Ethoxyquin

Ethoxyquin is an antioxidant that may cause reproductive disorders, dermatological problems, and immune-mediated diseases.

From: Handbook of Small Animal Practice (Fifth Edition), 2008

Related terms:

Butylated Hydroxytoluene, Iodine, Vitamin E, Metabolite, DNA Adduct, Granule Cell, Bladder, Fischer 344 Rat, Chicken

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Assessment of Nongenotoxic Mechanisms in Carcinogenicity Test of Chemicals; Quinone, Quinone Imine, and Quinone Methide as Examples

Yasushi Yamazoe, Kunitoshi Mitsumori, in Thresholds of Genotoxic Carcinogens, 2016

Genotoxicity

Ethoxyquin is not mutagenic to Salmonella TA100 and TA98 [85]. Ethoxyquin induced DNA damage in human lymphocytes in a dose-dependent manner; the observed DNA fragmentation induced by ethoxyquin in the presence of the metabolic activation system was always significantly lower, as compared to cells treated with the same doses of ethoxyquin alone [86]. Ethoxyquin-induced DNA damage is caused by the free radical generated [87]. In vivo, ethoxyquin gave a weak positive response in the liver micronucleus test in juvenile rats, but negative responses in the mouse bone marrow micronucleus test and in the UDS test using rat liver [88]. Although ethoxyquin and/or its metabolite(s) induce chromosomal aberration, the influence on the chromosomal aberration is likely to be associated with ethoxyquin's action on the functional protein component rather than the direct DNA damage.

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Common Nutrition Myths and Feeding Practices

Linda P. Case MS, ... Melody Foess Raasch DVM, in Canine and Feline Nutrition (Third Edition), 2011

ETHOXYQUIN CAUSES HEALTH PROBLEMS IN DOGS AND CATS

Ethoxyquin is a synthetic antioxidant that is included in some animal and human foods as a preservative to protect fats and fat-soluble vitamins from oxidative degradation (see Section 3, pp. 155-158 for a complete discussion of antioxidant preservatives). Without the inclusion of antioxidants in pet foods, oxidative processes lead to rancidity of the food. Rancid fat is offensive in odor and flavor and includes compounds that are toxic when consumed. The inclusion of antioxidants in commercial pet foods ensures the food's safety, nutritional integrity, and flavor. Starting in the late 1980s ethoxyquin was identified by dog breeders and owners as a potentially dangerous synthetic preservative. Depending on the source of the information, ethoxyquin was believed to be responsible for reproductive problems, autoimmune disorders, behavior problems, and various types of cancers in dogs and cats.

Prior to approval by the Food and Drug Administration (FDA), ethoxyquin's safety in foods was studied in a variety of species, including rabbits, rats, poultry, and dogs. The original studies on which the FDA based approval for the inclusion of ethoxyquin in animal feeds included a 1-year chronic toxicity study in dogs. Data from this and other studies were used when ethoxyquin was first marketed to determine a "safe tolerance level" of 150 parts per million (150 mg/kg) of food.[57] Subsequent studies failed to show any adverse health or reproductive effects of ethoxyquin when it was fed to several generations of dogs and at levels of up to 360 mg/kg of the diet (the highest concentration that was tested).[58] However, data from another study showed that feeding high levels of ethoxyquin may result in pigment accumulation in the liver and an increase in serum levels of certain liver enzymes.[59] A series of in vitro studies also reported that ethoxyquin had both cytotoxic and genotoxic effects upon cultured human lymphocytes and that these effects were dose dependent.[60,61] Because there is also evidence that direct exposure to high levels of ethoxyquin cause health problems in human workers, a search for new types of antioxidants or improved forms of ethoxyquin has been undertaken in recent years.[62-64]

In response to the new data, pet food manufacturers have voluntarily limited ethoxyquin concentrations in pet foods to 75 parts per million or less in foods that use this preservative. No harmful effects of ethoxyquin have been demonstrated at these levels. Most manufacturers have also developed foods that contain no ethoxyquin. In addition, numerous foods are now available that are preserved entirely or primarily using naturally derived antioxidants (see Section 3, pp. 156-157). Consumers should always read labels to ensure that the product that they select includes ingredients that they feel confident about feeding to their dog or cat. As with other additives and ingredients, ethoxyquin must be included in the list of ingredients and should be identified as a preservative.

