

***trans*-Resveratrol**  
**[501-36-0]**

**Review of Toxicological Literature**

**March 2002**

***trans*-Resveratrol  
[501-36-0]**

**Review of Toxicological Literature**

*Prepared for*

**Scott Masten, Ph.D.  
National Institute of Environmental Health Sciences  
P.O. Box 12233  
Research Triangle Park, North Carolina 27709  
Contract No. N01-ES-65402**

*Submitted by*

**Karen E. Haneke, M.S.  
Integrated Laboratory Systems  
P.O. Box 13501  
Research Triangle Park, North Carolina 27709**

**March 2002**

## Executive Summary

### Nomination

*trans*-Resveratrol was nominated for toxicology studies by the National Institute of Environmental Health Sciences (NIEHS) based on the widespread human exposure to resveratrol through natural dietary sources and dietary supplement use, and concern that it has not been sufficiently evaluated for potential toxicological effects.

### Non-Toxicological Data

General Description: *trans*-Resveratrol is a polyphenol that occurs naturally in grapes, peanuts, and a number of other plants. It is found in foods/drinks made from grapes and peanuts, and also in a number of herbal remedies, both alone and as part of plant extracts.

Commercial Availability, Production, and Uses: *trans*-Resveratrol is produced commercially by several companies. A commercial extraction method involves using alcohol and water to produce *trans*-resveratrol from *Polygonum cuspidatum*. Resveratrol compounds may be produced or extracted for research purposes by treating cell suspension cultures of grapes with a natural substance from a fungus.

Resveratrol compounds have long been found in herbal medicines. Health claims of oral dietary supplements containing *trans*-resveratrol include protection from free-radical damage, inhibition of arthritic inflammation, inhibition of the cyclooxygenase-2 enzyme, protection of blood vessels, protection against cardiovascular disease and cancer, and alleviation of menopausal symptoms. A patent exists for the use of resveratrol to prevent and to treat restenosis after coronary disease treatment, and a patent application was filed for using resveratrol compounds with nucleoside analogs for treating HIV-1 infections.

Environmental Occurrence and Persistence: Plants that produce *trans*-resveratrol include grapes, peanuts, eucalyptus, spruce, lily, mulberries, groundnut, and members of the knotweed and hellebore genera. Plants synthesize *trans*-resveratrol when infected by microbes exposed to ultraviolet radiation, or when injured or subjected to stress; *trans*-resveratrol levels peak upon exposure to such stress.

Human Exposure: Human exposure to resveratrol compounds is mainly through ingestion, particularly of grapes, peanuts, and their products. Levels are higher in grapes and in grape products than in peanut products. The highest levels in grape products were found in red wine ( $\leq 0.02$ -13.4 mg/L [0.09-58.7  $\mu$ M]). The highest levels in peanut products were found in boiled peanuts (0.02-1.79  $\mu$ g/g [0.09-7.84 nmol/g]).

Exposure through dietary supplements is primarily oral, although one source provided information on a topical cream containing resveratrol. Recommended dosages for oral dietary supplements range from 2.495 mg to 1 g (0.01091  $\mu$ mol to 4 mmol).

Regulatory Status: Manufacturers and distributors must notify the U.S. Food and Drug Administration (FDA) when they plan to market dietary supplements that contain "new dietary ingredients" (Section 413b of the Food, Drug, and Cosmetic Act [FDCA], 21 U.S.C. 350b). Other regulations that apply include 21CFR Section 190.6(b)(4), regarding safety; and Section 403(r)(6) of FDCA, 21, U.S.C. 343 (r)(6), regarding evaluation as a drug.

### Toxicological Data

Note: When specified by the author(s), isomers were named. In most studies, "resveratrol" was used.

Human Studies: Adverse effects of resveratrol have not been reported; a recommended dosage of 5-10 mg (22-44  $\mu\text{mol}$ ) per day was stated to be "entirely safe." Recently, the National Cancer Institute (NCI) initiated preclinical toxicity studies on *trans*-resveratrol; clinical trials may also be conducted.

Chemical Disposition, Metabolism, and Toxicokinetics: In an isolated rat small intestine perfusion model, the majority of absorbed *trans*-resveratrol (administered doses of 28, 34, and 57  $\mu\text{M}$  [6.4, 7.8, and 13  $\mu\text{g/mL}$ ]) was found in the luminal effluent (53.9%). Of this amount, free resveratrol was the dominant product (39.7%). At the vascular side, 20.5% of the administered resveratrol appeared, with the major product being the glucuronide (16.8%). Small amounts of unmetabolized resveratrol were absorbed across the enterocytes of the jejunum and ileum, while significant amounts of its glucuronide were found in the serosal fluid.

In human partially hepatectomized liver microsomes, the highest rate of *trans*-resveratrol glucuronidation (up to 1 mM [228  $\mu\text{g/mL}$ ] resveratrol and 1 mM uridine 5'-diphosphoglucuronic acid [UDPGA] in incubation mixture) occurred at neutral pH, and the resveratrol-glucuronide amount increased linearly with time up to 40 minutes. The reaction of resveratrol sulphation (up to 2  $\mu\text{M}$  [0.5  $\mu\text{g/mL}$ ] resveratrol and 0.4  $\mu\text{M}$  3'-phosphoadenosine-5'-phosphosulphate [PAPS]) showed similar effects. The rates of resveratrol sulphation, similar in the human liver and duodenum, were inhibited by quercetin, fisetin, myricetin, kaempferol and apigenin; the inhibition was mixed and non-competitive. Flavonoids also inhibited resveratrol glucuronidation, but to a lesser extent. The addition of wine to the incubation mixture decreased both the rate of resveratrol sulphation and the rate of glucuronidation.

In human intestinal epithelial cell line Caco-2 cultured in Transwell, the permeability constant for resveratrol suggested that it could be orally absorbed.

In rats, resveratrol (single administration of 86  $\mu\text{g/kg}$  [0.38  $\mu\text{mol/kg}$ ] or 43  $\mu\text{g/kg}$  [0.19  $\mu\text{mol/kg}$ ] for 15 days) in red wine was rapidly absorbed at the intestinal level, immediately entering the blood and reaching a maximum level around one hour after oral administration. The liver contained the highest concentrations (20.7 and 53.5 ng/g following single and repeated administration, respectively). Kinetic studies showed equilibrium between the absorbed resveratrol and the eliminated resveratrol. Significant cardiac bioavailability has also been observed. Given intraperitoneally (i.p.), *trans*-resveratrol (2 mg/kg [9  $\mu\text{mol/kg}$ ]) was rapidly absorbed and the concentration in rat blood declined in a "two-exponential" manner.

Short-term and Subchronic Exposure: In rats, daily oral administration of resveratrol (300, 1000, and 3000 mg/kg [1.31, 4.381, and 13.14 mmol/kg]) for 28 days produced nephrotoxicity, dehydration, labored breathing, hunched posture, decreased activity, rough coat, diarrhea, soft stool, and red material around the nose at the high dose. Males also had leukocytosis, and both sexes may have had anemia. Based on the results, the no observed adverse effect level (NOAEL) was 300 mg/kg/day.

In hypercholesterolemic rabbits, *trans*-resveratrol (0.06 mg/kg [0.3  $\mu\text{mol/kg}$ ] during days 1-5 and 1.0 mg/kg [4.4  $\mu\text{mol/kg}$ ] from days 6-60) promoted atherosclerosis.

Synergistic/Antagonistic Effects: Resveratrol produces a synergistic effect, as well as increased potency and availability, when combined with other antioxidants or compounds having antimutagenic or cardioprotective properties (e.g., anthocyanadins, indole-3-carbinol, and green tea extracts). A recent discovery is resveratrol's potential role in the control of HIV-1 (human immunodeficiency virus-1) replication; it may synergize with existing drugs, potentiating their antiviral effects.

In several mammary cancer cell lines, resveratrol showed mixed estrogen agonist/antagonist activities, whereas in the presence of 17 $\beta$ -estradiol, it was an antiestrogen. For example, in MCF-7 and S30 cells,

resveratrol alone showed weak estrogenic response, but when combined with estradiol (1 nM), a dose-dependent antagonism occurred. In addition, progesterone receptor (PR) protein expression was induced with the compound alone, but when combined with estradiol, the expression was suppressed. Administered at pharmacological doses, resveratrol (52-74  $\mu\text{M}$  [12-17  $\mu\text{g}/\text{mL}$ ]) suppressed the growth of estrogen receptor (ER)-positive breast cancer cells (KPL-1 and MCF-7) and ER-negative breast cancer cells (MKL-F) stimulated by linoleic acid. Resveratrol (1 pM-1  $\mu\text{M}$  [ $2.28 \times 10^{-7}$ -0.2  $\mu\text{g}/\text{mL}$ ]) was also an agonist of steroid receptors. In MCF-7 and T47-D cells, it interacted with estradiol (at the nanomolar range) simultaneously with PRs (at the picomolar range). A significant increase in the growth of MCF-7 cells also occurred with *cis*-resveratrol (10 and 25  $\mu\text{M}$  [2.3 and 5.7  $\mu\text{g}/\text{mL}$ ]). In MVLN cells, *trans*-resveratrol (10 and 25  $\mu\text{M}$ ) and *cis*-resveratrol (25  $\mu\text{M}$ ) significantly increased luciferase activity compared to estradiol. In the presence of estradiol, both isomers at the same doses functioned as superagonists of estradiol. In the MCF-7 and MVLN cell lines, *cis*-resveratrol was less effective than *trans*-resveratrol. Resveratrol also exhibited estradiol antagonist activity for ER- $\alpha$  with select estrogen response elements and no such activity with ER- $\beta$ .

In contrast to *in vitro* tests, results of an *in vivo* study using weanling rats suggested that resveratrol (oral; 1, 4, 10, 40, and 100  $\mu\text{g}$  [0.004, 0.02, 0.044, 0.18, and 0.438  $\mu\text{mol}$ ] per day for six days) was not an agonist at the ER (e.g., it had no effect on bone formation and mineralization rates versus the estrogen 17 $\beta$ -estradiol). However, when resveratrol and 17 $\beta$ -estradiol were administered in combination (1000 and 100  $\mu\text{g}$  [4.381 and 0.438  $\mu\text{mol}$ ], respectively), a synergistic effect was observed—i.e., a significant decrease in cholesterol levels was seen in the animals. Oral or subcutaneous (s.c.) administered of *trans*-resveratrol (0.03-575 mg/kg [0.1  $\mu\text{mol}/\text{kg}$  - 2.5 mmol/kg]) produced no estrogenic response in the uterine tissue of the animals.

**Cytotoxicity:** In bovine capillary endothelial (BCE) cells stimulated with fibroblast growth factor-2 (FGF-2), resveratrol inhibited capillary endothelial cell growth in a dose-dependent manner (1-10,000 nM [0.0002-2.2825  $\mu\text{g}/\text{mL}$ ]), the phosphorylation of mitogen-activated protein kinases (MAPKs) (10 and 20  $\mu\text{M}$  [2.3 and 4.6  $\mu\text{g}/\text{mL}$ ]), and FGF-2 and vascular endothelial growth factor (VEGF)-induced proliferation of porcine aortic cell lines expressing PAE/FGFR-1 and PAE/VEGFR-2, respectively, in a dose-dependent manner (0.5-10  $\mu\text{M}$  [0.1-2.3  $\mu\text{g}/\text{mL}$ ]). In human gingival epithelial Smulow-Glickman (S-G) cells, toxicity leveled off between day 2 and 3 for a 3-day continuous exposure to resveratrol (5-150  $\mu\text{M}$  [1-34.2  $\mu\text{g}/\text{mL}$ ]). At concentrations >75  $\mu\text{M}$  (17  $\mu\text{g}/\text{mL}$ ), irreversible damage to cell proliferation occurred, and the presence of an hepatic S9 microsomal fraction did not potentiate or improve the cytotoxicity. Additionally, the cytotoxicity of hydrogen peroxide or nitrogen oxide to S-G cells was not affected by resveratrol.

In mice with skin wounds, resveratrol (5.7  $\mu\text{g}/\text{mL}$  [25  $\mu\text{M}$ ]) was an angiogenesis inhibitor. In corneal micropockets of the animals, resveratrol (oral; 0.4  $\mu\text{g}/\text{mL}$  [2  $\mu\text{g}/\text{mL}$ ] given three days before growth factor implantation and for 15 days after surgery) significantly inhibited VEGF- and FGF-2-induced corneal neovascularization compared with controls.

In HL-60 cells, resveratrol (2.5, 5, 10, 20, 40, and 80  $\mu\text{g}/\text{L}$  [0.011, 0.02, 0.044, 0.088, 0.18, and 0.35  $\mu\text{M}$ ]) dose-dependently inhibited [ $^3\text{H}$ ]thymidine incorporation into DNA (by 30, 56, 67, 81, 83, and 87%, respectively) and [ $^3\text{H}$ ]uridine incorporation into RNA (by 43, 54, 72, 85, 90, and 93%, respectively).

**Reproductive and Teratological Effects:** In developing white Leghorn chick embryos, resveratrol (1, 10, 25, 50, and 100  $\mu\text{g}/\text{disk}$  [0.004, 0.044, 0.11, 0.22, and 0.438  $\mu\text{mol}/\text{disk}$ ] incubated for 48-72 hours) induced vascular zones in the developing chorioallantoic membrane.

**Initiation/Promotion Studies:** Resveratrol and 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) were observed to equally antagonize toxicity when combined together. Resveratrol (10  $\mu\text{M}$  [2.3  $\mu\text{g}/\text{mL}$ ])

was toxic to Chinese hamster V79MZ cells (nonmetabolically competent); coincubation with PhIP (100  $\mu\text{M}$ ) inhibited this effect. In contrast, resveratrol inhibited PhIP-induced mutation in V79MZh1A2 (expresses human CYP1A2) cells. Resveratrol (10  $\mu\text{M}$ ) in combination with PhIP (100  $\mu\text{M}$ ) increased the colony survival of V79MZh1B1 (expresses human CYP1B1) cells, whereas alone, neither compound was toxic.

**Anticarcinogenicity:** Using the mouse mammary gland organ culture model, resveratrol (1-10  $\mu\text{M}$  [0.2-2.3  $\mu\text{g}/\text{mL}$ ]) inhibited formation of estrogen-dependent preneoplastic ductal lesions induced by 7,12-dimethylbenz[*a*]anthracene (DMBA).

In human breast cancer cell lines (KPL-1, MCF-7, MKL-F, T47-D, and MDA-MB-231), resveratrol (1 pM-180  $\mu\text{M}$  [ $2 \times 10^{-7}$ -40  $\mu\text{g}/\text{mL}$ ]) inhibited the growth of cell lines in a time- and/or dose-dependent manner. In addition, resveratrol (1 pM-1  $\mu\text{M}$  [ $2 \times 10^{-7}$ -0.2  $\mu\text{g}/\text{mL}$ ]) produced inhibition in the growth of prostate cancer cell lines PC3 and DU145. In LNCaP prostate cancer cells, resveratrol (100  $\mu\text{M}$  [22.8  $\mu\text{g}/\text{mL}$ ]) inhibited cell growth in the presence of androgens.

In mice, oral administration of resveratrol (5.7  $\mu\text{g}/\text{mL}$  [25  $\mu\text{M}$ ]; 1 mg/kg/day) significantly inhibited the growth of T241 fibrosarcomas. In rats, resveratrol (200  $\mu\text{g}/\text{kg}$  [0.876  $\mu\text{mol}/\text{kg}$ ] body weight per day for 100 days) inhibited the number of azoxymethane (AOM)-induced aberrant crypt foci (ACF) and their multiplicity. When rats were treated with resveratrol (100 mg/kg [0.438 mmol/kg] body weight 5 days/week for >120 days) before *N*-methyl-*N*-nitrosourea (NMU) administration, a delay in tumorigenesis occurred; resveratrol increased tumor latency by 28 days. Additionally, the multiplicity of tumors and the total number of tumors were decreased compared to controls.

**Genotoxicity:** In the presence and absence of metabolic activation, *trans*-resveratrol (0.02-5000  $\mu\text{g}/\text{plate}$  [0.09 nmol/plate – 21.91  $\mu\text{mol}/\text{plate}$ ]) was not mutagenic in *Salmonella typhimurium* strains TA98 and TA100 and in *Escherichia coli* strain WP2uvrA. In the Chinese hamster lung, structural chromosome aberrations (CAs) (mainly chromatid breaks and exchanges) were induced dose-dependently at 2.5-20  $\mu\text{g}/\text{mL}$  (11-88  $\mu\text{M}$ ). Furthermore, resveratrol (same doses) induced micronuclei (MN), polynuclei (PN), and karyorrhectic cells after a 48-hour treatment and sister chromatid exchanges (SCEs) in a dose-dependent manner at concentrations up to 10  $\mu\text{g}/\text{mL}$ . Cell cycle analysis showed that resveratrol caused S phase arrest and induced apoptosis after a 48-hour treatment.

*trans*-Resveratrol (1, 5, 10, 25, 50, and 100  $\mu\text{M}$  [0.2, 1, 2.3, 5.7, 11, and 22.8  $\mu\text{g}/\text{mL}$ ]) strongly cleaved plasmid DNA (i.e., strand excision or relaxation of pBR322) in the presence of  $\text{Cu}^{2+}$  at neutral pH and under aerobic conditions. Under anaerobic conditions, however, increasing the concentration of resveratrol failed to enhance the efficiency of DNA cleavage. Resveratrol was also found to be capable of binding to DNA. In the presence of ascorbic acid or glutathione, resveratrol (0.1 mM) lost its ability to promote hydroxyl-radical ( $\cdot\text{OH}$ ) formation by DNA-bound  $\text{Cu}^{2+}$  and was instead a powerful antioxidant. Resveratrol (10  $\mu\text{M}$  [2.3  $\mu\text{g}/\text{mL}$ ]) significantly stimulated DNA strand breaks induced by adenosine 5'-diphosphate (ADP)- $\text{Fe}^{3+}$  in the presence of hydrogen peroxide. By reducing ADP- $\text{Fe}^{3+}$ , resveratrol acted as a prooxidant of DNA.

**Other Data:** In CD2F1 mice, *cis*- and *trans*-resveratrol (oral; 1000  $\mu\text{g}/\text{kg}$  [4.381  $\mu\text{mol}/\text{kg}$ ] per day for five or ten days) caused almost complete inhibition of 7-ethoxyresorufin-*o*-dealkylation (EROD) activity (CYP1A2). No effect was observed on ethoxycoumarin-*o*-deethylation (ECOD) activity (CYP1A2/2E1) or benzo[*a*]pyrene metabolism. It was an effective inhibitor of recombinant human estrogen sulfotransferase (EST) ( $\text{IC}_{50}$  = 1.6  $\mu\text{M}$ ) and recombinant human P form phenolsulfotransferase (PST), an enzyme involved in carcinogen bioactivation ( $\text{IC}_{50}$  = 0.2  $\mu\text{M}$ ). In intact human hepatoma Hep G2 cells, inhibition of P-PST decreased fourfold ( $\text{IC}_{50}$  = 0.8  $\mu\text{M}$ ).

In rats orally administered resveratrol (8 mg/kg [0.04 mmol/kg]), CYP2E1 (chlorzoxazone 6-hydroxylation) and protein level in liver microsomes were significantly reduced 24 hours after administration. In human microsomes incubated with resveratrol (low micromolar levels), CYP1A2 (methoxyresorufin *O*-demethylation) and CYP3A4 (erythromycin demethylation) were inhibited, while CYP2E1 activity was moderately increased. Resveratrol also induced Phase 2 biotransformation.

Resveratrol (6-100  $\mu\text{M}$  [1-22.8  $\mu\text{g/mL}$ ]) inhibited the growth and tube formation of bovine aorta endothelial (BAE) cells in a dose-dependent manner. In addition, DMBA metabolism by liver microsomes was inhibited *in vitro* in a dose-dependent manner by the compound.

### Structure-Activity Relationships

Several compounds show structural similarities to *trans*-resveratrol. Kaempferol, for example, has a 4'-hydroxyl group in the B-ring and a 2,3-double bond in the C-ring, which allows conjugation across the A-ring containing the meta dihydroxy structure. *trans*-Resveratrol is also structurally similar to the synthetic estrogenic agent diethylstilbestrol (DES). In contrast to resveratrol, DES induced polyploidy *in vitro*. Like resveratrol, DES strongly inhibited nicotinamide adenine dinucleotide phosphate (NADPH)- and ADP-Fe<sup>3+</sup>-dependent microsomal lipid peroxidation; an IC<sub>50</sub> of 1.1  $\mu\text{M}$  was obtained versus 4.8  $\mu\text{M}$  for resveratrol. Both compounds strongly inhibited the reaction at the initiation and propagation stages. Other flavonoids, including quercetin, are very effective inhibitors of iron-dependent lipid peroxidation; their extent of reduction of ADP-Fe<sup>3+</sup>, however, was less than that of resveratrol. DES, on the other hand, caused no reduction of ADP-Fe<sup>3+</sup> or EDTA-Fe<sup>3+</sup>. It also had no effect on DNA damage.

In several chemical disposition and toxicokinetic studies, the activity or effect of resveratrol was compared to that of quercetin. In the human intestinal epithelial cell line Caco-2, the permeability constant for quercetin was similar to that of resveratrol. In addition, quercetin, like resveratrol, was a strong inhibitor of P-PST (IC<sub>50</sub> = 0.1  $\mu\text{M}$ ). In intact human hepatoma Hep G2 cells, this decreased by 25-fold (IC<sub>50</sub> = 2.5  $\mu\text{M}$ ); the hepatocyte had a greater metabolism of quercetin than of resveratrol.

Resorcinol produced Cu<sup>2+</sup>-dependent DNA strand excision under oxidative conditions. Having the same structural elements as this compound, the DNA-cleaving ability of resveratrol has been studied.

The NTP has conducted short-term toxicity, carcinogenicity, and/or genotoxicity tests on the above three chemicals. Below is a summary of available tests and their results.

Diethylstilbestrol (DES): In female mice, "continuous exposure" (duration not specified) to 50 ppb DES in feed produced decreases in the fertility index, the number of litters, the number of live pups, and the proportion of pups born alive per litter. In addition, females had almost a 30% increase in pituitary weight and a majority (>75%) had no clear estrous cycle. Males given the same dose also showed a significant increase in pituitary weight as well as decreases in the weight of the epididymis, cauda epididymis, and prostate.

Mice given 2.5-100  $\mu\text{g/kg}$  DES daily on gestation days 9-16 showed a decreased corrected maternal body weight gain. At 5  $\mu\text{g/kg}$ , an increase in skeletal malformations was observed. At  $\geq 10$   $\mu\text{g/kg}$ , the number of corpora lutea per dam was decreased and the percent resorptions per litter was increased. At the high dose, gravid uterine weight and live litter size were decreased, while relative maternal liver weight and the incidence of malformation per litter were increased.

Short-term toxicity tests have been conducted in mice; no results were available.

Resorcinol: In 17-day gavage studies, rats given 27.5-450 mg/kg resorcinol all survived and had no chemical-related gross or microscopic lesions. Mice, however, dosed with 37.5-600 mg/kg, had one

death (male) at 300 mg/kg, and all females (5 of 5) and 4 of 5 males died at the high dose. In a 13-week gavage study, all female (10 of 10) and 8 of 10 male rats died given 520 mg/kg. For mice, a dose of 420 mg/kg resulted in 80% death for both sexes. Other short-term toxicity tests (e.g., 24-week topical study in mice and a 14-day gavage study in rats) have been conducted; no results were available.

