CHAPTER 4

RESULTS

4.1 The studied populations

The details of relevant information for characterizations of the studied populations and the evaluation of confounding factors of healthy subjects from Saraphi and Chom Thong populations participated in the studies for detection of the basal levels of chromosomal aberration and micronuclei frequency, and for DNA damage detected by comet assay, were shown in Appendix G and H respectively.

As previously mentioned, some of subjects denied participating in the comet study which consequently decreased the number of the studied subjects. However, the difference in the numbers of studied subjects gave no significance in the details of relevant information of the comet assay comparing to those in the chromosomal aberration and micronucleus tests. Briefly, more than 95% of the subjects residing in both Saraphi and Chom Thong were between 51-80 years old. Approximately 50 % of them had no profession due to their old ages while about 23 and 37% of the subjects in Saraphi and Chom Thong were agriculturists. However, only 25% of the subjects working in the fields used pesticides. Most of the subjects residing in the two populations had burning activities both in and around their houses.

Regarding to smoking habit, the number of subjects who smoked cigarettes were 21 and 50% in Saraphi and Chom Thong respectively. The former were only half of the latter. Most of smokers smoked Khiyo, a northern local cigarette, about 2-3 pieces per day. Approximately 65 % or more of the two populations chewed Miang which made of fermented tea leaf about 2-3 pieces per days.

Nevertheless, the perfect matching on all factors between studied populations from Saraphi and Chom Thong districts could not be achieved. The details of characteristics were restricted on age and gender. But there were significant differences of Chi square P-value analyzed from some confounding factors on cigarette smoking and fermented tea leaf or betel nut chewing between the two populations as shown in Table 3.

Table 3 Chi-square test of confounding factors between Saraphi and Chom Thong populations

Factor	**Chi-square P-value**	
	chromosomal aberration and micronucleus tests	**comet assay**
Gender	0.4187	0.6139
Age	0.0602	0.2724
Present pesticide exposure	0.3534	0.8311
Smoking habit	<0.0001***	<0.0001***
Alcohol drinking	0.0947	0.0529
Chewing habit	0.0016*	0.0025*

Significant difference between the studied populations indicates with asterisk

*P<0.05, **P<0.001, ***P<0.0001

4.2 Chromosomal aberration analysis

4.2.1 Chromosomal aberration endpoints and mitotic indices

The average (mean ± SD.) percentages of aberrant cells and aberrations and mitotic indices measured from the populations residing in the areas with high and low incidences of lung cancer, Saraphi and Chom Thong, were statistically analyzed as shown in Table 4. There were no differences either on the percentages of aberrant cells or aberrations when the gaps were included as aberrations, or mitotic indices between studied populations. When gaps were excluded from aberrations, however, subjects residing in the high risk area, Saraphi, had significantly lower aberrations, both the percentage of aberrant cells ($P=0.0001$) and the percentage of aberrations ($P<0.0001$). The mean mitotic index in Saraphi subjects was also significantly lower ($P=0.0246$) than in Chom Thong subjects. Furthermore, the significant differences on the chromosome aberrations were observed only in males when the comparison of male and female subjects between the two populations was performed. The frequency distributions of aberrant cells and aberrations in both populations were in between 1 to 5% as shown in Fig. 1. It also indicated a shift towards higher frequencies in Chom Thong population.

The observed normal chromosomes and various chromosomal aberrations, both of chromatid- and chromosome-types were shown in Fig. 2-9 respectively. The majority of aberrations observed were chromatid breaks which were higher in the Chom Thong population, especially in males ($P=0.0346$) which were approximately five times higher than those from Saraphi as shown in Table 5. Nevertheless, there were no significant differences among

the chromosome-type aberrations, acentric fragments and dicentric chromosomes, between the two populations, while breaks were more observed and rings were seen only in Chom Thong population.

4.2.2 The effects of confounding factors on chromosomal aberrations in total population

It is evident that genetic risk assessment is not only based on the potency of genotoxic chemicals but also the influence of some other factors on chromosomal aberrations. Therefore, the effects of the factors that potentially influenced the chromosomal aberrations in total population were analyzed by ANOVA and shown in Table 6. Cigarette smoking and fermented tea leaf or betel nut chewing influenced the percentage of aberrant cells as well as the percentage of aberrations. However, the potential of an enhance frequency of the chromosomal aberrations with increasing age range was observed but not statistically different. Nonetheless, the other factors, including gender, pesticide exposure, and alcohol drinking, did not affect chromosome damage in total populations.

Table 4 Comparison of chromosome aberrations separated by gender between studied populations

Population	N	Aberrant cells, included gap(%)	Aberrant cells, excluded gap(%)	Aberrations, included gap(%)	Aberrations, excluded gap (%)	Mitotic index
Saraphi						
All	107	1.907 ± 1.278	0.486 ± 0.905	2.178 ± 1.810	0.486 ± 0.905	1.251 ± 0.439
Females	55	2.036 ± 1.465	0.673 ± 1.090	2.036 ± 1.465	0.673 ± 1.090	1.333 ± 0.473
Males	52	1.769 ± 1.041	0.288 ± 0.605	1.769 ± 1.041	0.288 ± 0.605	1.165 ± 0.385
Chom Thong						
All	118	1.907 ± 1.278	1.042 ± 1.180***	2.220 ± 1.836	1.076 ± 1.214***	1.331 ± 0.329*
Females	67	2.179 ± 1.723	1.045 ± 1.296	2.194 ± 1.725	1.060 ± 1.301	1.382 ± 0.316
Males	51	2.176 ± 1.936	1.039 ± 1.019***	2.255 ± 1.988	1.098 ± 1.100***	1.265 ± 0.338

Significant difference on chromosomal aberration between the studied populations indicates with asterisk

*P≤0.05, **P≤0.001, ***P≤0.0001

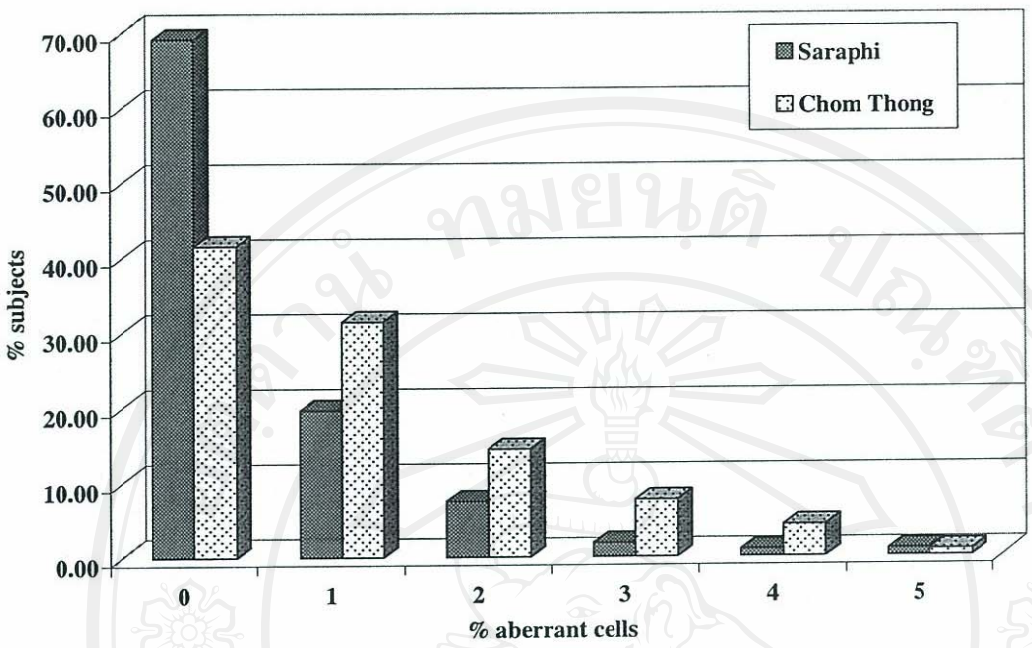
Table 5 Types and frequencies of chromosome aberrations observed in each population

