

CHRONIC TOXICITY STUDIES ON FOOD COLOURS

PART III. OBSERVATIONS ON THE TOXICITY OF MALACHITE GREEN, NEW COCCINE AND NIGROSINE IN RATS

BY M. G. ALLMARK, W. A. MANNELL AND H. C. GRICE

*From The Food and Drug Laboratories, Department of National Health and Welfare,
Ottawa, Canada*

Received April 29, 1957

New Coccine and Nigrosine, at levels of 0.03, 0.3 and 3.0 per cent in the diet, had no effect on mortality in male or female rats. In groups receiving Malachite Green at levels of 0.3 and 3.0 per cent in the diet there was 100 per cent mortality within the first week of the experiment. Female rats receiving Malachite Green at 0.03 per cent or Nigrosine at 3.0 per cent in the diet showed a significant decrease in growth rate. For the group on Malachite Green this was accompanied by a decrease in food consumption. In both male and female rats receiving Nigrosine at 3.0 per cent in the diet, there was an increase in the organ-body weight ratio for liver and kidneys. Histo-pathological changes attributed to the toxic effects of the colours were noted in kidneys and testes.

The work reported here is part of a continuing study on the chronic toxicity of food colours. Reports have been presented previously on the toxicity of Oil Yellow AB, Oil Yellow OB, Light Green SF Yellowish Orange SS and Oil Red XO^{1,2}. Malachite Green, New Coccine, and Nigrosine are not included in the permitted list of food colours for Canada but are in use in some countries.

METHODS

The methods used were similar to those described in the first two papers of this series^{1,2}. The colours were added to the normal laboratory diet in dry form to give concentrations of 0.03, 0.3 and 3.0 per cent for each dye. The rats, which were five to six weeks of age at the start of the experiment, were kept in individual cages and were given free access to food and water. Groups of ten males and ten females were assigned to each concentration level of each food colour. Ten rats of each sex were also used as control groups. Weight and food consumption were recorded weekly. Post-mortem examinations were made where possible on rats which died on test. At the termination of the experiment, the surviving rats were killed and post-mortem examinations made. Some of the organs were weighed and prepared for histological examination.

RESULTS AND DISCUSSION

Mortality

The mortality of control and test rats during the period of the experiment (64 weeks) is indicated in Table I. It seems logical to conclude that

TABLE I
CUMULATIVE NUMBER OF DEATHS

Treatment	Conc. of colour (per cent in diet)	No. rats on test	Time in weeks on test															
			1	4	8	12	16	20	24	28	32	36	40	44	48	52	56	60
Males																		
Control	..	10	0	0	0	0	0	0	1	2	3	4	4	4	4	4	4	5
Malachite Green	0.03	10	1	1	2	2	2	3	4	4	4	4	5	5	6	6	6	7
	0.3	10	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	3.0	10	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
New Coccine	0.03	10	0	0	0	0	0	2	2	3	4	4	5	5	6	6	6	7
	0.3	10	1	1	2	2	2	2	2	2	2	2	4	4	4	4	4	6
	3.0	10	0	0	0	1	1	1	1	2	3	4	4	4	4	4	4	4
Nigrosine	0.03	10	0	0	0	0	1	1	1	2	2	2	2	2	2	3	3	4
	0.3	10	0	0	0	0	2	2	2	2	2	2	3	3	3	4	5	5
	3.0	10	0	0	0	0	0	0	0	0	0	0	0	2	3	3	3	6
Females																		
Control	..	10	0	0	0	0	0	0	0	1	1	1	1	1	1	3	3	3
Malachite Green	0.03	10	0	0	0	0	0	0	0	2	2	2	3	4	4	4	4	4
	0.3	10	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	3.0	10	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
New Coccine	0.03	10	0	1	2	2	2	2	2	2	2	2	3	3	4	4	4	6
	0.3	10	0	0	0	0	0	0	0	1	1	1	1	2	2	2	2	3
	3.0	10	0	0	0	0	0	0	0	0	1	1	1	1	2	2	2	3
Nigrosine	0.03	10	0	0	0	1	1	1	1	3	3	4	4	4	4	4	5	5
	0.3	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1
	3.0	10	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	4

neither New Coccine nor Nigrosine, in the dose range used, had any effect on mortality. There was no correlation of mortality rate and concentration of dye for these two colours. All rats on the two higher levels of Malachite Green died within the first week of the experiment. Obviously these dosages were not suitable for a chronic toxicity study for this dye. There is some indication that Malachite Green at 0.03 per cent in the diet affected the mortality rate in the first half of the experiment, especially for male rats.

