Scientific Edition

JOURNAL OF THE AMERICAN PHARMACEUTICAL ASSOCIATION

A. G. DuMez, Editor, Baltimore, Maryland

VOLUME XXIX | *OCTOBER, 1940* | NUMBER 10 CONSECUTIVE No. 19

On the Toxicity of Vanillin and Ethyl Vanillin for Rabbits and Rats*

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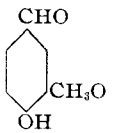
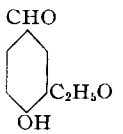
According to Preusse (1) the ingestion of 2 Gm. of vanillin in one dose by a rabbit produced no harmful effects, but repeated doses caused loss of appetite and weight, muscular weakness and death in coma. Dyson (10) set the toxic dose for intravenous administration to white rats at 1.5 Gm. per Kg. A human adult is cited by Kobert (2) as having ingested vanillin in amounts up to one gram without feeling symptoms of discomfort. Despite such evidence that a high dosage is required to produce systemic effects, reports of "vanillin mass poisoning," supposedly due to food flavored by vanilla, found their way into the literature (Kobert (2), Flury and Zangger (3)). The symptoms, which appeared 5–12 hours after the ingestion of food (Rasser (4)), were those of acute gastroenteritis followed, in some instances, by disorders of the central nervous system, and occasionally by death. The mystery of these sudden attacks of poisoning was solved by the discovery of their origin from degradation products of proteins (Vaughan (5)), or from the direct action of microorganisms, primarily those of the paratyphoid group (Wassermann (6), v. Vagdes (7), Curschmann (8), Rasser (4) and Gersbach (9)).

EXPERIMENTAL

The experiments described herein were carried out to determine the acute fatal concentration as well as the effects of the repeated administration of vanillin and ethyl vanillin. The compounds employed for the observations were obtained in a high degree of purity from commercial sources. Chemically, vanillin is 4-hydroxy, 3-methoxy benzaldehyde. Ethyl vanillin, commercially known as Ethavan, is 4-hydroxy, 3-ethoxy benzaldehyde. Its flavoring capacity is approximately 3.5 times that of vanillin.



Vanillin



Ethyl Vanillin

Acute Toxic Dosages of Vanillin and Ethyl Vanillin.—Vanillin and ethyl vanillin were dissolved to the extent of 4% or 5% in milk, by heating slowly to 90°, and cooling to 37°, after which they were administered by stomach tube to albino rabbits or injected subcutaneously into young albino rats. (One rabbit was given a small oral dose in the form

* Presented at the meeting of the American Society for Pharmacology and Experimental Therapeutics, New Orleans meeting, 1940.

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of a 4% solution in olive oil and 2 others received small doses as 0.5% solutions in 10% aqueous glycerol.) The minimal fatal dose of either vanillin or ethyl vanillin, when administered orally to rabbits, was 3.0 Gm. per Kg. of body weight (Table I). Subcutaneous dosages of 2.6 Gm. of vanillin or 2.0 Gm. of ethyl vanillin, per Kg. of body weight, killed 50% of the rats to which they were administered (Table II).

Table I.—Results of Oral Administration of Single Doses of Vanillin and Ethyl Vanillin to Rabbits

Rabbit	Dose, Gm./Kg.	Material Administered	Fate
		Vanillin	
D-338	0.15	0.5% in 10% glycerol	No illness
D-372	0.20	0.5% in 10% glycerol	No illness
N- 24	2.0	5% in milk	Increased respiration, recovery
N- 25	2.5	5% in milk	Increased respiration, collapse, recovery
D-642	3.0	5% in milk	Died in 45 minutes
N- 53	3.5	5% in milk	Died in 45 minutes
D-648	4.0	5% in milk	Died in 110 minutes
		Ethyl Vanillin	
D-337	0.15	4% in olive oil	No illness
D-150	2.5	5% in milk	Increased respiration, recovery
D-721	3.0	5% in milk	Increased respiration, recovery
D-722	3.0	5% in milk	Died in 90 minutes
D-148	4.0	5% in milk	Died in 48 hours
D-149	4.0	5% in milk	Died in 36 hours

