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Provisional translation

Evaluation Report of Food Additives

Polysorbates
(Polysorbates 20, 60, 65 and 80)

June 2007

Food Safety Commission

Contents

〈History of deliberation〉

October 8, 2003	Request for the food safety risk assessment related to designation of food additives by the Minister of Health, Labour and Welfare, and acceptance of relevant documents
October 16, 2003	15th Meeting of the Food Safety Commission (requested items were explained)
October 29, 2003	1st Meeting of the Expert Committee on Food Additives
April 27, 2004	8th Meeting of the Expert Committee on Food Additives
July 28, 2004	11th Meeting of the Expert Committee on Food Additives
September 8, 2004	12th Meeting of the Expert Committee on Food Additives
April 13, 2006	31st Meeting of the Expert Committee on Food Additives
March 23, 2007	42nd Meeting of the Expert Committee on Food Additives
April 12, 2007	186th Meeting of the Food Safety Commission (report)
April 12 - May 11 2007	Hearing of public opinions and information
May 29, 2007	44th Meeting of the Expert Committee on Food Additives
June 5, 2007	Report from the chairman of the Expert Committee on Food Additives to the chairman of the Food Safety Commission
June 7, 2007	193rd Meeting of the Food Safety Commission (report) (Notified to the Minister of Health, Labour and Welfare as of the same date)

〈Members of the Food Safety Commission〉

Expires on June 30, 2006

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From September 25, 2003 to September 30, 2005

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Masato Naya

Evaluation Report of Food Additives “Polysorbate 20, Polysorbate 60, Polysorbate 65 and Polysorbate 80”

June, 2007

Food Safety Commission

[Summary]

Assessment of the effect of food on health of “Polysorbate 20, Polysorbate 60, Polysorbate 65 and Polysorbate 80” (CAS number.: 9005-64-5, 9005-67-8, 9005-71-4 and 9005-65-6), an food additive used as the emulsifier, was performed by using various study data.

The data used for the assessment included those of repeated-dose toxicity, carcinogenicity, reproductive and developmental toxicity, genotoxicity and etc..

No essential differences in disposition or adverse effects were observed among 4 polysorbates, and they were therefore treated as one group.

Study results of the polysorbates, showed no carcinogenicity and genotoxicity. In repeated-dose toxicity studies, diarrhea was observed as a major symptom.

In a single-dose neurodevelopmental toxicity study with rats by Brubaker et al., behavioral changes were observed in litters of treated rats and an additional study to confirm these effects was therefore carried out. Maternal toxicity occurs in a 7.5 vol% treated group, and in litter decreased weight gain, a lower avoidance rate in a conditioned avoidance response study, and etc. were observed. In a ≤1% treated group, there was no effect on the mother rats and their subsequent generations (F1).

The lifetime cancer risk which was in turn calculated from the estimated consumption in the U.S. Levels and lifetime cancer risk due to impurities contained in the polysorbates was considered to be very low, based on the estimated consumption in Japan, were estimated to be less than one millionth of the genotoxic carcinogen level that can generally be ignored. The risk management organization should, however, continue to reduce the residues at a technically possible level.

Based on the occurrence of diarrhea in rats fed polysorbate 60 in a 13 week, dietary administration study, the no observed adverse effect level (NOAEL) of polysorbates was calculated at 2% (equivalent to 1,000 mg/kg body weight/day). The acceptable daily intake (ADI) of Polysorbates (Polysorbate 20, Polysorbate 60, Polysorbate 65 and Polysorbate 80) was set at 10 mg/kg body weight/day as a group, using a safety factor of 100.

1. Introduction

Polysorbate, a substance prepared by the reaction of sorbitan fatty acid ester (a nonionic surfactant) with ethylene oxide[1)], is widely used in many foreign countries, including the U.S. and the EU, where it acts as an emulsifier, dispersant or solubilizer in many foods, including bread, cake mix, salad dressing, shortening oil and chocolate[2) 3)].

At the 17th Meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in 1973, an ADI of 0-25 mg/kg body weight/day was set for polysorbate 40 (not included in this designation study), as well as for polysorbate 20, polysorbate 60, polysorbate 65 and polysorbate 80, as a group of compounds[4)]. A draft of standards for use of polysorbates was proposed by the Codex Alimentarius Commission.

In the U.S., polysorbates were approved as food additives at the beginning of the 1960s, and their use in foods, the maximum concentrations, and the other ingredients allowed were specified for polysorbate 60, polysorbate 65 and polysorbate 80. Polysorbate 20, polysorbate 60 and polysorbate 80 can also be used as synthetic flavoring substances or auxiliary substances[4)]. In the EU, polysorbates 20, 40, 60, 65 and 80 were approved by the Directives for Food Additive (1995), and standards for their use were established.

2. Background

The Ministry of Health, Labour and Welfare, in accordance with the matters approved by the Subcommittee of the Pharmaceutical Affairs and Food Sanitation Council in July 2002, is indicating a policy to start, without waiting for requests of designation from companies and others, a study initiated by the government for the designation of food additives, (1) for which a safety evaluation was completed internationally in the Joint FAO/WHO Expert Committee on Food Additives (JECFA) to ensure their safety to a certain extent, (2) whose use has been approved widely in the U.S., European Union (EU) member states, etc., and for which are considered to have high demands internationally.This time, evaluation data of Polysorbate 20, Polysorbate 60, Polysorbate 65 and Polysorbate 80 (hereinafter, "Polysorbates") as the emulsifier was collected,

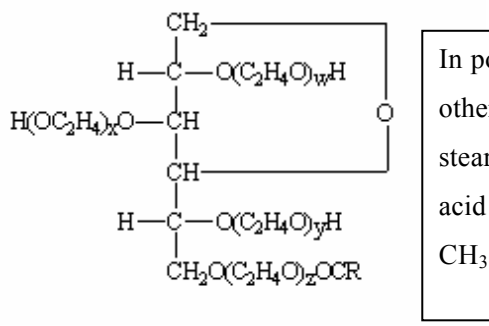
and the assessment of the effect of food on health was requested to the Food Safety Commission according to the Food Safety Basic Law (The Related documents were received on December 8, 2003).

3. Outline of the designation of food additives

In order to designate polysorbates as new food additives, standards for their use in milk, cream replacement, salad dressings, etc. will be established and specifications for their use will be laid down with reference to the existing specifications of the JECFA, etc..

4. Name, etc.

Polysorbates are hydrophilic emulsifying agents, of which polysorbate 20 is more hydrophilic than polysorbate 60 and polysorbate 80, and polysorbate 65 is more lipophilic than the others.



In polysorbate 20, RCO- is mainly lauric acid, $CH_3(CH_2)_{10}CO$-, and other fatty acids are included. In polysorbate 60, RCO- is mainly stearic acid, $CH_3(CH_2)_{16}CO$-, and other fatty acids, such as palmitic acid are included. In polysorbate 80, RCO- is mainly oleic acid, $CH_3(CH_2)_7CH=CH(CH_2)_7CO$-, and other fatty acids are included.

Fig. 1 Polysorbates 20, 60, and 80 (w + x + y + z = about 20)

(1) Polysorbate 20

English name: Polyoxyethylene (20) Sorbitan Monolaurate or Polysorbate 20

Code name: Polyoxyethylene sorbitan monolaurate (Tween 20)

CAS number: 9005-64-5

Chemical formula: w + x + y + z = 20; Fatty acid is $C_{58}H_{114}O_{26}$ as lauric acid.

Molecular weight: 1,227.72

Some hydroxyl groups of sorbitol and anhydrous sorbitol are esterified, mainly with lauric acid, followed by condensation of about 20 ethylene oxide molecules (fatty acids are mainly lauric acid bound to sorbitan at 1:1 molar ratio).

Oxyethylene (OCH_2CH_2) is included at 70.0-74.0%.

(2) Polysorbate 60

English name: Polyoxyethylene (20) Sorbitan Monostearate or Polysorbate 60

Code name: Polyoxyethylene sorbitan monostearate (Tween 60)

CAS number: 9005-67-8

Chemical formula: w + x + y + z = 20; Fatty acid is $C_{64}H_{126}O_{26}$ as stearic acid.

Molecular weight: 1311.90

Some hydroxyl groups of sorbitol and anhydrous sorbitol are esterified, mainly with stearic acid, followed by condensation of about 20 ethylene oxide molecules (fatty acids are mainly stearic acid bound to sorbitan at 1:1 molar ratio).