Growth, Food Consumption and Food Efficiency

Weight, food consumption and food efficiency data for the various test groups are presented in Tables II, III and IV. From the results

TABLE II
MEAN BODY WEIGHT OF RATS FED MALACHITE GREEN, NEW COCCINE, AND NIGROSINE

Treatment	Dosage (per cent of diet)	No. rats surviving No. rats on test	Mean body weight (g ± S.E.)						
			Initial	16 weeks	32 weeks	48 weeks	64 weeks		
Males									
Control		5/10	63 ± 2	286 ± 9	321 ± 6	315 ± 10	314 ± 12		
Malachite Green	0.03	3/10	60 ± 3	259 ± 12	323 ± 12	336 ± 12	346 ± 11		
New Coccine	0.03	3/10	64 ± 2	270 ± 9	317 ± 18	327 ± 20	352 ± 36		
	0.3	4/10	62 ± 4	285 ± 11	327 ± 18	374 ± 22*	354 ± 12		
	3.0	6/10	63 ± 3	271 ± 12	310 ± 10	333 ± 10	326 ± 14		
Nigrosine	0.03	5/10	61 ± 4	263 ± 10	307 ± 15	324 ± 15	294 ± 13		
	0.3	5/10	65 ± 4	268 ± 18	300 ± 17	329 ± 14	327 ± 28		
	3.0	4/10	65 ± 3	290 ± 5	346 ± 10	358 ± 9*	337 ± 5*		
Females									
Control		7/10	62 ± 4	184 ± 5	220 ± 6	236 ± 5	246 ± 8		
Malachite Green	0.03	6/10	59 ± 2	169 ± 2*	197 ± 6*	214 ± 6*	197 ± 2*		
New Coccine	0.03	4/10	61 ± 3	177 ± 6	202 ± 9	243 ± 5	254 ± 12		
	0.3	4/10	61 ± 3	176 ± 8	198 ± 9	233 ± 9	241 ± 2		
	3.0	7/10	60 ± 3	167 ± 3*	203 ± 6	227 ± 5	232 ± 5*		
Nigrosine	0.03	5/10	61 ± 3	178 ± 2	203 ± 7	225 ± 5	231 ± 7		
	0.3	9/10	59 ± 1	175 ± 2	207 ± 2	228 ± 5	233 ± 5		
	3.0	5/10	60 ± 2	165 ± 6*	194 ± 8*	215 ± 3*	227 ± 3*		

* Significant at P = 0.05 or less

in Table II the consistent effect on growth rate occurred only in female rats receiving 0.03 per cent Malachite Green and 3.0 per cent Nigrosine. These groups showed a significant decrease in growth rate throughout

CHRONIC TOXICITY STUDIES ON FOOD COLOURS. PART III

the course of the experiment. For the group on Malachite Green this retardation was partly due to a decrease in food consumption as shown in Table III. There was no significant change in food consumption for the 3 per cent Nigrosine group. Table III also shows that female rats receiving 3.0 per cent New Coccine and 0.03 per cent Nigrosine had a lower mean food consumption throughout the experiment than did the