Population	N	Chromatid-type aberration			Chromosome-type aberration				
		gap	break	deletion	gap	break	acentric	dicentric	ring
Saraphi									
All	107	1.374 ± 0.927	0.215 ± 0.514	0.000 ± 0.000	0.047 ± 0.253	0.075 ± 0.298	0.121 ± 0.381	0.121 ± 0.381	0.000 ± 0.000
Females	55	1.309 ± 0.920	0.309 ± 0.635	0.000 ± 0.000	0.055 ± 0.299	0.091 ± 0.348	0.218 ± 0.498	0.127 ± 0.338	0.000 ± 0.000
Males	52	1.442 ± 0.938	0.115 ± 0.323	0.000 ± 0.000	0.038 ± 0.194	0.058 ± 0.235	0.019 ± 0.139	0.115 ± 0.379	0.000 ± 0.000
Chom Thong									
All	118	0.949 ± 1.053**	0.483 ± 0.814*	0.008 ± 0.092	0.195 ± 0.559	0.263 ± 0.576	0.195 ± 0.476	0.102 ± 0.330	0.025 ± 0.205
Females	67	0.985 ± 0.945*	0.433 ± 0.743	0.000 ± 0.000	0.149 ± 0.435	0.313 ± 0.656	0.179 ± 0.424	0.090 ± 0.288	0.045 ± 0.272
Males	51	0.902 ± 1.188**	0.549 ± 0.901*	0.020 ± 0.140	0.255 ± 0.688	0.196 ± 0.448	0.216 ± 0.541	0.118 ± 0.382	0.000 ± 0.000

Significant difference between the studied populations indicates with asterisk

*P≤0.05, **P≤0.001, ***P≤0.0001

a)



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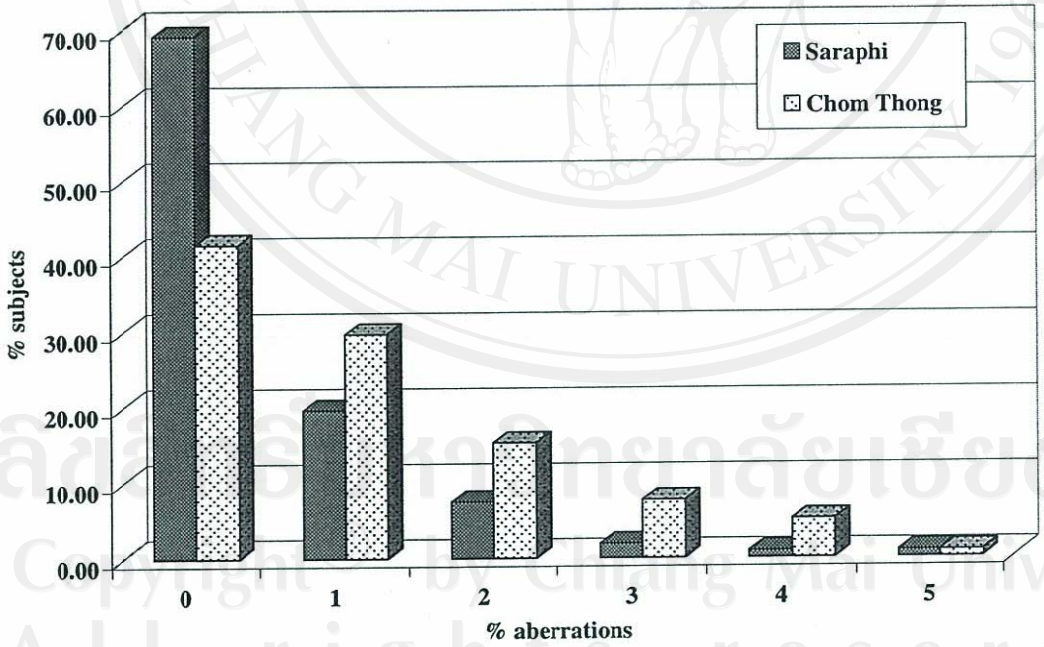


Figure 1 Distributions of a) % aberrant cells and b) % aberrations of both studied populations