In two-year studies, rats (males received 225 mg/kg five days per week; females received 150 mg/kg for 15 months) exhibited decreased mean body weights and survival compared to controls. For mice, only females (receiving 225 mg/kg five days per week) showed reduced mean body weights. In both species, effects on the central nervous system—ataxia, recumbency, and tremors—were observed.

There was no evidence of carcinogenic activity in rats or mice.

Resorcinol was not mutagenic in *S. typhimurium* strains TA98, TA100, TA1535, and TA1537 in the presence and absence of metabolic activation (S9). In mouse L5178Y lymphoma cells, it induced trifluorothymidine resistance in the absence of S9. With and without S9, resorcinol induced SCEs in Chinese hamster ovary (CHO) cells, and only with S9 did it induce CAs. In *Drosophila melanogaster*, no induction of sex-linked recessive lethal mutations was seen, but an equivocal response was observed when resorcinol was administered by injection. Positive results were obtained in the MN test.

Quercetin: Studies showed some evidence of carcinogenicity. Male rats given 100-40,000 ppm quercetin in feed for two years had an increased incidence of renal tubule hyperplasia and an increased severity of nephropathy. Parathyroid hyperplasia was also seen. At the high dose, renal tubule adenomas were found in three rats and adenocarcinomas in one other rat.

In *S. typhimurium* strains TA98 and TA100, quercetin induced mutations with and without S9. In CHO cells, both SCEs and CAs were induced. For the dihydrate, negative results were obtained in the MN test.



# Table of Contents

Executive Summary .....	i
1.0 Basis for Nomination.....	1
2.0 Introduction.....	1
2.1 Chemical Identification and Analysis .....	1
2.2 Physical-Chemical Properties of Resveratrol .....	2
2.3 Commercial Availability.....	2
3.0 Production Processes.....	3
4.0 Production and Import Volumes .....	4
5.0 Uses .....	4
6.0 Environmental Occurrence and Persistence .....	5
7.0 Human Exposure.....	5
8.0 Regulatory Status.....	7
9.0 Toxicological Data.....	7
9.1 General Toxicology .....	7
9.1.1 Human Data .....	7
9.1.2 Chemical Disposition, Metabolism, and Toxicokinetics .....	7
9.1.3 Acute Exposure .....	14
9.1.4 Short-term and Subchronic Exposure.....	14
9.1.5 Chronic Exposure .....	16
9.1.6 Synergistic/Antagonistic Effects .....	16
9.1.7 Cytotoxicity .....	17
9.2 Reproductive and Teratological Effects.....	21
9.3 Carcinogenicity .....	21
9.4 Initiation/Promotion Studies .....	21
9.5 Anticarcinogenicity.....	21
9.6 Genotoxicity .....	24
9.7 Cogenotoxicity .....	24
9.8 Antigenotoxicity.....	24
9.9 Immunotoxicity.....	24
9.10 Other Data .....	26
10.0 Structure-Activity Relationships .....	26

<b>11.0</b>	<b>Online Databases and Secondary References .....</b>	<b>31</b>
<b>11.1</b>	<b>Online Databases .....</b>	<b>31</b>
<b>11.2</b>	<b>Secondary References.....</b>	<b>32</b>
<b>12.0</b>	<b>References.....</b>	<b>32</b>
<b>13.0</b>	<b>References Considered But Not Cited .....</b>	<b>45</b>
	<b>Acknowledgements .....</b>	<b>53</b>
	<b>Appendix: Units and Abbreviations.....</b>	<b>53</b>
<b>Tables:</b>		
<b>Table 1</b>	<b>Concentration of Resveratrol in Wines .....</b>	<b>6</b>
<b>Table 2</b>	<b>Concentration of Resveratrol in Various Herbal Products .....</b>	<b>6</b>
<b>Table 3</b>	<b>Chemical Disposition, Metabolism, and Toxicokinetics of Resveratrol .....</b>	<b>8</b>
<b>Table 4</b>	<b>Short-term and Subchronic Exposure to Resveratrol .....</b>	<b>15</b>
<b>Table 5</b>	<b>Cytotoxicity Studies of Resveratrol .....</b>	<b>18</b>
<b>Table 6</b>	<b>Anticarcinogenicity Studies of Resveratrol.....</b>	<b>22</b>
<b>Table 7</b>	<b>Genotoxicity Studies of Resveratrol .....</b>	<b>25</b>
<b>Table 8</b>	<b>NTP Studies: Structurally Related Chemicals of Resveratrol.....</b>	<b>28</b>

## 1.0 Basis for Nomination

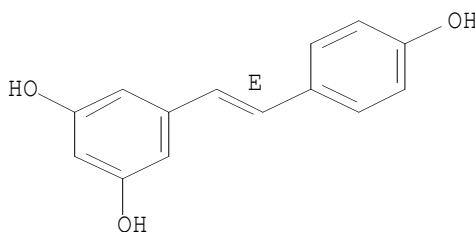
*trans*-Resveratrol was nominated for toxicology studies by the National Institute of Environmental Health Sciences (NIEHS) based on the widespread human exposure to resveratrol through natural dietary sources and dietary supplement use, and concern that it has not been sufficiently evaluated for potential toxicological effects.

## 2.0 Introduction

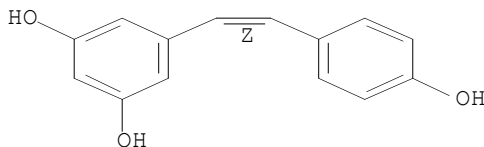
This report includes information on the *cis*-form of resveratrol, in addition to the *trans*-form, as well as *trans*-piceid ( $\beta$ -D-Glucopyranoside, 3-hydroxy-5-[(*E*)-2-(4-hydroxyphenyl)ethenyl]-phenyl), a glucoside of *trans*-resveratrol. These compounds are often found in combination and are sometimes not identified specifically in the literature.

## 2.1 Chemical Identification and Analysis

*trans*-Resveratrol  
[501-36-0]



*cis*-Resveratrol  
[61434-67-1]



*trans*-Resveratrol (C<sub>14</sub>H<sub>12</sub>O<sub>3</sub>; mol. wt. = 228.25) is also called:  
1,3-Benzenediol, 5-[(*E*)-2-(4-hydroxyphenyl)ethenyl] (9CI)  
3, 5, 4'-Trihydroxystilbene  
CA 1201  
(*E*)-Resveratrol  
3,4', 5-Stilbenetriol (7CI, 8CI)  
(*E*)-5-[2-(4-Hydroxyphenyl)ethenyl]-1,3-benzenediol  
(*E*)-5-(*p*-Hydroxystyryl)resorcinol  
Resveratrol (6CI)

*cis*-Resveratrol (C<sub>14</sub>H<sub>12</sub>O<sub>3</sub>; mol. wt. = 228.25) is also called:

- 1,3-Benzenediol, 5-[1Z]-2-(4-hydroxyphenyl)ethenyl (9CI)
- 1,3-Benzenediol, 5-[2-(4-hydroxyphenyl) ethenyl], (Z)-  
(Z)-Resveratrol

Several methods have been used to extract resveratrol and related compounds from wine and to isolate the *trans*- and *cis*- isomers of resveratrol. They include high-performance liquid chromatography (HPLC) (Goldberg et al., 1997; Lamuela-Raventós et al., 1997; McMurtrey, 1997), liquid chromatography (LC) (McMurtrey et al., 1994; cited by McMurtrey, 1997), gas chromatography (GC), ([Barlass et al., 1987; Blache et al., 1997; both cited by Lin and Chen, 2001]; Goldberg et al., 1997), gas chromatography-mass spectrometry (GC-MS) (Soleas et al., 2001), and capillary electrophoresis (CE) ([Beras Nevado et al., 1999; Cartoni et al., 1995; Gu et al., 1999; all cited by Lin and Chen, 2001]; Lin and Chen, 2001; Gu et al., 2000).

High-speed counter-current chromatography—with the solvents chloroform, methanol, and water—was found to be an effective method for separating resveratrol from *Polygonum cuspidatum* Sieb. et Zucc.; this extraction method yields greater than 98% purity (as measured by HPLC) (Yang et al., 2001).

## 2.2 Physical-Chemical Properties of Resveratrol

Property	Information	Reference(s)
Physical State	Solid, powder	Budavari (1996)
Color	Off white	Budavari (1996)
Melting Point (°C)	253-255	Budavari (1996)
Octanol-Water Partition Coefficient (LogP)	3.139±0.343	Registry (2001)
pKa (of the most acidic H-donor)	9.14±0.20	Registry (2001)
Solubility in Water (mol/L)	<0.01	Registry (2001)

## 2.3 Commercial Availability

Several companies produce *trans*-resveratrol commercially. Pharmascience of Montreal, Canada, produces a pure form of *trans*-resveratrol (PCT Gazette, 2001; Agriculture and Agri-Food Canada, undated). InterHealth of Concord, CA, (InterHealth, undated-a), produces a standardized extract of *trans*-resveratrol. Pharmascience calls its patented product Resverin<sup>®</sup> (PCT Gazette, 2001; Pharmascience, undated; Agriculture and Agri-Food Canada, undated; Food and Beverage America, 2000). InterHealth manufactures Protykin<sup>™</sup>, a standardized extract containing *trans*-resveratrol and emodin, also a polyphenol, from the dried rhizome of *P. cuspidatum* (InterHealth, undated-a,b,c,d). Laboratorio Italiano Biochimico Farmaceutical Lisapharma has patented a pharmaceutical composition of grape and wine polyphenols, particularly resveratrol, with yeast (Osterwalder, 1999). Other manufacturers of *trans*-resveratrol include TCI America of Portland, OR; LKT Laboratories of St. Paul, MN; and Samlong Chemical Co., Ltd. of China (Block, 2000; LKT Laboratories, Inc., undated; Samlong Chemical Co., Ltd., undated; TCI America, 1999). Moravek Biochemicals of Brea, CA produces radiolabeled resveratrol (Moravek Biochemicals, 2001).

Other companies incorporate Protykin<sup>™</sup>, Resverin<sup>®</sup>, and other formulations of resveratrol and related compounds or natural products containing it into dietary supplements (CCNow, undated; Enrich Corporation, 2000a, b; IHerb.com, undated; LaSasso, 2000; Life Extension Foundation,

2000; Mineral Connection, 2001). In dietary supplements, the isomer is not always specified; however, when the form is specified, it is typically *trans*-resveratrol.

### 3.0 Production Processes

Plants synthesize *trans*-resveratrol when infected by microbes or exposed to ultraviolet (UV) radiation ([Creasy and Coffee; 1988; Langcake and Pryce, 1976; Roggero and Garciaparilla, 1995; all cited by Daniel et al. 1999]; Deffieux et al., 2000; Stockley, 1996). It is also produced in response to injury and stress (Frémont, 2000; Nutrition for a Living Planet, undated). In response to these factors, plants synthesize one molecule of *trans*-resveratrol from one molecule *p*-coumaroyl-CoA and three molecules of malonyl-CoA (Daniel et al., 1999; Nutrition for a Living Planet, undated; Soleas et al., 1997).

Fresh grape skin contains 50 µg/g to 100 µg/g (0.22 µmol/g to 0.44 µmol/g) of *trans*-resveratrol (Hendler and Rorvik, 2001). As they ripen, grapes produce less resveratrol (Jeandet et al., 1995a; [Vrhovsek et al., 1995; cited by Daniel et al., 1999]).

Resveratrol forms are freed from the skin as wine is made ([Mattivi et al., 1995; Siemann and Creasy, 1992; both cited by Frémont, 2000]; Roggero, 1996). *cis*-Resveratrol is found in wine, but at lower levels than the *trans* isomer (Roggero and Garciaparilla, 1995; Romero-Perez et al., 1996; both cited by Daniel et al., 1999). These forms are probably created during the winemaking process (Goldberg et al., 1997; [Romero-Perez et al., 1996; Roggero and Garciaparilla, 1995; cited by Daniel et al., 1999]). *trans*- to *cis*-Resveratrol conversion occurs when the wine must (i.e., the juice from grapes) is exposed to light and oxygen (Cantos et al., 2000; Goldberg et al., 1997). The ratios of *cis*- to *trans*-resveratrol in wines vary by region (Goldberg et al., 1997).

Winemaking technique, the type of grape used, climate, and other factors all influence the levels of resveratrol found in wine. The most important factor is the length of time the skin is kept with the grape must during the winemaking process; longer times increase resveratrol concentration. In the case of white wine production, the skin is always removed prior to fermenting, giving these wines a lower resveratrol concentration than red wines. Rose wines (a combination of red and white wines) have an intermediate concentration (Frémont, 2000; Goldberg et al., 1997; Lamuela-Raventós et al., 1997; McMurtrey, 1997; Stockley, 1996; Roggero, 1996).

Commercial producers of resveratrol induce plants to produce greater quantities by adding aluminum chloride or aluminum sulfate to grape shoots and vines (Adrian et al., 1996; Jeandet et al., 2000). Production of resveratrol in harvested grapes increased twofold with irradiation by UVB light and threefold with irradiation by UVC light (Cantos et al., 2000).

Resveratrol has been produced by treating cell suspension cultures of grapes with Onozuka R-10, a cellulase derived from the fungus *Trichoderma viride* (Calderon et al., 1993). *trans*-Piceid, a glucoside of resveratrol, can be produced by growing grape plant cells in fermenters (Decendit et al., 1996). Also, resveratrol synthase genes have been isolated (Hain et al., 1996, 1997, 2000; Schroder et al., 1999) and inserted into plants, creating transgenic varieties of tobacco, grape, tomatoes, potatoes, rice, and alfalfa with higher *trans*-resveratrol concentrations (Stark-Lorenzen

et al., 1997; cited by Daniel et al., 1999; Hain et al., 1990; Paiva, 1999 abstr.; Soleas et al., 1997; Thomzik et al., 1997).

Grape plants excreted *trans*-resveratrol from leaves' wounds touching a cellulosic substratum, such as filter paper, soaked with inducers in aqueous solution. These inducers included monosaccharides, disaccharides, some polysaccharides, and Cu<sup>2+</sup> ions. Alginate and mucic acid, a *Botrytis cinerea* metabolite, were the most potent inducers (Blaich and Bachmann, 1980). Resveratrol can be extracted from these plants with water and alcohol (InterHealth, undated-c), or with methanol and ethyl acetate (Vastano et al., 2000).

#### 4.0 Production and Import Volumes

No data were available.

#### 5.0 Uses

Traditional Asian medicine has long used the root of *P. cuspidatum*, a source of resveratrol, as a circulatory tonic, among other uses (Frémont, 2000; Hendler and Rorvik, 2001; Satchell, 2000). It is also a product of *Erythrophleum lasianthum*, a tree used in traditional medicine in South Africa (Orsini et al., 1997). Darakchasava, an herbal remedy containing resveratrol, is used as a heart tonic in Ayurvedic medicine (Hendler and Rorvik, 2001). Currently, several dietary supplements available in the United States contain resveratrol.

Health claims of supplements incorporating resveratrol include protection from free-radical damage, inhibition of inflammation such as in arthritis, inhibition of the cyclooxygenase-2 (COX-2) enzyme, enhancement of the elasticity and flexibility of muscles, relaxation and protection of blood vessels (Life Extension Foundation, 2000; Richards, 1999; Graves, 2000a; b; InterHealth, undated-b; Jarrow Formulas, 2001; Agriculture and Agri-Food Canada, undated; Natural Ways to Health, undated), improvement of cardiovascular health (Cosgrove, 2000; Enrich Corporation, 2000a; Howard, 2000 abstr.; InterHealth, undated-b), and reduction of the appearance of wrinkles (in a topically applied cream and in a form to be ingested) (Best Skin Care, undated; Healthy Living Intl.com, 2000). Other claims include preventing cancer (Natural Ways to Health, undated; Cosgrove, 2000), enhancing the immune system (Enrich Corporation, 2000a), and slowing the process of aging (Natural Ways to Health, undated). *trans*-Resveratrol is marketed as a phytoestrogen to maintain estrogen levels and help alleviate menopausal symptoms (Cosgrove, 2000; Inno-Vite, undated), as well as to promote healthy bone density (Inno-Vite, undated; Cosgrove, 2000).

Pharmascience has a patent for use of *trans*-resveratrol to prevent and to treat restenosis after coronary disease treatment (AML Information Services, 2000), and the Institute for Human Virology, funded by Pharmascience, has filed a patent application for the use of resveratrol with nucleoside analogs for treating HIV-1 infections (IHV, 2001a,b).

Many sources say resveratrol's benefits come primarily from their antioxidant effects or from their estrogenic effects (Hendler and Rorvik, 2001; Gehm et al., 1997; Agriculture and Agri-Food Canada, undated; Soleas et al., 1997; [Rice-Evans et al., 1997; cited by Frémont, 2000]; Howard, 2000 abstr.; Paiva, 1999 abstr.). In demonstrations of its antioxidant properties, *trans*-resveratrol is better at inhibiting oxidation of LDL (low density lipoprotein) than was

$\alpha$ -tocopherol (Frankel et al., 1993; Arichi et al., 1982; both cited by Soleas et al., 1997). In demonstrations of its estrogenic properties, resveratrol acts as a mixed agonist-antagonist for estrogen receptors  $\alpha$  and  $\beta$  (ER- $\alpha$  and ER- $\beta$ ) (Hendler and Rorvik, 2001) and increased native-regulated gene expression and stimulated growth of estrogen-dependent breast cancer cells (Gehm et al., 1997).

## 6.0 Environmental Occurrence and Persistence

Resveratrol is a polyphenol that is found in more than 70 common plant species (Turner, 1999). Plants that contain *trans*-resveratrol include grapes, peanuts, eucalyptus, spruce, and lily ([Langcake and Pryce, 1976; cited by Daniel et al., 1999]; Sobolev and Cole, 1998 abstr.; McElderry, 1997), mulberries (Hendler and Rorvik, 2001), groundnut (Schroder et al., 1999; Hain et al., 1990), members of the knotweed and hellebore genera (*Polygonum* and *Helleborus*), and fescue grass (Budavari, 1996).

Resveratrol in grapes is found in lignified plant tissues, in leaves, and in berries (Langcake and Pryce, 1976; cited by Daniel et al., 1999). In *Vitis vinifera*, *trans*-resveratrol was detected in vines, and leaf tissues produced the compound when infected with fungi or when exposed to UV light (Langcake and Pryce, 1976; cited by Frémont, 2000). When a grape becomes infected with the fungus *B. cinerea*, known as gray mold, the concentration of resveratrol rises in nearby grapes (Sbaghi, 1994; Jeandet et al., 1995b). Stimulating a grape plant's production of resveratrol and other defense chemicals can increase its resistance to *B. cinerea*, enabling it to limit fungal infection (Jeandet et al., 1998). Once a plant has made resveratrol to defend itself, the concentration declines (Creasy and Creasy, 1998; Thomzik et al., 1997).

## 7.0 Human Exposure

Human exposure to resveratrol is mainly through ingestion, particularly of peanuts, grapes, and their products ([Langcake and Pryce, 1976; Goldberg, 1995; both cited by Daniel et al., 1999]; Sobolev and Cole, 1998 abstr.). In 1998, per-capita wine consumption in the United States was 7.88 L or 1.97 gallons (Wine Institute, 2000). Details about the levels of resveratrol in wines are in **Table 1**.

Resveratrol levels in peanuts and peanut products are lower than in grape products. Resveratrol concentrations were 0.055  $\mu\text{g/g}$  (0.24 nmol/g) for roasted peanuts, 0.324  $\mu\text{g/g}$  (1.42 nmol/g) for peanut butter, and 5.138  $\mu\text{g/g}$  (22.51 nmol/g) for boiled peanuts (Sobolev and Cole, 1998 abstr.). Hendler and Rorvik (2001) reported the levels of *trans*-resveratrol in peanuts to be 0.02 -1.79  $\mu\text{g/g}$  (0.09-7.84 nmol/g).

Exposure through dietary supplements is mostly oral. However, exposure would be dermal in the case of a resveratrol-containing cream (Best Skin Care, undated). For dietary supplements, amounts found in products and dosage recommendations vary. Information in a document from Protykin's<sup>TM</sup> manufacturer mentions that resveratrol supplements contain <1-10 mg (<4-44  $\mu\text{mol}$ ) resveratrol per tablet (InterHealth, 1998). An online article recommends a dosage of 200-600  $\mu\text{g}$  (0.876-2.63  $\mu\text{mol}$ ) resveratrol per day for atherosclerosis or cancer prevention (Micromedex Thomson Health Care, 2000). Additional details about the concentrations of resveratrol in herbal products, as well as recommended dosages, are shown in **Table 2**.

**Table 1. Concentration of Resveratrol in Wines**

Wine Type (Grape Species Used)	Compound	Concentration Range	References
White ( <i>V. vinifera</i> )	<i>trans</i> -Resveratrol	≤0.02 mg/L (0.09 μM)	McMurtrey et al. (1994); cited by McMurtrey (1997)
White ( <i>V. rotundifolia</i> )	<i>trans</i> -Resveratrol	0.29-1.18 mg/L (1.3-5.17 μM)	McMurtrey (1997)
Spanish rosé (grape species n.p.)	<i>trans</i> - and <i>cis</i> -Isomers of resveratrol, piceid	~4.5-7.0 μM (1.0-1.6 μg/mL)	Lamuella-Raventós et al. (1997)
	<i>trans</i> -Resveratrol	~1.2-2.2 μM (0.27-0.50 μg/mL)	
Red (muscadine [where noted]; otherwise, species n.p.)	<i>trans</i> - and <i>cis</i> -Isomers of resveratrol, piceid	175 ng/L - 0.5 mg/L (0.77 nM - 2.2 μM)	[Roggero and Archier (1994); cited by Frémont (2000)]; Lamuella-Raventós et al. (1997)
	<i>trans</i> -Resveratrol	≤0.02-13.4 mg/L (0.09-58.7 μM) (upper concentration from muscadine grapes)	McMurtrey et al. (1994); Lamikanra et al. (1996); both cited by Frémont (2000)

Abbreviation: n.p.=not provided

**Table 2. Concentration of Resveratrol in Various Herbal Products**

Product	Resveratrol Concentration	Recommended Dosage	Source
Bio Vin Full Spectrum Grape Seed and Skin Extracts	463 ppm (23.2 μg [0.102 μmol]) resveratrol per 50 mg capsule	1 to 2 capsules daily in divided dosages	Life Extension Foundation (2000)
Biochem Olive Leaf Extract	5 mg (0.02 mmol) resveratrol per capsule	2 capsules daily	Mineral Connection (2001)
Cardio Cholestamax™	1.9% (1.9 mg [8.3 μmol]) resveratrol per 100 mg tablet	2 tablets daily	Organix-South, Inc. (undated)
ORAC+ Biosynergistic Super Fruit Powder	20% (1 g [4 mmol]) resveratrol in 5 g of powder	1 heaping teaspoon (~5 g)	Natural Ways to Health (undated)
ActiVin™ + Resveratrol	100 μg (0.438 μmol) <i>trans</i> -resveratrol <sup>a</sup>	1 tablet twice daily	InterHealth (undated-a)
Protykin®	5-10 mg (0.02-0.04 mmol) <i>trans</i> -resveratrol per 25-50 mg tablet	1 tablet daily	InterHealth (1998; undated-a)
	2.495 mg (0.01091 μmol) <i>trans</i> -resveratrol per 50 mg tablet	1 to 2 tablets daily	LaSasso (2000)
Resveratrol Synergy™	16 mg (70 μmol) resveratrol per tablet	1 to 2 tablets daily	Jarrow Formulas (2001)

Abbreviation: n.p.=not provided

<sup>a</sup> This product is also listed to contain 100 mg grape seed extract and 500 μg of *P. cuspidatum* root extract without indicating the quantity of resveratrol in these extracts.



