone closely related to growth hormone. Prolactin stimulates the mammary glands for its development and milk production. Its role in male is not understood clearly.

Luteinizing Hormone (LH) and Follicle Stimulating Hormone (FSH): LH and FSH are two gonadotropic hormones (*i.e.*, hormones concerned with the regulation of the activity of the gonads, or sex glands) produced by the pituitary gland. FSH, a glycoprotein operating in conjunction with LH, stimulates development of the graafian follicle, a small egg-containing vesicle in the ovary of the female mammal. In the male, it promotes the development of the tubules of the testes and the differentiation of sperm. Though in the male the presence of FSH is necessary for the maturation of spermatozoa, additional FSH may not be required for months because testosterone can maintain this activity. In the female, however, there is a rhythmic or cyclical, increase and decrease of LH and FSH, which is essential for monthly ovulation. In the male, LH stimulates the development of the interstitial cells of the testes, which secrete testosterone, a male sex hormone.

Testosterone is responsible for development of the male sex organs and masculine characteristics such as facial hair and deepening of the voice.

Estrogen and Progesterone: These two hormones are secreted by the ovaries and are important for the cyclic changes occurring during menstrual cycle. The most potent natural estrogen is estradiol. The levels of these hormones vary widely with age and

during the menstrual cycle. Many synthetic chemicals including pesticides have been shown to mimic the action of estrogens.

Hormone Levels in Serum:

Thyroid Hormones: Table 23 shows the mean (± SD) levels of Tri-iodo thyronine (T_3), thyroxin (T_4) and thyroid stimulating hormone (TSH) in two groups of subjects according to sex. Levels of T_3 and TSH were higher in female subjects as compared to male subjects in both the groups. However there were no statistically significant differences in the mean serum levels of these hormones when compared to the subjects of the same sex in two groups. Serum T_3, T_4 and TSH levels were available in five of the seventeen subjects who had goiter and they were normal.

Table 23. Levels of Thyroid Hormones in Study and reference groups Subjects according to sex.

	Reference Group		Study Group	
	Male	Female	Male	Female
T3 (ng/dl)	1.52 ± 0.48 (47)	1.80 ± 0.34 (34)	1.62 ± 0.32 (96)	1.89 ± 0.36 (63)
T4 (µg/dl)	13.0 ± 2.63(46)	11.4 ± 2.88(34)	13.7 ± 2.85(96)	11.4 ± 3.81 (63)
TSH mu/L	0.95 ± 0.30 (48)	1.58 ± 0.92 (34)	1.01 ± 0.33 (93)	1.46 ± 0.78 (60)

Growth Hormone: Table 24 shows the levels of growth hormone in study and reference groups of subjects according to sex. The levels were higher in female subjects as compared to the male in both groups. However, there were no significant differences in the levels of this hormone between study and reference groups when compared in same sex.

Table 24. Levels of Growth Hormone (ng/L) in Study and reference groups Subjects according to sex.

Sex	Reference Group	Study Group
Female	1.27 ± 2.24 (38)	1.41 ± 2.19 (38)
Male	0.55 ± 0.80 (47)	0.52 ± 1.09 (69)

Prolactin: Mean and SD levels of serum Prolactin levels are shown in table 25.

The levels of this hormone were higher female subjects as compared to the ma*** both groups. However, there were no significant differences in the levels of this hormones between study and reference groups.

Table 25. Levels of Prolactin (µg/L) in Study and reference groups Subjects according to sex.

Sex	Reference Group	Study Group
Female	13.8 ± 8.60 (38)	12.3 ± 4.95 (38)
Male	7.47 ± 6.12 (47)	8.88 ± 4.13 (69)

Table 26. Mean ± SD Levels (IU/L) of Luteinizing hormone (LH)

	Reference Group	Study Group
Female	1.66 ± 1.83 (38)	2.08 ± 1.81 (38)
Male	0.27 ± 0.47 (47)	0.62 ± 0.74 (69)

Table 27. Mean ± SD Levels (IU/L) of FSH

	Reference Group	Study Group
Female	1.90 ± 1.13 (38)	2.05 ± 1.51 (38)
Male	0.66 ± 0.79 (47)	0.93 ± 1.06 (69)

Table 26 depicts mean ± SD Levels (IU/L) of LH according to age (completed years) and sex in two groups. The mean levels generally increases with age. LH levels are higher in the female subjects as compared to the male in both the groups. The levels of this hormone were higher in the study subjects in the same age group in both the sexes. (table 27). ANOVA (analysis of variance) showed that the levels were significantly higher in the study group after taking age into account.

Table 28 depicts mean ± SD Levels (IU/L) of testosterone according to age (completed years) in two groups. The levels of testosterone were lower in the study group as compared to reference population in the same age group. The multiple regression analysis showed that testosterone levels are significantly related to age and residential area (i.e. stay in study or reference village).

Table 28 Mean ± SD Levels (ng/ml) of Testosterone according to age (completed years) and sex in two groups

	Reference Group	Study Group
BELOW 10	0.03 ± 0.00 (1)	0.02 ± 0.00 (2)
10	0.12±0.10(4)	0.06±0.05(5)
11	0.16±0.15(15)	0.14±0.12(8)
12	0.24±0.16(5)	0.29±0.33(12)
13	1.32 ± 1.02 (5)	0.73 ± 1.20 (16)
14	1.69±1.32(7)	1.17±1.18(8)
15	1.30±1.44(6)	1.93±1.10(8)
16	5.65±4.18(3)	2.36±2.14(2)
17	-	4.80±1.06(5)
18and above	5.98 ± 0.00 (1)	3.31 ± 0.58 (2)

Table 29 and 30 shows the levels of progesterone and estradiol in study and reference groups and they were higher in the study group

Table 29 Mean SD ± levels of Progesterone (ng/dl)

	Reference Group	Study Group
Female	0.12 ± 0.46 (38)	1.43 ± 4.65 (37)

Table 30 Mean SD ± levels of Estradiol (Pcg/ml)

	Reference Group	Study Group
Female	32.6 ± 53.2 (38)	38.3 ± 63.2 (37)

Table 31 shows statistical significance of regression coefficients (b value) of SMR and testosterone levels by multiple regression analysis in Study and reference groups of male subjects. It may be noted that the regression coefficient for public hair, testes, penis and testosterone were positively related to the age meaning that the age has significantly positive effect. However, the regression analysis taking into account the place of residence (designated as group in the table) showed the negative effect of stay in study area on regression co-efficient which was statistically significant. These findings seem together indicate the slower rate of SMR in study area boys associated with lower levels of testosterone in the serum.

Table 31 Multiple regression equation parameters in SMR and Testosterone

Study Parameter	**Public hair**		**Testes**		**Penis**		**Testosterone**	
	b	S.S	b	S.S	b	S.S	b	S.S
Age	0.44	P<0.001	0.40	P<0.001	0.40	P<0.001	0.60	P<0.001
Group*	-0.56	P<0.001	-0.43	P<0.001	-0.48	P<0.001	-0.58	P<0.001
Constant	-3.60	P<0.001	-3.08	P<0.001	-3.03	P<0.001	-6.48	P<0.001

* Study or reference group

Interpretation: Most of the hormones are secreted in short pulses and therefore their levels vary during different times of the day in the same individual. Moreover, their levels vary

with the age and sex. The levels of female hormones (estrogen, progesterone, LH and FSH) depend on the time of menstrual cycle. It is therefore important to analyze and interpret after considering age and sex.

The analysis of the serum hormone levels showed following features.

The levels of thyroid hormones, growth hormone, prolactin, FSH and LH were higher in female subjects as compared to the male subjects in both the groups. These hormones are related to somatic and reproductive growth. During adolescent period, there is a growth spurt in both sexes. In female subjects this spurt is earlier and the observed differences could be explained on the basis of age and composition of the subjects.

Information about families:

Ethnicity of the study population: The study population in study and reference areas consisted of a mixture of tribal and non-tribal population. Majority of them (about 75%) were Hindus and the rest were Muslims and Christians. The predominant cast amongst Hindus was Nayak (mostly tribal), Brahmin, Moolya and Harijans.

Occupations: There were no striking differences in the pattern of occupations in study and reference populations. About 70% of the men worked as farmers. Other major occupations were labour work, shop keeping, government service, bidi rolling etc. In addition to domestic work majority of the women in study and reference population were engaged in bidi rolling. About 40% of the families in reference area and 51% in study area had their own land. The major crops in both the areas were arecanut, cashew, paddy, coconut, banana and pepper.

Sources of water for Domestic Purpose: The annual rain fall in the area is about 140" giving rise to a number of options for water sources. Table 32 shows family wise major source of drinking water. The commonest source of water for drinking and cooking purpose was dug well in study population. Surangam is another source of water, which was found more often in study population. Surangam literally means a tunnel where water percolating through a cleft in the rocks is tapped by a pipe. Some rarely used sources of water used by study population were tap water and pond water.

Table 32 Family wise source of Drinking and Cooking water

Water Source	Study area		Reference area	
	Number	%	Number	%
Dug well	278	77.4	301	93.5
Surangam	41	11.4	8	2.5
Bore well	9	2.5	6	1.9
Pipe	3	0.8	-	-
Pond	3	0.8	-	-
No information	25	7.3	7	2.2
Total	358	100.0	322	100.0

Health Problems in Cattle: Some of the reports in the press have shown congenital malformations and other problems in the cattle. Our preliminary inquiry in this matter is showed that no significant differences in health problems were reported by the subjects in the two areas. (table 33).

Age and sex wise composition of the subjects belonging to the families of the students examined is depicted in table 34. In both the groups, children and young adults (below 20) consituted the major group.

Table 33. Health Problems in cattle belonging to the families.

	Reference population	Study Population
Families having cattle	182 (56.50%)	223 (62.30%)
Convulsions	3 (1.60%)	4 (1.80%)
Birth deformity	3 (1.60%)	3 (1.30%)
Infertility	4 (2.20%)	1 (0.40%)
Abortions	0 (0.00%)	3 (1.30%)

Table 34. Age and sex wise distribution of subjects belonging to the families of the students examined

Age group	Male				Female			
	Reference population		Study population		Reference population		Study population	
	No.	%	No.	%	No.	%	No.	%
Below 10	243	32.9	229	25.9	225	22.8	188	16.6
11-20	340	46.1	404	45.7	297	30.0	322	28.5
21-30	82	11.1	121	13.7	151	15.3	204	18.0
31-40	23	3.1	40	4.5	187	18.9	202	17.9
41-50	7	0.9	29	3.3	70	7.1	95	8.4
51-60	12	1.6	15	1.7	25	2.5	50	4.4
Above 60	31	4.2	46	5.2	34	3.4	70	6.2
Total	738	100.0	884	100.0	989	100.0	1131	100.0

Bronchial Asthma: Table 35 shows age wise prevalence of bronchial asthma cases in study and reference populations. The overall prevalence of bronchial asthma in two groups is comparable.

