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Research Section

Food Flavourings and Compounds of Related Structure. II. Subacute and Chronic Toxicity

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Abstract—The subacute and/or chronic toxicity of 48 food flavourings was studied in rats. The toxicity of five of these flavourings (coumarin, 6-methylcoumarin, dihydrocoumarin, methyl salicylate and safrole) was also studied in dogs. A summary of the studies is presented. The loss of 21 flavourings from laboratory animal diets during a 7-day period was also determined.

INTRODUCTION

For a number of years the Food and Drug Administration has been investigating the toxicity of food flavourings, both natural and synthetic. The toxicity studies consist of (1) determination of the acute oral effects, (2) subacute studies in which the flavouring agents are mixed in the diet or administered by stomach tube, and (3) chronic feeding studies on those flavouring agents having wide use, having chemical structures implying toxicity and/or showing toxic effects in the subacute studies. The data on the acute oral toxicity of these substances have been reported (Jenner, Hagan, Taylor, Cook & Fitzhugh, 1964). The present report is a summary of the subacute and chronic studies. Full reports on some of the chronic studies have been published for methyl salicylate (Webb & Hansen, 1963), safrole (Long, Nelson, Fitzhugh & Hansen, 1963; Hagan, Jenner, Jones, Fitzhugh, Brouwer & Webb, 1965) and dihydrosafrole (Long & Jenner, 1963; Hagan *et al.* 1965).

Commercially-available materials, rather than pure chemicals, were used since the purpose of these studies was to evaluate the toxicity of these materials in relation to their use as food additives.

EXPERIMENTAL

Rat studies. Weanling Osborne–Mendel rats were used in the chronic and most of the subacute experiments. In a few subacute experiments young adult rats of the same strain were used. Litter mates were used except for a few subacute studies in which the weanlings were randomized by weight (every level having animals of equal weight). A control group containing the same number of rats as the test groups was included with each test compound except in the following studies in which a common group was used for a number of test compounds:

(1) Allyl caproate, allyl cyclohexane propionate, anisaldehyde, benzaldehyde, carvone, dimethylbenzyl carbinol, ethyl sebacate, geraniol extra, piperonal and vanillin fed at 1000 ppm for 27–28 wk (control group 10 males and 10 females).

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(2) Anethole, anisaldehyde, benzaldehyde, carvone, dimethylbenzyl carbinol, Dolcourin, ethyl methylphenyl glycidate, ethyl pelargonate, ethyl sebacate, geraniol extra, isoeugenol, piperonal and vanillin fed at 10,000 ppm for 16 wk (control group 10 males and 10 females).

(3) Benzyl cinnamate, eugenol and thymol fed at 1000 and 10,000 ppm for 19 wk (control group 5 males and 5 females).

(4) Anethole, carvone and ethyl methylphenyl glycidate fed at 2500 ppm for 1 yr (control group 5 males and 5 females).

(5) Allyl caproate and allyl cyclohexane propionate fed at 2500 ppm for 1 yr (control group 5 males and 5 females).

(6) Ethyl vanillin and vanillin fed at 5000, 10,000 and 20,000 ppm for 2 yr (control group 12 males and 12 females).

(7) Ethyl vanillin and vanillin fed at 20,000 and 50,000 ppm for 1 yr (control group 5 males).

(8) Dihydrosafrole, isosafrole and safrole fed at 1000, 2500, 5000 and 10,000 ppm for 2 yr (control group 35 males and 35 females).

The animals were housed individually in wire cages and had access to food and water at all times. Most of the compounds were fed in the diet, but a few were administered by stomach tube. Fresh diets were made and distributed weekly except in the study with allyl isothiocyanate in which the diet was prepared and fed daily*. When the compound was administered by stomach tube, corn oil solutions of the flavouring were prepared. The concentration of the solution was adjusted for each level so that all rats received a constant volume of 1 ml of solution/kg daily. The control animals were given an equal volume of corn oil. The rat's weight, food intake and general condition were recorded every week. Haematological examinations were made at termination of the subacute studies, and at 3, 6, 12 and 22 months in the chronic experiments. These examinations included white cell counts, red cell counts, haemoglobins and haematocrits.

At the termination of the experiments the rats were sacrificed and exsanguinated. The tissues of all the rats were examined macroscopically at the time of sacrifice. The viscera were removed and the liver, kidneys, spleen, heart, and testes were weighed. These organs, the remaining abdominal and thoracic viscera, and one hind leg, for bone, bone marrow, and muscle, were preserved in 10% buffered formalin-saline solution for histopathological examination. For routine histopathology, sections were embedded in paraffin wax and stained with haematoxylin and eosin. Tissues from rats dying during the experiment were examined for gross changes and were preserved if autolysis was not advanced. Organs were not weighed but abnormalities and the suspected reason for death were noted.

