Comparison between BHT and Ethoxyquin.

REGULATORY STATUS:

Substance	GRAS (FDA, USA)	Food Additive (FDA status)	Feed additive	ADI	Organism which allocated the ADI
BHT	Yes (CFR 182.3173)	Yes (CFR 172.115)	Yes	0 - 0.25 mg/kg/day	EFSA
Ethoxyquin	No	(CFR 172.140)** With restrictions	Yes ^ with restrictions	0 - 0.005 mg/kg/day. (As pesticide)	JMPR
				0 - 0.0083 mg/kg/day	FSCJ

GRAS: Generally recognized as safe
FDA: Food and drug administration (USA)
ADI: Acceptable daily intake
EFSA: European Food Safety Authority
JMPR Joint Food and Agriculture Organization/World Health Organization Meeting on Pesticide Residues.
FSCJ: Food Safety Commission of Japan

** Permitted as an antioxidant for preservation of color in the production of **chili powder, paprika, and ground chili** at levels not in excess of 100 parts per million.

^ In order to provide for the safe use of the additive in feed prepared in accordance with CFR 573.380 and 573.400, tolerances are established for residues of ethoxyquin in or on edible products of animals as follows:
5 parts per million in or on the uncooked fat of meat from animals except poultry.
3 parts per million in or on the uncooked liver and fat of poultry.
0.5 part per million in or on the uncooked muscle meat of animals.
0.5 part per million in poultry eggs.
Zero in milk.

Toxicological comparative:

ETHOXYQUIN

Ethoxyquin is a common name for: IUPAC name: 1,2-dihydro-2,2,4-trimethylquinolin-6-yl ethyl ether , EC number: 202-075-7, CAS number 91-53-2. Ethoxyquin belong to the group of quinolone compounds and it is used primarily as antioxidant preservative in animal feed, an antiscald agent in pears and apples may also have a fungicidal effect and it is also used as a colour preservative in some spices. The regular composition contains: ≥ 91 % ethoxyquin, ≤ 8 % ethoxyquin polymers and ≤ 3 % *p*-phenetidine

It can be considered that Ethoxyquin it selfs has little acute toxicity based on different unpublished reports which results are summarised in the 1998 Ethoxyquin report by JMPR, all of them giving an oral LD_{50} of 1700 mg/kg in rats **The problem comes with *p*-Phenetidine as a recognised possible mutagen.**

Moreover, the studies showed that dogs were more sensitive to toxic effects of Ethoxyquin than rats.
In a chromosome aberration assay performed by Blaszczyk et al. (2003) with human cultured lymphocytes Ethoxyquin induced different kinds of chromosome aberrations at the tested concentrations (0.1 to 0.5 mM) with and without metabolic activation. This result is supported by an additional chromosomal aberration test from an unpublished report referenced in the Ethoxyquin addendum (JMPR, 2005) where positive results at concentrations ranging from 15 to 30µg/mL in CHO cells with or without metabolic activation were recorded. In addition an Ames test (strains: TA98, TA 100, TA 1535and TA1537 and concentrations 10 – 5000 µg/plate) and an in vivo micronucleus formation (375-1500mg/kg) in mice gave negative results.
In a cytotoxicity test (MTT) Ethoxyquin and 2 of its salts were evaluated in human cultured lymphocytes and Ethoxyquin resulted to be more cytotoxic than its salts with an IC_{50} of 0.09mM. This cytotoxicity study included a TUNEL assay in order to obtain information about the ability of the test substance to induce apoptosis and it was seen that Ethoxyquin can induce apoptosis at 0.25 and 0.50 mM., Blaszczyk et al. 2005.
Regarding repeated dose studies, diverse studies, 2 of them performed with Ethoxyquin at 0.5 % in diet up to 18 months, showed the potential of Ethoxyquin to cause renal damage, and even exert a carcinogenic effect.
Anyway, in the Ethoxyquin evaluation of JMPR for acceptable daily intake of 1998 and its posterior addendum (2005), diverse unpublished sub-chronic and chronic studies with rats and dogs are cited. A multigenerational study with dogs is selected to be used in order to determine the ADI because the result was considered to be the minimal effect level among all (including rat studies).

In 1998 the JMPR established an ADI (acceptable daily intake) of 0 to 0.005 mg/kg body weight for Ethoxyquin, based on an unpublished multigenerational study with dogs (which gave a minimal-effect level of 2.5 mg/kg) and applying a safety factor of 500. In the posterior Ethoxyquin addendum (2005), JMPR confirmed this ADI.
The FSCJ (Food Safety Commission of Japan) conducted a risk assessment (2013) of Ethoxyquin

based on JMPR assessment and others derived an ADI of 0.0083 mg/kg bodyweight based on the same multigenerational study with dogs but applying a safety factor of 300.

