DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service
Food and Drug Administration

Memorandum

Date: MAR 22 2001

From: Director, Division of Standards and Labeling Regulations, Office of Nutritional Products, Labeling and Dietary Supplements, HFS-820

Subject: 75-Day Premarket Notification for New Dietary Ingredients

To: Dockets Management Branch, HFA-305

90 4 8 '01 APR 3 P2:51

New Dietary Ingredient:	Isoquercetin (quercetin-3-glocoside)
Firm:	Merck KGaA
Date Received by FDA:	December 28, 2000
90-Day Date:	March 28, 2001

In accordance with the requirements of section 413(a) of the Federal Food, Drug, and Cosmetic Act, the attached 75-day premarket notification for the aforementioned new dietary ingredient should be placed on pubic display in docket number 95S-0316 after March 28, 2001.

Felicia B. Satchell

95S-0316

RPT 89

MAR 13 2001

VIA FACSIMILE AND MAIL

Najib Sehat, Ph.D.
Merck KgaA
CHN-BS
Regulatory Affairs, C11/243
Frankfurter Str. 250
64271 Darmstadt, Germany

Dear Dr. Sehat:

This is in response to your letter to the Food and Drug Administration dated December 20, 2000, making a submission of a new dietary ingredient pursuant to 21 U.S.C. 350b(a)(2) (section 413(a)(2) of the Federal Food, Drug, and Cosmetic Act (the act)). Your letter notified FDA of your intent to market a dietary supplement product containing the new dietary ingredient described as "isoquercetin."

21 U.S.C. 350b(a)(2) requires that a manufacturer or distributor of a dietary supplement that contains a new dietary ingredient submit to FDA, at least 75 days before the dietary ingredient is introduced or delivered for introduction into interstate commerce, information that is the basis on which the manufacturer or distributor has concluded that a dietary supplement containing such new dietary ingredient will reasonably be expected to be safe. FDA reviews this information to determine whether it provides an adequate basis for such a conclusion. Under section 350b(a)(2), there must be a history of use or other evidence of safety establishing that the dietary ingredient, when used under the conditions recommended or suggested in the labeling of the dietary supplement, will reasonably be expected to be safe. If this requirement is not met, the dietary supplement is deemed to be adulterated under 21 U.S.C. 342(f)(1)(B) because there is inadequate information to provide reasonable assurance that the new dietary ingredient does not present a significant or unreasonable risk of illness and injury.

FDA has carefully evaluated the information in your submission. Your submission contains evidence of history of use and other information that you assert is an adequate basis to conclude that a dietary supplement product containing isoquercetin will reasonably be expected to be safe. To support the safety of isoquercetin, you have submitted information concerning the safety of quercetin. FDA recognizes that quercetin is a component of several foods and is marketed in dietary supplements. However, the agency has concerns about the use of quercetin at the exposures you intend to recommend in your product's labeling, up to 600 milligrams per day. First, quercetin is a known mutagen. In addition, there is some evidence suggesting that quercetin may be a

carcinogen. Recognizing these unresolved safety concerns, the agency's safety concerns surrounding the use of isoquercetin would be mitigated if the daily exposure were reduced to be more consistent with current daily dietary exposure.

Should you have any questions concerning this matter, please contact me at (202) 205-4168.

Sincerely yours,

Felicia B. Satchell

Felicia B. Satchell
Director
Division of Standards
and Labeling Regulations
Office of Nutritional Products, Labeling
and Dietary Supplements

Food and Drug Administration
Washington, DC

JAN 18 2001

Dr. Najib Sehat
Merck KGaA
CHN-BS
Regulatory Affairs, C11/243
Frankfurter Str. 250
64271 Darmstadt, Germany

Dear Dr. Sehat:

This is to inform you that the notification, dated December 20, 2000, you submitted pursuant to 21 U.S.C. 350b(a)(2) was received and filed by the Food and Drug Administration (FDA) on December 28, 2000. Your notification concerns the substance called "isoquercetin (quercetin-3-glocoside)" that you assert is a new dietary ingredient.