TABLE III
MEAN FOOD CONSUMPTION OF RATS FED MALACHITE GREEN, NEW COCCINE,
AND NIGROSINE

Treatment	Dosage (per cent of diet)	Mean food consumption g./rat/day \pm S.E.			
		16 weeks	32 weeks	48 weeks	64 weeks
Males					
Control		15.7 \pm 0.4	16.4 \pm 0.7	16.6 \pm 0.3	16.9 \pm 0.4
Malachite Green	0.03	15.2 \pm 0.2	16.9 \pm 0.5	17.1 \pm 0.4	17.3 \pm 0.7
	0.03	16.0 \pm 0.3	17.2 \pm 0.7	17.5 \pm 0.4	18.5 \pm 0.4*
New Coccine	0.3	15.7 \pm 0.4	17.2 \pm 0.4	17.9 \pm 0.5*	18.1 \pm 0.3*
	3.0	15.2 \pm 0.4	15.3 \pm 0.4	15.5 \pm 0.4*	15.9 \pm 0.4
	0.03	15.5 \pm 0.4	16.9 \pm 0.6	17.5 \pm 0.6	16.9 \pm 0.6
Nigrosine	0.3	15.4 \pm 0.3	16.4 \pm 0.6	17.9 \pm 0.6	18.4 \pm 0.6
	3.0	16.4 \pm 0.4	17.8 \pm 0.4	18.1 \pm 0.5*	17.6 \pm 0.3
Females					
Control		13.2 \pm 0.2	14.1 \pm 0.2	14.5 \pm 0.3	14.9 \pm 0.3
Malachite Green	0.03	11.8 \pm 0.4*	12.4 \pm 0.3*	13.0 \pm 0.2*	13.2 \pm 0.4*
	0.03	12.7 \pm 0.4	13.2 \pm 0.3*	14.0 \pm 0.4	13.9 \pm 0.9
New Coccine	0.3	13.1 \pm 0.2	14.2 \pm 0.4	15.9 \pm 0.4*	16.0 \pm 0.4
	3.0	11.3 \pm 0.2*	12.5 \pm 0.1*	13.4 \pm 0.3*	13.7 \pm 0.4*
	0.03	12.2 \pm 0.4*	12.3 \pm 0.4*	13.1 \pm 0.2*	13.5 \pm 0.3*
Nigrosine	0.3	13.0 \pm 0.3	14.1 \pm 0.2	14.8 \pm 0.4	15.0 \pm 0.4
	3.0	12.6 \pm 0.4	13.6 \pm 0.3	14.2 \pm 0.4	14.0 \pm 0.4

* Significant at P = 0.05 or less

control group. This did not produce any significant change in body weight except at 16 weeks for the rats on New Coccine. There were a few other instances where the food consumption differed significantly from the control values but only two of these were accompanied by corresponding changes in body weight.

There was little effect of the colours on food efficiency (expressed as g. body weight gained per hundred g. of food consumed) as shown in Table IV. In only two cases of all the data tested was there a significant P value found and neither of these occurred in groups receiving the highest concentration of dye.

TABLE IV

MEAN FOOD EFFICIENCY DATA FOR RATS FED MALACHITE GREEN, NEW COCCINE AND NIGROSINE

Treatment	Dosage (per cent of diet)	Mean food efficiency g. gained/100 g. food consumed \pm S.E.			
		16 weeks	32 weeks	48 weeks	64 weeks
Males					
Control		13.0 \pm 0.5	7.2 \pm 0.2	4.6 \pm 0.1	3.4 \pm 0.1
Malachite Green	0.03	12.0 \pm 0.6	7.3 \pm 0.2	4.8 \pm 0.3	3.7 \pm 0.4
	0.03	11.8 \pm 0.4	6.6 \pm 0.3	4.4 \pm 0.3	3.5 \pm 0.5
New Coccine	0.3	13.2 \pm 0.5	7.0 \pm 0.4	5.3 \pm 0.3	3.7 \pm 0.3
	3.0	12.5 \pm 0.5	7.2 \pm 0.3	5.2 \pm 0.3	3.7 \pm 0.2
Nigrosine	0.03	11.9 \pm 0.4	6.6 \pm 0.1*	4.5 \pm 0.2	3.3 \pm 0.2
	0.3	12.2 \pm 0.8	6.5 \pm 0.3	4.4 \pm 0.3	3.2 \pm 0.3
	3.0	12.6 \pm 0.2	7.2 \pm 0.1	4.9 \pm 0.1	3.5 \pm 0.3
Females					
Control		8.7 \pm 0.3	5.2 \pm 0.1	3.6 \pm 0.2	2.8 \pm 0.1
Malachite Green	0.03	8.3 \pm 0.4	5.1 \pm 0.3	3.7 \pm 0.1	2.4 \pm 0.2
	0.03	8.3 \pm 0.2	4.8 \pm 0.2	3.8 \pm 0.2	3.0 \pm 0.1
New Coccine	0.3	8.1 \pm 0.3	4.3 \pm 0.3*	3.2 \pm 0.1	2.5 \pm 0.2
	3.0	8.7 \pm 0.5	5.2 \pm 0.1	3.7 \pm 0.2	2.7 \pm 0.1
Nigrosine	0.03	8.8 \pm 0.2	5.1 \pm 0.2	3.7 \pm 0.2	2.7 \pm 0.2
	0.3	8.2 \pm 0.3	4.8 \pm 0.2	3.4 \pm 0.1	2.6 \pm 0.2
	3.0	7.8 \pm 0.5	4.5 \pm 0.4	3.4 \pm 0.2	2.8 \pm 0.2