Oxyethylene (OCH_2CH_2) is included at 65.0-69.5%.

(3) Polysorbate 80

English name: Polyoxyethylene (20) Sorbitan Monooleate or Polysorbate 80

Code name: Polyoxyethylene sorbitan monooleate (Tween 80)

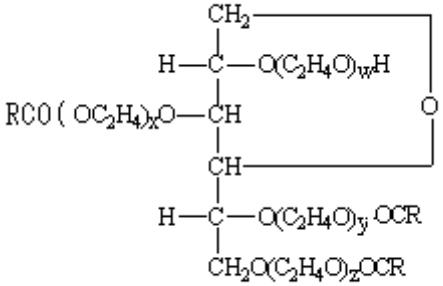
CAS number: 9005-65-6

Chemical formula: w + x + y + z = 20; Fatty acid is $C_{64}H_{124}O_{26}$ as oleic acid.

Molecular weight: 1,309.68

Some hydroxyl groups of sorbitol and anhydrous sorbitol are esterified, mainly with oleic acid, followed by condensation of about 20 ethylene oxide molecules (fatty acids are mainly oleic acid bound to sorbitan at 1:1 molar ratio).

Oxyethylene (OCH_2CH_2) is included at 65.0-69.5%.



RCO- is mainly stearic acid, and other fatty acids such as palmitic acid are included.

Fig. 2 Polysorbate 65
(w + x + y + z = about 20)

(4) Polysorbate 65

English name: Polyoxyethylene (20) Sorbitan Tristearate or Polysorbate 65

Code name: Polyoxyethylene sorbitan tristearate (Tween 65)

CAS number: 9005-71-4

Chemical formula: w + x + y + z = 20; Fatty acid is $C_{100}H_{194}O_{26}$ as stearic acid.

Molecular weight: 1,842

Some hydroxyl groups of sorbitol and anhydrous sorbitol are esterified, mainly with stearic acid, followed by condensation of about 20 ethylene oxide molecules (fatty acids are mainly stearic acid bound to sorbitan at 3:1 molar ratio).

Oxyethylene (OCH_2CH_2) is included at 46.0-50.0%.

5. Safety

(1) Disposition (absorption, distribution, metabolism, excretion and degradation)

Following oral administration in rats, the ester bond sites of polysorbates are hydrolyzed, within the digestive tract, by pancreatic lipase. Free fatty acids are then absorbed from the digestive tract and oxidized and excreted, mainly as carbon dioxide in exhaled breath. These kinetics are presumed to be similar to those demonstrated in ordinary fatty acid metabolism[5) 6)]. The rates of hydrolysis of the polysorbates within the digestive tract are 100% (polysorbate 80), 98% (polysorbate 60) and 84% (polysorbate 65)[7)]. Absorption of polyoxyethylene sorbitan, generated by hydrolysis, from the digestive tract is very low; approximately 87% of polysorbate 20 and approximately 91% of polysorbate 80 are excreted in the feces and 8.5% and 2.1% in the urine, respectively[6)]. No migration of the of polyoxyethylene sorbitan into the thymus lymph nodes has been demonstrated[8)]. At 24 hours after administration, only a

small amount of dissociated fat sites and polyoxyethylene sorbitan structural sites remain within the body[9].

In twenty four hours following oral administration of polysorbate 20 (whose fatty acid [lauryl acid] was ^{14}C-labeled) in rats, the radioactivity was distributed in expired carbon dioxide (80%), carcass (12%), feces and digestive tract contents (4%), urine (2.5%) and liver (1.2%). In twenty four hours after oral administration of polysorbate 20 (whose polyoxyethylene group was ^{14}C-labeled), the radioactivity was distributed between feces (90%) and urine (8%), but no radioactivity was detected in the liver, carcass or expired carbon dioxide[5) 9)].

No sex difference has been detected in the disposition of polysorbates in rats[5)].

Following oral ingestion of polysorbate 20 in humans, 90% or more of the administered substance was excreted in the feces as metabolites, with the polyoxyethylene sorbitan structure maintained, and 2-3% of these metabolites were excreted in the urine[10)].

Polysorbate 40 capsules (4.5 g/day or 6 g/day for 12 days (54 g or 72 g)) and polysorbate 20 capsules (4.5 g/day for 12 days (54 g)) were administered to subjects in order to investigate excretion into the urine and feces 6 days after administration. The total recovery rates were 92.7-99.2% and 96.2-102.2%, respectively, while the amounts of polysorbate excreted in the urine maintaining the polyoxyethylene structure were 2.3-3.1% and 3.9-5.8%, and 90.2-96.6% and 90.4-98.3%, respectively, in the feces[10)].

(2) Toxicity

(1) Acute toxicity

The toxicity following a single oral administration of polysorbates was very weak. No abnormal symptoms developed in rats administered polysorbate 80 (22 g/kg body weight), and there was no evidence of liver or kidney dysfunction[11)]. Symptoms such as blepharoptosis (mouse), continuous decreased blood pressure (dog), myotony (rat) and vomiting (dog) developed when polysorbates were injected intravenously or intraperitoneally[11)-14)].

As for the effects of single-dose administration of polysorbates in humans, there were no clear harmful effects following the oral administration of about 4.4 g of polysorbate 20 to 2 children[15)], and when 5% polysorbate 20 solution was intradermally injected in 6 people, local effects were comparable with those experienced with saline[16)]. No toxic effects occurred to 6 people administered 20 g of polysorbate 60 orally, though the amount of gastric acid was slightly reduced [17)].

(2) Repeated-dose toxicity

In an 8-week dietary administration of polysorbate 20 (3% or 5%) to immature rats, mild diarrhea and growth suppression were observed in both treated groups[18)] and in a

28-39-week dietary administration of polysorbate 20 (5% (approximately 5,000 mg/kg body weight/day)) to hamsters, marked diarrhea and suppression of weight gain were observed[19]. In a 22-month dietary administration of polysorbate 20 (5% or 10% (approximately 7.5 or 15 g/kg body weight/day, respectively)) to mice, mild diarrhea was observed in the 10% treated group[20]. Following 17-months of oral administration of 1 g /day of polysorbate 20 (approximately 50 mg/kg body weight/day) to 4 monkeys, no harmful effects were observed, and after 20 days of intramuscular, intravenous or subcutaneous administration (275 mg/kg body weight/day) to 4 monkeys, no histopathological changes were observed in the liver or kidney[16].

When mixed feed including polysorbate 60 (1%, 2% or 5% (equivalent to 500, 1,000 or 2,500 mg/kg body weight/day)) was administered to both male and female rats for 13 weeks, diarrhea and distention of the cecum were seen in the 5% group, and distention of the cecum in the 1% and 2% groups[21]. When mixed feed including polysorbate 60 (2%, 5%, 10% or 25%) was administered to both male and female rats (12 male rats and 12 female rats for each group) for 2 years, marked diarrhea was observed in the 10% and 25% groups, slight to moderate distention of the cecum in the 25% group, more slight distention of the cecum in the 10% group and slightly suspicious changes in fat in the liver in the 25% group[22]. Development of diarrhea in rats was observed following 5% polysorbate 60-supplemented feeding in other experiments[23], where it was reported that the development of symptoms changed with the basal diet; diarrhea was caused by a casein diet, but not by a soybean diet[23]. It was also reported that the development of diarrhea due to polysorbate 60 was inhibited by an increase in fiber volume of the feed[24]. No adverse effects were seen in a 3-12-month dietary administration study of polysorbate 60 to mice (2.5% (3.8 g/kg body weight/day), 5% and 10%)[25]. In hamsters, renal disorders (interstitial nephritis), probably due to chronic diarrhea and dehydration, and an increase in mortality were seen in a 12-13-month dietary administration of polysorbate 60 (5% (4 g/kg body weight/day)), but no adverse effects were observed in the 1% administration group[25]. No adverse effects such as diarrhea were observed in a 1-year dietary administration of polysorbate 60 to dogs (5% and 10%)[25].

No adverse effects were observed in a 2-year study of dietary administration of polysorbate 65 to rats (2% (approximately 1 g/kg body weight/day))[26] and there was no effect on the growth and survival in a 2-year multigeneration study by administration of 5% polysorbate 65 supplemented feed to rats. No abnormalities were observed in blood, urine or liver or kidney histological studies, but slight diarrhea was observed in 1 male rat out of a total of 12 male rats[7) 27) 28)].