In accordance with 21 C.F.R. § 190.6(c), FDA must acknowledge its receipt of a notification for a new dietary ingredient. For 75 days after the filing date (i.e., after March 13, 2001), you must not introduce or deliver for introduction into interstate commerce any dietary supplement that contains "isoquercetin (quercetin-3-glocoside)"

Please note that the acceptance of this notification for filing is a procedural matter and thus, does not constitute a finding by FDA that the new dietary ingredient or the dietary supplement that contains the new dietary ingredient is safe or is not adulterated under 21 U.S.C. 342. As another procedural matter, your notification will be kept confidential for 90 days after the filing date. After March 28, 2001, your notification will be placed on public display at FDA's Docket Management Branch in docket number 95S-0316. However, any information that is trade secret or otherwise commercial confidential information in the notification will not be disclosed to the public.

Please contact us at (202) 205-4168, if you have any questions concerning this matter.

Sincerely,

Margaret C. Carlson
(Acting) Leader
Dietary Supplements Team
Division of Standards
and Labeling Regulations
Office of Nutritional Products, Labeling
and Dietary Supplements
Center for Food Safety
and Applied Nutrition

Datum December 20, 2000
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December 20, 2000

Food and Drug Administration
Center for Food Safety and Applied Nutrition, CFSAN
Office of Nutritional Products, Labeling, and Dietary Supplements
HFS-820
Division of Standards and Labeling Regulations

200 C Street, S.W.
Washington, DC 20204
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Received NLA
DEC 28

Attention: Ms. Margaret C. Carlson, Acting Team Leader

Dear Ms. Carlson:

Pursuant to Section 8 of the Dietary Supplement Health and Education Act of 1994, Merck KGaA ("Merck"), located at Darmstadt, Frankfurter Str. 250, 64293, Darmstadt, Germany, submits this new dietary ingredient notification to the Food and Drug Administration ("FDA") for isoquercetin (quercetin-3-glocoside) to be manufactured by Merck.

Merck's isoquercetin product is intended for use as a dietary ingredient in dietary supplements. The product was critically evaluated by a panel of independent recognized experts, which published its expert opinion on May 31, 2000. The panel unanimously concluded that under the conditions of intended use as a dietary ingredient in dietary supplements, Merck's isoquercetin product, meeting food grade specifications and manufactured in accordance with current good manufacturing practices, would not present a significant or unreasonable risk of illness or injury, and would reasonably be expected to be safe.

Attached is a discussion of the scientific data and information demonstrating that Merck's isoquercetin product, when used under the conditions suggested in the labeling of the dietary supplement, is reasonably expected to be safe. Included in the attachment are the following:

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(1.0) chemistry, manufacturing, and

(2.0) a description of the proposed use; and

(3.0) safety data, and

(4.0) a conclusion, and

(5.0) a list of references.

Sincerely,

Merck KGaA

ppa. i. V.

Dr. Roland Martin Dr. Najib Sehat

Dr. Najib Sehat
Merck KGaA
CHN-BS
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Enclosures:

- Expert Panel Opinion Statement: The Safety of Isoquercetin Dietary Supplement Product Manufactured by Merck KGaA.

- Copy of cited references

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New Dietary Ingredient Notification Attachement

(Isoquercetin)

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1.0 Chemistry Considerations Concerning Isoquercetin

1.1 Chemical Name

2-(3,4-Dihydroxyphenyl)-3-(-D-glucofuranosyloxy)-5,7-dihydroxy-4H-1-benzopyran-4-one; 3,3',4' ,5,7-pentahydroxyflavone-3-glucoside

