Table V. Methyl Esters 2, 3, 4, and 5 Derived from Catechol Units (Mole Percent of Total Yields)

Methyl esters	Turnip rapeseed cultivars		
	Bele	Lute	Sv. 72/60029
2	81	66	7
3	3	7	3
4	51	35	4
5	94	91	44

Table VI. Methyl Esters 2, 3, 4, and 5 Derived from Polyphenols and Lignin[a]

Methyl esters	Turnip rapeseed cultivars		
	Bele	Lute	Sv. 72/60029
(A) From Lignin			
2	1.9	2.3	3.5
3	1.6	2.0	2.3
4	0.5	0.6	0.9
5	0.2	0.2	0.2
(B) From Polyphenols			
2	7.9	4.4	0.3
3	0.1	0.1	0.1
4	0.6	0.3	0
5	3.4	2.0	0.1

[a] Yields in milligrams per gram of plant material.

cultivars have high contents of polyphenols, Bele containing nearly twice as much as Lute (esters **2**, **4**, and **5**). The low yield of **3** indicates negligible amounts of hydrolyzable tannins (yielding gallic acid on hydrolysis). Very little tri-*O*-methyl gallate (**3**) is derived from *O*-methoxycatechol structures and this material may have been formed by sulfide ion promoted cleavage of some aromatic methoxyl groups during the alkaline hydrolysis preceding oxidative degradation (Sarkanen et al., 1963). White mustard and Sv. 72/60029 hulls contain very little polyphenols. The lignin content shows a moderate increase going in the opposite direction (dark → yellow).

The major chemical difference between yellow-hulled and dark-hulled seeds is thus in the polyphenol (condensed type) content.

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d-α-Tocopheryl Poly(ethylene glycol) 1000 Succinate. Acute Toxicity, Subchronic Feeding, Reproduction, and Teratologic Studies in the Rat

Walter J. Krasavage* and Clarence J. Terhaar

The acute oral LD_{50} of *d*-α-tocopheryl poly(ethylene glycol) 1000 succinate (TPGS) and either of its moieties, poly(ethylene glycol) 1000 and *d*-α-tocopheryl acid succinate, is >7000 mg/kg for young adult rats of both sexes. When tested in 2-day old neonates, mechanical injury at the time of gavage made calculation of an LD_{50} impractical. However, the data did indicate that TPGS is no more toxic than the materials from which it was made and is somewhat less toxic in female rats than in males. When incorporated in the diet and fed for 90 days in concentrations of 0.002, 0.2, and 2.0% to male and female rats, TPGS had no adverse effects on body weight gain, food consumption, hematology, organ weights, serum chemistries, or micromorphology. When these animals were bred for two litters of a one-generation reproduction study, the reproductive indexes of the treated groups were unaffected and the offspring developed normally. A separate teratology study in which pregnant females ingested 0.002, 0.2, and 2.0% TPGS in the diet during the period of organogenesis revealed no congenital abnormalities attributable to TPGS.

d-α-Tocopheryl poly(ethylene glycol) 1000 succinate (Eastman Vitamin E TPGS) (TPGS) is a water-soluble form of vitamin E prepared from crystalline *d*-α-tocopheryl acid succinate by esterification of the acid group with poly(ethylene glycol) having an average molecular weight of 1000. It is a pale yellow waxy substance which provides 260 mg of *d*-α-tocopherol per g (387 IU) and it forms a

Health, Safety, and Human Factors Laboratory, Eastman Kodak Company, Rochester, New York 14650.

clear solution in water at concentrations up to 20%. It is practically tasteless and stable in air and the solutions do not hydrolyze on normal handling and storage. TPGS can be used in the preparation of oral solutions of vitamin E for humans and farm animals, aqueous dispersions of vitamin E containing TPGS and additional *d*-α-tocopheryl acetate, aqueous dispersions of vitamins E and A, and aqueous multivitamin dispersions of A, D, and E with water-soluble vitamins. It may also be used to fortify water-solubilized vitamin preparations with vitamin E ("Eastman Vitamin E TPGS, d-alpha-Tocopheryl Polyethylene Glycol 1000 Succinate, A Water Soluble Vitamin E for Oral Use", 1973).

