This is provisional English translation of an excerpt from the original full report.

Risk Assessment Report

Dibutylhydroxytoluene

(Feed Additives)

Food Safety Commission of Japan (FSCJ)
September 2020

ABSTRACT

FSCJ conducted a risk assessment of dibutylhydroxytoluene (CAS No. 128-37-0), an antioxydant, using documents such as assessment reports from JECFA and EFSA.

Genotoxicity of the assessed item has been shown to be almost negative by *in vitro* and *in vivo* genotoxicity studies. Although some positive results were observed in some of *in vitro* and *in vivo* toxicity studies, these results are presumably due to indirect effects from reactive oxygen species derived from oxidative metabolite or quinone compound produced through in vivo metabolism of dibutylhydroxytoluene (BHT). Therefore, FSCJ judged that BHT has a threshold for genotoxicity, and that BHT has no genotoxicity relevant for human health. Hence, FSCJ considered it possible to specify the ADI.

In subacute toxicity study and chronic toxicity study, adverse effects were mainly observed in the blood coagulation system, and in the liver and thyroid. The effects on the blood coagulation system are due to inhibition of vitamin K-dependent blood coagulation factors by vitamin K antagonistic action of BHT, and bleeding tendency was observed in short term studies in mice and rats where the NOAEL was determined to be 125 mg/kg bw/day. On the other hand, adverse effects on the blood coagulation system was not observed in long term studies. Adverse effects on the liver and kidney accompanied by biochemical and histological changes were observed mainly in rats.

Regarding carcinogenicity, increased incidence of lung tumors or liver tumors was observed by some of carcinogenicity studies in mice, and that of liver tumors was observed in a reproductive toxicity study in rats and in chronic toxicity/carcinogenicity study in rats of F_1 generation. Although the mechanism for tumor induction is unknown, BHT has no genotoxicity relevant for human health as was described. Therefore, FSCJ considered that tumor induction by BHT is attributable to nongenotoxic mechanisms having the threshold of dose.
In a reproductive developmental toxicity study, decrease in the number of fetuses per litter and suppressed body weight were observed in offspring and infants. Developmental toxicity studies in rats and rabbits showed no teratogenicity.
The lowest NOAEL of all the tests referred was 25 mg/kg bw/day observed in reproductive toxicity study in rats and in chronic toxicity/carcinogenicity study in F1 generation of rats. FSCJ specified an ADI of 0.25 mg/kg bw/day for BHT based on the NOAEL of 25 mg/kg bw/day, applying a safety factor of 100.

Table 1. *Levels relevant to toxicological evaluation of Dibutylhydroxytoluene*

Species	Study	Dose (mg/kg bw/day)	NOAEL (mg/kg bw/day)
			FSCJ
Mouse	30-day subacute toxicity study	0, 1 570, 1 980, 2 630, 3 370, 4 980, 5 470	1 570 (LOAEL) Decreases in thromboplastin index and kaolin APTT, Renal tubular lesions in the kidney
	96-week carcinogenicity study	0, 30, 120, 600[1]	120 (M) Suppressed body weight, Increased AST 30 (F) Suppressed body weight
	108-week carcinogenicity study	0, 450, 900[2]	450 (M) (LOAEL) Increased findings of liver injury, Cannot be judged (F)
	Three-generation reproductive toxicity study	0, 22.5, 67.5, 202.5, 607.5[1]	202.5 Suppressed body weight (F_1 Offspring)
	Developmental toxicity study (7-day dietary administration)	0, 70, 240, 800	240 Increased organ weight of the spleen and decreased organ weight of the kidney No teratogenicity
Rat	76-week chronic toxicity/carcinogenicity study	0, 7.5, 23, 75, 225, 450[2]	75 Decreased body weight No carcinogenicity
	24-month chronic toxicity study	0/0, 2.14/2.49, 9.61/10.26, 144.9/170.9 (M/F)	144.9/170.9 (M/F) No carcinogenicity
	108-week chronic toxicity/carcinogenicity study	0, 150, 600[1]	150 (LOAEL) (M) Decreased TG, increased γ-GTP (F) Increased T Chol, increased organ weight of the liver, decreased organ weight of the spleen No carcinogenicity
	105-week carcinogenicity study	0, 225, 450[2]	225 Increased incidence of alveolar cell histiocytosis No carcinogenicity

	Reproductive toxicity study and F_1 generation used Chronic toxicity/carcinogenicity study (the 1st study)	F_0: 0, 25, 100, 500 F_1: 0, 25, 100, 250	Dams, infants, F_1: 25 Suppressed body weight
	Reproductive toxicity study and F_1 generation used Chronic toxicity/carcinogenicity study (the 2nd study)	②F_0: 0, 25, 100, 500 F_1: 0,25, 100, 250	Suppressed body weight, decreased organ weight of the liver, disappearance of hepatic glycogen, increased fat droplet in the adrenal zona fasciculata 25 Increased expression of γ-GTP in the periportal hepatocytes, hyperthyroidism imaging
	Study of effects on the thyroid (for 90-day)	0, 25, 250	25 Increased number of follicular cells in the thyroid
Toxicological ADI (mg/kg bw/day)			0.25
The critical study for setting toxicological ADI			Reproductive toxicity study and F_1 generation used Chronic toxicity/carcinogenicity study (the 1st)・(the 2nd), Study of effects on the thyroid (rat) NOAEL: 25 mg/kg bw/day SF: 100
ADI (mg/kg bw/day)			0.25

[1] Calculated by conversion formula of JECFA
[2] Converted by OECD SIDS (Reference 24)