Dermatitis: Table 36 depicts age wise distribution of cases of dermatitis as reported by the family members. The overall prevalence of dermatitis was higher in the reference population. The difference were statistically not significant.

Convulsive Disorders: Table 37 shows age wise prevalence of cases of convulsive disorders in two groups as reported by the family members. In all the age groups, the prevalence of convulsive disorder was higher in the study group. These differences are particularly more obvious in age groups “10 years and below” and “11-20 years”. The differences in overall prevalence were statistically significant ($p<0.01$).

Table 35. Distribution of cases of bronchial asthma according to age in study and reference group families

AGE GROUP	Reference population	Study Population
Below 10	18/469 (3.8)	8.417 (1.9)
11-20	7/637 (1.1)	15/726 (2.1)
21-30	4.233 (1.7)	2/325 (0.6)
31-40	5/210 (2.4)	7/242 (2.9)
41-50	2/77 (2.6)	7/124 (5.6)
51-60	0/37 (0.)	3/65 (4.6)
ABOVE 60	5/65 (7.7)	13/116 (11.2)
Total	41/1728 (2.4)	55/2015 (2.7)

Table 36. Agewise distribution of cases of dermatitis as reported by family members

AGE GROUP	Reference population	Study Population
Below 10	3 (0.6)	4 (1.0)
11-20	10 (1.6)	2 (2.03)
21-30	2 (0.9)	2 (0.6)
31-40	3 (1.4)	1 (0.4)
41-50	1 (1.3)	1 (0.8)
51-60	1 (2.7)	0
ABOVE 60	0	2 (1.7)
Total	20 (1.2)	12 (0.6)

Table 37. Agewise distribution of cases of convulsive disorders in study and reference group

AGE GROUP	Reference population	Study Population
Below 10	2 (0.4)	4 (1.)
11-20	3 (0.5)	9 (1.2)
21-30	1 (0.4)	5 (1.5)
31-40	0	3 (1.2)
41-50	0	1 (0.8)
51-60	0	2 (3.1)
ABOVE 60	0	0
Total	6 (0.3)	24 (1.2)

Table 38. Agewise prevalence of subnormal or delayed mental development as reported by family members

AGE GROUP	Reference population	Study Population
Below 10	4 (0.9)	9 (2.2)
11-20	1 (0.2)	9 (1.2)
21-30	1 (0.4)	5 (1.5)
31-40	1 (0.5)	2 (0.8)
41-50	0	0
51-60	0	1 (1.5)
ABOVE 60	0	0
Total	7 (0.4)	26 (1.3)

Table 38 shows age wise prevalence of subnormal or delayed mental development reported by family members. In all age groups the prevalence of these conditions are higher. These differences are particularly more obvious in age groups “10 years and below” and “11-20 years”. The overall prevalence was significantly higher in study group ($p<0.01$).

Interpretation of Results:

I. Endosulfan residues in environmental samples and serum samples:

1. Endosulfan residues were found in serum samples and environmental samples in the study as well as reference areas. However, the serum endosulfan residue levels were several times higher in study population as compared to the reference group and they were statistically highly significant. This signifies that the study population had much higher exposure than reference population. This is supported by our finding of endosulfan in water bodies and sediments only in the study area. However, it was found in soil samples in both areas, but the levels were higher in the study area. The translocation of endosulfan in water from the soil on the hilltops and subsequent exposure of subjects in study area could be easily explained on the basis of topography. This is supported by a report published by Frank et al. 1982 which says that endosulfan appeared in water samples throughout the year (outside the spray season); it entered water with storm runoff throughout the season because of its persistence in soil. The results of several laboratory and greenhouse studies indicate that α- and β-endosulfan are strongly adsorbed to soil. (Bowman et al. 1965; El Beit et al. 1981 a, b) and endosulfan has a half life of 60 -800 days in soil. (ATSDR, 2000).

2. Comparison of endosulfan residue levels reported by FIPPAT in March 2001, our study in Sept./Oct. 2001 and June 2002 show a significant fall in endosulfan levels in soil in study area. This indicates the gradual loss of endosulfan in the environment after cessation of aerial spray. The presence of endosulfan in soil in the reference area is most likely to be from manual spray.

3. The prominent residue was a endosulfan. This can be because of conversion of β endosulfan to α endosulfan. An experiment by Rice et al showed that when pure β endosulfan was allowed to equilibrate in the apparatus, the ratio of the β-isomer to the α-isomer in the gas phase became 8:92 at 20° C, suggesting that the β–isomer converts to the α-isomer (Rice et al. 1997). Presence of high proportion of α endosulfan in biological samples have been reported earlier also. For example, Novak and Ahmad (1989) found maximum tissue concentrations in mosquito fish (933 µg/kg; (α-isomer) exposed to 16 µg technical-grade endosulfan/ L for 2 hours.

4. The serum concentrations of endosulfan were much higher as compared to the residues in the environmental samples. This could be because of multiple so of environmental exposure which need to be explored. Another reason could accumulation of endosulfan in fatty tissue which may subsequently result dynamic equilibrium with blood as is reported with organochlorine compounds Until recently it was held that endosulfan does not accumulate in fatty tissue however, a recent study has shown that isomers and metabolites of endosulfan were detected in the fat of 30-40% of children hospitalized in agricultural regions of Spain, demonstrating that endosulfan accumulates in adipose tissue of children after presumably repeated dietary exposure (Olea et al. 1999).

5 Our major criteria for the selection of reference population was the similarity ethnic background, climate, food habits, occupations, crops and socio-economic status and absence of aerial spray of endosulfan. We therefore selected Meenja Panchayat area as control. As the endosulfan was also found in this population which was presumed to be not exposed to significant amounts of endosulfan, the term "control" used in first report has been replaced by the term reference group". Nevertheless, the endosulfan levels in the reference group were much lower as compared to the study population. This might have a small masking effect when two populations are compared.

II. Correlation between Various Endpoints in the study and Endosulfan Exposure:

Neurobehavioral problems, birth defects and delayed sexual maturation in males was found in study population as compared to the reference group. These type of effects are possible with endosulfan exposure as demonstrated by animal experiments and human studies as narrated below.

Neurobehavioral Problems: Nervous system is the target organ for endosulfan toxicity. Higher prevalence of learning disability and neurobehavioral disorders were found in study population. Paul et al. (1994) found significant increases in serotonin concentration in the cerebrum and midbrain of rats after 90 days of treatment with 2 mg/kg/day endosulfan, and in this study, spontaneous motor activity was significantly increased in the

treated animals. Furthermore, they also found a correlation between the increase in serotonin and inhibition of a learning paradigm.

Lakshmana and Raju (1994) also reported changes in the concentrations of dopamine, noradrenaline, and serotonin in various brain areas of endosulfan-treated rats. In this case, treated rats took 29 % more time to learn a behavioral task; however, it was not determined which neurotransmitter(s) change may have been responsible for the behavioral change.

Birth Defects: Daily administration of endosulfan at doses of 5 or 10 mg/kg/day during Gd 6-14 produced a statistically significant increase in the percentage of resorptions and skeletal variations in the fetuses (e.g., absent fifth sternebrae) (Gupta et al. 1978). Statistically significant skeletal variations (e.g., bipartite and misaligned sternebrae) were observed in fetuses following daily administration of doses of 0.66 mg/kg/day to pregnant rats during Gd 6-19 (FMC.' 1980)

Male Reproductive System: Garcia-Rodriguez et al. 1996 reported high frequency of cryptorchidism in the Granada region of Spain where there was extensive use of endosulfan. Endosulfan exposure levels were unavailable. Subsequent study reported endosulfan isomers and/or metabolites in adipose tissue of 20 of 50 children (40%) who were hospitalized in the Granada hospital for a variety of reasons (Olea et al. 1999), indicating that significant endosulfan exposures occurred in the region. A study by Singh and Pandey (1990) indicated a dose-related decrease in testicular testosterone, plasma testosterone, luteinizing hormone (LH), and follicular stimulating hormone (FSH) in groups of male Wistar rats orally administered endosulfan at 0, 7.5, or 10 mg/kg/day for 15 or 30 days. In addition, activities of steroidogenic enzymes and testicular cytochrome P450-dependent monooxygenases were depressed after the 30-day exposure at 7.5 mg/kg/day. All of the effects from 30 days of exposure were reversible during a 7-day recovery period, except for decreased testicular testosterone, which remained depressed; no recovery period was utilized fond the 15-day exposures. Reduced sperm count and altered testicular enzyme activities, indicating altered spermatogenesis, were reported in mature rats treated by gavage with 2.5 mg technical endosulfan/kg/day (the lowest dose tested), 5 days/week for 70 days (Singa et al. 1995). Additional effects seen at higher doses (5 and 10 mg/kg/day) included reduced intratesticular spermatid count and daily sperm production, and increased incidence of abnormal sperm. All of these effects were also observed in young male rats (3 weeks old) treated by gavage with 2.5 mg technical

endosulfan/kg/day (the lowest dose tested), 5 days/week for 90 days, suggesting that the younger animals were more sensitive than the older ones (Sinha et al, 1997). Altered spermatogenesis was also reported in male mice treated by gavage with 3 mg technical endosulfan/kg/day for 35 days (Khan and Sinha 1996).

Conclusions of the Study

The first report submitted in Jan. 2002, included data on the medical findings in children from study and reference areas and some of the results of endosulfan analysis in serum and environmental samples. In this final report in addition to the previous data, data of the families of the children from both the areas have also been included as also the results of analysis of environmental and serum samples. On the basis of our findings and as per the objectives defined in the beginning of the study, following are the main conclusions:

1. There is significantly higher prevalence of neurobehavioural disorders, congenital malformations in female subjects and abnormalities related to male reproductive system in the study group (Padre village, Enmakaje Panchayat) as compared to the reference group (Miyapavadu village of Meenja Panchayat).

2. Regarding the aetiological factors, responsible for these health problems, various factors were compared and it was found that the two groups differed mainly with respect to aerial spray of endosulfan. Therefore the most probable cause fond the health problems in the study area could be relatively high and continued exposures to endosulfan through various environmental media such as food, water, soil and air.

3. The physiography of Padre village has been a major factor responsible fond continued exposure of the population.

4. There is a close similarity between the spectrum of health effects observed in the study population and those described in animal experiments. This supports the hypothesis of endosulfan as a causative factor fond the endpoints observed in the study. It need to be stressed that the animal experiments were carried out with much higher dosages and the exposure was mostly acute or subacute. Animal toxicity studies are carried out to identify the target organs of toxicity and possible spectrum of effects. The effects of any chemical are determined by the dose, duration and the time of exposure. It has been demonstrated that much lower doses of toxicants may result in adverse health effects manifesting as functional or organic disorders in later life if the exposure takes place during the early developmental phase.

5. The detection of endosulfan residues in the reference area does not antagonize or hypothesis and in fact this may have masking effect on the observed health effects.

6. Our analysis of family data do not support the observations of dr. Mohan Kumar with respect to high incidence of cancers, asthma, dermatitis and and suicides. This should be investigated by studies involving larger populations.

