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Toxicological Evaluation of some Combinations of Food Preservatives

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Abstract—Short- and long-term studies are reported in which preservatives were administered orally, singly or in pairs, to rats and mice. Daily doses of nisin (2 or 4 mg/kg), sorbic acid (40 or 80 mg/kg), benzoic acid (40 or 80 mg/kg) and sodium bisulphite (80 or 160 mg/kg), and mixtures of benzoic acid/sodium bisulphite (40/80 or 80/160 mg/kg) and sorbic acid/nisin (40/2 or 80/4 mg/kg) were given to mice or rats for 3, 17 or 18 months, and in a further test nisin (0·4–400 mg/kg/day), sorbic acid (40 mg/kg/day) and a polymeric impurity derived from the sorbic acid were tested in mice for 2 months. In addition to the effects of these substances on growth and survival, haematology and blood chemistry (in rats), kidney function (in rats) and reproduction (in mice), the effects of certain stress factors, including dietary restriction, low temperature and carbon tetrachloride administration, on the potential toxicity of the preservatives were studied in both species.

In general, these doses showed some adverse effects in both rats and mice, particularly on growth, survival and susceptibility to stress, but sorbic acid appeared to be the least toxic. The effects of the benzoic acid/sodium bisulphite mixture were generally greater than those of the compounds given singly, while the reverse was the case with the sorbic acid/nisin mixture. The antibiotic, nisin, increased the growth rate when given alone. The authors' recommendation that the use of benzoate and sulphite as preservatives should be further restricted and that sorbic acid should be used instead where possible is being followed in the USSR.

INTRODUCTION

Despite the development of newer techniques, chemical preservatives still retain their economic importance for extending the shelf-life of foods and conserving their nutritional value. Until recently sulphites and benzoates found fairly wide use in the USSR as food preservatives, but now their use is restricted as much as possible. Limited amounts of sulphurous acid are permitted in certain foods, including some wines, jams, fruit candies, starch, tomato puree, dried fruits, fruit juices and some fresh and semi-processed fruits. Benzoic acid is also used, for preserving gelatin, fruit candy and paste, pastilles, margarine, egg mayonnaise, salmon caviare, spiced sprats and some berries and fruit products, and sodium benzoate is permitted in fruit juices, fish preserves and margarine.

Recently sorbic acid and the antibiotic, nisin, have been proposed for extensive use as preservatives, but their safety-in-use had to be established before they could be added to the existing list of permitted food additives. It was decided, therefore, that the technological and economic questions concerning these materials should be studied at the All Union Institute of the Food Canning Industry, while we undertook the toxicological studies.

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Toxicological data are available for the administration of each of these compounds individually to experimental animals (Joint FAO/WHO Expert Committee on Food Additives, 1962), but not for their administration in combination. According to the literature, sodium bisulphite fed at dietary levels of 0·05% for up to 2 yr had no adverse effects but levels of 0·1% or higher reduced weight gain and induced histopathological changes in some organs (Fitzhugh, Knudsen & Nelson, 1946). The authors attributed these effects to avitaminosis B_1 following destruction of this vitamin in the diet by the sodium bisulphite. Intubated doses of up to 2·5 g sodium bisulphite given daily to dogs for up to 419 days had no toxic effects. Sodium benzoate had no adverse effects in rats given dietary levels of up to 4% for 90 days, but levels of 8% increased mortality, reduced weight gain and caused hypertrophy of the liver and kidneys (Deuel, Alfin-Slater, Weil & Smyth, 1954). No toxic effects occurred in four generations of rats fed 0·5 or 1·0% in the diet (Kieckebusch & Lang, 1960). High levels of protein in the rat diet have a protective action against this preservative (Lang, 1961). Sorbic acid had no toxic effects when fed in the diet of rats at levels of up to 8% for 90 or 1000 days (Deuel *et al.* 1954; Lang, 1960). No toxic effects were detected in rats fed 0·1–0·3% nisin in the diet, with cheese, for periods of 12 wk–1 yr, survival, weight gain, reproduction indices, haematology, gastro-intestinal and kidney function and organ histology being within the limits of the control animals (Hawley, 1958; Frazer, 1960).