Tocopherols in general are found in many organic materials including foods. Consequently α-tocopherol is a regular part of the human diet. The majority of feeding studies done with α-tocopherol in experimental animals are concerned with vitamin deficiency. Few studies have been done on the effects of feeding an excess of vitamin E. None of these were long-term studies and only a limited amount of data are available on the acute toxicity of tocopherol. Short-term feeding studies and a few reproduction studies have been reported. An excellent review of these studies and the available scientific literature from 1920 to 1972, related to the "safety" of tocopherols, is presented in a report compiled by Informatics, Inc. for the Food and Drug Administration ("GRAS (Generally Recognized as Safe) Food Ingredients—Tocopherols", 1973).

The studies described here were initiated to determine the acute and subchronic toxicity and the effects on reproduction in the rat by *d*-α-tocopheryl poly(ethylene glycol) 1000 succinate (TPGS).

MATERIALS AND METHODS

Acute Toxicity. Mature rats (Charles River COBS, CD) of both sexes were fasted for 16 h prior to administration by gavage of 7000 mg/kg body weight of either TPGS, poly(ethylene glycol) 1000 (PG1000), or *d*-α-tocopheryl acid succinate NF (DTS). This was the highest dose practicable. The PG1000 and DTS were tested to ascertain if TPGS was more toxic than its moieties. The treatment groups consisted of ten animals of each sex. All animals were observed for mortality and/or clinical signs of toxicity for 2 weeks after treatment at which time gross necropsies were performed. The acute toxicity of TPGS and either of its moieties was also tested in neonates. Untreated mature male and female rats were mated to provide the animals for this study. The pregnant females were observed near parturition and the day (8:30 a.m.) pups were first seen was considered day 0. A randomization scheme, set up prior to any births, was designed to provide 14 to 20 pups of each sex to each of 12 groups, 4 treatments at 3 dose levels each. The pups were assigned to compounds and dose level regardless of dam.

At 2 days of age, the pups were sexed, weighed, individually identified by toe amputation, and treated.

Treatment consisted of administration by gavage of 0.15, 0.1, or 0.075 ml/pup of either TPGS, PG1000, DTS, or corn oil (control). The size of the pups at 2 days of age and the syringes available made dosage on a milligrams per kilogram basis impracticable. The intubation was accomplished using a 0.25-ml syringe fitted with a 1.0 in., 22 gauge needle with a 1.25-ml ball tip (Popper and Sons, Inc., New Hyde Park, N.Y.). All animals were observed daily and body weights were recorded at 3, 10, and 17 days after treatment.

Subchronic Feeding. Charles River (COBS, CD) albino weanling rats were randomly assigned to four groups, each containing 30 rats per sex. TPGS was fed at concentrations of 0.002, 0.2, and 2.0% in a basal diet of ground Purina Laboratory Chow supplemented with 5.0% corn oil. A control group received the ground chow–corn oil diet only.

The Food and Nutrition Board of NAS ("Recommended Dietary Allowances", 1974) recommended a daily allowance of vitamin E for adult humans of 12–15 IU/day for the female and male, respectively; consequently, an adult may consume up to 0.2 mg/kg per day of vitamin E depending on sex and the form (natural or synthetic) of α-tocopherol ingested. The recommended daily intake for infants, birth to 1 year of age, is from 0.3 to 0.6 mg/kg.

The 0.002% dose level of TPGS fed in this study provided a 200-g rat, which ingested 20 g of diet per day, with a daily intake of 0.5 mg/kg of vitamin E. The mid and high dose levels provided 100 and 1000 times this amount. In addition, the Purina Laboratory Chow which contains 29 IU of natural and synthetic α-tocopherol (Shelton, 1976) provided the 200-g rat ingesting 20 g of diet per day with 5.2 mg/kg per day of vitamin E.

TPGS was obtained from Distillation Products Industries (DPI), a division of the Eastman Kodak Company, and material from the same lot was used in all studies. The experimental diets were prepared by solubilizing the TPGS in the corn oil with the aid of stirring and gentle heat (55–60 °C). This solution was added to the ground chow and the mixture was homogenized in a cafeteria-type food mixer. The Product Development and Quality Control Laboratory of DPI analyzed the compound for impurities by thin-layer chromatography and, periodically, representative samples of the various batches of the experimental diets were analyzed for total α-tocopherol and the level of TPGS present was calculated.

The animals were housed five per cage in suspended wire-bottom cages. Water and the diets were available ad libitum for 91 days.