Detailed microscopic examinations in the subacute studies were generally done on 6 or 8 rats, evenly divided by sex, from the high dose group and the control group. If changes attributable to the test compound were found in the high dose group, additional animals on lower dosage levels were examined as indicated. For animals from the long-term studies, more extensive histopathological study was done.

Dog studies. Pure-bred beagles were used in the studies with safrole and methyl salicylate. Mongrels and beagles were used in the coumarin, dihydrocoumarin, and 6-methylcoumarin studies. The dogs were wormed and immunized against distemper, rabies, and infectious

*Two other exceptions were the safrole rat study (Long *et al.* 1963) and the chronic rat feeding study of methyl salicylate (Webb & Hansen, 1963) in which the diets were prepared every other week and stored under refrigeration in sealed containers.

hepatitis before treatment was begun. They were housed in individual cages with food (commercial dry dog food) and water available at all times. The test compound was administered by capsule daily for 6 days a week. The animals were weighed weekly and the dosages were recalculated at this time. Haematological examinations were made three times prior to the start of treatment and at 2 wk, 1 month, 3 months, 6 months and 1 yr after treatment began and then at yearly intervals until termination of the study.

At the end of a study, survivors were killed with an overdose of an intravenous barbiturate and exsanguinated. The major organs were weighed. Sections of brain (four levels), pituitary, salivary gland, thyroid, parathyroid, thymus, lymph nodes, lung, heart, stomach, spleen, pancreas, kidney, adrenal, liver, gall bladder, small intestine (three levels), large intestine, urinary bladder, rib, skeletal muscle, and testis and prostate (or ovary and uterus) were preserved in 10% buffered formalin–saline for histological examination. In addition liver, kidney and adrenal sections and a femoral marrow plug were fixed in Zenker's solution. The sections were embedded in paraffin wax and stained with haematoxylin and eosin. Frozen sections of the liver and kidney were stained with Oil Red O for fat. A rib marrow smear was air-dried and stained with the Wright–Giemsa stain. Dogs found in a moribund condition during the experiment were sacrificed and necropsied. Dogs dying during the experiment were necropsied and tissue sections preserved if autolysis was not too advanced. All the tissues that were preserved were examined microscopically.

RESULTS

Table 1 shows the compounds tested; the dose levels; number, sex and species of test animals; duration; and effects. When the dose level is given in ppm, the test compound was mixed in the diet. When the dose is given in mg/kg, the test compound was administered daily by stomach tube to rats and (6 days/wk) by capsule to dogs. Only those effects believed to be due to the administration of the compound are listed. The designation "no effect" indicates that there was no effect on growth or haematology, and no macroscopic or microscopic change in the tissues. The designation "no macroscopic effect" indicates that there was no effect on growth or haematology, and no macroscopic change in the tissues, and that microscopic examination of the tissues was not performed. Pathological changes attributable to disease or age have not been included. For those compounds on which the toxicity studies have been reported previously the reference is given in Table 1 with the name of the compound.

In the evaluation of the data from the feeding studies with flavouring agents, the possibility of loss of the test compound from the experimental diet must be considered. Many of the flavouring agents used in these studies were quite volatile. Studies in our laboratory have shown that a decrease in the concentration of some of the flavourings occurred when the experimental diet was exposed in the open feed cups (Jones, Taylor, & Hagan, 1962). In order to determine the actual amount of the flavouring consumed by the animals, many of the experimental diets were analysed for the flavouring immediately after preparation and again after being exposed to laboratory conditions for 7 days. The flavourings on which analytical studies have been made and the percentage loss calculated over a 7-day period are presented in Table 2.

Table 1. *Summary of subacute and chronic toxicity of food flavourings and related compounds*

Compound	Dose level (ppm)[a]	Species, group size and sex	Duration of test	Effects
Acetophenone	10,000	Rat 10M & 10F	17 wk	No effect
(methyl 1-phenyl ketone)	2500	do.	do.	No macroscopic effect
	1000	do.	do.	do.
Allyl butyrate (allyl butanoate)	90 mg/kg	do.	18 wk	Growth retardation in males. Liver: macroscopic—rough or granular surface, firm consistency, nutmeg appearance; microscopic—slight to moderate bile-duct proliferation and fibrosis with pseudolobule formation. Necrosis with polymorphonuclear infiltration and swollen, foamy liver cells in 2 of 8 rats examined
	50 mg/kg	do.	17 wk	Lung—slight to marked peribronchial lymphocytic infiltration[b]. Liver—no effect
Allyl caproate	2500	5M & 5F	1 yr	No effect
(allyl hexanoate)	1000	do.	28 wk	do.
	100 mg/kg	10M & 10F	18 wk	Liver: macroscopic — somewhat nodular and wrinkled with granular or rough surface; microscopic—slight to moderate bile-duct proliferation, some lobular architectural disarrangement, slight fibrosis, pigment in macrophages; necrotic foci in 2 of 8 rats examined
	65 mg/kg	do.	do.	Liver: microscopic—very slight bile-duct proliferation in 2 of 8 rats examined[c]
	15 mg/kg	do.	do.	No effect[c]
Allyl cyclohexane propionate	2500	5M & 5F	1 yr	No effect
(allyl 3-cyclohexylpropionate)	1000	do.	27–28 wk	do.
Allyl isothiocyanate[d]	10,000	do.	26 wk	do.
	2500	do.	do.	No macroscopic effect
	1000	do.	do.	do.