Since mixtures of food additives are ingested in a normal diet, it was considered appropriate to carry out short- and long-term oral toxicity tests in rats and mice on combinations of benzoic acid and sodium bisulphite and of sorbic acid and nisin, as well as on the individual compounds.

EXPERIMENTAL

Materials. Benzoic acid and sodium bisulphite were analytical-grade reagents obtained commercially. Sorbic acid, obtained commercially, contained an unknown polymeric impurity (0·02–0·07% ashed residue) which was insoluble in water, sparingly soluble in acids or alkali and moderately soluble in chloroform. Nisin with an activity of 1×10^6 IU/g was purchased from Aplin & Barrett Ltd., Yeovil, Somerset, UK. It was of largely unknown composition but contained a stabilizer.

Animals. Both sexes of cross-bred white mice and Wistar rats were used, the initial body weights varying according to the nature of the experiment. Mice were housed in groups of 10–25 and rats in groups of five.

Experimental design and conduct

2-Month study in mice. Groups of 25 male and 25 female mice (initial body weight 8–10 g) received for 2 months by oral intubation daily doses of 0·4, 4·0 or 400 mg nisin/kg/day (approximately equivalent to 0·002, 0·02 and 2·0% in human food). Groups of 25 male and 25 female mice (initial body weight 15–20 g) received the same doses of nisin for a similar period. Sorbic acid (40 mg/kg/day) and the polymeric impurity obtained from it (0·8 or 800 mg/kg/day) were given to similar groups of male and female mice from each body-weight range. The mice were observed for general condition and behaviour, and survival, food consumption and weight gain were recorded daily. Tests were carried out to determine the effects of hunger, physical stress and poisoning with carbon tetrachloride on the test animals, compared with untreated controls.

3-Month study in mice. Groups of 50 male and 50 female mice (initial body weight 8–10 g)

received, by oral intubation, sorbic acid or benzoic acid (in doses of 80 mg/kg/day), sodium bisulphite (160 mg/kg/day) or nisin (4 mg/kg/day). Similar groups received mixtures of the same levels of benzoic acid and sodium bisulphite and of sorbic acid and nisin. The observations and tests performed on these mice were the same as those described for the 2-month study, except that a co-carcinogenicity test with Ehrlich ascites carcinoma was added.

17-Month study in mice. Groups of 25 male and 25 female mice (initial body weight 10–15 g) and groups of 25 male and 25 female mice (initial body weight 16–20 g) were given sorbic acid or benzoic acid in doses of 40 mg/kg/day, sodium bisulphite (80 mg/kg/day) or nisin (2 mg/kg/day). Similar groups received mixtures of the same levels of benzoic acid and sodium bisulphite and of sorbic acid and nisin. The test compounds were fed in a paste prior to the main feed, feed and water were provided *ad lib.* and a control group received only the basic diet. The parameters studied on the animals in this test were those listed for the 2-month study, except that poisoning by carbon tetrachloride was not studied and in addition organ weights were recorded. After 8 months on the preservative-combination or control diets some animals were mated and reproduction was studied over five generations. The weight gain of the litters after weaning (at 8–10 g body weight) was recorded for 3·5 months.

18-Month study in rats. Groups of ten male and ten female rats (initial body weight 100–120 g) received sorbic acid or benzoic acid (40 mg/kg/day), sodium bisulphite (80 mg/kg/day) or nisin (2 mg/kg/day). Groups of 50 male and 50 female rats (initial body weight 100–120 g) received sorbic acid (40 mg/kg/day) in combination with nisin (2 mg/kg/day), or benzoic acid (40 mg/kg/day) in combination with sodium bisulphite (80 mg/kg/day). Test compounds were fed in a paste prior to the main feed, while control groups received the basic diet alone. Food and water were provided *ad lib.* Food and water consumption, weight gain and the effects of stress factors were recorded, blood morphology was studied and the following were estimated: titre of serum complement, by our micro-modification of the classical method; phagocytic activity of leucocytes, by the classical method of Kost and Stenco as described by Predtechenskiĭ (1959), serum-ceruloplasmin level by Ravin's method (Petrun'kina, 1959); blood alkalinity (Panisyak, 1952); blood ketones by Natelson's method (Todorov, 1960); blood morphology; erythrocyte sedimentation rate, by a modification of Efmann's method (Mukhorina, 1962); C-reactive protein in serum (Pashinin, 1962).

