

From the above discussions we can obtain the following conclusions:

(1) In addition to the spatial extent (both vertical and horizontal) of the high clouds, its albedo also increases with SST. As a result, HCA was found to influence the LWCRF and SWCRF more than the other cloud types.

(2) Over tropical Indian Ocean at SSTs above 26.4°C, the HCA and the components of CRF increase linearly with SST, and at certain SST threshold (~27.6°C during July). When the probability of occurrence of deep convective activity becomes > 50%, this increase becomes very rapid. This feature is observed over tropical Pacific Ocean also. However this increase with SST does not always sustain at very warm SSTs. Over tropical Indian (west Pacific) Ocean during July and October (January and April) due to the suppressed deep convection at SSTs above ~29°C (29.6°C) the HCA and the components of CRF are found to decrease with increasing SST.

(3) In general, during April, there is a near-cancellation between LWCRF and SWCRF over the tropical Indian Ocean. During October, over lower SST (26.4°C) and during January and July months mainly over warm SST (above and around the SST threshold values) regions, the SWCRF is found to be greater than LWCRF, resulting in the lack of cancellation between LWCRF and SWCRF. Except in April months the differences between area weighted monthly SWCRF and LWCRF over tropical Indian Ocean are significantly large and negative.

(4) Over tropical Indian (west Pacific) Ocean during January and July (January and October) when HCA becomes very large (~40–50%), the cloud albedo seems to increase much faster than the longwave absorption, resulting in net cloud radiative cooling of the region by the clouds.

But we would like to put a word of caution to these results particularly of January and July because of seasonal changes in the incoming solar radiation and the solar zenith angle, both of which have complex and largely unknown effects on the cloud forcings.

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**ACKNOWLEDGEMENTS.** We are grateful to DGM, India Meteorological Department for permitting us to submit the paper to this journal, to Dr U. S. De for his encouragement and useful suggestions and to Dr S. K. Dikshit for his encouragement and for providing facilities to carry out this research work. Our sincere thanks are also due to Prof. J. Srinivasan for going through this manuscript and for giving very useful suggestions. The data sets used in this study were obtained from the NASA Langley Research Center EOSDIS Distributed Active Archive Center. M.R. thanks NASA for supplying these data sets. We also thank the anonymous referees for their valuable suggestions.

Received 24 November 1997; revised accepted 9 June 1998

## Toxicity of *tuibur*, a unique form of tobacco smoke extract used in Mizoram, India

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A unique form of water extract of tobacco smoke called *tuibur* is used by some people in Mizoram. The toxicity of *tuibur* was studied using modified version of *Allium* test. Even dilute solutions of *tuibur* exhibited significant toxicity by reducing the root growth of *Allium* bulbs and inducing tumour formation in the roots. Microscopical features revealed reduction of mitotic index, formation of micronuclei, lagging chromosome and c-mitosis in the root tip cells treated with different concentrations of *tuibur*. EC<sub>50</sub> value of *tuibur* for root growth was also estimated.

TOBACCO use by people is an ancient practice, probably started in the early 1400s. It is estimated that the use of tobacco kills about three million people globally every

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year<sup>1</sup>. A number of smoking and smokeless tobacco products are in use all over the world. In India tobacco is mostly smoked or chewed but other forms of tobacco use are also prevalent. The smokeless tobacco products used in India are: *khaini*, *mishri*, *zarda*, *kiwam*, *pan masala*, etc. Unlike other smokeless tobacco products, a unique water (liquid preparation) containing the extracts of tobacco smoke is used in Mizoram and is locally known as *tuibur*. This product is made locally by passing smoke, generated by burning tobacco, through water till the preparation turns cognac in colour and has a pungent smell. Indigenous crude devices are used for the production of *tuibur* on small scale. Users take about 5 to 10 ml *tuibur* orally and keep it in the mouth for some time and then spit it out. Most of the users take it several times a day.

