CHRONIC TOXICITY OF BREAD ADDITIVES TO RATS

By W. Donald Graham, H. Teed and H. C. Grice

From the Food and Drug Laboratory, Department of National Health and Welfare Ottawa, Canada

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Currently there is much interest in the use of "chemical" additives in the food industry. Probably a considerable proportion of this interest stems from the report of Sir Edward Mellanby in 1946[1] that running fits had been induced in dogs by including in their diets flour that had been bleached and improved by treatment with nitrogen trichloride. The offending agent was finally isolated and found to be a derivative of methionine, methionine sulphoximine.[2] As a result of these discoveries several countries, Canada included, switched to the use of chlorine dioxide as a flour improving and bleaching agent. More recently, a great many compounds have been investigated in an effort to establish whether they might be added to food with a reasonable surety that no harm would result. At the time this investigation was planned a great deal of interest was being shown in the additives used in bread manufacture. Of the many compounds intentionally added to bread, chlorine dioxide, sodium propionate, the antioxidants *n*-propyl gallate and butylated hydroxyanisole, and the emulsifier polyoxyethylene (8) monostearate* were selected for study. The number of compounds which could be tested in a chronic toxicity trial was limited by the facilities available and it was considered that these 4 additives were most deserving of study.

Most investigations of this nature have been made by adding such compounds singly, in abnormally large amounts, to otherwise normal experimental diets. This may result in unequal concentrations of proteins, vitamins, minerals, and calories among the diets and the difficulty of providing adequate controls under these conditions is obvious. It appeared that a sounder method of checking for chronic toxicity would be to incorporate the additives in bread at abnormally high levels and, maintaining the levels of the nutritive elements as close to those of the control diets as possible, to give this bread as a major part of a good experimental diet. It was decided to reverse the usual procedure and to check the toxicity of the additives in combination rather than singly since the toxicity of the finished bread was of major concern.

In view of the popular "100-fold margin of safety"[3] or "margin of uncertainty" as it is rather aptly termed by Oser[4], it was decided to incorporate the additives under test in bread at 50 times the normal concentration and to give the finished bread as 75 per cent. of the experimental diet. It was considered that giving this amount would correspond to over 100 times the usual rate of intake for man.

The seven diets used are shown in Table I. The emulsifier, polyoxyethylene (8) monostearate, and the mould inhibitor, sodium propionate,

* The use of this material in bread had not been prohibited in Canada at the time these investigations were started.

were taken as approximately equivalent, on a caloric basis, to carbohydrate and additions of these ingredients were made at the expense of glucose. On this basis all 6 bread diets were approximately isocalorific and were as near alike in all respects, except for the additives under test, as could be achieved.

Diet I contained bread made to an essentially normal formula with the exception that the glucose content was elevated to permit substitution of high levels of emulsifier and mould inhibitor in other diets. Diet II contained all 4 test ingredients at 50 times the level used in diet I. With the flour and antioxidants this was accomplished conveniently with little weight change in the diet ingredients. Diets III, IV, V and VI were similar to diet II with the exception that one ingredient in each, emulsifier, propionate, antioxidants, or chlorine dioxide, respectively, was reduced to the control concentration. Diet VII consisted of a commercial cubed diet* ground to a consistency similar to that of the other diets. The basal diet used as 25 per cent. of diets I to VI supplied adequate supplementary amounts of all the required vitamins as well as high quality protein, bulk, minerals, and fat.

Technical difficulties were encountered in the preparation of the various breads, which are referred to here by the same numbers as the corresponding diets. Those mixes which contained the high concentration of propionate showed very poor yeast action. Increasing the quantity of yeast had little beneficial effect. To overcome this difficulty to some extent the dry propionate was incorporated only into the surface of the dough so that fair yeast action in the bulk of the material was obtained. With this modification of the mixing scheme it was possible to produce breads which had been proofed approximately equivalent lengths of time. As might be expected, loaf volume and texture on numbers II, III, V and VI were poor. To a lesser extent bread I was inferior to normal quality because of the inhibitory effect of the high glucose content on yeast action. Bread IV was excessively friable and open textured.