Effect of stress factors on preservative toxicity in mice and rats

Food restriction. At the termination of the feeding studies, surviving mice were subjected to a restricted dietary intake for up to 5 days, the degree of restriction being 50% after the 2-month, 90% after the 3-month and 100% after the 17-month test. Rats (ten/group) were starved for up to 13 days. Both rats and mice received water *ad lib.* and administration of the preservatives was continued at the same dose levels by oral intubation. Weight loss, mortality rate and, in the case of the rats, the rate of weight-recovery after the return of survivors to a normal diet, were recorded.

Swimming test. All mice surviving at the end of the 2- and 17-month studies were fitted with a 2-g weight on the tail and left to swim in a water bath at 15–18°C. The time until they sank exhausted was recorded.

Low temperature. Groups of 15 rats from those fed combinations of preservatives were exposed to cold (−2–+8°C) for 12–24 hr at the end of the 18-month study.

Centrifugation. To study the functional efficiency of the rat's circulatory–respiratory system

after the prolonged consumption of mixtures of preservatives, groups of 15 rats were subjected to a rotation rate of 90–300 revs/min for 15–30 min.

Carbon tetrachloride detoxication test. This test was carried out to determine the effect of feeding preservatives on the detoxifying action of the liver. Groups of 7–32 mice that had received single preservatives for 2 or 3 months were given carbon tetrachloride by oral intubation in a single dose of 0·1 ml/mouse in sunflower oil (1 : 1 solution). After receiving one of the combinations of preservatives for 18 months groups of 9–15 rats were treated with orally-intubated carbon tetrachloride either in a single dose of 0·6 ml/rat or in daily doses of 0·2–0·3 ml/rat for 15 days. The mortality rate in these animals was compared with those from the feeding-test control groups given similar doses of solvent.

Kidney function test. Groups of 15 rats that had been given combinations of preservatives were given a kidney function test at the end of the 18-month period of feeding. A saturated solution of K_2HPO_4 was administered in doses of 4 ml/rat twice daily for 6 wk, and haematological studies and urinary protein determinations were carried out.

RESULTS

2-Month study in mice

Weight gain after 15, 30 and 60 days on test showed little divergence from the controls in any group, except in males receiving 0·4, 4·0 or 400 mg nisin/kg/day, in which the weight gain was significantly increased in the group with the lower initial body weight, and in males and females of the group with the higher initial body weight given the polymeric impurity from sorbic acid in a dose of 800 mg/kg. Survival and food consumption in the test groups did not differ significantly from those of the controls. The effect of food restriction on body weight and survival is shown in Table 1. The weight loss was highest in groups receiving the higher doses of nisin and the lower level of polymeric impurity, but survival time was highest in the two groups given the impurity. The same tendency was found in the effects of physical stress and carbon tetrachloride poisoning (Table 2), with mice given nisin being more susceptible than the animals of the other groups, and those given sorbic acid or the lower level of sorbic acid impurity showing a lower degree of mortality than the controls.

3-Month study in mice

Highest mortality rates at 2·5 months were recorded in mice given nisin or the benzoic acid/sodium bisulphite combination (Table 3). Animals given benzoic acid or sodium bisulphite separately showed about twice as high a survival rate as was seen with the combination of the two.

With the exception of males of the nisin group, test animals did not grow as well as the controls at 3 months, but this finding was not associated with a reduced feed intake (Table 4). The weight gain of mice fed the benzoic acid/sodium bisulphite combination was higher than that of mice fed either of the components, whereas in the case of nisin and sorbic acid the reverse was the case.

Under conditions of restricted feed intake mice on benzoic acid, the benzoic acid/sodium bisulphite combination or nisin showed the highest mortality rate (Table 1). The effect of carbon tetrachloride on the mortality of mice fed the preservatives for 3 months can be seen from Table 2. Mice given benzoic acid or sodium bisulphite showed a higher mortality rate than controls but those given sorbic acid were not susceptible to the dose of carbon tetrachloride given. The results of the co-carcinogenicity test are reported below.