Recommendations:

1. Considering the physiography of the study area, use of any pesticide should not be permitted in future. This recommendation should also apply to other crops grown on similar terrains in other parts of the country.

2. The possibility of endocrine disrupting effect of endosulfan observed in the study has great relevance to the health of the future generations. Considering the potentiality of grave consequences, the Principle 15 of the Rio - Declaration of the Earth Summit should be followed. This relates to the precautionary principle and emphasizes that lack of scientific certainty is no reason to postpone action to avoid potentially serious or irreversible harm to the environment.

3. Extensive epidemiological studies in the areas where endosulfan has been sprayed should be carried out to investigate occurrence of illnesses similar to those reported in Padre village.

4. High exposure groups such as workers engaged in formulations, application and manufacturing of endosulfan and areas with high usage of endosulfan should be studied to determine the long term health effects of the compound.

5. The populations included in the present study should be followed up for the detection of endocrine related cancers.

6. The pesticide industry must follow the "Cradle to Grave" policy with regard to pesticide, starting from manufacture to the end users.

7. There is a strong need for continued training and awareness of pesticide dealers, retailers and farmers to promote safe use of pesticides. Because pesticides are basically toxic chemicals, there is every likelihood of long term health effects unless, all measures of safe use are taken and all regulations are strictly implemented.

8. The affected persons should be provided relief.

9. Finally, we request the Commission to take note of the recommendations of Achyuthan Committee appointed by Government of Kerala. (Annexure 7).

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ANNEXURES

List of Annexures

Annexure 1

Report of visit of I.C.M.R. Team to Kasaragod District, Kerala State for preliminary investigation of the health hazards related to spraying of Endosulfan in the Cashewnet Plantations, from 9-11 August 2001

As per the directives of Director-General ICMR, the following team visited Kas District of North Kerala from 9-11 August, 2001.

Dr. H.N.Saiyed, Director, N.1.O.H., Ahmedabad

Dr. Aruna Dewan, DD(SG), N.I.O.H., Ahmedabad

Dr. H.R. Rajmohan, Officer-in-charge, R.O.H.C., Bangalore

Before and during the visit, the scientists scrutinized reports published in various popular and scientific magazines (India Today, 23 July, 2001, Down to Earth, February,2001) the national dailies (The Hindu, 22 July 2001), and local newspapers about diseases reported from various villages of Kasaragod taluka which are being associated with Aerial spray of Endosulfan on cashew nut plantations in the area. Scientific published data on Toxicity profile of Endosulfan was also obtained and scrutinized.

During the visit, the team had discussions with Shri C. K. Viswanathan, District Collector, Kasaragod District, Dr. Abdul Salam, District Medical Officer, and Venkatgiri, Deputy D.MO. Talks were also held with Shri Padre, Dr. Mohana Kumar, Dr. Sripathy Kajampady and other members of ESPAC (Endosulfan Spray Protest Action Committee). The team also met teachers of two schools situated in Vaninagar and Swarga village of Enmakaje Panchayath.

Information collected

1. Dr. Mohana Kumar, MBBS, who has been practicing in Vaninagar for 20 years, has noticed a high prevalence of cancers of different organs, neurological disorders like epilepsy, cerebral palsy, psychiatric disorders, congenital malformations and reproductive problems, asthma and skin diseases during the past 10 years among villagers living near the cashew nut plantations. He has been writing to various Medical Associations to get an answer. He has collected 10 years data from his own records which has been published in different

forms in newspapers and magazines. He thinks that the diseases are due to aerial spray of Endosulfan, which has been going on fond more than 25 years.

2. Shri padre gave copies of reports which he had published way back in 1981 on congenital malformations of limbs noticed in cattle and also gave electronic copies of some recent documentaries prepared by a group from Manipal and another by a German group.

3. The school teachers of Vaninagar (Primary school 1-4th standard, Kannada medium) showed many children who were either mentally or physically handicapped. 50% of the children attending the school were tribals called "Naiks". They also showed minutes of the School Resource Group (SRG) meeting held on 3-I-2000 where it was written that the teachers were perplexed that students coming from the plantation side seemed to have low IQ, frequent illness and many had physical deformaties. It was also remarked by one teacher that in the secondary school, the results of 10th standard examination have been very poor in the last few years.

4. In the school at Swarga (1-7th Standard) , there were no physically abnormal children but there were many children with low IQ.

5. The District Collector and D.M.O. were very much concerned about the health problems in the Taluka. The State Government had asked the DMO to undertake a health surveillance study in the affected areas but the funds provided were very meager.

Conclusions:

After this preliminary visit and first hand discussions with various authorities, and available information on Endosulfan, it is felt that there is a need to undertake a well designed epidemiological study to find out the disease pattern in the affected areas and compare the same with a control village. The diseases observed do not fall in any specific category, but they could be linked to some factors causing genotoxicity. The causative agent may or may not be endosulfan or it may be something in addition to or other than endosulfan.

Future Plan of Action:

The I.C.M.R. is planning to undertake a cross-sectional Environmental Epidemiological Study, through its National Institute of Occupational Health, Ahmedabad. This study will be carried out to investigate the disease pattern in the affected villages and control population.

The study will be carried out in villagers, school children and plantation workers, and is expected to be completed within a period of a months.

It is requested that the National Human Rights Commission may write to the concerned Health authorities of the State to provide fullest co-operation to the I.C.M.R. to undertake this study.

Annexure 2 (Proforma for examination of School Children)

National Institute of Occupational Health

(Indian Council of Medical Research)

Health check-up for school children in Kasaragode

Part I

Date of Interview Individual ID

Name

Date of Birth (Cross check from school record)

Age (If date of birth is not available)

Family ID

Sex Male Female

Name of the School _____

_____

Standard in which studying

Have you been retained in any class Yes No

If Yes, give details _____

Residential Address (Name of the village & Location of the house in the village)

_____

_____

Have you ever worked in the cashew nut plantation during your vacation or otherwise?

Yes No

If Yes

year	Duration	
	Months	Days

Clinical history

The examining physician should record the presence or absence of each sympton and in the duration and explore further history to give any provision/possible diagnosis for each symptom complex after clinical examination.

Present History

Do you suffer from any of the following symptom

Symptom	Yes	Duration		
		Years	Months	Days
Cough	☐			
Expectoration	☐			
Breathlessness	☐			
Palpitation	☐			
Convulsions	☐			
Frequent cold and coughs	☐			
Jaundice	☐			
Skin Problem	☐			
Any other symptom	☐			

Past History

Did you suffer from any of the following symptom

Symptom	Yes	Duration		
		Years	Months	Days
Cough	☐			
Expectoration	☐			
Breathlessness	☐			
Palpitation	☐			
Convulsions	☐			
Frequent cold and coughs	☐			
Jaundice	☐			
Skin Problem	☐			
Any other symptom	☐			

Describe any other symptom if present try also to give probable diagnosis

_____

_____

_____

History of hospitalization

Have you ever been hospitalized?

No Yes

If yes, the reason for hospitalization

Duration of hospitalization Name of the hospital

Any case papers available Give details

PHYSICAL EXAMINATION

ANTHROPOMERTRY

Height (Cm.) ☐☐☐

.Upper Segment (Cm.) ☐☐☐

.Lower Segment (Cm.) ☐☐☐

.Weight (Kg.) ☐☐☐

Head circumference (Cm.) ☐☐☐

Skin fold thickness over tricepts (mm). ☐☐☐

.Arm Span (Cm.) ☐☐☐

PHYSICAL EXAMINATION

B.P. Systolic ☐☐☐ Diastolic ☐☐☐

Anemia ☐

Oedema ☐

Nails Teeth

Lymphadenopathy Eyes

Tongue Nails

Respiratory system

Normal Abnormal If abnormal, Give details below

_____

_____

Cardiovascular system

Normal Abnormal If abnormal, Give details below

_____

_____

Skin

Evidence of allergy No Yes Give details

_____

Mental status (IQ)

Normal Abnormal Give details

Sexual Maturity Rating

PROFORMA FOR GIRLS

A. Menstrual history:
Do you menstruate? Yes. No.

If yes, answer following questions

Age at menarche _____

Cycle: Regular Irregular (give details)

_____

Duration : (No. of days)

Flow: Scanty Moderate Excessive

Associated symptoms : 1. Abdominal pain

2. Premenstrual tension

Axillary hair: Absent Present

If present (a) Site and distribution _____

(b) Colour _____

(c) Amount _____

Distribution of fat: Hips Lumber area

Axilla Back

Femine characteristics:

Skin of the face: Normal Oily Dry Prsence of acne

Broadening of pelvis: Yes No.

Sexual Maturing Rating (SMR) by Tanner's classification

1. SMR of breast changes _____

2. SMR of public hair _____

3. SMR scoring (Grade 1 to V)_____

PROFORMA FOR BOYAS

Axillary hair: Absent Present

If present (a) Site and distribution _____

(b) Colour _____

(c) Amount _____

Facial hair: Present Absent

If present Site and distribution _____

Amount _____

Distribution of hair on other parts of body:

Chest : Yes No

Hair line (from umblicus to pub lic symphysis): Yes No

If Yes, whether Thin Thick

Voice: Normal Childhood type Feminine type

Masculine Characters:

Breaking of voice	Yes	No
Appearance of cricoid cartilage	Yes	No
Broadening of shoulders	Yes	No
Acne on the face:	Present	Absent

Orchidometry: _____

Sexual Maturing Rating (SMR) by Tanner's classification :

1. SMR of public hair _____

2. SMR of external genitalia and testes _____

3. SMR scoring (Grade 1 to V)_____

Congenital malformations

Skeletal No Yes If yes, describe

_____

Hypospadias No Yes

If yes, Mild Moderate Severe

Cryptorchidism No Yes

Congenital Cardiac disease No Yes

If yes, describe _____

Neural. Tube defects No Yes

If yes, describe _____

On the basis of history and clinical exaination please give a provisional diagnosis

Bronchial asthma	Yes	No	
Allergic dermatitis	Yes	No	
Congenital Malformations	Yes	No	
Neurological problems	Yes	No	
Cancer	Yes	No	
Hormonal imbalance	Yes	No	
Does the child need further referral speciality	Yes	No	If yes, which

Neurologist Physchologist Oncologist Dermatologist

Endocrinologist

Annexure 3 Proforma for History Collection of the families of School children

Family Proforma

Family ID

Name of the Father

Caste Non-Tribal Tribal

Residential Address

_____

_____

Is the house in your name Yes No

If no, who is the owner?