Diarrhea occurred in female rats in the 10% treated group in 2-year dietary administration studies of polysorbate 80 in rats (5%, 10% and 20%)[7), 27), 28)], while in a 13-week dietary administration study of polysorbate 80 (0.31%, 0.62%, 1.25%, 2.5% and

5.0%) in male and female F344 rats, no abnormal symptoms were observed, and no macroscopic or histological changes were seen in any organs. In a similar 13-week study conducted in male and female B6C3F1 mice, no abnormalities were observed[29]. A 2-year dietary administration study of polysorbate 80 (2.5% and 5.0%) in male and female F344 rats and B6C3F1 mice was conducted, and no effects on clinical symptoms, survival or growth were seen in either species[29]. It was reported that degenerative lesions in the heart, liver and kidney developed following daily, forced oral administration of 1.5 ml of 1%, 2% and 3% polysorbate 80 solution to rats for 3 months[29], however, no similar effects could be observed in other studies, including the NTP report.

In humans, one study found no adverse effects of feeding a diet containing 2 g of polysorbate 20 three times a day for 1 week (approximately 100 mg/kg body weight/day) to 5 adults[30], and another study reported no adverse effects after administration of 0.12-1.0 g of polysorbate 20 (used as a pharmaceutical additive) 4 times a day to 13 premature babies (250-2,000 mg/kg body weight/day for the premature babies) and 2 infants[31].

Thirty four patients and 10 healthy subjects who took 6 g of polysorbate 60 for 28 days experienced no adverse effects[32], and other reports found no adverse effects of 13-34-time oral administration of polysorbate 60 (1 g/day) to 4 children[33] or of 5 months oral administration (0.2 g/day) to 9 infants (an additional 0.4 g was orally administered to 3 of the 9 infants for 1-2 months)[34].

No digestive system disorders were observed following the daily oral ingestion of 9 g/day polysorbate 65 (equivalent to 0.15 g/kg body weight/day) for 13 days in 8 male and 4 female volunteers[35].

Mild diarrhea was observed in a 4-month-old baby who took polysorbate 80 (20 g/kg body weight/day) for 2 days[36]. No symptoms developed in the gastrointestinal tracts of 12 healthy volunteers who took 9 g/day polysorbate 80 (0.15 g/kg body weight/day) for 13 days[37]. A further study reported no effects on hemograms, blood pressure, or renal or hepatic functions following oral administration of polysorbate 80 (4.5-6 g/day) to 46 patients for 1-4 years [38].

(3) Genotoxicity

Detailed reports of experiments on polysorbate 20 are unavailable, though 1 review states that it is not mutagenic.

As for polysorbate 60, a *Bacillus subtilis* rec-assay was performed as a DNA repair study, and while Kada et al.[39] found the assay to be positive for reverse mutation, Kawachi et al.[40] and Morita et al.[41] obtained negative results. Ames studies using 2 strains of *Salmonella typhimurium* (TA98 and TA100) were performed 3 times, and the results were negative both with and without S9 mix[40)-42)]. Several other studies, including

clastogenicity studies, were conducted, but results were negative[40].

In reverse mutation studies on polysorbate 65 using bacteria (TA98, TA100, TA1535, TA1537 and *Escherichia coli* WP2*uvr*A), results were negative up to a threshold dose of 5,000 μg/plate, with or without a metabolic activation system[43]. In clastogenic studies using doses up to those found to cause cytotoxicity in Chinese hamster cells (CHL/IU), weak induction of structural chromosomal abnormalities was observed only at doses resulting in significant cytotoxicity in a short-term treatment group in the absence of a metabolic activation system. No induction of structural abnormalities was observed in a long-term continuous treatment group in the absence of a metabolic activation system or in a short-term treatment group in the presence of a metabolic activation system. In addition, weak induction of numerical chromosome abnormalities was observed in both the short-term treatment group and the long-term continuous treatment group, in the absence of a metabolic activation system. Testing of doses up to the threshold of 5,000 μg/ml in the presence of a metabolic activation system, resulted in a statistically significant difference in induction of abnormalities, though this was still markedly weaker when compared with the positive control in the absence of a metabolic activation system[44]. In a micronucleus assay using mice, no micronuclei were induced 24 hours after forced oral administration of 2 threshold doses of 2,000 mg/kg administered at 24 hour intervals[45].

In rec-assays of polysorbate 80 using *B. subtilis* and *E.coli*, the results were negative for DNA damage repair[40), 46]. In a reverse mutation assay using *S. typhimurium* strains (TA98, TA100, TA1535 and TA1537), all results were negative, with or without S9 mix[40), 41]. In a chromosome aberration study using mammalian cells, the results were negative, with or without a metabolic activation system[40), 47]. In addition, two micronucleus assays using rodents were conducted, and the results were negative in both assays[48), 49]. Negative results were also obtained in a dominant lethal study to examine the effects on mammalian germ cells[50].

(4) Carcinogenicity

There are few reports of studies examining the carcinogenicity of polysorbate 20. The result of an oral administration study was published in a paper in 1956, but the methodology did not conform to the standards of present carcinogenicity studies with respect to numbers of animals and duration of administration, and therefore no conclusions can be drawn.

No tumorigenesis was observed in a 9-week dietary administration study of polysorbate 20 (5%, 10% and 15%) in 10 hamsters (6 males, 4 females), in a 21-week 25% dietary administration study in 10 male rats[51], in a 68-day 5% dietary administration study in 36 hamsters and a 59-day 25% dietary administration to 14 rats[52]. No tumors

developed following transdermal administration of 0.18 mol polysorbate 20 to mice (once daily, 6 days a week for 30 days; twice daily, 6 days a week for 30 days) or transdermal administration of diluted solution (once daily, 6 days a week for 24 weeks). One benign skin tumor was, however, observed at 36 weeks during transdermal administration of 100% solution (approximately 3 mg/kg body weight/day) (once daily, 6 days a week for 52 weeks)[53].

As for polysorbate 60, few studys have been performed that meet the standards of the present carcinogenicity study.

No carcinogenicity was observed in a 2-year study of dietary administration study of polysorbate 60 (5%, 10% and 20%) in 20 female and 12 male Wistar rats in each group[54], nor in a 2-year dietary administration study of polysorbate 60 (2%, 5%, 10% and 25%) in 24 Osborne-Mendel male or female rats[22]. No carcinogenicity was detected in a 13-month dietary administration study (1% and 5%) in 12 male hamsters, a 4-month dietary administration study (2.5, 5 and 10%) in 10-12 male and female mice nor in a 1-year dietary administration study (10%) in dogs (beagles)[25]. The application of undiluted polysorbate 60 solution to mouse skin 2 or 6 times a week resulted in the development of benign skin tumors in 40-50% animals at 30 weeks or later, however, continued administration induced regression of most of the tumors[55)-57].

No carcinogenicity was observed in a 2-year dietary administration study of polysorbate 65 (5%, 10%, and 20%) in 12 male and 20 female Wister rats[54].

In a 2-year dietary administration carcinogenicity study of polysorbate 80 (5%, 10% and 20%) in male and female rats, a slight increase in the development of fibroadenomas as sporadic tumors was observed in the mammary glands of the female rats, including those in the control group, but no carcinogenicity was demonstrated[54]. In another 2-year dietary administration carcinogenicity study of polysorbate 80 (25,000 ppm and 50,000 ppm) in 50 male or female F344/N rats, half of them survived, and the incidence of adrenal pheochromocytomas was increased in the 50,000 ppm fed male group, though this increase was not significant by Fisher's exact study. No differences in rates of tumorigenesis in other organs were found between the experimental group and control groups. In a 2-year dietary administration carcinogenicity study of polysorbate 80 (25,000 ppm and 50,000 ppm) in 50 male or female B6C3F1 mice, approximately 50-60% of the animals survived, and hyperplastic lesions of the anterior gastric mucosa were significantly increased in male and female mice in the 50,000 ppm group, but no carcinogenicity was demonstrated[29]. No tumorigenesis was detected in an experiment in which a polysorbate 80-supplemented experimental diet was given to G57BL mice for 10 weeks (100 mg/mouse/day), followed by basal diet for the next 8 weeks and then commercial diet until 51 weeks[58]. In a skin-painting study of 80 mg of undiluted polysorbate 80 solution in 50 mice (6 times a week for 52 weeks), a benign skin tumor

developed in one mouse[53] and in a subcutaneous injection study of 2 ml of 6% polysorbate 80 solution in 20 rats (3 times a week for 40 weeks), fibrosarcomas were formed at the injection sites in 11 mice[59]. Intratracheal injection study of 0.2 ml of 5% polysorbate 80 in 50 Syrian hamsters (once a week for lifetime) failed to demonstrate any tumorigenesis[59].