## 8.0 Regulatory Status

Resveratrol available in dietary supplements is regulated under the U.S. Food, Drug, and Cosmetic Act (FDCA). Manufacturers and distributors must notify the Food and Drug Administration (FDA) when they plan to market dietary supplements that contain "new dietary ingredients" (Section 413b of the Act, 21 U.S.C. 350b)(FDA, 2001).

Solgar Vitamin & Herb filed a new dietary ingredient notification for resveratrol extract from *P. cuspidatum* on Sept. 13, 2000 (Docket #95S-0316) (FDA, 2001). Its product, however, was considered possibly adulterated under 21 U.S.C. 342 (f)(1)(B) because of inadequate information regarding reasonable expectation of its safety (21CFR Sec. 190.6(b)(4)). The submission contained contradictory information about the amount of *trans*-resveratrol in the supplement (Satchell, 2000; LaSasso, 2000). In March 2001, the company gave official notification to FDA that it would not market a product containing resveratrol (LaSasso, 2001).

An FDA regulatory letter informed Natural Balance, Inc. that it was not allowed to claim that its products containing *trans*-resveratrol treat inflammatory disorders of joint, back, and muscles. Under FDA regulation 21 U.S.C. 343(r)(6), manufacturers are not allowed to claim a dietary supplement can "diagnose, mitigate, treat, cure, or prevent a specific disease or class of diseases" (Graves, 2000a,b; Foret, 2000).

## 9.0 Toxicological Data

When specified by the author(s), isomers were named. In most instances, "resveratrol" was used.

### 9.1 General Toxicology

#### 9.1.1 Human Data

Adverse effects of resveratrol in humans have not been reported. InterHealth (Concord, CA) reported that the recommended dosage of 5 to 10 mg per day was "entirely safe" (Turner, 1999). Recently, the National Cancer Institute (NCI) initiated preclinical toxicity studies on *trans*-resveratrol; clinical trials may follow (AIM, 2000).

#### 9.1.2 Chemical Disposition, Metabolism, and Toxicokinetics

The details of the following studies, except where noted, are presented in **Table 3**.

##### *In Vitro* Assays

In an isolated rat small intestine perfusion model, the majority of absorbed *trans*-resveratrol (administered doses of 28, 34, and 57  $\mu$ M [6.4, 7.8, and 13  $\mu$ g/mL]) was found in the luminal effluent (53.9%). Of this amount, free resveratrol was the dominant product (39.7%). At the vascular side, 20.5% of the administered resveratrol appeared, with the major product being the glucuronide (16.8%) (Andlauer et al., 2000). In a separate study, small amounts of unmetabolized resveratrol were absorbed across the enterocytes of the jejunum and ileum. In contrast, significant amounts of its glucuronide (1.19 nmol/cm jejunum and ~0.45 nmol/cm ileum [100  $\mu$ M administered resveratrol]) were found in the serosal fluid (Kuhnle et al., 2000).

Table 3. Chemical Disposition, Metabolism, and Toxicokinetics of Resveratrol

Test System and/or Species, Strain, and Age, Number, and Sex of Animals	Chemical Form and Purity	Route, Dose, Duration, and Observation Period	Results/Comments	Reference																								
<b><i>In Vitro</i> Assays</b>																												
Small intestine (Rats, Sprague-Dawley, age n.p., 6M [3 test, 3 controls])	<i>trans</i> -resveratrol, purity n.p.	single-pass perfusion for 60 min; 28, 34, and 57 $\mu\text{mol/L}$ (6.4, 7.8, and 13 $\mu\text{g/mL}$ ) or 837.1, 1006.6, and 1704.4 nmol in 7 mL bolus of luminal media (flow rates of 5 mL/min vascularily and 0.5 mL/min lumenally)	<p>There were no significant differences in viability data (e.g., oxygen consumption and arterial pressure) between the test and control perfusion. In the luminal perfusate, resveratrol degradation was 16.0<math>\pm</math>3.8% after 2 h at 37 <math>^{\circ}\text{C}</math>; in the vascular perfusate, no degradation occurred.</p> <p>The recoveries of resveratrol (mean<math>\pm</math>SD%) were as follows:</p> <table border="1"> <thead> <tr> <th></th> <th>free resveratrol</th> <th>glucuronide</th> <th>sulfate</th> </tr> </thead> <tbody> <tr> <td>Luminal effluent</td> <td>39.7<math>\pm</math>7.6</td> <td>11.2<math>\pm</math>5.7</td> <td>3.0<math>\pm</math>4.4</td> </tr> <tr> <td>Vascular side</td> <td>3.4<math>\pm</math>2.2</td> <td>6.8<math>\pm</math>0.6</td> <td>0.3<math>\pm</math>0.5</td> </tr> <tr> <td>Intestinal tissue</td> <td>1.5<math>\pm</math>1.4</td> <td>0.1<math>\pm</math>0.1</td> <td>0.3<math>\pm</math>0.3</td> </tr> <tr> <td>Blood vessels</td> <td>0.0<math>\pm</math>0.0</td> <td>0.0<math>\pm</math>0.0</td> <td>0.0<math>\pm</math>0.0</td> </tr> <tr> <td>Total Recovery</td> <td colspan="3">= 76.3<math>\pm</math>6.7</td> </tr> </tbody> </table>		free resveratrol	glucuronide	sulfate	Luminal effluent	39.7 $\pm$ 7.6	11.2 $\pm$ 5.7	3.0 $\pm$ 4.4	Vascular side	3.4 $\pm$ 2.2	6.8 $\pm$ 0.6	0.3 $\pm$ 0.5	Intestinal tissue	1.5 $\pm$ 1.4	0.1 $\pm$ 0.1	0.3 $\pm$ 0.3	Blood vessels	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	Total Recovery	= 76.3 $\pm$ 6.7			Andlauer et al. (2000)
	free resveratrol	glucuronide	sulfate																									
Luminal effluent	39.7 $\pm$ 7.6	11.2 $\pm$ 5.7	3.0 $\pm$ 4.4																									
Vascular side	3.4 $\pm$ 2.2	6.8 $\pm$ 0.6	0.3 $\pm$ 0.5																									
Intestinal tissue	1.5 $\pm$ 1.4	0.1 $\pm$ 0.1	0.3 $\pm$ 0.3																									
Blood vessels	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0																									
Total Recovery	= 76.3 $\pm$ 6.7																											
Small intestine (Rats, Sprague-Dawley, age and number n.p., M)	resveratrol, purity n.p.	single-pass perfusion for 90 min; 100 $\mu\text{M}$ (22.8 $\mu\text{g/mL}$ )	<p>Significant amounts of resveratrol glucuronide (1.19 nmol/cm; 96.5% <math>\pm</math> 4.6 of the amount absorbed) were found on the serosal side of the enterocytes of the jejunum versus the amount of unmetabolized resveratrol (0.03 mol/cm). For the ileum, the combined transfer of resveratrol and its glucuronide was lower. In the serosal fluid, the amount of resveratrol glucuronide was ~38% of that transferred across the jejunum; the amount of unmetabolized resveratrol was undetectable.</p>	Kuhmle et al. (2000)																								
<b>Resveratrol Glucuronidation</b>																												
Partially hepatectomized liver microsomes (Humans, 41- to 71-yr-old, 7M and 3F)	<i>trans</i> -resveratrol, >99% pure	incubation for 30 min; 1 mM (228 $\mu\text{g/mL}$ ) in 50 $\mu\text{L}$ incubation mixture (1 mM UDPGA)	The highest rate of resveratrol glucuronidation occurred at pH 7. At pH 7.4, the resveratrol-glucuronide amount increased linearly with time up to 40 min and 0.2 mg/mL protein concentration. The coefficients of intra- and interassays variabilities were 1.0 and 1.5%, respectively. The rate of resveratrol glucuronidation ranged from 0.23 to 1.2 nmol/min/mg (mean = 0.69 $\pm$ 0.34; median = 0.80); it did not correlate with age or sex.	De Santi et al. (2000a)																								

Table 3. Chemical Disposition, Metabolism, and Toxicokinetics of Resveratrol (Continued)

Species, Strain, and Age, Number, and Sex of Animals	Chemical Form and Purity	Route, Dose, Duration, and Observation Period	Results/Comments	Reference
Partially hepatectomized liver microsomes (Humans, 47- to 71-yr-old, 3M and 2F)	<i>trans</i> -resveratrol, >99% pure	incubation for 30 min; 0.0625, 0.125, 0.25, 0.5, and 1 mM (14.3, 28.5, 57, 114, and 228 µg/mL) in incubation mixture (1 mM UDPGA)	Glucuronosyl transferase toward resveratrol followed Michaelis-Menten kinetics. mean $K_m$ = 0.15±0.09 mM mean $V_{max}$ = 1.3±0.3 nmol/min/mg intrinsic clearance = 11±0.004 mL/min/mg	De Santi et al. (2000a)
Partially hepatectomized liver microsomes (Humans, 47- to 71-yr-old, 2M and 1F)	<i>trans</i> -resveratrol, >99% pure	incubation for 30 min; 0.0625, 0.125, 0.25, 0.5, and 1 mM (14.3, 28.5, 57, 114, and 228 µg/mL) in incubation mixture (1 mM UDPGA) with 0, 1.25, 2.5, 5, 10, 20, and 40 µM quercetin	Quercetin effectively inhibited resveratrol glucuronidation; the mean $IC_{50}$ value was 10±1 µM. mean $K_i$ = 10±4 µM mean $K_{ics}$ = 9±2 µM mean $K_m$ = 0.15±0.09 mM (control), 0.06±0.04 mM (5 µM quercetin), and 0.13±0.06 mM (10 µM quercetin) $V_{max}$ = 1.3±0.3 nmol/min/mg (control), 0.73±0.07 (5 µM quercetin), and 0.46±0.23 (10 µM quercetin) The inhibition was mixed, non-competitive.	De Santi et al. (2000a)
Partially hepatectomized liver microsomes (Humans, 47- to 71-yr-old, 2M and 1F)	<i>trans</i> -resveratrol, >99% pure	incubation for 30 min; 1 mM (228 µg/mL) in incubation mixture with 20 µM myricetin, catechin, kaempferol, fisetin, or apigenin	The flavonoids inhibited resveratrol glucuronidation; the percents of control value were 50±3, 46±2, 55±13, 72±14, and 66±8, respectively.	De Santi et al. (2000a)
Partially hepatectomized liver microsomes (Humans, 47- to 71-yr-old, 2M and 1F)	<i>trans</i> -resveratrol, >99% pure	incubation for 30 min; 1 mM (228 µg/mL) in incubation mixture with 4 µL red Chianti wine (1998 year, 12% [v/v] alcohol, pH 6)	The rate of resveratrol glucuronidation decreased to 65.3% of the control value.	De Santi et al. (2000a)
<b>Resveratrol Sulphation</b>				
Partially hepatectomized liver microsomes (Humans, 47- to 71-yr-old, 3M and 1F)	<i>trans</i> -resveratrol, >99% pure	incubation for 20 min; 2 µM (0.5 µg/mL) in 150 µL incubation mixture (0.4 µM PAPS); reaction linear up to at least 40 min	The mean rate of resveratrol sulphation was 80±22 pmol/min/mg.	De Santi et al. (2000b)

Table 3. Chemical Disposition, Metabolism, and Toxicokinetics of Resveratrol (Continued)

Species, Strain, and Age, Number, and Sex of Animals	Chemical Form and Purity	Route, Dose, Duration, and Observation Period	Results/Comments	Reference
Partially hepatectomized liver microsomes (Humans, 60- to 71-yr-old, 3M and 2F)	<i>trans</i> -resveratrol, >99% pure	incubation for 20 min; 0.12, 0.25, 0.5, 1, and 2 $\mu$ M (0.027, 0.057, 0.1, 0.2, and 0.5 $\mu$ g/mL) in incubation mixture (0.4 $\mu$ M PAPS)	mean $V_{max}$ = 125 $\pm$ 31 pmol/min/mg cytosolic protein mean $K_m$ = 0.60 $\pm$ 0.08 $\mu$ M  The rate of resveratrol sulphation did not correlate with the activity of phenol sulphotransferase or of catechol sulphotransferase.	De Santi et al. (2000c)
Partially hepatectomized liver microsomes (Humans; age, number, and sex n.p.)	<i>trans</i> -resveratrol, >99% pure	incubation for 20 min; 2 $\mu$ M (0.5 $\mu$ g/mL) in incubation mixture (0.4 $\mu$ M PAPS) with the following inhibitors: 3.1, 6.2, 12.5, 25, and 50 pM quercetin; 0.62, 1.2, 2.5, 5, and 10 $\mu$ M kaempferol or apigenin; or 0.31, 0.62, 1.2, 2.5, and 5 $\mu$ M fisetin or myricetin	The following $IC_{50}$ values were obtained:  quercetin 12 $\pm$ 2 pm fisetin 1.0 $\pm$ 0.04 $\mu$ M myricetin 1.4 $\pm$ 0.1 $\mu$ M kaempferol 2.2 $\pm$ 0.1 $\mu$ M apigenin 2.8 $\pm$ 0.2 $\mu$ M	De Santi et al. (2000b)
Partially hepatectomized liver microsomes (Humans, 47- to 71-yr-old, 2M and 1F)	<i>trans</i> -resveratrol, >99% pure	incubation for 20 min; 0.12, 0.25, 0.5, 1, and 2 $\mu$ M (0.027, 0.057, 0.1, 0.2, and 0.5 $\mu$ g/mL) in incubation mixture with 0, 5, and 20 pM quercetin	Inhibition of resveratrol sulphation was mixed and non-competitive.  mean $K_i$ = 3.7 $\pm$ 1.8 pM mean $K_{ies}$ = 12.1 $\pm$ 1.7 pM mean $K_m$ = 0.23 $\pm$ 0.07 $\mu$ M (control), 0.40 $\pm$ 0.08 $\mu$ M (5 pM quercetin), and 0.56 $\pm$ 0.09 $\mu$ M (10 pM quercetin) $V_{max}$ = 99 $\pm$ 11 pmol/min/mg (control), 73 $\pm$ 15 pmol/min/mg (5 pM quercetin), and 57 $\pm$ 10 pmol/min/mg (10 pM quercetin)	De Santi et al. (2000b)
Partially hepatectomized liver microsomes (Humans, 47- to 71-yr-old, 2M and 1F)	<i>trans</i> -resveratrol, >99% pure	incubation for 20 min; 2 $\mu$ M (0.5 $\mu$ g/mL) in incubation mixture (0.4 $\mu$ M PAPS) with the following inhibitors: 3.1, 6.2, 12.5, 25, and 50 pM quercetin; 7.8, 15.6, 31.2, 62.5, and 125 nM mefenamic acid; or 12, 25, 50, 100, and 200 $\mu$ M salicylic acid	The following $IC_{50}$ values were obtained.  quercetin 12.4 $\pm$ 2 pM mefenamic acid 24 $\pm$ 3 nM salicylic acid 53 $\pm$ 9 $\mu$ M	De Santi et al. (2000c)

**Table 3. Chemical Disposition, Metabolism, and Toxicokinetics of Resveratrol (Continued)**

Species, Strain, and Age, Number, and Sex of Animals	Chemical Form and Purity	Route, Dose, Duration, and Observation Period	Results/Comments	Reference
Partially hepatectomized liver microsomes (Humans; age, number, and sex n.p.)	<i>trans</i> -resveratrol, >99% pure	incubation for 20 min; 2 $\mu$ M (0.5 $\mu$ g/mL) in incubation mixture with 2, 4, and 8 $\mu$ L red Chianti wine (1998 year, 11.5% [v/v] alcohol, pH 6)	The rate of resveratrol sulphation was decreased to 47 $\pm$ 9% with the low dose, 26 $\pm$ 7% with the mid dose, and 9 $\pm$ 0.4% with the high dose of the control value.	De Santi et al. (2000b)
Duodenal samples (Humans; age, number, and sex n.p.)	<i>trans</i> -resveratrol, >99% pure	incubation for 20 min; 2 $\mu$ M (0.5 $\mu$ g/mL) in incubation mixture with the following inhibitors: 3.1, 6.2, 12.5, 25, and 50 pM quercetin; 0.62, 1.25, 2.5, 5, and 10 $\mu$ M kaempferol or myricetin; or 0.31, 0.62, 1.2, 2.5, and 5 $\mu$ M fisetin or apigenin	The following IC <sub>50</sub> values were obtained: quercetin 15 $\pm$ 2 pM fisetin 1.3 $\pm$ 0.5 $\mu$ M myricetin 2.5 $\pm$ 0.3 $\mu$ M kaempferol 2.3 $\pm$ 0.1 $\mu$ M apigenin 1.3 $\pm$ 0.1 $\mu$ M	De Santi et al. (2000b)
Duodenal samples (Humans, 67- to 68-yr-old, 1M and 2F)	<i>trans</i> -resveratrol, >99% pure	incubation for 20 min; 2 $\mu$ M (0.5 $\mu$ g/mL) in incubation mixture (0.4 $\mu$ M PAPS) with the following inhibitors: 3.1, 6.2, 12.5, 25, and 50 pM quercetin; 1.9, 3.9, 7.8, 15.6, and 31.2 nM mefenamic acid; or 12, 25, 50, 100, and 200 $\mu$ M salicylic acid	The following IC <sub>50</sub> values were obtained: quercetin 15.2 $\pm$ 2 pM mefenamic acid 11 $\pm$ 0.6 nM salicylic acid 66 $\pm$ 4 $\mu$ M	De Santi et al. (2000c)
Duodenal samples (Humans; age, number, and sex n.p.)	<i>trans</i> -resveratrol, >99% pure	incubation for 20 min; 2 $\mu$ M (0.5 $\mu$ g/mL) in incubation mixture with 2, 4, and 8 $\mu$ L red Chianti wine (1998 year, 11.5% [v/v] alcohol, pH 6)	The rate of resveratrol sulphation was decreased to 32.2 $\pm$ 1% with the low dose, 16.5 $\pm$ 1% with the mid dose, and 10 $\pm$ 1% with the high dose.	De Santi et al. (2000b)

Table 3. Chemical Disposition, Metabolism, and Toxicokinetics of Resveratrol (Continued)

Species, Strain, and Age, Number, and Sex of Animals	Chemical Form and Purity	Route, Dose, Duration, and Observation Period	Results/Comments	Reference
<b><i>In Vivo</i> Assays</b>				
Rats, Wistar, age n.p., 42M (36 test, 6 control)	Cabernet Sauvignon red wine (from central Italy) containing 6.5 mg/L resveratrol, purity n.p.	gastric intubation; single dose of 4 mL red wine, corresponding to 86 µg/kg (0.38 µmol/kg) resveratrol; rats sacrificed before wine administration (controls) and after 30 min and 1, 2, 4, 8, and 12 h	Maximum resveratrol concentration was reached ~1 h after administration in plasma (20.2±1.55 ng/mL). Maximum resveratrol concentrations in tissues were as follows: 20.7±1.61 ng/g in the liver, 2.2±0.5 ng/g in the heart, and 20±1.15 ng/g in the kidney.	Bertelli et al. (1998a)
Rats, Wistar, age n.p., 42M (36 test, 6 control)	Cabernet Sauvignon red wine (from central Italy) containing 6.5 mg/L resveratrol, purity n.p.	gastric intubation; 2 mL red wine, corresponding to 43 µg/kg (0.19 µmol/kg) resveratrol, for 15 days; rats sacrificed after 15 days	Maximum resveratrol concentrations were as follows: 7.6±0.55 ng/mL in plasma, 53.5±1.46 ng/g in the liver, 3.1±0.3 ng/g in the heart, and 44.1±1.52 ng/g in the kidney. Kinetic studies showed that an equilibrium was reached between the absorbed resveratrol and the eliminated resveratrol.	Bertelli et al. (1998a)
Rats, Wistar, age n.p., 42M (36 test, 6 control)	Cabernet Sauvignon red wine (from central Italy) containing 7.06 mg/L resveratrol ( <i>trans</i> and <i>cis</i> ), purity n.p.	gastric intubation; 4 mL red wine corresponding to 28.24 µg (0.1237 µmol) resveratrol; rats sacrificed before wine administration (controls) and after 30 min and 1, 2, 4, 6, and 12 h	Plasma resveratrol concentrations were measured only at 30 min, 1 h, and 2 h, and data were, therefore, analyzed using a one-compartment model. The following values were obtained: clearance = 739 mL/h, $V_1$ = 533 mL, and $K_a$ = 1.46/h. Analysis of plasma and tissue (heart, kidney, and liver) concentration data used a two-compartment model. From the plasma+kidneys model, the following results were obtained: half-life of absorption = 0.46 h half-life of distribution, $\alpha$ = 0.48 h half-life of elimination from kidneys = 0.50 h half-life of elimination from plasma = 0.50 h half-life of terminal plasma, $\beta$ (last time point where authors were able to detect resveratrol in the plasma) = 25 h Tissue bioavailability of resveratrol was higher in the kidneys and liver (295 and 218% AUC plasma) and lower in the heart (24.7% AUC plasma) compared to plasma.	Bertelli et al. (1998b)

Table 3. Chemical Disposition, Metabolism, and Toxicokinetics of Resveratrol (Continued)

Species, Strain, and Age, Number, and Sex of Animals	Chemical Form and Purity	Route, Dose, Duration, and Observation Period	Results/Comments	Reference
Rats, Sprague-Dawley, age n.p., 3, sex n.p.	<i>trans</i> -resveratrol, purity n.p.	i.p.; single dose of 2 mg/kg (9 µmol/kg); Rats were connected to sterile tubing on the Culex™ and then dosed with resveratrol; blood concentration measured up to 300 min post-dosing	Resveratrol was rapidly absorbed. Blood concentration declined following a "two-exponential" pathway. The elimination rate constant for phase 1 ( $k_{e1}$ ) was 0.185/min. The half-life ( $t_{1/2}$ ) was 3.74 min, and the AUC was 9917 min-ng/mL.	Zhu et al. (2000)

Abbreviations: AUC = area under curve (time concentration curves); EROD = 7-ethoxyresorufin-*o*-dealkylation; F= female(s); h = hour(s); IC<sub>50</sub> = 50% inhibitory concentration; i.p. = intraperitoneal(dy); K<sub>a</sub> = absorption rate; K<sub>i</sub> = ([E]x[I])/[EI], where [E] = concentration of enzyme, [I] = concentration of inhibitor, and [EI] = concentration of enzyme-inhibitor complex; K<sub>ies</sub> = ([ES]x[I])/[EIS], where [ES] = concentration of enzyme-substrate complex and [EIS] = concentration of enzyme-inhibitor-substrate complex; K<sub>m</sub> = Michaelis constant; M = male(s); min = minute(s); n.p. = not provided; PAPS = 3'-phosphoadenosine-5'-phosphosulphate-[<sup>35</sup>S]; SD = standard deviation; UDPGA = uridine 5'-diphosphoglucuronic acid; V<sub>1</sub> = central volume; V<sub>max</sub> = maximum reaction velocity; wk = week(s); yr = year(s)

In human partially hepatectomized liver microsomes, the highest rate of *trans*-resveratrol glucuronidation (up to 1 mM [228 µg/mL] resveratrol and 1 mM uridine 5'-diphosphoglucuronic acid [UDPGA] in incubation mixture) occurred at neutral pH, and the resveratrol-glucuronide amount increased linearly with time up to 40 minutes. The reaction of resveratrol sulphation (up to 2 µM [0.5 µg/mL] resveratrol and 0.4 µM 3'-phosphoadenosine-5'-phosphosulphate [PAPS]) was also linear up to at least 40 minutes. The rates of resveratrol sulphation, similar in the human liver and duodenum, were inhibited by quercetin, fisetin, myricetin, kaempferol and apigenin; quercetin was the most effective inhibitor. The inhibition was mixed and noncompetitive. Flavonoids also inhibited resveratrol glucuronidation, but the extent of inhibition was less than that for sulphation. The addition of wine to the incubation mixture decreased both the rate of resveratrol sulphation and the rate of glucuronidation (De Santi et al., 2000a,b,c).