Since how many years you are staying in this area

Family occupation

Do you have your own land No Yes

If yes, What are the crops in your land

Do you use any pesticide No Yes

If Yes, name _____

Occupation
Farmer Plantation workers Others

Father

Mother

Other family members

Food habits Vegetarian Non-Vegetarian

Any special food habits peculiar to the family _____

Any ayurvedic herbs used routinely _____

Any storage methods for foods _____

Source of drinking water

Stream Name of the stream

Well Suranga

Any cattle in the family

No Yes If yes, how many & which type

Any cattle with physical abnormalities Yes No

Any cattle suffering from convulsions Yes No

Any deaths of cattles Yes No

Details of the family members

S.No.	Name	Age	Sex	R*	Any disease	Staying with family

Details in the family

S.No.	Name	Age	Sex	R*	Any disease	Staying with family

R* relation

Maternal history

Present age Age at marriage

Consanguineous marriage Yes No

Obstetrical history:

Serial number of pregnancy	1	2	3	4	5	6
Age at pregnancy						
FTND						
Premature						
Stillbirth						
Abortion						
Congenital malformation						
Neonatal death						
Any special diet during pregnancy						
Any illness or fever during pregnancy						

SMR RATING OF MARSHALL AND TANNER

CLASSIFICATION OF SMR IN GIRLS

SMR	Public Hair	Breasts
1.	Preadolescent	Preadolescent
2.1	Sparse, lightly pigmented straight, medical border of labia	Breast and papillae elevated as small mound; areolar diameter increased
3.1	Darker, beginning to curl, increased amount	Breast and areola enlarged, No contour separation
4.1	Coarse, curly, abundant but amount less than in adult	Areola and papillae form secondary mount
5.1	Adult ferminine traingle, spread to medical surface of thights	Mature, nipple projects, aerola part of general breast contour

CLASSIFICATION OF SEX MATURITY STAGES IN GIRLS

(a)	Stages of breast development
Stage-1	Pre-adolescent, elevation of papilla only.1
Stage-2	Elevation of breast and papilla as a small mould. Enlargement of areolar diameter
Stage-3	Further enlargement of breast and areola, with No separation of their contours1
Stage-4	Projection of areola and papilla to form a secondary mound above the level of the breast.1
Stage-5	Mature stage. Projection of papilla only, due to recession of areola to the general contour of the breast.
(B)1	STAGES OF DEVELOPMENT OF PUBLIC HAIR1
Stage-1	Pre-adolescent, No public hair1
Stage-2	Sparse growth of long, slightly pigmented dawny hair, straight or only slightly curled, appearing chiefly along labia.
Stage-3	Hair considerably darker, coarser and more curled, spreading sparsely on the junction of the pubis.1
Stage-4	Hair Now considerably adult type, but the area coverd by it still smaller than in adult. No spread to medial surface of thigh
Stage-5	Hair adult in quantity and type, with the distribution of classically feminine pattern i.e. horizontal. Hair may spread upto medial surface of the thighs.1

Annexure 4 Physiography of the exposed and control areas through satellite Imaging

भारत सरकार
अन्तरिक्ष विभाग
भारतीय अन्तरिक्ष अनुसंधान संगठन
प्रादेशिक सुदूर संवेदन सेवा केन्द्र
40, वां मुख्य मार्ग, ईश्वर नगर, बनशंकरी,
बेंगलूर - 560 070, भारत
दूरभाष : 6661003 / 6662995
फैक्स : 6661059 तार : इसरो

Department of Space
Government of India
Indian Space Research Organization
Regional Remote Sensing Service Centre
40th Main, Eawar Nagar, Benashankari
Bangalore 560 070, India
Telephone 6661003/6662995
Fax: 6661059, Grams: ISRO

Dr. P.P. Nagesware Rao
Head, RRSSC-B

No. RC:BG20.91 November 07, 2001

Dear Dr Saiyed,

In continuation of our letter dated September 19, 2001 please find enclosed satellite based assessment of physiographic disposition of the villages affected by neurological health problem in asargod district. The satellite images (A4 size) are also enclosed for the study area. Your feeback on the report would be very valuable for us.

We will be very glad to provide any further clarifications in this regard.

With regards,

Yours sincerely,

Dr.H.N. Saiyed
Director
National Institute of Occupational Health
Meghani Nagar
Ahmedabad-380 016

(P.P. Nageswara Rao)

Co. Officer-in-charge, ROHC (S), Bangalore

Annexure 4 Satellite-based assessment of Physiographic disposition of a few villages affected by neurological health problems in Kasargod district, Kerala

A quick analysis, done using I :25,000 scale toposheets, showed a favourable disposition of the croplands and villages to easily come in contact with any persistent toxicant. This was further examined in detail for a few specifically identified villages (as per the list given by Regional Occupational Health Centre, Bangalore vide their letter No.ROHCS/700/881 dated 4-10-01). This report gives salient findings of the assessment done using satellite remote sensing and field survey.

2.0 Satellite-data used:

Linear Imaging Self Scanning Sensor (LISS)-ill data of path 98 and row 65 of Indian Remote Sensing Satellite (IRS) -ID acquired on January I8, I999 was used. Digital data was analyzed using the computer facilities at the Regional Remote Sensing Service Centre (RRSSC), Bangalore.

3.0 Field visit:

A field visits and "ground truth" collection was carried out during November 2-4,2001

4.0 Analysis of satellite data:

5.0 Analysis outputs:

5.1 False Colour Composite (FCC):

In the FCC prepared for the study area (Plate-1), live vegetation is represented in shades of orange, red and magenta depending on its condition and type. Deep and clean/clear water appear black, progressively changing to blue shades depending on depth and sediment loads. Lateritic rock surface and bare soil appear in shades of cyan and grey depending on area exposed and soil moisture. Natural vegetation (reserved forest) is bright red in colour, Arecanut and Coconut plantations in the valleys are dark red and Cashew plantations on hill slopes are pink in tone and fine textured. A portion of the major road from Vittol to Hosdurg passing through Perla town, road from Perla to Bettampadi via Sorga village, road to

Yetteduka, Kumbadje, Kajmpadi villages are shown in white. The watershed boundaries of the micro- watersheds covering villages are shown in blue.

5.2 Computer classified output:

Plate-2 is the computer-classified output showing the different land cover/use water source and built-up land. Cashew plantations are yellow in colour, natural vegetation (reserved forest) in dark green, Arecanut and Coconut in the valleys are light green in colour. The white tones are built-up areas (villages, towns, houses etc.,) located on lateritic hillocks (bluish green) and along the valleys. The dark red tones in the valleys are also mixed plantations of Arecanut plus Coconut with Banana.

6.0 Salient findings:

The entire study area was divided into physiographic units (watersheds) which collect precipitation and serve as storage for water and sediment (micro-watersheds of size 500 to 1500 ha.). Such an approach is necessary to know how aerial-sprayed toxicant moves through the drainage system. The aerial sprayed toxicant that falls on the hill-slopes move down the slopes as surface and sub-surface flow and get collected in the streams. The smaller streams (first: order) feed water to the larger stream and eventually the water exits the drainage basin at the basin mouth. The volume of water that exits the drainage basin per unit time (stream discharge) is much faster from the circular or fan shaped watersheds than in an elongated watershed.

Circularity of the watersheds was calculated as follows:

F =

A = Area of the basin

p = Perimeter of the basin F = Shape factor

As F approaches I, the basin shape approaches a circle. As F becomes 0, the basin shape tends to be linear.

The rainfall of the study area was found to be 20-30cms per month during spraying season (October to December) and does not seem to vary much between the villages affected.

The only prominent difference could be in the topography, vegetation distribution and watershed size and shape. Hence, further analysis was carried out on these characteristics.

Slope characteristics

The watershed covering Kumbadaje and Bellur villages has an average slope of 10% moderately steep) that of Kajampadi and Sorga villages (Padre) has steep slopes (average 25%) Whereas the watershed covering Vaninagar and Nattanige have 20% slope (steep).

Circularity of watersheds:

It was found that the micro-watersheds covering Kumbadaje village has a circulatory of 03, Belluru village- 0.8, Nattanige village-1.0, Vaninagar village-0.8, Sorga village-0.8, Kajampady village-0.84.

Vegetation characteristics:

In the micro-watershed covering the Kumbadaje village, the Cashwnut plantations are about 2 to 3 ha/sq.km. and has 4 first order streams originating from cashew plantations.

.In the micro-watershed covering Belluru village, the Cashew nut plantations are about I5 to 20 ha/sq.lcm and have I6 first order streams originating from cashew plantations.

.Kajampady micro-watershed has 6 to I0 ha of Cashew nut plantations per sq.km of geographical area and has 5 to 6 first order streams originating from the cashew plantations.

.Padre village micro-watershed has about 6 to 10, ha of cashew per sq.km of geographical area. It has 12 first order streams originating from cashew plantations.

Naninagar village and Nattanige village micro-watershed has about 5 to 8ha of cashew per sq.km of geographical area. About 6 first order streams originating from cashew plantations.

Bantaje reserved forest at an altitude of 280-312 meters above MSL acts as a barrier to NE winds thus allowing the sprayed chemical to settle down on the narrow valleys.

Conclusions:

The physiographic analysis and vegetation type distribution shows that the village affected by the neurological health problems are located in the microwatershed that has steep slopes, nearly circular in shape, high run-off potential, with predom inantly**** crops (viz., Cashew, Arecanut, Coconut etc.)

The soils of these watersheds are lateritric and porous ill nature with an impervious clay layer at the lithomerge.

The first order streams of the drainage system are originating from the cashew planted on the steep to moderate hill slops of these watersheds.

Majority of the habitations (built-up areas) are along the valleys and close to the stream banks.

Most of the inhabitants depend on run-off water for their domestic and irrigation purposes.

The watershed characteristics are favourable for any aerially sprayed toxicant to reach the soil-water-plant continuum in a very short span of time and get accumulated.

Therefore, there is an urgent need for stopping any form of spraying of toxicants on the

cash crops of these watersheds.