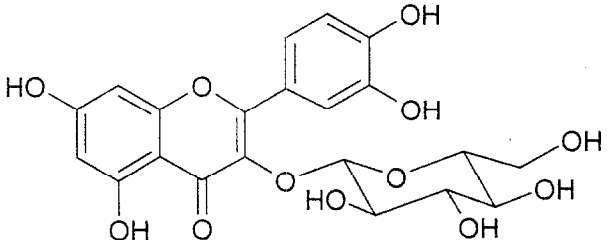
1.2 Chemical Abstract Service (CAS) Registry Number

21637-25-2

1.3 Chemical Synonyms

quercetin-3-glocoside
quercetin-3-O-glucose
isotrifoliin
trifoliin

1.4 Chemical Structure



1.5 Molecular Formula

$C_{21}H_{20}O_{12}$

1.6 Molecular Weight

464.37

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1.7 Physical and Chemical Properties

Physical state, color: Yellow needles from water.
pH: Isoquercetin > 98% dissolved in demineralized water (saturated solution) has a pH = 4,85.
Degradation: Between 225-227°C.
Solubility: Sparingly soluble in boiling water, but virtually insoluble in cold; soluble in alkaline solutions with a deep yellow tint.

1.8 Specifications and Manufacturing Method

2.0 Proposed Use

Merck's Isoquercetin product is intended for use as a dietary ingredient in dietary supplements. Such dietary supplements are expected to contain 200 mg isoquercetin per tablet or capsule, and to be consumed one to three times daily.

3.0 Safety Data

As noted above, Merck's isoquercetin dietary supplement product consists of isoquercetin (85%), quercetin (<15%) and rutin (<15%). Quercetin, isoquercetin and rutin (quercetin-3-rutinoside) are bioflavonoids found in nature and are very similar chemically and biologically. The aglycone quercetin is common to all three. Glycosides that contain quercetin have been shown to liberate quercetin in the gastro-intestinal tract. Therefore, the biological effects of these three bioflavonoids would be expected to be identical; that is, the biological effects of quercetin would be seen following ingestion of quercetin, isoquercetin, or rutin.

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The estimated total daily consumption of flavonoids from a normal U.S. diet is one gram/day (Pierpoint, 1986). Most flavonoids are ingested as glycosides

having a relatively high molecular weight. Absorption from the small intestine is very limited due to the molecular weight and the hydrophilicity of the glycosides. The flavonoids pass unchanged into the large intestine where the microflora produce glycosidases that hydrolyze the flavonoid into the aglycone and its sugar. If the intact aglycone is able to resist cleavage of the pyrone ring, the aglycone could be absorbed. However, absorption is very limited. For example, Gugler, et al. (1975) administered quercetin orally to humans at doses of 50-65 mg/kg body weight and did not find unaltered quercetin in the plasma but recovered 53% of the dose as the aglycone in the feces; the remainder was presumably degraded by the microflora in the lower bowel. Quercetin was administered orally to rats at doses of 1 or 2 g/kg bw, and only 0.3% and 0.5% of quercetin was absorbed and excreted unchanged in the urine (MacGregor, 1979 and Crebelli, et al., 1987). The data indicate that the bioavailability of flavonoids, including quercetin, is very low.

A comprehensive summary of the toxicological data for quercetin is presented as Table 1. The acute oral toxicity is very low (>5000 mg/kg bw in the rat and >16000 mg/kg in the mouse). A number of long-term/oncogenicity studies in rates, mice and hamsters have been reported. Stavric (1984) cited 17 feeding studies in which quercetin was administered to rats, mice and hamsters as a dietary admixture in the range of 0.25% to 10%. There was no evidence of carcinogenicity. Quercetin also was reported to induce renal tubular cell ademomas in male Fischer 344 N male rats at the highest level fed, 40000 ppm but not at the lower levels (1000 and 10,000 ppm) (NTP, 1991). Quercetin was not carcinogenic in F344/DuCrj rats as dietary concentrations of 1.25% and 5.0% for two years, nor in mice or golden hamster (at dietary concentrations up to 10%, the highest concentration tested). Quercetin has been reported to be protective against certain chemical carcinogens; it slows the growth of various human cancer cell lines and it may enhance the effect of certain antitumor drugs. The weight of the evidence supports the non-carcinogenicity of quercetin.