[a]Unless otherwise stated.
[b]Controls had slight to marked peribronchial lymphocytic infiltration not as severe as with the 50 mg/kg/day dose.
[c]Microscopic examinations were made on the liver only.
[d]The diet was prepared daily because of the extreme volatility of this compound. A control group pair-fed with the 10,000 ppm group was included in this study in addition to the usual untreated control group.

Table 1 continued

Compound	Dose level (ppm)[a]	Species, group size and sex	Duration of test	Effects
Allyl isothiocyanate[d]	50 mg/kg	Rat 5M & 5F	20 days	Stomach: macroscopic—non-glandular part of the stomach was thickened, with lining occasionally roughened; microscopic—slight to moderate epithelial hyperplasia of the non-glandular part of the stomach, with acute to subacute ulcers from 2 to 6 1/2 mm across observed in all animals. Liver: microscopic—minor inflammatory foci
	20 mg/kg	do.	20 days	Stomach: macroscopic—same as seen in the 50 mg/kg animals; microscopic—the same type of ulceration as seen in the higher dose, occurring in 50% of the animals
Amyl butyrate	10,000	10M & 10F	16 wk	No effect
(pentyl butyrate)	2500	do.	do.	No macroscopic effect
	1000	do.	do.	do.
Amyl valerianate	10,000	do.	13 wk	No effect
(pentyl pentanoate)	1000	do.	do.	No macroscopic effect
Anethole	10,000	5M & 5F	15 wk	Liver: microscopic—slight hydropic changes of hepatic cells in males only
(1-methoxy-4-propenylbenzene)				
	2500	do.	1 yr	No effect
Anisaldehyde	10,000	do.	15 wk	No effect
(*p*-methoxybenzaldehyde)	1000	do.	27–28 wk	do.
Benzaldehyde	10,000	do.	16 wk	do.
	1000	do.	27–28 wk	do.
Benzyl cinnamate	10,000	do.	19 wk	do.
(cinnamein, benzyl β-phenylacrylate)	1000	do.	do.	No macroscopic effect
Calamus oil	10,000[e]	10M & 10F	18 wk	Dose-related growth depression, greater in males than in females. Increased mortality. Large amounts of clear fluid in the abdominal cavity. Liver: macroscopic—variable in appearance with some lobes reduced in size with rough surfaces and others smooth, abnormally rounded, and enlarged; microscopic—variation in cell size, capsular thickening, proliferation of bile duct epithelium, portal area fibrosis with haemosiderin deposition (females more severely affected than males). Heart—minimal to slight myocardial degeneration characterized by varying degrees of necrosis of muscle fibres, early fibrosis, and infiltration with mononuclear cells

[e]Young adult animals were used.

Compound	Dose level (ppm)[a]	Species, group size and sex	Duration of test	Effects
Calamus oil	5000	Rat[e] 10M & 10F	18 wk	Growth depression. Moderate amounts of clear fluid in the abdominal cavity. Liver—same changes as with 10,000 ppm but less severe. Heart—same changes as with 10,000 ppm but lower incidence
do.	2500	do.	do.	Growth depression. Liver—same changes as with 5000 and 10,000 ppm but less severe. Heart—same changes seen with the 10,000 ppm dose were seen in 1 of 6 rats examined
	1000	5M & 5F	do.	Growth depression
Carvone	10,000	do.	16 wk	Growth retardation, testicular atrophy
(*p*-mentha-6,8-dien-2-one)	2500	do.	1 yr	No effect
	1000	do.	27–28 wk	do.
Cinnamic aldehyde (cinnamaldehyde)	10,000	10M & 10F	16 wk	Liver—slight hepatic cell swelling. Stomach—slight hyperkeratosis of squamous portion
	2500	do.	do.	No effect[f]
	1000	do.	do.	No macroscopic effect
Citral	10,000	do.	13 wk	No effect
(3,7-dimethyl-2,7-octadienal)	2500	do.	do.	No macroscopic effect
	1000	do.	do.	do.
Coumarin[g,h] (2*H*-1-benzopyron-2-one)	10,000[e]	3M & 3F	4 wk	Marked growth retardation, testicular atrophy. Liver: microscopic—slight to moderate damage consisting of dead and dying cells, decrease in oxyphilia and cytoplasm in the centrolobular cells, and proliferation of bile ducts
	10,000	10M & 10F	1–8 wk	Marked growth retardation. No animal survived beyond 8 wk. Liver: microscopic—slight to moderate damage of the type seen with the 10,000 ppm dose for 4 wk

[f] Microscopic examinations were made on the stomach only.
[g] Toxicity study will be presented in a separate report.
[h] Not acceptable for food use because of toxic effects observed in chronic feeding studies.