Sdl- Dr. P.P. NAGESWARARAO Head RRSSC

Regional Remote Sensing Service Centre

Govt.of India

Department of Space, ISRO, Bangalore

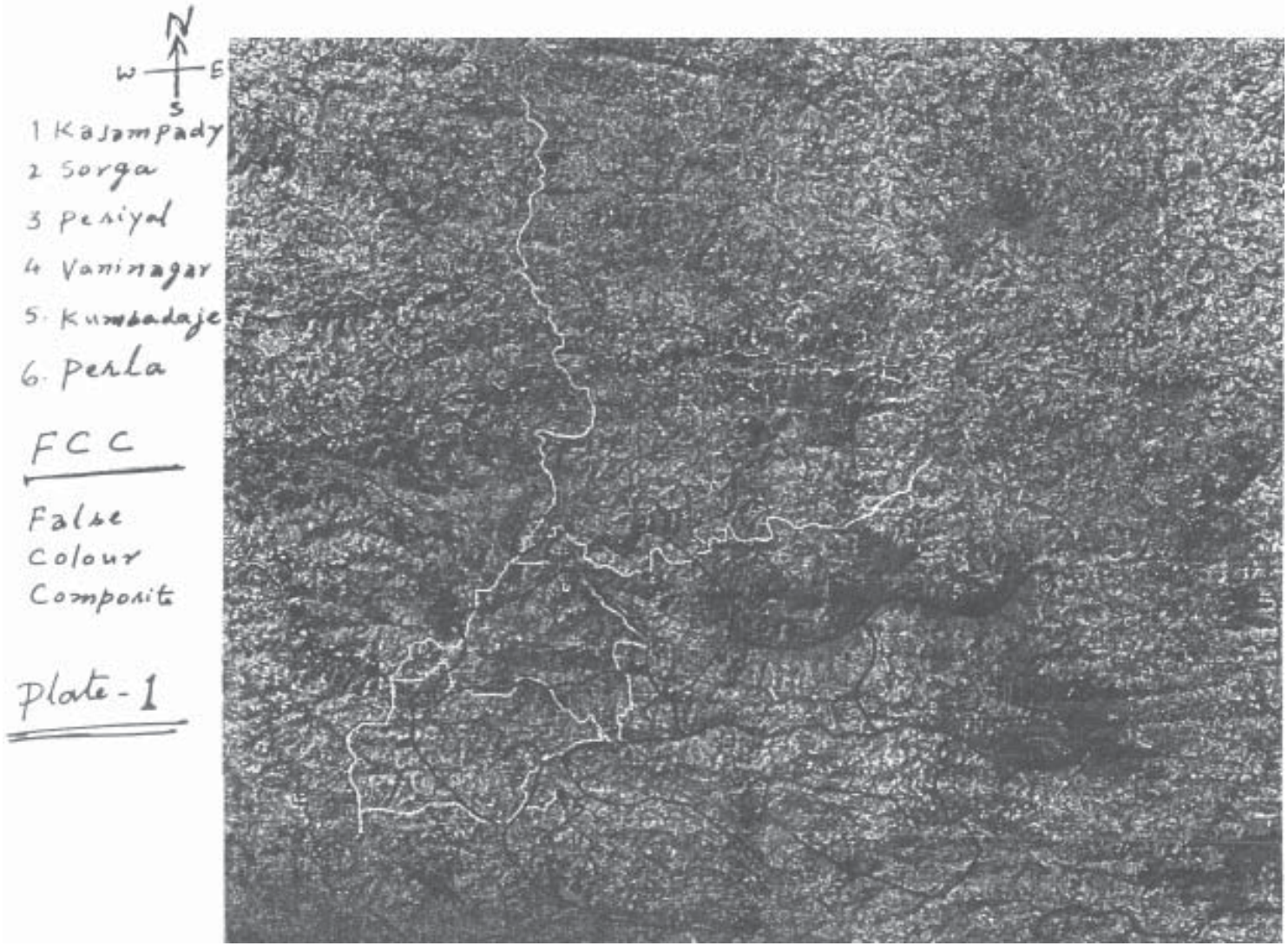
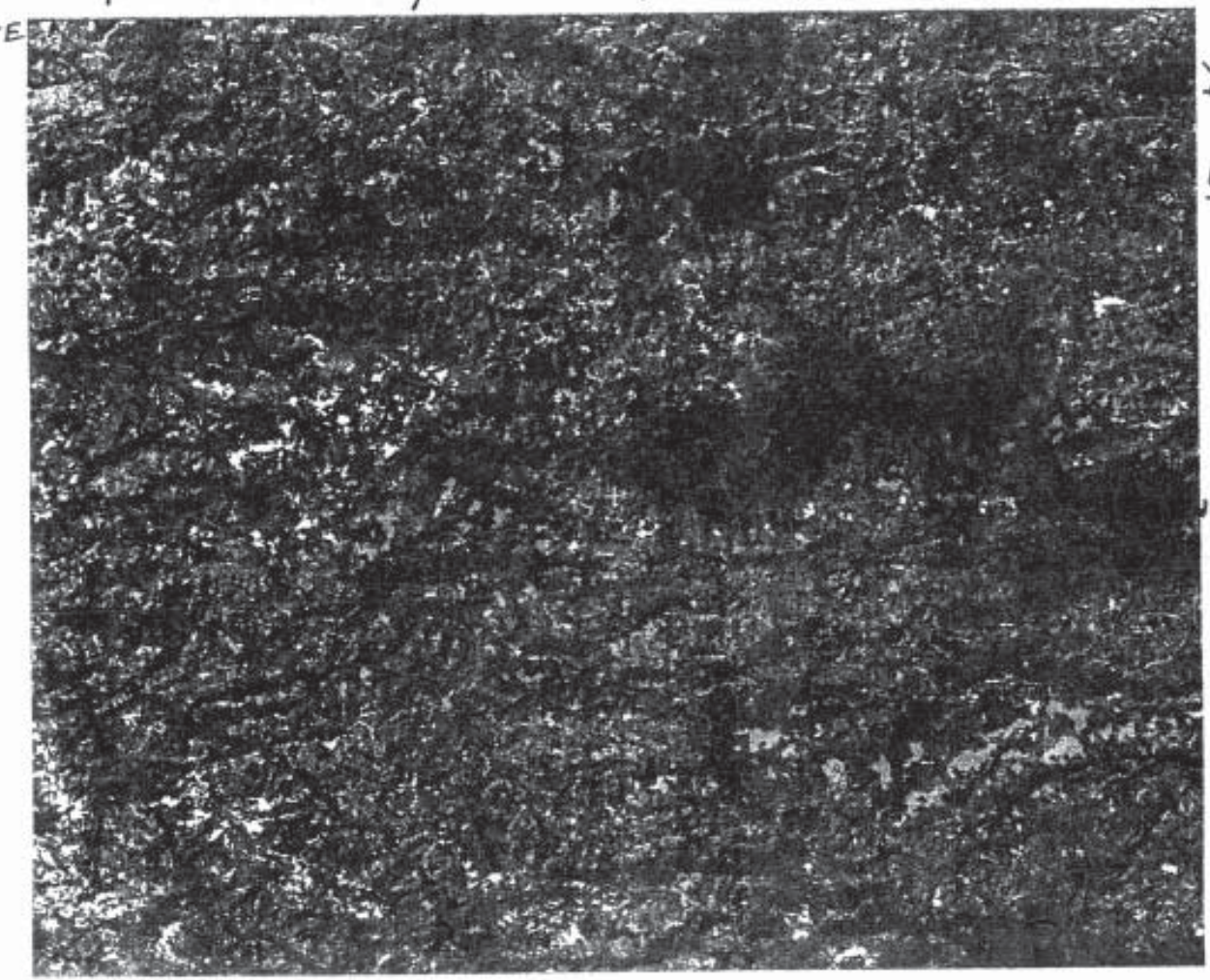

N
W
E
S
1 Kajampady
2 Sorga
3 Periyal
4 Vaninagar
5. Kumbadaje
6. Perla
FCC
False
Colour
Composite
Plate - 1

Plate - 2 : Computer classified output



Annexure 5 List of Participating Institutes and members

Principal Investigator **Dr. H.N. Saiyed, Director,**

Co-Investigator Dr. (Mrs.) A. Dewan, Dy. Director (S.G.), NIOH, Ahmedabad

Participating staff

National Institute of Occupational Health, (NIGH), Ahmedabad.

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	Dr. V.K. Bhatnagar, S.R.O.
	Dr. Rekha Kashyap, S.R.O.
	Dr. V. Krishnamurthy
	Dr. S.N. Sinha, R.O.
	Mr. M.R. Varia, R.A.
	Mr. N.M. Desai, R.A.
	Mr. M. M. Mansoori, Lab. Asst.
Cytogenetic Assay	Mr. B.C. Lakkad, Asst. Director
	Mr. A.M. Suthar, T.O
	Mr. M.M. Patel, R.A.
	Mr. A.K. Gautam, R.A.
Hormone Assay	Dr. K. G. Patel.
	Dr. S.S.A. Zaidi, Asst. Director
	Mrs. S.J. Gandhi, T.O.
	Mr. K.A. Patel, Lab. Tech.

Mr. J.K. Dabhi, T.A.

Mr. R.C. Kushwah, Field Attendant

Statistical Analysis: Mr. P.K.Kulkami, Dy. Director

Participating staff from ROHC (Bangalore)

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Mr. B.K. Rajan, Asst. Director

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Mrs. M. Ramasubbama, Field Asst.

Mr. H.C. Rangaswamy, Lab. Attndt.

Participating staff from Department of Pediatrics, Kasturba Medical College, Mangalore.

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Dr. Rathika D..Shenoy

Dr. Roshan Ann Oommen

Dr. Sangeetha Mahesh

Dr. Duggabatti Anjaneya Prasad

Dr. Vasanth

Dr. Prathiba Kamath S

Dr. Siddharthan S.

Dr. E. Venkatakamalakar Rao

Dr. Habib Alam Raza

Dr. Bodla Hari Prasad

Dr. Syed Mohamed Omran

Dr. Venkatagiri Praveen Kumar

Dr. Podalakur Madhusudhan Rao

Dr. Venkata Ravanamma P.

Dr. Vadlamudi Radha Madhavi

Dr. Maya Menon

Annexure 6 Methodology for Endosulfan Analysis and Cytogenetic Studies

Blood samples were collected for the following investigations.

Endosulfan residues

Hormonal analysis

Cytogenic studies

5-7 ml blood sample was collected using vacutainer syringes under aseptic conditions and in randomly selected cases, 0.5 ml of the blood sample taken in heparinized sterile vials for tissue culture to study chromosomal aberrations and SCE. The remaining blood sample was centrifuged at 5,000 rpm for 5 min and serum was separated. The serum samples were transported every day and stored at -20°C at Kasturba Medical College, ' Mangalore. A total of 262 blood samples (170 exposed + 92 control) were collected from children and coded. The serum samples were carried by air under dry ice (-80°C) to NIOH, Ahmedabad for further analysis.

Endosulfan Analysis:

Soil samples:

Soil samples were collected for estimation of endosulfan residues in the polythene bags from the different locations in the cashew nut plantation on Periyar near Kajampady hill and sediments from the ponds of Kodenkiri and residential area. Eight soil samples wer collected in polythene bags from the exposed area (Village Vaninagar Padre) and three samples from the control area (Miyapadavu, Meenja Gram Panchayat.). The soil sample were collected after digging to a depth of 1 foot.

Water samples:

A total of seven water samples were collected from the exposed area (Village Vaninagar Padre) and three samples from the control (Miyapadavu, Meenja Gram Panchayat). The collection sources were the well, hand - pump, Kodenkiri stream's water and residential area. The samples were collected in 1L brown coloured glass bottle and HgC12 was used as preservative.

Extraction, Cleanup and Quantification of Endosulfan:

The method for endosulfan analysis was based on EPA method Section 5, A, (3), (a)

Blood

5 ml serum was taken in a graduated round-bottom centrifuge tube and extracted with 6.0 .ml of Hexane (HPLC grade, Qualigen). The contents were mixed and extraction conducted for 2 hours on a slow speed roto rac shaker. After the settlement of the contents, 5.0 ml upper layer of hexane extract was taken in a separate tube and concentrated to dryness under the stream of N_2. The residue was made up to appropriate volume in hexane and a suitable aliquot was analysed by GC-ECD.