(5) Other carcinogenicity (combined administration with a known carcinogen)

In a 26-week oral administration study of 50 ppm N-methyl-N'-nitro-N-nitorosoguanidine (MNNG) and 0.4% polysorbate 20 (equivalent to approximately 100 mg/kg body weight/day) in drinking water of male Wistar rats, the incidence of adenocarcinomas in the glandular stomach of the rats was increased compared with that in rats treated with MNNG alone, and development of gastric sarcomas were also observed[60]. Repeated skin-painting with 0.2 ml of 0.3%-3% polysorbate 20 solution, following transdermal administration of 0.125 mg of 7,12-dimethylbenz[a]anthracene (DMBA) to ICR and Swiss mice, resulted in slight increases in skin tumor development, while in a 1-year skin-painting study of undiluted polysorbate 20 solution after transdermal administration of DMBA, skin tumors were observed in 5 mice, whereas none developed in mice treated with DMBA alone[53].

In a combined carcinogenicity study in which male Wistar rats had free access to drinking water containing 0.4% polysorbate 60 and 100 ppm MNNG for 36 weeks, and were then kept for a subsequent 63 weeks, a slight increase in the development of well-differentiated adenocarcinomas of the glandular stomach was observed, compared with the MNNG alone group. Poorly-differentiated adenocarcinomas and the occurrence of sarcomas with adenosarcomas were seen in the polysorbate group, but not in the MNNG group, demonstrating an enhancement of carcinogenicity and malignancy[61]. Additionally, in a combined carcinogenicity study in which male Wistar rats had free access to drinking water containing 50 ppm MNNG and 0.4% polysorbate 60 for 26 weeks and were then supplied with tap water for the subsequent 80 weeks, an increased incidence of adenocarcinomas, including poorly-differentiated adenocarcinomas of the glandular stomach (1 in 9 cases), and development of sarcomas were observed[60].

Many combined administration studys of polysorbate 80 and known carcinogens have been reported, most of them designed to study skin or gastric tumorigenicity.

In a combined carcinogenicity study in which 3-methylcholanthrene (MC) (0.6 mg/mouse/day) and 100 mg polysorbate 80 were administered to male C57BL mice for 10 weeks, and the mice then had been fed basal diet for the next 8 weeks and commercial diet for the subsequent 51 weeks, the incidence of lung tumors in the MC group was 44.4%, while that in the combined administration group was 74.1%. The incidence of papillomas in the anterior stomach was increased from 4.8% to 25.9%, and that of

squamous cell cancers from 3.7% to 7.4%. The total number of tumors that developed and frequently-occurred cases of primary tumors were increased [58]. In a further combined carcinogenicity study in which male Wistar rats had free access to drinking water containing 50 ppm MNNG and 0.4% polysorbate 80 for 26 weeks, followed by tap water for the next 80 weeks, no increase in the incidence of adenocarcinomas of the glandular stomach, no increase in malignancy of adenocarcinomas and no development of gastric sarcomas were reported, compared with the MNNG group[60]. Application of DMBA to mouse skin, followed by the continuous application of polysorbate 80 to the skin, increased the incidence of skin tumors compared with DMBA alone, but the characteristics of the tumors were not specified[59].

In a report on cancer inhibition, when benzpyrene (B(a)P) and polysorbate 80 were transtracheally administered to hamsters, 24 of the 50 hamsters in the B(a)P single administration group developed tracheal tumors, but the number was reduced to 12 of the 50 in the combined administration group[59]. In addition, male SJL/J mice frequently develop spontaneous reticulosarcomas, but intraperitoneal injection of polysorbate 80 reduced their incidence by about half. One study reported that injection of polysorbate 80, together with the anticancer agent, cyclophosphamide, to SJL/J mice once a week lowered the incidence of spontaneous reticulosarcomas from 85%, seen in the control group, to 0%[29].

(6) Reproductive and developmental toxicity

Oral administration of polysorbate 20 to 24-25 pregnant rats for 6-15 days (500 and 5,000 mg/kg body weight/day) suppressed weight gain in the 5,000 mg/kg body weight/day group. No changes were observed in ovary weights in both administration groups, there was no difference in the number of corpus lutea and implantations and the death rate of preimplantation embryos per mother animal and no marked difference in the growth and development of fetuses compared with those in the control group[62].

When mixed feed containing polysorbate 60 (0.1%, 1% and 10% (99, 960 and 7,693 mg/kg body weight/day)) was administered to 10-12 female rats at 7-14 days gestation period, a statistically significant increase in the death rate of embryos/fetuses was observed in the 1% administration group, but not in the 0.1% and 10% groups. No differences in number of resorptions, dead fetuses, live fetuses, sex or weight of fetuses, and morphological abnormalities of fetuses were seen between the administration group and the control group[63]. In a forced oral administration study of polysorbate 60 in 22-26 female Wistar or SD rats at 1-19 or 7-15 days gestation period, (one drop (approximately 150 mg/kg body weight/day)), there was no effect on the number of live fetuses and weights of fetuses[64]. In a forced oral administration study of polysorbate 60 in mice at 6-13 days gestation (5.2 g/kg body weight/day), no effects on the survival of mother

animals, the number of live litter and the birth weights of newborns were seen, but an increase in body weight of newborns was suppressed at day 3 or later after birth[65].

When mixed feed containing polysorbate 65 was administered to 12 male and 20 female rats for 3 generations (5%, 10% and 20%; mating after administration of mixed feed of each concentration to male/female rats of F0 generation for 12 weeks), there was no effect on the fertility, the number of live fetuses, birth rate and survival of newborns in the 2nd generation in the 5% (approximately 2.5 g/kg body weight/day) group, but the 4-day survival of newborns was slightly reduced in the 3rd generation. The 4-day survival of newborns was more markedly reduced in all the generations in the 10% and 20% administration groups, and the observer considered that this was due to weakening of the mother animals. In the 20% administration group, the mortality of the parent animals was increased, and the 4-day and longer survival of newborns was reduced in all generations. There were slight changes in other parameters, but the association between these and the polysorbate administration is unknown[28].

Mixed feed containing polysorbate 80 was administered to 12 male and 20 female rats for 3 generations (5%, 10% and 20% (approximately 2.5, 5 and 10 g/kg body weight/day)). Each concentration was administered to the F0 generation for 12 weeks, followed by mating. In the 20% group, the fertility of the rats and the survival of newborns at day 4 after birth was slightly reduced. The observer considered that the reduced survival might be due to weakening of the mother animals. However, there were no obvious effects on reproductive functions in the 5% and 10% administration groups[28]. In a 3-generation study dietary administration of polysorbate 80 to rats (2% (approximately 1 g/kg body weight/day)), there were no effects on fertility and growth[66]. In the study by Brubaker et al., in which polysorbate 80 (1.35 g/L (approximately 100 mg/kg body weight/day)) was administered in drinking water to female rats for 14 days before pregnancy, during the mating period, the pregnancy period, and for 21 days of lactation, and to male rats at day 5 before mating, an increase in exercise was observed in male litter prior to weaning[67]. In forced oral administration of polysorbate 80 (500 and 5,000 mg/kg body weight/day) to 2 groups of 25 pregnant rats for 6-15 days, there was no difference in the increase in body weight between the administration group and the control group. In addition, there were no differences in the number of lutea and implantations, and the death rate of preimplantation embryos per mother animal compared with the control group and no obvious differences in morphological development, survival and growth of fetuses were seen, compared with those of the control group[68]. In forced oral administration of polysorbate 80 (2.5 g/kg body weight/day) to 30 female mice at 8-12 days gestation, there were no significant effects on the number of newborns and their mean weights. It was noted that this dose caused minimal toxicity in mother animals in a preliminary study[69].