Table 1. *Effect of dietary restriction on body weight and survival of preservative-treated mice*

Test compound(s)	Daily intake of preservative (mg/kg)	Group size*	Mortality (%) after 5 days on restricted diet	Weight loss† (%)	Days required to regain lost weight	Survival of fasted animals (days)
50% Food restriction after 2 months on preservative treatment						
None (control)	—	28	43	20		3·37
Nisin	0·4	58	34·5	19·5		3·5
	4·0	54	48·4	24·4		2·7
	400·0	46	70	25·0		3·7
Sorbic	40·0	42	19	14·6		3·5
Sorbic acid impurity	0·8	32	37·5	27·8		5·2
	800·0	28	21·4	20·0		4·25
90% Food restriction after 3 months on preservative treatment						
None (control)	—	16	56·3	41·2		4·6
Benzoic acid	80	14	85·7	31·7		4·3
Sodium bisulphite	160	14	57·2	36·9		4·6
Sorbic acid	80	14	55·7	41·5		4·5
Nisin	4	13	84·6	34·7		4·1
Benzoic acid/sodium bisulphite	80/160	12	83·3	37·9		4·2
Sorbic acid/nisin	80/4	14	64·3	43·8		5·4
100% Food restriction after 17 months on preservative treatment						
None (control)	—	32	12·5	17·8	1·6	
Benzoic acid	40	12	50·0	26·0	2·7	
Sodium bisulphite	80	17	0·0	17·8	2·3	
Sorbic acid	40	10	30·0	26·4	2·1	
Benzoic acid/sodium bisulphite	40/80	33	51·5	22·4	2·6	
Sorbic acid/nisin	40/2	40	7·5	16·7	2·0	

* Values for males and females are pooled together.
Groups of mice were used with body weights in the range 23·5–33·3 g before feed restriction.

Table 2. *Effect of 0·1 ml carbon tetrachloride on the mortality of preservative-treated mice*

Test compound	Daily intake (mg/kg)	Mortality (%)
None (control)*	—	28·6
Benzoic acid*	80	62·5
Sodium bisulphite*	160	45·0
Sorbic acid*	80	0·0
Control†	—	22·3
Nisin†	0·4	46·7
	4·0	56·4
	400·0	70·0
Sorbic acid†	40·0	14·8
Sorbic acid impurity†	0·8	0·0
	800·0	26·6

* Groups of ten mice fed the control or preservative diet for 3 months.
† Groups of 7–32 mice fed the control or preservative diet for 2 months.
The effects of carbon tetrachloride were not tested on mice fed the combinations of preservatives.

Table 3. *Survival rate at 2·5 and 8 months in mice given preservatives orally, alone or in combination*

Test compound(s)	Daily intake (mg/kg)	Survival (%) in Males at month 2·5*	Males at month 8†	Males at month 8‡	Females at month 2·5*	Females at month 8†	Females at month 8†
None (control)	—	60	72·5	78·5	62	—	—
Benzoic acid	80	68			68		
Sodium bisulphite	160	70			68		
Sorbic acid	80	98			52		
Nisin	4	12			32		
Benzoic acid/sodium bisulphite	80/160	30			38		
	40/80		44·4	35·4		28·5	55·3
Sorbic acid/nisin	80/4	48			74		
	40/2		22·4	9·4		37·9	73·0

* Groups initially comprised 50 male or 50 female mice, weighing 8–10 g in either case.
† Groups initially comprised 9–29 mice of both sexes, weighing 10–15 g. Controls were males only.
‡ Groups initially comprised 26–56 mice of both sexes, weighing 16–25 g. Controls were males only.

17-Month study in mice

Growth and survival. The survival rate of these mice at 8 months can be seen in Table 3. Animals in the control groups had a better survival rate than those receiving the preservative

combinations. The poorest survival was in males receiving sorbic acid in combination with nisin, but this group registered the greatest weight gain as shown in the reproduction study data (Table 5). This was especially marked in the group with initial body weights between 10 and 15 g. The weights of liver, kidney and testes relative to body weight in mice sacrificed at the end of the test period were lower in the group given sorbic acid alone than in all the other groups.