Single or multiple doses of 2-2000 mg quercetin/kg bw/day during organogenesis (gestation days 6-15) failed to elicit adverse developmental (teratological) effects in S-D rats. Quercetin was reported to be mutagenic in most in vitro tests but was not mutagenic in most in vivo tests.

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A comprehensive search of the scientific literature for toxicological data for isoquercetin failed to identify any studies. Since isoquercetin is metabolized to quercetin and glucose, one can assume that the biological effects of isoquercetin and quercetin are very similar or identical.

A critical analysis of the available toxicological information on orally administered quercetin indicates that quercetin is very poorly absorbed from the gastrointestinal tract (<1% of an orally administered dose), has a low order of acute and chronic toxicity, is not mutagenic or carcinogenic in appropriate in vivo assays, and is neither a reproductive nor a developmental toxin.

TABLE 1. QUERCETIN PRECLINICAL STUDIES

MUTAGENICITY IN VITRO

TEST	RESULT	REFERENCE
Bacterial mutagenicity (unactivated)	Positive	Bjeldanes, 1977, MacGregor, 1978 Brown, 1979, Rueff, 1986
Bacterial mutagenicity (metabolic activation)	Positive	Bjeldanes, 1977, Brown, 1979, MacGregor, 1978; Rueff, 1986
Gene mutation	Negative	Carver, 1983, Van der Hoeven, 1984
Gene mutation	Positive	Maruta, 1979, Meltz, 1981 Nakayasu, 1986
Chromosome aberration	Positive	Carver, 1983; Yoshida, 1980;
Sister chromatid exchange	Positive	Carver, 1983, Ishida, 1988
Sister chromatid exchange	Negative	Van der Hoeven, 1984
Micronucleus test	Positive	Popp, 1991

Mutagenicity in Vivo

TEST	SPECIES	RESULT	REFERENCE
Sister chromatid exchange	Rabbit	Negative	MacGregor, 1983
Micronucleus test	Mouse	Negative	Aeschbacher, 1982; MacGregor, 1983 Caria, 1995; Ngomuo, 1996
Micronucleus test	Mouse	Positive	Sahu, 1981
Micronucleus test	Rat	Negative	Utesch, 1998
Host-mediated assay		Negative	Aeschbacher, 1982

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Acute Toxicity

SPECIES	STRAIN/SEX	ROUTE	DOSE/CONC.	RESULTS/LD_{50}	REF
Rat		Oral (Gavage)		>5,000 mg/kg bw	Covance, 1998 unpublished
Mouse		Oral (Gavage)		>16,000 mg/kg bw	Merck, 1976 unpublished
Mouse	Albino farm	Oral (Gavage)		160 mg/kg bw	Sullivan, 1951
Mouse		IP		4064 mg/kg	Merck, 1976 unpublished
Mouse	Albino farm	SC		98 mg/kg bw	Sullivan, 1951

Reproductive Toxicity

SPECIES	STRAIN/SEX	ROUTE	DOSE/CONC.	RESULTS/NOAEL	REF
Rat	S-D/Female (pregnant)	Oral	0, 2, 20, 200, 2000 mg/kg bw daily on days 6-15 of preg; or single dose on day 9	Negative	Willhite, 1982
Mouse	(C57xBALB/B) F1 / Male	IP	0, 3.2, 6.4, 16, 32 mg/kg bw for 5 consecutive days (total doses 0, 16, 32, 80, 160 mg/kg)	Paternal Effects (spermatogenesis; testes, epididymis, sperm duct)/ 16 mg/kg total dose	Rastogi, 1987

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Developmental Toxicity

SPECIES	STRAIN/SEX	ROUTE	DOSE/CONC.	RESULTS/NOAEL	REF
Mouse	Swiss/Male	IP	0; 200, 300, 400 mg/kg bw in DMSO	Reduction in fertility of untreated females paired with males/200 mg/kg bw	Aravindakshan, 1985