The animals were observed for deviations from normal with respect to general behavior, appearance of coat, stool, urine, etc. Individual body weights were recorded prior to treatment and body weights and food consumption were recorded twice during the initial week of feeding and weekly thereafter.

Hematologic and clinical chemistry determinations were made on 15 rats of each sex in the high dose and control groups at 42 and 84 days of treatment. These animals were selected at random, prior to beginning treatment, to be autopsied at termination of the study. The remaining animals in each group were used to begin a reproduction study (see below). The hematologic and clinical chemistry determinations made were hematocrit, hemoglobin concentration, total and differential white cell counts, serum glutamic oxalacetic transaminase (SGOT), serum alkaline phosphatase (SAP), urea nitrogen (UN), lactic acid dehydrogenase (LDH), serum glucose, serum total protein, triglyceride, and cholesterol.

The animals necropsied at termination were killed by CO_2 inhalation and the following tissue samples were taken for examination by light microscopy: lung, bronchus, heart, kidney, bile duct, liver, spleen, brain, meninges, stomach, colon, duodenum, ileum, jejunum, pancreas, esophagus, adrenal, pituitary, thyroid, trachea, lymph node, testis, bladder, ovary, uterus, bone marrow, and tongue. Liver, spleen, brain, pituitary, kidneys, gonads, adrenals, and thyroids were weighed for organ weight comparisons.

Reproduction Study. After 90 days of treatment, half of the animals from each group in the feeding study were designated the parent generation (F_0) and were mated to

produce two groups of first generation litters (F_{1a} and F_{1b}). All animals, including the offspring, were maintained on their respective diets prior to and throughout this study.

F_0 had ingested the diets for 112 days when mated to produce the F_{1a} offspring. The males were randomly assigned to the females within dose groups and paired 1:1 for 2 weeks. Insemination was confirmed by daily vaginal smears and the day sperm was found in the vagina was considered day 0 of gestation. When a female was inseminated, the male was removed from the cage, a nesting pan was introduced and the female was allowed to litter. After the last litter of F_{1a} was weaned, this same procedure was used to produce the litters of F_{1b} except the males were mated to a different female within the group. The F_0 had been on the experimental diets for 175 days at the time of this mating. The litters were not culled and all pups were weaned at 21 days of age.

Mean gestation period, litter size, sex ratio, mortality of pups and parents, and the mean body weights per litter of the pups at 4 days of age, weaning, 1 and 2 weeks after weaning, and at necropsy were recorded along with indexes of insemination, fertility, gestation, viability, and lactation.

All offspring were killed by CO_2 inhalation and necropsied 5 weeks after weaning. Tissues for micropathology were taken from 4 animals of each litter. Where possible, these 4 rats were 2 of each sex. All other pups were examined for gross pathology.

The parent generation was maintained on the diet until it was certain no additional matings were necessary. At this time, days 265–268 of treatment, they were killed with CO_2 and tissues from all major organ systems were collected for microscopic examination.

The organ weights recorded in the feeding study were also recorded at necropsy. Also, 2 weeks prior to sacrifice (255 days of treatment) the same hematologic and blood chemistry determinations made in the feeding study were made on the animals in the high dose and control groups of this study.

Teratology Study. Fifty male and 100 female Charles River COBS, CD albino rats, 90–110 days old, were caged 2:1 to obtain 75 pregnant females of known date of insemination. Insemination was verified by vaginal smears and the day sperm was found in the vagina was considered day 0 of gestation. The inseminated females were randomly assigned to one of five groups and caged singly with food and water available ad libitum. The groups consisted of a negative and a positive control and three treatment groups of 0.002, 0.2, and 2.0% TPGS. The compound was administered via the diet from the 6th to the 16th days of gestation. Prior to this period and on all other days of gestation, all groups received the negative control diet. Except for the positive control, all diets fed in the teratology study were from the same batches used in the feeding study.

Body weight of the females was recorded daily and food consumption was monitored from days 0 to 6, 6 to 16, and 16 to 20 during gestation. On gestation day 20, the females were killed by inhalation of CO_2 and the uteri were exposed by laparotomy. Implantation sites were counted and categorized as being live fetuses, dead fetuses, or resorptions. The fetuses, upon removal of the placentae, were blotted dry, sexed, examined for gross anomalies, and weighed. Half from each litter were preserved in Bouin's fixative and examined for internal soft tissue anomalies according to the free-hand razor blade method of Wilson (1965). The other half was fixed in 95% ethanol, eviscerated, cleared, and stained with alizarin red using a modification of the procedure described by Walker and Wirschafter (1957) and examined for skeletal abnormalities.

