Carcinogenesis vol.16 no.5 pp.1095–1099, 1995

# Occupational exposure to unburnt bidi tobacco elevates mutagenic burden among tobacco processors

**Aparna N.Bagwe and Rajani A.Bhisey[1]**

Carcinogenesis Division, Cancer Research Institute, Tata Memorial Centre, Parel, Bombay-400 012, India

[1]To whom correspondence should be addressed

**The nature of mutagenic burden due to occupational exposure to tobacco flakes and dust was determined among 20 female tobacco processors (TP) and 20 matched controls (C) by testing urinary mutagenicity in the Ames assay. In addition, urinary cotinine was estimated as a marker of tobacco absorption. Workers and controls were sub-divided into those with no tobacco habit (NH) and those habituated to the use of masheri (a pyrolysed form of tobacco) as a dentifrice (MH). Cotinine was not detected in samples from C−NH while the mean urinary cotinine levels in TP−NH and TP−MH were significantly higher than that in C−MH (3.46 ± 0.95 and 3.57 ± 0.46 versus 1.80 ± 0.58 mM/M creatinine; $P < 0.02$). The majority of the urine samples from C−NH were non-mutagenic in the presence or absence of rat liver S9 while those from C-MH were mutagenic to TA98 and TA102 strains upon metabolic activation. On the other hand, direct mutagenicity to TA98, TA100 and TA102 strains respectively was noted in 6/10, 5/10 and 8/10 samples from TP−NH and 7/10, 4/10 and 3/10 samples from TP−MH. Generally, β-glucuronidase treatment reduced or abolished the mutagenic potential of workers' urine samples indicating that glucuronide conjugates may have partially contributed to direct mutagenicity. Experiments using scavengers of reactive oxygen species revealed that direct mutagenicity in TA102 strain was mediated mainly via hydroxyl radicals. The results clearly demonstrate that tobacco processors are exposed to a wide spectrum of mutagens that cause frame-shift, base pair substitution and oxidative damage.**

## Introduction

In India, the bulk of the tobacco crop is processed to obtain blends which are used for the manufacture of 'bidis'−indigenous substitutes for cigarettes (1). Inside tobacco processing plants, sun-cured tobacco leaves (*Nicotiana tabacum*) are manually shredded, pounded, winnowed and sieved to obtain flakes of the desired size. These activities generate particulates and respirable dust, and expose the workers to tobacco by cutaneous and nasopharyngeal routes. We reported earlier that extracts of processed, unburnt bidi tobacco contain nitrosatable precursors to potent mutagens (2) and the aqueous bidi tobacco extract was found to exhibit moderate mouse skin tumor promoting activity (3). Further studies on bidi industry workers revealed a marked increase in urinary thioether excretion (4) and micronucleated buccal epithelial cell frequency (5). These findings necessitated an evaluation of the nature of mutagenic exposure among tobacco processors. Data on urinary cotinine levels and mutagenicity in the Ames test among workers and controls are presented in this communication.

***Abbreviations:** DMSO, dimethyl sulfoxide; G6P, glucose-6-phosphate; β-gluc, β-glucuronidase; NADP, β-nicotinamide adenine dinucleotide phosphate; SOD, superoxide dismutase; TP, tobacco processors; C, controls; NH, no tobacco habit; MH, masheri habit; UC, urine concentrate/s; SR, spontaneous reversion; ROS, reactive oxygen species; ND, not detectable; NM, non-mutagenic.

## Materials and methods

*Chemicals*

Amberlite XAD-2 resin, D-biotin, catalase, chloramine-T, dimethyl sulfoxide (DMSO*), glucose-6-phosphate (G6P), β-glucuronidase (β-gluc), L-histidine, β-nicotinamide adenine dinucleotide phosphate (NADP) and superoxide dismutase (SOD) were purchased from Sigma Chemical Co. Inc., USA, while bacto agar and bacto nutrient broth were from Difco Labs, USA. Cotinine and diethyl thiobarbituric acid (Sigma) were gifts from Dr N.C.Mishra, USA. All the other chemicals were of analytical or guaranteed reagent grade.

*Bacterial strains*

*Salmonella typhimurium* TA98, TA100 and TA102 strains, kindly provided by Prof. B.N.Ames, USA, were maintained in the laboratory as per the revised protocol of Maron and Ames (6).