To confirm the results of Brubaker et al., polysorbate 80 was administered in drinking water to mother rats (n=22) from the start of gestation (day 0) to delivery (day 21) (0, 0.018, 0.13, 1.0 and 7.5 vol% solution (0, 38, 265, 2,013 and 18,126 mg/kg body weight/day)) and the effects on the mothers and newborns during gestation/lactation was examined. Maternal toxicities such as loose stools, suppression of weight gain and reduced food intake were seen, as well as poor lactation in some mother animals, though in the ≤1.0 vol% administration group, no maternal toxicities were observed. Effects on the subsequent generation (F1) included a smaller number of live births, suppression of weight gain and a low avoidance rate in a conditioned avoidance response study at 23-27 days old in the 7.5 vol% administration group. No effects on survival, development, differentiation, reflex responses, motor activity, sensory function, necropsy, organ weight and a neural histopathological study were demonstrated in the subsequent generation (F1).. Based on the above results, the no observed adverse effect level (NOAEL) of polysorbate 80 for mother animals and the subsequent generation (F1) was considered to be 1.0 vol% (2,013 mg/kg body weight/day) as a level in drinking water [70].

(7) Local irritation

In a daily skin-painting study of 5% polysorbate 60 aqueous solution in rabbits for 30 days, there was moderate irritation, while skin necrosis occurred with 10% solution[71]. In a further study on rabbits[72], there was no effect of 15% aqueous solution applied for 60 consecutive days, though there was mild irritation after application of an undiluted solution. Local inflammation also occurred after long-term application of undiluted polysorbate 60 solution to mouse skin[13].

In a study of 30% polysorbate 65 aqueous solution applied to mouse skin for 6 days a week for 30 days, local inflammation and epidermal hyperplasia were observed[14].

Application of 5% polysorbate 80 aqueous solution to rabbit skin every day for 1 month, resulted in moderate irritation, but no marked irritation was seen after applying 5% aqueous solution every day for 10 days[71].

As a study on human skin, 10 drops of 25% polysorbate 60 aqueous solution applied to the scalps of 68 males twice a day for 16 weeks caused slight redness in 1 case[73].

Undiluted polysorbate 65 solution applied for 48 hours in a closed system caused no irritation to human skin[14] and no irritation was reported in a study of 2 applications of a 60% aqueous suspension to 50 healthy persons for 72 hours at a 7-day interval[74].

One report noted that application of 5% polysorbate 80 aqueous solution for 48 hours in a closed system caused mild irritation to human skin[75], but another report found no irritation following its application for 48 hours to 50 healthy people in a closed system[11]. To study the effect on human ocular-mucous membranes, an aqueous solution (pH 5-7) was applied, but no irritation was reported with solutions up to 20%[11].

(8) Sensitization

It has been reported that application of polysorbate 60-based cream or polysorbate 60 alone to the forehead skin caused urticaria 20 minutes after application[76]. According to this report, there was no effect of either the polysorbate 60 or the cream on the dorsal and arm skin.

When 10% polysorbate 80 mineral oil solution was applied for 48 hours in a closed patch to 737 patients who were suspected of having contact dermatitis caused by cosmetics, positive reactions were observed in 4 cases, 3 of which also demonstrated positive reactions to polysorbate 40[77]. In a patch study with eczema patients, positive reactions were observed in 3 out of 330 cases for undiluted polysorbate 80 solution[78], 1 of 590 cases for 10% polysorbate 80 mineral oil solution[75], and 2 of 1,206 cases for 5% polysorbate 80 + 5% polysorbate 40 mineral oil solution[79]. When 5 g of polysorbate 80 was administered orally to 21 patients with chronic rhinitis, nasal mucosal polyps, and asthma, who has a history of hypersensitivity to polysorbate 80, deterioration of nasal symptoms was reported. In the same study, no reactions were observed in 19 healthy people given the same treatment[80].

The unpublished results of a study on guinea pigs, referred to in a review, indicated a moderately or strongly positive result for polysorbate 20[12] and a negative result for polysorbate 65, using the maximization method[14].

6. Evaluation in international organizations

(1) Evaluation in JECFA

JECFA evaluated the effects of polysorbates 20, 40, 60, 65 and 80 at their 17th Meeting (1973)[9]. Based on the findings that there were no adverse effects in the 5% administration group (equivalent to 2,500 mg/kg body weight/day) in a polysorbate dietary adoministration chronic toxicity study, JECFA set the NOAEL at 2,500 mg/kg body weight/day, and the group ADI of polysorbates at 0-25 mg/kg body weight/day, using a safety factor of 100.

(2) Evaluation in the Science Committee on Food (SCF)

In 1978, the SCF evaluated the effects of polysorbates, mainly based on the chronic toxicity study of polysorbate 60, which was reviewed by JECFA[81]. The SCF took into account the development of diarrhea as supporting data for the determination of ADI as well as JECFA, and set the value at 0-25 mg/kg body weight/day as an interim group ADI for polysorbates in consideration of mild diarrhea in 5% administration rat group, which JECFA had not considered as an adverse effect. The SCF requested data from a 90-day oral administration study and a metabolism study with 1 animal species as data required for final evaluation. In 1983, the SCF conducted a reevaluation based on a 13-week oral

administration study with rats[21] using polysorbate 60 supplemented feed (1%, 2% and 5% (equivalent to 500, 1,000 and 2,500 mg/kg body weight/day)) and found diarrhea to occur in the 5% administration group, and 0-10 mg/kg body weight/day was therefore determined as the group ADI for polysorbates. Subsequently, the SCF further reevaluated the ADI, based on results from a 2-year carcinogenicity study, performed by the U.S. NTP (1992), using polysorbate 80 in rats and mice[29], and concluded that the ADI need not to be changed[82].

(3) Evaluation in the U.S. Food and Drug Administration (FDA)

The FDA set the group ADI of polysorbates at 1,500 mg/person/day (0-25 mg/kg body weight/day)[83]. The FDA also used the NOAEL of diarrhea in a repeated-dose toxicity study as a basis for setting the ADI, and emphasized the fact that hamsters were apparently more sensitive (development of marked diarrhea with 5.0% supplemented feed; no effect with 1% supplemented feed) than rats and dogs (no effect with 5% supplemented feed) [84]. They concluded that polysorbates themselves presented no carcinogenic risk to humans. In the reevaluation of polysorbate 60 in 1999, a small amount of residual 1,4-dioxane and ethylene oxide residues were recognized, but the exposure of humans to these residues was significantly low (1,4-dioxane: ≤19 ng/person/day, ethylene oxide: ≤7.7 ng/person/day). They therefore concluded that there was no risk of adverse effects in humans so long as they were appropriately used as food additives, with due consideration given to the human lifetime cancer risks (6.7×10^{-10} and 1.5×10^{-8}, respectively)[83]. The effects of diarrhea following the oral administration of large amounts of polysorbates were considered to be responsible for the development of poorly-absorbable polyol and local irritation of the gastrointestinal mucosa by polysorbates.

(4) Evaluation in the International Agency for Research on Cancer (IARC)

Evaluation of the effects of the contaminants, 1,4-dioxane and ethylene oxide, was conducted.

In 1994, it was concluded that 1,4-dioxane belonged to “Group 2B (possibly carcinogenic to humans),” based on adequate evidence of carcinogenesis in experimental animals, but not in humans[85].

In 1999, they concluded that ethylene oxide belonged to “Group 1 (carcinogenic to humans),” based on considerable evidence of carcinogenesis in experimental animals, but limited evidence in humans[86].

7. Estimation of the daily intake

In Western countries, the daily intake of polysorbates, based on their usage in food, was estimated at 12-111 mg/person/day.

In Japan, the intake of polysorbates should be estimated by a market basket survey,

after their designation as food additives, but it is unlikely that the intake exceeds that in Western countries and affects human health.

Table. Estimated market sizes of polysorbates (Tween) (2002)

	EU (ton)[*1]	U.S. (ton)[*2]
Polysorbate 20 (Tween 20)	10-20	10-20
Polysorbate 65 (Tween 65)	10-20	10-20
Polysorbate 60 (Tween 60)	1,500-2,500	4,000-7,000
Polysorbate 80 (Tween 80)	200-400	2,500-5,000

Source: Data from Quest International (polysorbate manufacturer in Netherlands)

[*1]Daily consumption per capita in a population of 377 million people: 12-21 mg/person/day

[*2]Daily consumption per capita in a population of 298 million people: 60-111 mg/person/day

8. Evaluation results

Since there were no essential differences in disposition and adverse effects among the 4 substances (polysorbates 20, 60, 65 and 80) evaluated here, it was considered appropriate to evaluate them as one group, when setting the ADI.

