

## EFFECTS OF NITROSAMINES, NITRITE AND SECONDARY AMINES ON TUMOR DEVELOPMENT IN MICE

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## INTRODUCTION

ALTHOUGH preformed nitrosamines have been found in nearly all cured meats, the amount has been reduced or eliminated in all cured products except for bacon (Gray and Randall, 1979). In the case of bacon, N-nitrosopyrrolidine (N-Pyr) has consistently been found after cooking, although it has never been reported in raw bacon (Gray, 1976). Even though Greenberg (1976) has reported a small but consistent decline in N-Pyr levels in cooked bacon, it is still considered to be a serious meat industry problem (Gray and Randall, 1979). An even more important safety consideration than the level of preformed nitrosamines in meat products, however, is the production of nitrosamines by the interaction of nitrites and secondary amines in the acid environment of the stomach, as has been reviewed by Walters (1976).

The present study was undertaken to compare the carcinogenicity of N-Pyr, nitrite and nitrite plus a secondary amine (pyrrolidine - Pyr) with a control group of mice. All additives were administered in the drinking water. Results should indicate not only whether N-Pyr produced malignant tumors, but also whether nitrite alone or the combination of nitrite plus Pyr, which theoretically should produce N-Pyr in the stomach, were carcinogenic to mice.

## MATERIALS AND METHODS

Four groups of 10 weanling mice per group were fed a complete laboratory chow diet and given the following additives in the drinking water:

- Group 1 - Control - ordinary tap water ad libitum
- Group 2 - N-Pyr - 100 mg per liter of water
- Group 3 - Nitrite - 1 g NaNO<sub>2</sub> per liter of water
- Group 4 - Nitrite (100 mg/liter water) + Pyr (100 mg/liter of water)

During the first year, 4 mice from each group were killed and examined for tumors after 8 weeks on experiment. Since there was no evidence of tumors at this time, the remaining mice and all subsequent animals were kept on the diets for 12 months. The study was replicated four times to give a total of 36 mice per group during a 4-year period of time.

Records of feed and water consumption were kept throughout and weight gains were determined for each group. Survival data were also maintained throughout the study.

At the end of the experimental period of 12 months, the surviving mice were sacrificed by etherizing. The internal organs and glands were then grossly examined for tumors and other abnormalities. Samples of the liver, lungs, spleen, heart and stomach were then placed in 10% neutral formalin and preserved for subsequent detailed histological examination. All the fixed tissues were embedded in paraffin, sectioned, mounted and stained after which they were examined for tumors. All tumors were then classified as benign or malignant.

The data were subjected to statistical analysis by two-way analysis and comparing treatment means with Duncan's multiple range test as described by Steel and Torrie (1960).

## RESULTS

Table 1 presents the data on feed consumption. Although group 3 (Nitrite) ate less feed than the other groups, the differences between groups were not statistically significant. Table 2 shows the same information on water consumption. It reveals that group 3 drank significantly less water than groups 1 and 2, but was not significantly different ( $P < .05$ ) from group 4, which in turn was not different from groups 1 and 2.

Table 3 summarizes the total intake of the various additives by entire groups and shows the average intake per mouse per day. Group 2 consumed an average of 0.75 mg/mouse/day of N-Pyr. Group 3 had an average intake of 6.02 g of nitrite/mouse/day, while group 4 had an intake of 6.61 g of nitrite and 0.66 mg of N-Pyr/mouse/day.

The average weight gains per mouse during the experimental period of 12 months ranged from a low of 31.7 g for group 3 to a high of 34.0 for group 1. The difference in weight gains is not significant ( $P < .05$ ).

The mortality data are summarized in Table 5. Survival ranged from 94.4% in group 1 to 80.5% in group 2. Although the differences were quite large, they were not significant ( $P < .05$ ). However, the differences approached significance ( $P < .05$ ).

Upon gross examination of the mice for tumors upon autopsy, it was evident that group 2 (N-Pyr) had more total tumors than any other group. Approximately 58 and 66% of group 2 had tumors of the liver and lungs, respectively. No tumors of the liver were found in any of the other groups, while tumors of the lungs were only 2.7,

5.5 and 2.8% in groups 1, 3 and 4, respectively. Statistical analysis revealed that group 2 (N-Pyr) had significantly more tumors than all other groups. Otherwise, there were no differences.