Tobacco has at least 2549 chemical constituents<sup>2</sup> which include aliphatic hydrocarbons, isoprenoids, phytosterols, polynuclear aromatic hydrocarbons, alcohols, phenols and phenolic acids, carboxylic acids, amides and amines, alkaloids, N-nitrosamines, metals, radio elements and agricultural chemicals. Many of them are mutagenic and carcinogenic. Mutagenic potency of tobacco extract has been reported by Bhide *et al.*<sup>3</sup>. Shirname *et al.*<sup>4</sup> reported micronuclei formation with tobacco extract in bone marrow cells of Swiss albino mice. The tobacco alkaloids, anatabine, nicotine and nor-nicotine induce sister chromatid exchanges in the Chinese Hamster Ovary (CHO) cells<sup>5</sup>. Tobacco products used in 'pan masala' have genotoxic effect on Chinese Hamster Ovary (CHO) cells<sup>6</sup>. The practice of consuming tobacco products is associated with various diseases including malignancy. Therefore this unique form of smokeless tobacco abuse assumes importance from a public health point of view. In the present study, toxicity of *tuibur* is evaluated in a modified version of *Allium* test<sup>7</sup>.

For the present study, four samples of *tuibur* were collected from Aizawl, Mizoram, in clean sterilized glass bottles and pooled together for analysis. The pH of the undiluted *tuibur* was 9.7. Five different concentrations ranging from 1 to 3% were prepared (v/v) in tap water (pH ranged from 8.9 to 9.3). Random sample of *Allium* bulbs (8–10 g) were chosen from a population of common onion, *Allium cepa* L (2n = 16). After removal of the outer scales of the bulbs, the brownish bottom plates were also removed without injuring the ring of root primordia. Twelve bulbs for each concentration were placed on top of the test tubes (28 ml) filled with test solutions. Two control series in tap water were run concurrently at room temperature (29–31°C) away from direct sunlight. Control series I was at original pH (7.2) whereas in control series II, pH was adjusted at 9.4. Since no statistically significant difference was observed in root growth response or mitotic index between the two series of controls, the data pertaining to control se-

ries I only are presented. After 48 h, root tips from each bulb were sampled, fixed in Carnoy's fixative and preserved at 4°C in 70% ethanol. Maceration and hydrolysis of the root tips were done in a 9:1 mixture of 45% acetic acid and 1N HCl at 55°C for 10 min followed by squashing in 2% aceto orcein. After 72 h, root length of each of the 10 best grown root bundles was measured. Presence of tumours on roots, their size and number were also noted. The mean and 95% confidence interval value of 10 measurements of root length at each concentration was expressed as percentage of control. The data were plotted and a second degree polynomial equation was fitted for the root growth curve. One-way analysis of variance (ANOVA) was used to find out whether there was statistically significant difference in the mean response of root length and mitotic index in treated and control groups. However, prior to one way ANOVA test, data on mitotic index were transformed to a variate  $\sin^{-1} \sqrt{(\text{Proportion of mitotic cells})}$  in order to stabilize variance<sup>8</sup>. However, for presentation in the table the data were back transformed to the original values. For multiple comparisons between different pairs of treatments, Tukey-Kramer honestly significant difference (HSD) test is used for its more conservative nature than Fisher's least significant difference (LSD) test<sup>9</sup>. The EC<sub>50</sub> value (effective concentration permitting 50% growth in relation to control) was estimated from the growth curve using second degree polynomial equation (Figure 1) and was found to be 2.48%. The microscopic parameters noted in the study were mitotic index, lagging chromosome, c-mitosis and micronuclei.