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ANTIOXIDANTS | Synthetic Antioxidants, Characterization and Analysis

G. Ramis-Ramos, in Encyclopedia of Food Sciences and Nutrition (Second Edition), 2003

Ethoxyquin

EQ can be determined by extracting the samples with hexane, followed by HPLC with spectrophotometric (absorption bands at 230 and 365 nm), fluorimetric (emission at 435 nm with excitation at 365 nm), or electrochemical (oxidation wave at + 0.45 V) detection, or by GC with a FID or with thermionic detection, or using TLC on fluorescent plates. In a comparative study, significant differences among the RDSs were not found for EQ in fish meals using different techniques, which was attributed to the large variability in the extraction process.

EQ is decomposed by light, which should be taken into account in storing samples. It has been found that EQ is stable for at least 9 days by keeping the apple extracts in deep freeze, and for at least 20 days by storing the pieces of apple in hexane in the dark. Further, its degradation products are found in significant concentrations in the food samples. EQ and up to 12 of its degradation products have been resolved by HPLC. Also, EQ and its two major oxidation products, 1,8'-di-1,2-dihydro-6-ethoxy-2,2,4-trimethylquinoline (DM) and 2,6-dihydro-2,2,4-trimethyl-6-quinolone (QI), have been determined in fish meals by HPLC (DL = 5 mg kg^{-1} for EQ and DM, and 0.5 mg kg^{-1} for QI) and gas chromatography (GC), and the changes in the EQ, DM, and QI concentrations during storage have been studied.

HPLC procedures for EQ in meat meals and extruded pet foods (with fluorimetric detection), apples, skins of apples, pears, citrus fruit, and bananas, chilli powder, and paprika have been developed. For paprika, and using spectrophotometric detection, the DL has been found to be 2 μg ml^{-1}, and the RSDs for 20 and 40 μg ml^{-1}, 2.4 and 1.9%, respectively; however, with fluorimetric detection, the DL has been found to be 0.2 μg ml^{-1}, and the RSD for 0.5 μg ml^{-1}, 3.2%. EQ in apples has been also determined using electrochemical detection (DL = 0.03 ng) [69]. The levels of EQ residues in apple varieties stored under different conditions have been tabulated (with spectrophotometric detection, DL = 0.25 μg ml^{-1} in the extract and 0.2 mg kg^{-1} in the fruit). EQ has also been determined in apples by GC (DL = 50 μg kg^{-1}, □ 74% recovery). The use of guaiacol as an internal standard in the GC determination of phenolic antioxidants and EQ has been described.

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ANTIOXIDANTS | Synthetic Antioxidants

G. Ramis-Ramos, in Encyclopedia of Food Sciences and Nutrition (Second Edition), 2003

Ethoxyquin

Ethoxyquin (EQ) (see Figure 5) is a clear, viscous, light yellow to dark brown liquid, with an unpleasant, mercaptan-like smell, that causes irritation of the skin and eyes. It is absorbed through the skin, and is moderately toxic by ingestion. It darkens on exposure to light and air and tends to polymerize, particularly at temperatures above 160 °C (hazardous exothermic reaction). EQ is considered a highly effective antioxidant, is cheap and has a very long shelf-life. It has been the antioxidant of choice in pet food for many years, although it has also been added to other animal feeds and human food. Concern about its toxicity (not yet sufficiently investigated) has promoted the alternative use of tocopherols in pet food, in spite of the higher prices and increasing demand of tocopherols for human consumption. EQ is also used as an insecticide, herbicide, fungicide, postharvest dip to prevent scald on apples and pears, plant growth regulator, and antidegradation agent for rubber.

Figure 5. Molecular structure of ethoxyquin.