Table 1 continued

Compound	Dose level (ppm)[a]	Species, group size and sex	Duration of test	Effects
Coumarin[g,h] (2*H*-1 benzopyron-2 one)	1000	Rat 10M & 10F	14 wk	No effect
	1000	5M & 5F	28 wk	do.
	2500	6M & 6F	29 wk	Growth retardation. Liver: macroscopic—slight mottling in the males; microscopic—slight mid-zonal fatty change in both sexes but more marked in the males
	5000[i]	do.	2 yr	Growth retardation. Normal food utilization (measured for first year). Haemogram-decrease in haemoglobin. Liver: macroscopic—enlargement, distortion by poorly to well delineated masses, pale pin-point foci; microscopic—focal proliferation of bile ducts of atypical appearance with associated fibrosis (cholangiofibrosis) situated in the masses described grossly and not clearly delineated from the surrounding hepatic parenchyma; slight degree of fatty metamorphosis, very slight to slight architectural irregularity, very slight to slight variation in cell size, very slight proliferation of bile ducts of the normal type, minimal focal necrosis
	2500[i]	5M & 7F	2 yr	Liver—slight damage of the same type as seen with the 5000 ppm dose; cholangiofibrosis was not seen
	2500	6M & 6F	do.	Liver—minimal damage of the same type as seen with 5000 ppm dose; cholangiofibrosis was not seen
	1000[i]	do.	do.	No effect

[i]Corn oil added to diet at a level of 3% (w/w). Two control groups were included in the study. Corn oil was added to the diet of one control group; the diet of the other group was untreated.

Compound	Dose level (ppm)[a]	Species, group size and sex	Duration of test	Effects
Coumarin[g,h] (2*H*-1-benzopyron-2-one)	100 mg/kg	Dog 1M & 1F	9–16 days	Male killed in extremis after 9 days. Female found dead on day 16. Marked emaciation, slight dehydration, and slight jaundice. Liver: macroscopic—moderate to marked yellow colour and moderate "nutmeg" appearance; microscopic—marked disorganization of the lobular pattern, moderate increase in size of liver cells (with variation ranging from slight atrophy to a great increase), slight to moderate vacuolation, moderate to large amount of diffusely distributed fat, slight to moderate focal necrosis, fibrosis ranging from slight (centrolibular) to moderate to marked (portal) and very slight to moderate bile-duct proliferation. Spleen—macroscopic moderately pale. Gastro-intestinal tract—intestinal contents thick, dark, and tarry. Bone marrow—thin and fatty. Gall bladder—moderately distended
	50 mg/kg	2M & 1F	35–277 days	Emaciation; slight to moderate jaundice in the two female dogs. One death at 35 days; remaining dogs sacrificed at intervals between 45 and 277 days. Liver: macroscopic—moderately yellow colour with "nutmeg" appearance; microscopic—the same changes as with the 100 mg/kg dose except that the liver cell vacuolation was greater. Spleen—large amount of haemosiderin. Bone marrow—pale
	25 mg/kg	do.	133–330 days	Moderate emaciation and slight jaundice in one female. Weight gain in remaining dogs. Liver—slightly pale in two dogs, dark red in a third and yellow, markedly "nutmeg" in the fourth; microscopic—liver changes like those seen on the 50 mg/kg dose to the same degree in one dog and less severe in the other three. Spleen—moderate haemosiderosis. Gall bladder—moderately distended in two animals
	10 mg/kg	2M & 2F	297–350 days	No definite effect
Dihydroanethole (1-methoxy-4-propylbenzene)	10,000	Rat 10M & 10F	19 wk	Bone—slight osteoporosis of the tibia and femur (6/10)
	2500	do.	do.	Bone—slight to very slight osteoporosis (6/10)
	1000	do.	do.	Bone—very slight osteoporosis[j] (4/10)

[j]In the control group slight osteoporosis was seen in one of 10 animals.