In human intestinal epithelial cell line Caco-2 cultured in Transwell, resveratrol had a permeability constant of  $7.4 \times 10^{-6}$  cm/s, suggesting that it could be orally absorbed (study details, including dose, not provided) (Walle et al., 1998 abstr.).

#### In Vivo Assays

In rats, resveratrol (single administration of 86 µg/kg [0.38 µmol/kg] or 43 µg/kg [0.19 µmol/kg] for 15 days) in red wine was rapidly absorbed at the intestinal level, immediately entering the blood and reaching a maximum level around one hour after oral administration. The liver contained the highest concentrations (20.7 and 53.5 ng/g following single and repeated administration, respectively), while the "main excretion pathways appear to be renal." Kinetic studies showed an equilibrium between the absorbed resveratrol and the eliminated resveratrol (Bertelli et al., 1998a). In a separate study, significant cardiac bioavailability was observed, as well as a strong affinity for the liver and kidneys (Bertelli et al., 1998b). Given intraperitoneally (i.p.), *trans*-resveratrol (2 mg/kg [9 µmol/kg]) was rapidly absorbed and the concentration in rat blood declined in a "two-exponential" manner (Zhu et al., 2000).

#### **9.1.3 Acute Exposure**

No data were available.

#### **9.1.4 Short-term and Subchronic Exposure**

The details of the following two studies are presented in **Table 4**.

In rats, daily oral administration of resveratrol (300, 1000, and 3000 mg/kg [1.31, 4.381, and 13.14 mmol/kg]) for 28 days produced nephrotoxicity, dehydration, labored breathing, hunched posture, decreased activity, rough coat, diarrhea, soft stool, and red material around the nose at the high dose. Males also had leukocytosis, and both sexes may have had anemia. Based on the results, the no observed adverse effect level (NOAEL) was 300 mg/kg/day (Korytko et al., 2002). [The same test was carried out on dogs; no results, however, were available (CRISP, 2002).]

In hypercholesterolemic rabbits, *trans*-resveratrol (0.06 mg/kg [0.3 µmol/kg] during days 1-5 and 1.0 mg/kg [4.4 µmol/kg] from days 6-60) promoted atherosclerosis. On day 60, the percentage area of stained aortic surface was ~67% in treated animals compared to ~41% in controls (95% ethanol only) (Wilson et al., 1996).



**Table 4. Short-term and Subchronic Exposure to Resveratrol**

Species, Strain, and Age, Number, and Sex of Animals (If Given)	Chemical Form and Purity	Route, Dose, Duration, and Observation Period	Results/Comments	Reference
Rats, strain and age n.p., 20M and 20F/group	resveratrol, purity n.p.	gavage; 300, 1000, and 3000 mg/kg (1.31, 4.381, 13.14 mmol/kg) daily for 28 days	At the high dose, nephrotoxicity (elevated serum BUN and creatinine levels, increased kidney weights, and gross and microscopic renal lesions) and dehydration (reduced bw gain and hyperalbuminemia) were observed. The animals also exhibited labored breathing, hunched posture, decreased activity, a rough coat, diarrhea, soft stool, and red material around the nose. M rats had leukocytosis. Anemia in F and possibly M may have been a direct effect on red blood cells (increased total bilirubin) or secondary to renal injury (decreased erythropoietin synthesis). Mild liver toxicity, indicated by increased serum ALT, ALKP, and possibly total bilirubin, was not seen histologically. Liver QR, GST, UGT, and 2E1 were increased, while 1A1 was slightly decreased.  At the mid dose, dehydration, labored breathing, and reduced bw gains were seen in all rats. Additionally, M had an increased white blood cell count. (NOAEL = 300 mg/kg/day)	Korytko et al. (2002)
Rabbits, New Zealand white (specific-pathogen-free), 72-days-old, 18M	resveratrol, purity n.p.	oral; 0.06 mg/kg [0.3 µmol/kg] during days 1-5 and 1.0 mg/kg [4.4 µmol/kg] from days 6-60). (Rabbits were first fed a cholesterol-supplemented diet that contained 0.5% cholesterol by weight for 60 days after 7-day acclimatization period.) Blood samples and plasma lipoproteins and triglycerides were collected and observed on days 0, 40, and 60.	No adverse effects on health were observed other than the promotion of atherosclerosis. The largest increase in cholesterol concentration occurred between days 0 to 40. On day 60, Sudan-IV dye showed that the percentage area of stained aortic surface was 66.87±18.92% in treated animals compared to 40.81±24.63% in controls (95% ethanol only).	Wilson et al. (1996)

Abbreviations: ALKP = alkaline phosphatase; ALT = alanine aminotransaminase; BUN = blood urea nitrogen; bw = body weight; F = female(s); GST = glutathione-S-transferase; M = male(s); n.p. = not provided; QR = quinone reductase; UGT = uridylyl diphosphate (UDP)-glucuronyltransferase

### 9.1.5 Chronic Exposure

No data were available.

### 9.1.6 Synergistic/Antagonistic Effects

Resveratrol produces a synergistic effect, as well as increased potency and availability, when combined with other antioxidants or compounds having antimutagenic or cardioprotective properties (e.g., anthocyanadins, indole-3-carbinol, and green tea extracts) (Turner, 1999). A recent discovery is resveratrol's potential role in the control of HIV-1 (human immunodeficiency virus-1) replication; the compound may synergize with existing drugs, potentiating their antiviral effects (IHV, 2000).

Resveratrol and 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) were observed to equally antagonize toxicity when combined together. Resveratrol (10  $\mu$ M [2.3  $\mu$ g/mL]) was toxic to Chinese hamster V79MZ cells (nonmetabolically competent); cocubation with PhIP (100  $\mu$ M) inhibited this effect. In contrast, resveratrol inhibited PhIP-induced mutation in V79MZh1A2 (expresses human CYP1A2) cells. Resveratrol (10  $\mu$ M) in combination with PhIP (100  $\mu$ M) increased the colony survival of V79MZh1B1 (expresses human CYP1B1) cells, whereas alone, neither compound was toxic (Boyce et al., 2000 abstr.).

In some mammary cancer cell lines, resveratrol showed mixed estrogen agonist/antagonist activities, whereas in the presence of 17 $\beta$ -estradiol, it was an antiestrogen (Bhat et al., 2001; Gehm et al., 1997). For example, in MCF-7 and S30 cells, resveratrol alone showed weak estrogenic response, but when combined with estradiol (1 nM), a dose-dependent antagonism occurred. In addition, progesterone receptor (PR) protein expression was induced with the compound alone, but when combined with estradiol, the expression was suppressed. In T47-D and LY2 cells, resveratrol was a pure estrogen antagonist, and it significantly down-regulated steady-state and estradiol-induced PR protein levels. With LY2 and S30 cells, presnelin 2 protein expression was down-regulated (Bhat et al., 2001). [Resveratrol competes with 17 $\beta$ -estradiol to bind to the human estrogen receptor (ER) (Calabrese, 1999).]

Resveratrol, administered at pharmacological doses (52-74  $\mu$ M [12-17  $\mu$ g/mL]), was able to suppress the growth of ER-positive breast cancer cells (KPL-1 and MCF-7) and ER-negative breast cancer cells (MKL-F) stimulated by linoleic acid, a potent stimulator of these cells (Nakagawa et al., 2001). Resveratrol (1 pM-1  $\mu$ M [ $2.28 \times 10^{-7}$ -0.2  $\mu$ g/mL]) was also an agonist of steroid receptors. In the MCF-7 cells, resveratrol interacted with estradiol (at the nanomolar range) simultaneously with PRs (at the picomolar range). In T47-D cells (hormone-sensitive breast cancer cell line), the same interactions were seen but to a lesser extent; both occurred at the nanomolar range. In MDA-MB-231 cells (hormone-independent breast cancer cell line), no steroid binding was observed (Damianaki et al., 2000).

In a study of both isomers, *trans*- and *cis*-resveratrol (10 and 25  $\mu$ M [2.3 and 5.7  $\mu$ g/mL]) significantly increased the growth of MCF-7 cells. At a high dose of 50  $\mu$ M (11  $\mu$ g/mL), cell growth was decreased, and this concentration was determined to be cytotoxic. In the presence of estradiol and at 25 and 50  $\mu$ M *trans*-resveratrol and 50  $\mu$ M *cis*-resveratrol, significant reduction in cell proliferation was observed. In MVLN cells, *trans*-resveratrol (10 and 25  $\mu$ M) and *cis*-resveratrol (25  $\mu$ M) significantly increased luciferase activity compared to estradiol. In the

presence of estradiol, both isomers at the same doses functioned as superagonists of estradiol. In both cell lines, *cis*-resveratrol was less effective than *trans*-resveratrol (Basly et al., 2000).

Resveratrol was observed to exhibit estradiol antagonist activity for ER- $\alpha$  with select estrogen response elements and no such activity with ER- $\beta$  (Bowers et al., 2000). For example, in human endometrial adenocarcinoma (Ishikawa) cells at concentrations as high as 10  $\mu$ M (2.3  $\mu$ g/mL), it mediated antiestrogenic effects by selective down-regulation of ER- $\alpha$  but no ER- $\beta$  (Bhat and Pezzuto, 2001).

In contrast to *in vitro* tests, an *in vivo* study using weanling rats suggested that resveratrol (oral; 1, 4, 10, 40, and 100  $\mu$ g [0.004, 0.02, 0.044, 0.18, and 0.438  $\mu$ mol] per day for six days) was not an agonist at the ER (e.g., it had no effect on bone formation and mineralization rates versus the estrogen 17 $\beta$ -estradiol). But when resveratrol and 17 $\beta$ -estradiol were given together (1000 and 100  $\mu$ g [4.381 and 0.438  $\mu$ mol], respectively), a synergistic effect was observed—i.e., a significant decrease in cholesterol levels was seen in the animals. The inability of low doses (1 and 10  $\mu$ g [0.004 and 0.044  $\mu$ mol], respectively) to lower serum cholesterol levels suggested antagonism by resveratrol at the ER (Turner et al., 1999). In rats orally or subcutaneously administered *trans*-resveratrol (0.03-575 mg/kg [0.1  $\mu$ mol/kg - 2.5 mmol/kg]), no estrogenic response was observed in uterine tissue (Ashby et al., 1999; Freyberger et al., 2000 abstr.).

### 9.1.7 Cytotoxicity

The details of the following studies are presented in **Table 5**.

#### In Vitro Assays

In bovine capillary endothelial (BCE) cells stimulated with fibroblast growth factor-2 (FGF-2), resveratrol inhibited capillary endothelial cell growth in a dose-dependent manner (1-10,000 nM [0.0002-2.2825  $\mu$ g/mL]), the phosphorylation of mitogen-activated protein kinases (MAPKs) (10 and 20  $\mu$ M [2.3 and 4.6  $\mu$ g/mL]), and FGF-2 and vascular endothelial growth factor (VEGF)-induced proliferation of porcine aortic cell lines expressing PAE/FGFR-1 and PAE/VEGFR-2, respectively, in a dose-dependent manner (0.5-10  $\mu$ M [0.1-2.3  $\mu$ g/mL]) (Bråkenhielm et al., 2001).

Using the neutral red uptake (NRU) assay, the following sequence of sensitivity to resveratrol (doses up to 500  $\mu$ M [114  $\mu$ g/mL]) was determined: tongue squamous carcinoma SCC-25 cells > Smulow-Glickman (S-G) human gingival epithelial cells > RHEK-1 keratinocytes >> fibroblasts (i.e., gingival, periodontal ligament, and pulp). In S-G cells, toxicity was found to level off between day 2 and 3 for a 3-day continuous exposure to resveratrol (5-150  $\mu$ M [1-34.2  $\mu$ g/mL]). At concentrations >75  $\mu$ M (17  $\mu$ g/mL), irreversible damage to cell proliferation occurred, and the presence of an hepatic S9 microsomal fraction did not potentiate or improve the cytotoxicity. Additionally, the cytotoxicity of hydrogen peroxide or nitrogen oxide to S-G cells was not affected by resveratrol. Other cytotoxicity endpoints were noted (see table) (Babich et al., 2000).

In HL-60 cells, resveratrol (2.5, 5, 10, 20, 40, and 80  $\mu$ g/L [0.011, 0.02, 0.044, 0.088, 0.18, and 0.35  $\mu$ M]) inhibited [ $^3$ H]thymidine incorporation into DNA (by 30, 56, 67, 81, 83, and 87%, respectively) and [ $^3$ H]uridine incorporation into RNA (by 43, 54, 72, 85, 90, and 93%, respectively) in a dose-dependent manner (Dubash et al., 1999).

Table 5. Cytotoxicity Studies of Resveratrol

Test System or Species, Strain, Age, Number, and Sex	Chemical Form and Purity	Route, Dose, and Duration	Results/Comments	Reference
<i>In Vitro</i> Assays				
BCE cells stimulated with FGF-2 (1 ng/mL)	resveratrol, >99% pure	incubation with 1, 10, 100, 1000, and 10,000 nM (0.2, 2.3, 22.8, 228, 2282.5 ng/mL) for 72 h	Resveratrol inhibited capillary endothelial cell growth in a dose-dependent manner. [EC <sub>50</sub> value was 10x lower compared with several other tumor cell lines (e.g., murine B16 melanoma cells, T241 fibrosarcoma cells, and Lewis lung carcinoma cells).]	Bräkenhielm et al. (2001)
BCE cells stimulated with FGF-2 (1 ng/mL)	resveratrol, >99% pure	incubation with 10 and 20 µM (2.3 and 4.6 µg/mL)	Resveratrol inhibited FGF-2-induced phosphorylation of MAPK <sup>p42</sup> and MAPK <sup>p44</sup> .	Bräkenhielm et al. (2001)
BCE cells stimulated with FGF-2 (1 ng/mL)	resveratrol, >99% pure	incubation with 0.5, 1, 2.5, 5, and 10 µM (0.1, 0.2, 0.57, 1, and 2.3 µg/mL) for 72 h	Resveratrol inhibited the FGF-2 and VEGF-induced proliferation of PAE/FGFR-1 and PAE/VEGFR-2 cells, respectively, in a dose-dependent manner. At 1 µM, significant inhibition of the VEGF-induced PAE/VEGFR-2 endothelial cell migration was observed.	Bräkenhielm et al. (2001)
S-G human gingival epithelial cell line	resveratrol solubilized in ethanol, purity n.p.	incubation with up to 500 µM (114 µg/mL) for 24 h	<u>Initial toxicity (µM)</u> 100	Babich et al. (2000)
periodontal ligament fibroblasts			206	
dental pulp fibroblasts			457	
normal human gingival GN56 fibroblasts			432	
nontumorigenic human epidermal RHEK-1 keratinocytes			462	
human tongue squamous carcinoma SCC-25 cells			150	216
			25	154

Table 5. Cytotoxicity Studies of Resveratrol (Continued)

Test System or Species, Strain, Age, Number, and Sex	Chemical Form and Purity	Route, Dose, and Duration	Results/Comments	Reference
S-G human gingival epithelial cell line, seeded at a density of $1.5 \times 10^4$ cells/well	resveratrol solubilized in ethanol, purity n.p.	<i>NRU assay</i> : incubation with 10, 25, 50, 75, 100 and 150 $\mu\text{M}$ (2.3, 5.7, 11, 17, 22.8, and 34.2 $\mu\text{g/mL}$ ) continuously for 3 days	The toxicity of resveratrol gradually increased to day 2 of exposure and leveled off between day 2 and 3. $\text{NRU}_{50}$ values were 154 $\mu\text{M}$ for a 1-day exposure, 93 $\mu\text{M}$ for a 2-day exposure, and 94 for a 3-day exposure.	Babich et al. (2000)
S-G human gingival epithelial cell line, seeded at a density of $1.5 \times 10^4$ cells/well	resveratrol solubilized in ethanol, purity n.p.	<i>NRU assay</i> : incubation with 5-150 $\mu\text{M}$ (1-34.2 $\mu\text{g/mL}$ ) for 2 days, refed with recovery medium (without resveratrol), and incubated for an additional 3 days	At doses up to 50 $\mu\text{M}$ resveratrol, cells resumed normal growth kinetics during the recovery period. Concentrations between 75-150 $\mu\text{M}$ caused a steady decrease in cell numbers during the period, possibly indicating irreversible damage.	Babich et al. (2000)
S-G human gingival epithelial cell line, seeded at a density of $1.5 \times 10^4$ cells/well	resveratrol solubilized in ethanol, purity n.p.	<i>Bioactivation assay</i> : incubation with 100, 150, and 200 $\mu\text{M}$ (22.8, 34.2, and 45.7 $\mu\text{g/mL}$ ) in the presence of an hepatic S9 microsomal fraction, derived from Aroclor-induced rats, for 24 h	Cytotoxicity was not potentiated or improved.	Babich et al. (2000)
S-G human gingival epithelial cell line, seeded at a density of $1.5 \times 10^4$ cells/well	resveratrol solubilized in ethanol, purity n.p.	<i>AlamarBlue reduction assay</i> : incubation with up to 400 $\mu\text{M}$ (91.3 $\mu\text{g/mL}$ ) for 24 h, followed by refeeding with phenol red-free medium containing 10% Alamar Blue solution and incubation for 3 h	Initial toxicity occurred with 100 $\mu\text{M}$ resveratrol, and the $\text{NRU}_{50}$ value at 24 h was 256 $\mu\text{M}$ .	Babich et al. (2000)
S-G human gingival epithelial cell line, seeded at a density of $1.5 \times 10^4$ cells/well	resveratrol solubilized in ethanol, purity n.p.	<i>WST-1 assay</i> : incubation with up to 400 $\mu\text{M}$ (91.3 $\mu\text{g/mL}$ ) for 24 h, followed by washing with PBS, refeeding with exposure medium containing 4% WST-1 reagent, and incubation for 20 min	Initial toxicity occurred with 150 $\mu\text{M}$ resveratrol, and the $\text{NRU}_{50}$ at 24 h was 282 $\mu\text{M}$ .	Babich et al. (2000)
S-G human gingival epithelial cell line, seeded at a density of $1.5 \times 10^4$ cells/well	resveratrol solubilized in ethanol, purity n.p.	<i>BrdU ELISA assay</i> : incubation with 0.01-100 $\mu\text{M}$ (0.002-22.8 $\mu\text{g/mL}$ ) for 24 h, followed by treatment with BrdU for 4 h	Inhibition of DNA synthesis was initially detected at 50 $\mu\text{M}$ resveratrol, and the $\text{NRU}_{50}$ was 100 $\mu\text{M}$ .	Babich et al. (2000)

Table 5. Cytotoxicity Studies of Resveratrol (Continued)

Test System or Species, Strain, Age, Number, and Sex	Chemical Form and Purity	Route, Dose, and Duration	Results/Comments	Reference
S-G human gingival epithelial cell line, seeded at a density of $1.5 \times 10^4$ cells/well	resveratrol solubilized in ethanol, purity n.p.	incubation with or without 25 $\mu$ M and either H <sub>2</sub> O <sub>2</sub> or NO for 24 h	Resveratrol had no effect on the toxicity of H <sub>2</sub> O <sub>2</sub> or NO to the cells.	Babich et al. (2000)
human promyelocytic HL-60 cells	resveratrol, purity n.p.	incubation with 2.5, 5, 10, 20, 40, and 80 $\mu$ g/L (0.011, 0.02, 0.044, 0.088, 0.18, and 0.35 $\mu$ M) for 90 min	In a dose-dependent fashion, [ <sup>3</sup> H]thymidine incorporation into DNA was inhibited by 30, 56, 67, 81, 83, and 87%, respectively, and [ <sup>3</sup> H]uridine incorporation into RNA was inhibited by 43, 54, 72, 85, 90, and 93%, respectively. [Stilbenes and piceid (at the same doses) also inhibited both processes dose-dependently.]	Dubash et al. (1999)
<b><i>In Vivo</i> Assays</b>				
Mice, C57B16/J, having full thickness skin wounds created by surgery on the backs, 5- to 6-wk-old, 6F/group	resveratrol, >99% pure	oral; 5.7 $\mu$ g/mL (25 $\mu$ M) in 1% ethanol in drinking water 2 days before the operation and for 15 days after surgery	Resveratrol significantly delayed wound healing. Wound sizes were significantly larger from day 2 and throughout the experiment in test animals.	Bräkenhielm et al. (2001)
Corneal micropockets (Mice, C57B16/J, 6- to 7-wk-old, 5/group, sex n.p.)	resveratrol, >99% pure	oral; 0.4 $\mu$ g/mL (2 $\mu$ M) in 1% ethanol (final amount of 1.2 $\mu$ g/mouse/day [48 $\mu$ g/kg]) given 3 days before growth factor implantation and "throughout the experiment" (duration n.p.). Animals were examined on day 5 after pellet implantation.	Resveratrol significantly inhibited corneal neovascularization induced by VEGF and FGF-2 compared with controls (ethanol). Vessel density was significantly reduced in the FGF-2-implanted corneas.	Bräkenhielm et al. (2001)

Abbreviations: BCE = bovine capillary endothelial; BrdU = 5-bromo-2'-deoxyuridine; DNA = 2'-deoxy-5'-ribonucleic acid; EC<sub>50</sub> = concentration needed to reach 50% inhibition; FGF-2 = fibroblast growth factor-2; h = hour(s); H<sub>2</sub>O<sub>2</sub> = hydrogen peroxide; MAPK = mitogen-activated protein kinase; min = minute(s); NO = nitric oxide; NRU = neutral red uptake; NRU<sub>50</sub> = midpoint cytotoxicity, NRU=neutral red uptake; PAE/VEGFR-2 and PAE/FGFR-1 = porcine aortic cell lines expressing VEGFR-2 and FGFR-1, respectively; PBS = phosphate buffer solution; RNA = ribonucleic acid ;S-G = Smulow-Glickman; VEGF = vascular endothelial growth factor; wk = week(s); WST-1 = 4-[3-(4-iodophenyl)-2-(4-nitrophenyl)-2H-5-tetrazolio]-1,3-benzene disulfonate

### In Vivo Assays

In mice with skin wounds, resveratrol (5.7 µg/mL [25 µM]) was an angiogenesis inhibitor. In corneal micropockets of the animals, resveratrol (oral; 0.4 µg/mL [2 µg/mL] given three days before growth factor implantation and throughout the experiment) significantly inhibited VEGF- and FGF-2-induced corneal neovascularization compared with controls (Bråkenhielm et al., 2001).