500 ml of water was taken in a separatory funnel. The sample was partitioned with 50 ml portions of methylene chloride (twice). The aqueous layer was then discarded. The combined layer of methylene chloride was dried on anhydrous sodium sulphate and concentrated to dryness. The residues were then transferred in hexane and finally quantified by GC - ECD.

Instrument Conditions

The GC (HP Model 6890 equipped with Electron capture detector).

Instrument conditions were

Oven initial temp 80°C, Ramp rate 20°C per min to 200°C;

Capillary column	:	HP5, 60 m, 0.25 mm id, 250 p,m;
Injector Port temp	:	220°C; Splitless mode
Detector temp	:	275°C;
Carrier gas	:	N_2 (UHP grade)
Standerd refrence material	:	α- Endosulfan (99.0%)
		β, - Endopsulfan (99.0%)
		Endosulfan sulphate (99.0%)

These standards were procured from CCSRI,

Excel Estate, Mumbai.

Minimum detection limit

The minimum detection limits of a- Endosulfan, (3 - Endopsulfan and Endosulfan sulphate were 1, 1 and 3 pg/ml respectively.

Confirmation Tests

(1) A case of acute poisoning due to Endosulfan was referred to NIO Poisoning Information Centre on 04-12-2001, by Civil Hospital, Ahmedabad In th patient, the blood samples were collected at the interval of 6 , 30, 54, and 78 hours. The patient died on the 5th day due to multiple organ failure. Tissue samples will be obtaine from FSL and analysed for endosulfan. The various residues (α-Endosulfan, β–Endopsulfan and Endosulfan sulphate) and standard chromatogram were furthe confirmed by GC-MS (Varian GC 3800, MS detector Saturn) (Figure 1, 2 and 3).

The recovery tests were performed to check the efficiency of the extraction procedure o the estimation of endosulfan residues in the samples. Such recovery tests were performed(from time to time.

Analysis of the samples (biological and environmental) for the endosulfan residues concentration is in progress.

Analytical resolution of various residues of endosulfan (α Endosulfan , β - Endopsulfan and Endosulfan sulphate) in the study samples collected from the population of Kasargod, Kerala by GC-ECD requires further confirmation by GC-MS technique. We performed confrmatory tests employing standard endosulfan samples and serum sample of a positive control (an acute poisoning patient who consumed endosulfan) and study population with following objectives.

To confirm the residues of α- Endosulfan, β - Endopsulfan and Endosulfan sulphate in serum samples of acute poisoning case and study population using GC-MS.

To establish the minimum detection limit of a-endosulfan, B - endosulfan and endosulfan sulphate by GC-MS.

Method

The standards α-endosulfan, β - endosulfan and endosulfan sulphate were procured from CCSRI, Excel Estate, Mumbai. Standard stock solutions were prepared in Hexane (HPLC grade, Qualigen) and further diluted of required strength. A case of acute poisoning due to Endosulfan was referred to NIGH Poisoning Information Centre on 0412-2001, by Civil Hospital, Ahmedabad. In this patient, the blood samples were collected at the interval of 6 , 30, 54, and 78 hours. 0.5 ml serum was taken in a graduated round-bottom centrifuge tube and extracted with 6.0 ml of Hexane (HPLC grade, Qualigen). The contents were mixed and extraction conducted for 2 hours on a slow speed roto rac shaker. After the settlement of the contents, 5.0 ml upper layer of hexane extract was taken in a separate tube and concentrated to dryness under the stream of N_2. The residue was made up to appropriate volume in hexane and a suitable aliquot (1Ml) was analyzed by GC-MS. (Varian GC- MS Saturn 2000 system).

Instrumental conditions were

DB-5 capillary column (30 m X 0.25 mm (id) of 0.25mm particle size) at a flow rate of 1ml / min of He gas. MS was operated in El Auto ionization mode. The detail instrumental condition are as.

Column oven

	Temp °C	Rate (°C/min)	Hold (min)	Total (min)
Initial	80	0.0	0.0	0.0
Final	25 0	5.0	6.0	40.0

INJECTOR: 250°C

INJECTION MODE

Time	Split mode	Split ratio
Initial	On	10
0.00	Off	Off
5.00	On	10

RESULTS

Spectrochromatograms of the standard mixture of α-endosulfan, β - endosulfan and endosulfan sulphate shown in spectrochromatogram 1 a, b & c clearly defines that the molecular ion peaks were obtained at M/Z 339, 339 and 422 respectively, and also the retention time at 28.8, 30.909 and 32.453 were obtained for a-endosulfan, B - endosulfan and endosulfan sulphate as confirmed by NIST and Saturn MS library search. The results on the three peaks for a positive control and standard samples as confirmed by GC-MS also stands for the residues of endsulfan present in the study population But a the minimum detection limit of α-endosulfan, β- endosulfan and endosulfan sulphate by GC-MS were 100 pg/µl for each and is higher as compared to GC ECD detection limit,(for α-endosulfan 1 pg/µl, β - endosulfan 1 pg/µl and endosulfan sulphate 3 pg/µl) the results as confirmed by GC-MS may reflect indirect confirmation for the sample of the study population. The spectro chromatograms of serum sample are given in spectrochromatogram 2 which confirm the

presence of α endoslufan, β endosulfan and endosulfan sulphate in the sample. The relevant parameters used for identification and confirmation of these compounds are given in table

TABLE - 1
Identification Parameters of Serum sample of acute poisoning case

Compound	Base peak	Molecular ion peak	Retention time (min)
α-Endosulfan	85	339	28.808
β-Endosulfan	85	339	30.909
Endosulfan Sulfate	272	422	32.453

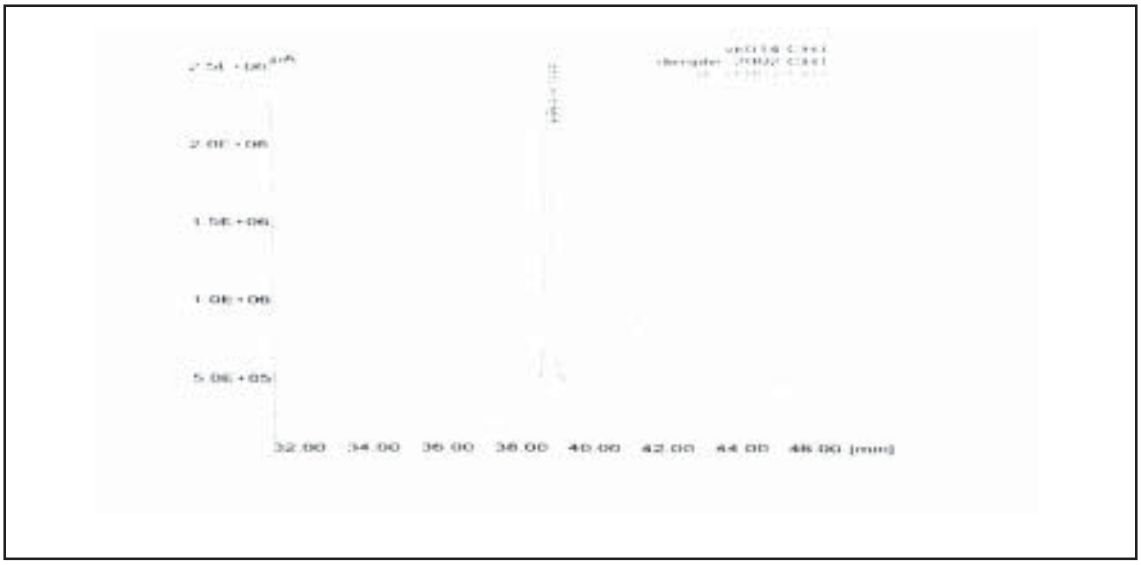


Figure 1 Chromatograph of alpha Endosulfan in standard, a poisoning case and a study subject.

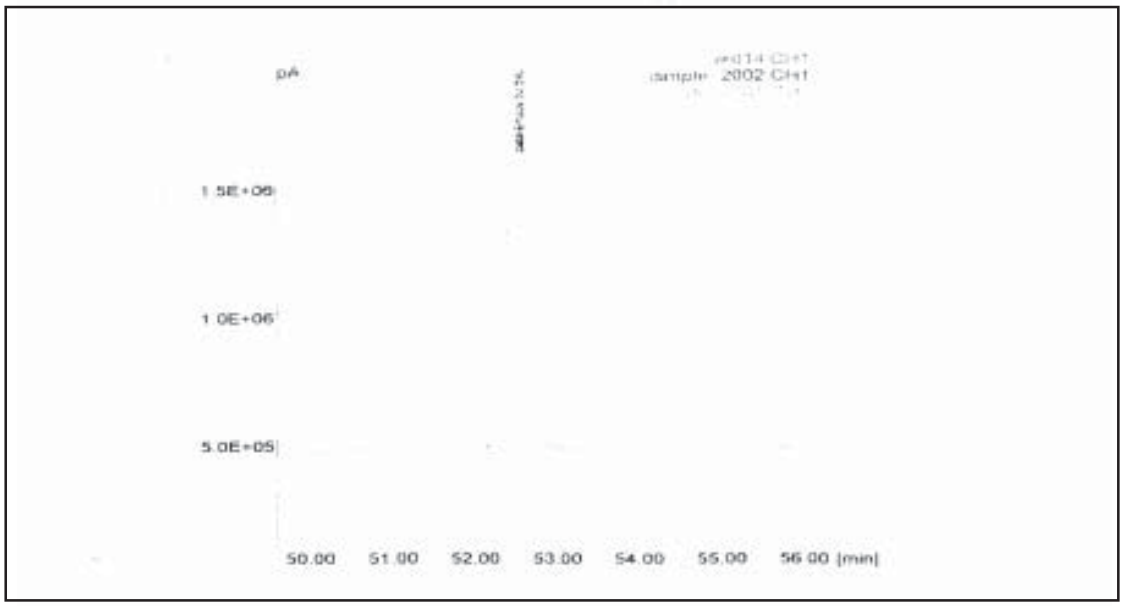


Figure 2 Chromatograph of beta Endosulfan in standard, a poisoning case and a study subject.