Table 1 shows root length of *Allium* bulbs and presence of tumours in different groups. There was significant

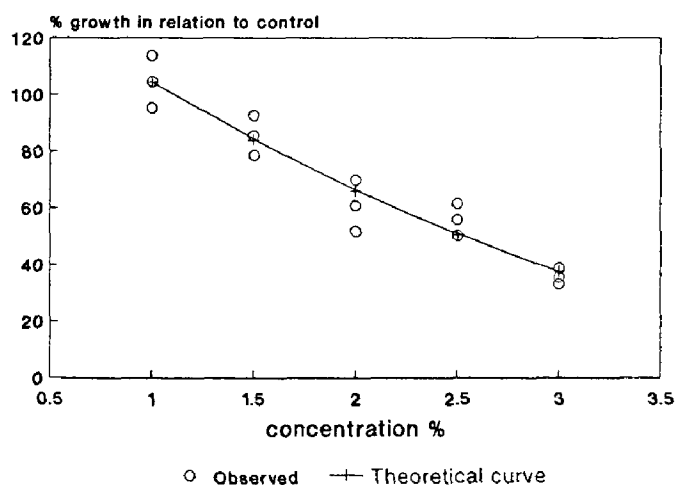
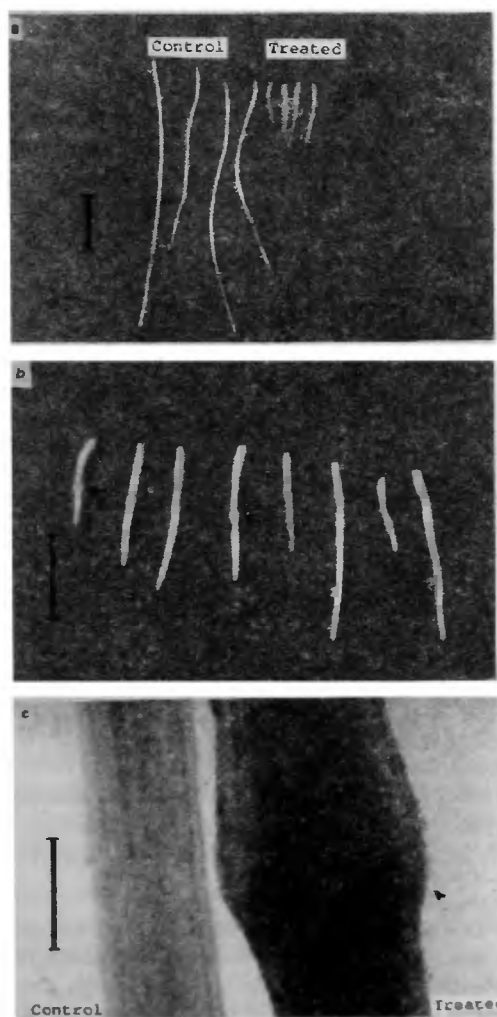


Figure 1. Growth of *Allium* roots in relation to control after treatment with different concentrations of *tuibur*. The values shown are mean and 95% CI. The relationship between root growth response and different concentrations of *tuibur* was found to be curvilinear and was best explained by 2nd degree polynomial equation:  $(Y = 152.662 - 53.276X + 4.972X^2)$ , where  $Y$  = response of root growth expressed as % of control and  $X$  = % concentration of *tuibur*.

**Table 1.** Root length and tumour frequency in *Allium* bulbs treated with *tuibur* ( $n = 10$  bulbs for each treatment group)

% Concentration	Root length (cm) mean $\pm$ SD	95% CI	% of roots with tumour
0.0 (control)	4.98 $\pm$ 0.64	4.52–5.44	–
1.0	5.20 $\pm$ 0.65	4.74–5.66	–
1.5	4.26 $\pm$ 0.49	3.91–4.61	–
2.0	3.03 $\pm$ 0.62	2.58–3.48	12.5 ( $n = 128$ )
2.5	2.78 $\pm$ 0.39	2.50–3.06	26.8 ( $n = 194$ )
3.0	1.79 $\pm$ 0.20	1.65–1.93	43.3 ( $n = 120$ )

95% confidence interval (CI) were based on:  $\bar{X} \pm ts(\bar{X})$ , where  $t = t(1-\alpha/2; n-1)$ .