### **9.2 Reproductive and Teratological Effects**

In developing chick embryos of white Leghorn, resveratrol (1, 10, 25, 50, and 100 µg/disk [0.004, 0.044, 0.11, 0.22, and 0.438 µmol/disk] incubated for 48-72 hours) induced vascular zones in the developing chorioallantoic membrane (Bråkenhielm et al., 2001).

### **9.3 Carcinogenicity**

No data were available.

### **9.4 Initiation/Promotion Studies**

No data were available.

### **9.5 Anticarcinogenicity**

Studies have shown the blocking ability of resveratrol on the process of multistep carcinogenesis—that is, tumor initiation, promotion, and progression via mitotic signal transduction blockade, removal of reactive oxygen species by resveratrol, etc. (Lin and Tsai, 1999). The anticancer activity of resveratrol and its molecular mechanisms have been recently reviewed (Gusman et al., 2001).

The details of the following studies are presented in **Table 6**.

### In Vitro Assays

Using the mouse mammary gland organ culture model, resveratrol (1-10 µM [0.2-2.3 µg/mL]) inhibited formation of estrogen-dependent preneoplastic ductal lesions induced by 7,12-dimethylbenz[a]anthracene (DMBA) (Bhat et al., 2001).

In human breast cancer cell lines (KPL-1, MCF-7, MKL-F, T47-D, and MDA-MB-231), resveratrol (1 pM-180 µM [ $2 \times 10^{-7}$ -40 µg/mL]) inhibited the growth of cell lines in a time- and/or dose-dependent manner (Damianaki et al, 2000; Nakagawa et al., 2001). In addition, resveratrol (1 pM-1 µM [ $2 \times 10^{-7}$ -0.2 µg/mL]) inhibited growth of prostate cancer cell lines PC3 and DU145 (Kampa et al., 2000). In LNCaP prostate cancer cells, resveratrol (100 µM [22.8 µg/mL]) inhibited cell growth in the presence of androgens (Mitchell et al., 1999).

### In Vivo Assays

In mice, oral administration of resveratrol (5.7 µg/mL [25 µM]; 1 mg/kg/day) significantly inhibited the growth of T241 fibrosarcomas (Bråkenhielm et al., 2001).

**Table 6. Anticarcinogenicity Studies of Resveratrol**

Test System or Species, Strain, and Age, Number, and Sex of Animals	Chemical Form and Purity	Route, Dose, Duration, and Observation Period	Results/Comments	Reference												
<i>In Vitro</i> Assays																
Mammary glands of mice, BALB/c, 3- to 4-wk-old, number n.p., F	resveratrol, purity n.p.	incubation with 1, 2.5, 5, and 10 $\mu$ M (0.2, 0.57, 1, and 2.3 $\mu$ g/mL) for the first 10 days of 14-day culture (Ductal lesions were induced with 2 $\mu$ g/mL DMBA on day 3 for 24 h.)	The incidence of hyperplastic and aggressive ductal lesions induced by DMBA was reduced by resveratrol in a dose-dependent manner ( $IC_{50}$ ~3 $\mu$ M).	Bhat et al. (2001)												
Human breast cancer cell lines: ER-positive KPL-1 and MCF-7 and ER-negative MKL-F	<i>trans</i> -resveratrol, 99.8% pure	incubation with 0.01-40 $\mu$ g/mL (0.04-180 $\mu$ M) for 24, 48, 72, and 96 h	At $\geq$ 44 $\mu$ M, the growth of all cell lines was inhibited in time- and dose-dependent manners. The $IC_{50}$ for the 72-h treatment ranged from 105 to 149 $\mu$ M. At lower concentrations of resveratrol, moderate inhibition of the growth of MKL-F and stimulation of KPL-1 and MCF-7 in a time-dependent manner were seen. At 72 h, the cells were stimulated by up to 132 and 115% of control level, respectively.	Nakagawa et al. (2001)												
Human breast cancer cell lines: hormone-sensitive MCF-7 and T47-D and hormone-resistant MDA-MB-231	(+)-resveratrol, >99% pure	incubation with $10^{-12}$ - $10^{-6}$ M (1 pM-1 $\mu$ M [ $2 \times 10^{-7}$ -0.2 $\mu$ g/mL]) for a total of 6 days; applied on day 2 (one cell cycle) and day 5 (three cell cycles)	Cell proliferation was inhibited in a dose-dependent manner in all cell lines; the effect after day 5 was more apparent than at day 2. The $IC_{50}$ and maximum inhibition of resveratrol were as follows:  <table border="1" style="margin-left: auto; margin-right: auto;"> <tr> <td></td> <td><math>IC_{50}</math> (pM)</td> <td>Inhibition</td> </tr> <tr> <td>MCF-7</td> <td>13.7<math>\pm</math>8.3</td> <td>0.42</td> </tr> <tr> <td>T47-D</td> <td>0.1<math>\pm</math>1.2</td> <td>0.56</td> </tr> <tr> <td>MDA-MB-231</td> <td>5.2<math>\pm</math>9.1</td> <td>0.30</td> </tr> </table>		$IC_{50}$ (pM)	Inhibition	MCF-7	13.7 $\pm$ 8.3	0.42	T47-D	0.1 $\pm$ 1.2	0.56	MDA-MB-231	5.2 $\pm$ 9.1	0.30	Damianaki et al. (2000)
	$IC_{50}$ (pM)	Inhibition														
MCF-7	13.7 $\pm$ 8.3	0.42														
T47-D	0.1 $\pm$ 1.2	0.56														
MDA-MB-231	5.2 $\pm$ 9.1	0.30														
Prostate cancer cell lines: hormone-sensitive LNCaP, PC3, and DU145	(+)-resveratrol, >99% pure	incubation with $10^{-12}$ - $10^{-6}$ M (1 pM-1 $\mu$ M [ $2 \times 10^{-7}$ -0.2 $\mu$ g/mL]) given one day after seeding (day 0) and cultured for 6 days	Resveratrol had no effect in LNCaP cells ( $IC_{50} = >10^{-6}$ M). At $>10^{-7}$ M, resveratrol produced partial inhibition of growth in the PC3 cell line ( $IC_{50} = 0.11 \pm 1.23 \times 10^{-6}$ M; maximum inhibition at 0.48). In DU145 cells, it was a potent inhibitor of cell growth, which was time- and dose-dependent ( $IC_{50} = 0.57 \pm 0.58 \times 10^{-12}$ M; maximum inhibition at 0.82). In LNCaP cells, resveratrol was a very weak competitor of androgen binding.	Kampa et al. (2000)												
Prostate cancer cell line LNCaP	resveratrol, purity n.p.	incubation with up to 200 $\mu$ M (45.7 $\mu$ g/mL) for 24 or 32 h with or without Mib 2 days after cells were seeded	At 100 $\mu$ M, Mib-stimulated cell growth was inhibited and very little apoptosis was observed. At 200 $\mu$ M, massive apoptotic cell death was seen.	Mitchell et al. (1999)												



Table 6. Anticarcinogenicity Studies of Resveratrol (Continued)

Test System or Species, Strain, and Age, Number, and Sex of Animals	Chemical Form and Purity	Route, Dose, Duration, and Observation Period	Results/Comments	Reference
<b><i>In Vivo</i> Assays</b>				
Mice, C57B16/J (implanted s.c. with a murine T241 fibrosarcoma in the middle dorsum [tumors visible after 72 h]), 5- to 6-wk-old, 6-7M/group	resveratrol, >99% pure	oral; 5.7 µg/mL (25 µM) or 1 mg/kg/day in absolute ethanol added to drinking water for 25 days	Resveratrol significantly inhibited the growth of T241 fibrosarcomas in the animals.	Bräkenhielm et al. (2001)
Rats, F344, 2-mo-old, 10M/group	resveratrol, purity n.p.	oral; 200 µg/kg (0.876 µmol/kg) bw/day in drinking water for 100 days beginning 10 days before s.c. injection of 2 doses of 15 mg/kg AOM 1 wk apart	The number of ACF in the colorectal mucosa (25.7±3.6 vs. 39.4±3.3 in controls) and mean multiplicity (2.7±0.3 vs. 4.9±0.6 in controls) were significantly reduced. Resveratrol also reduced the number of small and medium ACF and stopped the development of large ACF.  Compared to controls, <i>bax</i> was significantly expressed in ACF of treated rats (53±1.3% and 57±1.3%, respectively) but not in the surrounding mucosa. In addition, <i>p21</i> was expressed in ACF of treated rats but to a lower degree compared to controls (1.5±0.1% and 2.2±0.1%, respectively) but not in the normal mucosa.	Tessitore et al. (2000)
Rats, Sprague-Dawley, 42-days-old, 20F/group	resveratrol, purity n.p.	intra-gastric; 10 and 100 mg/kg (0.044 and 0.438 mmol/kg) bw 5 days/wk starting 7 days before NMU administration and terminating 120 days after administration of NMU	By day 21, tumors were palpable in the control group after NMU administration. By day 111, 100% incidence was reached. The high dose of resveratrol delayed tumorigenesis: on day 40, 0% incidence was observed versus 42% incidence in the control group; the median time for appearance of the first tumor was 79.5 days in the treated group versus 51.5 days in the control group; at termination, the multiplicity of tumors was 3.9 versus 6.0 in control animals. There was also a decrease in the total number of tumors. Morphologically, there was an increase in differentiated alveolar structures among tumor parenchyma, focal reduction of cell layers and numerous luminal openings within alveolar structures, and necrosis and apoptotic cells in small areas of some tumors.	Bhat et al. (2001)

Abbreviations: ACF = aberrant crypt foci; AOM = azoxymethane, bw = body weight; DMBA = 7,12-dimethylbenz[*a*]anthracene; ER = estrogen receptor; h = hour(s); IC<sub>50</sub> = inhibitory concentration for 50% of cells; M = male(s); Mib = anti-hormone blockade (nonmetabolizable, synthetic androgen); NMU = *N*-methyl-*N*-nitrosourea; mo = month(s); n.p. = not provided; s.c. = subcutaneous(ly); wk = week(s)

In rats, resveratrol (200 µg/kg [0.876 µmol/kg] body weight per day for 100 days) inhibited the number of azoxymethane (AOM)-induced aberrant crypt foci (ACF) and their multiplicity, suggesting a protective role in colon carcinogenesis. In ACF but not the surrounding mucosa, *bax* and *p21* were expressed (Tessitore et al., 2000). When rats were treated with resveratrol (100 mg/kg [0.438 mmol/kg] body weight 5 days/week for >120 days) before *N*-methyl-*N*-nitrosourea (NMU) administration, a delay in tumorigenesis occurred; resveratrol increased tumor latency by 28 days. Additionally, the multiplicity of tumors and the total number of tumors were decreased compared to controls (Bhat et al., 2001).

## 9.6 Genotoxicity

The details of the following studies by Matsuoka et al. (2001) are presented in **Table 7**. In the presence and absence of metabolic activation, *trans*-resveratrol (0.02-5000 µg/plate [0.09 nmol/plate – 21.91 µmol/plate]) was nonmutagenic in *Salmonella typhimurium* strains TA98 and TA100 and *Escherichia coli* strain WP2uvrA. In the Chinese hamster lung, structural chromosome aberrations (CAs) (mainly chromatid breaks and exchanges) were induced dose-dependently at 2.5-20 µg/mL (11-88 µM); in addition, weak aneuploidy induction was observed. Furthermore, resveratrol (same doses) induced micronucleus (MN), polynuclear (PN), and karyorrhectic cells after a 48-hour treatment and sister chromatid exchanges (SCEs) in a dose-dependent manner at concentrations up to 10 µg/mL. Cell cycle analysis showed that resveratrol caused S phase arrest and induced apoptosis after a 48-hour treatment.

*trans*-Resveratrol (1, 5, 10, 25, 50, and 100 µM [0.2, 1, 2.3, 5.7, 11, and 22.8 µg/mL]) strongly cleaved plasmid DNA (i.e., strand excision or relaxation of pBR322) in the presence of Cu<sup>2+</sup> at neutral pH and under aerobic conditions. Under anaerobic conditions, however, increasing the concentration of resveratrol failed to enhance the efficiency of DNA cleavage, suggesting the cleavage to be "absolutely" dependent on the presence of both Cu<sup>2+</sup> and oxygen. Resveratrol was also found to be capable of binding to DNA (Fukuhara and Miyata, 1998). Studying the mechanism of the DNA-damaging properties of *trans*-resveratrol, the compound's effects were found to be of no importance under physiological conditions. In the presence of ascorbic acid or glutathione, resveratrol (0.1 mM) lost its ability to promote hydroxyl-radical (•OH) formation by DNA-bound Cu<sup>2+</sup> and was instead a powerful antioxidant (Burkitt and Duncan, 2000).

In addition, resveratrol (10 µM [2.3 µg/mL]) significantly stimulated DNA strand breaks induced by adenosine 5'-diphosphate (ADP)-Fe<sup>3+</sup> in the presence of hydrogen peroxide. By reducing ADP-Fe<sup>3+</sup>, resveratrol acted as a prooxidant of DNA (Miura et al., 2000).

## 9.7 Cogenotoxicity

No data were available.

## 9.8 Antigenotoxicity

No data were available.

## 9.9 Immunotoxicity

No data were available.

Table 7. Genotoxicity Studies of Resveratrol

Test System	Biological Endpoint	Chemical Form and Purity	Dose and Duration	Comments	Reference
<i>Salmonella typhimurium</i> strains TA98 and TA100 and <i>Escherichia coli</i> strain WP2uvrA	bacterial reverse mutation	<i>trans</i> -resveratrol, purity n.p.	incubation with 0.02 to 5000 µg/plate (0.09 nmol/plate – 21.91 µmol/plate) for 20 min	Resveratrol was negative in all strains.	Matsuoka et al. (2001)
	cytotoxicity				
Chinese hamster lung cells	CA		cells seeded at 1.5 x 10 <sup>5</sup> /plate incubated for 17 h and then treated with 2.5, 5, 10, and 20 µg/mL (11, 22, 44, and 99 µM) for 24, 29, 36, 48, 54, or 72 h	Cell survival decreased with dose with both the 24- and 48-h treatments. Survival calculations were greater for cell density than for cell count. Cytotoxicity was observed at the high dose. Structural CAs (chromatid breaks and exchanges [majority], chromatid and chromosome gaps, and chromosome breaks) were induced dose-dependently. The modal chromosome number of 25 in 80% of control cells was reduced to ~60% and aneuploid cells increased at 10 µg/mL with the 48-h treatment and at 5.0 and 10 µg/mL with the 72-h treatment.	
	MN and/or PN				
	SCE			A slight increase in MN occurred with the 24-h treatment and a dose-dependent increase in MN, PN, and karyorrhectic cells occurred with the 48-h treatment up to the 10 µg/mL dose. Mitotic cells did not increase significantly with either time of treatment and had ~2% tripolar anaphase cells at 10 µg/mL with the 48-h treatment. At 24 h, almost all control cells had reached the second metaphase, while cells given 5 and 10 µg/mL reached the first metaphase. At 48 h, control cells had passed through the fifth metaphase, while cells treated at 10 µg/mL reached the second metaphase. At 54 h, cells given 20 µg/mL were still in the first metaphase.	
	S phase arrest			SCEs were induced dose-dependently. At 10 µg/mL, peak frequency of SCEs per cell was 71.6±21.79 versus 10.36±3.52 at baseline. The number of cells in G1 phase was decreased, while the number in S phase was increased, particularly early to mid-S phase. At high concentrations, apoptosis was induced with the 48-h treatment.	

Abbreviations: CA = chromosome aberration; h = hour(s); min = minute(s); MN = mononuclei; n.p. = not provided; PN = polynuclei; SCE = sister chromatid exchange

## 9.10 Other Data

### Modulation of Enzyme Activity

In CD2F1 mice (four- to six-weeks-old), *cis*- and *trans*-resveratrol (oral; 1000 µg/kg [4.381 µmol/kg] per day for five or ten days) caused almost complete inhibition of 7-ethoxyresorufin-*o*-dealkylation (EROD) activity (CYP1A2). No effect was observed on ethoxycoumarin-*o*-deethylation (ECOD) activity (CYP1A2/2E1) or benzo[a]pyrene metabolism (Boyce and Gooderham, 2000 abstr.).

Resveratrol was an effective inhibitor of recombinant human estrogen sulfotransferase (EST); the IC<sub>50</sub> was 1.6 µM. In intact cultured human mammary epithelial cells, a more physiologically relevant condition, the inhibition (1.3 µM) was similar to that with EST (Otake et al., 2000). In recombinant human P form phenolsulfotransferase (PST), an enzyme involved in carcinogen bioactivation, resveratrol was a potent inhibitor; its IC<sub>50</sub> was 0.2 µM. In intact human hepatoma Hep G2 cells, inhibition of P-PST decreased fourfold (IC<sub>50</sub> = 0.8 µM) (Walle et al., 1998 abstr.).

### Phase 1 (Cytochrome P450) Enzymes

In rats orally administered resveratrol (8 mg/kg [0.04 mmol/kg]), CYP2E1 (chlorzoxazone 6-hydroxylation) and protein level in liver microsomes were significantly reduced 24 hours after administration. In human microsomes incubated with resveratrol (low micromolar levels), CYP1A2 (methoxyresorufin *O*-demethylation) and CYP3A4 (erythromycin demethylation) were inhibited, while CYP2E1 activity was moderately increased. Resveratrol also induced Phase 2 biotransformation (Delaporte and Wilkinson, 1998 abstr.).

### Miscellaneous Studies

Resveratrol (6-100 µM [1-22.8 µg/mL]) inhibited the growth and tube formation of bovine aorta endothelial (BAE) cells in a dose-dependent manner (Igura et al., 2001). In addition, DMBA metabolism by liver microsomes was inhibited *in vitro* in a dose-dependent manner; at 10, 20, 40, and 80 µg/mL, resveratrol produced inhibitory effects of 37, 48, 61, and 69%, respectively (Dubash et al., 1999).

## 10.0 Structure-Activity Relationships

Several compounds show structural similarities to *trans*-resveratrol. Kaempferol (3,5,7-trihydroxy-2-(2-hydroxyphenyl)-4*H*-1-benzopyran-4-one), for example, has a 4'-hydroxyl group in the B-ring and a 2,3-double bond in the C-ring, which allows conjugation across the A-ring containing the meta dihydroxy structure (Kuhle et al., 2000). The bioavailability of resveratrol and other polyphenols, such as enterodiol, isoflavone, and anthocyanidin, has been reviewed (Scalbert and Williamson, 2000). Comparative studies regarding beneficial effects and mechanism of resveratrol commonly use the compounds below. A table summarizing studies conducted by the National Toxicology Program (NTP) occurs at the end of this section.

### Diethylstilbestrol

*trans*-Resveratrol is structurally similar to the synthetic estrogenic agent diethylstilbestrol (DES), also called α,α'-diethylstilbenediol. In contrast to resveratrol, DES induced polyploidy *in vitro* (Sawada and Ishidate, 1978; Sofuni, 1998; both cited by Matsuoka et al., 2001).

Like resveratrol, DES strongly inhibited nicotinamide adenine dinucleotide phosphate (NADPH)- and ADP-Fe<sup>3+</sup>-dependent microsomal lipid peroxidation; an IC<sub>50</sub> of 1.1 µM was

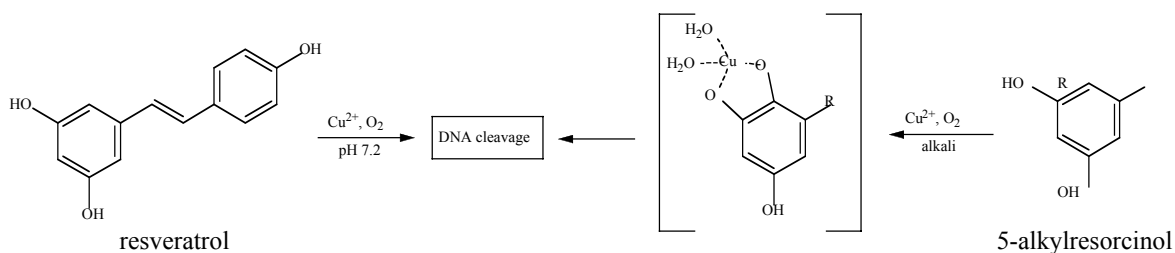
obtained versus 4.8  $\mu\text{M}$  for resveratrol. In addition, both compounds strongly inhibited the reaction at the initiation and propagation stages (Miura et al., 2000). Other flavonoids, including quercetin (see below), are very effective inhibitors of iron-dependent lipid peroxidation; their extent of reduction of  $\text{ADP-Fe}^{3+}$ , however, was less than that of resveratrol. DES, on the other hand, caused no reduction of  $\text{ADP-Fe}^{3+}$  or  $\text{EDTA-Fe}^{3+}$ . It also had no effect on DNA damage (Afanas'ev et al., 1989; cited by Miura et al., 2000).

### Quercetin

In several studies, the activity or effect of resveratrol was compared to that of quercetin (2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4*H*-1-benzopyran-4-one) (e.g., see Section 9.1.2). In the human intestinal epithelial cell line Caco-2, the permeability constant for quercetin was similar to that of resveratrol. In addition, quercetin, like resveratrol, was a strong inhibitor of P-PST ( $\text{IC}_{50} = 0.1 \mu\text{M}$ ). In intact human hepatoma Hep G2 cells, this decreased by 25-fold ( $\text{IC}_{50} = 2.5 \mu\text{M}$ ); the hepatocyte had a greater metabolism of quercetin than of resveratrol (Walle et al., 1998 abstr.).

### Resorcinol

Resorcinol (*m*-dihydroxybenzene) produced  $\text{Cu}^{2+}$ -dependent DNA strand excision under oxidative conditions (Barr et al., 1988; Scannell et al., 1988; Hecht, 1989; Lytollis et al., 1995; all cited by Fukuhara and Miyata, 2001). Having the same structural elements as this compound, resveratrol was then studied for its DNA-cleaving ability (see Section 9.6) (Fukuhara and Miyata, 2001).



### Other Stilbene Analogs

Many other compounds containing a stilbene moiety have been tested for estrogenicity. These include 4,4'-stilbenediol and 4-stilbenol; 1,1,2-triphenylbut-1-ene derivatives such as tamoxifen, droloxifene, nafoxidine, and clomiphene; and 2-phenylindene derivatives (in which the moiety is part of a fused ring structure) (Fang et al., 2001). [An ILS project A086-003 report with test results will be published on the ICCVAM/NICEATM web site.]

