UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF PREVENTION,
PESTICIDES, AND TOXIC SUBSTANCES

DATE: May 17, 2006

ACTION MEMORANDUM

SUBJECT: Inert Reassessment: Tertiary butylhydroquinone (CAS Reg. No 1948-33-0)

FROM: Pauline Wagner, Chief Pauline Wagner 5/19/06
Inert Ingredient Assessment Branch
Registration Division (7505C)

TO: Lois A. Rossi, Director
Registration Division (7505C)

I. FQPA REASSESSMENT ACTION

Action: Reassessment of one inert exemption from the requirement of a tolerance. The reassessment decision is to maintain the inert ingredient tolerance exemption "as-is."

Chemical: Tertiary butylhydroquinone

CFR: 40 CFR part 180.920

CAS Registry Number and Name: CAS Reg. No. 1948-33-0; CAS Name: 1,4-Benzenediol, 2-(1,1-dimethylethyl)- (9CI)

Use Summary: TBHQ is widely used as an antioxidant food additive in edible fats and oils under FDA. The inert ingredient use of TBHQ is as an antioxidant in agricultural and residential pesticide products.

List Reclassification Determination: The current List Classification for tertiary butylhydroquinone is 3. Because EPA has determined that there is a reasonable certainty that no harm to any population subgroup will result from aggregate exposure to tertiary butylhydroquinone used as inert ingredients in pesticide formulations, the List Classification for tertiary butylhydroquinone will change from List 3 to List 4B.

II. MANAGEMENT CONCURRENCE

I concur with the reassessment of the one exemption from the requirement of a tolerance for the inert ingredient tertiary butylhydroquinone (CAS Reg. No. 1948-33-0)

and with the List reclassification determination(s), as described above. I consider the one exemption established in 40 CFR part 180.920 to be reassessed for purposes of FFDCA's section 408(q) as of the date of my signature, below. A Federal Register Notice regarding this tolerance exemption reassessment decision will be published in the near future.

Lois A. Rossi

Lois A. Rossi, Director
Registration Division

May 23, 2006
Date:

cc: Debbie Edwards, SRRD
Joe Nevola, SRRD

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF PREVENTION,
PESTICIDES, AND TOXIC SUBSTANCES

May 17, 2006

MEMORANDUM

SUBJECT: Reassessment of the One Exemption from the Requirement of Tolerance for *tertiary*-Butylhydroquinone (CAS Reg. No. 1948-33-0)
Decision No. 363670
Barcode No. 325384
P.C. Code: 900812:

FROM: Byron T. Backus, Ph.D., Toxicologist
Technical Review Branch
Registration Division (7505C)

TO: Pauline Wagner, Chief
Inert Ingredient Assessment Branch
Registration Division (7505C)

BACKGROUND

Attached is the science assessment for *tertiary*-Butylhydroquinone (TBHQ; CAS Reg. No. 1948-33-0). The purpose of this document is to reassess the existing exemption from the requirement of a tolerance for residues of TBHQ (used pre-harvest) as required under the Food Quality Protection Act (FQPA). This assessment summarizes available information on the use, physical/ chemical properties, toxicological effects, exposure profile, and environmental fate and ecotoxicity of TBHQ.

EXECUTIVE SUMMARY

This report evaluates *tertiary*-Butylhydroquinone (TBHQ), a pesticide inert ingredient, and its exemption from the requirement of a tolerance for residues when used in accordance with good agricultural practice as an inert ingredient in pesticide formulations applied pre-harvest to growing crops (40 CFR 180.920).

Exposure to TBHQ associated with its use as an inert ingredient in pesticides is minor, particularly in comparison to that from its extensive use as an antioxidant (at concentrations up to 0.02%) in certain foods such as oils, fats, and meat products to slow oxidization and prevent rancidity (refer to 21 CFR 172.185). For this purpose, TBHQ shows significantly better anti-oxidant protection than propyl gallate, especially with soybean and safflower oils, which are highly unsaturated and particularly susceptible to oxidation. TBHQ was approved by the FDA for food use in the U.S. in 1972. It is also used as an antioxidant in cosmetic products such as lipsticks, eye shadows, perfumes and skin care preparations at concentrations ranging from 0.1 to 1.0%.

A considerable number of developmental, reproductive, chronic, carcinogenic and mutagenic studies have been conducted on TBHQ because of its use as an antioxidant in edible oils and fats. The findings from these studies have been comprehensively evaluated and peer-reviewed. Based on the available studies, TBHQ is of low toxicity in rodent studies which is useful for extrapolating human health effects. No quantitative or qualitative susceptibility was observed in reproductive and developmental rodent studies for TBHQ. Also, the effects seen in the offspring were at levels where effects were also seen in the adults.