* Significant at $P = 0.05$ or less*Organ Weights*

Certain organs of the rats killed upon termination of the test were weighed. The mean weights (in mg./g. of weight) of these organs are given in Table V. Significant weight changes occurred most often in liver and kidneys but were also recorded for heart, spleen and testes. The effect on liver weight occurred with four groups and in each case was an increase over the corresponding control value. The increase was marked in both male and female rats on the diet containing 3 per cent of Nigrosine. It may be mentioned that no pathological changes in liver were recorded for any group. Of four significant changes in kidney weights, three were in groups receiving Nigrosine. One of these, on the low dose level, was a decrease in weight, whereas the other two, both at the 3 per cent level of Nigrosine, were increases over the control values.

Histopathology

A detailed examination was made of the haematoxylin-eosin stained paraffin sections of a number of organs including lung, heart, liver,

CHRONIC TOXICITY STUDIES ON FOOD COLOURS. PART III

spleen, thyroid, pancreas, stomach, small intestine, kidney, urinary bladder, adrenal, testes, ovaries and thymus. A summary of the findings is given in Table VI.

The histopathological changes that were attributed to the toxic or adverse effects of the colours were those noted in the kidneys and testes.

TABLE V
ORGAN WEIGHTS OF RATS FED MALACHITE GREEN, NEW COCCINE AND NIGROSINE

Treatment	Dosage (per cent of diet)	No. rats sacrificed	Mean organ weight (mg./g. rat \pm S.E.)					
			Heart	Liver	Spleen	Kidneys	Gonads	Adrenals
Males								
Control ..		5	4.5 \pm 0.6	31.1 \pm 1.3	2.3 \pm 0.1	8.2 \pm 0.2	8.1 \pm 0.3	0.11 \pm 0.02
Malachite Green ..	0.03	3	3.7 \pm 0.4	34.7 \pm 1.7	2.4 \pm 0.1	8.1 \pm 0.5	7.3 \pm 0.9	0.11 \pm 0.00
New Coccine ..	0.03	3	3.7 \pm 0.1	31.1 \pm 1.1	2.1 \pm 0.1	7.6 \pm 0.3	7.9 \pm 0.5	0.11 \pm 0.01
	0.3	4	3.9 \pm 0.2	29.6 \pm 1.3	2.1 \pm 0.2	7.1 \pm 0.7	7.0 \pm 0.5	0.11 \pm 0.01
	3.0	6	3.8 \pm 0.1	32.0 \pm 1.1	2.1 \pm 0.1	7.8 \pm 0.3	8.0 \pm 0.6	0.12 \pm 0.01
Nigrosine ..	0.03	5	3.8 \pm 0.1	28.1 \pm 0.7	2.5 \pm 0.2	7.1 \pm 0.3*	6.3 \pm 0.7*	0.12 \pm 0.02
	0.3	5	3.6 \pm 0.3	32.7 \pm 1.3	2.5 \pm 0.2	7.7 \pm 0.1	7.0 \pm 0.4	0.11 \pm 0.02
	3.0	4	4.2 \pm 0.4	40.1 \pm 0.8*	2.9 \pm 0.2*	9.5 \pm 0.3*	7.5 \pm 0.7	0.12 \pm 0.03
Females								
Control ..		7	4.6 \pm 0.2	34.0 \pm 1.0	3.3 \pm 0.2	8.1 \pm 0.3	0.44 \pm 0.02	0.27 \pm 0.04
Malachite Green ..	0.03	6	5.1 \pm 0.2	37.3 \pm 0.6*	3.4 \pm 0.4	8.5 \pm 0.4	0.46 \pm 0.05	0.26 \pm 0.04
New Coccine ..	0.03	4	4.6 \pm 0.2	36.1 \pm 1.1	3.2 \pm 0.1	8.9 \pm 0.4	0.46 \pm 0.05	0.21 \pm 0.01
	0.3	4	4.8 \pm 0.3	35.3 \pm 1.1	3.1 \pm 0.2	8.6 \pm 0.6	0.42 \pm 0.05	0.32 \pm 0.02
	3.0	7	5.3 \pm 0.2*	37.8 \pm 1.3*	3.2 \pm 0.2	10.4 \pm 0.4*	0.45 \pm 0.03	0.34 \pm 0.02
Nigrosine ..	0.03	5	4.6 \pm 0.2	36.6 \pm 2.4	3.6 \pm 0.4	9.1 \pm 0.5	0.45 \pm 0.00	0.26 \pm 0.03
	0.3	9	4.5 \pm 0.2	34.5 \pm 1.7	2.9 \pm 0.2	8.2 \pm 0.2	0.38 \pm 0.06	0.26 \pm 0.01
	3.0	5	4.7 \pm 0.1	44.8 \pm 2.2*	4.0 \pm 0.4	9.6 \pm 0.2*	0.49 \pm 0.02	0.27 \pm 0.01

* Significant at P = 0.05 or less.