Table I. TPGS, Percent Mortality in Neonates

	Dose, ml/pup					
	Males			Females		
Compound	0.15	0.10	0.075	0.15	0.10	0.075
Corn oil	19	0	13	7	21	25
TPGS	67	47	35	42	59	29
PG1000	73	27	7	21	20	38
DTS	60	50	47	47	35	24

Data in all studies were analyzed statistically by analysis of variance, Duncan's multiple range test, or Student's t test $p \leq 0.05$.

RESULTS AND DISCUSSION

Acute Toxicity. Six of 60 mature rats died, 5 within 24 h and 1 within 48 h after treatment. All deaths were attributed to mechanical injury and were distributed as follows: 3 females and 1 male from the DTS group, 1 female from the PG1000 group, and 1 male from the TPGS group. All other animals ate well and gained weight normally. After an initial transient (24 h) period of lassitude and diarrhea, no gross changes in behavior, coat, or stools were noted. Therefore, the acute LD_{50} of TPGS and of each of its moieties for young adult rats of either sex is in excess of 7000 mg/kg body weight.

It was apparent by observation that many of the deaths of the neonates were also due to mechanical injury at the time of intubation. Because of this, no attempt was made to calculate LD_{50}'s from these data. However, the data do indicate that TPGS is more toxic than corn oil, but no more toxic than its moieties (DTS and PG1000) and that the females showed better survival than the males (Table I). The treatments had no adverse effects on the body weight gain and general well being of the survivors during the 2.5 weeks following treatment.

Subchronic Feeding. Thin-layer chromatographic analysis of the lot of TPGS used in all studies revealed that it was 99+% pure. Analysis of the treatment diets revealed levels comparable to those calculated to be present in the high and middle dose levels, while the small quantity of TPGS added to the low dose level was undetectable because of the high level of background α-tocopherol present in the basal diet.

A summary of body weight gain, feed intake, diet and feed efficiency, and dosage is shown in Table II.

The ingestation of TPGS in quantities of 0.32–0.48, 31.5–45.6, and 316.8–443.1 mg/rat per day for 91 days had no deleterious effects on the body weight gain or food consumption of male and female rats. General behavior and appearance were normal.

No significant toxicological effects were revealed by hematologic and clinical chemistry determinations made on the high dose (2.0%) and control animals after 42, 84, and 255 (see Reproduction Study) days of treatment.

No significant effects were seen on the mean body and organ weights of the high dose animals of either sex at necropsy. The absolute liver and kidney weights of the low dose level males (20.85 and 3.67 g) were statistically greater than the control weights (17.77 and 3.35 g); however, the mean body weight of these animals was also increased (512 g vs. 466 g) and when analyzed as percent of body weight, these values did not differ significantly from the controls. The adrenal and pituitary weights of the low dose (0.002%) males as percent of body weight were statistically greater than the controls; however, these differences were not seen at the higher dose levels and

Table II. TPGS Feeding Study; Summary of Feed Intake, Weight Gain, and Dosage

	Males				Females			
	Control	0.002%	0.2%	2.0%	Control	0.002%	0.2%	2.0%
Rat days	2715	2715	2715	2715	2715	2715	2715	2715
Wt gain, g								
Total	12 098	12 802	12 720	11 707	5884	6123	5988	6247
Mean	403	427	424	390	196	204	200	208
Diet eaten, g								
Total	60 959	65 184	61 923	60 152	44 504	42 801	42 765	42 994
g/rat per day	22.5	24.0	22.8	22.2	16.4	15.8	15.8	15.8
Diet effic., %	19.8	19.6	20.5	19.5	13.2	14.3	14.0	14.5
Dose, mg/rat per day		0.48	45.6	443.1		0.32	31.5	316.8

