Safety Evaluation and Biochemical Behavior of Monotertiarybutylhydroquinone[1]

B.D. ASTILL, C.J. TERHAAR, W.J. KRASAVAGE, G.L. WOLF, R.L. ROUDABUSH, D.W. FASSETT, Health and Safety Laboratory, Eastman Kodak Company, Rochester, New York 14650 and K. MORGAREIDGE, Food and Drug Research Laboratories, East Orange, New Jersey 07019

ABSTRACT

The safety of monotertiarybutylhydroquinone as an oil-soluble food grade antioxidant was evaluated in acute studies with rats and dogs, in subacute feedings in rats, in rat reproductive efficiency and placental transfer studies, and in subacute feedings with monotertiarybutylhydroquinone heated in vegetable oil. In lifetime feedings in rats and 2 year feedings in dogs, wt gain, feed consumption, behavior, mortality, hemograms, clinical chemistries, gross, microscopic, and electron microscopy were evaluated. There were no toxic or untoward effects. Monotertiarybutylhydroquinone was handled similarly by rats, dogs, and humans. Rats eliminated single oral 0.1-0.4 g/kg doses mostly in the urine in 3-4 days as the 4-0-sulfate (57-80%) and the 4-0-glucuronide (4%) and with 4-12% unchanged. 2,3,5,6-^{14}C-Monotertiarybutylhydroquinone was eliminated similarly with <0.1% as $^{14}CO_2$ and <0.2% remaining in the animal after 4 days. Oral 0.1 g/kg doses to dogs gave a somewhat higher glucuronide contribution. The elimination pattern was little altered in long term feedings. Humans eliminated 0.002 g/kg single doses (high fat vehicle) almost completely in the urine in 2-3 days, with <0.1% unchanged, 73-88% as the 4-0-sulfate, and 15-22% as the 0-glucuronide. Monotertiarybutylhydroquinone residues in most tissues of long term animals were below lower detection limits or negligible. Monotertiarybutylhydroquinone did not induce liver microsomal mixed function oxidases in short and long term rat and dog feedings. The feeding studies and comparative biochemical studies showed monotertiarybutylhydroquinone to be safe for its intended use.

INTRODUCTION

Monotertiarybutylhydroquinone (TBHQ), 2-t-butylhydroquinone, was approved as a food grade oil-soluble antioxidant in a food additive regulation (CFR 21.121.1244) published in 1972 (1). The total concentration in food based upon the fat and oil content was not to exceed 200 ppm (0.02%) including other fat stabilizers such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), and propyl gallare (PG). This paper reviews the extensive toxicology and biochemical studies in the petition for the regulation (Table I).

EXPERIMENTAL PROCEDURES

Feeding Studies

TBHQ was fed to 275 male and 275 female Sprague-Dawley rats for 20 months at 0.016%, 0.05%, 0.16%, and 0.5% of the diet. Animals were sacrificed and studied at 6, 12, and 20 months. In addition, a 3-generation, 6 mating (2/generation) reproductive study was made with rats receiving 0.5% TBHQ in their diet. The F_0 group (15 males and 15 females) was derived from 30 male and 30 female Sprague Dawley rats. Littering throughout the study produced 2090 rats. A 6 month feeding study also was made to test the effect of heated TBHQ. Groups of 15 male and 15 female rats at levels of 0.02, 0.10, and 0.50% of TBHQ in oil were used. Cottonseed oil with added TBHQ was either unheated or heated during 1 hr to 375 F and held at 375 F for 3 hr. These oils then were incorporated into the diet at a level of 5.0%.

Male and female beagles (4 each) at each level were fed diets containing 0.05%, 0.158%, or 0.5% TBHQ. Males and females (8 each) served as controls, amounting to 40 dogs in all. Feedings were continued for 2 years, when the dogs were sacrificed and studied. Parameters in dog and rat feedings are reported in "Results and Discussion."