Chronic Toxicity

SPECIES	STRAIN/SEX	ROUTE	DOSE/CONC.	RESULTS/NOAEL	REF
Rat	Albino/MF	Oral (Diet)	0, 0.25, 0.5, 1%, 410 days	No effect/0.25%	Ambrose, 1952

Carcinogenicity

SPECIES	STRAIN/SEX	ROUTE	DOSE/CONC.	RESULTS/NOAEL	REF
Rat	F344/Female	Oral (Diet)	0; 1.25; 5.0% for 104 wk	Negative/1.25%	Ito, 1989
Rat	F344/Male	Oral (Diet)	0; 1.25; 5.0% for 104 wk	Negative/1.25%	Ito, 1989
Rat	F344/Male	Oral (Diet)	0; 1000; 10000; 40000 ppm for 104 wk	Kidney (renal tubule): adenoma	NTP, 1991
Rat	F344/Female	Oral (Diet)	0; 1000; 10000; 40000 ppm for 104 wk	Negative/1000	NTP, 1992
Rat	F344/Male	Oral (Diet)	0; 0.1; 0.2% for 64 wk	Negative	Stoewsand, 1984
Rat	F344/Female	Oral (Diet)	0; 0.1; 0.2% for 64 wk	Negative	Stoewsand, 1984
Rat	ACI	Oral (Diet)	0; 1.0; 5.0% for 540 days	Negative	Hirono, 1981
Rat	F344/Male	Oral (Diet)	0; 5% in diet for 4 weeks, followed by ± BHBN in diet for 29 weeks	Negative (only urinary bladder examined)	Hirose, 1983

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Hamster	Non-inbred golden	Oral (Diet)	0; 10% for 735 days	Negative	Morino, 1982
Rat	Non-inbred albino	Oral (Diet)	0; 1% for 58 weeks	Intestinal (adenoma, fibro-adenoma, adenocarcinoma) Bladder (papillary, sessile transitional cell carcinoma)	Pamukcu, 1980
Mouse	Strain A	Oral (Diet)	0; 5% for 23 weeks	Negative (only lung tumor assessed)	Hosaka, 1981
Mouse	ddY M/F	Oral (Diet)	0: 2% for 842 days	Negative (high mortality)	Saito, 1980
Rat	Fischer 344	Oral (Diet)	0; 0.1% for 540 days	Negative	Takanashi, 1983
Mouse	ICR/Ha Swiss/Female	Topical	0; 25 mg in 0.1 ml DMSO to dorsal skin 3x/week for 368 days	No skin tumors induced	Van Duuren, 1976
Mouse	Albino Swiss/Female	Bladder implant	20 mg cholesterol pellets containing 4 mg quercetin for 175 days – 1 yr	No significant difference in incidence of bladder carcinoma	Wang, 1976

4.0 Conclusion

Merck's Isoquercetin product, intended for use as a dietary ingredient in dietary supplements providing up to 600 mg isoquercetin daily, and meeting food grade specifications and manufactured in accordance with current good manufacturing practices, would not present a significant or unreasonable risk of illness or injury, and would reasonably be expected to be safe.

5.0 References

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EXPERT PANEL OPINION STATEMENT: THE SAFETY OF ISOQUERCETIN DIETARY SUPPLEMENT PRODUCT MANUFACTURED BY MERCK KGaA

May 31, 2000

The undersigned, an independent panel of recognized experts (the Expert Panel), qualified by their scientific training and experience to evaluate the safety of food and food ingredients, was asked by Merck KGaA (Merck) of Darmstadt, Germany, to evaluate the safety of Merck's isoquercetin product intended for use as a dietary ingredient in dietary supplements. The qualifications of the Expert Panel members are evidenced in the attached *curricula vitae*.