Summary of available toxicity studies for **Ethoxyquin:**

Study	Test object	Concentration tested/time	Comments	Result	Reference
Chromosome aberrations	Human lymphocytes	0.01 to 0.5 mM.	Stability studies by HPLC showed EQ stable under conditions.	Ethoxyquin induces chromosome aberrations: gaps and breaks as well as dicentrics and atypical translocation chromosomes. (with or without metabolic activation)	Blaszczyk et al. 2003Mutat Res. 2003 Dec 9;542(1-2):117-28
Apoptosis and Citotoxicity (MTT assay)	Human lymphocytes	72-h incubation	2 salts were also tested; Ethoxyquin was the most cytotoxic compound.	MTT: IC_{50}= 0.09mM TUNEL method = ability to induce apoptosis at 0.25 and 0.50 mM	Blaszczyk et al. 2005. Cell Mol Biol Lett. 2005;10(1):15-21.
	Fischer 344 rats	0.5 % in diet.	Ethoxyquin prevented preneoplasic liver lesions by aflatoxin B1.	Ethoxyquin alone caused severe damage to kidney, may be exerting carcinogenic effect.	Manson et al. 1987 Carcinogenesis. 1987 May;8(5):723-8.
	Fischer 344 rats Male/female 3weeks old	0.5 % in diet	4 weeks up to 18 months	Primary lesion: renal papillary necrosis in male rats. No evidence Ethoxyquin directly induced preneoplasic renal tubule hyperplasia.	Toxicological Sciences. Vol 18, issue 2, 278-287

Carcinogenicity				Ethoxyquin enhanced kidney and urinary bladder carcinogenesis, but inhibited liver carcinogenesis	Ito N et al. 1985Crit Rev Toxicol. 1985;15(2):109-50.

BHT

Butylhydroxytoluene, EC number 204-881-4, CAS number 128-37-0 and IUPAC name: 2, 6-di-tert-butyl-4-methylphenol is a chemical substance commonly used as antioxidant, approved for food, feed and food contact. Related to BHT we can find much more studies and toxicological information than Ethoxyquin.

BHT has GRAS (generally recognized as safe) status according to the FDA (Food and Drug Administration, USA) and it is allowed to direct addition to food for human consumption (CFR 172.115) under different limitations.
Diverse studies have shown the low acute toxicity of BHT. (Yamamoto et al. , 1980: DL_{50} =3550 mg/kg , mice; Sax NI, 1984: oral DL_{50}=890 mg/kg, rats; Miyakawa et al., 1986: dermal DL5> 2000 mg/kg, mice)

Regarding the genotoxic potential of BHT, the table 1 summarises different in vitro and in vivo studies.

Table 1

Endpoint	Test objetct	Concentration/dose	Results	Reference
In vitro				
Reverse mutation	*S. thyphimurium* TA102 TA 2638 *E. Coli* WP2/pkM101 and WP2 uvrA/pKM101	313-5000 µg per plate in DMSO	**Negative** with metabolic activation	Mutation Research 416: 169-181
Reverse mutation	*S. thyphimurium* TA98, TA100, TA1535, TA1537	100 – 10000 µg per plate in DMSO	**Negative**[a]	Williams GM et al.[b]
Chromosomal aberration	CHO	1.6 – 16 µg/mL in DMSO ± S9	**Negative** without metabolic activation. Slight increase and	National Toxicology Program

			positive trend but the authors considered the overall results as negative.	(USA) 549686 (1992)
In vivo				
Micronucleus formation	Female mice (C57BL/6 x C3H/He) Bone-marrow cells	125 – 1000 mg/kg bw by intraperitoneal injection, daily for 5 consecutive days	**Negative**	Bruce WR and Heddle JA 1979.
Chromosome aberration/ dominant lethal assay	Male rats	50-500mg/kg bw/day, 10 weeks in diet	**Negative** according to guideline OECD 1984 criteria	Environmental Mutagenesis 8:357-367

[a] with and without metabolic activation

[b] Compliant with GLP.

As it can be seen all in vitro and in vivo assays are negative, therefore, using a weight of evidence method it can be conclude that BHT lacks genotoxic potential.
This conclusion is supported by the Scientific Opinion on the re-evaluation of BHT (E321) as a food additive. (EFSA Journal 2012; 10(3): 2588).

From the study conducted by Olsen et al. (1986) the EFSA (European Food Safety Authority) derived an **ADI (acceptable daily intake) for BHT of 0.25 mg/ kg bw/ day** applying a uncertainty factor of 100. (EFSA Journal 2012; 10(3):2588

Summarizing:

Concerning genotoxicity, the exposure to Ethoxyquin in 2 different in vitro tests (chromosome aberration) from CHO cells and human lymphocytes resulted in significant increases of frequency of chromosomal aberrations, despite being in vitro tests, it is important to notice that both test used mammalian cells. In addition an Ames test and an in vivo micronucleus formation gave negative results, which make the relevance of the chromosomal aberration tests questionable. In addition Ethoxyquin showed cytotoxic potential and the ability to induce apoptosis in human cultured lymphocytes.
In contrast BHT did not show genotoxic potential in the in vitro and in vivo studies.
There are numerous published studies concerning repeated-dose toxicity of BHT but there is limited information on repeated-dose toxicity of Ethoxyquin.
Taking as reference the ADIs it is concluded that BHT is less toxic than Ethoxyquin. It can be noticed that the safety margin used in order to derive ADI for BHT is 100 (EFSA),

FINAL CONCLUSION:

Based on the available information on the public domain , it can be concluded that BHT it is approved for food , feed , food contact by EFSA(EU) , GRAS(USA) , and Ethoxyquin it is just approved for FEED with restrictions and not allowed to direct addition to food for human consumption Ethoxyquin has greater genotoxic potential than BHT and based on actual ADIs BHT is less toxic than Ethoxyquin.