After a comprehensive review of the available toxicity data, the European Food Safety Authority (EFSA; 2004) concluded that TBHQ, when used as an antioxidant in edible oils and fats, would not pose a serious health risk to the general population under normal exposure conditions, as the daily oral for all population subgroups would fall below the ADI of 0.7 mg/kg bw/day. TBHQ is readily metabolized in the body. Based on the available toxicity information at various durations, concentrations, and routes of exposure along with the anticipated inert uses of TBHQ, dietary and residential exposures of concern are not likely from the use of TBHQ as an inert ingredient in pesticide formulations.

Taking into consideration all available toxicity and exposure information on TBHQ, EPA has determined that there is a reasonable certainty that no harm to any population subgroup will result from aggregate exposure to TBHQ from its use as an inert ingredient in pesticide formulations when considering dietary exposure and all other nonoccupational sources of pesticide exposure for which there is reliable information. Therefore, it is recommended that the exemption from the requirement of a tolerance established for residues of TBHQ under 40 CFR 180.920 can be considered reassessed as safe under section 408(q) of the Federal Food, Drug, and Cosmetic Act (FFDCA).

I. Introduction

This report evaluates *tertiary*-Butylhydroquinone (TBHQ), a pesticide inert ingredient for which an exemption from the requirement of a tolerance exist for its

residues when used in accordance with good agricultural practice as an inert ingredient in pesticide formulations applied pre-harvest to growing crops (40 CFR 180.920).

II. Use Information

A. Pesticides

As an inert ingredient, TBHQ is an antioxidant in a number of pesticide formulations, including some with agricultural uses. The tolerance exemption for TBHQ is shown in Table 1 below.

Table 1. Pesticide Uses of TBHQ

CFR Citation				CAS Reg. No. 9CI Name
40 CFR §	Inert Ingredients	Limits	Uses	
180.920*	*Tertiary*-butylhydroquinone	(none)	Antioxidant	1948-33-0 1,4-Benzenediol, 2-(1,1-dimethylethyl)-

*Residues listed in 40 CFR 180.920 are exempted from the requirement of a tolerance when used in accordance with good agricultural practice as inert (or occasionally active) ingredients in pesticide formulations applied to growing crops only.

B. Other Uses

TBHQ is extensively used as an FDA-approved antioxidant (at concentrations up to 0.02% in terms of oil and fat content) in certain foods such as oils, fats, and meat products to slow oxidization and prevent rancidity (refer to 21 CFR 172.185). It is also used in cosmetic products such as lipsticks, eye shadows, perfumes and skin care preparations at concentrations from 0.1 to 1.0%. It is also used as a polymerization inhibitor for styrene, butadiene, and other alkenes.

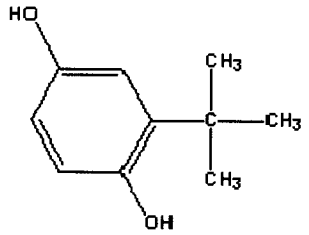
Table 2. FDA Approved Food Uses for TBHQ

Name	21 CFR	Use Pattern
Tertiary-Butylhydroquinone	172.185	To be used as an antioxidant alone or in combination with BHA and/or BHT, with the stipulation that the total antioxidant content of a food containing the additive(s) will not exceed 0.02 percent of the oil or fat content of the food, including the essential (volatile) oil content of the food.

III. Physical and Chemical Properties

Some of the physical and chemical characteristics of TBHQ, along with its structure and nomenclature, are given in Table 3.

Table 3. Physical and Chemical Properties of *tert*-Butylhydroquinone

Parameter	Value	Reference
Structure		TOXNET SIS, 2004
CAS #	1948-33-0	
Molecular Formula	$C_{10}H_{14}O_2$	
Molecular Weight	166.22	
CAS Name	1,4-Benzenediol, 2-(1,1-dimethylethyl)-(9CI)	
Physical State	White to light tan crystalline solid, with very slight odor	
Melting Point	126.5-128.5°C	
Boiling Point	NA	
Flammability	NA	
Density/Specific Gravity	NA	
Vapor Pressure	NA	
Solubility in organic solvents	60% in ethanol, 60% in ethyl acetate, 30% in propylene glycol	National Toxicology Program, 1997
Water Solubility	Less than 1% at 25°C	

Under normal conditions of storing and cooking, the main decomposition product is 2-*tertiary*-butyl-p-benzoquione or TBBQ (European Food Safety Authority, 2004).

IV. Hazard Assessment

To assess the toxicity posed by the use of TBHQ as an inert ingredient in pesticide formulations, the Environmental Protection Agency (EPA or the Agency) has largely relied on information contained in the following documents: a Department of Health and Human Services National Toxicology Program Technical Report (1997; No. 459; NIH Publication No. 97-3375) titled: "Toxicology and Carcinogenesis Studies of t-Butylhydroquinone (CAS No. 1948-33-0) in F344/N Rats and B6C3F$_1$ Mice (Feed Studies)" (National Toxicology Program, 1997); a document from the World Health Organization (WHO) International Programme on Chemical Safety (IPCS) prepared by

the forty-ninth meeting of the Joint FAO/WHO Expert Committee on Food Additives (1998); and an article by the European Food Safety Authority (EFSA) (2004) titled: "Opinion of the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food on a request from the Commission related to *tertiary*-Butylhydroquinone (TBHQ)."