*Subjects*

Twenty workers employed in a tobacco processing plant located in a small town in Southern India and 20 controls (C) from the same locality, matched for age, sex and socio-economic conditions, were monitored. The study was restricted to female tobacco processors (TP), as they constitute > 80% of the workforce. Detailed information on diet, type and frequency of tobacco habit, duration of occupational exposure, health status and medical history was collected from each subject. None of the workers smoked or consumed alcohol; however, habitual use of masheri (a pyrolysed tobacco product) as a dentifrice was commonly reported. For data analysis, workers and controls were sub-grouped (10/sub-group) as: those with no tobacco habit (NH) and those with masheri habit (MH).

*Sample collection and processing*

Eight-hour urine samples (~500 ml) starting with the early morning voided sample, were collected in polypropylene bottles and stored over ice during transportation to the laboratory. Aliquots were set aside for cotinine estimation, routine analysis as well as creatinine estimation by the alkaline picrate method (7), and the rest of the urine was frozen without preservative at −20°C. Within a week of collection, urine samples were concentrated using XAD-2 resin columns (8) and the urine concentrates (UC) were lyophilized and solubilized in DMSO to achieve 250-fold concentration.

*Urinary cotinine estimation*

The spectrophotometric method of Peach *et al.* (9) which detects cotinine and related metabolites of nicotine (hereinafter referred to as 'cotinine') was used as described earlier (4). Samples in which cotinine was not detectable (< 0.5 μg/ml) were re-tested after 100-fold concentration, bringing the detection limit to 5 ng/ml. Quantitation was done using cotinine as standard and values were expressed as mM/M creatinine.

*Mutagenicity testing*

Mutagenicity of each UC was tested at three doses−1.25, 2.5 and 5.0 μl corresponding to 0.31, 0.63 and 1.25 ml of the original volume, using the modified liquid pre-incubation protocol (10). Each UC (triplicate plates per dose) was tested : (i) without modification (−S9), (ii) upon metabolic activation with Aroclor-1254 induced rat liver S9 (+S9) and (iii) after pre-treatment with 150 units of β-glucuronidase (+β-gluc) for 20 min at 37°C. Zero dose plates for spontaneous reversion (SR) contained 20 μl of DMSO instead of the UC. The assay mix consisted of 100 μl of 2-fold concentrated bacterial culture, 100 μl of phosphate buffer (pH 7.4) and different volumes of the unmodified or β-gluc-treated UC. In experiments with S9, the buffer was replaced by 100 μl of the S9 mix comprising of 10 μl each of 80 mM magnesium chloride, 8 mM NADP and 50 mM G6P, 30 μl of 0.25 M Sorensen's phosphate buffer (pH 7.4), 35 μl of distilled water and 5 μl of rat

liver S9. After incubation for 90 min at 37°C , 2 ml of histidine–poor soft agar was added and the mixture was overlayed on minimal glucose agar plates. After 48 h incubation, revertant colonies were enumerated.

The criteria used to label a sample as mutagenic were: (i) a dose-dependent increase in revertant number and (ii) minimum induced reversion ≥ 3 times the standard deviation value of the historic mean SR (i.e. beyond the upper 99% confidence limit).

*Scavenging studies*

Six UC eliciting direct mutagenic response in TA102 were co-incubated with scavengers of reactive oxygen species (ROS) and their effect on mutagenicity was determined. Briefly, the overnight culture of *S. typhimurium* TA102 was centrifuged and resuspended in phosphate buffered saline (pH 7.2) to achieve 2-fold concentration. After mixing with 2 × concentration of each scavenger (25 μg/plate), the cultures were equilibrated on ice for 30 min and used for mutagenicity testing (11). The enzymes SOD and catalase were used as scavengers of superoxide anion and hydrogen peroxide respectively, while mannitol and benzoate served as scavengers of radicals such as hydroxyl, peroxyl, etc. (12).

*Statistical analysis*

Data on urinary cotinine were evaluated using the Student's *t*-test for comparison of means and the Chi-square test was used to determine the differences in the proportion of samples positive for cotinine or mutagenic activity, in worker and control groups. The influence of occupational tobacco exposure on induced reversion was assessed using the Wilcoxon rank sum test. In the final group-wise analysis of mutagenicity data, equal weighting was given to a significant increase being noted in revertant number as well as in the proportion of samples eliciting a mutagenic response.