Reproduction study. In the four generations of offspring, the weight gain of the litters was studied for 3·5 months after weaning (at 8–10 g body weight) and the effects of the preservative combinations on the weight of the animals at 3·5 months are shown in Table 5. Those fed sorbic acid in combination with nisin grew better on the whole than mice given benzoic acid in combination with sodium bisulphite.

Effects of stress. In the swimming test a better time was recorded for the groups of mice given sorbic acid alone or in combination with nisin than for mice given benzoic acid alone or in combination with sodium bisulphite. The effect of feed restriction on body weight and survival can be seen in Table 1. Percentage mortality after 5 days of feed restriction was highest in groups receiving benzoic acid alone or in combination with sodium bisulphite. The time required to regain lost weight was also slightly greater in these groups.

Tumour incidence. After the feeding of preservative diets for 17 months, 8/100 mice in the benzoic acid/sodium bisulphite group of the first generation and 1/8 in the third generation of the same group were found to have malignant tumours. No tumours were found in the control or sorbic acid/nisin groups. In connexion with these findings we decided to study the possible co-carcinogenicity of the preservatives using the tumour transplantation test. Erlich ascites mouse carcinoma was transplanted intraperitoneally into mice which had been fed for 3-months on the test diets. The tumour growth was greatest in mice that had received sodium bisulphite, and was less marked in mice given bisulphite in combination with benzoic acid or benzoic acid alone. The tumour growth in mice given sorbic acid alone or in combination with nisin was comparable with that in the control group.

Long-term effects in rats

Rats fed the sorbic acid/nisin mixture grew better than those fed benzoic acid/sodium bisulphite over an 18-month period (Table 6). The titre of serum complement and the phagocytic activity of the leucocytes were also higher in the former than in the latter group, but the level of serum ceruloplasmin was higher in the latter than in the former. Cold stress, centrifugation and kidney loading with K_2HPO_4 had most effect in rats given the benzoic acid/sodium bisulphite combination.

Blood alkalinity, C-reactive protein levels and blood morphology were comparable in all groups including the controls. But the erythrocyte sedimentation rate was increased in rats fed the benzoic acid/sodium bisulphite combination, in which group the level of blood ketones was 1·97 mg/100 ml, compared with 2·5 mg/100 ml in the control group and 3·4 mg/100 ml in the sorbic acid/nisin group.

The results in Table 7 indicate that rats fed benzoic acid for 18 months developed some tolerance to a lethal dose of the compound given terminally. Rats on a sorbic acid/nisin diet tolerated carbon tetrachloride better than did those on the sodium bisulphite/benzoic acid diet. In the latter group, 20% of the animals showed signs of haemorrhagic keratoconjunctivitis, which developed 2 months after the beginning of the study. The effects of starvation (with water available *ad lib.*) were inconclusive, rats in all the test groups surviving longer than the controls.

Table 4. *Mean values of weight gain and daily intake of feed and water in mice given preservatives orally, singly or in combination for 3 months*

Test compound(s)	Daily intake (mg/kg)	Weight gain (% of initial body weight)		Daily feed intake (g)		Daily water intake (ml)	
		Males	Females	Males	Females	Males	Females
None (control)	—	199	167	2·33	2·81	3·67	4·14
Benzoic acid	80	125	118	2·57	2·65	3·85	3·52
Sodium bisulphite	160	143	124	2·43	1·98	3·33	3·13
Sorbic acid	80	145	144	2·17	2·13	3·86	3·31
Nisin	4	227	143	3·36	4·31	5·92	4·34
Benzoic acid/sodium bisulphite	80/160	174	141	3·24	2·32	3·54	2·63
Sorbic acid/nisin	80/4	129	115	2·34	2·48	3·32	4·42

Groups of 50 males and 50 females (initial body weight 8–10 g) were used.
Feed and water consumption were recorded daily throughout the test period.