Table 8. NTP Studies of Chemicals Structurally Related to Resveratrol

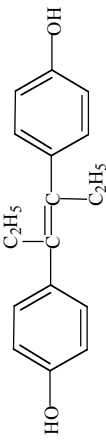
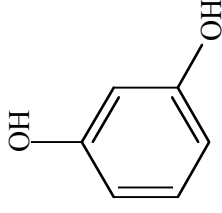
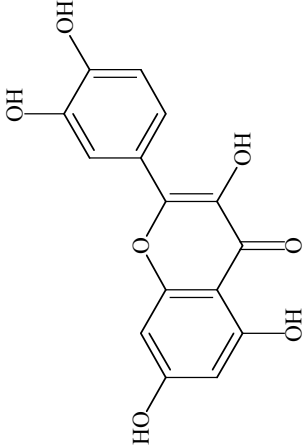
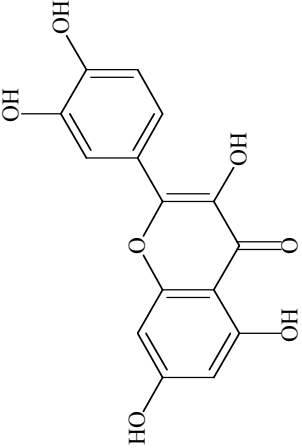
Chemical Name and [CASRN]	Structure	Toxicity Tests*	Reference(s)
Diethylstilbestrol (DES) [56-53-1]		<p><b>Short-term carcinogenicity (<i>transgenic models</i>):</b> 24- and 26-wk topical [Tg.AC], 25-wk s.c. [p53<sup>+/+</sup>] and 26-wk gavage [Tg.AC] studies have been conducted in transgenic mice; results were not available.</p> <p><b>Reproductive Toxicity:</b> [mice: 1, 10, and 50 ppb in feed] At the high dose, continuous exposure (duration not specified) produced decreases in the fertility index, the number of litters, the number of live pups, and the proportion of pups born alive per litter in F mice.</p> <p>At the high dose, F had almost a 30% increase in pituitary weight and &gt;75% had no clear estrous cycle (versus 25% of controls). In M, a significant increase in pituitary weight (~15%) and 13-18% reductions in the weight of the epididymis, cauda epididymis, and prostate were observed.</p> <p><b>Teratology:</b> [mice: s.c.; 2.5, 5, 10, or 100 µg/kg/day in corn oil on gd 9-16] Corrected maternal bw gain was decreased in all dose groups. At 5 µg/kg/day, there was an increase in skeletal malformations (scrambled sternbrae, perforated sternum, and fused ribs [which have not been shown to be DES-treatment specified]). At ≥10 µg/kg/day, the number of corpora lutea per dam was decreased, and the percent resorptions per litter was increased. At the high dose, gravid uterine weight and live litter size were decreased, while relative maternal liver weight and the incidence of malformation per litter (F more severely affected than M) were increased. LOAEL = 10 µg/kg/day; NOAEL = 5 µg/kg/day</p> <p>Comparison test: 100 µg/kg/day DES in trioctanoin had similar effects.</p>	<p>NTP (2002d)</p> <p>NTP (1983)</p> <p>NTP (1984)</p> <p>NTP (1994)</p>
Resorcinol [108-46-3]		<p><b>Short-term or Subchronic Toxicity:</b> 17-day gavage studies [M and F rats: 27.5, 55, 110, 225, or 450 mg/kg; M and F mice: 37.5, 75, 100, 300, or 600 mg/kg] No rats died, and no chemical-related gross or microscopic lesions were found. For mice, 1/5 M from the 300 mg/kg group died, and all F (5/5) and 4/5 M from the high-dose group died. 13-wk gavage studies [M and F rats: 32, 65, 130, 260, or 520 mg/kg; M and F mice: 28, 56, 112, 225, or 420 mg/kg] All F rats (10/10) and 8/10 M rats from the high-dose group died. For mice, 8/10 M and 8/10 F from the high-dose group died.</p> <p><b>Short-term carcinogenicity (<i>transgenic models</i>):</b> 24-wk topical [Tg.AC] or gavage [p53<sup>+/+</sup>] and 26-wk gavage [rasH2] studies have been conducted in transgenic mice; results were not available.</p>	<p>NTP (1992b)</p> <p>NTP (2002c,e)</p>

Table 8. NTP Studies of Chemicals Structurally Related to Resveratrol (Continued)

Chemical Name and [CASRN]	Structure	Toxicity Tests*	Reference(s)
Resorcinol [108-46-3] (continued)	(See above row.)	<p><b>Chronic Toxicity:</b> [M rats: 112 or 225 mg/kg 5 days/wk for 2 yr; F rats: 50, 100, or 150 mg/kg for 15 mo; M and F mice: 112 or 225 mg/kg 5 days/wk for 2 yr] At the high dose, mean body weights of rats were decreased compared to those of controls (10-15% lower in M from wk 87 to study termination and 11-14% lower in F from wk 95 to study termination). Additionally, survival was significantly lower than controls. For mice, mean body weights of F were 10-15% lower compared to controls from wk 85 to study termination. In both rats and mice, effects on the CNS were observed—ataxia, recumbency, and tremors.</p> <p><b>Carcinogenicity:</b> [M rats: 112 or 225 mg/kg 5 days/wk for 2 yr; F rats: 50, 100, or 150 mg/kg for 15 mo; M and F mice: 112 or 225 mg/kg 5 days/wk for 2 yr] Studies showed no evidence of carcinogenicity in rats and mice. There were no treatment-related increased incidences of neoplasms or nonneoplastic lesions in the animals. In all F rats, significantly reduced incidences of mammary gland fibroadenomas were seen. In high-dose M mice, the incidence of s.c. fibroma or sarcoma was significantly reduced compared to controls.</p> <p><b>Genotoxicity:</b> In <i>S. typhimurium</i> strains TA98, TA100, TA1535, and TA1537, no mutagenic activity was seen in the presence and absence of S9. In the absence of S9, induction of trifluorothymidine resistance in mouse L5178Y lymphoma cells was seen; no tests were done with S9. With and without S9, resorcinol induced SCEs in CHO cells. Induction of CAs was seen in CHO cells with S9; an equivocal response was found in the absence of S9. In <i>Drosophila melanogaster</i>, no induction of sex-linked recessive lethal mutations was seen, but an equivocal response was observed when resorcinol was administered by injection.</p> <p>Positive results were obtained in the MN test.</p>	NTP (1992b)
			NTP (2002b)

Table 8. NTP Studies of Chemicals Structurally Related to Resveratrol (Continued)

Chemical Name and [CASRN]	Structure	Toxicity Tests*	Reference(s)
Quercetin [117-39-5]	 <p>The structure shows a flavan-3-ol core. It consists of a central chromane ring system. At position 2, there is a hydroxyl group and a penta-2,4-dien-3-ylidene side chain. At position 3, there is a hydroxyl group and a hydroxyl group. At position 4, there is a hydroxyl group. At position 5, there is a hydroxyl group. At position 7, there is a hydroxyl group. At position 8, there is a hydroxyl group. At position 9, there is a hydroxyl group. At position 10, there is a hydroxyl group.</p>	<p><b>Carcinogenicity:</b> [F344/N M rats: 1000, 10,000, and 40,000 ppm in feed for 2 yr] Studies showed some evidence of carcinogenicity: incidence of renal tubule hyperplasia and severity of nephropathy were increased. Parathyroid hyperplasia (indicative of renal secondary hyperparathyroidism) was also observed. At the high dose, renal tubule adenomas were found in three rats and adenocarcinomas in one other rat. In addition, there was accumulation of yellow-brown granular pigment adsorbed to or absorbed by the epithelial cells of the glandular stomach, ileum, jejunum, duodenum, and colon.</p> <p><b>Genotoxicity:</b> In <i>S. typhimurium</i> strains TA98 and TA100, mutations were induced with and without S9. In CHO cells, SCEs and CAs were induced.</p>	NTP (1992a)
Quercetin dihydrate [6151-25-3]	 <p>The structure is identical to Quercetin, but with a water molecule of hydration (•2H<sub>2</sub>O).</p>	<p><b>Genotoxicity:</b> Negative results were obtained in the MN test.</p>	NTP (2002a)

\* Study details (e.g., dose of compound) have been reported if provided in the NTP abstract/report.

Abbreviations: bw = body weight; CA = chromosome aberration; CHO = Chinese hamster ovary; CNS = central nervous system; F = female(s); gd = gestation day(s); LOAEL = lowest observed adverse effect level; M = male(s); MN = micronucleus; mo = month(s); NOAEL = no observed adverse effect level; s.c. = subcutaneous; SCE = sister chromatid exchange; wk = week(s); yr = year(s)



**11.0 Online Databases and Secondary References****11.1 Online Databases**Dialog Files

DIOGENES

Chemical Economics Handbook

STN International Files

AGRICOLA	LIFESCI
BIOSIS	MEDLINE
CA	NIOSHTIC
CABA	PROMT
CANCERLIT	Registry
CAPLUS	RTECS
EMBASE	TOXLINE

TOXLINE includes the following subfiles:

Toxicity Bibliography	TOXBIB
International Labor Office	CIS
Hazardous Materials Technical Center	HMTC
Environmental Mutagen Information Center File	EMIC
Environmental Teratology Information Center File (continued after 1989 by DART)	ETIC
Toxicology Document and Data Depository	NTIS
Toxicological Research Projects	CRISP
NIOSHTIC <sup>®</sup>	NIOSH
Pesticides Abstracts	PESTAB
Poisonous Plants Bibliography	PPBIB
Aneuploidy	ANEUPL
Epidemiology Information System	EPIDEM
Toxic Substances Control Act Test Submissions	TSCATS
Toxicological Aspects of Environmental Health	BIOSIS
International Pharmaceutical Abstracts	IPA
Federal Research in Progress	FEDRIP
Developmental and Reproductive Toxicology	DART

Databases Available on the Internet

CFR (Code of Federal Regulations, National Archives and Records Administration)

CRISP (Computer Retrieval of Information on Scientific Projects)

In-House DatabasesCurrent Contents on Diskette<sup>®</sup>

The Merck Index, 1996, on CD-ROM

## 11.2 Secondary References

Block, J., Ed. 2000. Chemcyclopedia 2001. Vol. 19. American Chemical Society, Washington, DC, p. 278.

Budavari, S., Ed. 1996. The Merck Index, 12<sup>th</sup> ed. Merck & Co., Inc., Whitehouse Station, NJ.

Hendler, S.S., and D. Rorvik. 2001. Resveratrol. In: PDR for Nutritional Supplements. Medical Economics™ Thomson Healthcare, Montvale, NJ, pp. 397-401.

Registry. 2001. Registry. American Chemical Society, Columbus, OH.

## 12.0 References

Adrian, M., P. Jeandet, R. Bessis, and J. M. Joubert. 1996. Induction of phytoalexin (resveratrol) synthesis in grapevine leaves treated with aluminum chloride (AlCl<sub>3</sub>). J. Agric. Food Chem. 44(8):1979-1981. Abstract from CAB International 97-01 970300413.

Afanas'ev, I.B., A.I. Dorozhko, A.V. Brodskii, V.A. Kostyuk, and A.I. Potapovitch. 1989. Chelating and free radical scavenging mechanisms of inhibitory action of rutin and quercetin in lipid peroxidation. Biochem. Pharmacol. 38:1763-1769. Cited by Miura et al. (2000).

Agriculture and Agri-Food Canada. Undated. Functional foods and nutraceuticals: Pharmascience Inc. Internet address: <http://www.agr.ca/food/markets/nutraceu/Profiles2000E/phrmasci.html>. Last accessed on April 9, 2001.

AIM (Alcohol In Moderation). 2000. Researchers closer to understanding "French paradox." Internet address: <http://www.aim-digest/gateway/pages/heart/articles/French.htm>. Last updated on November 13, 2000. Last accessed on July 17, 2001.

AML Information Services. 2000. Biomed devices patent update January/February 2000. Internet address: [http://www.amlinfo.com/documents/Jan00D\\_M.pdf](http://www.amlinfo.com/documents/Jan00D_M.pdf).

Andlauer, W., J. Kolb, K. Siebert, and P. Fürst. 2000. Assessment of resveratrol bioavailability in the perfused small intestine of the rat. Drugs Exp. Clin. Res. 26(2):47-55.

Arichi, H, Y. Kimura, H. Okuda, K. Baba, M. Kozawa, and S. Arichi. 1982. Effects of stilbene components of the roots of *Polygonum cuspidatum* Sieb et Zucc. on lipid metabolism. Chem. Pharm. Bull. 30:1766-1770. Cited by Soleas et al. (1997) and InterHealth (1998).

Ashby, J., H. Tinwell, W. Pennie, A.N. Brooks, P.A. Lefevre, N. Beresford, and J.P. Sumpter. 1999. Partial and weak oestrogenicity of the red wine constituent resveratrol: Consideration of its superagonist activity in MCF-7 cells and its suggested cardiovascular protective effects. J. Appl. Toxicol. 19:39-45.

Babich, H., A.G. Reisbaum, and H.L. Zuckerbraun. 2000. *In vitro* response of human gingival epithelial S-G cells to resveratrol. Toxicol. Lett. 114(1-3):143-153.

- Barlass, M., R.M. Miller, and T.J. Douglas. 1987. Development of methods for screening grapevines for resistance to infection by downy mildew. *Am. J. Enol. Vitic.* 38(1):65-68. Abstract from Wine Database 7-03 87-1-03-g0001-VITI. Cited by Lin and Chen (2001).
- Barr, J.R., V.S. Murty, K. Yamaguchi, D.H. Smith, and S.M. Hecht. 1988. [Title not provided.] *Chem. Res. Toxicol.* 1:204 ff. Cited by Fukuhara and Miyata (1998).
- Basly, J.-P., F. Marre-Fournier, J.-C. Le Bail, G. Habrioux, and A.J. Chulia. 2000. Estrogenic/ antiestrogenic and scavenging properties of (*E*)- and (*Z*)-resveratrol. *Life Sci.* 66(9):769-777.
- Bertelli, A., A.A.E. Bertelli, A. Gozzini, and L. Giovannini. 1998a. Plasma and tissue resveratrol concentrations and pharmacological activity. *Drugs Exp. Clin. Res.* 24(3):133-138. [Similar results appear in Bertelli et al. (1996a); see Section 13.0.]
- Bertelli, A.A.E., L. Giovannini, R. Stradi, S. Urien, J.-P. Tillement, and A. Bertelli. 1998b. Evaluation of kinetic parameters of natural phytoalexin in resveratrol orally administered in wine to rats. *Drugs Exp. Clin. Res.* 24(1):51-55. [Similar results appear in Bertelli et al. (1996b); see Section 13.0.]
- Berzas Nevado, J.J., A.M. Contento Salcedo, and G. Castaneda Penalvo. 1999. [Title not provided.] *Analyst* 124:61-66. Cited by Lin and Chen (2001).
- Best Skin Care. Undated. Best skin care natural cosmetics and makeup. Internet address: [http://www.bestskincare.com/best\\_skin\\_care.htm](http://www.bestskincare.com/best_skin_care.htm). Last accessed on April 9, 2001.
- Bhat, K.P.L., and J.M. Pezzuto. 2001. Resveratrol exhibits cytostatic and antiestrogenic properties with human endometrial adenocarcinoma (Ishikawa) cells. *Cancer Res.* 61(16):6137-6144.
- Bhat, K.P.L., D. Lantvit, K. Christov, R.G. Mehta, R.C. Moon, and J.M. Pezzuto. 2001. Estrogenic and antiestrogenic properties of resveratrol in mammary tumor models. *Cancer Res.* 61(20):7456-7463.
- Blache, D., I. Rustan, P. Durand, G. Lesgards., and N. Loreau. 1997. [Title not provided.] *J. Chromatogr. B* 702:103-110. Cited by Lin and Chen (2001).
- Blaich, R., and O. Bachmann. 1980. The resveratrol synthesis in *Vitaceae*: Induction and cytological observations (*m. engl. Zus.*) *Vitis* 19:230-240. Abstract from Wine Database 84-1-01-g3772-VITI.
- Bowers, J.L., V.V. Tyulmenkov, S.C. Jernigan, and C.M. Klinge. 2000. Resveratrol acts as a mixed agonist/antagonist for estrogen receptors  $\alpha$  and  $\beta$ . *Endocrinology* 141(10):3657-3667.
- Boyce, A.P., and N.J. Gooderham. 2000 abstr. Treatment of mice with the phytoalexin resveratrol inhibits hepatic CYP1A2 mediated metabolism. *Toxicol. Lett.* 116(Suppl. 1):81. Abstract #297.

Boyce, A.P., J. Doehmer, and N.J. Gooderham. 2000 abstr. The toxicity of the anti-mutagen resveratrol and the mutagen 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) is mutually antagonised in V79 cells. *Toxicol. Lett.* 116(Suppl. 1):81. Abstract #296.

Bråkenhielm, E., R. Cao, and Y. Cao. 2001. Suppression of angiogenesis, tumor growth, and wound healing by resveratrol, a natural compound in red wine and grapes. *FASEB J.* 15(8):U568-U581. Express article 10.1096/fj.01-0028fje. Published online on June 8, 2001.

Burkitt, M.J., and J. Duncan. 2000. Effects of *trans*-resveratrol on copper-dependent hydroxyl-radical formation and DNA damage: Evidence for hydroxyl-radical scavenging and a novel, glutathione-sparing mechanism of action. *Arch. Biochem. Biophys.* 381(2):253-263.

Calabrese, G. 1999. Nonalcoholic compounds of wine: The phytoestrogen resveratrol and moderate red wine consumption during menopause. *Drugs Exp. Clin. Res.* 25(2-3):111-114.

Calderon, A.A., J.M. Zapata, R. Munoz, M.A. Pedreno, and A. Ros Barcelo. 1993. Resveratrol production as a part of the hypersensitive-like response of grapevine cells to an elicitor from *Trichoderma viride*. *New Phytol.* 124(3):455-463. Abstract from CAB International 95-05 950308403.

Cantos, E., C. Garcia Viguera, S. De Pascual Teresa, and F.A. Tomas Berberan. 2000. Effect of postharvest ultraviolet irradiation on resveratrol and other phenolics of cv. Napoleon table grapes. *J. Agric. Food Chem.* 48(10):4606-4612. Abstract from Life Sciences.

Cartoni, G., F. Coccioli, and R. Jasionowska. 1995. [Title not provided.] *J. Chromatogr. A* 709:209-214. Cited by Lin and Chen (2001).

CCNow. Undated. Products: Resveratrol the Dietary Supplement. Internet address: <http://www.aaa-reports.com/resver4.htm>. Last accessed on April 9, 2001.

Cosgrove, J. 2000. Nutritional supplements for women. *Nutrabid*. Internet address: <http://www.nutrabid.com/info/NutritionalOutlookMag/Dec22000/forwomen>. Last accessed on September 18, 2001.

Creasy, L.L., and M. Coffee. 1988. Phytoalexin production potential of grape berries. *J. Am. Soc. Hort. Sci.* 113:230-234. Cited by Daniel et al. (1999).

Creasy, L.L., and M.T. Creasy. 1998. Grape chemistry and the significance of resveratrol: An overview. *Pharm. Biol.* 36 (Suppl.):8-13. Pezzuto. Abstract from CAB International 99-02 990301752.

Damianaki, A., E. Bakogeorgou, M. Kampa, G. Notas, A. Hatzoglou, S. Panagiotou, C. Gemetzi, E. Kouroumalis, P.-M. Martin, and E. Castanas. 2000. Potent inhibitory action of red wine polyphenols on human breast cancer cells. *J. Cell. Biochem.* 78(3):429-441.

Daniel, O., M.S. Meier, J. Schlatter, and P. Frischknecht. 1999. Selected phenolic compounds in cultivated plants: Ecologic functions, health implications and modulation by pesticides. *Environ. Health Perspect.* 107, (Suppl. 1): February 1999. Internet address: <http://ehpnet1.niehs.nih.gov/members/1999/Suppl-1/109-114daniel/Daniel-full.html>. Last accessed on November 22, 2000.

Deffieux, G., J.M. Merillion, and J. Rosenbaum. 2000. Wine polyphenols and cancer. *Vins et Sante* Web site. Internet address: <http://www.vinsetsante.com/a9e.html>. Last accessed on December 3, 2001.

De Santi, C., A. Pietrabissa, F. Mosca, and G.M. Pacifici. 2000a. Glucuronidation of resveratrol, a natural product, present in grape and wine, in the human liver. *Xenobiotica* 30(11):1047-1054.

De Santi, C., A. Pietrabissa, R. Spisni, F. Mosca, and G.M. Pacifici. 2000b. Sulphation of resveratrol, a natural compound present in wine, and its inhibition by natural flavonoids. *Xenobiotica* 30(9):857-866.

De Santi, C., A. Peitrabissa, R. Spisni, F. Mosca, and G.M. Pacifici. 2000c. Sulphation of resveratrol, a natural product present in grapes and wine, in the human liver and duodenum. *Xenobiotica*. 30(6):609-617.

Delaporte, E., and G.R. Wilkinson. 1998 abstr. Modulation of cytochrome P450 enzyme activity by resveratrol. *Clin. Pharmacol. Ther.* 63(2):149. Abstract No. PI-48.

Dubash, B.D., B.L. Zheng, C.H. Kim, K. He, Y. Shao, Q.Y. Zheng, C.-T. Ho, and M.-T. Huang. 1999. Inhibitory effect of resveratrol and related compounds on the macromolecular synthesis in HL-60 cells and the metabolism of 7,12-dimethylbenz[a]anthracene by mouse liver microsomes. In: Shahidi, F., and C.-T. Ho, Eds. *Phytochemicals and Phytopharmaceuticals*. AOCS Press, Champaign, IL, pp. 314-320.

Enrich Corporation. 2000a. LifePath 50: Super Antioxidant—with Resveratrol. Internet address: [http://www.enrich.com/us/prod\\_cat\\_eng/prod\\_11310.htm](http://www.enrich.com/us/prod_cat_eng/prod_11310.htm). Last accessed on April 9, 2001.

Enrich Corporation. 2000b. LifePath 50: Super Antioxidant – with Resveratrol. Internet address: [http://www.enrich.com/us/prod\\_cat\\_eng/prod\\_13620.htm](http://www.enrich.com/us/prod_cat_eng/prod_13620.htm). Last accessed on April 9, 2001.

FDA (U.S. Food and Drug Administration). 2001. Center for Food Safety and Applied Nutrition, Office of Nutritional Products, Labeling, and Dietary Supplements. *New Dietary Ingredients in Dietary Supplements*. Internet address: <http://vm.cfsan.fda.gov/~ms/ds-ingrd.html>. Last accessed on January 7, 2002.

Fang H, Tong W, Shi LM, Blair R, Perkins R, Branham W, Hass BS, Xie Q, Dial SL, Moland CL, Sheehan DM. 2001. Structure-activity relationships for a large diverse set of natural,

synthetic, and environmental estrogens. *Chem. Res. Toxicol.* 14(3):280-94.

Food and Beverage America. 2000. Canadian Health Food Manufacturers. Internet address: [http://www.foodandbeverageamerica.com/Canadian\\_health\\_food%20cos.htm](http://www.foodandbeverageamerica.com/Canadian_health_food%20cos.htm). Last accessed July 18, 2001.

Foret, J. 2000. Letter dated September 8, 2000, from FDA Division of Compliance and Enforcement, Office of Nutritional Products, Labeling and Dietary Supplements, Center for Food Safety and Applied Nutrition, to Nancy C. Graves, Quality Manager, Natural Balance, Inc.  
Frankel, E. N., A. L. Waterhouse, and J. E. Kinsella. 1993. Inhibition of human LDL oxidation by resveratrol. *Lancet* 341:1103-1104. Cited by Soleas et al. (1997).