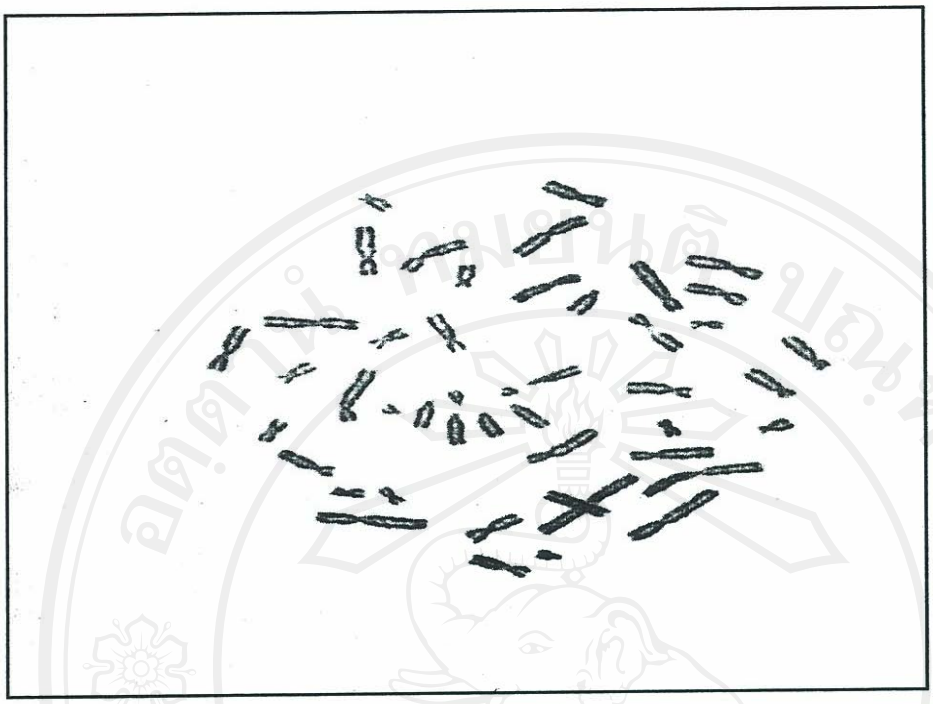


Figure 2 Normal metaphase with 46 chromosomes

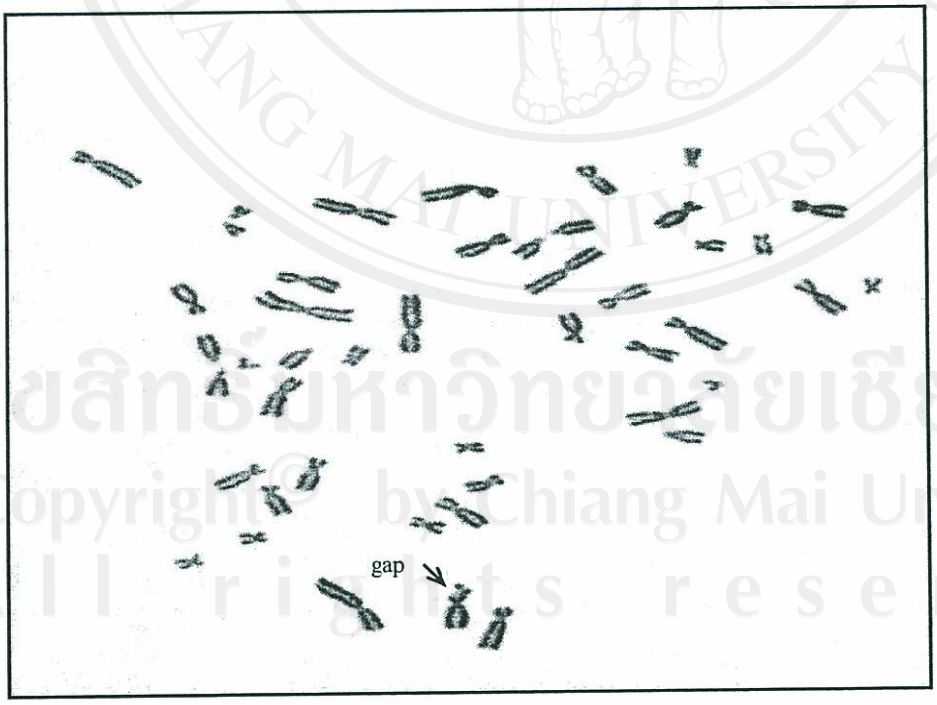


Figure 3 Chromatid-type aberration, gap

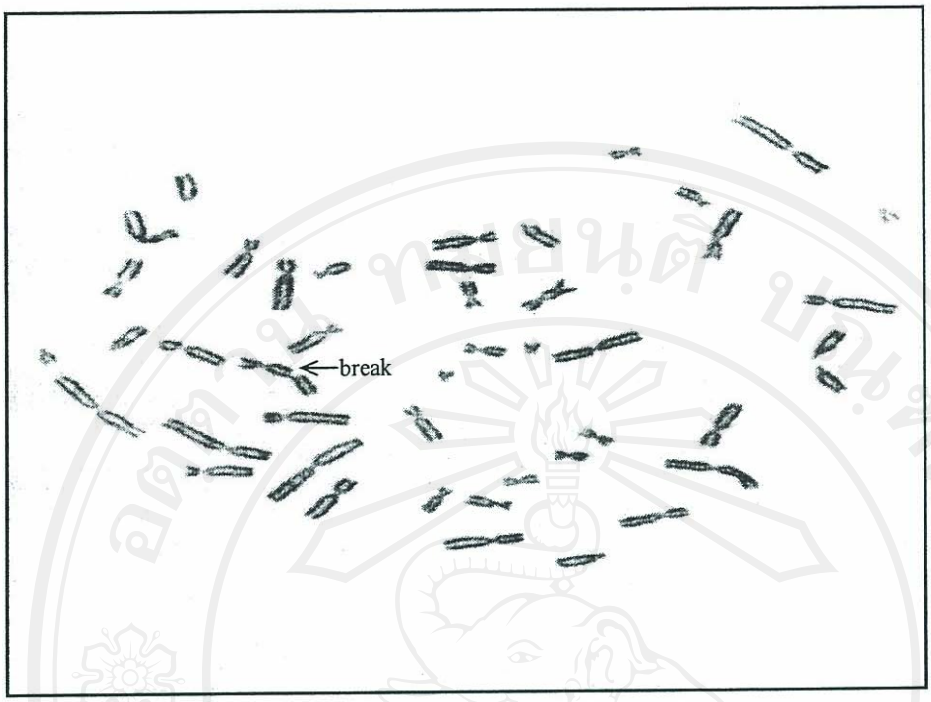


Figure 4 Chromatid-type aberration, break

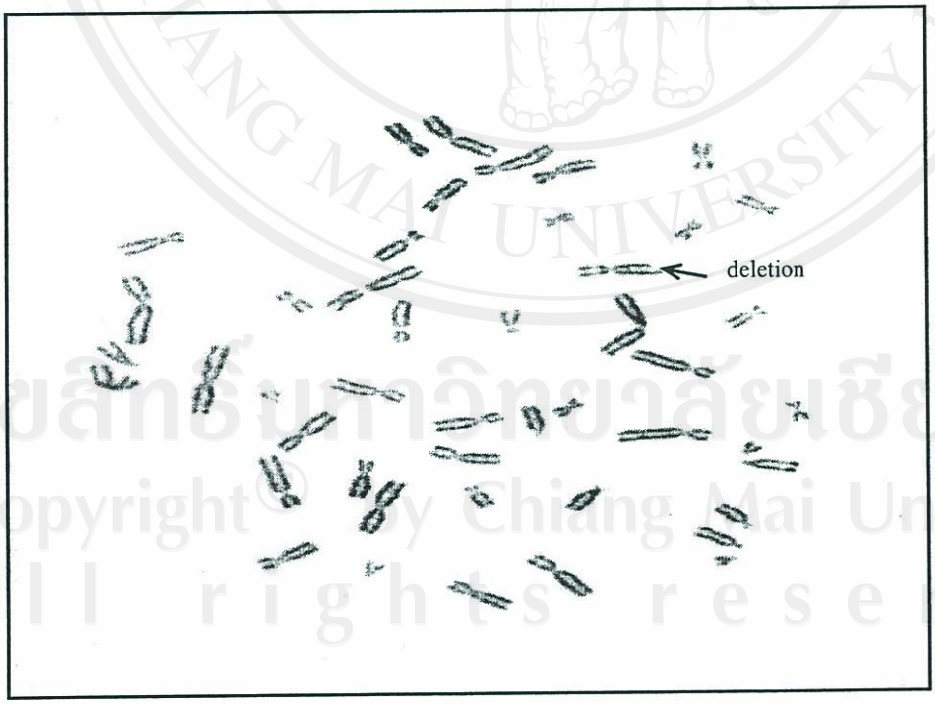


Figure 5 Chromatid-type aberration, deletion

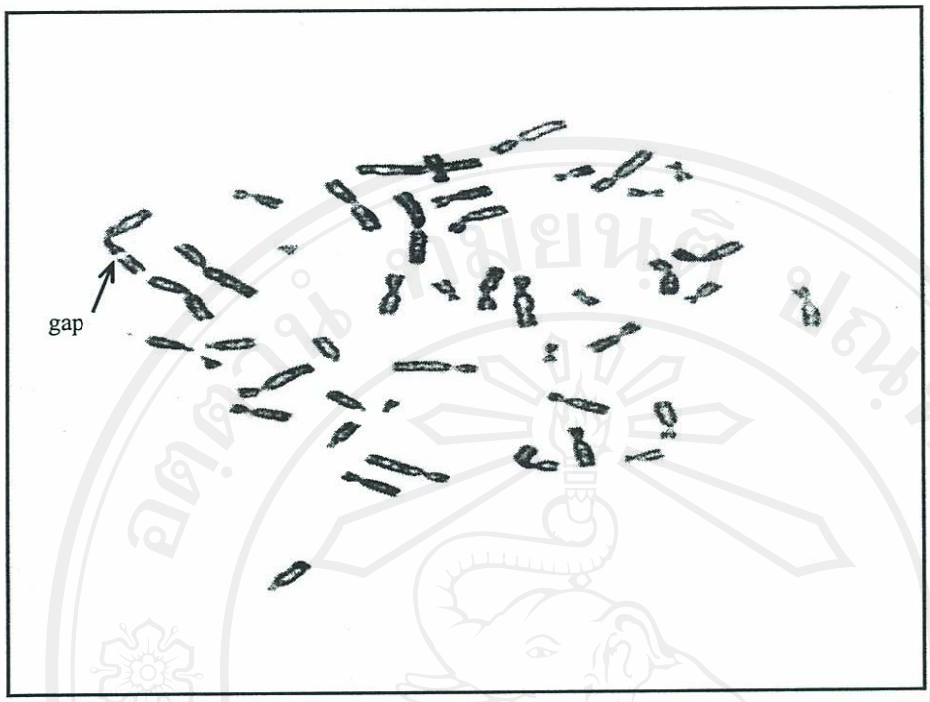


Figure 6 Chromosome-type aberration, gap

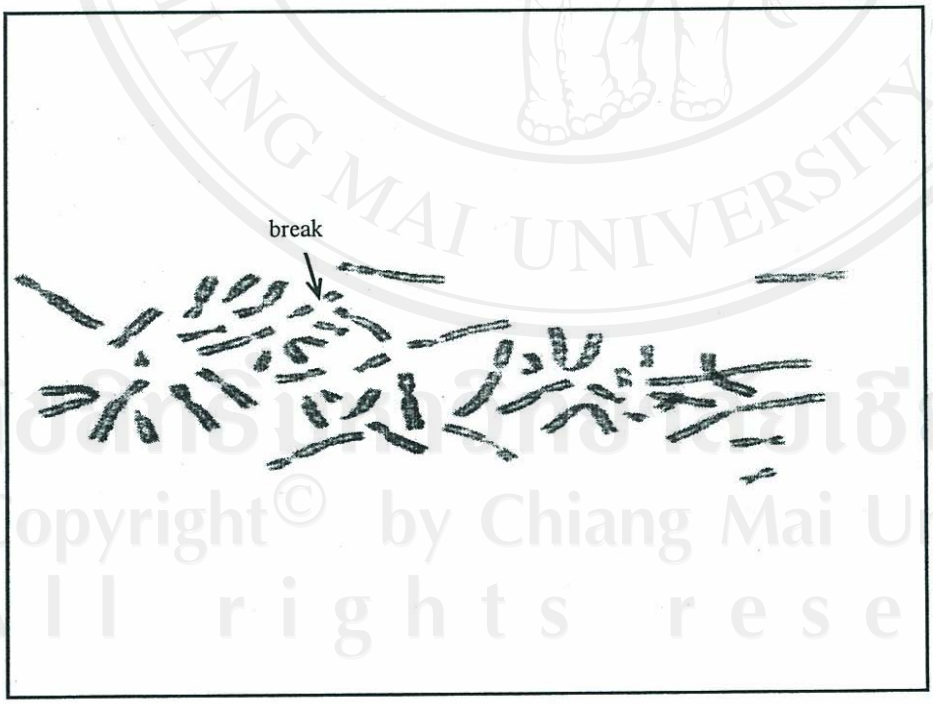


Figure 7 Chromosome-type aberration, break

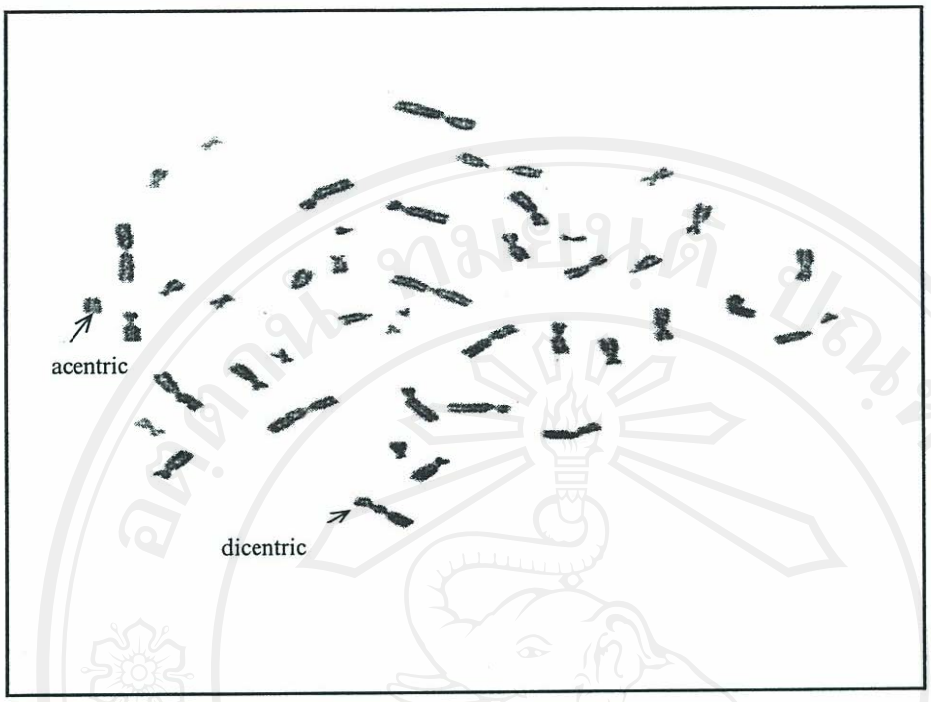


Figure 8 Chromosome-type aberrations, acentric and dicentric

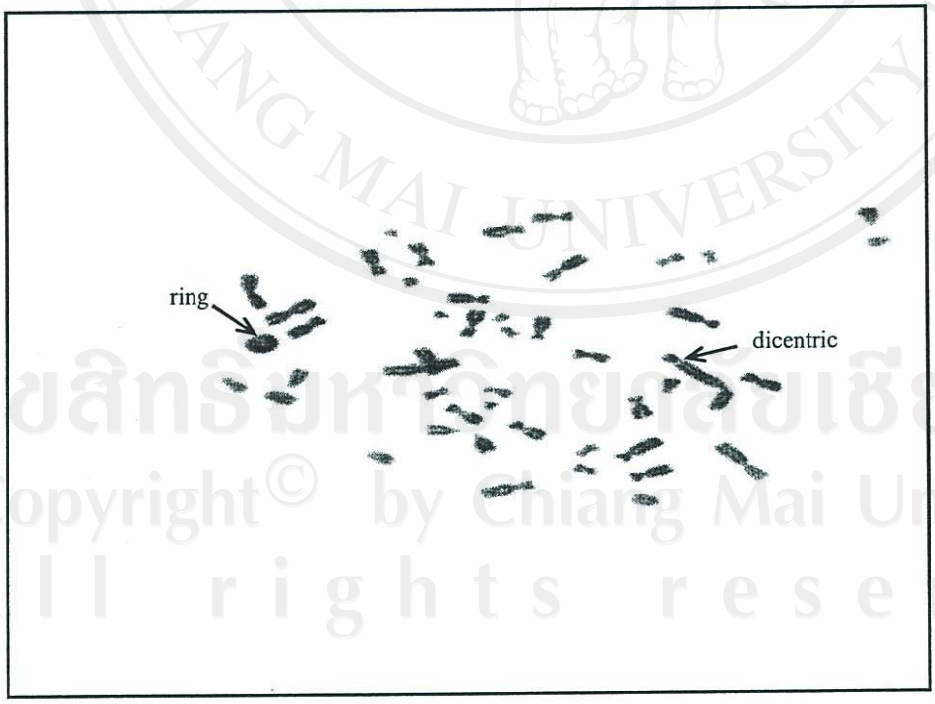


Figure 9 Chromosome-type aberrations, ring and dicentric

Table 6 The effect of confounding factors on chromosomal aberrations evaluated from total population

Factor	% Aberrant cells	P-value	% Aberrations	P-value
Gender		0.1380		0.1898
females	0.877 ± 1.217		0.885 ± 1.221	
males	0.660 ± 0.913		0.689 ± 0.970	
Age range		0.7594		0.8432
41-50	0.600 ± 0.699		0.700 ± 0.823	
51-60	0.697 ± 0.960		0.712 ± 0.973	
61-70	0.841 ± 1.167		0.841 ± 1.167	
71-80	0.750 ± 1.193		0.800 ± 1.285	
81-90	1.500 ± 0.707		1.500 ± 0.707	
Present pesticide exposure		0.9021		0.8989
no	0.783 ± 1.155		0.801 ± 1.182	
yes	0.763 ± 0.897		0.780 ± 0.911	
Smoking habit		0.0147*		0.0068*