Fate and Biochemistry

Fate studies were performed on rats, dogs, and human volunteers. The latter were selected and dosed under medical supervision. Rats and dogs were maintained in stainless steel metabolism cages, including selected animals from long term feedings. In labeled studies, rats were kept in all glass metabolism cages. Urinary O-sulfate, glucuronide, and chro-

TABLE I

Toxicology and Biochemistry Studies on Monotertiarybutylhydroquinone (TBHQ)

Acute and short term in rats
Long term rat feedings
Reproductive performance, 3 generations in rats
Heated fat feedings to rats
Two year dog feedings
Fate and metabolism in rats and dogs
Fate and metabolism in humans
Effect upon liver microsomal enzyme systems
Residues in long term feedings

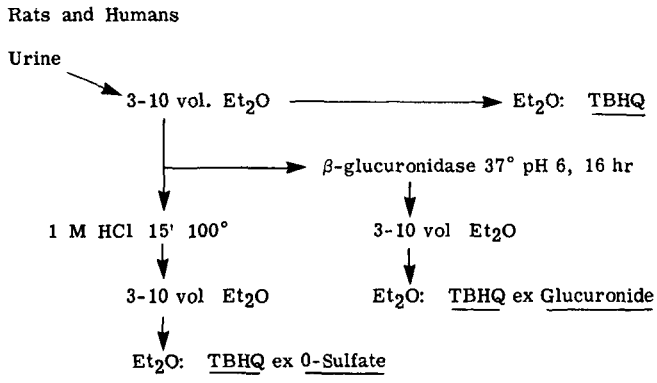

FIG. 1. Extraction scheme for quantitation of monotertiarybutylhydroquinone (TBHQ) and metabolites in urine. TBHQ in extracts or hydrolysates was estimated by isotope dilution for labeled studies or gas chromatographically.

[1]One of nine papers presented in the symposium, "Toxicology and Biochemistry of Food Additives Used in Fats and Oils," at the AOCS Fall Meeting, Chicago, September 1973.

matographic procedures have been described (2). Metabolite isolation employed the acridinium salt technique (3).

Radioactivities were measured by liquid scintillation spectrometry. Quantitation TBHQ and its metabolites in urine was achieved with the scheme in Figure 1, applied to labeled studies in rats and unlabeled studies in humans. Recoveries were worked out with labeled TBHQ and usually exceeded 95%. TBHQ was determined in extracts of hydrolysates by reverse isotope dilution or by gas chromatography of the trimethylsilyl ether of TBHQ on 3% of SE-30 on Chromport XXX (80-90 mesh) with flame ionization detection.

The procedure for cleanup and assay of tissues in long term studies for residual TBHQ involved homogenizing in $CHCl_3$ followed by hexane extraction and transfer to 80% ethanol; removal of solvent; and taking up in benzene. Benzene extracts were chromatographed on silica gel, when TBHQ was eluted from the column in ether and estimated spectrophotofluorometrically. Recoveries were worked out with labeled TBHQ. The procedure as used with liver, kidney, brain, and fat gave sensitivities down to the ppm level in wet tissues.

Liver microsomal enzyme specific activities were measured as follows: glucose-6-phosphatase (4), p-nitroanisole demethylase (4), bilirubin glucuronyl transferase (4), and aniline hydroxylase (5).

RESULTS AND DISCUSSION

Acute and Short Term Feedings

The acute oral LD_{50} of TBHQ as a 5.0 or 10% solution in corn oil in rats was between 700-1000 mg/kg, and the IP LD_{50} was between 300-400 mg/kg. No estimate of the oral LD_{50} in dogs was possible, since doses of 400 mg/kg or higher caused regurgitation with loss of the dose. There were no other effects. TBHQ did not cause skin irritation, nor was it a skin sensitizer. In a preliminary 22 day feeding study in rats at a level of 1.0% of the total diet, TBHQ produced a slight wt gain impairment but no associated mortality or pathology.