A. Toxicological Data

Acute Toxicity

Available acute oral LD_{50} data indicate technical TBHQ after a single dose for several rodent species ranges from > 756 mg/kg to 1040 mg/kg). Inhalation toxicity data in rodents revealed ataxia and dyspnea at 2,900 mg/m3 for 4 hours. No information is available as to the technical's dermal irritation potential, but its use in cosmetic products indicates that it is not a primary dermal irritant at concentrations of up to 1%. However, occasional dermal sensitization reactions have been reported from its use in lipgloss. It is practically insoluble in water, but its solubility in fats and oils suggests some potential for dermal absorption. TBHQ, however, is a relatively minor constituent (<1%) in pesticide formulations limits its availability for absorption.

The acute toxicity data available for TBHQ are summarized below in Table 4.

Table 4. Summary of Acute Toxicity Data for TBHQ

Parameter	Toxicity	Reference
Oral LD_{50}	Oral LD_{50} values for several rodent (rat, mouse, guinea pig) species range between 756 and 1040 mg/kg body weight. At 400 mg/kg dogs consistently regurgitated, but not until approximately 10 hours after dosing. Toxic effects following a single exposure not reported. *Toxicity Category III*	Terhaar et al., 1968
Dermal LD_{50}	No information	
Inhalation LC_{50}	Rat LC_{Lo} 2,900 mg/m^3/4H (1/6 rats died following exposure to this concentration; 2/6 died following exposure to 4,200 mg/m^3/4H) Toxic effects: Ataxia, dyspnea *Toxicity Category IV*	
Skin Irritation	No specific study is available. According to representative Material Safety Data Sheets (see, for example: http://www.coleparmer.com/catalog/Msds/01325.htm): "May cause skin irritation."	
Eye Irritation	No specific study is available. According to representative Material Safety Data Sheets (see, for example: http://www.coleparmer.com/catalog/Msds/01325.htm): "May cause eye irritation."	

Subchronic Toxicity

Dermal Irritation

In a 13-week study (Eastman Kodak Co., 1989), TBHQ was dermally administered to outbred black guinea pigs (5/sex/group) at concentrations of 0.1, 1.0, or 5.0% in 1 ml each scheduled working day for 13 weeks, for a total of 63 applications. The test substance was spread evenly on the shaved flank each day of dosing. At 0.1%, the test substance produced weak irritant responses and did not produce depigmentation. At 1.0%, 20% of animals had spotty or uniform loss of pigment. At 5%, 40% of animals were hypopigmented.

Developmental and Reproductive Effects

In a study conducted by Krasavage (1977), groups of 20 female SD rats were fed a basal diet with 0, 0.125, 0.25 or 0.5% TBHQ from days 6 to16 of gestation. During the mating period and on all other days of gestation, all treatment groups received control diet only. The experiment was terminated on day 20 of gestation. Total TBHQ doses from the 11 days of exposure of 970, 1880 or 3600 mg/kg bw had no effect on mean body weight gain or feed consumption of the dams. Average corpora lutea, implantation sites, viable fetuses, resorptions, fetal body weights and mortality did not differ between the control and treatment groups. An unusually large number of skeletal variations such as rudimentary and extra fourteenth ribs were seen in all groups, but the overall incidence was double in controls from that of any treated group. It was concluded that TBHQ did not cause developmental toxicity in rats at any of the tested doses.

In a 2-generation rat study (Fassett et al., 1965), Holtzman albino rats were fed ground Purina chow with 0 or 0.5% TBHQ (equivalent to 250 mg/kg/day). After 36 days on treatment, groups of 24 female and 10 male F_0 rats were mated, until 10 were inseminated. At 100 days of age, F_1 animals were mated 15 females to 5 males, until eight TBHQ-treated females were inseminated. In both generations, body weights and feed consumptions were recorded weekly during the pre-mating period. For mating, parturition and weaning, the following data were recorded: mating index, fertility index, dead births, and pup survival to weaning. Pup weights were recorded at weaning and at 1 and 2 weeks post-weaning. Adults from the F_0 and F_1 generations were necropsied, and liver and kidney weights reported. F_1 generation and offspring showed poor survival due to pneumonia. TBHQ-exposed parental animals in both generations had lower body weight than their controls and this was significant in F_1 females. TBHQ did not affect mating, fertility or gestation indices, average litter side or number of live births in either generation. Absolute

but not relative liver and kidney weights were decreased. No histological alterations were seen.