smoking	1.050 ± 1.050 a		1.100 ± 1.176 a	
ex-smoking	0.681 ± 1.080 b		0.681 ± 1.080 b	
non-smoking	0.529 ± 0.987 b		0.529 ± 0.987 b	
Alcohol drinking		0.6099		0.3688
drinking	0.761 ± 0.955		0.761 ± 0.955	
ex-drinking	0.966 ± 1.210		1.069 ± 1.334	
non-drinking	0.744 ± 1.134		0.752 ± 1.139	
Fermented tea leaf or betel nut chewing		0.0076*		0.0100*
chewing	0.903 ± 1.175 a		0.921 ± 1.200 a	
ex-chewing	0.706 ± 0.920 a		0.706 ± 0.920 a	
non-chewing	0.326 ± 0.606 b		0.349 ± 0.650.b	

Significant difference of the factors indicates with asterisk

*P≤0.05

In addition, the details of effects of confounding factors influencing the chromosome aberration were investigated both within and between studied populations as described follow.

4.2.2.1 Effect of cigarette smoking on chromosome aberration

Although cigarette smoking elevated chromosome aberration, measured by the percentages of aberrant cells and aberrations in total populations but there were no difference on the chromosome aberration among the smoking habits within Saraphi and Chom Thong populations as summarized in Table 7. However, the significant difference on chromosome aberration of smokers between both populations was observed. The result showed that only male smokers from Chom Thong had higher chromosome aberration than those from Saraphi.