Frémont, L. 2000. Biological effects of resveratrol. *Life Sci.* 66(8):663-673.

Freyburger A., E. Hartmann, H. Hildebrand, and F. Krötlinger. 2000 abstr. Organ toxicity and mechanisms: Differential response of immature rat uterine tissue to ethinylestradiol and the red wine constituent resveratrol. Presented in part at the 41<sup>st</sup> Spring Meeting of the German Society for Experimental and clinical Pharmacology and Toxicology in 2000. Abstract available at Internet address: <http://link.springer-ny.com/link/service/journals/00204/contents/0...-/s002040000186ch002.htm>. Last accessed on July 17, 2001.

Fukuhara, K., and N. Miyata. 1998. Resveratrol as a new type of DNA-cleaving agent. *Bioorg. Med. Chem. Lett.* 8(22):3187-3192.

Gehm, B.D., J.M. McAndrews, P.-Y. Chien, and J.L. Jameson. 1997. Resveratrol, a polyphenolic compound found in grapes and wine, is an agonist for the estrogen receptor. *Proc. Natl. Acad. Sci.* 94(25):14138-14143.

Goldberg, D.M. 1995. Does wine work? *Clin. Chem.* 41:14-16. Cited by Goldberg (1997) and by Daniel et al. (1999).

Goldberg, D.M., G.J. Soleas, S.E. Hahn, E.P. Diamandis, and A. Karumanchiri. 1997. Identification and assay of trihydroxystilbenes in wine and their biological properties. In: Watkins, T. R., Ed. *Wine: Nutritional and Therapeutic Benefits*. American Chemical Society, Washington, DC. pp. 24-43.

Graves, N.C. 2000a. Letter dated August 25, 2000, to John B. Foret, Director, FDA Division of Compliance and Enforcement, Office of Nutritional Products, Labeling and Dietary Supplements, Center for Food Safety and Applied Nutrition, FDA.

Graves, N.C. 2000b. Letter dated Aug 31, 2000, to John B. Foret, director, Division of Compliance and Enforcement, Office of Nutritional Products, Labeling and Dietary Supplements, Center for Food Safety and Applied Nutrition, FDA.

Gu, X., L.L. Creasy, A. Kester, M.G. Zeece. 1999. [Title not provided.] *J. Agric. Food Chem.* 47:3223-3227. Cited by Lin and Chen (2001).

Gu, X.L., Q.Y. Chub, M. O'Dwyer, and M. Zeece. 2000. Analysis of resveratrol in wine by capillary electrophoresis. *J. Chromatogr. A* 881(1-2):421-481. Abstract from Life Sciences.

Gusman, J., H. Malonne, and G. Atassi. 2001. A reappraisal of the potential chemopreventive and chemotherapeutic properties of resveratrol. *Carcinogenesis* 22(8):1111-1117.

Hain, R., B. Bieseler, H. Kindl, G. Schroder, and R. Stocker. 1990. Expression of a stilbene synthase gene in *Nicotiana tabacum* results in synthesis of the phytoalexin resveratrol. *Plant Molec. Biol.* 15(2):325-335. Abstract from Food Science and Technology Abstracts 91-1-05-b0019-FSTA.

Hain, R., H. Reif, and K. Stenzel. 1996. Stilbene synthase genes from grapevine. U.S. Patent 05500367. Leverkusen, DEX. Bayer Aktiengesellschaft. Abstract, U.S. Patent Bibliography Database.

Hain, R., H.-J. Reif, and K. Stenzel. 1997. Stilbene synthase genes from grapevine. U.S. Patent 05500367. Leverkusen, DE. Bayer Aktiengesellschaft. March 19, 1996. Abstract from U.S. Patent Bibliographic Database.

Hain, R., H. Reif, and K. Stenzel. 2000. Stilbene synthase gene of grapevine. Patent 00464461. Leverkusen, DEX. Bayer Aktiengesellschaft. Abstract from European Patent Granted.

Healthy Living Int.com. 2000. Where can I find the best antioxidant vitamin supplement? Internet address: <http://www.healthylivingintl.com/antioxidant>. Last accessed on April 9, 2001.

Hecht, S.M. 1989. [Title not provided.] *Pure Appl. Chem.* 61:577 ff. Cited by Fukuhara and Miyata (1998).

Howard, D. 2000 abstr. The biological effects of resveratrol, a red wine constituent. *Nutrition Bytes*. Abstract available at Internet address: <http://www.medsch.ucla.edu/som/ddo/biolchem/nut-1998/Bits9798/b00howard.htm>. Last accessed on July 20, 2001.

Igura, K., T. Ohta, Y. Kuroda, and K. Kaji. 2001. Resveratrol and quercetin inhibit angiogenesis in vitro. *Cancer Lett.* 171(1):11-16.

IHerb.com. Undated. Resveratrol Synergy, Jarrow, 60 Tabs. Internet address: <http://www.iherb.com-/resveratrol.html>. Last accessed on April 9, 2001.

IHV (Institute of Human Virology). 2000. IHV Snapshot 2000 (October issue). IHV, Center of the University of Maryland Biotechnology Institute (UMBI), Baltimore, MD. Internet address: <http://www.ihv.org/ihv%20pdfs/oct2000.pdf>. Last date accessed not available.

IHV. 2001a. Centerip: Institute of Human Virology. Internet address: <http://www.umbi.umd.edu/budget/centeripihv.html>. Last accessed on July 17, 2001.

IHV. 2001b. [Profile of the organization.] RB.35, University of Maryland Biotechnology Institute, University System of Maryland, Operating Budget Analysis, Program Description, the University of Maryland Biotechnology Institute. Internet address: [http://mlis.state.md.us/2001RS/budget\\_docs/All/Operating/R00B35\\_UM\\_Biotechnology\\_Institute.pdf](http://mlis.state.md.us/2001RS/budget_docs/All/Operating/R00B35_UM_Biotechnology_Institute.pdf). Not accessible as of January 14, 2002.

Inno-Vite. Undated. Inno-Vite – Protykin™ (IH727) *trans*-Resveratrol 60 Caps. Internet address: <http://www.inno-vite.com/sp0105.html>. Last accessed on July 18, 2001.

InterHealth. 1998. Protykin™ Dosage Recommendations. No longer available on the Web as of September 18, 2001.

InterHealth. Undated-a. Product labeling guidelines. [Availability not indicated.]

InterHealth. Undated-b. Protykin™ (IH727) product highlights. Internet address: <http://207.181.234.98/cfdocs/Interhealth/assets/pdf/ACF14C.pdf>. Last accessed on September 18, 2001.

InterHealth. Undated-c. Protykin™ (IH727) product overview. Internet address: <http://207.181.234.98/cfdocs/Interhealth/assets/pdf/HIGHLITS.pdf>. Last accessed on September 18, 2001.

InterHealth. Undated-d. Protykin™ (IH727) Product Specifications. Internet address: <http://207.181.234.98/cfdocs/Interhealth/assets/pdf/ProductLabelingGuidelines.pdf>. Last accessed on September 18, 2001.

Jarrow Formulas. 2001. Resveratrol Synergy. Internet address: <http://www.jarrow.com/products/Resveratrol.htm>. Last accessed on April 9, 2001.

Jeandet, P., M. Sbaghi, and P. Meunier. 1995a. The potential relationship of stilbene (resveratrol) synthesis to anthocyanin content in grape berry skins. *Vitis* 34(2):91-94. Abstract from CAB International 95-08 950312860.

Jeandet, P., R. Bessis, M. Sbaghi, P. Meunier. 1995b. Production of the phytoalexin resveratrol by grapes as a response to *Botrytis* attack under natural conditions. *J. Phytopathol.* 143(3):135-139. Abstract from CAB International 95-07 952308292.

Jeandet, P., M. Adrian, A.C. Breuil, M. Sbaghi, J.M. Joubert, L.A. Weston, R. Harmon, and R. Bessis. 1998. Chemical stimulation of phytoalexin synthesis in plants as an approach to crop protection. *Recent Res. Develop. Agr. Food Chem.* 2(2):501-511. Abstract from CAB International, 99-09 991006083.

Jeandet, P., R. Bessis, M. Adrian, J. Yvin, and J. Joubert. 2000. Use of aluminum chloride as a resveratrol synthesis elicitor. U.S. Patent 06080701. Saint Malo Cedex, FRX. Laboratoires Goemar S.A. Abstract U.S. Patent Bibliography Database. Last accessed on December 5, 2000.



Kampa, M., A. Hatzoglou, G. Notas, A. Damianaki, E. Bakogeorgou, C. Gemetzi, E. Kouroumalis, P.-M. Martin, and E. Castanas. 2000. Wine antioxidant polyphenols inhibit proliferation of human prostate cancer cell lines. *Nutr. Cancer* 37(2):223-233.

Korytko, P.J., R.L. Morrissey, V. Hebbar, G. Shen, T. Kong, J.A. Crowell, and B.S. Levine. 2002. Four week oral toxicity study of the cancer chemopreventive agent resveratrol in rats. Supported by NCI Contract No. NO1-CN-95132. Abstract No. 1580. To be presented at the Society of Toxicology (SOT) 2002 41<sup>st</sup> Annual Meeting, March 17-21, 2002, Nashville, TN. Abstract available at Internet address: [http://sot.abstractcentral.com/itin/main.html?new\\_page\\_id=76&abstract\\_id=51959&is\\_tech=](http://sot.abstractcentral.com/itin/main.html?new_page_id=76&abstract_id=51959&is_tech=). Last accessed on February 11, 2002.

Kuhnle, G., J.P.E. Spencer, G. Chowrimootoo, H. Schroeter, E.S. Debnam, S.K.S. Srail, C. Rice-Evans, and U. Hahn. 2000. Resveratrol is absorbed in the small intestine as resveratrol glucuronide. *Biochem. Biophys. Res. Commun.* 272(1):212-217.

Lamikanra, O., C.C. Grimm, J.B. Rodin, and I.D. Inyang. 1996. [Title not provided.] *J. Agric. Food Chem.* 44:1111-1115. Cited by Frémont (2000).

Lamuela-Raventós, R.M., and A.L. Waterhouse. 1993. [Title not provided.] *J. Agric. Food Chem.* 41:521-523. Cited by Frémont (2000).

Lamuela-Raventós, R.M., A.I. Romero-Perez, and M.C. de la Torre-Boronat. 1997. Resveratrol and piceid levels in wine production and in finished wines. In: Watkins, T. R., Ed. *Wine: Nutritional and Therapeutic Benefits*. American Chemical Society, Washington, DC. pp. 56-68.

Langcake, P., and R.J. Pryce. 1976. The production of resveratrol by *Vitis vinifera* and other members of the vitaceae as a response to infection or injury. *Physiol. Plant Pathol.* 9:77-86. Cited by Daniel et al. (1999) and Frémont (2000).

LaSasso, K. 2000. Letter dated September 8, 2000, from Solgar Vitamin & Herb to FDA Division of Compliance and Enforcement, Office of Nutritional Products, Labeling and Dietary Supplements, Center for Food Safety and Applied Nutrition about establishing "resveratrol" as a dietary ingredient. Internet address: [http://www.fda.gov/ohrms/dockets/dockets/95s0316/rpt0085\\_01.pdf](http://www.fda.gov/ohrms/dockets/dockets/95s0316/rpt0085_01.pdf). Last accessed on January 6, 2002.

LaSasso, K. 2001. Letter dated April 5, 2001, from Solgar Vitamin & Herb to FDA Office of Special Nutritionals (HFS-450) Center for Food Safety and Applied Nutrition, stating that the company will not sell a product with the ingredient resveratrol. Internet address: <http://www.fda.gov/ohrms/dockets/dockets/95s01316/let0003.pdf>. Last accessed on January 7, 2002.

Life Extension Foundation. 2000. Proanthocyanidins. Internet address: [http://www.lef.org/prod\\_desc/item00222.html](http://www.lef.org/prod_desc/item00222.html). Last accessed on April 9, 2001.

Lin, J.-K., and S.-H Tsai. 1999. Chemoprevention of cancer and cardiovascular disease by resveratrol. *Proc. Natl. Sci. Counc. ROC(B)* 23(3):99-106.

Lin, C., and Y. Chen. 2001. On-line identification of *trans*- and *cis*-resveratrol by nonaqueous capillary electrophoresis/fluorescence spectroscopy at 77K. *Electrophoresis* 22:2574-2579.

LKT Laboratories, Inc. Undated. LKT Laboratories, Inc.: Your Specialty Chemical Resource. Internet address: <http://www.lktlabs.com/About%20LKT/description.html>. Last accessed on January 2, 2002.

Lytollis, W., R.T. Scannell, H. An, V.S. Murty, K.S. Reddy, J.R. Barr, and S.M. Hecht. 1995. [Title not provided.] *J. Am. Chem. Soc.* 117:12683 ff. Cited by Fukuhara and Miyata (1998).

Matsuoka, A., A. Furuta, O. Masayasu, K. Fukuhara, and N. Miyata. 2001. Resveratrol, a naturally occurring polyphenol, induces sister chromatid exchanges in a Chinese hamster lung (CHL) cell line. *Mutat. Res.* 494(1-2):107-113.

Mattivi, F., F. Reniero, and S. Korhammer. 1995. [Title not provided.] *J. Food Chem.* 43:1820-1823. Cited by Frémont (2000).

McElderry, M.Q.B. 1997. Resveratrol hype. *Nutrition Forum* 1997. Internet address: [http://www.findarticles.com/cf\\_0/m0GCU/5\\_16/56881798/print.jhtml](http://www.findarticles.com/cf_0/m0GCU/5_16/56881798/print.jhtml). Last accessed on April 9, 2001.

McMurtrey, K.D. 1997. Resveratrol in wine. In: Watkins, T. R., Ed. *Wine: Nutritional and Therapeutic Benefits*. American Chemical Society, Washington, DC, pp. 44-55.

McMurtrey, K.D., J. Minn, K. Pobantz, and T.P. Schultz. 1994. Analysis of wines for resveratrol using direct injection high-pressure liquid chromatography with electrochemical detection. *J. Agric. Food Chem.* 42:2077-2080. Cited by Frémont (2000) and McMurtrey (1997).

Micromedex Thomson Health Care. 2000. Herbs, vitamins & minerals: Resveratrol. Thriveonline. Internet address: <http://thriveonline.oxygen.com/medical/library/herbs/-ame0340.html>. Last accessed on April 9, 2001.

Mineral Connection. 2001. Olive Leaf Extract (*Olea europa*). Internet address: <http://www.mineralconnection.com/oliveleaf.htm>. Last accessed April 9, 2001. Last updated March 12, 2001.

Mitchell, S.H., W. Zhu, and C.Y.F. Young. 1999. Resveratrol inhibits the expression and function of the androgen receptor in LNCaP prostate cancer cells. *Cancer Res.* 59(23):5892-5895.

Miura, T., S. Muraoka, N. Ikeda, M. Watanabe, and Y. Fujimoto. 2000. Antioxidative and prooxidative action of stilbene derivatives. *Pharmacol. Toxicol.* 86(5):203-208.

Moravek Biochemicals. 2001. Resveratrol. Internet address: <http://www.moravek.com/catalogfiles/resveratrol.htm>. Last accessed on April 9, 2001.

Nakagawa, H., Y. Kiyozuka, Y. Uemura, H. Senzaki, N. Shikata, K. Hioki, and A. Tsubura. 2001. Resveratrol inhibits human breast cancer cell growth and may mitigate the effect of linoleic acid, a potent breast cancer cell stimulator. *J. Cancer Res. Clin. Oncol.* 127(4):258-264.

Natural Ways to Health. Undated. ORAC+ for Anti-Aging. Internet address: <http://www.naturalways.com/orac.htm>. Last accessed on April 9, 2001.

NTP (National Toxicology Program). 1983. Reproductive and fertility assessment of trans-diethylstilbestrol (DES) (CAS No. 56-53-1) in CD-1 mice when administered in the feed. NTP Report No. RACB83094. NTIS No. PB84136746. Abstract available at Internet address: <http://ntp-server.niehs.nih.gov/htdocs/RT-studies/RACB83094.html>. Last accessed on February 12, 2002.

NTP. 1984. Diethylstilbestrol (CAS No. 56-53-1): Fertility assessment in CD-1 mice when administered in feed. NTP Report No. RACB83046. NTIS No. PB85167674/AS. Abstract available at Internet address: <http://ntp-server.niehs.nih.gov/htdocs/RT-studies/RACB83046.html>. Last accessed on February 12, 2002.

NTP. 1992a. Toxicology and carcinogenesis studies of quercetin (CAS No. 117-39-5) in F344 rats (feed studies). Technical Report No. 409. NTIS No. PB93-147478. Available at Internet address: <http://ntp-server.niehs.nih.gov/htdocs/LT-studies/tr409.html>. Last accessed on February 12, 2002.

NTP. 1992b. Toxicology and carcinogenesis studies of resorcinol (CAS No. 108-46-3) in F344 rats and B6C3F<sub>1</sub> mice (gavage studies). Technical Report No. 403. NTIS No. PB93-126381. Available at Internet address: <http://ntp-server.niehs.nih.gov/htdocs/LT-studies/tr403.html>. Last accessed on February 12, 2002.

NTP. 1994. Developmental toxicity of diethylstilbestrol (CAS No. 56-53-1) in Swiss (CD-1<sup>®</sup>) mice. NTP Study No. TER93137. NTIS No. PB95-130175. Abstract available at Internet address: <http://ntp-server.niehs.nih.gov/htdocs/TT-studies/TER93137.html>. Last accessed on February 12, 2001.

NTP. 2002a. Testing status: Quercetin dihydrate. Internet address: [http://ntp-server.niehs.nih.gov/htdocs/Results\\_Status/Resstatq/M940124.html](http://ntp-server.niehs.nih.gov/htdocs/Results_Status/Resstatq/M940124.html). Last updated on January 15, 2002. Last accessed on February 12, 2002.

NTP. 2002b. Testing status: Resorcinol. Internet address: [http://ntp-server.niehs.nih.gov/htdocs/Results\\_Status/Resstatr/10163-Y.html](http://ntp-server.niehs.nih.gov/htdocs/Results_Status/Resstatr/10163-Y.html). Last updated on January 15, 2002. Last accessed on February 12, 2002.

NTP. 2002c. Testing status: Transgenic LEP (resorcinol). Internet address: [http://ntp-server.niehs.nih.gov/htdocs/Results\\_Status/Resstatt/M970008.html](http://ntp-server.niehs.nih.gov/htdocs/Results_Status/Resstatt/M970008.html). Last updated on January 15, 2002. Last accessed on February 12, 2002.

NTP. 2002d. Testing status: Transgenic model evaluation (DES). Internet address: [http://ntp-server.niehs.nih.gov/htdocs/Results\\_Status/Resstatt/M950080.html](http://ntp-server.niehs.nih.gov/htdocs/Results_Status/Resstatt/M950080.html). Last updated on January 15, 2002. Last accessed on February 12, 2002.

NTP. 2002e. Testing status: Transgenic model evaluation (resorcinol). Internet address: [http://ntp-server.niehs.nih.gov/htdocs/Results\\_Status/Resstatt/M950084.html](http://ntp-server.niehs.nih.gov/htdocs/Results_Status/Resstatt/M950084.html). Last updated on January 15, 2002. Last accessed on February 12, 2002.

Nutrition for a Living Planet. Undated. Resveratrol. Internet address: <http://www.geocities.com/nutriflip/supplements/Resveratrol.html>. Last accessed on April 9, 2001.

Organix-South, Inc. Undated. Biotech Corporation: Product #BICC Cardio CholestaMax. Internet address: <http://organix.net/organix/shenc.htm>. Last accessed on April 9, 2001.

Orsini, F., F. Pelizzoni, L. Verotta, T. Aburjai, and C.B. Rogers. 1997. Isolation, synthesis, and antiplatelet aggregation activity of resveratrol 3-*O*-beta-*D*-glucopyranoside and related compounds. *J. Nat. Prod.* 60 (11):1082-1087. Abstract from CAB International 98-01 980300487.

Osterwalder, N. 1999. Pharmaceutical composition containing polyphenols from grapes, in particular resveratrol, and yeast extracts. Laboratorio Italiano Biochimico Farmaceutico Lispharma, Erba, Italy. Cited by PCT Database. 01-14-99 09901148.

Otake, Y., A.L. Nolan, U.K. Walle, and T. Walle. 2000. Quercetin and resveratrol potently reduce estrogen sulfotransferase activity in normal human mammary epithelial cells. *J. Steroid Biochem. Molec. Biol.* 73(5):265-270.

Paiva, N.L. 1999 abstr. Engineering resveratrol accumulation into alfalfa and other food plants. In: Abstract Book: International Molecular Farming Conference, London, Ontario, Canada, August 29 to September 1, 1999.

PCT Gazette. 2001. Published International Applications: Resveratrol. p. 7299 of May 3, 2001.

Pharmascience. Undated. Resverin<sup>®</sup>. Internet address: [http://www.pharmascience.com/pms\\_en/health/3\\_1\\_6.ASP](http://www.pharmascience.com/pms_en/health/3_1_6.ASP). Last accessed on July 18, 2001.

Rice-Evans, C.A., J. Miller, and G. Pagana. 1997. [Title not provided.] *Trends Plant Sci.* 2:152-159. Cited by Frémont (2000).

Richards, R.L. 1999. Notification Pursuant to Section 6 of DSHEA and 21 CFR101.93. Filed on behalf of Kaire Nutraceuticals, Inc. about the product Ginkgo Shield. Received by FD on August 10, 1999. Internet address: <http://www.fda.gov/ohms/dockets/dailys/113099/let-4211.pdf>. Last accessed April 9, 2001.

Roggero, J.P. 1996. Changes in resveratrol and piceid contents in wines during fermentation or ageing: Comparison of *Grenache* and *Mourvedre* varieties. *Sci. Alim.* 16(6):631-642. Abstract from Food Science and Technology Abstracts 97-05 97-1-05-h0061-FSTA.

Roggero, J.P., and P. Archier. 1994. [Title not provided.] *Sci. Alim.* 14:99-107. Cited by Frémont (2000).

Roggero, J.P., and C. Garciaparilla. 1995. Effects of ultraviolet irradiation on resveratrol and changes in resveratrol and various of its derivatives in the skins of ripening grapes. *Sci. Alim.* 15:411-422. Cited by Daniel et al. (1999).

Romero-Perez, A.I., R.M. Lamuela-Raventós, A.L. Waterhouse, and M.C. De La Torre-Boronat. 1996. Levels of *cis*- and *trans*-resveratrol and their glucosides in white and rose *Vitis vinifera* wines from Spain. *J. Agric. Food Chem.* 44:2124-2128. Cited by Daniel et al. (1999) and Frémont (2000).

Samlong Chemical Co., Ltd. Undated. New offerings. Internet address: <http://www.samlong-chemical.com>. Last accessed on April 9, 2001.

Satchell, F.B. 2000. Letter dated November 27, 2000, to K. LaSasso of Solgar Vitamin and Herb from FDA Division of Compliance and Enforcement, Office of Nutritional Products, Labeling and Dietary Supplements, Center for Food Safety and Applied Nutrition questioning evidence that resveratrol product is safe.