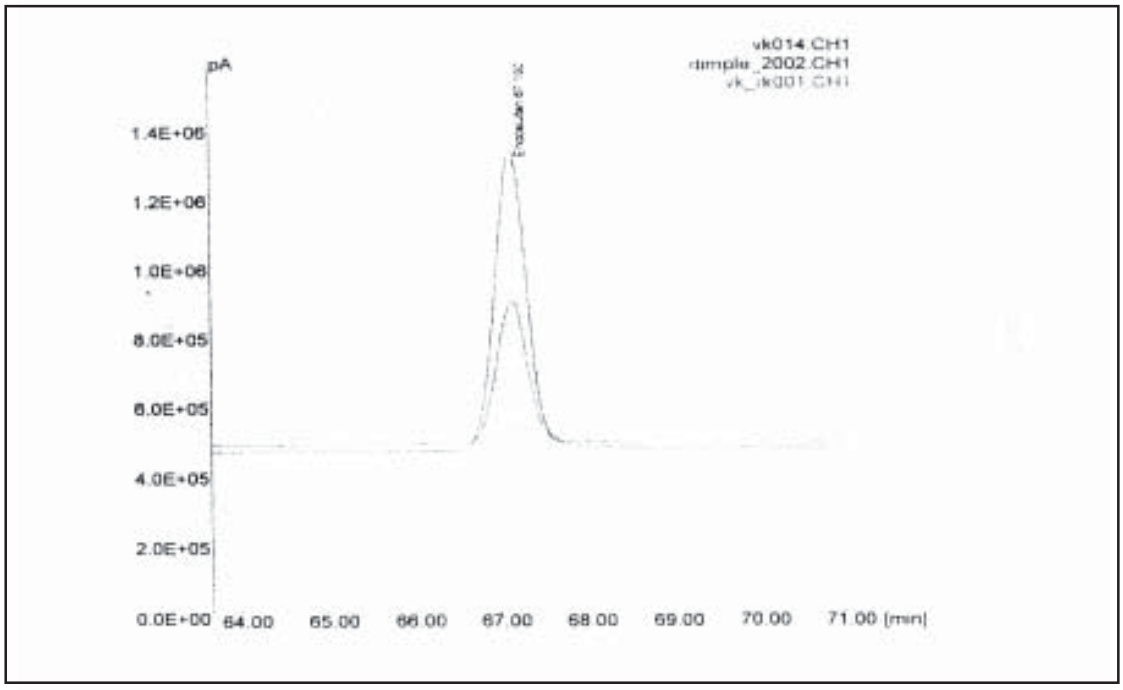
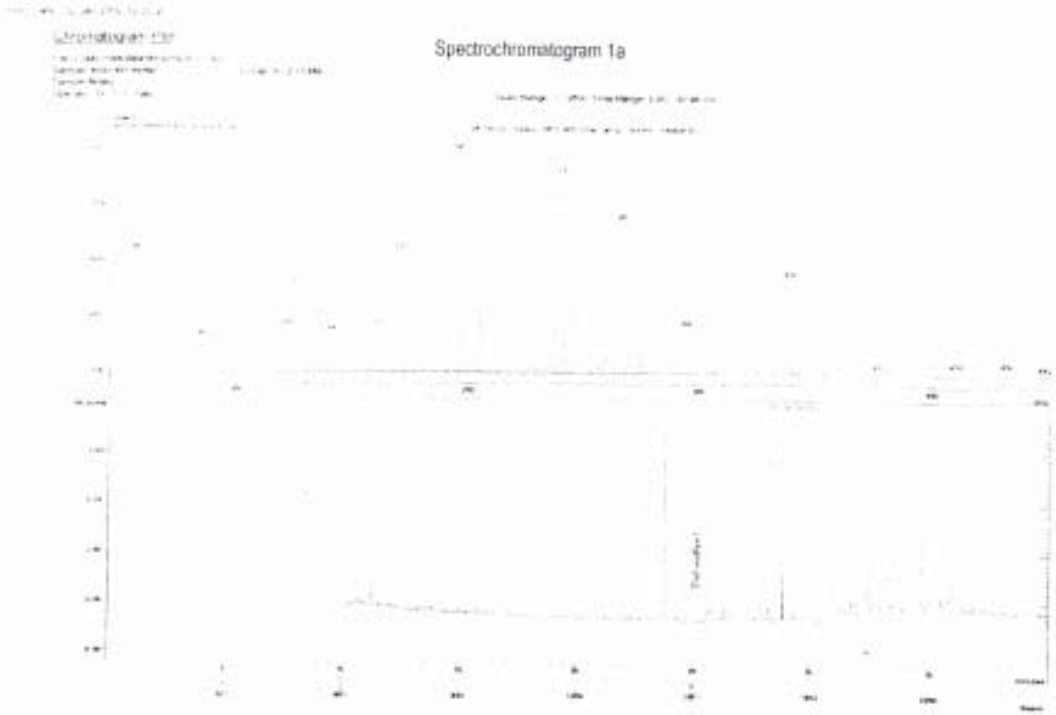
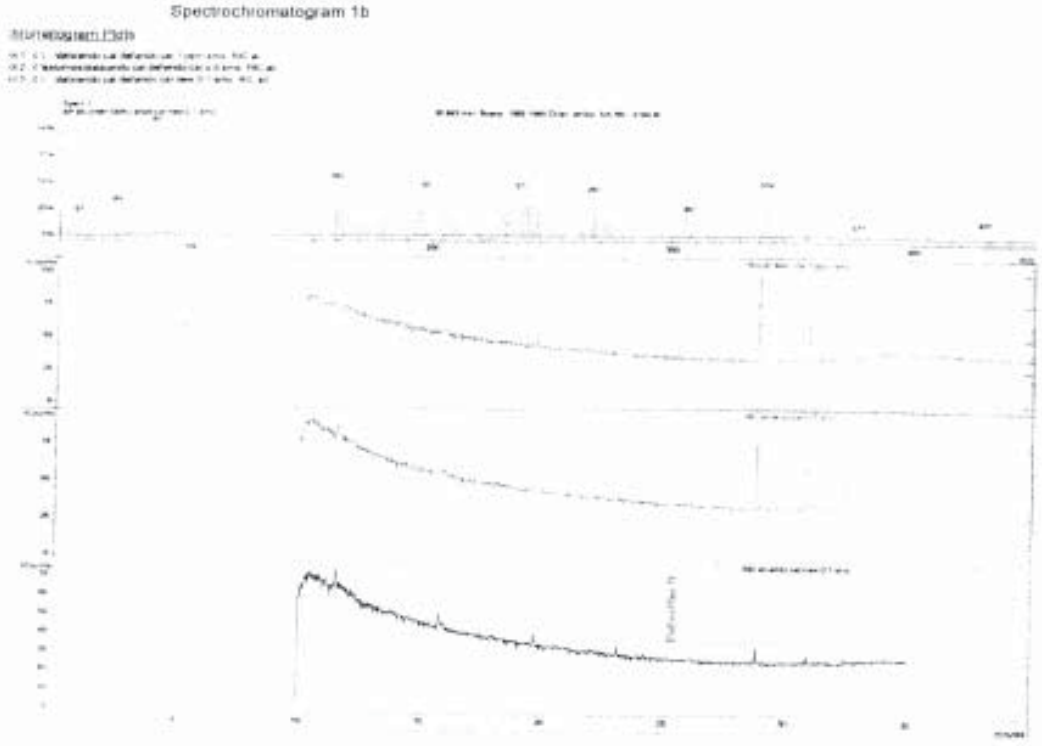


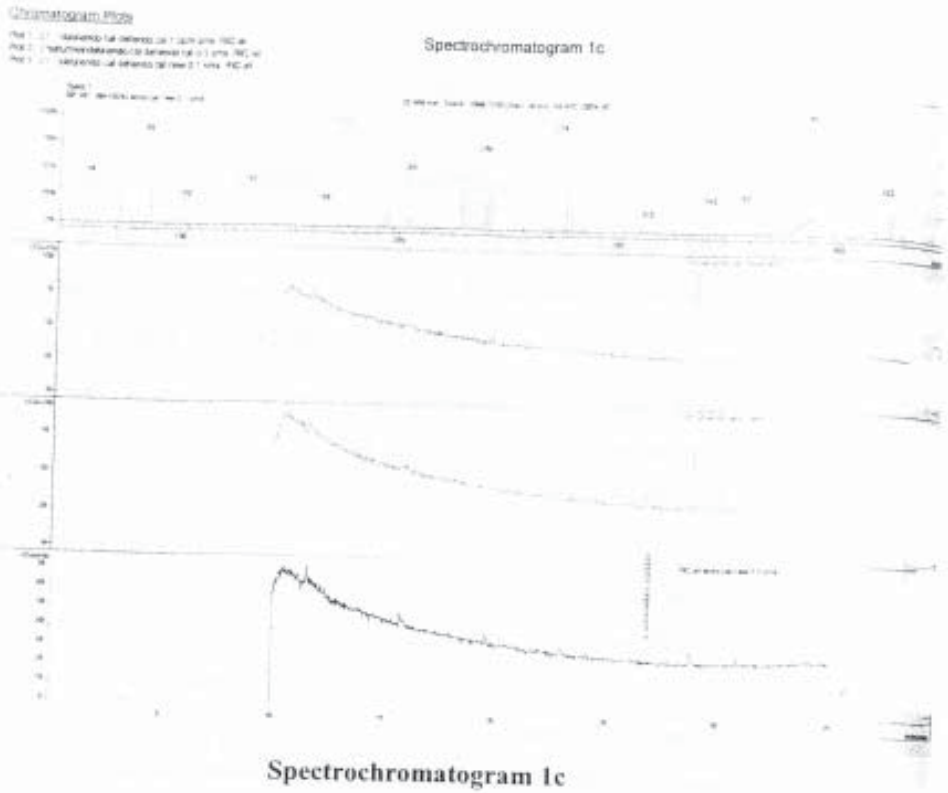
Figure 3 Chromatograph of Endosulfan Sulfate in standard, a poisoning case and a study subject.



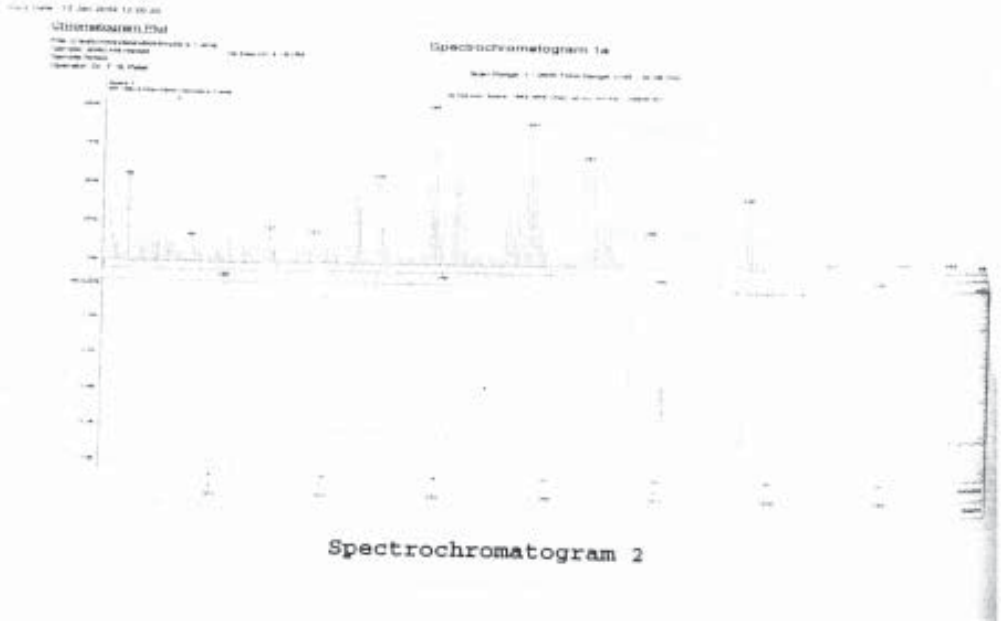
Spectrochromatogram 1a



Spectrochromatogram 1b



Spectrochromatogram 1c



Spectrochromatogram 2

Cytogenetic Studies:

Method for Peripheral Leucocyte Culture for Chromosomal Aberration:

Sample Collection: A total of 48 Blood samples were collected from exposed and control subject by vein puncture, in heparinized sterile syringe. The blood samples will be transported to the laboratory, set up in the field, in a well-insulated ice-box.