Long Term Rat Feedings

TBHQ produced no differences from controls in behavior, growth rate, feed intake, diet efficiency, or mortality. At necropsy, there were no differences in absolute or relative wt of lung, liver, kidney, heart, adrenals, testes, or spleen as compared with controls. There was a slight decrease from controls in the absolute brain wt of the male rats fed 0.5%, occurring only in male rats sacrificed at 20 months. Thus, absolute brain wt were (intake, wt in g) 0%, 2.35; 0.016%, 2.35; 0.05%, 2.20; 0.16%, 2.19; and 0.5%, 2.19. There was no statistical difference between the means at intake levels 0-0.16%. The slight decrease at 0.5% was not associated with any pathology or behavioral change. The apparent contradiction, that the 0.5% and 0.16% groups had mean absolute wt of 2.19 g yet were significantly different, is a result of rounding. Also, in Duncan's Multiple Range Test used in analyzing these results, significance is related to the number of means spanned.

Hemograms consisting of hemoglobin, hematocrit, white cells and differential counts, were made every 5 months, and all were normal. Clinical chemistries, consisting of serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), serum alkaline phosphatase (SAP), blood urea nitrogen (BUN), and total protein done at the same time were all normal. Serum lipids were not examined. Urinalyses, consisting of specific gravity, pH, albumin, sugar, and microscopic examinations, were done at termination on the male and female survivors in the two high intake groups and the controls. The results of all were normal.

At the time of death or sacrifice, a complete autopsy was made. Gross and microscopic examinations were made of trachea, lung, heart, tongue, esophagus, stomach, small intestine, large intesting, liver, kidney, bladder, adrenal, pancreas, thyroid, gonads, uterus, spleen, bone marrow, cerebrum, cerebellum, and eyes. There were no compound-related changes in any of the organs examined. The most prevalent tumors seen were mammary gland fibroadenomas or cystic fibroadenomas; all neoplasms occurred randomly in all groups, including the controls, with no predilection to intake level.

Reproductive Performance in Rats

Gonadal function, estrus, mating, conception rates, gestation times, parturition, and lactation of all experimental groups were similar to the controls. There was a slight, questionable increase in the F_1 pup mortality and a slight, questionable decrease in F_1 pup growth wt. In a second experiment designed to investigate these results in more detail, no effect could be seen.

The F_{3b} pups were taken by Caesarian section and examined grossly and for skeletal and soft tissue abnormalities. There were no differences between the experimental groups and their controls. The F_{3a} groups remained on their diets for 11 months, at which time they were sacrificed, and livers of some individuals were examined under the electron microscope. No abnormalities were seen.

Pregant females from the F_{1a} generation were given ^{14}C-labeled TBHQ prepared for fate studies (see below) 1 day prior to littering. Elimination of the label was similar to other experimental rats described below. Traces of ^{14}C were found in fetuses, amounting to ca. 0.2% of the dose at 7.6 hr and 0.02% at 16.7 hr after treatment. These were removed rapidly, thus indicating the pregnant females and their fetuses handle TBHQ similarly to nonpregnant rats. Levels in amniotic fluid and uteri were similar to those in the fetus and decreased at ca. the same rate.

Rat Feedings with TBHQ in Heated Fats

In its use as an oil antioxidant, TBHQ may be heated prior to ingestion. In feeding heated oils containing TBHQ to rats, the same parameters as described in the 20 month rat feeding study were evaluated in comparison with controls.

The growth, feed use, behavior, urinalyses, hemograms, clinical chemistries, and mortality were almost all normal, as were the absolute and relative organ wt at autopsy. A slight increase in relative mean liver wt was noted in the 0.5% group, which was 3.58% compared with a control 3.32%. To show a significant difference in this statistic, the heated and nonheated groups had to be combined. Even so, the difference was of a low order. In addition, there was a slight depressed SGOT value in the male group receiving 0.5% TBHQ heated in cottonseed oil at both 3 and 6 months, amounting to ca. 190 Karmen Units compared with 223 in controls. This was not correlated with any changes in alkaline phosphatase or any pathological changes. This effect was not seen at the lower intake levels of oil heated TBHQ nor in the female groups. A slight statistical decrease in SGOT is not evidence of cellular damage.