**Figure 2.** Control and *tuibur*-treated roots of *Allium* bulbs. *a*, Photograph showing control versus treated roots (scale bar = 1 cm); *b*, Enlarged view of 72 h *tuibur*-treated roots showing tumour formation (the number of tumours per root varies from 1 to 2 and the mean diameter of tumours is  $1017.4 \pm 79.71 \mu\text{m}$ ). (Scale bar = 1 cm); *c*, Photomicrograph showing one control and one treated root with tumour (Scale bar =  $650 \mu\text{m}$ ).

reduction of root length with increase of *tuibur* concentration from 1.5% onwards. Tumour formation on the roots was seen in groups treated with 2–3% concentration of *tuibur* after 72 h (Figure 2*a–c*). At 2%

**Table 2.** One way ANOVA table for onion root length response to *tuibur* in *Allium* test

Source of variation	ss	df	ms	F-ratio
Treatments	91.41133	5	18.28227	66.32019*
Error	14.88600	54	0.27567	
Total	106.29733			

\* $P < 0.001$ .

**Table 3.** Pairwise comparison of mean root length between control and different treatment groups of *tuibur*

% Concentration	Mean value difference
0.0–1.0	$4.98 - 5.20 = 0.220$
0.0–1.5	$4.98 - 4.26 = 0.720^*$
0.0–2.0	$4.98 - 3.03 = 1.950^*$
0.0–2.5	$4.98 - 2.78 = 2.200^*$
0.0–3.0	$4.98 - 1.79 = 3.190^*$
1.0–1.5	$5.20 - 4.26 = 0.940^*$
1.0–2.0	$5.20 - 3.03 = 2.170^*$
1.0–2.5	$5.20 - 2.78 = 2.420^*$
1.0–3.0	$5.20 - 1.79 = 3.410^*$
1.5–2.0	$4.26 - 3.03 = 1.230^*$
1.5–2.5	$4.26 - 2.78 = 1.480^*$
1.5–3.0	$4.26 - 1.79 = 2.470^*$
2.0–2.5	$3.03 - 2.78 = 0.250$
2.0–3.0	$3.03 - 1.79 = 1.240^*$
2.5–3.0	$2.78 - 1.79 = 0.990^*$

\*Significant at  $P < 0.05$  (based on Tukey–Kramer HSD test).

concentration, 16 out of 128 (12.5%) roots examined showed presence of tumour whereas at 3% concentration 52 out of 120 (43.3%) roots examined showed presence of tumour. The size of tumours on the roots varied from  $894.7$  to  $1105.3 \mu\text{m}$  (mean =  $1017.54 \mu\text{m}$ ; 95% CI =  $975.05$  to  $1060.04 \mu\text{m}$ ) in diameter whereas the diameter of control roots ranged from  $578.9$  to  $894.7 \mu\text{m}$  (mean =  $748.5 \mu\text{m}$ ; 95% CI =  $700.12$  to  $796.95 \mu\text{m}$ ). One-way analysis of variance (Table 2) shows highly significant difference in mean response of root length between control and different treatment groups ( $P < 0.001$ ). The results of pairwise comparison between different treatment groups are summarized in Table 3. 1.5% of *tuibur* was the minimum concentration at which there was a statistically-significant difference in mean response of root length as compared to control. Similarly, there was highly significant ( $P < 0.001$ ; F-ratio 9.657; ANOVA test) difference in mitotic index between *tuibur*-treated and control roots. There was inverse relationship between mitotic index and concentration of *tuibur* (correlation  $r = -0.66$ ;  $P < 0.01$ ), i.e. with increase in the concentration of *tuibur* there was decrease in the mitotic index, the minimum mitotic index being 26.9 at 2.5% concentration of *tuibur* (Table 4). Highest abnormality in microscopic parameters was seen in groups treated with 2.0 to 3.0% *tuibur*. 7.1% of cells showed