## Results

*Personal information*

The age of controls and TP ranged from 20–58 years and 18–65 years respectively, mean age being similar in each of the sub-groups (Table I). The frequency of masheri use was 1–7 times/day in controls as well as workers, while the duration of occupational tobacco exposure ranged from 1–42 years. Most of the subjects were vegetarian and shared a similar dietary pattern. The frequency of consumption of non-vegetarian food was rarely more than once a week.

*Urinary cotinine*

As shown in Table I, cotinine was not detected in urine samples from C–NH, while it was present in 8/10 samples from C–MH. In contrast, all the samples from TP contained fairly high amounts of cotinine and the mean cotinine levels in TP–NH and TP–MH were found to be significantly higher than that in the C–MH group (3.46 ± 0.95 and 3.57 ± 0.46 respectively versus 1.80 ± 0.58 mM/M creatinine; $P < 0.02$).

*Urinary mutagenicity*

*TA98: Controls.* As shown in Table II, none of the samples from C–NH were directly mutagenic while only one C–MH sample exhibited direct mutagenicity. Addition of S9 rendered three C–NH and seven C–MH samples mutagenic while β-glucuronidase treatment rendered four C–NH samples mutagenic but abolished the mutagenicity of the C–MH sample.

**Table I.** Baseline information and tobacco-specific exposure[a]

| Parameters | C–NH | C–MH | TP–NH | TP–MH |
|---|---|---|---|---|
| Age (yrs) | 32±3 | 42±2 | 40±3 | 39±2 |
| Habit frequency (daily) | – | 3±0.5 | – | 2±0.3 |
| Occupational exposure (yrs) | – | – | 18±3 | 12±2 |
| Urinary cotinine (mM/M creatinine) | Nd | 1.80±0.58 | 3.46±0.95 | 3.57±0.46 |

[a]Mean ± SE.
Nd, Not detectable; C, controls; TP, tobacco processors; NH, no tobacco habit; MH, masheri habit.

*Workers.* Direct mutagenicity was noted in six TP–NH and seven TP–MH samples (Table II). Upon metabolic activation with rat liver S9, nine TP–NH and all 10 TP–MH samples were mutagenic, whereas treatment with β-glucuronidase reduced the number of mutagenic samples in both groups.

*TA100: Controls.* There was negligible or no mutagenic activity in samples from C–NH and C–MH groups both in the absence or presence of S9, and only three samples each were mutagenic after β-glucuronidase treatment (Table III).

*Workers.* Five and four samples each from TP–NH and

**Table II.** Urine mutagenicity[a] in TA98

| Habit | | Controls | | | Workers | | |
|---|---|---|---|---|---|---|---|
| | | −S9 | +S9 | +β-gluc | −S9 | +S9 | +β-gluc |
| NH | 1 | NM | NM | NM | NM | 0.32 | NM |
| | 2 | NM | NM | NM | NM | 0.20 | NM |
| | 3 | NM | 0.25 | NM | NM | 2.06 | NM |
| | 4 | NM | NM | 0.36 | 0.49 | 0.35 | NM |
| | 5 | NM | NM | 1.07 | 0.61 | 0.53 | NM |
| | 6 | NM | 2.24 | NM | NM | 0.78 | 0.35 |
| | 7 | NM | 2.13 | 0.92 | 2.13 | NM | NM |
| | 8 | NM | NM | 0.20 | 1.45 | 0.54 | 0.40 |
| | 9 | NM | NM | NM | 1.42 | 2.12 | NM |
| | 10 | NM | NM | NM | 0.72 | 0.75 | 0.42 |
| MH | 1 | NM | 2.52 | NM | NM | 0.77 | NM |
| | 2 | NM | 0.47 | NM | NM | 0.54 | NM |
| | 3 | 0.56 | 1.03 | NM | 2.46 | 0.80 | NM |
| | 4 | NM | NM | NM | 2.06 | 1.40 | 0.37 |
| | 5 | NM | 1.04 | NM | 1.38 | 0.87 | 0.22 |
| | 6 | NM | NM | NM | 1.25 | 0.31 | 0.73 |
| | 7 | NM | 0.32 | NM | 0.35 | 1.05 | 0.20 |
| | 8 | NM | 0.43 | NM | NM | 1.22 | NM |
| | 9 | NM | NM | NM | 0.48 | 0.95 | 0.53 |
| | 10 | NM | 0.33 | NM | 0.83 | 0.96 | 0.75 |

[a]Values represent induced revertants per M creatinine×$10^{-6}$.
NH, no tobacco habit; MH, masheri habit; NM, non-mutagenic.