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Glutathione Transferases

Shigeki Tsuchida, in Encyclopedia of Cancer (Second Edition), 2002

III.B Induction of Glutathione Transferases by Drugs

Rat GST subunits are preferentially induced by various drugs, including carcinogens and anti-carcinogenic agents such as butylated hydroxyanisole (BHA) and ethoxyquin. Although subunits 1 and 3 are inducible by almost all drugs examined, the induction of subunit 3 by 3-methylcholanthrene or □-naphthoflavone is not remarkable. Preferential induction of Alpha class GST is also reported in primary cultured human hepatocytes. Antioxidants induce mouse liver GST to a much higher extent than in rat liver. The major mouse forms induced by BHA are GST-9.3 in the Mu class and GST-10.3 in the Alpha class, both being undetectable in normal liver (Table IV). GST-MI, a constitutive form in normal liver, is not significantly induced.

Table IV. Induction of Mouse Liver Glutathione Transferases by Drugs[a]

	Molecular form				
	Alpha		Mu		Pi
Inducer	**10.6 (MI, Ya3Ya3)**	**10.3 (YaiYa2)**	**8.7 (MIII)**	**9.3**	**9.0 (MII)**
tert- Butylhydroxy anisole	→[b]	↑↑	↑	↑↑	→
Bisethylxanthogen	→	↑↑	↑	↑↑	→
□-N aphthoflavone	→	↑	↑	↑	→
Phenobarbital	→	↑	↑	↑	→

a Adapted from Stao and Tsuchida (1991).

b Single and double arrows pointing upward indicate slight (within fivefold) and strong (above fivefold) induction, respectively. A horizontal arrow indicates no change.

The gene structure of rat subunit 1 provides the molecular basis for its induction by drugs. This gene possesses at least two enhancers, a xenobiotic-responsive element (XRE) and an antioxidant-responsive element (ARE), in the 5□-flanking region (Fig. 2). The former element contains the XRE core sequence found in the 5□-flanking region of the cytochrome P450 IA1 gene, while the ARE contains the 12-*O*--tetradecanoylphorbol-13-acetate (TPA) responsive element (TRE)-like sequence. An

enhancer analogous to the rat ARE is also present in the mouse Ya (possibly identical to GST-10.3) gene and is named the electrophile-responsive element (EpRE). The oncogene products, *Jun* and *Fos* protein families, have been suggested to bind to ARE and EpRE and to be involved in the basal and inducible activities of these related elements. □-Naphthoflavone, a planar aromatic compound, activates the gene through either XRE or ARE, but the presence of *Ah* receptors and metabolism of □-naphthoflavone by cytochrome P450 IA1 are required for its transcriptional activation. On the other hand, *t*-butylhydroquinone, a phenolic antioxidant, activates the gene only through ARE, independently of *Ah* receptors or cytochrome P450 IA1. In the presence of *Ah* receptors, XRE reacts with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, but ARE does not. These results support the conclusion that Phase II drug-metabolizing enzymes, including GST, are induced by monofunctional and bifunctional inducers by different mechanisms, and that induction by monofunctional inducers such as *t*-butylhydroquinone is mediated by an electrophilic signal, independent of *Ah* receptors. Mouse EpRE is responsive to a wide variety of GST inducers, and transcriptional activation through this element seems to account for most of the enzyme elevations produced by these inducers. The element contains two repeats of the TRE-like sequence, while one base is replaced in rat ARE. This difference may be responsible for the higher extent of GST induction in mouse liver than in rat liver.

Fig. 2. Regulatory elements of the rat subunit 1 gene. XRE, xenobiotic-responsive element-like sequence; ARE, antioxidant-responsive element; TCDD, 2,3,7,8-tetrachlorodibenzo-p-dioxin. The XRE core sequence is shown in the left-hand box. ARE contains a 12-O-tetrade-canoylphorbol-13-acetate response element-like sequence as shown in the right-hand box. Adapted from Tsuchida and Sato (1992).

Administration of clofibrate and other peroxisome proliferators to rats results in diminished GST subunits 1 and 3 in livers. Peroxisome proliferator receptors are supposed to mediate the biological effects of these agents and act as a transcription factor. The receptors belong to the steroid hormone receptor superfamily. *Ah* receptors are stimulatory for induction of GST, while peroxisome proliferator receptors are inhibitory.