Table 4. Mitotic index in *Allium* test with *tuibur* ( $n = 10$ )

% Concentration	Mitotic index (mean; $\bar{x}$ )	$\pm$ SD	95% CI**
0.0 (control)	41.1	$\pm 4.91$	37.59–44.61
1.0	37.5	$\pm 10.15$	30.24–44.76
1.5	33.3	$\pm 7.04$	28.26–38.34
2.0	28.1*	$\pm 4.58$	24.82–31.38
2.5	26.9*	$\pm 3.84$	24.15–29.65
3.0	27.5*	$\pm 2.51$	25.71–29.29

\*Significantly different ( $P < 0.05$ ) when compared to control (based on Tukey–Kramer HSD test).

\*\*95% confidence interval (CI) were based on:  $\bar{x} \pm ts(\bar{x})$ , where  $t = t(1 - \alpha/2; n - 1)$ .

Table 5. Microscopic parameters of *Allium* test with *tuibur*. (Time interval of treatments = 48 h)

% Concentration	Total mitotic cells counted	Percentage of cells showing:		
		Lagging chromosome	C-mitosis	Micronuclei
0.0 (control)	481	0.6	1.0	–
1.0	478	0.2	1.0	–
1.5	519	4.2	2.1	–
2.0	502	5.0	3.6	0.2
2.5	509	7.1	2.9	0.8
3.0	500	6.0	3.4	1.0

Cells were counted from 10 slides for each treatment concentration.

lagging chromosomes at 2.5% concentration of *tuibur*. Similarly, c-mitosis was noted in 3.6% of cells at 2.0% treatment concentration and 1% of cells showed presence of micronuclei at 3% concentration of *tuibur* (Table 5).

Results generated by *Allium* test system or other test systems using eukaryotic or prokaryotic cells largely give similar results<sup>10</sup>. Grant<sup>11</sup> also observed that compounds which have c-mitotic effect in plants will induce the same effect in animal tissues. Hence results obtained in *Allium* test can indicate the effect on the human body.

In the present study, *tuibur* showed dose-dependent inhibition of root growth and mitotic index and the effect was significant even at low concentrations. Another significant finding was formation of tumours in roots proximal to the tips. Fiskesjö<sup>7</sup> reported tumours in the root tips in his study using *Allium* test with toxic metal ions (methyl mercury chloride).

The ability of *tuibur* to cause mitotic damage by inducing micronuclei formation, c-mitosis and lagging chromosomes indicates that it acts as a mitotic poison during the process of cell division. It can be mentioned

that people use undiluted (100%) *tuibur* regularly whereas even its dilute solutions showed significant toxic effect. Therefore, impact of this unique form of smokeless tobacco product can have greater implications on human health.

Tobacco-related cancers account for about half of all cancers among men and one fourth among women in the world<sup>1</sup>. There is no cancer registry in Mizoram and consequently no information is available on the incidence of different cancers in the state. However, data collected for the year 1997 from the Directorate of Health Services of Mizoram revealed that stomach cancer is the leading tumour in this state and constitutes 43.7% of all cancer cases and oral cancer accounts for 2.7% of all cancer cases. The highest frequency of stomach cancer in India is reported from Madras where it constituted only about 13.32% of all cancer cases among men in 1989 (ref. 12). It is pertinent to mention here that while keeping *tuibur* in the mouth for sometime, some portion of it is also swallowed. Therefore, association of *tuibur* with cancers of stomach and mouth cannot be ruled out and further epidemiological as well as experimental studies are required to elucidate the risks of *tuibur* use in Mizoram.

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ACKNOWLEDGEMENTS. We thank Mr Arun Gogoi for his assistance in conducting the experiment. We also thank the Director, Health Services, Govt. of Mizoram for providing necessary information about cancers in Mizoram and for help during collection of samples.

Received 18 March 1998; revised accepted 30 June 1998