In a 3-generation rat study (Terhaar and Krasavage, 1968a) with 15 male and 15 female SD rats, animals were fed 0 or 0.5% TBHQ. Pairs of rats were mated to produce two litters per generation with the next generation selected from the second litter. Data recorded for each included: number of inseminations, number of pregnancies, gestation period, average litter size, mortality of young from birth to weaning and from weaning to sacrifice. Average body weight per pup was measured at weaning and at one and two weeks after weaning. Tissues were collected from breeders. F_{1b} non-breeders were sacrificed at 7 weeks of age. All were examined for gross pathology and micropathology on at least four animals from each litter. Organs examined included trachea, lung, heart, tongue, urinary bladder, pituitary, adrenal, pancreas, thyroid, parathyroid, gonads, uterus, spleen, bone marrow, cerebrum and cerebellum, and eye. The litters were delivered on the 19th day of gestation. One-third of the litters were stained with alizarin red for skeletal defects, while the other two-thirds were fixed, sectioned and examined for abnormalities. The reproductive indices were normal. Deaths in F_{1a} and F_{1b} litters exceeded control values. Mean body weights were lower in treated animals. Minor skeletal abnormalities were observed in two test animals. No effect on histology was seen in any of the treated animals.

In a 13-week reproductive study in F344/N rats (National Toxicology Program, 1997), groups of females (the F_0 generation) were fed 0; 2,500; 5,000; 10,000; 20,000; or 40,000 ppm TBHQ from 2 weeks before breeding until their pups (the F_1 generation) were weaned. Females exposed to 20,000 or 40,000 ppm did not litter. Pup survival at 28 days in the 5,000 ppm group (88%) and in the 10,000 ppm group (62%, $p<0.01$) was reduced compared to that of the control group (100%). Pup weight per litter in the 5,000 and 10,000 ppm groups (45.1 and 42.0 g, respectively) was lower than that of the control (49.2 g). TBHQ did not affect gestation length, the average number of pups born per litter, or the number of dams with stillborn pups for dams exposed to 2,500; 5,000 or 10,000 ppm.

In another study, groups of 10 male and 10 female F_1 rats continued to receive diets containing 0, 2,500, 5,000 or 10,000 ppm (equivalent to 200, 400 or 800 mg/kg/day for males; 200, 400 or 750 mg/kg/day for females) for 13 weeks following weaning. All rats survived to the end of the study. The final mean body weights of males (307 and 279 g) and females (185 g and 175 g) in the 5,000 and 10,000 ppm groups were significantly lower than those of the controls (328 g for males and 199 g for females), as was the mean body weight gain of males exposed to 10,000 ppm. However, interpretation of these findings was complicated by the significantly lower initial mean body weights of the 10,000 ppm groups (69 g in the 10,000 ppm males vs. 96 g in control males, and 64 g in the 10,000 ppm females vs. 82 g in control females). Differences in initial body

weights were reported to be due to *in utero* exposure to TBHQ. Feed consumption of the groups receiving TBHQ was lower than that of controls at week 2, and feed consumption of the 5,000 and 10,000 ppm males and 10,000 ppm females was slightly lower than that of controls at the end of the study. Hair discoloration in all exposed groups of rats, except females at 2,500 ppm, was the only clinical observation considered related to chemical exposure. The mean spermatid count, spermatid heads per testis, and spermatid heads per gram of testis were significantly decreased in males at 5,000 ppm. The estrous cycles of females at 2,500 and 5,000 ppm were significantly longer than that of controls. Increased incidences of hyperplasia of the nasal respiratory epithelium were seen in males at 5,000 ppm and in both sexes at 10,000 ppm. Increased incidences of pigmentation were observed in the spleen of both sexes at 5,000 and 10,000 ppm. Because the pigment was golden brown and present within the phagocytic cells, it was considered to be hemosiderin. Otherwise, there were no biologically significant changes in clinical pathology parameters (including bone marrow and hematology) or in organ weights. Serum bile acids were generally significantly increased in 5,000 and 10,000 ppm male and female rats at day 5, at week 3, and at the end of the study. Serum alanine aminotransferase activity levels were increased on day 5 in females exposed to 10,000 ppm, at week three in males and females exposed to 2,500, 5,000 or 10,000 ppm, and at the end of the study in males at 2,500 ppm. However, because the increases observed in these two parameters were marginal, and since histopathologic evaluation did not reveal evidence of liver toxicity, these marginal increases were not considered to be biologically significant.

Based on the results given above, the NOAEL for the F_1 generation of F334/N rats was a dietary level of 2,500 ppm TBHQ (200 mg/kg/day for both males and females), and the LOAEL was 5,000 ppm TBHQ (400 mg/kg/day for both sexes).

In a 13-week study in $B6C3F_1$ mice (National Toxicology Program, 1997), groups of males and females were fed diets containing 0; 2,500; 5,000; 10,000; 20,000 or 40,000 ppm (equivalent to 440; 880; 1,950; 4,000; and 8,400 mg/kg for males; 500; 1,100; 2,200; 4,600; and 9,000 mg/kg for females). There were no exposure-related deaths. Final mean body weights and body weight gains for males and females exposed to 10,000 or more ppm were significantly less than those of controls. There was excessive feed scatter by mice fed diets containing 10,000 or more ppm, so food consumption in these groups was probably less than that of controls, but could not be accurately quantitated. Significant increases in segmented neutrophil counts were observed at week three and at the end of the study in females exposed to 10,000 ppm and in both sexes at 20,000 and 40,000 ppm. Increased incidences and severities of mucosal hyperplasia were seen in the forestomach of females at 10,000 ppm and in both sexes at 20,000 and 40,000 ppm. Increased incidences of hyperplasia of the nose and skin occurred in both sexes at 10,000; 20,000; and 40,000 ppm.