Table III. TPGS Reproduction Study; Mating and Fertility Indexes

		F_{1a}				F_{1b}			
		Control	0.002%	0.2%	2.0%	Control	0.002%	0.2%	2.0%
Indexes	M/F per group	15/15	15/15	15/15	15/15	15/15	15/15	15/15	15/15
Insemination[a]		100.0	93.3	80.0	93.3	93.3	80.0	80.0	93.3
Fertility[b]		100.0	85.7	100.0	100.0	92.9	91.7	83.3	92.9
Gestation[c]		100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Viability[d]		99.4	98.5	99.3	97.7	97.5	99.2	100.0	99.3
Lactation[e]		96.6	91.7	97.9	95.2	98.0	96.9	100.0	98.7
Av. gestation, days		22.0	21.8	21.8	22.1	22.1	21.9	22.0	21.9
Av. litter size		12.1	11.3	12.3	12.5	12.6	11.9	13.0	12.1
Sex, M/F		89/92	72/63	67/81	87/88	82/82	59/72	70/60	86/71
Mean mortality									
Birth		3.3	0.7	4.1	2.3	4.3	1.5	3.1	3.2
0-8 weeks		7.2	9.7	6.8	9.1	8.5	5.3	3.1	6.4

[a] Inseminated females/males × 100. [b] Pregnant females/inseminated females × 100. [c] Live litters/pregnant females × 100. [d] Live pups at 4 days/live pups at birth × 100. [e] Live pups at weaning/live pups at 4 days × 100.

Table IV. TPGS Teratology Study; Body Weight and Food Consumption during Gestation

	Negative control	Positive control	TPGS		
			0.002%	0.2%	2.0%
Dose per rat, mg		232	4.6	475	4613
Mean dose per rat, mg/kg		853	15	1513	15 174
Mean body wt gain, g					
0-6 days	19	20	21	27	18
6-16 days	51	−31	59	53	55
16-20 days	43	33	56	50	43
Food consumption, g/rat per day					
0-6 days	18.1	19.2	18.2	19.7	17.5
6-16 days	22.5	11.6	23.2	23.7	23.1
16-20 days	24.6	22.6	26.4	26.2	25.2

microscopic examination of these tissues in the 2.0% males revealed no pathologic changes.

Both the absolute and relative liver weights of the mid-dose (0.2%) females were statistically lighter than the controls. Again, this weight difference was not seen at the high dose level and microscopic examination of liver tissue from the high dose females revealed no pathology.

Only the tissues collected at necropsy from the high dose and control groups of both sexes were examined for micropathology. Except for subclinical murine bronchitis and focal interstitial mononuclear cell infiltration in the liver, kidney, and heart, which appeared to a comparable degree in both the control and treated animals, all organ systems were histologically unremarkable.

Reproduction Study. Data collected from the two-litter one-generation reproduction study are presented in Table III. No apparent differences in reproductive indexes were seen between the control and treated groups. Mean gestation period, litter size, sex ratio, and mortality of pups and parents were unaffected by TPGS treatment at both matings. The ingestion of TPGS by the parents for 112 and 175 days and by their offspring for 5 weeks after weaning had no effect on the body weight gain of the pups. Hematologic and clinical chemistry determinations done on the F_0 10 days prior to necropsy (255 days of treatment) revealed no toxicologic differences between the control and high dose animals due to the ingestion of TPGS.

None of the organ weights, whether analyzed as absolute or relative weight, of the treated groups were significantly different than the controls.

Microscopic examination of the tissues collected from the F_0 high dose and control animals and of those tissues collected from the F_{1b} pups (high dose and controls) killed at 8 weeks of age revealed no morphological changes due to the ingestion of TPGS.

Teratology Study. Mean body weight gain and food consumption of the females in all treated groups during the period of gestation prior to treatment (0–6 days) were similar or slightly better than the controls (Table IV). During the treatment period (6–16 days), the feed consumption of the positive control group was reduced with a loss of body weight in these animals. None of the other treated groups differed from the controls. From the cessation of treatment to laparotomy (16–20 days), all treated groups consumed a normal amount of feed and all except the positive control group had weight gains similar to the negative control.