Two Year Dog Feeding

The 2 year dog feeding program was conducted at the Food and Drug Research Laboratories, Waverly, N.Y. Blood and urines were examined twice prior to initiation of the study, and at five intervals throughout the study. Blood studies included hemoglobin, hematocrit, white cell and differential counts, platelets, BUN, glucose, SAP, lactic dehydrogenase (LDH), protein, total and direct bilirubin, albumin, albumin-globulin (A/G) ratio, SGPT, and reticulocytes. A slight depression of hemoglobin and hematocrit

TABLE II

Disposition of Single Oral Doses of Monotertiarylbutylhydroquinone (TBHQ) by Rats, Dogs, and Male Humans[a]

Species	Dose g/kg	No. of animals	Percent of dose eliminated in urine[b]				In feces	As CO_2	During (days)	Total accounted for
			Unchanged	O-SO_2O^-	O-gluc'de	Total				
Rat	0.10	2 x 6	(12?)	80	4				3	96
	0.20	2 x 6	0.6-8.0	60, 61	2, 8.5				3	70
		1 x 8								
	0.40	2 x 6	4	57	4				4	65
Rat (TBHQ-^{14}C)	0.015-0.92	3	0.8-1.3[c]	57-74[c]	0.9-3.3[c]	82-88	2.4-3.7	<1	4	86-92[d]
	0.38, 0.40	2				74-80	6.0, 6.0	<1	4	80, 86[d]
Dog	0.10	2 x 3	3	69, 85	31, 24				3	100, 112
Humans (HF)	0.002	2	<0.1	73,88	15, 22				2-3	95, 103
(LF)	0.001-0.002	8	<0.1	18-51	0-6				2-3	18-51

[a]Values are percent of given dose as averages or ranges. Rats received oral intubations of a 10% corn oil solution of TBHQ, dogs received TBHQ as a meat capsule. Humans received TBHQ incorporated in high fat (HF) or low fat (LF) content food.

[b]O-SO_2O^- = as increased O-sulfate output; O-gluc'de = O-glucuronide output.

[c]In 24 hr after dose.

[d]Tissues, organs, and carcass account for <0.2% of the dose.

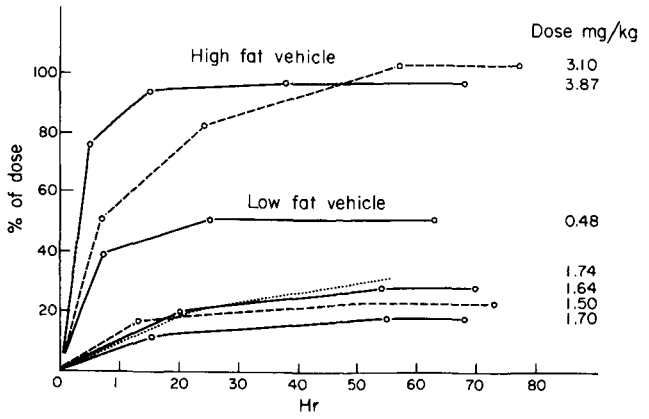

FIG. 2. Total urinary cumulative excretions of monotertiarybutylhydroquinone (TBHQ) by humans. Each curve represents total TBHQ elimination by one subject receiving the indicated dose in food containing 30% of corn oil (high fat vehicle) or 10% (low fat vehicle). Total TBHQ includes unchanged TBHQ and TBHQ eliminated in urine as O-sulfate or O-glucuronide conjugates. The extraction scheme is given in Figure 1.

values in the 0.5% group was within the normal range for these animals. During the ninety-ninth week and one-hundred-fourth week, the high intake group exhibited an increased reticulocyte count. Two female control dogs also had an increased reticulocyte count, and the final reticulocyte counts revealed no definite pattern with respect to TBHQ intake. Urinalyses consisted of glucose, albumin, specific gravity, pH, occult blood, ketone bodies, urobilinogen, and microscopic examination and were normal.

