

ii) 90- Day Oral Toxicity study in Rats (1973):

Test substance: Thiodiethylene bis (3,5-di-tert-butyl-4-hydroxyhydro cinnamate)
CAS No. 41484-35-9 Assumed Purity >98%

Method: In this study, 120 charles river strain albino rats (60 males and 60 females) were housed individually in standard, wire-bottomed steel cages. The dietary levels of the test substance are 0, 10000, 20000, and 30000 ppm. The feed and water was available ad libitum. Each animal was weighed on the first day of the test and weekly once. Food consumption data were collected individually from 5 rats of each sex in each group weekly during the study. Abnormal reactions and/or deaths were recorded daily during the study. Blood and urine samples were analyzed after 45 and 84 days of the 90-day feeding study. Pathological studies were conducted. In addition, the weights of the brain, gonads, heart, kidneys, liver, and spleen of each rat were recorded. Microscopic examination was conducted on tissues taken from 10 rats of each sex from the control group and the highest exposure group. Tissues studied included: urinary bladder, bone marrow, brain, colon, gonads, heart, kidneys, liver, lungs, aorta, cecum, esophagus, eye, optic nerve, peripheral nerve, pituitary, salivary glands, seminal vesicles, small intestine (duodenum, ileum, and jejunum), spinal cord, trachea, thymus, uterus, lymph node (cervical and mesentric), skeletal muscle, pancreas, parathyroid, prostate, spleen, stomach (cardia, fundus, and pylorus), and thyroid.¹

Species/strain: Charles River strain Albino rats

No. of animals per group: 15 males and 15 females/ group; total 120 rats

Route of administration: Dietary

Exposure period: 90 days

Dose: 0, 10000, 20000, and 30000 ppm in food

GLP: No

Year: 1973

Results:

The general behaviour of the animals in group HV was comparable to the control group. Body weight gains and health remained normal in control and test group animals.

No changes were attributed to the test material in any of the following parameters: body weight (growth), food consumption, food utilization, mortality, behavioural reactions, hematologic studies, clinical blood chemistry studies, and urine analysis.

No outstanding differences were noted between test and control rats upon gross pathological examination.

Organ weight and Organ to Body weight and Organ to Brain weight ratio data:

Statistically significant increases in liver weights and ratios were noted for the 20,000 and 30,000 ppm group. The mean liver weights of the 30,000 ppm females and of the 20,000 ppm males and females are within the normal range for the rats of this age and strain. The mean liver weight of the 30,000 ppm males is only slightly higher than the normal range but the difference was significant. The absence of any deleterious histopathologic changes and of clinical blood chemistry effects also suggests that the liver weight increases may not be related to the ingestion of the test substance. The number of other statistically significant inter-group differences noted were considered to be normal for a random population of albino rats of this age and strain. The NOEL < 10,000 ppm based on liver enlargement and > 30,000 ppm for other parameters.

Organ Weight and Ratio Data
Summary of Mean values

Organ - Liver

Dietary Levels (PPM)	Organ Weight (g)		Organ/Body Weight Ratio (g / 100 g)		Organ/Body Weight Ratio (g / 100 g)	
	Males	Females	Males	Females	Males	Females
0	13.602	7.519	2.6259	2.6327	6.2979	3.8442
10000	16.316**	9.108**	3.1536**	3.2258**	7.5019**	4.4954**
20000	17.968**	9.493**	3.3669**	3.3276**	8.3671**	4.7084**
30000	15.046	7.535	2.9368**	2.7583	6.9037	3.8321

* Statistically significant difference at the 95% confidence level.

** Statistically significant difference at the 99% confidence level.

No outstanding differences were noted between test and control rats upon gross pathological examination. Microscopic evaluation showed the lesions which are described as those of spontaneous diseases, and they are present in most instances among both the control and test animals.

Remarks:

This study was assigned a reliability code of 2c² (comparable to guideline study with acceptable restrictions)

Reference:

¹90-day sub-acute oral toxicity study in albino rats, Final Report, 19 December 1973. IBT No. 622-03561, Industrial BIO-TEST laboratories, Inc., Illinois

²Klimisch, H.J., Andreae, M and Tillman, U. A systemic approach for evaluating the quality of experimental toxicological and ecotoxicological data. *Regulatory Toxicology and Pharmacology*. 25:1-5, 1997.