Table II.—Results of Subcutaneous Injections of Single Doses of Vanillin and Ethyl Vanillin as 4% Solutions in Milk, into Rats

Number of Animals	Dose, Gm./Kg.	Per Cent Dead	Time till Death
		Vanillin	
10	1.0	..	....
10	1.4	..	....
10	1.8	20	2 hrs. to 4 days
10	2.2	40	2½ hrs. to 4 days
10	2.6	50	2½ hrs. to 4 days
		Ethyl Vanillin	
10	1.0	..	....
10	1.4	..	....
10	1.8	30	7 to 10 hrs.
10	2.0	50	7 to 24 hrs.
10	2.2	80	8 to 30 hrs.
10	2.6	80	3 to 24 hrs.

A fatal dose of vanillin or ethyl vanillin induced an increased rate of respiration followed by muscular weakness, lachrymation, dyspnea, collapse and death in coma. Convulsions were not observed. Sublethal doses produced these signs to a lesser extent, followed by prompt recovery.

Effect of Repeated Administration and Prolonged Feeding of Vanillin and Ethyl Vanillin to Rabbits and Rats.—Vanillin and ethyl vanillin in the form of 4% solutions in milk or olive oil, and 0.5% solutions in 10% aqueous glycerol, were administered to rabbits repeatedly by subcutaneous injection or by means of a stomach tube. No injury or discomfort resulted so long as the dose did not require the administration of toxic amounts of the solvent. Two rabbits died of acute glycerol poisoning evidenced by restlessness, tremor, convulsions and coma.

The effect of prolonged ingestion was studied as follows: To each of 12 rats approximately 300 mg./Kg. of either vanillin or ethyl vanillin, as 4% solutions in olive oil, were administered twice a week for 14 weeks, by means of a blunt hypodermic needle introduced into the esophagus; for 126 days, 16 rats were fed on a normal diet to which vanillin or ethyl vanillin (4% solutions in milk) had been added, each animal consuming about 20 mg./Kg. of vanillin or ethyl vanillin per day; another group of 16 rats was fed as those in Group 2, with the exception that each animal consumed approximately 64 mg./Kg. of vanillin or ethyl vanillin per day, for 70 days. The rats in the first two groups above showed no signs of illness. Their appearance, behavior and gain in weight were normal. Those in the last group appeared normal and lively, but their rate of increase

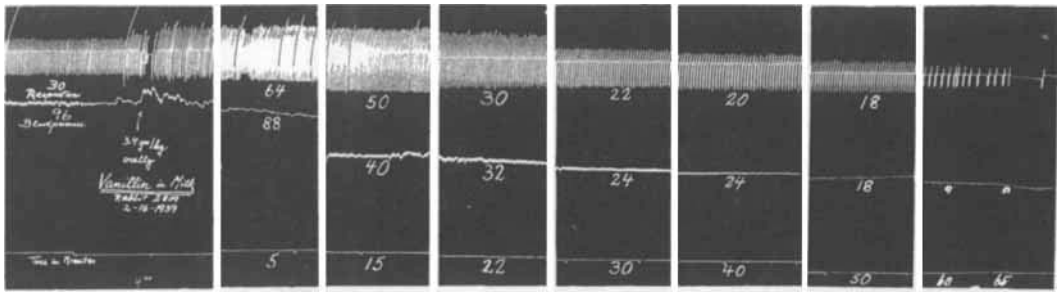

Fig. 1.—The Effect of Vanillin on the Blood Pressure and Respiration of a Rabbit. The Upper Tracing Represents the Respiration, While the Lower Shows the Blood Pressure in Mm. Hg. The Time in Minutes Is Shown on the Bottom Line.