4.2.2.2 Effect of chewing of fermented tea leaf or betel nut on chromosome aberration

The effect on the chewing of fermented tea leaf or betel nut both within and between studied populations was analyzed and summarized in Table 8. Although the elevation of chromosome aberration was observed in total population but the chewing habit did not influence chromosome aberration within both Saraphi and Chom Thong populations. Nevertheless, the significant difference of chromosome aberration between subjects who have chewing activity between these populations was observed in males. Males from Chom Thong had higher chromosome aberration than those from Saraphi.

Table 7 The basal levels of the percentages of aberrant cells and aberrations separated by smoking habits between studied populations

Population	N	% aberrant cells	% aberrations
Saraphi	107		
Smokers	23	0.478 ± 0.730	0.478 ± 0.730
females	11	0.545 ± 0.688	0.545 ± 0.688
males	12	0.417 ± 0.793	0.417 ± 0.793
Ex-smokers	50	0.520 ± 0.995	0.520 ± 0.995
Non-smokers	34	0.441 ± 0.894	0.441 ± 0.894
Chom Thong	118		
Smokers	57	1.281 ± 1.176*	1.351 ± 1.232*
females	34	1.176 ± 1.267	1.206 ± 1.274
males	23	1.435 ± 1.037*	1.565 ± 1.161*
Ex-smokers	44	0.864 ± 1.153	0.864 ± 1.153
Non-smokers	17	0.706 ± 1.160	0.706 ± 1.160

Significant difference between the studied populations indicates with asterisk

*P≤0.05, **P≤0.001, ***P≤0.0001

Table 8 The basal levels of the percentages of aberrant cells and aberrations separated by chewing habit between studied populations

Population	N	% aberrant cells	% aberrations
Saraphi	107		
Chewing	69	0.594 ± 1.005	0.594 ± 1.005
females	42	0.786 ± 1.180	0.786 ± 1.180
males	27	0.296 ± 0.542	0.296 ± 0.542
Ex-chewing	7	0.286 ± 0.756	0.286 ± 0.756
Non-chewing	31	0.290 ± 0.643	0.290 ± 0.643
Chom Thong	118		
Chewing	96	1.125 ± 1.242*	1.156 ± 1.276*
females	61	1.066 ± 1.315	1.082 ± 1.320
males	35	1.229 ± 1.114**	1.286 ± 1.202**
Ex-chewing	10	1.000 ± 0.943	1.000 ± 0.943
Non-chewing	12	0.417 ± 0.515	0.500 ± 0.674

Significant difference between the studied populations indicates with asterisk

*P≤0.05, **P≤0.001, ***P≤0.0001

4.3 Micronucleus test

4.3.1 Micronucleus endpoints and nuclear division indices

The average (mean ± SD.) frequencies of total micronuclei (MN) in 1,000 binucleated cells (BN), the percentage of BN with MN and nuclear division indices (NDI) were also compared between Saraphi and Chom Thong populations as shown in Table 9. The results obtained from micronucleus test were contrarily with those from the chromosomal aberration assay which was previously shown in Table 4. Both total MN in 1,000 BN and the percentage of BN with MN were significantly higher in Saraphi population in both genders. The frequency distribution indicated a shift towards higher frequencies in Saraphi population. This can be seen from total MN in 1,000 BN as well as the percentage of BN with MN as shown in Fig. 10. But there was no significant difference when NDI of the two populations were compared.

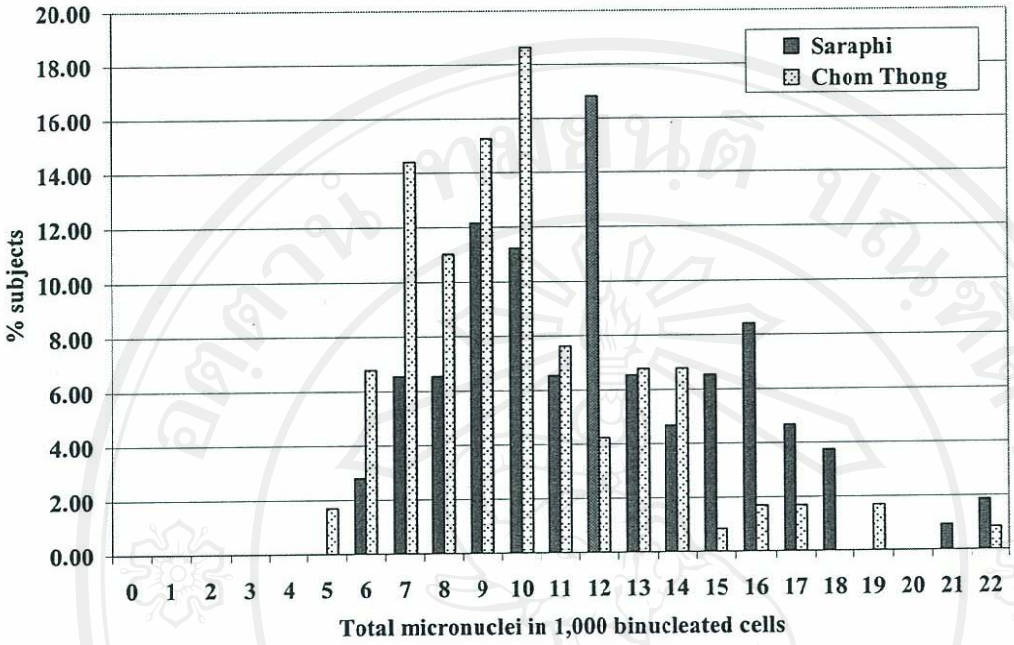
Table 9 Comparison of micronucleus induction between studied populations

Population	N	Total MN in 1,000 BN	BN with MN (%)	NDI
Saraphi				
Total	107	12.009 ± 3.570***	1.141 ± 0.311***	1.491 ± 0.074
Females	55	13.236 ± 3.595***	1.247 ± 0.303***	1.499 ± 0.074
Males	52	10.712 ± 3.076	1.029 ± 0.281*	1.483 ± 0.074
Chom Thong				
Total	118	9.992 ± 3.109	0.925 ± 0.227	1.471 ± 0.112
Females	67	10.194 ± 3.372	0.942 ± 0.310	1.486 ± 0.117
Males	51	9.725 ± 2.736	0.902 ± 0.227	1.450 ± 0.102

Significant difference between the studied populations indicates with asterisk

*P≤0.05, **P≤0.001, ***P≤0.0001

a)



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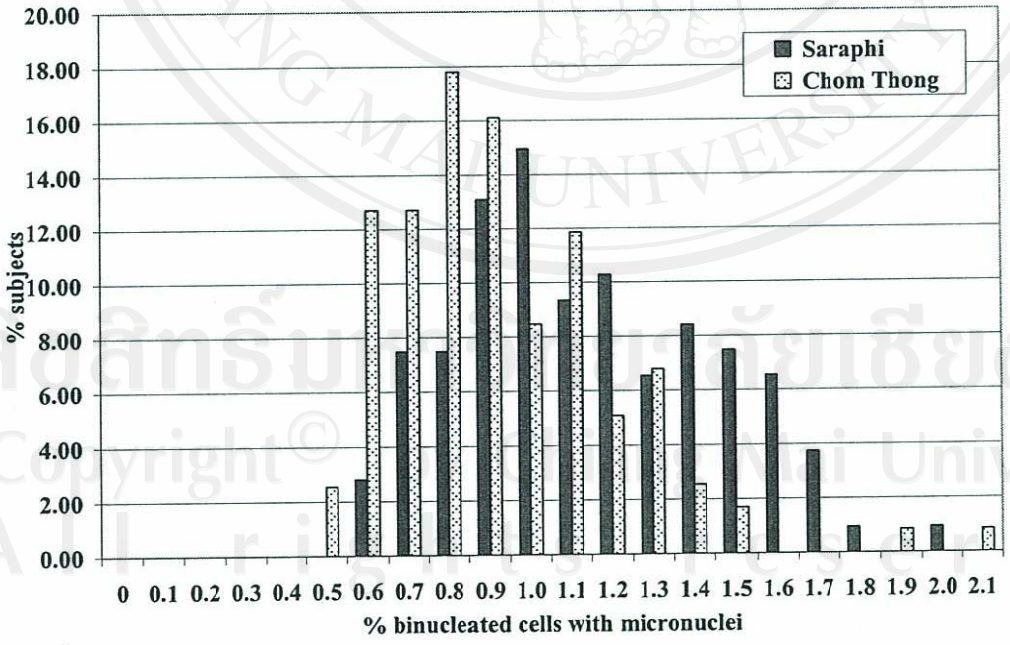