Table V. TPGS Teratology Study; Fertility Data

	Negative control	Positive control[b]	TPGS 0.002%	TPGS 0.2%	TPGS 2.0%
No. pregnant females	14	13	15	14	15
No. females with viable fetuses	13	10	15	14	15
No. females with complete resorption	1	3	0	0	0
Corpora lutea per pregnancy	13.4	14.8	14.5	14.0	13.8
Implantations per pregnancy	10.6	12.0	13.5	11.6	10.3
Resorptions per pregnancy	1.2	8.9	0.7	0.9	1.1
Dead fetuses per pregnancy	0	0.2	0	0	0.07
Viable fetuses per pregnancy	10.2	3.7	12.8	10.8	9.1
Mean body wt (g) per fetus[a]					
Male	4.00 (3.2-4.6)	2.05 (1.0-2.9)	4.06 (2.2-4.9)	4.05 (3.2-5.1)	3.98 (3.2-4.6)
Female	3.70 (2.6-4.6)	1.95 (1.2-3.0)	3.83 (3.0-4.7)	3.65 (2.5-4.5)	3.57 (2.8-4.6)
Males/females per group	80/52	18/22	104/88	78/73	60/78

[a] Figures in parentheses are ranges. [b] Technical Apholate, Olin Mathieson Chemical Corp.

Table VI. TPGS Teratology Study; Number of Fetuses with Abnormalities[a]

Anomalies	Negative control	Positive control	TPGS 0.002%	TPGS 0.2%	TPGS 2.0%
Gross external					
No. fetuses examined	132 (13)[b]	40 (10)	192 (15)	151 (14)	138 (15)
Curly tail	0	0	0	0	1[c]
Microagnathia	0	3 (3)	0	0	1[c]
Stubby trunk	0	2 (2)	0	0	0
Ectrodactyly	0	0	0	1	0
Skeletal					
No. fetuses examined	63 (11)	17 (7)	93 (15)	72 (14)	66 (14)
Ribs: Missing	0	0	1	0	0
Wavy	0	0	1	0	0
Fused	0	4 (3)	0	0	0
Retarded ossification	0	1	0	0	0
Extra	0	1	2 (2)	1	0
Rudimentary	24 (9)	10 (5)	26 (10)	19 (7)	10 (6)
Skull: Retarded ossification	0	2 (2)	0	0	0
Presphenoid missing	0	1	0	0	0
Palatine split	0	2 (2)	0	0	0
Vertebra: Retarded ossification	0	1	0	1	0
Fused	0	2 (2)	0	0	0
Centra: Split	1	3 (2)	0	0	0
Missing	0	5 (4)	0	0	1
Sternabra: Retarded ossification	0	1	0	0	0
Missing	0	4 (4)	0	0	0
Internal soft tissue					
No. fetuses examined	69 (13)	23 (10)	99 (15)	79 (14)	72 (15)
Cleft palate	0	1	0	0	0
Hydrocephalus	0	7 (5)	0	0	0
Microthalmia	0	1	0	0	0
Anophthalmia	0	4(3)	0	0	0
Edema	0	3 (3)	0	0	1[c]
Folded retina	0	0	0	0	1[c]
Testicle missing	0	0	0	1	0
Transposition great vessels	0	0	1	0	0

[a] Due to space requirements, some negative data are not included in this report. The authors will gladly supply these data to interested persons requesting them. [b] Numbers in parentheses are number of litters involved. [c] Same fetus; only one in litter; dead in utero.

Table V presents the fertility data relevant to the teratology study. None of the TPGS treated groups differed from the negative controls, while increases in resorptions and abnormal fetuses and decreases in viable fetuses and fetal body weights were seen in the positive control group. Gross anomalies were seen in only two fetuses from TPGS treated groups (Table VI). One of these from the mid-dose level had two digits missing on one front paw and the other, which was dead in utero, was edematous with a curly tail and microagnathia. This fetus also had a folded retina seen on cross-sectional examination. Also, cross-sectional examination revealed 1 low-dose fetus with transposition of the aorta and pulmonary vessels and a mid-dose fetus with a missing testicle. These anomalies plus a few skeletal abnormalities involved only 1 fetus in each litter affected, did not constitute a syndrome of abnormalities, and were not dose related. The anomalies seen in the high-dose group were seen in only 1 of 138 fetuses and this fetus was dead in utero. Therefore, the few anomalies seen in the TPGS treated groups are not attributed to the ingestion of TPGS and are considered to be spontaneous occurrences.

SUMMARY

The acute LD_{50} (po) for young adult male and female rats of TPGS and either of the materials from which it is made is in excess of 7000 mg/kg. When given orally to neonates, TPGS is no more toxic than any of its moieties.