Table III.—Results of Repeated Administrations of Vanillin and Ethyl Vanillin to Rabbits

Rabbit	Number of Doses	Dose, mg./Kg.	Duration of Treatment in Days	Total Amount Administered Gm./Kg.	Solvent Employed	Mode of Application	Fate
				Vanillin			
D- 387	6	153	6	0.9	Olive oil	Subcutaneous	No illness
D- 386	6	165	6	1.0	Olive oil	Subcutaneous	No illness
D- 334	11	83	14	1.16	10% glycerol	Oral	Died
D- 336	51	103	61	6.28	10% glycerol	Oral	Anemia, diarrhea, recovered
D-1299	25	240	56	6.00	Milk	Oral	No illness
D-1297	54	240	126	13.06	Milk	Oral	No illness
D-1298	54	240	126	13.06	Milk	Oral	No illness
				Ethyl Vanillin			
D- 388	6	148	6	0.89	Olive oil	Subcutaneous	No illness
D- 421	6	154	6	0.92	Olive oil	Subcutaneous	No illness
D- 331	13	15	15	0.23	10% glycerol	Oral	Died
D- 377	26	15	31	0.47	10% glycerol	Oral	No illness
D- 332	15	32	17	0.54	10% glycerol	Oral	No illness
D- 378	26	41	31	1.27	10% glycerol	Oral	No illness
D- 333	43	49	49	2.40	10% glycerol	Oral	Anemia, diarrhea, no gain in wt.
D-1302	25	240	56	6.00	Milk	Oral	No illness
D-1300	54	240	126	13.06	Milk	Oral	No illness
D-1301	54	240	126	13.06	Milk	Oral	No illness

Table IV.—Effect of Repeated Oral Administration and Prolonged Feeding of Vanillin and Ethyl Vanillin on the Increase in Weight of Rats

	Vanillin			Ethyl Vanillin		
Time	(6 Rats) 300 mg./Kg. Orally in Olive Oil Twice a Week for 14 Weeks	(8 Rats) 20 mg./Kg. Daily for 126 Days with Diet	(8 Rats) 64 mg./Kg. Daily for 70 Days with Diet	(6 Rats) 300 mg./Kg. Orally in Olive Oil Twice a Week for 14 Weeks	(8 Rats) 20 mg./Kg. Daily for 126 Days with Diet	(8 Rats) 64 mg./Kg. Daily for 70 Days with Diet
Control weight	168	165	129	178	158	133
Wt. after 2 weeks	188	208	141	196	196	155
Wt. after 6 weeks	253	261	166	259	253	167
Wt. after 10 weeks	296	284	173[a]	297	281	190[a]
Wt. after 14 weeks	334	305	182[a]	320	310	202[a]
Wt. after 18 weeks	..	323	193[a]	..	334	212[a]

[a] Animals on normal diet.

in weight was retarded. At the end of the 70-day period 8 of these animals were sacrificed for histopathological study, and the remaining 8 were changed to a normal diet. The rate of weight increase of the latter did not accelerate, and it would appear therefore that they had suffered some systemic damage from the daily dosage of approximately 64 mg./Kg. of one or other of these compounds.

The Effect of Vanillin and Ethyl Vanillin on the Blood Pressure and Respiration.—Rabbits anesthetized by an intraperitoneal injection of 0.2 Gm. per Kg. sodium barbital were employed to obtain tracings of the carotid blood pressure and the respiration. The compounds were administered as a 5% solution in milk (prepared as above) by means of a stomach tube. A lethal dose of vanillin produced a sudden (within the first 10 minutes) drop of blood pressure from an average of 100 to 40 mm. Hg. The rate of respiration usually doubled during the first 10 minutes, then fell just as quickly to the normal level and remained there until shortly before the animal died. The blood pressure continued to fall, slowly but persistently, over a period of from 40 to 110 minutes, finally causing death. Sublethal doses also produced sudden depression of the blood pressure and stimulation of the respiration.

Lethal doses of ethyl vanillin produced essentially the same results as vanillin except that the onset of change in the respiration and blood pressure was much more gradual. A barely sublethal dose had no effect on the respiration over a period of 5 hours, and there was no well-defined effect on the blood pressure.