At the time of sacrifice, the following organ wt of the 0.5% group were taken and compared to the controls: liver, kidney, spleen, heart, brain, lung, gonads, adrenal, thyroid, and pituitary. There were no differences between the control and experimental groups. Microscopic examination of liver, spleen, gall bladder, stomach, intestines, pancreas, kidney, urinary bladder, adrenal, gonads/adnexa, pituitary, thymus, thyroid, salivary glands, lymph nodes, heart, lung, marrow, aorta, skin, muscles, spinal cord, and brain from all experimental and control dogs showed them to be normal. Additionally, livers and kidneys of dogs on the high diet level were examined under the electron microscope and found to be normal.

Fate of TBHQ in Rats and Dogs

The earliest information on the biochemistry of TBHQ was reported from this laboratory in 1960 when TBHQ was identified as the major metabolite of the well known antioxidant BHA in dogs (2). The occurrence of TBHQ as a metabolic product of BHA, thus, implies the existence of a prior indirect evaluation of its behavior in animal feeding studies. Urinary chromatograms of dogs receiving BHA or TBHQ are almost identical when sprayed with Gibbs' reagent for phenols.

Conventional studies with orally administered unlabeled TBHQ in rats and dogs established urinary elimination as the principal route of elimination (Table II). In the rat, O-sulfate conjugation was the principal metabolic pathway, with a small contribution from O-glucuronide formation. A very small amount of the dose was accounted for as uncombined phenolic materials. Single oral doses (100 mg/kg) were almost entirely eliminated in the urine within 48 hr of dosing. Both the proportions eliminated in the urine and the speed of elimination decreased with higher doses, ca. 65% of 400 mg/kg doses being accounted for in the urine in 4 days.

Single 100 mg/kg oral doses to dogs, given in a meat capsule, were eliminated mostly in the urine as unchanged TBHQ and O-glucuronide and O-sulfate conjugates. Compared with the rat, less of the dose was accounted for as sulfate and more as glucuronide. At the 100 mg/kg intake level in both species, urinary excretion accounts for almost all of the dose of TBHQ in 24 hr.

The availability of long term rats and dogs allowed us to use periodic glucuronide and sulfate measurements to detect changes in overall metabolic pattern with prolonged feeding. After 20 months of feeding to rats and 2 years to dogs of up to 0.5% in the diet, TBHQ metabolic patterns in the urine remained unaltered in the dog at all levels. The proportion of glucuronide in the rat was elevated somewhat on prolonged feeding. Urinary chromatograms showed the same metabolites throughout.

Further insight into the disposition of TBHQ was obtained with carbon-14 labeled TBHQ, obtained by tert-butylation of 2,3,5,6-^{14}C hydroquinone. As with the unlabeled material, single doses to rats (Table II) were eliminated mostly in the urine in the first 1-2 days of dosing. Levels of carbon-14 labeled CO_2 in expired air were negligible, indicating that TBHQ was not handled by the intermediary metabolism. As with doses of unlabeled TBHQ, the proportion of the dose eliminated via the urine diminished with increasing intake. The balance of the dose appeared to be in the feces.

Metabolites of TBHQ in Rat and Dog

The major urinary metabolite of TBHQ in the dog, TBHQ sulfate, was isolated as the 9-amino-acridinium salt, followed by ion exchange and isolation of the potassium salt. Methylation of the isolated conjugate and hydrolysis to the 2-isomer of BHA indicated that sulfate conjugation occurred with the 4-hydroxyl. The glucuronide was isolated as the methyl O-tetraacetylglucuronide ester, and it is assumed by analogy that O-glucuronide formation occurs with the 4-hydroxyl group. The isolated sulfate conjugate had the chromatographic properties of the major metabolite detected by chromatography in dog and rat urines.

Isotope dilution studies with the appropriate extracts from the urine of rats receiving 15-92 mg/kg doses of TBHQ confirmed the unlabeled observations (Table II). The principal metabolite in rats, accounting for over 90% of the 24 hr urinary radioactivity was the O-sulfate conjugate, and the small glucuronide contribution was established conclusively. The radioactivity in the urine for the remaining days of elimination was distributed similarly.