After baking, the loaves were wrapped and stored in the refrigerator for short periods before being thinly sliced. The slices were then placed on edge in a special drier and dried in a forced draft hot air oven at 70° C. The drying process usually took 3 to 5 hours, and, for those loaves high in propionate, was accompanied, as was the baking, by a strong odour of propionate. Subsequently the dried bread was ground in a hammer mill and stored in sealed metal containers in the refrigerator (4° C.) until required for use. This dried, ground bread was rarely kept for more than 1 month. Diets were mixed at intervals of no greater than 2 weeks and were kept in closed cans in the refrigerator except during feeding periods.

A preliminary feeding trial of 3 weeks' duration indicated that there was little difference in the acceptability of the several diets to weanling rats. None of the ingredients was acutely toxic at the concentrations under test. Accordingly, a large scale experiment was started. A group of 182 male albino rats of Wistar strain ranging in age from 27 to 41 days

* Master Fox Cubes, Toronto Elevators, Toronto.

TABLE I

DIET COMPOSITIONS

Part A. 75 per cent. of the diet	Diet						
Ingredient	I	II	III	IV	V	VI	VII
Commercial bread flour[1]	70·5	—	—	—	—	70·5	GROUND MASTER
Bread flour 50 × ClO_2[1]	—	70·5	70·5	70·5	70·5	—	
Commercial lard[2]	1·8	—	—	—	1·8	—	
Lard 50 × Antioxidants[2]	—	1·8	1·8	1·8	—	1·8	
Sodium propionate[3]	0·1	5·0	5·0	0·1	5·0	5·0	
Polyoxyethylene (8) monostearate[4]	0·3	15·0	0·3	15·0	15·0	15·0	
Cerelose (glucose)	20·4	0·8	15·5	5·7	0·8	0·8	
Salt (NaCl)	1·8	as I	as I	as I	as I	as I	
Skim milk powder	2·2	"	"	"	"	"	
Malt flour	0·5	"	"	"	"	"	
Yeast food[5]	0·2	"	"	"	"	"	
Wytase[6]	0·9	"	"	"	"	"	
Yeast	1·3	"	"	"	"	"	
	100·0	100·0	100·0	100·0	100·0	100·0	

Part B. 25 per cent. of the diet	Diet						
Ingredient	I	II	III	IV	V	VI	FOX CUBES
Casein	60·0	as I	as I	as I	as I	as I	
Alphacel[7]	9·4	"	"	"	"	"	
Corn oil	12·8	"	"	"	"	"	
Mineral mix (a)	12·0	"	"	"	"	"	
Vitamins and excipient (b)	5·8	"	"	"	"	"	
	100·0	100·0	100·0	100·0	100·0	100·0	

(a)—Minerals—the 12·0 g. of mineral mix present in 100 g. of basal diet were made up as follows—

KCl	1·580	$FePO_4$	0·195
KH_2PO_4	4·100	$KAl(SO_4)\cdot 12H_2O$	0·001
$Ca_3(PO_4)_2$	1·970	$CuSo_4\cdot 5H_2O$	0·080
$MgSO_4$	1·190	NaF	0·076
$MnSO_4\cdot H_2O$	0·027	KI	0·001

(b)—Vitamins—the 5·8 g. of vitamins and excipient in 100 g. of basal diet were made up as follows—

Thiamin hydrochloride	0·0020		
Riboflavin	0·0040	Menadione	0·0004
Calcium pantothenate	0·0080	E concentrate*	0·0560
Pyridoxine	0·0020	A and D concentrate†	0·1600
Nicotinamide	0·0080	Corn oil	4·2830
l-Inositol	0·0800		
Choline chloride	0·4000		
Folic acid	0·0008		
p-Aminobenzoic acid	0·0040		
Liver fraction‡	0·8000		

* Vitamin E concentrate, 350 mg. of $d\alpha$-tocopheryl acetate equivalent per g.
† Navitol, 65000 A, 13000 D per g.
‡ Wilson's Liver fraction L.