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Cellular and Molecular Toxicology

J.D. Groopman, J.-S. Wang, in Comprehensive Toxicology, 2010

2.15.7.4 Determine Relation of Biomarker to Exposure and Disease in Experimental Animals

In the early 1980s a collaboration to identify effective chemoprevention strategies for aflatoxin carcinogenesis was initiated. The hypothesis was that reduction of aflatoxin–DNA adduct levels by chemopreventive agents would be mechanistically related to and therefore predictive of cancer-preventive efficacy. Preliminary studies with a variety of established chemopreventive agents demonstrated that after a single dose of aflatoxin, levels of DNA adducts were reduced (Kensler *et al.* 1985). Therefore, a more comprehensive study using multiple doses of aflatoxin and the chemopreventive agent ethoxyquin was carried out to examine effects on DNA adduct formation and removal and hepatic tumorigenesis in rats. Treatment with ethoxyquin reduced both area and volume of liver occupied by presumptive preneoplastic foci by >95%. This same protocol also dramatically reduced binding of AFB_1 to hepatic DNA, from 90% initially to 70% at the end of a 2-week dosing period. No differences in residual DNA adduct burden, however, were discernible several months after dosing. Thus, the efficacy of the intervention apparently depended on the time of analysis.

The experiment was then repeated with several different chemopreventive agents and in all cases aflatoxin-derived DNA and protein adducts were reduced; however, even under optimal conditions, the reduction in the macromolecular adducts always underrepresented the magnitude of tumor burden (Bolton *et al.* 1993; Roebuck *et al.* 1991). These macromolecular adducts can track disease outcome on a population basis, but in the multistage process of cancer the absolute level of adduct provides a necessary but insufficient measure of tumor formation. Indeed, it is reasonable to envision a situation where a chemopre-ventive agent could suppress adduct formation, but through other actions promote tumors, leading to a dichotomous outcome of fewer adducts and more tumors. Finally, because the design of these DNA adduct studies requires serial sacrifice of the animals, it is not possible to track the fate of an individual's adduct burden with tumor outcome. Hence, these investigations could only be used to predict the protective effects of an intervention at the level of the group, but not individual risk of disease.

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Poisonous plants

Kip E. Panter, ... Dale R. Gardner, in Biomarkers in Toxicology, 2014

Prevention and treatment

There are no effective methods of prevention or treatment except avoidance of the plant and controlling plant populations with herbicides or biological control. Resistance to PA toxicosis in some species suggests that the possibility may exist to increase resistance to PAs. Dietary factors such as increased protein, particularly those high in sulfur amino acids, had minor protective effects in some species. Antioxidants such as BHT and ethoxyquin induced increased detoxifying enzymes such as glutathione S-transferase and epoxide hydrolase. Zinc salts have been shown to provide some protection against hepatotoxicosis from sporidesmin or lupinosis in New Zealand and Australia, and zinc supplementation reduced toxicity in rats from Senecio alkaloids (Knight and Walter, 2001; Burrows and Tyrl, 2013).

Many of these plants were introduced either inadvertently or intentionally. Without natural predators to keep populations in check, they experienced explosive growth and distribution followed by epidemic proportions of toxicity. Introduction of biological controls and natural population controls have reduced many of the plant populations and thus toxicoses have declined. Sheep, a resistant species, have been used to graze down some of these plants, particularly *S. jacobaea*.

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FISH MEAL

S.M Barlow, in Encyclopedia of Food Sciences and Nutrition (Second Edition), 2003

Storage of the Finished Meal

Most fish meal, except white-fish meal, contains a residue of 8–12% of lipid which could not be pressed out of the product economically. This lipid reacts with oxygen and produces heat, which if not controlled properly can result in scorching or even ignition of the fish meal during storage. Thus most of the world's fish meal contains added antioxidant. This is normally ethoxyquin added at a level of about 750–1000 p.p.m. (*See* ANTIOXIDANTS | Synthetic Antioxidants.)