**Table III.** Urine mutagenicity[a] in TA100

| Habit | | Controls | | | Workers | | |
|---|---|---|---|---|---|---|---|
| | | −S9 | +S9 | +β-gluc | −S9 | +S9 | +β-gluc |
| NH | 1 | NM | NM | 2.50 | NM | NM | NM |
| | 2 | 3.17 | NM | NM | NM | NM | NM |
| | 3 | NM | NM | 0.41 | NM | NM | NM |
| | 4 | NM | NM | 3.53 | NM | 2.21 | 11.01 |
| | 5 | NM | NM | NM | 2.48 | NM | NM |
| | 6 | NM | NM | NM | NM | NM | 2.22 |
| | 7 | NM | NM | NM | 1.98 | 0.94 | NM |
| | 8 | NM | NM | NM | 1.45 | 2.53 | 1.51 |
| | 9 | NM | NM | NM | 3.34 | 9.20 | 3.44 |
| | 10 | NM | NM | NM | 4.32 | 1.80 | NM |
| MH | 1 | NM | NM | NM | NM | NM | NM |
| | 2 | NM | NM | 1.04 | NM | NM | NM |
| | 3 | NM | NM | 0.79 | NM | NM | NM |
| | 4 | NM | NM | NM | 1.50 | NM | 0.56 |
| | 5 | NM | NM | NM | NM | NM | NM |
| | 6 | NM | NM | NM | 1.62 | 0.81 | NM |
| | 7 | NM | NM | NM | NM | 0.50 | NM |
| | 8 | NM | NM | 1.65 | 1.36 | 0.56 | 0.57 |
| | 9 | NM | 0.32 | NM | NM | NM | NM |
| | 10 | NM | NM | NM | 1.14 | 1.76 | NM |

[a]Values represent induced revertants per M creatinine×$10^{-6}$.
NH, no tobacco habit; MH, masheri habit; NM, non-mutagenic.

TP−MH groups respectively exhibited mutagenicity in the absence and presence of S9, while after β-glucuronidase treatment, four TP−NH and only two TP−MH samples were mutagenic (Table III).

*TA102: Controls.* As shown in Table IV, direct mutagenicity was absent in C−NH and negligible in C−MH samples. Treatment with S9 rendered only two C−NH and six C−MH samples mutagenic. Deconjugation with β-glucuronidase increased the mutagenic activity in C−NH group rendering seven samples mutagenic, while only two C−MH samples were mutagenic after β-glucuronidase treatment.

*Workers.* Eight TP−NH and three TP−MH samples were directly mutagenic while six and nine samples respectively from these two groups elicited a mutagenic response after addition of S9. Treatment with β-glucuronidase led to a major reduction in mutagenic activity in TP−NH samples with only two samples being positive, whereas increased number of TP−MH samples exhibited mutagenicity.

*Studies using ROS scavengers*

As shown in Table V, SOD and catalase had no effect on the mutagenicity of five out of six UC tested, while addition of mannitol reduced the revertant number in four samples and abolished the mutagenic potential of the remaining two samples. However, co-incubation of UC with sodium benzoate rendered all six samples non-mutagenic.

Samples from both the control groups generally evoked similar responses except that the mutagenic potential of β-glucuronidase-treated C−MH samples to TA98 and TA102 was significantly lower than that of C−NH samples ($P < 0.05$). A comparative analysis of urinary mutagenicity in worker and control groups is provided in Figures 1 and 2. A group was considered to be significantly different from its control only if significant differences were noted in the number of mutagenic samples as well as in the induced reversion. TP−NH samples evoked a marked increase in direct mutagenicity in TA98 and TA102, and were mutagenic to TA100 only after S9 treatment (Figure 1). On the other hand, TP−MH samples exhibited a significant increase in direct mutagenicity in TA98 and TA100 strains. In addition, a significant increase in mutagenicity in TA98 and TA102 was also evoked by TP−MH samples upon treatment with β-glucuronidase (Figure 2).