NOTES

1. Chlorine dioxide.—Canadian number one patent flour was treated with 0·3 g. of chlorine dioxide per barrel (196 lb.) to yield commercial bread flour and with 15·0 g. of chlorine dioxide per barrel to yield high ClO_2 flour.
2. Antioxidants.—Tenox II, a commercial preparation containing 20 per cent. butylated hydroxyanisole, 6 per cent. *n*-propylgallate, 4 per cent. citric acid and 70 per cent. propylene glycol was used at the rates of 0·05 per cent. in commercial lard and 2·5 per cent. in high antioxidant lard.
3. Propionate.—The commercial sodium propionate sold by Du Pont de Nemours and Co., under the trade name Mycoban was used.
4. Polyoxyethylene monostearate.—The material sold by Atlas Powder Co. as polyoxyethylene (8) monostearate under the trade name Myrj 45 was used.
5. Yeast food.—The commercial material sold under the name RKD by Standard Brands was used.
6. Wytase.—This material is sold by J. R. Short Canadian Mills Limited, and is used commercially to improve whiteness.
7. Alphacel is a commercial cellulose supplying bulk without nutritive value.

and in weight from 41 to 91 g. was divided into 7 sub-groups by arranging the rats in order of decreasing weight and assigning consecutive lots of 7 rats by random selection to the sub-groups. The 182 female rats

aged 25 to 41 days and weighing 36 to 86 g. were grouped in a similar manner. The oldest rats of each sex had been maintained on diet VII from weaning at 22 days of age until placed on test. The 14 groups of rats were housed in cages with wire screen floors. Each cage held 1 group (26 rats). Food and water were allowed *ad libitum*. For the first 3 weeks, feed consumption and body weight were recorded twice weekly; thereafter the measurements were made once a week. Cages were moved in a regular rotation on the racks so that all spent approximately equal intervals at the 4 different levels above the floor. Mortality was recorded and the probable cause of death (autopsy) was noted for those rats which died and were found before autolysis was too far advanced.

At 13 and 26 weeks, 3 rats of each sex from each diet were chosen by random selection and killed. Tissue samples from the bladder, small intestine, spleen, stomach, pancreas, adrenal, kidney, liver, heart, lung, thyroid, brain, and either testicle or ovary were preserved in formalin-saline fixative and reserved for histopathological examination. At the 52nd week all surviving animals were killed and the heart, liver, spleen and both kidneys were weighed. In addition, the usual tissue samples were reserved for histopathological examination. Gross abnormalities which were observed were recorded during this work. It should be noted that the rats were group fed and that the food consumption figures include wastage. Every effort was made to keep wastage at a minimum and there is no reason to believe, on the basis of observations made, that any particular group wasted more food than other groups.

RESULTS

The mean body weights, at weekly intervals, of the rats in the 14 groups are presented graphically in Figures 1A (males) and 1B (females). The mean body weights of the survivors on each diet at 52 weeks and the standard error of the means are shown for males, females, and the sexes combined in Table II.

TABLE II

FINAL WEIGHT OF SURVIVORS AT 52 WEEKS

Weight in grams ± standard error

	Sexes combined		Males		Females	
Diet	Mean wt.	No.	Mean wt.	No.	Mean wt.	No.
I	248 ± 8·8	16	284 ± 9·8	6	226 ± 5·9	10
II	262 ± 10·8	17	288 ± 17·9	8	238 ± 6·9	9
III	266 ± 6·7	18	290 ± 7·5	8	251 ± 6·7	10
IV	251 ± 12·5	18	303 ± 25·7	6	226 ± 5·7	12
V	260 ± 9·4	22	295 ± 10·8	10	231 ± 7·5	12
VI	278 ± 16·7	14	333 ± 12·8	7	224 ± 6·8	7
VII	244 ± 7·8	19	266 ± 11·7	8	229 ± 7·7	11

The commercial diet (VII) was approximately equivalent to the bread diets in supporting the growth of female rats. Female rats on diet III surpassed in growth those on diet VII toward the end of the experiment. For male rats, with their more rapid growth, diet VII was inferior to

bread diet I in the earlier weeks of the experiment. At 10 weeks the mean weight of male rats on diet I was significantly greater than that of male rats on diet VII. At the end of the 52nd week this difference had disappeared and the mean body weight of male rats on diet VII was not significantly lower than that of male rats on diet I or on all 6 bread diets combined.