The testicular changes were similar to those previously described¹. The kidney changes in the 3 per cent Nigrosine group appeared more marked than in the other groups.

Haematology

Haemoglobin estimations were made on all surviving rats at the termination of the test. A slight modification of the pyridine-haemochromogen method of Rimington was used³. The only difference from control values occurred in the female rats on 0.03 per cent of Malachite Green, where an increased blood haemoglobin was recorded. The significance of this finding is not apparent.

TABLE VI
SUMMARY OF HISTOPATHOLOGICAL FINDINGS

Sex	MALE			FEMALE			MALE	FEMALE	MALE	FEMALE	Totals				
	0.03 per cent			0.03 per cent											
Dosage (per cent of diet)	0.03 per cent			0.03 per cent			0.3 per cent	0.3 per cent	3.0 per cent	3.0 per cent					
Treatment	Control	Nigrosine	New Coccine	Malachite Green	Control	Nigrosine	New Coccine	Malachite Green	Nigrosine	New Coccine	Nigrosine	New Coccine	Nigrosine	New Coccine	
Number of rats on test	10	10	10	10	10	10	10	10	10	10	10	10	10	10	160
Number of survivors	5	5	3	3	7	5	4	6	5	4	9	4	4	6	82
Number of rats examined	5	5	3	3	7	4	4	3	5	4	9	4	4	6	78
Bladder parasites	2	3	—	—	—	1	—	3	1	—	—	—	—	—	12
Hydronephrosis	—	—	—	—	—	4	—	—	—	3	1	—	—	—	10
Hydronephrosis	—	—	—	—	—	—	—	—	—	1	—	—	—	—	1
Glomerulosis	—	1	—	—	—	—	—	—	—	—	—	—	—	—	1
Hyaline casts kidney	2	—	2	2	2	—	—	—	1	—	1	4	—	4	20
Focal distal nephritis	—	—	—	—	—	—	—	—	—	—	—	—	1	—	1
Ephithelial crescents (glomerulus)	—	—	—	—	—	—	—	—	—	—	—	1	—	—	1
Nephritis	—	—	—	—	—	—	—	—	—	—	—	—	2	—	5
Altered spermatogenesis	1	2	—	1	—	—	—	—	3	1	—	—	2	—	10
Ovarian cysts	—	—	—	—	—	—	—	—	—	3	—	—	—	—	4
Focal necrosis adrenal	—	—	—	—	—	—	—	—	—	—	—	—	1	—	1
Tracheitis	—	—	—	—	—	1	—	—	1	—	—	—	—	—	3
Ectopic myelopoiesis	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Spleen	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1
T Uterine polypus	—	—	—	—	1	2	2	—	—	—	—	2	—	—	9
Fibro adenoma	—	—	—	—	—	1	—	—	—	—	—	—	—	1	2
U Adenoma (coagulating gland)	—	—	—	—	—	—	—	—	—	1	—	—	—	—	1
M Sertoli cell tumor	—	—	—	—	—	—	—	—	—	1	—	—	—	—	1
O Papilloma ureter	—	—	—	—	—	—	—	—	—	—	1	—	—	—	1
R Chromaffinoma	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Adrenal	—	—	—	—	—	—	—	—	—	—	—	—	—	1	1

Acknowledgements. The authors wish to acknowledge the technical assistance of Miss Rita Carioto and Miss Elaine Connell.

The Malachite Green used in these experiments was obtained from Dye Specialties Company, Jersey City, New Jersey. New Coccine and Nigrosine were supplied by Allied Chemical and Dye Corporation, New York, N.Y.

REFERENCES

1. Allmark, Grice and Lu, *J. Pharm. Pharmacol.*, 1955, 7, 591.
2. Allmark, Grice, and Mannell, *ibid.*, 1956, 8, 417.
3. Rimington, *Brit. med. J.*, 1942, 1, 177.