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SINGLE DOSE TOXICITY STUDY OF β-MYRCENE, A NATURAL ANALGESIC SUBSTANCE

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The present study was undertaken to provide data on acute toxicity of β-myrcene, a peripheral analgesic substance found in the essential oils of several plants. Although myrcene has long been used in perfumes and as a food additive, there is almost no information on its toxicological hazards. The acute oral toxicity of myrcene was low in rodents, with approximate lethal doses (ALD) of 5.06 g/kg body weight for mice and greater than 11.39 g/kg body weight for rats. Necropsy data did not reveal any relevant alteration in rats but histopathology findings in mice suggested that the liver and stomach may be target organs for myrcene toxicity after oral administration. Myrcene is highly irritant to the peritoneum, and deaths after intraperitoneal injection of this monoterpene in rats (ALD 5.06 g/kg body weight) and in mice (ALD 2.25 g/kg body weight) were probably due to drug-induced chemical peritonitis.

Key words: myrcene, monoterpenes, essential oils, peripheral analgesics, acute toxicity, food additives.

β-Myrcene (7-methyl-3-methylene-1,6-octadiene) is a monoterpene found in the essential oils of a large variety of plants (e.g. lemon grass, hop, verbena, bay laurel, and others) and has long been used in cosmetic fragrances and in food industries as a flavoring substance. Although many people are exposed to some extent to myrcene through edible plants, perfumes and food additives, information on its potential health hazards is scarce (1).

Recently, Lorenzetti et al. (2) reported that lemon grass (*Cymbopogon citratus*, Stapf) tea, a potion widely used in Brazilian folk medicine, induces dipyrone-like analgesia in rodents. In a subsequent study (3), β-myrcene proved to be the active substance of the lemon grass tea and a very potent peripheral analgesic drug. If myrcene is non-toxic or presents acceptable toxicity, it could be an alternative to the already available analgesic drugs (most of them with problems in terms of toxicity) and the first member of a new family of such drugs.

The present study was designed to provide data on the acute toxicity of myrcene and is the initial stage of a more comprehensive pre-clinical evaluation of this monoterpene.

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Albino Swiss mice of either sex (females, 20 ± 7 g and males, 24 ± 7 g) and Wistar rats (females, 186 ± 18 g and males, 236 ± 22 g) from our own breeding stock were used. The animals, aged about 3 months, were housed individually in plastic cages and kept in air-conditioned (21-23°C) and artificially lighted (12-h light and 12-h dark) rooms. All animals were fasted overnight (*ca.* 12 h) before and approximately 3 h after receiving a single dose of myrcene (Sigma, 90% pure) in corn oil. For all doses except the highest (higher than 5.06 g/kg for rats and 11.39 g/kg for mice), when undiluted myrcene was administered in volumes adjusted according to the dose, a constant volume of myrcene solution in oil (5 ml/kg for rats and 10 ml/kg for mice) was given either by the oral (intragastric tube) or intraperitoneal *(ip)* route. Doses were increased progressively so that each was 50% higher than the preceding one, as suggested by Deichmann and LeBlanc (4) for the determination of the approximate lethal dose (ALD). Animals were observed for 14 days after myrcene administration. All signs of toxicity and deaths and their time of occurrence were recorded. All animals that died during the observation period, and all surviving animals (sacrificed by ethyl ether inhalation and cervical dislocation) were subjected to necropsy and any organ showing macroscopic changes other than agonic alterations were fixed in buffered 10% formalin solution and stained with hematoxylin-eosin for histopathological examination.

As can be inferred from data summarized in Table 1, the acute

Table 1 - *Acute oral toxicity of* β*-myrcene in rats and in mice.*

β-myrcene was administered by gavage to male (M) and female (F) rodents. Latencies to death are indicated as follows: within 24 h (a), between 24 and 48 h (b), between 48 and 72 h (c) and between 3 and 6 days (d). The main symptoms observed were: hypoactivity (Ho), piloerection (Pi), palpebral ptosis (Pt) and ataxia (At). D/T, Number of deaths/number of treated animals; bw, body weight.

Dose (g/kg bw)	Sex	Rats		Mice		
		D/T	Symptoms	D/T	Latency	Symptoms
0.00	M	0/1		0/1		
	F	0/1		0/1		
0.67	M	–		–		
	F	–		–		
1.00	M	0/1	Pi	0/1		
	F	0/1		0/1		
1.50	M	0/1	Pi	0/1		
	F	0/1		0/1		
2.25	M	0/1	Pi	0/1		
	F	0/1		0/1		
3.38	M	0/1	Pi	0/1		
	F	0/1		0/1		
5.06	M	0/2	Pi	2/3	aa	Pt Ho At
	F	0/2		3/3	aab	
7.59	M	0/2	Pi	3/3	aab	Pt Ho At
	F	0/2		2/3	ab	
11.39	M	0/2	Pi	1/1	b	Pt Ho At
	F	0/2		1/1	b	