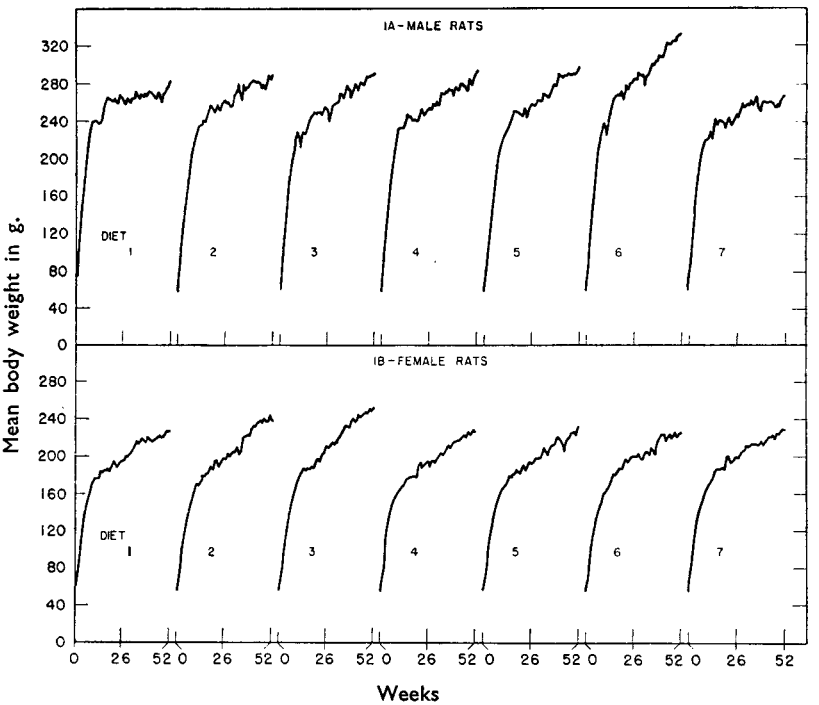

FIG. 1.

The final weights recorded in Table II were subjected to an analysis of variance based on the statistical methods of Snedecor[5] for disproportionate groups with significant interaction. The results for the commercial diet (VII) were excluded from these and subsequent calculations since the rats on this diet were included only to illustrate the usual growth, mortality, and pathology of animals from the laboratory colony. The results of the statistical analysis shown in Table III indicate that although sex had a significant effect on final weight, as was to be expected, diet was without significant influence.

The significant interaction apparently arose from the fact that male rats on the 4 diets high in polyoxyethylene (8) monostearate had a higher mean weight than those on the 2 diets low in this ingredient and female rats on the 4 diets high in chlorine dioxide had a higher mean weight than those on the 2 diets low in chlorine dioxide. These differences however were below the 5 per cent. point level of significance when the

"t" test was applied to the appropriate means. Since the effects of diet were not significant it may be concluded that none of the additives tested, in the amounts and manner used, had any deleterious effect on the growth of male or female rats during one year.