A comprehensive search of the scientific literature concerning relevant safety and toxicity information through 31March 2000 was conducted by George A. Burdock, Ph.D., and made available to the Expert Panel. The Panel independently and critically evaluated the pertinent articles identified in the literature search, the materials provided by Merck and other data and information deemed appropriate or necessary. Specifically, the Panel considered data and information concerning the method of manufacture including the enzyme source organism, *Penicillium decumbens*; product specifications; chemical analyses of the product; the conditions of intended use; and data and information relating to the safety of the bioflavonoids isoquercetin, quercetin and rutin.

Following independent, critical evaluation of such data and information, the Panel members conferred by conference call. The Expert Panel unanimously concluded that, under the conditions of intended use as a dietary ingredient in dietary supplements, Merck's isoquercetin product, meeting food grade specifications and manufactured in accordance with current good manufacturing practices, would not present a significant or unreasonable risk of illness or injury, and would reasonably be expected to be safe.

A summary of the basis for the Expert Panel's conclusion is provided below.

Manufacturing and Specifications

Intended Use

Merck's Isoquercetin product is intended for use as a dietary ingredient in dietary supplements. Such dietary supplements are expected to contain 200 mg. isoquercetin per tablet or capsule, and to be consumed one to three times daily.

Data Pertaining to the Safety of Merck's Isoquercetin Product

As noted above, Merck's isoquercetin dietary supplement product consists of isoquercetin (85%), quercetin (<15%) and rutin (<15%). Quercetin, isoquercetin (quercetin-3-glucoside) and rutin (quercetin-3-rutinoside) are bioflavonoids found in nature and are very similar chemically and biologically. The aglycone quercetin is common to all three. Glycosides that contain quercetin have been shown to liberate quercetin in the gastro-intestinal tract. Therefore, the biological effects of these three bioflavonoids would be expected to be identical; that is, the biological effects of quercetin would be seen following ingestion of quercetin, isoquercetin, or rutin.

The estimated total daily consumption of flavonoids from a normal U.S. diet is one gram/day (Pierpoint, 1986). Most flavonoids are ingested as glycosides having a relatively high molecular weight. Absorption from the small intestine is very limited due to the molecular weight and the hydrophilicity of the glycosides. The flavonoids pass unchanged into the large intestine where the microflora produce glycosidases that hydrolyze the flavonoid into the aglycone and its sugar. If the intact aglycone is able to resist cleavage of the pyrone ring, the aglycone could be absorbed. However, absorption is very limited. For example, Gugler, et al (1975) administered quercetin orally to humans at doses of 50-65 mg/kg body weight and did not find unaltered quercetin in the plasma but recovered 53% of the dose as the aglycone in the feces; the remainder was presumably degraded by the microflora in the lower bowel. Quercetin was administered orally to rats at doses of 1 or 2 g/kg bw, and only 0.3% and 0.5% of quercetin was absorbed and excreted unchanged in the urine (MacGregor, 1979 and Crebelli, et al, 1987). The data indicate that the bioavailability of flavonoids, including quercetin, is very low.

A comprehensive summary of the toxicological data for quercetin is presented as Table 1.

The acute oral toxicity is very low (>5000 mg/kg bw in the rat and >16000 mg/kg in the mouse). A number of long-term/oncogenicity studies in rates, mice and hamsters have been reported. Stavric (1984) cited 17 feeding studies in which quercetin was administered to rats, mice and hamsters as a dietary admixture in the range of 0.25% to 10%. There was no evidence of carcinogenicity. Erturk et al (1985) reported that quercetin produced bladder tumors in rats and Pabukeo (1980) reported that quercetin from bracken fern produced intestinal and bladder tumors. Quercetin also was reported to induce renal tubular cell ademomas in male Fischer 344 N male rats at the highest level fed, 40000 ppm but not at the lower levels (1000 and 10,000 ppm) (NTP, 1991). Quercetin was not carcinogenic in F344/DuCrj rats as dietary concentrations of 1.25% and 5.0% for two years, nor in mice or golden hamster (at dietary concentrations up to 10%, the highest concentration tested). Quercetin has been reported to be protective against certain chemical carcinogens; it slows down the growth of various human cancer cell lines and it may enhance the effect of certain antitumor drugs. The weight of the evidence supports the non-carcinogenicity of quercetin.