Mutagenicity

TBHQ was negative in an Ames assay (Zeiger et al., 1992) in which *Salmonella typhimurium* strains TA97, TA98, TA100 and TA102 were tested. Two types of S9 fractions were used in this assay, one from Aroclor 1254-induced induced male Sprague-Dawley rat liver, and one from Aroclor 1254-induced Syrian hamster liver. TBHQ has also been evaluated with negative results in four additional Ames assays (Société Kemin Europa, 1982a; Muellar & Lockhart, 1983; Hageman et al., 1988; and Matsuoka et al., 1990).

In a reverse mutation and mitotic gene conversion assay with *Sacharomyces cerevisiae* D7, TBHQ was negative at doses of up to 500 µg/mL in the absence of S9, and at doses of up to 200 µg/mL in the presence of S9 (Rogers et al, 1992).

In a mouse lymphoma L5178Y forward mutation assay (Litton Bionetics, 1982), TBHQ was positive (with a mutant frequency that just exceeded the minimum criterion for considering this a positive test) with S9 at the highest concentration tested (31.3 ng/L).

There are two negative *in vitro* point mutation assays with mammalian cell lines. In one, TBHQ was negative in a CHO/HGPRT assay at doses of up to 6 µg/mL in the absence of S9, and 250 µg/mL in the presence of S9 (Beilman and Barber, 1985). It was sporadically positive in a Chinese hamster V79 HGPRT locus assay, both with and without S9 activation at partially cytotoxic dose levels (0.17 – 3.4 µg/mL of medium), indicating the possibility of weak genotoxic activity (Rogers et al., 1992).

Positive results were obtained in two *in vitro* Chinese hamster ovary cell chromosomal aberration assays (Societé Kemin Europa, 1982c; National Toxicology Program, 1997). The National Toxicology Program (NTP) study was performed at Litton Bionetics, Inc. In this Litton study, no positive results were obtained in the absence of S9 activation, but in the presence of S9 and using a lengthened harvest time of 20 hours a highly significant ($p \leq 0.01$) and dose-dependent increase was observed in two separate trials, with evaluated doses ranging from 100.5 to 200 µg/mL in the first trial, and from 149.4 to 249 µg/mL in the second. These are extremely high concentrations of TBHQ, probably unattainable under *in vivo* conditions. According to the National Toxicology Program (1997), the addition of catalase to the hamster cell cultures without S9 resulted in a substantial decrease in the frequency of TBHQ-induced chromosomal aberrations (Phillips *et al.*, 1989), indicating that generation of H_2O_2 played a role in the observed induction of chromosomal damage. Because TBHQ autooxidizes in solution to *t*-butylquinone, forming superoxide and H_2O_2, the mutagenic effects that are observed in cells exposed to TBHQ are most likely the indirect result of the release of oxidative byproducts within the cell.

Experiments with various radical scavengers provided evidence that either singlet oxygen or a singlet oxygen-like entity (perhaps a copper-peroxide complex) rather than free hydroxyl radicals was responsible for DNA damage in phage induced by phenolic compounds such a TBHQ (Li and Trush, 1994).

In a rat dominant lethal assay (Krasavage and Faber, 1983) male rats were fed diets containing TBHQ for 83 days. The TBHQ treated males were mated with untreated females for two consecutive weeks immediately following treatment. Post implantation losses were increased in the 285 mg/kg group after the first mating (13.9% of total implantations compared to 6.1 for the control group, $p \leq 0.05$), and were also increased in the 145 mg/kg group after the second mating (11.9 compared to 6.1 for the control group, $p \leq 0.05$). No dose dependency could be observed for these effects. However, the assay is not clearly negative.

Carcinogenicity and Chronic Toxicity

Altmann (1985) investigated the incidence of forestomach lesions in rats fed diets with 2% TBHQ (equivalent to 1,000 mg/kg/day). Treated animals developed brown discoloration and mild hyperplasia of the forestomach mucosa with focally increased hyperplasia of basal cells; however, the local basal cell hyperplasia did not tend to differentiate.