Culture Technique: 48 Cultures in duplicate were set using Method of Morhead et al, (1960) for each of the subject using 7 ml of RPMI - 1640 medium (pH 7.4) supplmented with 15% fetal bovine serum, and 0.1 ml of phytohemagglutanin (PHA). 0.5 ml of blood was added to each culture vial and incubated at 37°C for 72 hrs. 2 hours prior to the harvesting 10 u l/ml colchicine was added to each culture vial. The culture vials were centrifuged at 1000 rpm for 10 mins. Than the medium was discarded. The cell button was suspended in 5 ml of 0.075 M KCl solution at 37°C for 10 to 15 mins. followed by centrifugation at 1000 rpm for 5 mins. Freshly prepared 1:3 acetic acid methanol fixative was added slowly to the cell button and then cell were suspended. The fixative was changed 2 times at an interval of 10 mins. each. After final centrifugation the cells were suspended in adequate fresh fixative to form a slightly milky suspension. Then 1-3 drops of cell suspension were dropped evenly from a distance on wet, clean grease free slide. The slide were kept in a standing position and allowed to dry at room temperature.

For studying the chromosomal aberration slides were stained with 4% Giemsa in Sorenson's buffer (pH 7) for 10-15 mins. About 50 well spread metaphases were scored for various types of aberrations in each of the subject from blindly coded slides. Statistical analysis done by using student `t' test.

Chromosomal aberration scoring Criteria:

1. ***Chromatid Gap*****: a non-staining, constricted region in the chromatid arm on alignment with damaged segments of the chromatid.**
2. ***Chromosome Gap*****: Chromatid gaps involving both chromatids at iso-locus points of the chromosome arm.**
3. ***Chromatid Break*****: a non-alinged broken chromatid or the attenuated region being wider than the diameter of the chromatid.**
4. ***Chromosome break*****: iso-chromatid breaks resulting in terminal deletion and intercalary fragments.**
5. ***Ring*****. intra-chromatid exchange giving rise to a ring like configuration.**

6. **Dicentric: chromatid exchange involving two chromosomes with intact centromeres.**
7. **Exchange: configuration involving inter-chromatid exchanges.**
8. ***Miscellanous Aberration*: unusual configuration, severely damaged (> 10 aberrations), endoreduplication (duplicated chromosome lying side by side) and chromatid saperation.**

Sister Chromatid Exchanges (SCEs):

Sister chromatid exchanges (SCEs) were evaluated using method of Perry and Wolff (1974). 5 bromo-deoxyuridine (BrdU),10 pg/ml of culture medium was added 24 hours after setting up of cultures. The cells were harvested after 72 hours of incubation. Metaphase chromosomes were prepared and stained with Hoechst 33258 and 4% Giemsa after 3 days of preparation of slides. The average number of exchanges per metaphase were determined through examination of well spread 25 metaphases for each subject. The result for SCE was statistically analyzed applying student 't' test.

References:

1. **Morhead, P.S.; Nowell, P.C.; Millman, W.J. and Hunderford D.A.: Chromosome preparation of leucocytes cultured from human peripheral blood. Exp. Cell Res. 1960, 20, 613-616.**
2. **Perry, P. and Wolff S.: New Giemsa method for differential staining of sister chromatids. Nature: 1974, 251, 156-158.**

Annexure 7 The recommendations of Achyuthan Committee appointed by Government of Kerala.

Date 30 -11-2001.

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To,

Dr. H. N. Saiyed

Director, National Inst. of Occupational Health,

Meghaninagar, Ahmedabad, 380 016.

Dear Sir,

Sub: Aerial Spray of Endosulfan in Cashew Plantation.

Ref. Your letter No. .3/4/10(1)14/2001 dated l I th Oct. 2001

As soon as I had received your letter referred to above, I had sent the acknowledgement letter through e-mail. But it was not delivered there. I am enclosing a copy of the letter.

The report was submitted on 22 11 200 1. 1 am sending you a copy of our Conclusions and Recommendations for your kind information. The detailed report is with the Director of Agriculture, Govt. of Kerala, Vikas Bhavan, Trivandrum 695 032.

Regards,

YoursTruly

Sd/-

Achyuthan

7. CONCLUSIONS & RECOMMENDATIONS

After a detailed study of the data, and the oral and written statements and site visits, the Committee has arrived at the following conclusions.

1. The cashew plantations of PCK Ltd. In Kasaragod District are all located in the undulating hilly areas (Refer Plan - Annexure 21). The plantations are spread in isolated patches and are intertwined with habitations. The topography of the area precludes the possibility of aerial spraying observing all the protocols.

2. There are a large number of wells inside and just outside the plantations area. Several streams originate there. The water from the plantations (situated on the hills) can run off into the valleys inhabited by local people. The rivulets Panathur and Karicheri, which are fed by streams originating from or passing through the area, are tributaries of Chandragiri river, which supplies drinking water to Kasaragod town and several Panchayaths. The surangams, from which the local people draw water, are cut deep into the hills forming the plantations. They are prone to contamination by chemicals applied in the estates. Therefore, the hydrology and morphology of the area are unsuited for aerial spraying.

3. The human settlement pattern of the area also makes the plantation area unsuitable for aerial spraying. The adjoining areas are thickly populated. There are large numbers of houses inside the plantations. There are pockets of human settlement surrounded on 3 sides by the plantations. There are large number of houses and wells inside and just outside the plantations. The local people allow their cattle to freely graze in the plantation area. There are several schools inside and just outside the plantation area.

   Even the Pesticide Manufacturers and Formulators Association has agreed to the view that the area is not ideal for aerial spraying of pesticides.

4. The PCK has not been following the rules prescribed for aerial spraying. This has been reported by the District Collector, all the functionaries of the Panchayaths who deposed before the Committee, the experts and the great majority of general

public. There was no effective supervision of spraying and no monitoring of the precautionary measures and the after-effects.

5. The same pesticide endosulfan was used continuously from 1981 onwards; in spite of the recommendations of Research organizations rotate the chemicals. The reason given by the PCK is that endosulfan is the most economic pesticide available in the market. Even the possibility of the bugs acquiring immunity to endosulfan due to long exposure has not been considered by the PCK.

6. As in the cases of most other pesticides, endosulfan can cause acute toxicity in animals and human beings due to over exposure. That is why strict protocol is prescribed for its use. Though chronic toxicity due to long term exposure has not been convincingly established, it cannot be ruled out.

7. There are reports of health problems free three Panchayath adjacent to the plantations. There is no direct evidence to attribute these directly to endosulfan pollution, but there is no evidence to completely deny it. Other usual causes like pollution from automobiles and industries are absent here. The only activity that is not normal is the aerial spraying of endosulfan. The pesticide is applied without observing the safety rules. The same chemical is used for 2 decades. Hence at this point of time, there is no evidence to implicate or esxonerate endosulfan as the causative factor of the health problems. But, the proof of absence cannot be taken as the absence of proof. In all environmental pollution problems, the onus of responsibility to prove or disprove the causeeffect relationship should be that of the polluter and not of the general public who are the victims of pollution. Since cashew is an important export item earning revenue to the State and a large number of workers are involved in it, publicity to the pollution from endosulfan spray can prove detrimental to the industry.

On the basis of the investigations and the above conclusions, the Committee recommends the following measures to be adopted.

1. Ban aerial spraying of pesticides in all the cashew plantations of PCK Ltd. In Kasaragod District.

2. Use of endosulfan in the PCK plantations of Kasaragod District should be frozen for 5 years.

3. In the cashew plantations in the Peria Division (which includes Enmakaje Panchayath),

a total pesticide holiday should be observed for 5 years. This plantations should be left to the nature during these 5 years. Detailed studie on tea-mosquito bug menace and its relation to the crop productivity should be made during this period.

4. In the other plantations of PCK in Kasaragod district, need based ground spraying, (manual or power-operated) of pesticides other than endosulfan may be resorted to, in consultation with research organizations.

5. The pesticide management and plant protection of PCK should be scientifically organized.

6. Research efforts to evolve integrated pest management (IPM) should be augmented.

7. Breeding programme to develop cashew strains resistant to tea-mosquito bug should be undertaken.

8. Since the cause of the human health problem could not be deduce conclusively, a detailed investigation involving scientists from all relate fields should be conducted to identify the risk factors for the high morbidity the Padre village and other affected areas. A detailed health survey should be conducted in the Padre village and other areas from which cases of abno health problems are reported. The health survey should cover the plantation workers also.

9. Since most of the people who complain about health problems are from poorer sections of the community, the Government should make arrangemen to provide special medical care to these persons.

10. The Government should take all steps to implement these recommendatio and dispel the fears regarding pesticide application.

11. The right to information of the use of pesticides should be respected. The Gram Panchayaths should be given all details, when requested. The apprehensions of the local people regarding the alleged pesticide problem should be cleared by awareness

programmes conducted through PCK, Agricultural Department and Research Institutions. In every division of the PCK, a committee consisting of the following members should be constituted for monitoring the proper application of pesticide.

1. President/presidents of the concerned Gram Panchayath/Panchayaths
2. Agricultural Assistant
3. A representative of the Health Department
4. The Regional Officer of PCK
5. A representative of the workers of the concerned division of the PCK.

ANNEXURE-8

SL.NO.	PRINTED	CORRECTED
Page #2 Line 4	Endosulf	Endosulfan
Page #7, Line 9	Lymphocyte	Leucocyte
Page # 8, Line 21	Lymphocyte	Leucocyte
Page # 15 Table # 3	Pond filtrate Study sample #2 Endosulfan sulfate (ppb) = 0.23	Pond filtrate Study sample #2 Endusulfan sulfate (ppb) =0.023
Page # 16 Table # 4 Mid soil (5"-7"), Study sample	*B*-endosulfan (ppb)= 0.005±0.001	*B*-endosulfan (ppb)= 0.0005±0.001
Page # 16 Table # 5 Lower Soil (10"-12") Study sample	Total endosulfan (ppb)= 0.106±0.085	Total endosulfan (ppb)= 0.14±0.087

Page # 16, Table # 5	Study		Reference		Study		Reference	
	Female	Male	Female	Male	Female	Male	Female	Male

Page #21 Para3, Line 4	Control female	Females in reference group
Page # 29, Para 1	Table 15	Table 19