Single or multiple doses of 2-2000 mg quercetin/kg bw/day during organogenesis (gestation days 6-15) failed to elicit adverse developmental (teratological) effects in S-D rats. Quercetin was reported to be mutagenic in most in vitro tests but was not mutagenic in most in vivo tests.

A comprehensive search of the scientific literature for toxicological data for isoquercetin failed to identify any studies. Since isoquercetin is metabolized to quercetin and glucose, one can assume that the biological effects of isoquercetin and quercetin are very similar or identical.

A critical analysis of the available toxicological information on orally administered quercetin indicates that quercetin is very poorly absorbed from the gastrointestinal tract (<1% of an orally administered dose), has a low order of acute and chronic toxicity, is not mutagenic or carcinogenic in appropriate in vivo assays and is neither a reproductive nor a developmental toxin.

Conclusion

We, the Expert Panel, have critically and independently evaluated the data and information summarized above and conclude that Merck's Isoquercetin product, intended for use as a dietary ingredient in dietary supplements providing up to 600 mg. isoquercetin daily, and meeting food grade specifications and manufactured in accordance with current good manufacturing practices, would not present a significant or unreasonable risk of illness or injury, and would reasonably be expected to be safe.

By:

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Joseph F. Borzelleca, Ph.D.
Toxicology and Pharmacology, Inc.

Date

Walter H. Glinsmann — 1 June 2000

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TABLE 1. QUERCETIN PRECLINICAL STUDIES

MUTAGENICITY IN VITRO

TEST	RESULT	REFERENCE
Bacterial mutagenicity (unactivated)	Positive	Bjeldanes, 1977, MacGregor, 1978 Brown, 1979, Rueff, 1986
Bacterial mutagenicity (metabolic activation)	Positive	Bjeldanes, 1977, Brown, 1977, MacGregor, 1978; Rueff, 1986
Gene mutation	Negative	Carver, 1983, Van der Hoeven, 1984
Gene mutation	Positive	Maruta, 1979, Meltz, 1981 Nakayasu, 1986
Chromosome aberration	Positive	Carver, 1983; Yoshida, 1980; Ishida, 1988
Sister chromatid exchange	Positive	Carver, 1983, Ishida, 1988
Sister chromatid exchange	Negative	Van der Hoeven, 1984
Micronucleus test	Positive	Popp. 1991
Unscheduled DNA synthesis	Negative	Poginsky, 1988

Mutagenicity in Vivo

TEST	SPECIES	RESULT	REFERENCE
Sister chromatid exchange	Rabbit	Negative	MacGregor, 1983
Micronucleus test	Mouse	Negative	Aeschbacher, 1982; MacGregor, 1983 Caria, 1995; Ngomuo, 1996
Micronucleus test	Mouse	Positive	Sahu, 1981
Micronucleus test	Rat	Negative	Utesch, 1998
Host-mediated assay		Negative	Aeschbacher, 1982

Acute Toxicity

SPECIES	STRAIN/SEX	ROUTE	DOSE/CONC.	RESULTS/LD_{50}	REF
Rat		Oral (Gavage)		>5,000 mg/kg bw	Covance, unpublished
Mouse		Oral (Gavage)		>16,000 mg/kg bw	Merck, unpublished
Mouse	Albino farm	Oral (Gavage)		160 mg/kg bw	Sullivan, 1951
Mouse		IP		4064 mg/kg	Merck, unpublished
Mouse		IP		3 g/kg	Ezaki, 1968 (RTECS)
Mouse		IV		18 mg/kg	NIOSH (RTECS)
Rabbit		IV		100 mg/kg	FAO, 1969 (RTECS)
Mouse	Albino farm	SC		98 mg/kg bw	Sullivan, 1951