Table 1 continued

Compound	Dose level (ppm)[a]	Species, group size and sex	Duration of test	Effects
Dihydroanethole (1-methoxy-4-propylbenzene)	Maximal dose 5000 mg/kg	Rat 20M	32 days	Initial dose 2000 mg/kg increased gradually to 5000 mg/kg. Seven of the animals survived the full 32 days of treatment and 16 lived long enough to receive the maximal daily dose of 5000 mg/kg Stomach: macroscopic—mucosa of the forestomach showed coalescent areas covered by thick flaky white material punctuated by minute ulcers; microscopic—moderately severe degree of hyperkeratosis of the stratified squamous epithelium in the forestomach. Bone—moderate degree of osteoporosis
Dihydrocoumarin (1,2-benzohydropyrone)	10,000	10M & 10F	14 wk	No effect
	1000	do.	do.	do.
	150 mg/kg	Dog 1F	2 yr	do.
	50 mg/kg	1M & 1F	do.	do.
Dihydrosafrole[b] (1,2-methylenedioxy-4-propylbenzene) (Long & Jenner, 1963) (Hagan *et al.* 1965)	10,000	Rat 10M & 10F	do.	Growth retardation in both sexes. Oesophagus—benign epidermoid papillomas and malignant papillary epidermoid carcinomas; 75% of the rats showed oesophageal tumours, 50% of the tumours were malignant. Liver: macroscopic—enlargement; microscopic—slight damage of the same type as that seen in the safrole-treated rats (see safrole rat—2-yr feeding study). Spleen—moderate follicular atrophy
	5000	25M & 25F	do.	Growth retardation in both sexes. Oesophagus—74% of the rats showed oesophageal tumours, 32% of the tumours were malignant. Liver: macroscopic—enlargement; microscopic—slight damage of the same type as seen in the safrole-treated rats. Spleen—moderate follicular atrophy
	2500	10M & 10F	do.	Growth retardation in the females. Oesophagus—20% of the rats showed oesophageal tumours, 5% of which were malignant. Liver: macroscopic—enlargement; microscopic—slight liver damage of the same type as seen in the safrole-treated rats. Kidney—moderate increase in chronic nephritis

Compound	Dose level (ppm)[a]	Species, group size and sex	Duration of test	Effects
Dihydrosafrole[b] (1 2-methylenedioxy 4-propylbenzene) (Long & Jenner, 1963) (Hagan *et al.*1965)	1000	Rat 10M & 10F	2 yr.	Growth retardation in the females. Liver: macroscopic—slight enlargement; microscopic—slight liver damage of the same type as that seen in the safrole-treated rats. Kidney—moderate increase in chronic nephritis
Dimethylbenzyl carbinol	10,000	5M & 5F	16 wk	No effect
(1,1-dimethyl-2-phenylethanol)	1000	10M & 10F	28 wk	do.
Dolcourin[k]	10,000	5M & 5F	16 wk	do.
Ethyl caprylate	10,000	10M & 10F	17 wk	do.
(ethyl penanoate)	2500	do.	do.	No macroscopic effect
	1000	do.	do.	do.
Ethyl formate	10,000	do.	17 wk	No effect
	2500	do.	do.	No macroscopic effect
	1000	do.	do.	do.
Ethyl methylphenyl glycidate (ethyl ester of 2,3-epoxy-3-methyl-3-phenylpropionic acid)	10,000	5M & 5F	16 wk	Growth retardation most obvious in males Testes—marked atrophy
	2500	do.	1 yr	No effect
Ethyl oenanthate	10,000	10M & 10F	13 wk	do.
(ethyl heptanoate)	1000	do.	do.	No macroscopic effect
Ethyl oxyhydrate[l]	10,000	do.	17–18 wk	Liver: microscopic—very slight swelling of hepatic cells in males
	2500	do.	do.	No macroscopic effect
	1000	do.	do.	do.
Ethyl pelargonate (ethyl nonanoate)	10,000	5M & 5F	16 wk	No effect
Ethyl sebacate	10,000	do.	17–18 wk	do.
	1000	do.	27–29 wk	do.
Ethyl vanillin	50,000[m]	5M	1 yr	do.
(3-ethoxy-4-hydroxybenzaldehyde)	20,000[m]	do.	do.	do.
	20,000[n]	12M & 12F	2 yr	do.
	10,000[n]	do.	do.	do.
	5000[n]	do.	do.	do.
Eugenol	10,000	10M & 10F	19 wk	do.
(1-hydroxy-2-methoxy-4-allylbenzene)	1000	do.	do.	No macroscopic effect

[k]Dolcourin is a trade mark name for a substance supplied by Dodge and Olcott, Inc. as a coumarin substitute.
[l]Ethyl oxyhydrate is a mixture whose composition varies with the method of manufacture. The material used in this study was obtained from Florasynth Labs., Inc., N.Y.
[m]3% (w/w) corn oil added to control and test diets as a binder to reduce evaporation of the flavouring.
[n]3% (w/w) propylene glycol added to control and test diets as a binder to reduce evaporation of the flavourings.