Gross Pathologic Changes Induced by Vanillin and Ethyl Vanillin.—The pathologic picture in acutely poisoned animals was that of pronounced congestion of the liver and a well-marked dilatation of the abdominal and mesenteric vessels, with little evidence of changes in other tissues, except for the edema at the site of subcutaneous injections and an intense irritation of the gastro-enteric tract in animals given fatal oral doses. The rats given repeated oral doses or fed vanillin and ethyl vanillin with the diet showed no gross evidence of injurious effects.

Microscopic Pathologic Changes.—The histopathologic tissue changes were essentially of the same type in both rabbits and rats regardless of the mode of administration of vanillin and ethyl vanillin. They were those of severe toxemia, involving chiefly the

myocardium, lung, liver and kidney, upon which were superimposed the effects of circulatory failure incident to myocardial damage, and, in many instances, secondary lobular pneumonia.

Lethal and slightly sublethal doses produced cloudy swelling, loss of cross striations and sometimes coagulation necrosis of the fibers of the myocardium, with swelling and pyknosis of the nuclei, edema and slight leucocytic infiltration of interstitial tissue, and edema of the vascular endothelium.

Similar effects, varying only in degree and in accord with the architecture of the organ, were seen in the lung, liver and kidney. Acute passive congestion was the most striking picture in the spleen, central degeneration (often of the fatty type) or necrosis was the most prominent lesion in the liver, while generalized cloudy swelling and degeneration of the tubal epithelium characterized the kidney changes.

Such damage as occurred in the stomach and small intestine resulted in edema and superficial desquamation of the mucosa, with some evidences of congestion and acute exudative reaction in the submucosa.

The animals to which repeated doses of vanillin and ethyl vanillin were administered or fed in dosages above 20 mg. per Kg. of body weight showed definite toxic changes of the foregoing type in their tissues. The rats given daily doses of 20 mg./Kg. for $4^1/_2$ months failed to develop pathologic processes of greater severity or frequency than those commonly found in control animals as the result of spontaneous disease or other unrecognized environmental influences.

Discussion.—The daily ingestion of 20 mg./Kg. of vanillin and ethyl vanillin by rats over a period of 126 days was found to be harmless. Higher doses produced histopathological changes of varying degrees of severity in the myocardium, kidney, liver, lungs, spleen and stomach of rats and rabbits.

It is evident that these compounds are capable of causing fatal poisoning but their relative insolubility in cold water or in cold milk and the bitter taste of concentrated extracts make the possibility of such an event very remote. Concentrations up to 0.5 Gm. of vanillin or 0.15 Gm. of ethyl vanillin per quart are added to foods, and in view of current dietary usage or even extreme individual predilections for flavored foods, injurious effects from prolonged consumption of these compounds are not to be anticipated.

Most of the vanillin is oxidized in the animal body to vanillic acid, which is excreted with the urine as conjugated sulfate. Traces of vanillin and vanillic acid are excreted as such (Preusse (1)).

CONCLUSION

When ingested in high concentration vanillin and ethyl vanillin induce an acute toxemia with death by circulatory failure. Experimental observations on rabbits and rats indicate that the quantities which are employed in foods as flavoring materials are harmless.

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A Study of Red Squill*

By Guilford C. Gross†

The use of red squill as a rat poison has become very popular in recent years. A great share of this popularity is due, no doubt, to the fact that while red squill is extremely toxic to rodents, it is relatively non-toxic to other wild and domestic animals and human beings. The other rodent poisons in general use to-day (arsenic, barium carbonate, phosphorus and strychnine) have the disadvantage of being toxic to all forms of animal life.

The development of red squill as a specific rat poison has been greatly retarded until recent years because of the difficulty which was experienced in obtaining a uniformly toxic preparation. However, recently there has been considerable investigation conducted with reference to the toxic nature of the rat-killing principle in red squill and as a result this difficulty has been largely overcome.

* Kilmer Paper, 1939.
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