Fate of TBHQ in Humans

The extraction and assay procedure was used to determine the fate of TBHQ in humans. A range of single doses 0.5-4 mg/kg was used with various modes of intake. No physiological effects were noted in any subject. Cumulative excretions of combined and free TBHQ (Fig. 2), showed urinary recoveries of TBHQ to depend upon the vehicle. Thus, in a medium which contained 30% corn oil, almost all of the dose was recovered in the urine within 40 hr. In a vehicle containing 10% corn oil, urinary elimination accounted for less than half of the intake. Levels of TBHQ in serum, measured spectrophotofluorometrically, reflected the urine concentration and the fall in urinary output of total TBHQ. It was concluded that TBHQ in a high fat vehicle was almost completely absorbed but that absorption was much lower in a low fat vehicle.

The metabolism of TBHQ by humans was markedly similar to that by rats and dogs (Table II), doses in either high or low fat vehicles producing mostly the O-sulfate conjugate of TBHQ. A much smaller varying proportion of the glucuronide, with no detectable free TBHQ, occurred. Thus, there are good reasons for extrapolating from these very extensive animal feeding studies to humans in evaluating the safety of TBHQ. In dogs, rats, and humans, TBHQ is handled by the same metabolic pathways, which are those commonly found for dihydric phenols in mammals (6).

TABLE III

Effect of Dietary Feeding of Monotertiarybutylhydroquinone (TBHQ) upon Rat Liver Microsomal Enzyme Activities[a]

Dietary intake	Feeding (days)	Specific activities of liver microsomal enzyme[b]		
		Glucose-6-phosphatase[c]	p-NO_2 anisole demethylase[d]	Aniline hydroxylase[d]
No additive	21	1.92 (1.73-2.35)	104 (97-114)	30 (12- 62)
100 mg/kg intraperitoneal injection phenobarbital/day	5	1.55 (1.37-1.78)	525 (437-634)	99 (98-106)
0.20% Butylated hydroxyanisole	21	1.31 (1.18-1.64)	219 (165-244)	51 (14- 60)
0.20% TBHQ	21	1.91 (1.70-2.24)	164 (137-194)	47 (38- 51)
0.05% TBHQ	21	1.44 (1.20-1.62)	94 (64-110)	34 (31- 38)

[a]Average values from eight rats at each level.

[b]Ranges in parenthesis.

[c]μM Pi/15 min/mg protein.

[d]Optical density x 10^5/min/mg protein.

TABLE IV

Monotertiarybutylhydroquinone (TBHQ) in Tissues and Organs of 2 Year Dogs and 20 Month Rats[a]

Species	Intake		Sex[b]	ng TBHQ in organ			Mg TBHQ in fat[c]
	Percent	Total (g)		Liver	Kidney	Brain	
Dog	0.05	110	M (4)	15	nd	31	4
			F (4)	100	4	nd	nd
	0.16	350	M (4)				5
			F (4)				5
	0.5	1100	M (4)	80	nd	nd	nd
			F (4)	2	nd	7	nd
Rat	0.16	21	M (5)	nd	nd	2	nd
		16	F (5)	9	nd	0	nd
	0.5	63	M (4)	nd	4	nd	nd
		47	F (4)	3	7	nd	nd

[a]Based upon average net fluorescence of extractives at sacrifice 24 hr after last intake.

[b]Numbers in parentheses are animals where tissues were analyzed at each level. Organs and fat samples were pooled for each assay.

[c]Estimated total body fat of rats, 30 g; of dogs, 1 kg.

Effect of TBHQ upon Mixed Function Oxidases

Many organic compounds produce the well known phenomenon of liver microsomal mixed function oxidase induction. The effects of TBHQ, BHA, and phenobarbital were compared on glucose-6-phosphatase, p-nitroanisole demethylase, and aniline hydroxylase activities in the liver microsomal fraction of rats (Table III).

Phenobarbital produced the expected depression in glucose-6-phosphatase, elevation of p-nitroanisole demethylase, and some elevation of aniline hydroxylase activities. As expected, 21 days of feeding BHA produced only a moderate induction of p-nitroanisole demthylase and aniline hydroxylase (7). At the 0.2% dietary level, TBHQ was without effect upon glucose-6-phosphatase and produced elevations in p-nitroanisole demethylase and aniline hydroxylase somewhat less than those due to BHA in 21 days. The effect of TBHQ upon glucose-6-phosphatase at the 0.05% level is comparable with that due to BHA at the 0.2% level and appears to be anomalous. TBHQ is also less active than BHT and BHA in inducing liver microsomal bilirubin glucuronyl transferase activity.