Table 5. *Weight gain at 3·5 months in four generations of mice given combinations of preservatives from weaning to mating*

Test compounds	Dosage level (mg/kg)	Weight gain (% weight at weaning) at 3·5 months for generation							
		F_1		F_2		F_3		F_4	
		Males	Females	Males	Females	Males	Females	Males	Females
None (control)	—	218	200	147	118	228	212	106	80·5
Benzoic acid/sodium bisulphite	40/80	150	145	103	99	180	217	171	212
Sorbic acid/nisin	40/2	257	202	138	118	219	199	242	187

Table 6. *Mean values of weight gain and daily intake of feed and water in rats fed various preservatives singly or in combination for 18 months*

Test compound(s)	Daily intake mg/kg)	Weight gain at 18 months (% of initial body weight)		Daily feed intake (g)		Daily water intake (ml)	
		Males	Females	Males	Females	Males	Females
None (control)	—	203·49	168·8	17·2	9·6	37·8	26·4
Benzoic acid	40			14·8	13·1	28·6	26·3
Sodium bisulphite	80			14·5	9·8	32·0	25·7
Sorbic acid	40			13·6	9·7	38·7	27·3
Nisin	2			16·8	9·2	39·5	32·6
Benzoic acid/sodium bisulphite	40/80	164·8	151·8				
Sorbic acid/nisin	40/2	253·7	203·0				

Weight gain was estimated in groups of 50 rats (initial body weight 100–120 g) and feed and water intakes in groups of ten.

Table 7. *Effect of carbon tetrachloride and sodium bisulphite and benzoate on mortality in rats fed preservatives singly or in combination for 18 months*

Test compound(s) fed for 18 months	Secondary compound and dose		Mortality (%)
None (control)	Sodium benzoate (mg/kg)	3600	100
Sorbic acid/nisin		3400	71·5
Sodium bisulphite/benzoic acid		3400	100
Benzoic acid		4000	25
None (control)	Sodium bisulphite (mg/kg)	860	100
		430	100
Sorbic acid/nisin		860	66·6
		430	33·3
Sodium bisulphite/benzoic acid		860	100
		430	60
Sorbic acid/nisin	Carbon tetrachloride (ml)	0·6	33·3
		0·2	11·0
Sodium bisulphite/benzoic acid		0·6	60·0
		0·2	27·0

Groups of 3–15 rats were used.

DISCUSSION

The data presented suggest that the preservatives tested had some slight adverse effects on rats or mice in a dosage range equivalent to the maximum levels at which these preservatives may occur in the human diet. The toxic effects of benzoic acid and sodium bisulphite when administered in combination appeared to be synergistic or additive to some extent, while there were indications that the reverse was the case with the sorbic acid/nisin combination. This is an important consideration in the control of the use of preservatives for extending the shelf-life of food for human consumption. In our experiments the sorbic acid showed the lowest toxicity and changes that were recorded in some tests were not considered to be pathological. Moreover, this unsaturated fatty acid has some beneficial effect on the animal organism, being not only a good preservative for food but a nutritional additive with some features of the vitamins. Therefore it was considered reasonable to recommend to the Food Hygiene Department of the Ministry of Health of the USSR a maximum restriction on the permitted list of foods preserved with sodium bisulphite (and other compounds of sulphurous acid) and with benzoates. This recommendation has been accepted.

As the data obtained for nisin were not convincing, it was decided to carry out a further study with nisin manufactured in the Soviet Union. This was recently completed (Shillinger, Bogoroditskaya & Osipova, 1969) and the home-produced nisin was found to be less toxic than the imported. However nisin is an antibiotic, a fact confirmed by our own studies (particularly the finding of weight stimulation in the test animals) and its use as a food preservative should therefore be limited. The high content of nisin occurring naturally in some dairy products is not a sound reason for unrestricted use of this compound as a food preservative, but there is some case for using nisin in combination with sorbic acid, as we have recommended for some products. In the present situation it is considered reasonable to restrict the use of benzoates and sulphites at the expense of extending the use of sorbates, a trend which is at present being followed in the USSR.