**Table IV.** Urine mutagenicity[a] in TA102

| Habit | | Controls | | | Workers | | |
|---|---|---|---|---|---|---|---|
| | | −S9 | +S9 | +β-gluc | −S9 | +S9 | +β-gluc |
| NH | 1 | NM | NM | 1.49 | 6.28 | 5.99 | NM |
| | 2 | NM | NM | 7.01 | 7.01 | 3.36 | NM |
| | 3 | NM | NM | 1.29 | 16.40 | 37.11 | NM |
| | 4 | NM | NM | NM | 4.70 | NM | NM |
| | 5 | NM | 24.29 | 3.02 | NM | NM | NM |
| | 6 | NM | NM | 10.93 | 19.32 | 7.72 | 19.16 |
| | 7 | NM | NM | 21.22 | 17.66 | NM | NM |
| | 8 | NM | NM | NM | 27.75 | NM | NM |
| | 9 | NM | 37.54 | NM | NM | 9.40 | 7.13 |
| | 10 | NM | NM | 1.85 | 4.12 | 7.82 | NM |
| MH | 1 | NM | NM | NM | NM | 7.11 | 17.14 |
| | 2 | NM | NM | NM | NM | 1.32 | 7.10 |
| | 3 | 2.50 | NM | 14.53 | NM | 5.94 | 19.72 |
| | 4 | NM | 1.41 | NM | 2.69 | 1.69 | NM |
| | 5 | NM | NM | NM | 2.43 | 1.70 | 0.94 |
| | 6 | NM | 2.90 | NM | NM | 5.01 | NM |
| | 7 | NM | 3.05 | NM | NM | 2.62 | 2.10 |
| | 8 | NM | 4.10 | NM | 4.90 | 4.09 | 3.37 |
| | 9 | NM | 1.28 | NM | NM | 11.52 | 12.05 |
| | 10 | NM | 3.94 | 1.11 | NM | NM | NM |

[a]Values represent induced revertants per M creatinine × $10^{-6}$.
NH, no tobacco habit; MH, masheri habit; NM, non-mutagenic.

**Table V.** Effect of ROS scavengers on urine mutagenicity[a] in TA102

| UC | Unmodified | +SOD | +Catalase | +Mannitol | +Benzoate |
|---|---|---|---|---|---|
| None | 295±6 | 298±12 | 305±6 | 305±5 | 296±5 |
| UC 1 | 611±11 | 603±7 | 620±10 | 416±5 | 300±11[b] |
| UC 2 | 518±8 | 488±2 | 509±4 | 337±18[b] | 305±15[b] |
| UC 3 | 510±9 | 511±11 | 504±8 | 420±10 | 325±5[b] |
| UC 4 | 593±10 | 293±7[b] | 564±13 | 465±15 | 305±6[b] |
| UC 5 | 434±5 | 407±4 | 314±13[b] | 374±25 | 320±12[b] |
| UC 6 | 427±5 | 425±10 | 412±11 | 339±9[b] | 292±6[b] |

[a]Mean ± SE.
[b]Abolition of mutagenicity.
UC, urine concentrate.

## Discussion

In this study, increased absorption of tobacco constituents due to occupational exposure was evident from the elevated cotinine levels in urine samples from tobacco processors. This finding is in agreement with earlier reports of high urinary cotinine levels in non-smoking farmers and harvesters (13) and bidi rollers (14). Moreover, the mean cotinine level in TP−NH