Microscopic examination of the tissues confirmed the effect of N-Pyr (group 2) in producing malignant tumors, with 50 and 47% having malignant tumors of the liver and lungs, respectively. The other groups ranged from a low of 5.50% of lung tumors in group 3 (nitrite) to a high of 8.33% for both of the other groups. Microscopic examination confirmed the absence of liver tumors in groups 1, 3 and 4. However, one mouse in group 3 had a lymphosarcoma and one mouse in group 4 had a malignant tumor of the uterus.

#### SUMMARY

Results showed that feed intake and gains were not significantly influenced by N-Pyr, nitrite or a combination of nitrite plus Pyr (a secondary amine). Addition of nitrite to the drinking water resulted in a significant ( $P < .05$ ) reduction in water intake for group 3 as compared to groups 1 (control) and 2 (N-Pyr), but the difference between groups 3 (nitrite) and 4 (nitrite plus Pyr) was not significant. Both gross observation and microscopic examination confirmed the carcinogenicity of N-Pyr. There were, however, no significant differences between the other groups. Thus, there was no evidence that a combination of nitrite and a secondary amine (Pyr) were carcinogenic to mice administered these additives for a period of 12 months.

#### REFERENCES

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Table 1. Effects of Nitrosamines, Nitrite and Secondary Amines on Feed Consumption<sup>1</sup> of Mice

Feed Consumption				
	g/mouse/year	S.D.	g/mouse/day	S.D.
Group 1	1549.1 <sup>a</sup>	± 108.1	4.24 <sup>a</sup>	± 0.30
Group 2	1595.2 <sup>a</sup>	± 183.1	4.37 <sup>a</sup>	± 0.50
Group 3	1467.9 <sup>a</sup>	± 77.1	4.02 <sup>a</sup>	± 0.21
Group 4	1544.2 <sup>a</sup>	± 93.2	4.23 <sup>a</sup>	± 0.26

<sup>1</sup> All values in the same column not having the same superscript are statistically significant at  $P < .05$ .

Table 2. Effect of nitrosamines, Nitrate and Secondary Amines on Water Consumption of mice<sup>1</sup>.

Water Consumption				
	ml/mouse/year	S.D.	ml/mouse/day	S.D.
Group 1	2913.6 <sup>a</sup>	± 462.3	7.98 <sup>a</sup>	± 1.27
Group 2	2748.4 <sup>a</sup>	± 141.6	7.53 <sup>a</sup>	± 0.39
Group 3	2195.5 <sup>b</sup>	± 142.9	6.02 <sup>b</sup>	± 0.39
Group 4	2411.8 <sup>a,b</sup>	± 527.2	6.61 <sup>a,b</sup>	± 1.44

<sup>1</sup> See Table 1.

Table 3. Data on Consumption of Nitrosopyrrolidine (NP), Nitrite (N) and Pyrrolidine (P) by Groups

Group	mg/NP/mouse/yr ± S.D.	mg/NP/mouse/day ± S.D.	mg/N/mouse/yr ± S.D.	mg/N/mouse/day ± S.D.	mg/P/mouse/yr ± S.D.	mg/P/mouse/day ± S.D.
1	0	0	0	0	0	0
2	274.8 ± 14.2	0.75 ± 0.04	0	0	0	0
3	0	0	2195.5 ± 142.9	6.02 ± 0.39	0	0
4	0	0	2411.8 ± 527.2	6.61 ± 1.44	241.1 ± 52.7	0.66 ± 0.14

Table 4. Data on Weight Gains by Groups

Group	Weight Gain (g) ± S.D.	
	Gain (g)	S.D.
1	34.0 <sup>a</sup>	4.09
2	32.3 <sup>a</sup>	5.07
3	31.7 <sup>a</sup>	4.24
4	33.7 <sup>a</sup>	4.27

Table 5. Effects of Nitrosopyrrolidine, Nitrite and Pyrrolidine on Mortality in Mice

Mortality Rate		
Group	No. of deceased mice/yr ± S.D.	% Survival (4 years)
1	0.5 ± 1 deaths/yr/10 mice	34/36 = 94.4%
2	1.75 ± 0.96 deaths/yr/10 mice	29/36 = 80.5%
3	0.75 ± 0.96 deaths/yr/10 mice	33/36 = 91.6%
4	1.5 ± 0.58 deaths/yr/10 mice	30/36 = 83.3%