In a repeated-dose toxicity study, diarrhea was observed as a major symptom. It is usual that the diarrhea after the administration of a large amount of a poorly absorbable substance, was deemed to be due to the physical cause and was therefore not considered to be toxic effect. However, local irritation of the gastrointestinal mucosa by polysorbates was also suspected to affect digestion, together with physical factors resulting from poorly absorbable polyol; and diarrhea was therefore evaluated as toxic effect from the viewpoint of safety.

Positive results were reported for polysorbates 65 and 80 in some *in vitro* chromosomal aberration studies, but genotoxicity was not considered to be a particular problem because of the low frequency of aberrations and the negative results obtained in an *in vivo* bone marrow micronucleus assay.

In a 2-year dietary administration study of polysorbate 80 in rats, an increased incidence of adrenal medullary pheochromocytomas was reported, mainly in male rats[29)], but this was associated with large amounts of poorly absorbable substances along with increased calcium absorption, and similar effects are known to develop in the male rat adrenal medulla with high-dose exposure to related compounds, such as sorbitol and alcohol. This result was therefore not considered to be indicative of carcinogenic risk to humans.

It was reported that concomitant administration of a potent carcinogen, MNNG (50

ppm and 100 ppm in drinking water), and polysorbate 60 caused an increased incidence of gastric adenocarcinomas, development of sarcomas, promotion of carcinogenesis and increased malignancy, but it was decided that these results need not to be considered in setting the ADI because of the small scale of the studies and the negative results of an *in vivo* genotoxicity study etc..

In a single-dose neurodevelopmental toxicity study with rats conducted by Brubaker et al., behavioral changes were observed in litters, and an additional study was therefore conducted to confirm the effects on the behavior of the litter. And further, maternal toxicity in a 7.5 vol% administration group were observed, including suppression of weight gain and reduced avoidance rate in a conditioned avoidance response study. No effect was observed in mother animals or the subsequent generation (F1) in the ≤1% administration group.

The calculation of lifetime cancer risk was based on the estimated intake of polysorbates in Japan, which was in turn calculated from the estimated intake in the United States. The risk due to contaminants contained in polysorbates was considered to be extremely low, since it was below the one-millionth level, a level at which genotoxic carcinogens can generally be ignored. The risk management organization should, however, make continued efforts to lower residues at a technically-possible level.

When diarrhea is considered as toxic effect, the minimum NOAEL in each study was 1% (approximately 800 mg/kg body weight/day) in a 12-13-month dietary administration study of polysorbate 60 in hamsters. However, JECFA decided that this study result would not be used as a ground for setting the ADI, since this was old data having a problem with the study method, and no other, reliable long-term data were available. The Food Safety Commission came to a similar conclusion. Therefore, the minimum NOAEL of polysorbates is set at 2% (1,000 mg/kg body weight/day) based on diarrhea observed in the 13-week dietary administration study of polysorbate 60 in rats. When the ADI is set based on the results of a short-term studies such as the 13-week repeated-dose study, an additional uncertainty factor is generally applied to a safety factor of 100. However, results from multiple long-term study, such as the 2-year repeated-dose toxicity study, are now available for polysorbates. After evaluation of all these results together, the NOAEL in the 13-week repeated-dose study was considered to be the lowest and thus application of the usual safety factor of 100 was determined.

Based on the above findings, the ADIs of polysorbates (polysorbate 20, 60, 65 and 80) were collectively set at 10 mg/kg body weight/day as a group.

Group ADI	10 mg/kg body weight/day
(Reffered data for ADI)	13-week dietary administration study of polysorbate 60
(Animal species)	Rat

(Administration route)	Dietary administration
(Finding for NOAEL setting)	Diarrhea
(NOAEL)	1,000 mg/kg body weight/day
(Safety factor)	100

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Polysorbate 20 Safety Test Results

Test type	Duration	Method of Dosing	Species (No./Group)	Dose/concentration	Test results	Ref. No.
Acute toxicity	Single	Oral	Human (2 children)	Approx. 4.4 g	No marked adverse effects were observed.	15
	Single	ID	Human (6)	5% solution	Local effects were comparable to those of saline.	16
Repeated-dose toxicity	8 w	MF	Rat (immature)	3 or 5% (3,000, 5,000 mg/kg/day[1])	Mild diarrhea and growth suppression were seen in both treated groups.	18
	28-39 weeks	MF	Hamster	5% (approx. 5,000 mg/kg/day)	Marked diarrhea and suppression of weight gain were seen.	19
	22 months	MF	Mouse (10 male)	5 or 10% (approx. 7.5, 15 g/kg/day)	Mild diarrhea was observed in 10% of the group.	20
	17 months	Oral	Monkey (4)	1 g/day (approx. 50 mg/kg/day)	No adverse effects were observed.	16
	20 days	IM, IV, SQ	Monkey (4)	275 mg/kg/day (5% aqueous solution)	No histopathological changes were observed in the liver and the kidney.	16
	One week	Oral	Human (5)	2 g/dose, 3 times/day (approx. 100 mg/kg/day)	No adverse effects were observed.	30
	13-53 days	Oral	Human (13 immature babies, 2 infants)	0.12-1.0 g/dose, 4 times/day (immature baby: 250-2,000 mg/kg/day)	No adverse effects were observed.	31
Carcinogenicity	21 weeks	MF	Male rat (10)	25% (12.5 g/kg/day[1])	No tumor development was observed.	51
	59 days	MF	Rat (14)	25% (12.5 g/kg/day[1])	No tumor development was observed.	52
	9 weeks	MF	Hamster (10)	5, 10 or 15% (4, 8, 12 g/kg/day[1])	No tumor development was observed.	51
	68 days	MF	Hamster (36)	5% (4 g/kg/day[1])	No tumor development was observed.	52
	30 days	PC	Mouse	0.18 mol, 1 dose/day, 6 days/week	No tumor development was observed.	53
	30 days	PC	Mouse	0.18 mol, 2 doses/day, 6 days/week	No tumor development was observed.	53
	24 weeks	PC	Mouse	Dilute solution, 1 dose/day, 6 times/week	No tumor development was observed.	53
	52 weeks	PC	Mouse	100% solution (approx. 3 mg/kg/day), 1 dose/day, 6 days/week	Development of one benign skin tumor was observed at the 36th week.	53
	26 weeks	Oral	Rat (male)	0.4% in water (approx. 100 mg/kg/day) + 50 ppm MNNG[2]	Compared with MNNG single administration, the incidence of adenocarcinoma of the glandular stomach of rats became higher, and development of gastric sarcoma was observed.	60
		Skin[5]	Mouse	0.3-3% 0.2 mL (after percutaneous administration of 0.125 mg of DMBA[3])	Weak skin-tumor promoting effect was observed.	53
	1 year	Skin[5]	Mouse	Stock solution (after percutaneous administration of DMBA[3])	Skin tumor was observed in 5 mice, but not in the DMBA single treated group.	53

Test type	Duration	Method of Dosing	Species (No./Group)	Dose/concentration	Test results	Ref. No.
Reproduction and developmental toxicity	6-15 day pregnancy	Oral	Rat (24-25)	500, 5,000 mg/kg/day	Weight gain was suppressed in the 5000 mg/kg/day treated group.	62
Sensitization		Maximization method	Guinea pig		Moderate to strong positive results were indicated.	12

[1]A value reduced by the bureau based on JECFA "Principles for the safety assessment of food additives and contaminants in food"[87)]

[2]N-methyl- N'-nitro-N-nitorosoguanidine

[3]7,12-dimethylbenz[a]anthracene

[4]Abbreviations:IV (intravenous injection), ID (intradernal injection), IM (intramuscular injection), PC (percutaneous), SQ (subcutaneous), MF (mixed feed)