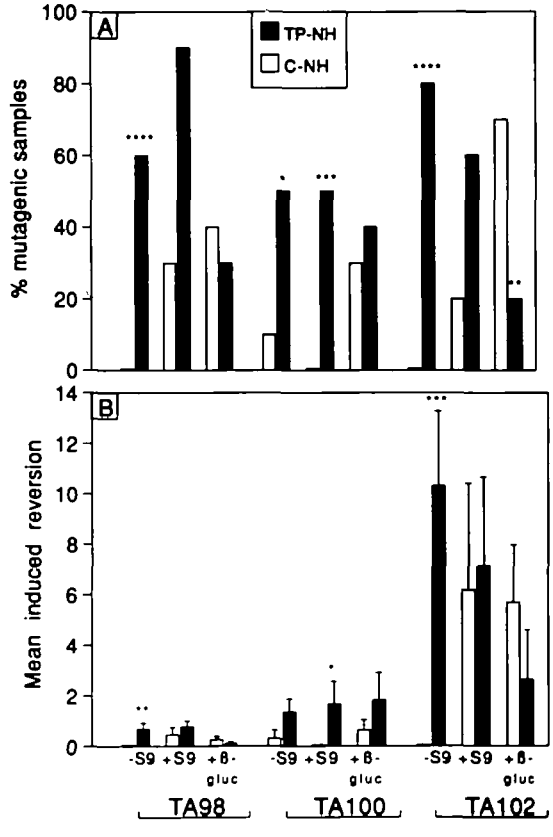


**Fig. 1.** Summary data on urinary mutagenicity in habit-free workers and controls. (A) Percentage of samples exhibiting mutagenic activity [*$P < 0.05$; **$P < 0.025$; ***$P < 0.01$; ****$P < 0.005$−Chi square test], (B) Mean + SE of induced revertants/M creatinine×$10^{-6}$ [*$P = 0.015$; **$P = 0.006$; ***$P = 0.0008$−Wilcoxon rank sum test].

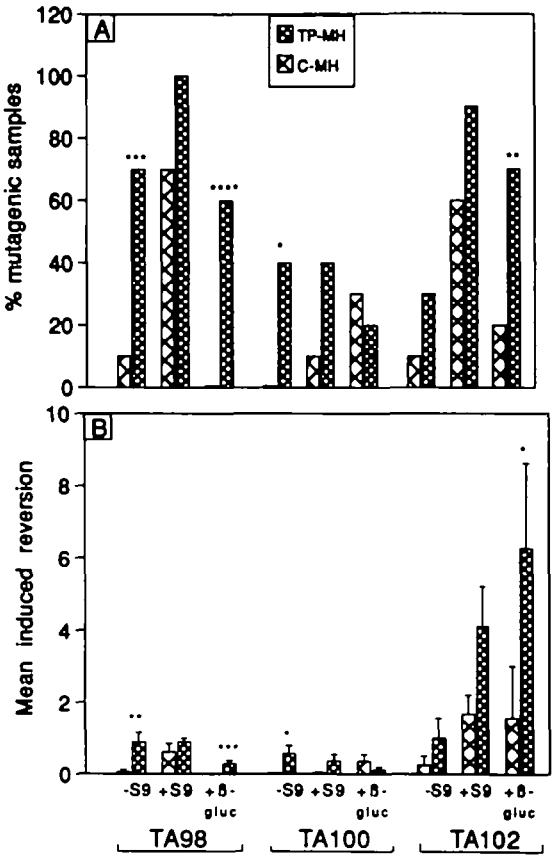


**Fig. 2.** Summary data on urinary mutagenicity in masheri habituated workers and controls. (**A**) Percentage of samples exhibiting mutagenic activity [$*P < 0.05$; $**P < 0.025$; $***P < 0.01$; $****P < 0.005$–Chi square test]. (**B**) Mean + SE of induced revertants/M creatinine$\times 10^{-6}$. [$*P = 0.035$; $**P = 0.008$; $***P = 0.006$–Wilcoxon rank sum test]

was nearly two-fold greater than that in masheri-habituated controls demonstrating that excessive absorption of tobacco constituents occurs among tobacco processors.

Urine samples from both C–NH and C–MH elicited a low or moderate degree of mutagenic response in TA98 and TA102 strains upon metabolic activation, and majority of C–NH samples treated with β-glucuronidase were mutagenic to TA102. These findings are indicative of the contribution of dietary mutagens (15,16) and concur with reports of urinary mutagenicity among non-smokers (17,10) and individuals with no tobacco habit (14). However, masheri use was found to be associated with modulation of urinary mutagenicity. Thus, the mutagenic potential of β-glucuronidase-treated C–MH samples in TA102 was lower than that of similarly treated C–NH samples suggesting that other detoxification pathway(s) may be effective in these individuals. Indeed our recent findings on elevated glutathione transferase activity in blood samples from masheri users (unpublished data), point to the activation of glutathione conjugation pathway among smokeless tobacco users.