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Evaluation toxicologique de certaines combinaisons de produits de conservation des aliments

Résumé—L'article décrit des études à court et à long terme, au cours desquelles des produits de conservation ont été administrés par voie orale, isolément ou par paires, à des rats et à des souris. Des rats ou des souris ont reçu pendant 3, 17 ou 18 mois des doses quotidiennes de nisine (2 ou 4 mg/kg), d'acide sorbique (40 ou 80 mg/kg), d'acide benzoïque (40 ou 80 mg/kg) ou de bisulfite de sodium (80 ou 160 mg/kg) ou de mélanges d'acide benzoïque et de bisulfite de sodium (40/80 ou 80/160 mg/kg) et de mélanges d'acide sorbique et de nisine (40/2 ou 80/4 mg/kg). La nisine (0,4–400 mg/kg/jour), l'acide sorbique (40 mg/kg/jour) et une impureté polymère dérivée de l'acide sorbique ont été testés pendant 2 mois sur des souris au cours d'un autre essai. En plus des effets de ces substances sur la croissance et sur la survie, sur l'hématologie et la chimie du sang (chez les rats), sur la fonction rénale (chez les rats) et sur la reproduction (chez les souris), on a étudié, chez les deux espèces animales, les effets de certains facteurs de stress: restriction alimentaire, basse température et administration de tétrachlorure de carbone, sur la toxicité potentielle des produits.

D'une manière générale, les doses précitées ont eu certains effets nocifs, autant chez les rats que chez les souris, plus particulièrement sur la croissance, la survie et la sensibilité au stress. L'acide sorbique s'est révélé le moins toxique. Les effets du mélange d'acide benzoïque et de bisulfite de sodium étaient généralement plus prononcés que ceux de ces produits administrés séparément, mais l'inverse a été observé pour le mélange d'acide sorbique et de nisine. Administré seul, l'antibiotique qu'est la nisine accélérait la croissance chez les rats. On recommande de restreindre davantage l'emploi du benzoate et du sulfite comme produits de conservation des aliments et de les remplacer autant que possible par l'acide sorbique. Cette recommendation est déjà suivie en URSS.

Toxikologische Prüfung einiger Kombinationen von Konservierungsmitteln für Nahrungsmittel

Zusammenfassung—Es wird über kurz- und langzeitige Untersuchungen berichtet, bei denen Konservierungsmittel einzeln oder paarweise Ratten und Mäusen oral verabreicht wurden. Tägliche Dosen von Nisin (2 oder 4 mg/kg), Sorbinsäure (40 oder 80 mg/kg), Benzoesäure (40 oder 80 mg/kg) und Natriumbisulfit (80 oder 160 mg/kg) und Mischungen von Benzosäure/Natriumbisulfit (40/80 oder 80/160 mg/kg) und Sorbinsäure/Nisin (40/2 oder 80/4 mg/kg) wurden 3, 17 oder 18 Monate Mäusen oder Ratten verabreicht, und bei einem weiteren Versuch wurden 2 Monate lang Nisin (0,4–400 mg/kg/Tag), Sorbinsäure (40 mg/kg/Tag) und eine aus der Sorbinsäure isolierte polymere Verunreinigung an Mäuse verabreicht. Ausser den Wirkungen dieser Substanzen auf das Wachstum und das Überleben, die Hämatologie und Blutchemie (bei Ratten), die Nierenfunktion (bei Ratten) und die Fortpflanzung (bei Mäusen) wurden die Wirkungen bestimmter Stressfaktoren einschliesslich der Futtereinschränkung, der Einwirkung niedriger Temperaturen und der Anwendung von Tetrakohlenstoff auf die mögliche Toxizität der Konservierungsmittel bei beiden Spezies untersucht.

Im allgemeinen zeigten diese Dosen einige nachteilige Wirkungen bei Ratten und Mäusen, besonders auf das Wachstum, das Überleben und die Stressempfindlichkeit, jedoch schien die Sorbinsäure am wenigsten toxisch zu sein. Die Wirkungen der Benzoesäure/Natriumbisulfit-Mischung war im allgemeinen grösser als die der einzeln verabreichten Verbindungen, während es im Fall der Sorbinsäure/Nisin-Mischung gerade umgekehrt war. Das Antibioticum Nisin erhöhte die Wachstumsgeschwindigkeit bei Einzelverabreichung. Der Autor empfiehlt, die Verwendung von Benzoat und Sulfit als Konservierungsmittel weiter einzuschränken. Stattdessen sollte wie in der UdSSR Sorbinsäure stets verwendet werden, wenn es möglich ist.