The meal is then cooled through a rotary cooling drum, or, more usually, stored directly in 50-kg bags or in heaps of several hundred tonnes on the ground or in warehouses. The bags are stacked in such a way as to allow a circulation of air in

the vicinity of each bag, so that any heat produced during storage as a result of residual oxidation of the meal is easily removed to the atmosphere. Bulk heaps of meal are turned by means of payloaders from time to time. This again allows any heat produced in the meal to escape. Most large storage areas of meal are equipped with thermocouples which constantly measure the temperature of the meal to insure that there is no risk of overheating.

Provided that the meal has been treated with antioxidant and maintained in a dry condition, and not allowed to overheat, it is possible to store the product for longer than 12 months without any change in nutritional quality. If moisture levels are allowed to rise above 15%, undesirable bacteria and mold growth may occur; this is true for most types of feedstuffs and organic material.

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Mycotoxins as Food Carcinogens

Ariane Vettorazzi, Adela López de Cerain, in Environmental Mycology in Public Health, 2016

Factors Affecting Carcinogenicity in Animal Studies

Modulating effects of different agents such as diets viruses, parasites, and other chemicals on aflatoxin-induced carcinogenicity have been widely studied. Some of the most relevant modulating agents and their principal effects are cited below (non-exhaustive list):

- Ammoniation. It has been demonstrated that decontamination of feed containing aflatoxins by ammoniation significantly reduced or completely eliminated the induction of hepatic tumors in trout[82] and rats,[61] respectively. Moreover, in trout fed nonfat dried milk from cows fed ammoniated or non-ammoniated aflatoxin-contaminated whole cottonseed, ammoniation almost eliminated the liver tumor response.[83]
- Interaction with other chemicals. Several studies evaluating the effects or interaction of aflatoxins with different substances have been carried out either for mechanistic purposes or to evaluate carcinogenic end points (detailed information in IARC[31]). In general, *N*-nitrosodimethylamine, nafenopin (a peroxisome proliferator), and ethanol demonstrated an increase in the carcinogenic effects of AFB1 in rats. In contrast, □-naphthoflavone, butylated hydroxyanisole or butylated hydroxytoluene, □-benzene hexachloride, ethoxyquin, oltipraz, lindane, 1-methyl-2-mercaptoimidazole, sodium selenite, and extracts of Rhi-

zopus delemar (edible yeast) diminished AFB1 carcinogenic potential when administered before, simultaneously, or after AFB1 administration in rats.

Different studies in trout have also demonstrated that Aroclor 1254 (polychlorinated biphenyl), □-naphthoflavone, and indole-3-carbinol (present in cruciferous vegetables) reduced the incidence of AFB1-hepatocellular carcinoma. However, in some studies in which doses of indole-3-carbinol were given after (not before) AFB1 exposure, a significant increase in tumor formation was observed compared with that in fish treated with AFB1 alone.[31] Taken into account the ability of indole-3-carbinol (depending on the exposure protocol) to both inhibit and promote AFB1-induced carcinogenesis,[84] other studies evaluated the influence of dietary indole-3-carbinol (0.2% w/w) on relative levels of CYP isozymes known to metabolize AFB1, the AFB1 glutathione detoxification pathway, and AFB1–DNA adduct formation.[85,86] Seven days of feeding the indole-3-carbinol diet increased microsomal concentrations of CYP1A1, 1A2, and 3A1/2, with a smaller effect on 2B1/2 and no effect on CYP2C11. Moreover, the liver glutathione *S*-transferase subunit (Yc2) appeared to be substantially elevated by a diet containing indole-3-carbinol. This effect was also observed, but to a lower extent, in a diet containing □-naphthoflavone. Indeed, the induction of this enzyme has been considered to have a major role in the resistance of rats to AFB1-induced hepatocarcinogenicity after treatment with enzyme inducers including oltipraz, ethoxyquin, and butylated hydroxyanisole, as well as in known mouse resistance to AFB1 carcinogenicity.[33]