Figure 10 Distributions of a) total MN in 1,000 BN and b) the percentage of BN with MN of both populations

4.3.2 The effects of confounding factors on micronucleus endpoints in total population

With regard to the influence of confounding factors on micronucleus frequencies in total subject, the results by ANOVA were shown in Table 10. Only gender and smoking habit significantly affected micronucleus frequencies whereas other confounding factors obviously mentioned did not influence the micronucleus induction. The details of effects of confounding factors which influenced micronucleus induction were investigated both within and between populations as described follow.

4.3.2.1 The effects of gender on micronucleus induction

The micronucleus induction, measured by total MN in 1,000 BN and percentage of BN with MN, was significantly higher in females than in males when the effect of gender in total population was determined as previously shown in Table 9. Moreover, the difference on micronucleus induction between males and females within and between the populations were observed. However, the effect of gender was only observed within Saraphi population. Females from Saraphi had the higher micronucleus frequency than males. Furthermore, both females and males from Saraphi had higher micronucleus induction than those from Chom Thong when the micronucleus inductions between these populations were compared.

Table 10 The effect of confounding factors on micronucleus induction evaluated in total population

Factor	Total of MN in 1,000 BN	P-value	% BN with MN	P-value
Gender		0.0037*		0.0063*
females	11.566 ± 3.779 a		1.080 ± 0.342 a	
males	10.223 ± 2.940 b		0.966 ± 0.262 b	
Age range		0.2428		0.3309
41-50	12.500 ± 4.696		1.150 ± 0.357	
51-60	11.197 ± 3.429		1.045 ± 0.294	
61-70	10.879 ± 3.406		1.026 ± 0.321	
71-80	10.550 ± 3.404		0.988 ± 0.308	
81-90	7.000 ± 0.000		0.700 ± 0.000	
Present pesticide exposure		0.5114		0.6542
no	11.042 ± 3.600		1.033 ± 0.317	
yes	10.695 ± 3.125		1.012 ± 0.301	
Smoking habit		0.0374*		0.0643
smoking	10.938 ± 4.020 ab		1.014 ± 0.354	
ex-smoking	10.415 ± 2.868 b		0.991 ± 0.269	
non-smoking	11.961 ± 3.435 a		1.116 ± 0.306	
Alcohol drinking		0.4811		0.5232
drinking	10.627 ± 3.571		1.000 ± 0.316	
ex-drinking	10.621 ± 3.099		1.000 ± 0.280	
non-drinking	11.194 ± 3.516		1.048 ± 0.318	
Fermented tea leaf or betel nut chewing		0.3608		0.3505
chewing	10.970 ± 3.594		1.027 ± 0.326	
ex-chewing	9.882 ± 2.522		0.935 ± 0.212	
non-chewing	11.302 ± 3.328		1.065 ± 0.289	

Significant difference ($P<0.05$) of the factors indicates with asterisk

*$P \leq 0.05$

4.3.2.2 The effects of cigarette smoking on micronucleus induction

Although the effect of cigarette smoking in total population was previously shown but there were no differences of micronucleus induction among smokers, ex-smokers and non-smokers within the Saraphi and Chom Thong populations as summarized in Table 11. However, the higher of micronucleus induction was observed in all groups of smokers, ex-smokers and non-smokers from Saraphi than those from Chom Thong. Both male and female smokers in Saraphi had significantly higher of both parameters, while ex-smokers and non-smokers had significantly higher percentage of binucleated cells with MN than those from Chom Thong.

Table 11 The basal levels of total MN in 1,000 BN and % BN with MN separated by smoking habits between studied populations

Population	N	Total MN in 1,000 BN	% BN with MN
Saraphi	107		
Smokers	23	13.552 ± 4.055**	1.270 ± 0.355***
females	11	13.909 ± 4.134*	1.291 ± 0.339*
males	12	13.167 ± 4.130*	1.250 ± 0.383*
Ex-smokers	50	10.960 ± 3.226	1.056 ± 0.296*
Non-smokers	34	12.529 ± 3.314	1.179 ± 0.271*
Chom Thong	118		
Smokers	57	9.895 ± 3.534	0.911 ± 0.300
females	34	10.000 ± 3.635	0.921 ± 0.320
males	23	9.739 ± 3.454	0.896 ± 0.274
Ex-smokers	44	9.795 ± 2.278	0.918 ± 0.215
Non-smokers	17	10.824 ± 3.486	0.988 ± 0.341

Significant difference between the studied populations indicates with asterisk

*P≤0.05, **P≤0.001, ***P≤0.0001

4.4 Single cell gel electrophoresis assay (comet assay)

Comet assay was performed under alkaline condition (pH>13). The experiment was divided into two parts consisting of estimation of internal standard ranges and detection of DNA damage in the studied populations residing in the areas with high and low incidence of lung cancer, Saraphi and Chom Thong districts. The results were described as follow.

4.4.1 Estimation of internal standard ranges and the exclusion of the outliers

The 113 couple slides prepared from one aliquot of untreated lymphocytes and one aliquot of gamma-irradiated (2 Gy) lymphocytes were included in each electrophoresis run and used as internal standards. After DNA damage of the internal standards was measured, the means of three parameters consisting of tail length, tail intensity and tail moment were plotted as the scattergrams for evaluation of acceptable ranges. The suitable range estimation both of internal standards should be the means with ± 2 SD. of all parameters because there were not too less or too much outliers as shown in Fig. 11 and Fig. 12. The data either of negative and positive reference standards which were lower and/or higher than the means with ± 2 SD. in at least one or more parameters were considered as outliers.

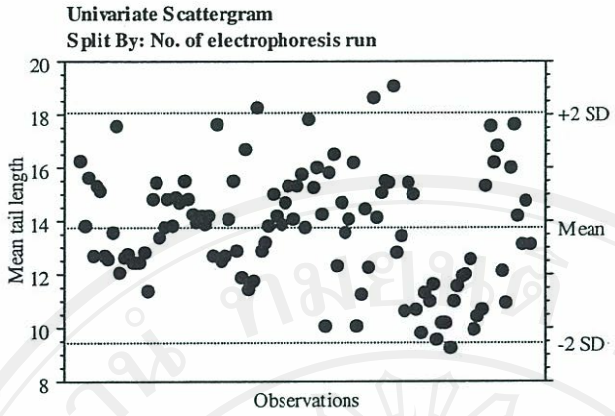
4.2.2 Detection of DNA damage in Saraphi and Chom Thong populations using three types of blood measured before and after exclusion of the outliers

The data from detection of DNA damage in studied subjects were divided into two groups. Firstly, the means of DNA damage measured by 3

parameters from all subjects were considered as data before exclusion of the outlier. Another group was the data after exclusion of the outlier which was the data derived from all studied subjected but not included data from the subjects whose run in the same electrophoresis section of the outliers. The subjects who had the outliers in at least one or more parameters were shown in Table 12. Most of outliers were found only in some parameters. Consequently, data of 18 and 8 subjects from Saraphi and Chom Thong were excluded from data from all subjects and be analyzed as data after exclusion of the outliers.