TABLE III

ANALYSIS OF VARIANCE OF FINAL WEIGHT DATA

Main effect	D.F.	Mean square	F
Sex	1	109908	47**
Diet	5	951	<1
Diet × sex ..	5	2346	2·56*
Error	93	919	

* significant at P = 0·05 ** significant at P = 0·01

Cumulative food consumption per rat per day over the 52-week period is shown in Figure 2A. It is readily observed that the commercial diet was eaten to a greater extent than the other diets, particularly in the case of the male rats, where significantly larger amounts of the diet VII were consumed. The cumulative food efficiency data, shown in Figure 2B, were plotted in a log relationship with time and straight lines were fitted

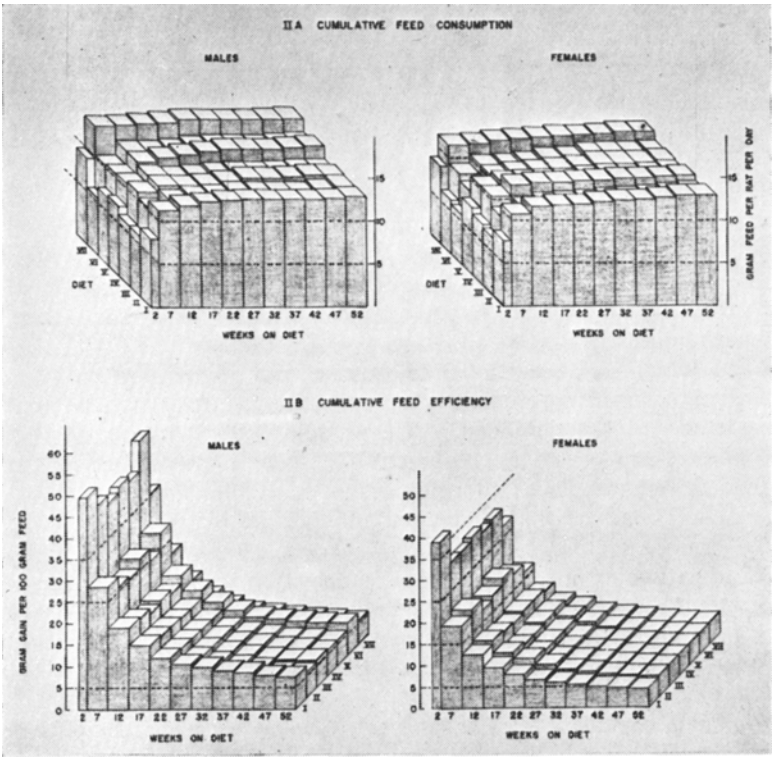

FIG. 2.

TABLE IV

A. HISTOPATHOLOGICAL FINDINGS

	Sex													
	Male							Female						
Diet	I	II	III	IV	V	VI	VII	I	II	III	IV	V	VI	VII
Number of rats examined*	12	14	14	12	16	13	14	16	15	16	18	18	13	17
Normal rats	9	7	10	9	11	8	10	13	7	8	10	14	9	14
Brain liquefaction necrosis (embolus)	—	—	—	—	—	—	—	—	—	—	—	1	—	—
Brain inflammation (cerebromeningitis)	—	—	—	—	—	1	—	—	—	—	—	—	—	—
Lungs—respiratory infection	1	2	2	1	1	1	2	2	—	3	1	—	2	2
Heart—fatty degeneration	—	—	—	—	—	—	1	—	—	—	—	—	—	—
Liver—cloudy swelling, fatty degeneration	—	1	—	—	—	1	1	—	2	—	—	—	—	—
Liver—excessive deposits of haemosiderin	—	—	—	—	—	—	—	—	—	—	2	—	—	—
Spleen—excessive deposits of haemosiderin	—	—	—	—	—	—	—	—	—	—	2	—	—	—
Stomach—inflammation of submucosa	—	—	—	—	1	—	—	—	—	—	—	—	—	—
Intestine—erosion	—	—	—	—	—	—	—	1	—	1	2	—	—	—
Kidney—degeneration of tubules	—	—	—	—	1	—	—	—	1	—	2	1	2	—
Kidney—glomerulonephritis	—	—	—	—	—	—	—	—	2	2	—	—	—	—
Kidney—hydronephrosis	—	—	—	—	—	—	—	—	1	1	—	1	—	—
Adrenal—cloudy swelling, necrosis in zona fasiculata	—	—	—	—	1	1	—	—	1	—	—	1	—	—
Bladder—parasite Trichosomoides crassicauda	2	5	2	3	3	1	1	—	1	3	—	1	2	2
Testicle—degeneration	—	—	1	—	—	—	—	—	—	—	—	—	—	—

* 3 rats were killed at 13 weeks, 3 at 26 weeks, and the remainder at 52 weeks.