Shibata et al. (1989) evaluated the effects of bladder tumor promoters on bladder epithelial tissue in rats. Ten male rats were administered either 0 or 2% TBHQ in the diet for 4 or 8 weeks. Five animals per group were killed at week 4 for estimation of DNA synthesis after interperitineal (i.p.) injections with 100 mg/kg body weight of 5-bromo-2'-deoxyuridine (BrdU). These animals also received a histopathologic examination by light microscopy and scanning electron microscopy in the urinary bladder. The remaining 5 rats were evaluated at the end of treatment (8 weeks). A significant decrease in body weight was observed in TBHQ-treated rats. TBHQ administration was associated with a decrease in urinary potassium, phosphorus and osmolality and increased pH and $MbNH_4PO_4$ crystals. DNA synthesis was significantly increased. SEM findings included leafy or ropy micro-ridges, and short uniform, or pleomorphic microvilli on the bladder epithelial surface. The authors interpreted these results as indicating a link between increased DNA synthesis, altered surface morphology, and promotion of bladder carcinogenesis.

In a long-term National Toxicology Program (1997) study, groups of 68-70 F344/N rats/sex following perinatal exposure were given ad libitum access to diets with 0; 1,250; 2,500; or 5,000 ppm TBHQ; corresponding to dose levels of 0; 50; 100; or 200 mg/kg bw per day for males, and 0; 60; 120; or 240 mg/kg bw per day for females. Dosing was 123 weeks post-weaning for males and 129 weeks for females. Survival rates were higher in high-dose rats of both sexes compared to respective controls with significance ($p<0.05$) for females. The

mean body weights of rats exposed to 5,000 ppm averaged 7 and 10% less than controls for high-dose males and females, respectively. Food consumption was not altered. Hair discoloration in all treated groups was the only clinical observation. Hematology was unaffected. A dose-related increase in hemosiderin in the spleen of females was seen (24/60, 27/60, 33/57, and 41/60).

At terminal sacrifice hepatocellular carcinoma was observed in 2/60 (3%) of high-dose males versus none in any other group. This was considered incidental. Various spurious increases in tumors were observed including interstitial cell adenoma of the testes in mid- and high-dose males, which was not statistically significant and within historical control ranges. Small increases in c-cell carcinoma (2/60) and follicular cell carcinoma (3/60) in high-dose males versus none in controls were not statistically significant. With the low incidences and lack of preneoplastic lesions, it was concluded that these male tumors were unrelated to treatment. Exposure of rats to TBHQ resulted in decreased incidences of mammary gland neoplasms in males and females, with significant negative trends.

Incidences of renal cysts and suppurative inflammation were increased in mid and high-dose male rats. In females, the incidence of chronic inflammation of the kidneys was slightly increased in mid- and high-dose females (1/60, 1/60, 3/60, and 5/60, not significant at the mid dose). Clinical findings of hair discoloration in exposed groups of males and females were considered to be related to TBHQ exposure. The NOAEL was 1250 ppm (50 mg/kg body weight) for males based on the incidence of cysts in the kidney at higher doses. The NOAEL for female rats was 2500 ppm (120 mg/kg/day) based on decreased body weight at 5000 ppm.

In a National Toxicology Program (1997) mouse chronic (2-year) bioassay, groups of 60 $B6C3F_1$ mice/sex/dose were fed *ad lib* diets with concentrations of 0, 1250, 2500 and 5000 ppm TBHQ. The dose levels were 0, 150, 300 and 600 mg TBHQ/kg bw/day for males and 0, 150, 300, and 700 mg TBHQ/kg bw/day for females. Mice were observed twice daily, 7 days a week. Individual body weights were recorded weekly for 13 weeks and then once a month. An interim sacrifice of 10 per group/sex was conducted at 15 months and included complete gross and microscopic evaluations, weights of kidney, liver and testis and hematological parameters. Complete necropsies and histological examinations were performed at termination and in animals dying during the course of the study. Terminal survival rates were 58-77%, and were not affected by TBHQ treatment. Food consumption was unaffected. High dose animals (male and female) exhibited an 8-11% lower body weight at termination, and their mean body weights were generally lower than those of the control group from week 13 to the end of the study. Feed consumption in the exposed groups for males and females was similar to what was observed in their controls. At 15 months, absolute and relative liver weights of treated animals were higher without a clear dose-response, but reaching significance ($p<0.05$) in high-dose

females versus their controls. Hematology was unremarkable. There were no biologically significant differences in clinical chemistry parameters between the exposed groups and their controls.

There was no evidence of carcinogenic activity for TBHQ. At termination, the incidences of hepatocellular adenoma and hepatocellular adenoma or carcinoma were significantly lower in high-dose males than controls. The incidence of follicular cell adenoma in the thyroid of females at 5000 ppm was higher than in controls (5/54 versus 1/51), but this was not statistically significant. This was associated with an increase in follicular cell hyperplasia in all treated groups (12/51, 19/51, 24/50 and 24/54, respectively) but no carcinoma. The adenoma rate was at the upper historical control range. No NOAEL could be assigned because of proliferative effects in the thyroid follicular cells in the females at the lowest dietary level.