Three types of blood samples, PB, SPB and SPB-APC, were prepared from each subject for DNA damage detection. DNA damage measured by the means of three parameters consisting of tail length, tail intensity and tail moment using data from subjects before, and after exclusion of internal standard outliers was shown in Table 13. It can be seen that the detected DNA damage was higher in SPB-APC than in other types of blood samples. However, there were only small differences in DNA damage in all parameters of the three types of blood between the studied populations, most of them not being statistically significant. A statistically significant difference was only measured between tail length of PB sample which showed a higher DNA damage, in Saraphi population than those in Chom Thong both before and after exclusion of the outliers. However, the higher of tail length in Saraphi population than those from Chom Thong s was observed only in females as shown in Table 14.

a)



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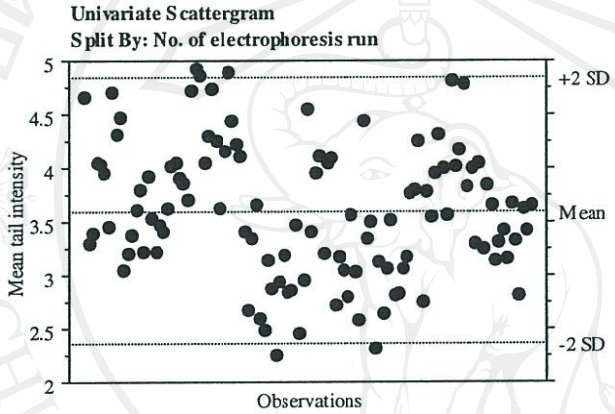


c)

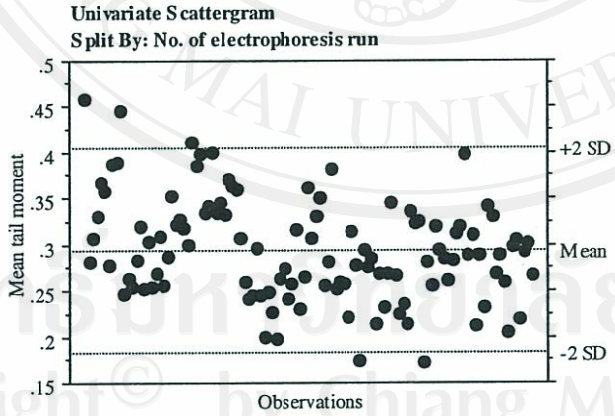
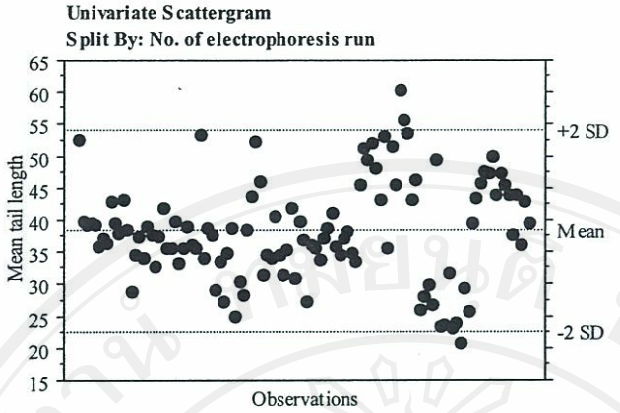
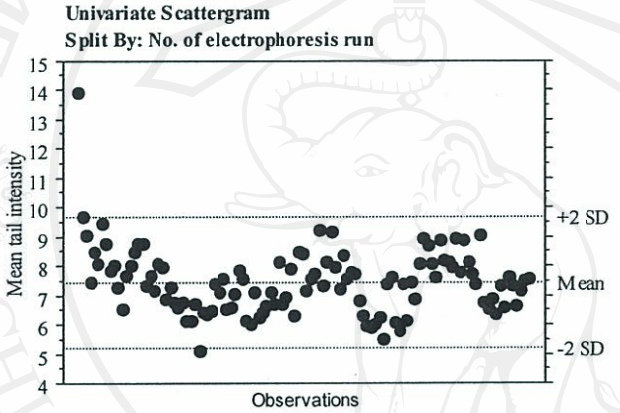


Figure 11 Internal standard for each of the experiments using untreated isolated lymphocytes and considering three image analysis parameters: a) tail length b) tail intensity and c) tail moment respectively

a)



b)



c)

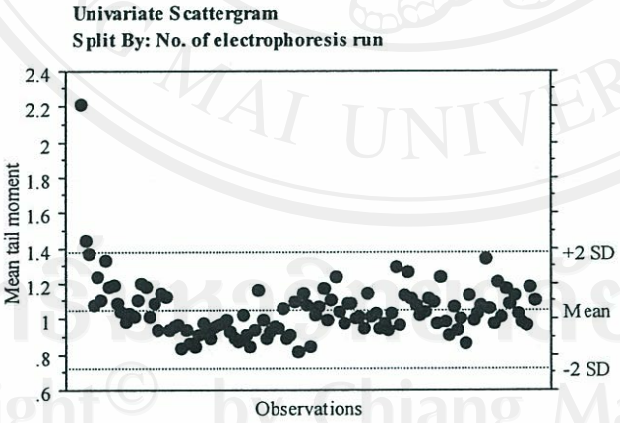


Figure 12 Internal standard for each of the experiments using irradiated isolated lymphocytes and considering three image analysis parameters: a) tail length; b) tail intensity and c) tail moment respectively

Table 12 Electrophoresis runs with the outliers (+) of internal standards which were outside the accepted ranges

No. of electrophoresis	Tail length				Tail intensity				Tail moment			
	Untreated cells		Irradiated cells		Untreated cells		Irradiated cells		Untreated cells		Irradiated cells	
	high	low	high	low	high	low	high	low	high	low	high	low
1	-	-	-	-	-	-	+	-	+	-	+	-
2	-	-	-	-	-	-	-	-	-	-	+	-
10	-	-	-	-	-	-	-	-	+	-	-	-
28	-	-	-	-	-	-	-	-	+	-	-	-
29	-	-	-	-	+	-	-	-	-	-	-	-
30	-	-	-	-	+	-	-	-	-	-	-	-
31	-	-	-	-	-	-	-	+	-	-	-	-
37	-	-	-	-	+	-	-	-	-	-	-	-
45	+	-	-	-	-	-	-	-	-	-	-	-
49	-	-	-	-	-	+	-	-	-	-	-	-
70	-	-	-	-	-	-	-	-	-	+	-	-
74	+	-	-	-	-	+	-	-	-	-	-	-
79	+	-	-	-	-	-	-	-	-	-	-	-
81	-	-	+	-	-		-	-	-	-	-	-
82	-	-	+	-	-	-	-	-	-	-	-	-
86	-	-	-	-	-	-	-	-	-	+	-	-
93	-	+	-	-	-	-		-	-	-	-	-
96	-	-	-	+	-	-		-	-	-	-	-

Table 13 The DNA damage measured by comet assay compared between two studied populations, using data from 3 types of blood a) before and b) after exclusion of the outliers

a)

Population	Types of blood	Tail length	Tail intensity	Tail moment
Saraphi (n=91)	PB	14.638 ± 4.650**	3.387 ± 0.867	0.297 ± 0.168
	SPB	25.538 ± 5.747	7.639 ± 7.639	1.060 ± 0.518
	SPB-APC	30.322 ± 6.502	9.057 ± 1.984	1.371 ± 0.493
Chom Thong (n=94)	PB	12.895 ± 1.944	3.368 ± 0.509	0.252 ± 0.054
	SPB	24.926 ± 5.618	7.372 ± 1.730	0.943 ± 0.356
	SPB-APC	30.959 ± 6.700	9.389 ± 2.053	1.384 ± 0.478

b)

Population	Types of blood	Tail length	Tail intensity	Tail moment
Saraphi (n=73)	PB	13.818 ± 2.610**	3.341 ± 0.788	0.277 ± 0.112
	SPB	25.205 ± 4.940	7.385 ± 2.144	1.005 ± 0.435
	SPB-APC	29.720 ± 6.151	8.869 ± 1.911	1.324 ± 0.463
Chom Thong (n=86)	PB	12.830 ± 1.926	3.387 ± 0.508	0.254 ± 0.055
	SPB	25.179 ± 5.770	7.473 ± 1.745	0.959 ± 0.364
	SPB-APC	31.339 ± 6.807	9.480 ± 2.076	1.406 ± 0.486

Significant difference between the studied populations indicates with asterisk

*P≤0.05, **P≤0.001, ***P≤0.0001

Table 14 Comparison of DNA damage detected from peripheral blood by comet assay between both studied populations.