B. MORTALITY DATA

Died on test	14	12	12	14	10	13	12	10	11	10	8	8	13	9
Undiagnosed	5	4	9	7	6	9	8	7	9	5	4	7	8	4
Respiratory infection	8	6	3	7	4	3	3	1	2	5	4	1	2	5
Middle ear disease	1	2	—	—	—	1	1	2	—	—	—	—	1	—
Tumor—fibroadenoma	—	—	—	—	—	—	—	—	—	—	—	—	2	—

to the points by the method of least squares. These lines were not significantly different for female rats but for the males the food efficiency on diet VII was significantly less than that on diet I.

The sections from the tissues of the 208 rats were stained with hæmatoxylin and eosin and examined microscopically. The group to which the various rats belonged was unknown to the pathologist (H.C.G.) at the time the tissues were examined. The histopathological findings are presented in Table IVA and the results have been summarised by the pathologist as follows:

"The tubular degeneration and perhaps the glomerulonephritis seen mainly in the kidneys of female rats are the only histopathological findings considered to be significant. The tubular degeneration is, in all probability, due to a toxic substance. The lack of coexisting glomerular change is remarkable as the glomerulus is usually affected before the tubules. It may be that there is a tubular specificity of the injurious agent or that there is an inherent weakness of the tubular epithelium in these particular rats. The former supposition is more probable. The hydronephrosis in each case was unilateral. Dilatation of the kidney pelvis may be caused by concretions, parasites, or tumors in the urinary bladder or it may be congenital. Concretions were not found in any of the bladders, there were no tumors present in the bladders of any of the animals examined, and there was no evidence of congenital atresia of the ureters. While parasites were not observed in the bladders of rats suffering from hydronephrosis, these could have been missed in histological

cross section (4 microns) and the known incidence of such parasites is sufficient to attribute the hydronephrosis to bladder parasites."

The significant kidney changes in female rats were found on diets II, III, IV and V. While the incidence is low, it should be noted that these were the rats receiving flour treated with the high concentration of chlorine dioxide. Tubular damage from propyl gallate has been demonstrated by Orten, Kuyper and Smith[6], and this may have been a contributing factor. Again, the incidence is so low that the distribution may be fortuitous.

The overall mortality data are tabulated in Table IVB. It will be noted that a considerable number of deaths occurred for which no explanation is given. These deaths occurred at night and by the time the bodies

TABLE V

EFFECT OF DIET ON THE WEIGHT OF VARIOUS ORGANS
Mean tissue weights in mg. per g. of rat

Diet	Number	Liver	Right kidney	Left kidney	Heart	Spleen
A.	Males					
I	6	32·7	3·56	3·55	3·96	2·82
II	8	31·0	3·66	3·83	3·81	2·51
III	8	32·5	3·72	3·71	3·65	2·71
IV	6	37·4	3·38	3·31	3·22	2·16
V	10	34·0	3·60	3·51	3·42	2·64
VI	7	34·2	3·47	3·35	3·38	2·59
VII	8	34·1	3·41	3·19	3·84	2·67
Over-all standard deviation		4·6	0·43	0·42	0·40	0·38
B.	Females					
I	10	35·0	4·10	4·07	4·48	3·47
II	9	39·5	4·95	5·06	4·29	3·31
III	10	41·5	4·82	4·81	4·70	4·01
IV	12	37·3	4·28	4·24	4·10	4·03
V	12	38·0	5·19	5·02	4·68	3·65
VI	7	35·6	4·46	4·61	4·47	3·43
VII	11	38·4	4·02	4·06	4·08	3·59
Over-all standard deviation		4·5	0·81	0·67	0·42	0·85