[5]Skin application

Polysorbate 60 Safety Test Results

Test type	Administration period	Method of Dosing[3]	Species No./group	Dose/concentration	Test results	Ref. No.
Genotoxicity	Rec-assay	Sorbic acid	*Bacillus subtilis* (M45 Rec$^-$)	100 μg /disk	A negative result was obtained.	39
	Rec-assay	Sorbic acid +$NaNO_2$	*Bacillus subtilis* (M45 Rec$^-$)	100 μg /disk	A positive result was obtained.	39
	Rec-assay		*Bacillus subtilis*		A negative result was obtained (without metabolic activation).	40 41
	Ames test		TA98, TA100		A negative result was obtained with or without S9 mix.	40, 41, 42
	Chromosome aberration		Chinese hamster cell		A negative result was obtained (without metabolic activation).	40
	Sister chromatid				A negative result was obtained (without metabolic activation).	40
Acute toxicity	Single	Oral	Human (6)	20 g/person	There were no toxic symptoms, but gastric juice volume was slightly reduced.	17
Repeated-dose toxicity	13 weeks	MF	Rat	1, 2 or 5% (500, 1,000, 2,500 mg/kg/day)	Diarrhea and distention of the cecum were seen in 5% treated group, and distention of the cecum in 1%, and 2% treated groups.	21
	2 years	MF	Rat (12 ea male and female)	2, 5, 10 or 25% (1,000, 2,500, 5,000, 12,500 mg/kg/day[1])	Marked diarrhea was observed in 10% and 25% groups, slight to moderate distention of the cecum in 25% group, milder distention of the cecum in 10% group and very slight suspicious change of fat in 25% group.	22
	3-12 months	MF	Mouse	2.5, 5 or 10% (3.75, 7.5, 15 g/kg/day[1])	No adverse effects were observed.	25
	12-13 months	MF	Hamster	1%, 5% (0.8, 4 g/kg/day [*1])	Kidney changes (interstitial nephritis), probably due to chronic diarrhea and dehydration, and mortality were increased in 5% group.	25
	1 year	MF	Dog	5 or 10% (1,250, 2,500 mg/kg/day[1])	No adverse effects such as diarrhea were seen.	25
	28 days	Oral	Human (34 patients, 10 healthy persons)	6 g/day	No adverse effects were observed.	32
		Oral	Human (4 children)	1 g/day, 13-34 times	No adverse effects were observed.	33
	5 months	Oral	Human (9 infants)	0.2 g/day	No adverse effects were observed.	34
Carcinogenicity	2 years	MF	Rat (20 female, 12 male)	5, 10 or 20% (2.5, 5, 10 g/kg/day[1])	No cancer induction was observed.	54

Test type	Administration period	Method of Dosing[3]	Species No./group	Dose/concentration	Test results	Ref. No.
Carcinogenicity (continued)	2 years	MF	Rat (24 ea male and female)	2, 5, 10 or 25% (1, 2.5, 5, 12.5 g/kg/day[1])	No cancer induction was observed.	22
	13 months	MF	Hamster (12 male)	1 or 5% (0.8, 4 g/kg/day[1])	No carcinogenicity was observed.	25
	4 months	MF	Mouse (10-12)	2.5, 5 or 10% (3.75, 7.5, 15 g/kg/day[1])	No carcinogenicity was observed.	25
	1 year	MF	Dog	10% (2.5 g/kg/day[1])	No carcinogenicity was observed.	25
	30 or more weeks	Skin[4]	Mouse	Stock solution, 2 or 6 times/week	Development of benign skin tumors were developed in 40-50% of animals at 30 or more weeks, but continued administration caused regression of most of the tumors.	55, 56, 57
	36 weeks	Oral (in drinking water)	Rat (male)	0.4% + 100 ppm MNNG[2]	Compared with MNNG single administration group, highly-differentiated adenocarcinoma of the glandular stomach was slightly increased, and some cases with poorly-differentiated adenocarcinoma and adenocarcinoma complicated by sarcoma, not seen in the single MNNG group, were observed; thus, tumor promotion and increased malignancy were observed.	61
	26 weeks	Oral (in drinking water)	Rat (male)	0.4% +50 ppm MNNG[2]	Increased incidence of adenocarcinoma, including 1/9 cases of poorly-differentiated adenocarcinoma in the glandular stomach, and development of sarcoma were observed.	60
Reproduction and developmental toxicity	7-14 day pregnancy	MF	Rat (10-12)	0.1, 1 or 10% (99, 960, 7,693 mg/kg/day)	Mortality of embryos/fetuses was statistically significantly increased in 1% treated group.	63
	1-19 or 7-15 day pregnancy	Oral	Rat (22-26)	1 drop (approx. 150 mg/kg/day)	There was no effect on the number of live fetuses and the fetal body weight.	64
	6-13 day pregnancy	Oral	Mouse	5.2 g/kg/day	Suppression of neonatal weight gain was observed at day 3 or later after birth.	65
Local irritation	30 days	Skin[4]	Rabbit	5 or 10% aqueous solution	Moderate irritation was observed with 5% solution, and necrosis of the skin was observed with 10% solution.	71
	60 days	Skin[4]	Rabbit	15% aqueous solution/stock solution	There was no effect of applying 15% aqueous solution, but application of stock solution caused mild irritation.	72
		Skin[4]	Mouse	Stock solution	Local inflammatory changes were observed.	13
	16 weeks	Scalp[5]	Human (68 males)	25% aqueous solution, 10 drops/dose, 2 times/day	Mild redness was observed in one case.	73

Test type	Administration period	Method of Dosing[3]	Species No./group	Dose/concentration	Test results	Ref. No.
Sensitization		Forehead[6]	Human	Cream containing polysorbate 60 as base or polysorbate 60 alone	Urticaria developed at application sites at 20 minutes.	76

[1]A value reduced by the bureau based on JECFA "Principles for the safety assessment of food additives and contaminants in food"[87)]

[2]N-methyl- N'-nitro-N-nitorosoguanidine

[3]Abbreviations:IV (intravenous injection), ID (intradernal injection), IM (intramuscular injection), PC (percutaneous), SQ (subcutaneous), MF (mixed feed)

[4]Skin application

[5]Scalp application

[6]Forehead skin application

Polysorbate 65 Safety Test Results

Test type	Administration period	Method of Dosing	Species No./group	Dose/concentration	Test results	Ref No.
Genotoxicity	Reverse mutation		TA98, TA100, TA1535, TA1537, WP2*uvr*A	Up to 5,000 μg/plate	Negative results were obtained with or without metabolic activation system.	43
	Chromosome aberration		Chinese hamster cell line (CHL/IU)	Up to 5,000 μg/plate	Weak structural abnormalities were induced only at the dose at which strong cytotoxicity was observed in a short-time treated group in the absence of a metabolic activation system. Numeric abnormalities of chromosomes were induced weakly in the short-time treated group and the log-time continuously-treated group in the absence of a metabolic activation system. Although there was a statistically significant difference, rather weakened induction in the presence of metabolic activation system indicated in comparison with the positive induction in the absence.	44
	Micronucleus test	Oral	Mouse	2,000 mg/kg, twice per 24 h interval	A negative result was obtained.	45
Repeated-dose toxicity	2 years	MF	Rat	2% (approx. 1 g/kg/day)	No adverse effect was observed.	26
	2 years (multigeneration)	MF	Rat	5% (2,500 mg/kg/day[1])	There was no effect on growth and survival, and no abnormality was observed in the blood test, urine test and histological test of the liver and the kidney. Mild diarrhea was observed in 1 of 12 male rats.	7, 27, 28
	13 days	Oral	Human (8 males, 4 females)	9 g/day (0.15 g/kg/day)	There was no symptom of gastrointestinal tract disorders.	35
Carcinogenicity	2 years	MF	Rat (12 male, 20 female)	5, 10 or 20% (2.5, 5, 10 g/kg/day[1])	No carcinogenicity was observed.	54
Reproduction and developmental toxicity	3 generations (F0: 12 weeks)	MF	Rat (12 male, 20 female)	5, 10 or 20% (approx. 2.5, 5.0, 10 g/kg/day)	The numbers of newborns surviving at 4 days of 3 generations were slightly decreased. The numbers of newborns surviving at 4 days were more markedly decreased for all the generations in the 10% and 20% groups. The dead parent animals increased in the 20% group. The 4-day or longer survival of newborns was reduced for all the generations.	28

Test type	Administration period	Method of Dosing	Species No./group	Dose/concentration	Test results	Ref No.
Local irritation	30 days	Skin[3]	Mice	30% aqueous solution, 6 days/week	There were local inflammation and hyperplasia of the epidermis.	14
	48 hours	Skin[3] (closed system)	Humans	Stock solution	No irritation was observed.	14
	72 hours	Skin[3]	Human (50 healthy persons)	60% aqueous suspension, 2 times at 7 day interval	No irritation was observed.	74