WHO (1998) reviewed a study conducted by Eastman Chemical Products (1968) in which groups of 4 beagle dogs/sex were fed diets containing 500, 1580 or 5000 ppm TBHQ (equivalent to 21, 72 and 260 mg/kg/bw in males and 22, 73 and 220 mg/kg/bw in females) for up to 117 weeks. At termination, all dogs were examined for gross and pathologic changes. The liver, kidneys, spleen, heart, brain, lungs, gonads, adrenals, thyroid and pituitary of each dog were weighed, and organs and tissues from control and high-dose animals were collected for gross and microscopic pathology. Samples of liver and kidney tissues were also examined by electron microscopy. Growth, food consumption, weight gain and behavior were similar in all groups. The only effects noted were in hematology parameters, with decreased RBC counts, slightly lower hemoglobin and hematocrit and elevated reticulocyte counts at weeks 99 and 104 (but not 117) in high dose animals. Peripheral blood smears from the high dose dogs also showed more normoblasts as well as occasional increases in erythrocyte basophilia. The NOAEL in this study was 1580 mg/kg/bw (equal to 72 mg/kg/bw/day) based on the hematologic findings. This NOAEL was used by WHO with a 100-fold safety factor to set an Acceptable Daily Intake (ADI) of 0.7 mg/kg/bw (EFSA, 2004).

B. Metabolism and Pharmacokinetics

TBHQ is readily metabolized. In mouse studies, metabolism primarily involved oxidation at the *tert*-butyl group, followed by formation of the glucuronide conjugate and excretion in the urine, or by excretion of the free acid in feces. In rats, 80-90% of the ^{14}C-radiolabel was excreted in urine or feces within 96 hours, mostly as the free acid in feces with smaller amounts in urine, and less than 0.3% in expired air. More than 43 metabolites were present in the urine and feces of mice and rats. In several studies with rats and dogs, TBHQ by the oral route was shown to be well absorbed and rapidly excreted, mainly in the urine. Primary urinary metabolites in both species are the 4-O-sulfate conjugate and the

4-O-glucuronide. Excretion seems to be essentially complete after 4 days (Astill et al., 1967a; 1967b, 1968).

C. Special Considerations for Infants and Children

Reproductive and developmental rodent studies for TBHQ inform the special considerations for infants and children. No quantitative or qualitative suspectibility was observed in these studies. Based on these available studies, TBHQ is of low toxicity for establishing human health endpoints. Results of the reproductive studies indicate no differences in toxicity between sexes and no difference in toxicity in the offspring compared to adults. Based on this information, there is no concern, at this time, for increased sensitivity to infants and children to TBHQ when used as an inert ingredient in pesticide formulations. For the same reason, a safety factor analysis has not been used to assess risk and, therefore, the additional tenfold safety factor for the protection of infants and children is unnecessary.

V. Environmental Fate Characterization and Drinking Water Considerations

Information in the open literature on the environmental fate of TBHQ was limited to a few basic physical-chemical properties in the Hazardous Substances Database. Therefore, the use of structure activity relationship (SAR) was used to estimate the potential chemical properties and environmental behavior of TBHQ. TBHQ is unlikely to be readily biodegradable in the environment. Estimates for primary degradation would be on the order of weeks and ultimate biodegradation (mineralization) on the order on months under acclimated conditions. Biodegradation in unacclimated conditions in both soil and water would likely be longer. TBHQ is soluble in water at concentrations that may be of concern ecologically. TBHQ is resistant to hydrolysis due to a lack of active hydrolysable functional groups, and photoylsis is not likely to be significant degradation pathway in natural waters or on soils. TBHQ is poorly volatile from soil and/or water, and will undergo rapid photolytic degradation in air should volatilization occur. Leaching to ground water may occur in some sandy soils based on an estimated K_{oc} of 3,000. TBHQ is not expected to bioaccumulate in the environment. Based on a projected half-life in soil and water of more than a month and other physical-chemical properties, application rates of 1 pound per acre will likely result in concentrations in the low parts per billion in untreated waters.

VI. Exposure Assessment

TBHQ is widely used as an antioxidant food additive in edible fats and oils under FDA. The World Health Organization (1999) gives the mean human dietary exposure in the United States from the food additive use (for 1995) as 0.07 mg/kg bw/day and the high (90^{th} percentile) as 0.14 mg/kg bw/day. These values represent 10% and 20% of the ADI value of 0.7 mg/kg assigned to TBHQ by the World Health Organization (1998).

The inert ingredient use of TBHQ is as an antioxidant in agriculture and residential pesticide products. Although the current tolerance exemption doesn't limit

the amount that can be used, the typical use of TBHQ in pesticide products tends to be small (<1.0%) and only pre-harvest applications are allowed. The following summarizes the dietary (food and drinking water) and residential (inhalation and dermal) exposures that are anticipated from the use of TBHQ in pesticide products.

Dietary

The primary potential human exposure would be via the oral route through consumption of food to which a TBHQ-containing pesticide product has been applied or exposure through drinking water. Residues of TBHQ from the inert use on pre-harvest crops are expected to low due to the small percentage of TBHQ in pesticide formulations and the low application rates. Based on a projected half-life in soil and water of more than a month and other physical-chemical properties, application rates of 1 pound per acre will likely result in concentrations in the low parts per billion in untreated waters. Therefore, dietary exposures of concern from food and drinking water are not likely from the use of TBHQ in pesticide formulations.