- Diet. Several studies have evaluated the effects of diet composition on AFB1 carcinogenicity.[31] In general, malnourishment, marginal lipotrope diets, or high-protein (casein) diets have been shown to increase the carcinogenic effects of AFB1 orally or intraperitoneally administered to rats. Fatty acids, such as cyclopropenoid fatty acids,[87] or different concentrations of vitamin A[88] had little or no modifying effect on the response to AFB1 in rats.
- Viruses. One of the most important agents that modulates AFB1 carcinogenicity is the hepatitis B virus (HBV). Several studies carried out in woodchucks,[89] tree shrews,[90,91] and transgenic mice (p53$^{+/-}$)[92,93] demonstrated that HBV-infected animals were more sensitive than uninfected ones. In general, combined HBV–AFB1 treatment not only reduced the time of appearance, but also resulted in a higher incidence of liver tumors. This agent is especially relevant for human studies, because HBV has been considering a confounding factor in many epidemiological studies performed for aflatoxins (see the section on cancer in humans).
- Hepatectomy. Certain liver insults such as partial hepatectomies may contribute to tumor formation. Indeed, mice are considered refractory to AFB1 tumor formation except under conditions of partial hepatectomy or HBV infection.[32]

- *Conclusions for Animal Studies*

Taking into account all of the information regarding aflatoxins in experimental animals, the IARC in its last evaluation[33] concluded that there was sufficient evidence in experimental animals for the carcinogenicity of naturally occurring mixtures of aflatoxins and of AFB1, AFG1, and AFM1. However, it was considered that there was limited evidence for AFB2 and inadequate evidence for AFG2.

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MYCOTOXINS | Toxicology

F.S. Chu, in Encyclopedia of Food Sciences and Nutrition (Second Edition), 2003

Dietary Modifications

As discussed elsewhere in this encyclopedia, dietary modification greatly affects carcinogenesis in experimental animals and in humans. Without exception, the carcinogenic and other toxic effects of mycotoxins, especially aflatoxins, are also affected by nutritional factors, dietary additives, and anticarcinogenic substances. Since the manifestation of carcinogenic/toxic effects of AFB1 depends on its ability to be absorbed and metabolized to an active form and on its subsequent interaction with DNA to form AFB1–DNA adducts, dietary modifications on any or all of these steps would lead to an increase or decrease in the carcinogenic effects of aflatoxin. Mycotoxins have a high affinity for the hydrated sodium calcium aluminasilicate (or NovaSil) and other related products. Diets containing NovaSil and related absorbers are effective in preventing absorption of AFB1 and several other mycotoxins in animals. Chemoprotective agents and antioxidants such as ascorbic acid, BHA, BHT, ethoxyquin, oltipraz, pentaacetyl geniposide, Kolaviron biflavonoids, and even green tea have also been found to inhibit carcinogenesis caused by AFB1 in test animals. Dietary administration of the naturally occurring chemopreventive agents, ellagic acid, coumarin or □-angelicalactone has been shown to cause an increase in the activity of glutamate–cysteine ligase, a key enzyme for the synthesis of glutathione. The mechanism of such protective effects was found to be the shifting of metabolism to a detoxification route by formation of a AFB1–glutathione conjugate rather than the formation of AFB1–DNA adducts. Ebselen exerts a potent protective effect against aflatoxin B-1-induced cytotoxicity; such a protective effect may be due to its strong capability in inhibiting intracellular reactive oxygen species formation and preventing oxidative damage.

Other absorbents such as zeolite, bentonite, and superactive charcoal are also effective in decreasing the toxicity of mycotoxins such as T-2 toxin. The toxicity of OA in test animals is minimized when antioxidants such as vitamins C and E are added to the diet. Aspartame, which has been found to be partially effective in decreasing the nephrotoxic and genotoxic effects of OA, may compete with OA for binding to serum albumin. l-Phenylalanine was found to protect the toxic effects of OA; administration of this amino acid prevented the OA's inhibitory effect on some of the enzymes discussed earlier. Vitamin E was found to efficiently prevent cytotoxicity induced by FB1. (*See* ASPARTAME.)

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