When fed to male and female albino rats for 90 days at levels of 0.002, 0.2, and 2.0% in the diet, TPGS had no untoward effects on the general behavior, appearance, feed consumption, or growth rate of these animals. Hemograms and clinical chemistry determinations were within normal ranges and organ weights recorded at necropsy were normal. Microscopic examination of tissues from all organ systems revealed no pathologic changes due to the ingestion of TPGS. Animals bred after 112 and 175 days of treatment had reproductive indexes similar to their controls and produced offspring that were normal. Microscopic examination of the tissue from the parents and offspring revealed no pathology due to ingestion of the compound. Fetuses collected from pregnant females treated with TPGS during the period of organogenesis had no congenital abnormalities which could be attributed to the compound.

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Extraction, Purification, and Partial Characterization of a Tea Metalloprotein and Its Role in the Formation of Black Tea Aroma Constituents

Philip Coggon,* Leo J. Romanczyk, Jr., and Gary W. Sanderson

Linolenic acid, the major fatty acid in tea leaves, is known to be partially oxidized during tea fermentation to yield 2(*E*)-hexenal, a constituent of black tea aroma. Evidence has now been obtained which suggests that this transformation is mediated by an endogenous, heat-stable, metal ion containing protein rather than a true lipoxygenase. This metalloprotein has been purified by isoelectric focusing and has been found capable of producing a number of volatiles other than 2(*E*)-hexenal. The activity of this nondialyzable metalloprotein was found to be stable for over 10 min at 100 °C. Inhibition was achieved with various metal ion chelators and respiratory enzyme inhibitors. The purified tea metalloprotein gave a radioactive volatiles pattern for the oxidation of [^{14}C]linolenic acid which was very similar to that of cytochrome *c* and chlorohemin. A total of ten radioactive volatiles were detected and identified during the extraction and purification of this tea metalloprotein. Eight of these compounds are aldehydes, which are predictable oxidation products of linolenic acid. Two alcohols were found which were presumed to be formed from two of the aldehydes by the action of an endogenous tea alcohol dehydrogenase.

2(*E*)-Hexenal, a constituent of black and green tea aroma (Sanderson, 1972, 1975), is known to be formed from linolenic acid during tea fermentation (Gonzalez et al., 1972; Saijo and Takeo, 1972). This aldehyde results from the isomerization of 3(*Z*)-hexenal (Hatanaka and Harada, 1973; Kazeniac and Hall, 1970; Kajiwara et al., 1975; Hatanaka et al., 1976) which is the first formed volatile product of linolenic acid breakdown, and is responsible for the characteristic flavor of various foodstuffs (Harper, 1975). In black tea, the 2(*E*)-hexenal content has been shown to relate to flavor quality (Gianturco et al., 1974), a fact which makes the mechanism by which this compound is formed in black tea of considerable interest.

Something is now known of several mechanisms in biological materials for the breakdown of linolenic (and linoleic) acid to 2(*E*)-hexenal and other products. First, there is the peroxidation catalyzed by the enzyme lipoxygenase (linoleic acid:oxygen oxidoreductase, EC 1.13.11.12), which is known (Axelrod, 1974; Gardner, 1975; Parsons, 1974) to initiate the formation of peroxidized linolenic acid which in turn breaks down via enzymatic and nonenzymatic pathways to form several volatile and nonvolatile oxidation products. Most of the adequately verified lipoxygenase preparations have come from plants in the *Leguminosae* family. However, there are reports of lipoxygenase activity having been detected in many different plant families (cf. Axelrod, 1974).

Next, it has been shown (Gardner, 1975; Tappel, 1961, 1962; Blain, 1970; Eriksson et al., 1969; Grosch et al., 1974) that hematin compounds, i.e. heme-containing proteins like the cytochromes and peroxidase, will catalyze the nonenzymatic peroxidation of unsaturated fatty acids.

Finally, Grosch et al. (Grosch and Schwartz, 1971; Grosch et al., 1974) have shown that singlet oxygen can react with unsaturated fatty acids producing a number of volatile compounds including 2(*E*)-hexenal from linolenic acid.

Of course, the above-mentioned mechanisms for the breakdown of unsaturated fatty acids in plant materials are not mutually exclusive and there may be other mechanisms not yet discovered. Our investigation was carried out to determine the mechanism by which unsaturated fatty acids, especially linolenic acid, are broken down during black tea manufacture with particular at-

Thomas J. Lipton, Inc., Englewood Cliffs, New Jersey 07632.