In contrast to the control groups, urine samples from both groups of workers were directly mutagenic to TA98. In addition, TP–MH samples exhibited significant direct mutagenicity in TA100 while TP–NH samples exhibited direct mutagenicity in TA102. Further studies using ROS scavengers revealed that oxidative damage was mediated mainly by hydroxyl radicals which are reported to be responsible for cigarette smoke-induced DNA damage in cultured human lung cells (18) and for mutagenicity of gastric fluid from tobacco chewers (11).

It has been hypothesized that smokers detoxify tobacco carcinogens in a more efficient manner than do non-smokers (19) and are reported to incur a lower level of DNA damage consequent to exposure to occupational genotoxicants (20). Cytogenetic studies from our laboratory have revealed a significant increase in chromatid breaks in lymphocytes of TP–NH but not in those of TP–MH (21). Reduced direct mutagenicity in TA102 of TP–MH samples observed in this study suggests that masheri habit may have resulted in more efficient detoxification of oxidative mutagens. Since ROS are effectively detoxified by conjugation with glutathione, analysis of glutathione conjugation pathway among TP–NH and TP–MH groups may help to understand the differences in internal exposure to oxidative mutagens and genotoxic manifestations in these groups.

In an earlier study, we reported that bidi tobacco extracts were mutagenic to the three tester strains after *in vitro* nitrosation (2). Increased endogenous nitrosation has been reported to occur in individuals habituated to tobacco (22,23). Hence it may be speculated that in tobacco processors too, this process is responsible for the conversion of nitrosatable precursors to direct–acting mutagens and their excretion in urine.

In conclusion, the present study emphasizes that tobacco processors receive elevated exposure to a wide spectrum of tobacco-derived mutagens that cause frame-shift, base pair substitution and oxidative damage. In view of the chronic exposure of these workers to tobacco—a known human carcinogen—it is necessary to determine the cancer incidence in this high-risk group.

## Acknowledgements

The authors acknowledge the whole-hearted co-operation received from the tobacco processors, control subjects and especially the proprietors of the tobacco processing plant. The technical assistance of Mr M.L.Jagtap and Mr M.D'Mello and the typography of Mr V.V.Pimpalkhare is acknowledged.

## References

1. Jaisani,B.G. (1989) Possible substitutes and alternate uses of bidi tobacco. In Sanghvi,L.D. and Notani,P. (eds), *Tobacco and health : the Indian scene*, Publ. Tata Memorial Centre, Bombay, pp. 207–215.
2. Bagwe,A.N. and Bhisey,R.A. (1991) Mutagenicity of processed bidi tobacco : possible relevance to bidi industry workers. *Mutat. Res.*, **261**, 93–99.
3. Bagwe,A.N., Ramchandani,A.G. and Bhisey,R.A. (1994) Skin-tumour–promoting activity of processed bidi tobacco in hairless S/RV Cri–ba mice. *J. Cancer Res. Clin. Oncol.*, **120**, 485–489.
4. Govekar,R.B. and Bhisey,R.A. (1992) Elevated urinary thioether excretion among bidi rollers exposed occupationally to processed tobacco. *Int. Arch. Occup. Environ. Health*, **64**, 101–104.
5. Bagwe,A.N. and Bhisey,R.A. (1993) Occupational exposure to tobacco and resultant genotoxicity in bidi industry workers. *Mutat. Res.*, **299**, 103–109.
6. Maron,D.M. and Ames,B.N. (1983) Revised methods for the Salmonella mutagenicity test. *Mutat. Res.*, **113**, 173–215.
7. Varley,H. (1967) *Practical Clinical Biochemistry*, 4th edn. Heinemann, London; Interscience, New York.
8. Clonfero,E., Zordan,M., Cottica,O., Venier,P., Pozzoli,L., Cardin,E.L., Sarto,F. and Levis,A.G. (1986) Mutagenic activity and polycyclic aromatic hydrocarbon levels in urine of humans exposed to therapeutical coal tar. *Carcinogenesis*, **7**, 819–823.
9. Peach,H., Morris,R.W., Ellard,G.A. and Jenner,P.J. (1986) A simple inexpensive urine test for smoking. In Zaridze,D.G. and Peto,R. (eds), *Tobacco : a major international health hazard*, International Agency for Research on Cancer, Lyon, IARC Sci. Publ. no. 74, pp. 183–193.
10. Malaveille,C., Vineis,P., Esteve,J., Ohshima,H., Brun,G., Hautefeuille,A.,