[1]A value reduced by the bureau based on JECFA "Principles for the safety assessment of food additives and contaminants in food"[87)]

[2]Abbreviations:IV (intravenous injection), ID (intradernal injection), IM (intramuscular injection), PC (percutaneous), SQ (subcutaneous), MF (mixed feed)

[3]Skin application

Polysorbate 80 Safety Test Results

Test type	Administration period	Method of Dosing[5]	Species No./group	Dose/concentration	Test results	Ref No.
Genotoxicity	Rec-assay		*Bacillus subtilis*		A negative result was obtained (without metabolic activation).	40
	Rec-assay		*E. coli*		A negative result was obtained.	46
	Reverse mutation test		TA98、TA100、TA1535, TA1537		All the results were negative with or without S9 mix.	40, 41
	Chromosomal aberration test		Mammalian cultured cells		A negative result was obtained with or without a metabolic activation system.	40, 47
	Micronucleus test		Rodent		A negative result was obtained.	48, 49
	Dominant lethal test		Mammalian germ cells		A negative result was obtained.	50
Acute toxicity	Single	Oral	Rat	22 g/kg	Abnormal symptoms were not developed, and there was no hepatic or renal impairment.	11
Repeated-dose toxicity	2 years	MF	Rat	5, 10 or 20% (2,500, 5,000, 10,000 mg/kg/day[1])	Diarrhea was observed in female rats of 10% treated group.	7, 27, 28
	13 weeks	MF	Rat/Mouse	0.31, 0.62, 1.25, 2.5 or 5.0% (155, -2,500/465-7,500 mg/kg/day[1])	No abnormal symptom was observed. No macroscopic or histological change was observed in organs.	29
	2 years	MF	Rats/mice	2.5 or 5% (1,250, 2,500/3,750, 7,500 mg/kg/day[1])	There was no effect of the administration on clinical symptoms, survival and growth.	29
	2 days	Oral	Infants	20 g/kg/day	Mild diarrhea was observed.	36
	13 days	Oral	Human (12 healthy persons)	9 g/day (0.15 g/kg/day)	No symptom of the gastrointestinal tract was observed.	37
	1-4 years	Oral	Human (46 patients)	4.5-6 g/day	There was no effect on hemogram, blood pressure, renal function, and hepatic function.	38
Carcinogenicity	2 years	MF	Rat	5, 10 or 20% (2,500, 5,000, 10,000 mg/kg/day[1])	The incidence of fibroadenoma as sporadic tumors was slightly increased in the mammary glands of female rats including those in the control group, but no carcinogenicity was demonstrated	54
	2 years	MF	Rat (50 ea male and female)	25,000, 50,000 ppm (1,250, 2,500 mg/kg/day[1])	About half of male/female rats survived, and an increased incidence of adrenal pheochromocytoma was demonstrated in the 50,000 ppm male group, although this was not significant.	29
	2 years	MF	Mouse (50 ea male and female)	25,000, 50,000 ppm (3,750, 7,500 mg/kg/day[1])	About 50-60% of animals survived. Hyperplastic lesions in the anterior stomach mucosa were significantly increased in the 50,000 ppm male/female mouse groups, but no carcinogenicity was demonstrated.	29
	10 weeks	MF	Mouse	100 mg/animal/day (5,000 mg/kg/day[1])	No tumor development was observed.	58

Test type	Administration period	Method of Dosing[5]	Species No./group	Dose/concentration	Test results	Ref No.
Carcinogenicity(continued)	52 weeks	Skin[6]	Mouse (50)	Stock solution 80 mg/time, 6 times/week	A benign skin tumor developed in 1 animal.	53
	40 weeks	SC	Rat (20)	2 mL of 6% aqueous solution, 3 times/week	Fibrosarcoma was formed in the injection sites of 11 animals.	59
	Lifetime	IT	Hamster (50)	0.2 mL of 5% aqueous solution, 1 time/week	No tumor development was observed.	59
	10 weeks	MF	Mouse (male)	100 mg/animal/day + 0.6 mg/animal/day MC[4]	The incidence of lung tumor in MC single administration group was 44.4%, while that in the multiple administration group 74.1%. The incidence of papilloma in the anterior stomach was increased from 4.8% to 25.9%, and that of squamous cell cancer from 3.7% to 7.4%; the total number of tumors that developed and frequently-occurred cases of primary tumors were increased	58
	26 weeks	Oral (in drinking water)	Rat (male)	0.4% +50 ppm MNNG[*2]	None of the increased incidence of adenocarcinoma in the glandular stomach, increased malignancy of adenocarcinoma and development of gastric sarcoma were especially seen compared with the MNNG single administration group	60
		Skin	Mouse	Stock solution (after percutaneous administration of DMBA[3])	Development of skin tumors increased compared with that in DMBA single administration.	59
Reproduction and developmental toxicity	3 generations (F0: 12 weeks)	MF	Rat (12 male, 20 female)	5, 10 or 20% (approx. 2.5, 5, 10 g/kg/day)	The fertility and 4-day survival after birth of newborns were slightly reduced.	28
	3 generations	MF	Rat	2% (approx. 1 g/kg/day)	There was no effect on fertility and growth.	66
	Female: Day 14 before pregnancy - 21 days of lactation Male: 5 days before mating	Oral (in drinking water)	Rat	1.35 g/L in water (approx. 100 mg/kg/day)	Newborns' exploratory behaviors and the amount of exercise were increased.	67
	6-15 day pregnancy	Oral	Rat (25)	500, 5,000 mg/kg/day	There was no marked difference in the body weight of the administration group, the numbers of corpus luteums and implantations, death rate of preimplantation embryos per mother animal, and survival, growth and morphological development of fetuses, compared with those of the control group	68

Test type	Administration period	Method of Dosing[5]	Species No./group	Dose/concentration	Test results	Ref No.
Reproduction and developmental toxicity (continued)	8-12 day pregnancy	Oral	Mouse (30)	2.5 g/kg/day	There was no significant effect on the number of newborns and the average weight of the newborns.	69
	0-day pregnancy - postpartum day 21	Oral	Rat (22)	0, 0.018, 0.13, 1.0, 7.5% vol liquid (0, 38, 265, 2,013, 18,126 mg/kg/day)	Mother animal: In 7.5% vol administration group, maternal toxicities such as loose stool, suppression of weight gain and reduced intake were seen, and poor lactation was observed in some mother animals. Subsequent generation (F_1): In the 7.5% vol administration group, suppression of weight gain and low avoidance rate in a conditioned avoidance response test in 23-27 days old were seen.	70
Local irritation	10 days or 1 month	Skin[6]	Rabbit	5% aqueous solution	Daily application for 1 month caused moderate irritation; however, there was no marked irritation by daily application for 10 days.	71
	48 hours	Skin[6] (closed system)	Human (50 healthy persons)	Stock solution	No irritation was observed.	75
		Instillation	Human	Up to 20% aqueous solution	No irritation was observed.	11
Sensitization	48 hours	Closed patch	Human (737 suspected of having contact dermatitis)	10% mineral-oil solution	Positive reaction was observed in 4 cases.	77
		Patch test	Human (with eczema)	5, 10 or 100%	Positive reaction was observed in 3 in 330 cases for undiluted polysorbate 80 solution, 1 in 590 cases for 10% polysorbate 80 mineral-oil solution and 2 in 1,206 cases for 5% polysorbate 80 + 5% polysorbate 40 mineral-oil solution	78, 79,75
		Oral	Human (21 patients, 19 healthy persons)	5 g	Deterioration of nasal symptoms was observed in patients with chronic rhinitis, nasal mucosal polyp and asthma, however, no reaction was observed in healthy persons.	80

[1]A value reduced by the bureau based on JECFA "Principles for the safety assessment of food additives and contaminants in food"[87)]

[2]N-methyl- N'-nitro-N-nitorosoguanidine

[3]7,12-dimethylbenz[a]anthracene

[4]3-methylcholanthrene

[5]Abbreviations:IV (intravenous injection), ID (intradernal injection), IM (intramuscular injection), PC (percutaneous), SQ (subcutaneous), MF (mixed feed), IT (Intratracheal injection)

[6]Skin application