Residential

TBHQ is used in residential pesticides such as insect sprays sold for use in and around the home (bug killers, treatments for ornamental plants), and flea and tick sprays for cats and dogs. While dermal and inhalation exposure from residential uses are possible, TBHQ is not volatile, which reduces the potential for exposures of concern

VII. Aggregate Exposures

In examining aggregate exposure, the FFDCA section 408 directs EPA to consider available information concerning exposures from the pesticide residue in food and all other nonoccupational exposures, including drinking water from ground water or surface water and exposure through pesticide use in gardens, lawns, or buildings (residential and other indoor uses).

For TBHQ, a qualitative assessment for all pathways of human exposure (food, drinking water, and residential) is appropriate given the lack of human health concerns associated with the low levels of exposure to this chemical when used as an inert ingredient in pesticide formulations.

VIII. Cumulative Exposure

Section 408(b)(2)(D)(v) of FFDCA requires that, when considering whether to establish, modify, or revoke a tolerance, the Agency consider "available information" concerning the cumulative effects of a particular pesticide's residues and "other substances that have a common mechanism of toxicity."

Unlike other pesticide ingredients for which EPA has followed a cumulative risk approach based on a common mechanism of toxicity, EPA has not made a common mechanism of toxicity finding as to TBHQ and any other substances and TBHQ does not appear to produce a toxic metabolite produced by other substances. For the purposes of this tolerance action, therefore, EPA has not assumed that TBHQ has a common mechanism of toxicity with other substances. For information regarding EPA's efforts to determine which chemicals have a common mechanism of toxicity and to evaluate the cumulative effects of such chemicals, see the policy statements released by EPA's Office of Pesticide Programs concerning common mechanism determinations and procedures for cumulating effects from substances found to have a common mechanism on EPA's website at http://www.epa.gov/pesticides/cumulative/.

IX. Human Health Risk Characterization

Based on the available studies, TBHQ is of low toxicity in rodent studies which is useful for extrapolating human health effects. No quantitative or qualitative susceptibility was observed in reproductive and developmental rodent studies for TBHQ Also, the effects seen in the offspring were at levels where effects were also seen in the adults.

After a comprehensive review of the available toxicity data, the European Food Safety Authority (EFSA; 2004) concluded that TBHQ, when used as an antioxidant in edible oils and fats, would not pose a serious health risk to the general population under normal exposure conditions, as the daily oral for all population subgroups would fall below the ADI of 0.7 mg/kg bw/day. TBHQ is readily metabolized in the body. Based on the available toxicity information at various durations, concentrations, and routes of exposure along with the anticipated inert uses of TBHQ, dietary and residential exposures of concern are not likely from the use of TBHQ as an inert ingredient in pesticide formulations.

Taking into consideration all available information on TBHQ, it has been determined that there is a reasonable certainty that no harm to any population subgroup will result from aggregate exposure to TBHQ when considering exposure through food commodities and all other non-occupational sources for which there is reliable information. Therefore, it is recommended that the one exemption from the requirement of a tolerance established for residues of TBHQ when used as an antioxidant under 40 CFR 180.920 can be considered reassessed as safe under section 408(q) of the FFDCA.

X. Ecotoxicity and Ecological Risk Characterization

Based on an SAR analysis of effects using the Quinone/Hydroquinone chemical class, TBHQ would be classified as very highly toxic to aquatic organisms. SAR predicted a freshwater fish LC_{50} of 80 µg/L and a saltwater fish LC_{50} of 67 µg/L. SAR predicted a freshwater invertebrate LC_{50} of 52 µg/L and a mysid shrimp LC_{50} of 67 µg/L.

SAR reported a freshwater algal EC_{50} of 92 µg/L and a saltwater algal EC_{50} of 8 µg/L. Chronic effects estimates were not predicted with the model for this class of compounds. There was a single study encompassing several aquatic species identified in the Agency's Ecotox Database (http://www.epa.gov/ecotox). The tests were run for 48 hours under static condition looking at mortality with the exception of Zebra mussels where one study looked at detachment from substrate. The most sensitive species tested was Bluegill sunfish, LC_{50} of 1507 µg/L. Additionally, the 48h LC_{50} for Rainbow trout and Channel was determined to be 370 µg/L. A Zebra mussel EC_{50} of 1000 µg/L for detachment and an LC_{50} of 118,000 µg/L for mortality was also determined in this study.

Considering the physical properties of the compound, aquatic exposures are possible. Acute effects to aquatic species (listed and non-listed) are likely if application rates exceed more than a pound per acre. Chronic effects are largely unknown. Effects due to TBHQ degradates are unknown. Terrestrial risks are likely to be lower than for aquatic species based on available mammalian data used as a surrogate for other terrestrial phase animals.

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