Table 1 continued

Compound	Dose level (ppm)[a]	Species, group size and sex	Duration of test	Effects
Eugenol (1-hydroxy-2-methoxy-4-allylbenzene)	Maximal dose 4000 mg/kg	Rat 20M	34 days	Initial dose 1400 mg/kg increased gradually to 4000 mg/kg. Eight of the animals survived the full 34 days of treatment and 15 lived long enough to receive the maximal dose of 4000 mg/kg Stomach: macroscopic—mucosa of the forestomach showed coalescent areas covered by thick flaky white material punctuated by minute ulcers; microscopic—moderately severe degree of hyperkeratosis of the stratified squamous epithelium in the forestomach. Bone—small degree of osteoporosis
Eugenyl acetate (1-acetoxy-2-methoxy-4-allylbenzene)	10,000	10M & 10F	19 wk	No effect
	2500	do.	do.	No macroscopic effect
	1000	do.	do.	do.
Geraniol extra (3,7-dimethyl-2,6-octadienol) (3,7-dimethyl-1,6-octadienol)	10,000	5M & 5F	16 wk	No effect
	1000	do.	27–28 wk	do.
Geranyl acetate	10,000	10M & 10F	17 wk	do.
	2500	do.	do.	No macroscopic effect
	1000	do.	do.	do.
Ionone standard [60% α-ionone,4-(2,6,6-trimethyl-2-cyclohexen-1-yl)-3-buten-2-one and 40% β-ionone, 4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-3-buten-2-one]	10,000	do.	do.	Liver: microscopic—slight to moderate swelling of parenchymal cells
	2500	do.	do.	Liver: microscopic—slight amount of swelling of parenchymal cells[c]
	1000	do.	do.	Liver: microscopic—very slight amount of swelling of the parenchymal cells[c]
Isoeugenol (1-hydroxy-2-methoxy-4-propenylbenzene)	10,000	5M & 5F	16 wk	No effect
Isosafrole[b]	10,000	10M & 10F	11 wk	Growth retardation in both sexes. No rats on this dose survived beyond 11 wk of treatment. Liver: macroscopic—enlargement; microscopic—slight damage of the same type as seen with safrole
(Hagan *et al.* 1965)	5000	25M & 25F	2 yr	Growth retardation in both sexes. Testes—increased number of interstitial cell tumours. Liver: macroscopic—enlargement; microscopic—slight damage of the same type as seen with safrole. Five rats had primary hepatic tumours (two of these had hepatic cell adenomas and three had hepatic cell carcinomas). Kidney—increased incidence of chronic nephritis. Thyroid—slight hyperplasia

Compound	Dose level (ppm)[a]	Species, group size and sex	Duration of test	Effects
Isosafrole[b] (Hagan *et al.* 1965)	2500	Rat 10M & 10F	2 yr	Slight growth retardation in females. Liver: macroscopic—slightly enlarged; microscopic—slight damage of the same type as seen with safrole, no primary hepatic tumours. Thyroid—slight hyperplasia
	1000	do.	do.	Slight growth retardation in females. Liver: microscopic—slight damage of the same type as seen with safrole
Linalyl cinnamate	10,000	10M & 10F	17 wk	No effect
	2500	do.	do.	No macroscopic effect
	1000	do.	do.	do.
Linalyl isobutyrate	10,000	do.	18 wk	No effect
	2500	do.	do.	No macroscopic effect
	1000	do.	do.	do.
Methyl anthranilate (*o*-aminobenzoic acid, methyl ester)	10,000	do.	13 wk	No effect
	1000	do.	do.	No macroscopic effect
6-Methylcoumarin (6-methyl-2*H*-1-benzopyran-2-one)	15,000	25M & 25F	2 yr	Depression of growth, severe in males and moderate in females, and paralleled by decrease in food intake. Liver: microscopic—slight fatty metamorphosis, and very slight bile-duct proliferation and focal telangiectasia. Testes—moderate atrophy, probably due to growth retardation
	7500	do.	do.	Depression of growth in males
	3500	do.	do.	No effect
	1000	do.	do.	do.
	500	do.	do.	do.
	10,000	10M & 10F	14 wk	do.
	1000	do.	do.	do.
	150 mg/kg	Dog 1M	39 days	Sacrificed because of weakness, emaciation, dehydration. Liver: microscopic—moderate to severe hepatitis. Skeletal muscle—slight to moderate atrophy
	50 mg/kg	1M & 1F	2 yr	No effect