Gallet,P., Ronco,G., Terracini,B. and Bartsch,H. (1989) Levels of mutagens in the urine of smokers of black and blond tobacco correlate with their risk of bladder cancer. *Carcinogenesis*, **10**, 577–586.
11. Niphadkar.M.P., Contractor,Q.Q. and Bhisey,R.A. (1994) Mutagenic activity of gastric fluid from chewers of tobacco with lime. *Carcinogenesis*, **15**, 927–931.
12. Brawn,K. and Fridovich,I. (1981) DNA strand scission by enzymatically generated oxygen radicals. *Arch. Biochem. Biophys.*, **206**, 414–419.
13. Ghosh,S.K., Saiyed,H.N., Gokani,V.N. and Thakker,M.U. (1986) Occupational health problems among workers handling Virginia tobacco. *Int. Arch. Occup. Environ. Health*, **58**, 47–52.
14. Bhisey,R.A. and Govekar,R.B. (1991) Biological monitoring of bidi rollers with respect to genotoxic hazards of occupational tobacco exposure. *Mutat. Res.*, **261**, 139–147.
15. Yano,M., Wakabayashi,K., Tahira,T., Arakawa,N., Nagao,M. and Sugimura,T. (1988) Presence of nitrosatable mutagen precursors in cooked meat and fish. *Mutat. Res.*, **202**, 119–123.
16. Hageman,G., Hermans,R., ten Hoor,F. and Kleinjans,J. (1990) Mutagenicity of deep-frying fat and evaluation of urine mutagenicity after consumption of fried potatoes. *Food Chem. Toxicol.*, **28**, 75–80.
17. Pasquini,R., Monarca,S., Scassellati-Sforzolini,G., Savino,A., Bauleo,F.A. and Angeli,G. (1989) Urinary excretion of mutagens, thioethers and D-glucaric acid in workers exposed to bitumen fumes. *Int. Arch. Occup. Environ. Health*, **61**, 335–340.
18. Leanderson,P. and Tagesson,C. (1992) Cigarette smoke-induced DNA damage in cultured human lung cells: role of hydroxyl radicals and endonuclease activation. *Chem.-Biol. Interactions*, **81**, 197–208.
19. Remmer,H. (1987) Passively inhaled tobacco smoke: a challenge to toxicology and preventive medicine. *Arch. Toxicol.*, **61**, 89–104.
20. Oesch,F., Hengstler,J.C. and Fuchs,J. (1994) Cigarette smoking protects mononuclear blood cells of carcinogen exposed workers from additional work exposure induced DNA single strand breaks. *Mutat. Res.*, **321**, 175–185.
21. Mahimkar, M.B. and Bhisey, R.A. (1995) Occupational exposure to bidi tobacco increases chromosomal aberrations in tobacco processors. *Mutat. Res.* (in press).
22. Ladd,K.F., Archer,M.C. and Newmark,H.L. (1984) Increased endogenous nitrosation in smokers. In O'Neill,I.K., von Borstel,R.C., Miller,C.T., Long,J. and Bartsch,H. (eds), *N-nitroso compounds: occurrence, biological effects and relevance to human cancer*, International Agency for Research on Cancer, Lyon, IARC Sci. Publ. no. 57, pp. 811–828.
23. Nair,J., Ohshima,H., Pignatelli,B., Friessen,M., Malaveille,C., Calmels,S. and Bartsch,H. (1986) Modifiers of endogenous carcinogen formation : studies on *in vivo* nitrosation in tobacco users. In *Mechanisms in tobacco carcinogenesis*, Cold Spring Harbor Laboratory Press, New York, Banbury Report 23, pp. 45–61.

*Received on October 19, 1994; revised on February 7, 1995; accepted on February 7, 1995*