oral toxicity of myrcene seems to be very low in rodents. Deaths or relevant signs of toxicity were not observed in rats even with oral doses as high as 11.39 g/kg body weight. Necropsy of these rats 14 days after myrcene administration did not reveal any salient findings. Mice were apparently more susceptible than rats to the toxic effects of myrcene. No deaths occurred with oral doses up to 3.38 g/kg, but 12 out of 14 mice which received 5.06 g/kg or higher doses died within 48 h of treatment. Most of these mice showed vacuolization of hepatic cells and histological evidence (Sudan III method) of an accumulation of lipids. One of them (treated with 11.39 g/kg body weight) presented a clear-cut picture of fatty liver with a widespread massive vacuolization of hepatocytes. In two of them (5.06 and 7.59 g/kg body weight), light microscopy examination permitted the detection of hepatocyte necrosis predominantly, but not solely, in the centrilobular region. In spite of the very large doses administered, the pattern of hepatocellular necrosis produced by myrcene was not so severe and extensive as that induced by acetaminophen (5) and can be regarded as a contributory cause rather than the only cause of death. Histopathological examination of surviving mice did not show any evidence of chemically induced liver injury, but hyperkeratosis in the non-glandular part of the stomach was observed in most animals treated with oral doses higher than 1.0 g/kg body weight. This suggests a possible gastric irritant effect of myrcene.

Administration of large volumes of undiluted myrcene (5.06 g/kg body weight or higher doses) into the peritoneal cavity of rats induced deaths (Table 2) with latencies of 24 h to 6 days after administration. Necropsy of these animals revealed extensive macroscopic lesions on the mesentery and on the surface of organs such as liver, kidneys, stomach, spleen and intestines exposed to myrcene. Surviving rats which had been treated with high doses of myrcene (higher than 1.00 g/kg body weight), also presented extensive adherence of diaphragm to the liver and between peritoneal cavity organs as well as granulation tissue on the outer surface of liver and kidneys. The symptomatology presented by animals treated with doses higher than 2.25 g/kg body weight by the *ip* route included: piloerection, palpebral ptosis, rapid breathing, hypertonus of abdominal wall muscles, nasal bleeding, hypoactivity, ataxia and eventually loss of righting reflex preceding death. Symptoms and necropsy data suggest that highly concentrated solutions of myrcene are quite irritant to the peritoneal membrane and may induce severe chemical peritonitis. Mice were apparently more susceptible than rats to the irritant effects of myrcene on the visceral peritoneum since signs of toxicity and deaths appeared after lower doses and shorter latencies (Table 2). Necropsy data for mice which received myrcene by the *ip* route were similar to those for rats, except that the mice did not show any adherence of peritoneal organs. Writhing reactions occurred in mice after *ip* injections of 5.06 g/kg and 7.59 g/kg body weight of myrcene but necropsy data suggesting peritoneal membrane irritation were observed with much lower doses (starting with 1.00 g/kg body weight), a discrepancy probably due to the peripheral analgesic effect of the substance.

In conclusion, acute oral toxicity of β-myrcene is low in rats (ALD higher than 11.39 g/kg body weight) and mice (ALD 5.06 g/kg body weight). Since the ED_{50} for the analgesic effect of myrcene was reported (3) to be around 16 mg/kg body weight for rats (modified Randall-Sellito method) and 294 mg/kg body weight for mice (writhing test), the

Table 2 - *Acute parenteral toxicity of β-myrcene in rats and in mice.*

β-Myrcene was administered intraperitoneally to male (M) and female (F) rodents. Latencies to death are indicated as follows: within 24 h (a), between 24 and 48 h (b), between 48 and 72 h (c) and between 3 and 6 days (d). The main symptoms observed were: hypoactivity (Ho), piloerection (Pi), palpebral ptosis (Pt), ataxia (At), writhing responses (Wr), hypertonus of abdominal wall muscles (Hm), nasal bleeding (Nb) and loss of righting reflex (Lr). D/T, Number of deaths/number of treated animals; bw, body weight.

Dose (g/kg bw)	Sex	Rats			Mice		
		D/T	Latency	Symptoms	D/T	Latency	Symptoms
0.00	M	0/1			0/1		
	F	0/1			0/1		
0.67	M	0/1			–		
	F	0/1			–		
1.00	M	0/1		Pi	0/1		Ho
	F	0/1			0/1		
1.50	M	0/1		Pi	0/1		Ho Pt
	F	0/1			0/1		
2.25	M	0/1		Pi Ho	2/3	ad	Ho Pt
	F	0/1			1/3	a	
3.38	M	0/1		Pi Ho Pt	1/2	a	Ho Pt At
	F	0/1			1/2	a	
5.06	M	1/2	c	Pi Ho Pt	3/3	aaa	Ho Pt Wr At
	F	2/2	bd		3/3	aaa	
7.59	M	1/1	b	Pi Ho Pt Nb At	2/2	aa	Ho Pt Wr At Lr
	F	1/1	c		2/2	aa	
11.39	M	2/2	ab	Pi Ho Pt Nb At Hm	–		
	F	1/1	b		–		

safety margin for a single oral dose seems to be wide. Histopathology findings in mice suggest that liver and stomach may be target organs for myrcene toxicity. Very concentrated myrcene solutions were irritant to the peritoneum, and deaths after *ip* injection of this substance into rats (ALD 5.06 g/kg body weight), and into mice (ALD 2.25 g/kg body weight) were probably due to drug-induced chemical peritonitis.

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