Table 1 continued

Compound	Dose level (ppm)[a]	Species, group size and sex	Duration of test	Effects
Methyl salicylate (*o*-hydroxybenzoic acid, methyl ester)	20,000	**Rat** 24M & 26F	49 wk	Growth retardation, rough coats. All dead by wk 49. Bone: microscopic—excess of cancellous tissue
(Webb & Hansen, 1963)	10,000	25M & 25F	2 yr	Growth retardation, rough coats. Bone: microscopic—slight excess of cancellous tissue
	5000	do.	do.	Bone: microscopic—slight excess of cancellous tissue
	1000	do.	do.	No effect
	10,000	10M & 10F	17 wk	Growth retardation
	1000	do.	do.	No macroscopic effect
	1200 mg/kg	**Dog** 1M & 1F	3 days	Died, weight loss. Liver: macroscopic—slightly pale, "nutmeg" in appearance; microscopic—moderate to marked amount of fat
	800 mg/kg	1M	29 days	Sacrificed, weight loss. Liver: microscopic—trace of fat
	800 mg/kg	1F	4 days	Died, weight loss. Liver: macroscopic—slightly pale, "nutmeg" in appearance; microscopic—moderate to marked amount of fat
	500 mg/kg	1M & 1F	8–9 days	Sacrificed—No effect
	250 mg/kg	do.	51–52 days	do.
	100 mg/kg	do.	52–53 days	do.
	50 mg/kg	do.	53–59 days	do.
	350 mg/kg	2M & 1F	2 yr	Weight loss. Liver: macroscopic—enlarged; microscopic—enlarged hepatic cells
	150 mg/kg	2M & 2F	do.	Growth retardation. Liver: macroscopic—enlarged; microscopic—enlarged hepatic cells
	50 mg/kg	do.	do.	No effect
Phenylethyl phenylacetate	10,000	**Rat** 10M & 10F	17 wk	do.
	2500	do.	do.	No macroscopic effect
	1000	do.	do.	do.
Piperonal (3,4-methylenedioxybenzaldehyde)	10,000	5M & 5F	15 wk	No effect
	1000	do.	27–28 wk	do.

Compound	Dose level (ppm)[a]	Species, group size and sex	Duration of test	Effects
Safrole[b] (1,2-methylenedioxy-4-allylbenzene) (Hagan *et al.* 1965)	10,000	Rat 10M & 10F	62 wk	Growth retardation in both sexes. All rats dead by wk 62. Testes—atrophy. Stomach—atrophy and atypical regeneration of the mucosa glands with associated fibrosis and hyalinization of the surrounding stroma most severe at the base. Liver: macroscopic—enlarged (2 to 3 times the normal size), mottled and irregularly nodular, with single or multiple tumour masses; microscopic—hepatic cell enlargement, which was usually focal and resulted in the formation of nodules. The enlargement was chiefly of the cytoplasm, which appeared normal in some cells but showed fatty metamorphosis or peripheral migration of the basophilic cytoplasmic granules in other cells. The nodules tended to progress in one of 3 ways (sometimes all 3 were seen in the same liver): (1) cystic necrosis; (2) cirrhosis; and (3) adenomatoid hyperplasia, leading to the formation of both benign and malignant tumours (hepatic cell adenoma, hepatic cell carcinoma, hepatocholangioma, hepatocholangiocarcinoma)
	5000	25M & 25F	2 yr	Growth retardation in both sexes. Increased mortality in the males. Liver—changes of the same type and severity as seen with the 10,000 ppm dose except for the occurrence of macroscopic cysts and a higher incidence of tumours in the lower dose, statistically-significant increase in malignant primary hepatic tumours. Stomach—same type of changes as with 10,000 ppm dose. Kidney—slight increase of chronic nephritis in the females. Thyroid—mild hyperplasia
	2500	10M & 10F	do.	Growth retardation in both sexes. Liver—moderate liver damage of the same type as seen with the 10,000 ppm dose with the exception of cirrhosis. Kidney—moderate increase of incidence of chronic nephritis.

Table 1 continued

Compound	Dose level (ppm)[a]	Species, group size and sex	Duration of test	Effects
Safrole[b] (1,2-methylenedioxy-4-allylbenzene) (Hagan *et al.* 1965)	1000	Rat 10M & 10F	2 yr	Growth retardation in the females. Liver—slight liver damage of the type seen with the 10,000 ppm dose, except that malignant tumours and cirrhosis were not seen with this dose. Kidney—moderate increase in chronic nephritis in the males.
(Long *et al.* 1963)	5000	25M & 25F	do.	Growth retardation in both sexes. Haemograms—mild anaemia, slight leucocytosis. Liver—marked liver changes (including malignant tumours but only rare cirrhosis) of the type seen with the 10,000 ppm dose. Statistically-significant increase in benign and malignant primary liver tumours.
	1000	25M & 25F	do.	Liver—slight to moderate damage of the same type seen with the 10,000 ppm dose except that malignant tumours and cirrhosis were not seen
	500	do.	do.	Liver—slight damage of the same type seen with the 10,000 ppm dose except that cirrhosis was not seen
	100	do.	do.	Liver—very slight damage of the same type seen with the 10,000 ppm dose except that cirrhosis was not seen
	80 mg/kg	Dog 2M & 2F	26–39 days	One dog died; the remaining dogs were sacrificed in a moribund condition. Marked weight loss. Skeletal muscle—moderate to severe atrophy in 2 of 4 dogs. Liver: macroscopic—slight enlargement in 2 of 4 dogs; microscopic—heavy infiltration of fat, occasional bile-duct proliferation and inflammatory-cell infiltration, slight to moderate streaking with necrobiosis and liver cell atrophy
	40 mg/kg	do.	91–116 days	Emaciation, general weakness. Liver: macroscopic—slight enlargement in 3 of 4 dogs; microscopic—slight fat infiltration, moderate hepatic cell atrophy and necrobiosis as at 80 mg/kg