The same liver microsomal enzyme activities in livers from high dose long term rats and dogs and in high dose rats from heated fat feedings, showed no differences from control levels. Similar enzyme activities for the F_{3a} generation in the rat reproduction study were determined after 11 months at the 0.5% level, and no significant differences from controls were found. The 21 day effects probably represent acclimation.

The absence of appreciable long term effects upon these enzyme systems agrees with the absence of functional liver enlargement in long term rats and dogs. Normal liver electronmicrographs were seen in livers from these animals with no proliferation of smooth surfaced endoplasmic reticulum.

Tissue Residues

Some indication that these might be inconsequential resulted from levels of radioactivity in liver, kidney, brain, and fat after single oral does of labeled TBHQ and after low level dietary feeding of labeled TBHQ for 17 days. Normal levels of radioactivity, expressed as μg of TBHQ/g wet tissues were liver, 0.06-0.34; kidney, 0.09-0.38; brain, 0.06-0.56; and fat, 0.06-0.37. Levels from experimental rats were within these background levels or were less than twice background. It was concluded that a short term feeding of TBHQ left no body burden.

Residues in liver, kidney, brain, and fat from selected long term rats (Table IV) were either less than background or negligible. In view of the size of the intake and the presence of low levels of TBHQ in serum at sacrifice, there is clearly no tendency for accumulation of significant levels of TBHQ in long term rats. Similarly, with long term dogs (Table IV), the small quantities of TBHQ in the liver are commensurate with the dogs being sacrificed a few hr after the last intake of TBHQ. The approximate totals of 4-5 ppm in fat are a very minute fraction of the total intake and probably originate from circulating TBHQ in the vasculature.

Dose Response Considerations

The feeding and metabolic studies indicate that the level at which untoward effects are produced by TBHQ in rats and dogs exceeds 0.5% in the diet (over 300 mg/kg/day). The maximum daily intake of TBHQ by man, assuming all the dietary fat to be stabilized by TBHQ as the sole anti-

oxidant at the maximum permitted level (200 ppm), would be ca. 20 mg or 0.3 mg/kg/day. The U.S. Food and Drug Administration has estimated that the current use of phenolic antioxidants in the American diet leads to a daily intake of ca. 4 ppm (8), i.e. <0.1 mg/kg. It is expected that this now will include TBHQ. Based upon the types of food likely to be stabilized by TBHQ, we estimate the probable daily intake of TBHQ to be 0.02-0.07 mg/kg. From these assessments of intake, the studies described above provide safety margins of 1000-10,000 for TBHQ.

ACKNOWLEDGMENT

Monotertiarybutylhydroquinone for these studies was provided by Tennessee Eastman Company, Kingsport, Tenn.

REFERENCES

1. Fed. Reg. 37:25357 (1972).
2. Astill, B.D., J. Mills, D.W. Fassett, R.L. Roundabush, and C.J. Terhaar, J. Agr. Food Chem. 10:315 (1962).
3. Dodgson, K.S., F.A. Rose, and B. Spencer, Biochem. J. 60:346 (1955).
4. Krasavage, W.J., K.S. Tischer, and R.L. Roudabush, Toxicol. Appl. Pharmacol. 22:400 (1972).
5. Kato, R., and J.R. Gillette, J. Pharm. Exp. Therap. 150:279 (1965).
6. Williams, R.T., "Detoxication Mechanisms," John Wiley & Sons, New York, N.Y., 1959, p. 303.
7. Gilbert, D., and L. Golberg, Fd. Cosm. Toxicol. 3:417 (1965).
8. U.S. Department of Health, Education, and Welfare, "FDA Fact Sheet," CSS-F19-870, U.S. Department of Health, Education, and Welfare, Rockville, Md., 1970.

[Received January 7, 1974]