Reproductive Toxicity

SPECIES	STRAIN/SEX	ROUTE	DOSE/CONC.	RESULTS/NOAEL	REF
Rat	S-D/Female (pregnant)	Oral	0, 2, 20, 200, 2000 mg/kg bw daily on days 6-15 of preg; or single dose on day 9	Negative	Willhite, 1982
Mouse	(C57xBALB/B)F 1 / Male	IP	0, 3.2, 6.4, 16, 32 mg/kg bw for 5 consecutive days (total doses 0, 16, 32, 80, 160 mg/kg)	Paternal Effects (spermatogenesis; testes, epididymis, sperm duct)/ 16 mg/kg total dose	Rastogi, 1987

Developmental Toxicity

SPECIES	STRAIN/SEX	ROUTE	DOSE/CONC.	RESULTS/NOAEL	REF
Mouse	Swiss/Male	IP	0; 200, 300, 400 mg/kg bw in DMSO	Reduction in fertility of untreated females paired with males/200 mg/kg bw	Aravindakshan, 1985

Chronic Toxicity

SPECIES	STRAIN/SEX	ROUTE	DOSE/CONC.	RESULTS/NOAEL	REF
Rat	Albino/MF	Oral (Diet)	0, 0.25, 0.5, 1%, 410 days	No effect/0.25%	Ambrose, 1952

Carcinogenicity

SPECIES	STRAIN/SEX	ROUTE	DOSE/CONCENTRATION	RESULTS/NOAEL	REF
Rat	F344/Female	Oral (Diet)	0; 1.25; 5.0% for 104 wk	Negative/1.25%	Ito, 1989
Rat	F344/Male	Oral (Diet)	0; 1.25; 5.0% for 104 wk	Negative/1.25%	Ito, 1989
Rat	F344/Male	Oral (Diet)	0; 1000; 10000; 40000 ppm for 104 wk	Kidney (renal tubule): adenoma	NTP, 1991
Rat	F344/Female	Oral (Diet)	0; 1000; 10000; 40000 ppm for 104 wk	Negative/1000	NTP, 1991
Rat	F344/Male	Oral (Diet)	0; 0.1; 0.2% for 64 wk	Negative	Stoewsand, 1984
Rat	F344/Female	Oral (Diet)	0; 0.1; 0.2% for 64 wk	Negative	Stoewsand, 1984
Rat	ACI	Oral (Diet)	0; 1.0; 5.0% for 540 days	Negative	Hirono, 1981
Rat	F344/Male	Oral (Diet)	0; 5% in diet for 4 weeks, followed by ± BHBN in diet for 29 weeks	Negative (only urinary bladder examined)	Hirose, 1983
Hamster	Non-inbred golden	Oral (Diet)	0; 10% for 735 days	Negative	Morino, 1982
Rat	Non-inbred albino	Oral (Diet)	0; 1% for 58 weeks	Intestinal (adenoma, fibro-adenoma, adenocarcinoma) Bladder (papillary, sessile transitional cell carcinoma)	Pamukcu, 1980
Mouse	Strain A	Oral (Diet)	0; 5% for 23 weeks	Negative (only lung tumor assessed)	Hosaka, 1981
Mouse	ddY M/F	Oral (Diet)	0: 2% for 842 days	Negative (high mortality)	Saito, 1980
Rat	Fischer 344	Oral (Diet)	0; 0.1% for 540 days	Negative	Takanashi, 1983
Mouse	ICR/Ha Swiss/Female	Topical	0; 25 mg in 0.1 ml DMSO to dorsal skin 3x/week for 368 days	No skin tumors induced	Van Duuren, 1976
Mouse	Albino Swiss/Female	Bladder implant	20 mg cholesterol pellets containing 4 mg quercetin for 175 days – 1 yr	No significant difference in incidence of bladder carcinoma	Wang, 1976

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