Compound	Dose level (ppm)[a]	Species, group size and sex	Duration of test	Effects
Safrole[h] (1,2-methylenedioxy-4-allylbenzene (Long *et al.* 1963)	20 mg/kg	Dog 2M & 2F	6 yr	Liver: macroscopic—slight to moderate enlargement, nodular surface; microscopic—mild post-necrotic cirrhosis characterized by focal or generalized nodules, consisting of enlarged hepatic cells with slight focal fatty vacuolization, separated by bands of atrophied hepatic cells and collagen; slight lymphocytic, Kupffer cell and bile-duct proliferation; minimal focal necrosis. Thyroid—hyperplasia
	5 mg/kg	do.	do.	Liver: microscopic—minimal focal necrosis, bile-duct proliferation, fatty metamorphosis, hepatic cell atrophy and leucocytic infiltration
Terpinyl acetate (*p*-menth-1-en-8-ol ester of acetic acid)	10,000	Rat 10M & 10F	20 wk	No effect
	2500	do.	do.	No macroscopic effect
	1000	do.	do.	do.
Thymol (*p*-cymen-3-ol; hydroxy-*p*-cymene)	10,000	5M & 5F	19 wk	No effect
	1000	do.	do.	No macroscopic effect
γ-Valerolactone (4-hydroxypentanoic acid lactone)	10,000	10M & 10F	13 wk	No effect
	5000	do.	do.	do.
	2500	11M & 9F	do.	do.
	1000	10M & 10F	do.	do.
Vanillin (4-hydroxy-3-methoxybenzaldehyde)	50,000[m]	5M	1 yr	do.
	20,000[m]	do.	do.	do.
	20,000[n]	12M & 12F	2 yr	do.
	10,000[n]	do.	do.	do.
	5000[n]	do.	do.	do.
	10,000	5M & 5F	16 wk	do.
	1000	do.	27–28 wk	do.

Table 2. *Loss of food flavourings from laboratory animal diets during a 7-day period*

Flavouring	Percentage loss*	Flavouring	Percentage loss*
Acetophenone	31	Eugenyl acetate†	6
Allylcaproate	31	Geranyl acetate†	4
Amyl butyrate†	35	Ionone†	1
Anethole	6	Isosafrole	5
Calamus oil	0	Linalyl cinnamate	4
Cinnamic aldehyde	14	Linalyl isobutyrate	7
Citral	58	Methyl salicylate†	3 & 5§
Dihydroanethole†	16	Phenylethyl phenylacetate	0
Dihydrocoumarin	—‡	Safrole	12
Dihydrosafrole	6	Terpinyl acetate	7
Ethyl caprylate†	33		

*100 − (100 × day 7 recovery/day 0 recovery).
†Reported by Jones *et al.* (1962). Recovery data on the remaining compounds were supplied by W. I. Jones.
‡Dihydrocoumarin was partially converted to melilotic acid when mixed with the laboratory diet; the test animals ingested a mixture of the two compounds.
§The two values for percentage loss indicate two different analytical procedures.

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Substances d'assaisonnement et composés de structure connexe. II. Toxicité subaiguë et chronique

Résumé—On a étudié sur des rats la toxicité subaiguë et chronique de 48 substances d'assaisonnement. On a également étudié sur des chiens la toxicité de cinq d'entre elles (coumarine, 6-méthylcoumarine, dihydrocoumarine, salicylate de méthyle et safrol). On a présenté un résumé de ces études. On a déterminé aussi la depérdition de 21 substances d'assaisonnement en 7 jours, dans des régimes pour animaux de laboratoire.

Lebensmittel-Geschmacksstoffe und Verbindungen verwandter Struktur. II. Subakute und chronische Toxizität

Zusammenfassung—Die subakute und bzw. oder chronische Toxizität von 48 Lebensmittel-Geschmackszusätzen wurde an Ratten untersucht. Die Toxizität von fünf dieser Geschmacksstoffe (Cumarin, 6-Methylcumarin, Dihydrocumarin, Methylsalicylat und Safrol) wurde auch an Hunden untersucht. Eine Zusammenfassung der Versuchsergebnisse wird vorgelegt. Der Verlust von 21 Geschmacksstoffen aus dem Futter von Laboratoriumstieren während einer 7-Tage-Periode wurde ebenfalls bestimmt.