TABLE VI

ANALYSES OF DATA ON TISSUE WEIGHTS

Main effect	D.F.	Liver		Right kidney		Left kidney		Heart		Spleen	
		m.s.	F.	m.s.	F.	m.s.	F.	m.s.	F.	m.s.	F.
Sex	1	3088	15*	15932	49**	16934	61**	11508	46**	25473	33**
Diet	5	212	1·0	618	2·1	714	2·6	299	1·2	361	<1·0
Sex × diet ..	5	203	1·4	324	<1·0	276	2·1	248	3·4**	765	1·5
Error	93	147		397		133		73		493	

* significant at P = 0·05 ** significant at P = 0·01

were retrieved in the morning autolysis was too far advanced to allow conclusive autopsy findings. It may be stated with a considerable degree of confidence that most of these deaths were due to respiratory infection. A χ^2 analysis of the data revealed no significant influence of diet on mortality.

The mean weights of the livers, kidneys, hearts, and spleens of the rats which survived to the time of sacrifice at 52 weeks are listed in Table V.

For statistical analysis the data in Table V were transformed into percentages of the mean weight for each tissue. The resulting data were analysed by Snedecor's methods for disproportionate subclass numbers. The results of these analyses are presented in Table VI.

As might be expected, sex had a significant influence on the weight of the tissues examined. Since the sex × diet interaction was significant in one instance and in 4 of 5 instances the mean square for interaction was of appreciable magnitude these interactions were used in the calculation of the F for diet and sex. In no instance did diet have a significant influence on tissue weight.

Discussion

Propionate.

Very few experimental data on the toxicity of sodium propionate are available. In a 3-week trial Harshbarger[7] fed concentrations of 1 and 3 per cent. sodium or calcium propionate in the diets of rats. No effect on weight gains was observed. Heseltine[8] reported that sodium propionate had a weak antihistamine action about 1/7·5 that of diphenhydramine. Daily oral doses of 6 g. to men led to the production of a fairly alkaline urine but no appreciable diuresis, catharsis, or other effects.

These findings are confirmed and extended by the present data which show that at concentrations of 5 per cent. in bread ingredients before baking, a finished product is obtained which exerts no discernible toxicity when fed as 3/4 of the diet of rats for 1 year; this in spite of the added stress of the presence of 3 other additives at high concentration in the diet.

Antioxidants.

n-Propyl gallate is a fat-soluble ester of the widely-occurring natural antioxidant gallic acid. It has been fairly intensively investigated by Orten *et al.*[6], who found that concentrations of 1·17 per cent. in the diet of male rats for 6 months caused growth depression, a decrease in hæmoglobin levels, and some damage to the kidney tubules. Guinea-pigs receiving 0·011 per cent. in the diet for 1 year and dogs receiving similar amounts for 14 months showed no ill-effects. Lehman, Fitzhugh, Nelson and Woodard[9] found this antioxidant even less toxic and, in rats, detected no growth inhibition at 1 per cent. of the diet of males or females. At 5 per cent. of the diet growth depression occurred in male rats, there was some increase in mortality over the 2-year feeding period, and there was evidence of patchy hyperplasia of the proventriculus. Allen and De Eds[10] and Van Sluis[11] reported very low toxicity for lauryl gallate given to rats.

Wilder and Kraybill[12] reported that butylated hydroxyanisole (the commercial material is usually a mixture of the 2- and 3-tertiary butyl esters) when given to rats for periods up to 21 months at concentrations up to 0·06 per cent. of the ration caused no toxic effects. At 0·12 per cent. palatability of the ration was lowered but no pathology was detected. Evidence accumulated by the U.S. Food and Drug scientists[13] indicated that this antioxidant was safe when used in the usual concentrations.

The amounts of the several ingredients of the antioxidant mixture fed in the various diets is shown in Table VII.

In the amounts in which they occurred there is no reason to suspect any direct toxic action from the propylene glycol or the citric acid. The high level of butylated hydroxyanisole was about 1/10 that found to cause no harmful effects and the high level of *n*-propyl gallate was about 1/50 of the maximum non-harmful amount. Even the combination of these 2 failed to exert any significant influence on the health of the rats.