Population	N	Data from PB before exclusion of the outliers			N	Data from PB after exclusion of the outliers		
		Tail length	Tail intensity	Tail moment		Tail length	Tail intensity	Tail moment
Saraphi								
Total	91	14.638 ± 4.650**	3.387 ± 0.867	0.297 ± 0.168	73	13.818 ± 2.610*	3.341 ± 0.788	0.277 ± 0.112
Females	46	14.363 ± 2.949**	3.417 ± 0.915	0.295 ± 0.131	39	14.465 ± 3.054**	3.480 ± 0.976	0.302 ± 0.139
Males	45	14.920 ± 5.930	3.357 ± 0.824	0.300 ± 0.200	34	13.077 ± 1.750	3.181 ± 0.456	0.248 ± 0.056
Chom Thong								
Total	94	12.895 ± 1.944	3.368 ± 0.509	0.252 ± 0.054	86	12.830 ± 1.926	3.387 ± 0.508	0.254 ± 0.055
Females	51	12.757 ± 1.644	3.354 ± 0.492	0.250 ± 0.053	49	12.780 ± 1.674	3.372 ± 0.492	0.252 ± 0.053
Males	43	13.059 ± 2.258	3.385 ± 0.535	0.255 ± 0.056	37	12.897 ± 2.239	3.407 ± 0.535	0.257 ± 0.059

Significant difference between the studied populations indicates with asterisk

*P≤0.05, **P≤0.001, ***P≤0.0001

4.4.3 The effects of confounding factors on DNA damage using data from PB measured before and after exclusion of the outliers in total population

To further evaluation, the possible effects of confounding factors on DNA damage measured by comet assay were determined by ANOVA using data from PB sample of subjects in total population, both before and after exclusion of the outliers. The effect of some factors were presented in Table 15 showing that pesticide exposure and chewing of fermented tea leaf or betel nut affected DNA damage from data before exclusion of the outliers but only on tail length and tail intensity respectively. Nevertheless, none of the factors significantly influenced DNA damage when the data after exclusion of the outliers was analyzed. As a result, the details of these effects were not continuously determined.

Table 15 The effect of confounding factors on DNA damage using data from PB of all subjects, a) before and b) after the exclusion of the outliers

a)

Factor	Tail length	P-value	Tail intensity	P-value	Tail moment	P-value
Gender		0.3599		0.9024		0.7253
females	11.566 ± 3.779		1.080 ± 0.342		1.080 ± 0.342	
males	10.223 ± 2.940		0.966 ± 0.262		0.966 ± 0.262	
Age range		0.3788		0.7345		0.6064
41-50	12.500 ± 4.696		1.150 ± 0.357		1.150 ± 0.357	
51-60	11.197 ± 3.429		1.045 ± 0.294		1.045 ± 0.294	
61-70	10.879 ± 3.406		1.026 ± 0.321		1.026 ± 0.321	
71-80	10.550 ± 3.404		0.988 ± 0.308		0.988 ± 0.308	
81-90	7.000 ± 0.000		0.700 ± 0.000		0.700 ± 0.000	
Present pesticide exposure		0.0120*		0.9647		0.2589
no	11.042 ± 3.600 a		1.033 ± 0.317		1.033 ± 0.317	
yes	10.695 ± 3.125 b		1.012 ± 0.301		1.012 ± 0.301	
Smoking habit		0.0567		0.9464		0.3165
smoking	10.938 ± 4.020		1.014 ± 0.354		1.014 ± 0.354	
ex-smoking	10.415 ± 2.868		0.991 ± 0.269		0.991 ± 0.269	
non-smoking	11.961 ± 3.435		1.116 ± 0.306		1.116 ± 0.306	
Alcohol drinking		0.4273		0.3449		0.3396
drinking	10.627 ± 3.571		1.000 ± 0.316		1.000 ± 0.316	
ex-drinking	10.621 ± 3.099		1.000 ± 0.280		1.000 ± 0.280	
non-drinking	11.194 ± 3.516		1.048 ± 0.318		1.048 ± 0.318	
Fermented tea leaf or betel nut chewing		0.5540		0.0387*		0.1452
chewing	10.970 ± 3.594		1.027 ± 0.326 a		1.027 ± 0.326	
ex-chewing	9.882 ± 2.522		0.935 ± 0.212 ab		0.935 ± 0.212	
non-chewing	11.302 ± 3.328		1.065 ± 0.289 b		1.065 ± 0.289	

Table 15 (continued)

b)

Factor	Tail length	P-value	Tail intensity	P-value	Tail moment	P-value
Gender		0.1407		0.2419		0.1102
females	11.566 ± 3.779		1.080 ± 0.342		1.080 ± 0.342	
males	10.223 ± 2.940		0.966 ± 0.262		0.966 ± 0.262	
Age range		0.9997		0.5913		0.8627
41-50	12.500 ± 4.696		1.150 ± 0.357		1.150 ± 0.357	
51-60	11.197 ± 3.429		1.045 ± 0.294		1.045 ± 0.294	
61-70	10.879 ± 3.406		1.026 ± 0.321		1.026 ± 0.321	
71-80	10.550 ± 3.404		0.988 ± 0.308		0.988 ± 0.308	
81-90	7.000 ± 0.000		0.700 ± 0.000		0.700 ± 0.000	
Present pesticide exposure		0.6449		0.3505		0.6231
no	11.042 ± 3.600		1.033 ± 0.317		1.033 ± 0.317	
yes	10.695 ± 3.125		1.012 ± 0.301		1.012 ± 0.301	
Smoking habit		0.0542		0.6067		0.8349
smoking	10.938 ± 4.020		1.014 ± 0.354		1.014 ± 0.354	
ex-smoking	10.415 ± 2.868		0.991 ± 0.269		0.991 ± 0.269	
non-smoking	11.961 ± 3.435		1.116 ± 0.306		1.116 ± 0.306	
Alcohol drinking		0.7484		0.2073		0.1235
drinking	10.627 ± 3.571		1.000 ± 0.316		1.000 ± 0.316	
ex-drinking	10.621 ± 3.099		1.000 ± 0.280		1.000 ± 0.280	
non-drinking	11.194 ± 3.516		1.048 ± 0.318		1.048 ± 0.318	
Fermented tea leaf or betel nut chewing		0.6314		0.0734		0.1670
chewing	10.970 ± 3.594		1.027 ± 0.326		1.027 ± 0.326	
ex-chewing	9.882 ± 2.522		0.935 ± 0.212		0.935 ± 0.212	
non-chewing	11.302 ± 3.328		1.065 ± 0.289		1.065 ± 0.289	

Significant difference (P<0.05) of the factors indicates with asterisk

*P≤0.05

4.5 The health risk assessment

The biomonitoring studies by using genetic endpoints consisting of chromosomal aberration, micronucleus and DNA damage detected by comet assays were compared between populations residing in the areas with high and low lung cancer incidences, Saraphi and Chom Thong, supposing that might be associated with the exposure of environmental pollution. In our study, a clear statement regarding potential differences in mutagen exposure between the two studied populations cannot be made because the differences on the biological effects were measured by these genetic biomarkers. For chromosomal aberration, Saraphi population had lower of both the percentages aberrant cells and aberrations than those from Chom Thong population. The contrarily results were however observed in micronucleus induction that Saraphi population had significantly higher both of total MN in 1,000 BN and the percentage of BN with MN. In addition, there were no significant differences on DNA damages measured by all parameters between Saraphi and Chom Thong populations. As a result, the precise biological monitoring on the populations residing in the areas with the differences of lung cancer incidence could not be drawn. However, the corresponding results between the number of micronucleus and DNA damage which was higher in Saraphi population, the area with high incidence of lung cancer, could be concluded that Saraphi population might expose to more environmental pollutant than those in Chom Thong.