TABLE VII

ANTIOXIDANT AND PROPYLENE GLYCOL CONTENT OF DIETS I–VI

	Diets I and V	Diets II, III, IV and VI
	mg./kg.	mg./kg.
Butylated hydroxyanisole ..	1·35	67·50
n-Propyl gallate	0·405	20·25
Citric acid	0·27	13·50
Propylene glycol carrier ..	4·725	236·25

Polyoxyethylene (8) *Monostearate.*

The literature on the toxicity of polyoxyethylene (8) monostearate is rather considerable. The chief conclusion reached after a careful scrutiny of these data is that, under certain conditions, polyoxyethylene (8) monostearate in fairly large amounts in the diet may cause harmful effects[14,15,16,17]. Whether or not these certain conditions are of such a nature as to lend practical significance to the observed toxicity is a matter of argument. The literature is too voluminous to review in detail here. Suffice to say that loss in weight, diarrhœa, bladder stones, etc., have been observed in rats fed diets containing 5 per cent. or more of this emulsifier. Other investigators have not demonstrated any harmful effects at concentrations up to 5 per cent. in the diet[18]. To quote Frazer[19] "Grossly excessive amounts of polyoxyethylene monostearate—some workers have used as much as 15 per cent. of the diet—may produce some effects. Some of the results of these experiments are not convincing and in any case they have little relevance to the normal dietary use of these materials."

Culver, Wilcox, Jones and Rose[20] have shown that in humans receiving polyoxyethylene (40) monostearate the polyoxyethylene was recovered almost completely in the urine and fæces, that this portion of the molecule was recovered free of combined fatty acid, and that there was no evidence of storage.

In the experiments reported here, polyoxyethylene (8) monostearate occurred as 0·3 or 15·0 per cent. of the breads which made up 75 per cent. of the diet. Under these conditions and in spite of 2 or 3 additional dietary stresses, no toxic effect of this material was observed.

Chlorine Dioxide.

The toxicity of chlorine dioxide-treated flour has been the subject of several investigations. Arnold[21] gave dogs, as 80 per cent. of their diet, flour treated with 5 g. of chlorine dioxide per 100 lb. for periods up to 28 days without detecting any ill-effect. Newell, Gershoff, Suckle,

Gilson, Erikson and Elvehjem[22], carried out similar test giving 60 per cent. flour in the ration with a maximum chlorine dioxide treatment of 4 g. per 100 lb. and continuing the trials for 13 weeks. The results of these studies on dogs were negative. Similar studies on rabbits (6 weeks) monkeys (5·5 months) and rats (5 weeks) also gave negative results. A group of 13 humans receiving 55 g. of chlorine dioxide-treated wheat gluten (20 g. of ClO_2 per 100 lb. of gluten) daily for 6 weeks showed no detectable injury. Nakamura and Morriss[23] gave to dogs flour treated with high concentrations of chlorine dioxide for prolonged periods without seeing any evidence of canine hysteria. With the exception of the last experiment most of the studies were of short duration and no long-term studies on rats have been reported.

In the present investigation it has been shown that rats receiving a diet containing 75 per cent. of bread made from flour treated with 15 g. of chlorine dioxide per barrel (196 lb.), 50 times the normal rate of treatment, for 1 year from weaning age exhibited no signs of toxic effect as evidenced by growth, mortality, gross observation, organ weights or histopathological examination of the tissues.

Summary

1. Bread containing 50 times the normal concentration of chlorine dioxide, propyl gallate and butylated hydroxyanisole, polyoxyethylene (8) monostearate, or sodium propionate as 75 per cent. of the diet for 1 year did not harmfully affect growth or mortality of rats.

2. The high concentrations of polyoxyethylene (8) monostearate, sodium propionate, antioxidants and chlorine dioxide had no detectable effect on organ weights or on histopathology of the tissues.

3. No evidence was obtained that the simultaneous presence of high concentrations of more than one potential toxicant in the diet led to any synergistic action.

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