Sawada, M., and M. Ishidate, Jr. 1978. Colchicine-like effect of diethylstilbestrol (DES) on mammalian cells *in vitro*. *Mutat. Res.* 57:175-182. Cited by Matsuoka et al. (2001).

Sbaghi, M. 1994. Physiological and biochemical aspects of the interaction between grapevines and *Botrytis cinerea*: Synthesis and degradation of resveratrol. Thesis, Univ. de Bourgogne. (French). Abstract from Wine Database 95-01 95-1-01-d0007-VITI.

Scalbert, A., and G. Williamson. 2000. Dietary intake and bioavailability of polyphenols. *J. Nutr.* 130(Suppl. 8):2073S-2085S.

Scannell, R.T., J.R. Barr, V.S. Murty, K.S. Reddy, and S.M. Hecht. 1988. [Title not provided.] *J. Am. Chem. Soc.* 110:3650 ff. Cited by Fukuhara and Miyata (1998).

Schroder, G., J. Schroder, R. Hain, and P. H. Schreider. 1999. Stilbene synthase gene. U.S. Patent 05985647. Bayer Aktiengesellschaft, Leverkusen, Germany. Abstract from U.S. Patent Bibliography Database.

Siemann, E.H., and L.L. Creasy. 1992. Concentration of the phytoalexin resveratrol in wine. *Am. J. Enol. Vitic.* 43(1):49-52. Cited by Frémont (2000) and Deffieux et al. (2000).

Sobolev, V.S., and R.J. Cole. 1998 abstr. Resveratrol content in commercial peanuts and peanut products. August 21, 1998. Internet address: <http://www.nal.usda.gov/ttic/tektran-/data/000009/51/0000095181.html>. Last accessed on April 9, 2001.

Sofuni, T., Ed. 1998. *Data Book of Chromosomal Aberration Test in Vitro*. Revised ed. LIC, Tokyo, Japan (1999), p. 184. Cited by Matsuoka et al. (2001).

Soleas, G.J., E.P. Diamandis, and D.M. Goldberg. 1997. Resveratrol: A molecule whose time has come? And gone? *Clin. Biochem.* 30:91-113.

Soleas, G.J., J. Yan, and D.M. Goldberg. 2001. Measurement of *trans*-resveratrol, (+)-catechin, and quercetin in rat and human blood and urine by gas chromatography with mass selective detection. *Methods Enzymol.* 335:130-145.

Stark-Lorenzen, P., B. Nelke, G. Hanssler, H.P. Muhlback, and J.E. Thomzik. 1997. Transfer of a grapevine stilbene synthase gene to rice (*Oryza sativa* L). *Plant Cell Rep.* 16:668-673. Cited by Daniel et al. (1999).

Stockley, C.S. 1996. Medically, is wine just another alcoholic beverage? Presented at Australian Society of Wine Education (ASWE) National Convention, Rydges Canberra Hotel, Canberra, Australia. October 5, 1996. Internet address: [http://www.aswe.org.au/6<sup>th</sup>\\_Cvn\\_Stockley.htm](http://www.aswe.org.au/6<sup>th</sup>_Cvn_Stockley.htm). Last accessed on July 17, 2001.

TCI America. 1999. Resveratrol. Internet address: [http://www.tciamerica.com/cgi-bin/tci-catalog.cgi?catalog.cgi?catalog\\_no=R0071](http://www.tciamerica.com/cgi-bin/tci-catalog.cgi?catalog.cgi?catalog_no=R0071). Last accessed on April 9, 2001.

Tessitore, L., A. Davit, I. Sarotto, and G. Caderni. 2000. Resveratrol depresses the growth of colorectal aberrant crypt foci by affecting *bax* and *p21<sup>CIP</sup>* expression. *Carcinogenesis* 21(8):1619-1622.

Thomzik, J.E., K. Stenzel, R. Stocker, P.H. Schreider, R. Hain, and D.J. Stahl. 1997. Synthesis of a grapevine phytoalexin in transgenic tomatoes (*Lycopersicon esculentum* Mill). *Physiol. Molec. Plant Pathol.* 51(4): 265-278. Abstract from CAB International, No. 98-04 981003742.

Turner, L. 1999. Supplement spotlight: Resveratrol. *Vitamin Retailer™: The Dietary Supplement Industry's Leading Magazine*. Internet address: <http://www.vitaminretailer.com/vr/archive/99/may/supsptresveratrol/htm>. Last updated on May 9, 1999. Last accessed on April 9, 2001.

Turner, R.T., G.L. Evans, M. Zhang, A. Maran, and J.D. Sibonga. 1999. Is resveratrol an estrogen agonist in growing rats? *Endocrinology* 140(1):50-54. Poster available from McPharmNet at Internet address: [http://www.fhs.mcmaster.ca/mcpharm/ejc/99may\\_pa.htm](http://www.fhs.mcmaster.ca/mcpharm/ejc/99may_pa.htm). Last accessed on July 17, 2001.

Vastano, B.C., C. Yong, Z. NanQu, H. ChiTang, Z. ZhengYi, and R.T. Rosen. 2000. Isolation and identification of stilbenes in two varieties of *Polygonum cuspidatum*. *J. Agric. Food Chem.* 48(2):253-256. Abstract from CAB International 00-06 20000309978.

Vrhovsek, U., R. Eder, and S. Wendelin. 1995. The occurrence of *trans*-resveratrol in Slovenian red and white wines. *Acta Aliment.* 24:203-212. Cited by Daniel et al. (1999).

Walle, T., A.A. Wilson, and U.K. Walle. 1998 abstr. Bioavailability of the chemopreventive agent resveratrol compared to quercetin using human cell models. *Clin. Pharmacol. Ther.* 63(2): 149. Abstract No. PI-49.

Wine Institute. 2000. Key facts: per capita wine consumption in selected countries August 2000. Internet address: [http://www.wineinstitute.org/communications/statistics-/keyfacts\\_worldpercapitaconsumption2kliter.htm](http://www.wineinstitute.org/communications/statistics-/keyfacts_worldpercapitaconsumption2kliter.htm). Last accessed on December 12, 2001.

Wilson, T., T.J. Knight, D.C. Beitz, D.S. Lewis, and R.L. Engen. 1996. Resveratrol promotes atherosclerosis in hypercholesterolemic rabbits. *Life Sci.* 59(1):15-21.

Yang, F., T. Zhang, and Y. Ito. 2001. Large-scale separation of resveratrol, anthraglycoside A and anthraglycoside B. from *Polygonum cuspidatum* sieb. et Zucc by high-speed counter-current chromatography. *J. Chromatogr. A* 919:443-448.

Zhu, Y., T. Huang, M. Cregor, H. Long, C.B. Kissinger, and P.T. Kissinger. 2000. Liquid chromatography with multichannel electrochemical detection for the determination of *trans*-resveratrol in rat blood utilizing an automated blood sampling device. *J. Chromatogr. B* 740(1):129-133.

### 13.0 References Considered But Not Cited

Ahmad, A., S.F. Asad, S. Singh, and S.M. Hadi. 2000. DNA breakage by resveratrol and Cu(II): Reaction mechanism and bacteriophage inactivation. *Cancer Lett.* 154:29-37.

Ahn, K.-S., J.-H. Kim, S.-R. Oh, S.-Y. Ryu, and H.-K. Lee. 2000. [Title not provided.] *Planta Med.* 7/2000. Internet address: [http://www.thieme.de/plantamedica/07\\_00-/planta641.html](http://www.thieme.de/plantamedica/07_00-/planta641.html). Last accessed on December 19, 2001.

AICR (American Institute for Cancer Research). 1999. American Institute for Cancer Research Grants – 1999 Grants. Internet address: <http://www.aicr.org/grants99.htm>. Last accessed on July 20, 2001.

Andlauer, W., J. Kolb, C. Stumpf, K. Siebert, and P. Fürst. 2000. Bioavailability of selected phytochemicals. *Clin. Nutr.* 19(Suppl. 1):21. Citation from BIOSIS 2000:446615.

Artemis. 1998. Complementary approaches: The healthful benefits of grapes. Internet address: <http://www.med.jhu.edu/breastcenter/artemis/199806/comp.html>. Last accessed on July 17, 2001.

Bavaresco, L., M. Fregoni, and D. Petegolli. 1994. Effect of nitrogen and potassium fertilizer on induced resveratrol synthesis in two grapevine genotypes. *Vitis* 33(3):175-176. Abstract from CAB International, 95-01 950300577.

Bertelli, A.A., L. Giovannini, R. Stradi, A. Bertelli, and J.P. Tillement. 1996a. Plasma, urine and tissue levels of *trans*- and *cis*-resveratrol (3,4',5-trihydroxystilbene) after short-term or prolonged administration of red wine to rats. *Int. J. Tissue Reac.* 18(2-3):67-71. Abstract available from MEDLINE 97217807. [Similar results appear in Bertelli et al. (1998a); see Section 12.0.]

Bertelli, A.A., L. Giovannini, R. Stradi, S. Urien, J.P. Tillement, and A. Bertelli. 1996b. Kinetics of *trans*- and *cis*-resveratrol (3,4',5-trihydroxystilbene) after red wine oral administration in rats. *Int. J. Clin. Pharmacol. Res.* 16(4-5):77-81. Abstract from 97316032. [Similar results appear in Bertelli et al. (1998b); see Section 12.0.]

Bertelli, A.A.E. 1998. Modulatory effect of resveratrol, a natural phytoalexin, on endothelial adhesion molecules and intracellular signal transduction. *Pharm. Biol.* 36:44-52.

Bertelli, A.A., L. Giovannini, R. Stradi, S. Urien, J.P. Tillement, and A. Bertelli. 1998. Evaluation of kinetic parameters of natural phytoalexin in resveratrol orally administered in wine to rats. *Drugs Exp. Clin. Res.* 24(1):51-55. Abstract from MEDLINE 1998:267507.

Blond, J.P., M.P. Denis, and J. Bezard. 1995. [Title not provided.] *Sci. Alim.* 15:347-358. Cited by Frémont (2000).

Bruder, J.L., H. T. Hsieh, K.M. Lerea, S.C. Olson, and J.M. Wu. 2001. Induced cytoskeletal changes in bovine pulmonary artery endothelial cells by resveratrol and the accompanying modified responses to arterial shear stress. *BMC Cell Biol.* 2(1). [Page numbers not provided.] Internet address: <http://www.biomedcentra.com/1471-2121/2/1>. Last accessed on July 20, 2001.

Chen, S., X.Z. Sun, Y.-C. Kao, A. Kwon, D. Zhou, and E. Eng. 1998. Suppression of breast cancer cell growth with grape juice. *Pharm. Biol.* 36:53-61.

Clydesdale, F.M., K.M. Kolasa, and J.P. Ikeda. 1994. All you want to know about fruit juice. *Nutr. Today* 29(2):14-18. Abstract from Food Science and Technology Abstracts.

Coblentz, B. 1997. Research reveals muscadines' potential. MAFES (Mississippi State University Office of Agricultural Communications) Research Highlights Winter 1997 60:01. Internet address: <http://www.mafes.msstate.edu/highlights/.6001.htm>. Last accessed on July 20, 2001.

Cornell University. 1998. September 2 press release. Internet address: [http://www.biospace.-com/news\\_rxtarget.cfm?rxtargetID=18&SR=16](http://www.biospace.-com/news_rxtarget.cfm?rxtargetID=18&SR=16). Last accessed on July 18, 2001.



- de Vries, J.H.M., P.C.H. Hollman, I. van Amersfoort, M.R. Olthof, and M.B. Katan. 2001. Red wine is a poor source of bioavailable flavonols in men. *J. Nutr.* 131(3):745-748.
- Decendit, A., K.G. Ramawat, P. Waffo, G. Deffieux, A. Badoc, and J.M. Merillon. 1996. Anthocyanins, catechins, condensed tannins and piceid production in *Vitis vinifera* cell bioreactor cultures. *Biotechnol. Lett.* 18(6):659-662. Abstract from U.S. Patent Bibliography Database. Last accessed on December 5, 2000.
- Folts, J. 1998. Antithrombotic potential of grape juice and red wine for preventing heart attacks. *Pharm. Biol.* 36(Suppl.):21-27.
- Frankel, E., and A. Meyer. 1998. Antioxidants in grape juices and their potential health effects. *Pharm. Biol.* 36(Suppl.):14-20.
- Frémont, L., L. Belguendouz, and S. Delpal. 1999. Antioxidant activity of resveratrol and alcohol-free wine polyphenols related to LDL oxidation and polyunsaturated fatty acids. *Life Sci.* 64(26):2511-2521.
- Freyberger, A., E. Hartmann, H. Hildebrand, and F. Krötlinger. 2001. Differential response of immature rat uterine tissue to ethinylestradiol and the red wine constituent resveratrol. *Arch. Toxicol.* 74(11):709-715.
- Hain, R., H.-J. Reif, and K. Stenzel. 1999. Stilbene synthase genes from grapevine. U.S. Patent 05689047. Leverkusen, DE. Bayer AG. November 16, 1999. Abstract from U.S. Patent Bibliographic Database.
- Han Y.N., S.Y. Ryu, and B.H. Han. 1990. Antioxidant activity of resveratrol closely correlates with its monoamine oxidase-A inhibitory activity. *Arch. Pharm. Res.* 13:132-135. Cited by Soleas et al. (1997).
- Heasman, M., and J. Mellentin. 1998. Nuts about nutraceuticals. *World of Ingredients*, October 1998, pp. 48, 50. Abstract from Food Science and Technology Abstracts.
- Holmgren, E. 1997. There's more to wine than good taste and antioxidants. *Wine Trader Magazine*. Internet address: <http://www.wines.com/winetrader/r3/r3hs.html>. Last updated on October 31, 1997. Last accessed on August 3, 2001.
- Holmgren, E. 2000. Health issues (wine consumption). *Wines & Vines*, June. Internet address: [http://www.findarticles.com/cf\\_0/m3488/6\\_81/64720708/print.jhtml](http://www.findarticles.com/cf_0/m3488/6_81/64720708/print.jhtml). Last accessed on July 17, 2001.
- Hsieh, T.-C., and J.M. Wu. 2000. Grape-derived chemopreventive agent resveratrol decreases prostate-specific antigen (PSA) expression in LNCaP cells by an androgen receptor (AR)-independent mechanism. *Anticancer Res.* 20(1A):225-228.
- Hung, L., J. Chen, S. Huang, R. Lee, and M. Su. 2000. Cardioprotective effect of resveratrol, a natural antioxidant derived from grapes. *Cardiovasc. Res.* 47:549-555.

Hursting, S. Undated. Division of Cancer Prevention, Office of Preventive Oncology. Internet address: <http://dcp.nci.nih.gov/pob/fellowship/preceptor.pdf>. Last date accessed not available.

Hursting, S. 1996. Chemoprevention modulation of p-cresidine-induced DNA adducts and bladder tumorigenesis in p53 –deficient mice. Pilot Projects, University of Texas, Smithville. Internet address: <http://www.niehs.nih.gov/centers/pilot/uts-pp.htm>. Last accessed on July 18, 2001.

Hursting, S. 2001. Tobacco smoke and bladder cancer. Mechanisms of Disease Prevention Research Core: University of Texas, Smithville. Internet address: <http://www.niehs.nih.gov/centers/res-core/uts-res5.htm>. Last updated on June 11, 2001. Last accessed on July 18, 2001.

InterHealth. 1998. Fight the good fight: Introducing Protykin™, the powerful new ingredient from InterHealth. Internet address: <http://207.181.234.98/cfdocs/Inter-health/assets/pdf/over-Add.pdf>. Last accessed on September 18, 2001.

InterHealth. 1998. Introducing Protykin™ the most potent all-natural resveratrol ingredient available. February 6, 1998, press release. Internet address: <http://207.181.234.98/cfdocs/Inter-health/assets/pdf/PK-Intro.pdf>. Last accessed on September 18, 2001.

Jang, M., L. Cai, G.O. Udeani, K.V. Slowing, C.F. Thomas, C.W. Beechers, H.H.S. Fong, N.R. Farnsworth, A.D Kinghorn, R.G. Mehta, R.C. Moon, and J.M. Pezzuto. 1997. Cancer chemopreventive activity of resveratrol, a natural product derived from grapes. *Science* 275:218-220.

Jang, M., and J.M. Pezzuto. 1998. Resveratrol blocks eicosanoid production and chemically-induced cellular transformation: Implications for cancer prevention. *Pharm. Biol.* 35 (Suppl.):28-34. Abstract from CAB International 99-02 991401947.

Jeandet, P., R. Bessis and B. Gautheron. 1991. The production of resveratrol, 3,5,4'-trihydroxystilbene, by grape berries in different developmental stages. *Am. J. Enol. Vitic.* 41-46. Abstract from Wine Database 91-04 91-1-04-h-0016-VITI.

Jeandet, P., M. Sbaghi, and R. Bessis. 1992. The production of resveratrol, 3,5,4'-trihydroxystilbene, by grapevine *in vitro* cultures, and its application to screening for grey mould resistance. *J. Wine Res.* 3 pp. 47-57. Abstract from Wine Database 92-04 92-1-04-c0013-VITI.

Johnson, C. 2002. Dexter Sport Science: Nutritional Supplements for the Active Lifestyle. Internet address: <http://www.dsportscience.com/powerstar.php>. Last updated January 2, 2002. Last accessed January 2, 2002.

Kane, J.R. 1999. The next wave of phytochemical antioxidants. *Chemical Market Reporter*. July 19, 1999. Internet address: [http://www.findarticles.com/cf\\_0/m0FVP/3\\_256/55220940/-print.jhtml](http://www.findarticles.com/cf_0/m0FVP/3_256/55220940/-print.jhtml). Last accessed on July 17, 2001.

Lifestar Millennium, Inc. 2001. A live cell-grown vitamin C, grapeseed complex food supplement for women. Internet address: <http://www.lifestar.com/Pages/LFCforWomen3.html>. Last accessed on April 9, 2001.

Loman, S. 1999. Resveratrol: Nieuw plantaardig extract bidet nieuwe therapeutische mogelijkheden. (Dutch.) De Orthomoleculaire Koerier Nr. 75. Internet address: <http://www.orthos.nl/media/perio/DOL75art.htm>. Last accessed on July 17, 2001.

Martinez, J., and J.J. Moreno 2000. Effect of resveratrol, a natural polyphenolic compound, on reactive oxygen species and prostaglandin production. *Biochem. Pharmacol.* 59(7):865-870. Abstract from CAB International 00-06 20001412427.

Martínez-Ortega, M.V., M.C. García-Parrilla, and A.M. Troncoso. 2001. Changes in phenolic composition of wines submitted to *in vitro* dissolution tests. *Food Chem.* 73(1):11-16.

Mizutani, K., K. Ikeda, Y. Kawai, and Y. Yamori. 2000. Resveratrol attenuates ovariectomy-induced hypertension and bone loss in stroke-prone spontaneously hypertensive rats. *Nutr. Sci. Vitaminol.* 46:78-83.

Mizutani, K., K. Ikeda, and Y. Yamori. 2000. Resveratrol inhibits AGEs-induced proliferation and collagen synthesis activity in vascular smooth muscle cells from stroke-prone spontaneously hypertensive rats. *Biochem. Biophys. Res. Comm.* 274:61-67.

Modern Brewery Age. 2000. New research finds cancer fighting compound in red wine. (Author anonymous.) July 17, 2000. Internet address: [http://www.findarticles.com/-cf\\_0/m3469/28\\_51/65021181/print.jhtml](http://www.findarticles.com/-cf_0/m3469/28_51/65021181/print.jhtml). Last accessed on July 17, 2001.

National Cancer Institute Division of Cancer Prevention. Undated. [Title not provided.] Internet address: <http://dec.nci.nih.gov/pob/fellowship/preceptor.pdf>. (No longer available.) Date accessed on not given.

National Institutes of Health. Undated. Search Clinical Trials. Internet address: <http://www.clinicaltrials.gov>. Last accessed on July 17, 2001.

Park, J.B. 2000. Inhibition of glucose and dehydroascorbic acid uptakes by resveratrol in human tumor cells. Agricultural Research Service TEKTRAN. Internet address: <http://www.nalusda.gov/ttic/tektran/data/000011/43/0000114312.html>. Last updated on September 6, 2000. Last accessed on July 17, 2001.

Pezet, R. 1990. Purification and characterization of a 32-kDa laccase-like stilbene oxidase produced by *Botrytis cinerea* Pers.:Fr. *Microbiol. Lett.* 167(2):203-208. Abstract from CAB International 99-05 991003489.

Pezet, R., and P. Cuenat. 1996. Resveratrol in wine: Extraction from skin during fermentation and post-fermentation standing of must from Gamay grapes. *Am. J. Enol. Vitic.* 47(3):287-290. Abstract from Food Science and Technology Abstracts 97-02 97-1-02-h0102-FSTA.

Pharmascience. Undated. Partnerships and Alliances. Internet address: [http://www.Pharmascience.com/pms\\_en/about/1\\_1\\_6.asp](http://www.Pharmascience.com/pms_en/about/1_1_6.asp). Last accessed on July 18, 2001.

Pharmascience. 1998. Pharmascience Inc.'s *trans*-resveratrol shows dual action against gene and enzyme responsible for tumor growth and inflammation. Internet address: [http://www.biospace.com/news\\_rxtarget.cfm?rxtargetID=18&SR=16](http://www.biospace.com/news_rxtarget.cfm?rxtargetID=18&SR=16). Last accessed on July 17, 2001.

Pharmascience. Undated. Our consumer products: General introduction. Internet address: [http://www.pharmascience.com/pms\\_en/health/3\\_1.asp](http://www.pharmascience.com/pms_en/health/3_1.asp). Last accessed on August 3, 2001.

PR Newswire. 1998. Study identifies red wine compound's activity against cancer and arthritis. September 2, 1998 press release. Internet address: [http://www.findarticles.com/cf\\_0/m4PRN/1998\\_Sept\\_2/21087697/print.jhtml](http://www.findarticles.com/cf_0/m4PRN/1998_Sept_2/21087697/print.jhtml). Last accessed on July 17, 2001.

RDInfo. Undated. A digest of health-related research funding and training opportunities. Internet address: <http://www.rdinfo.org.uk/queries>. Last accessed on August 3, 2001.

Reliv ReversAge. Undated. Resveratrol (Protykin™). Internet address: <http://pages.prodigy.net/villamar/Resveratrol.htm>. Last accessed on Jan. 7, 2002.

Reliv' International, Inc. 1998. Reliv' International, Inc. announces a natural contender in the fight for women's health. Press release, November 23, 1998. Newswire. Cited by Predicasts – New Product Announcements 98-48 53248400.

Sanders, T.H., R.W. McMichael, and K.W. Hendrix. 2000. Occurrence of resveratrol in edible peanuts. *J. Agric. Food Chem.* 48(4):1243-1246. Abstract from Life Sciences database. Last accessed on January 24, 2001.

Satchell, F.B. 2000. Letter dated October 18, 2000, from FDA Division of Compliance and Enforcement, Office of Nutritional Products, Labeling and Dietary Supplements, Center for Food Safety and Applied Nutrition informing K. LaSasso, Solgar Vitamin and Herb, that notification about resveratrol was received and filed by the FDA September 13.

Satchell, F.B. 2000. Letter dated December 21, 2000, from FDA Division of Compliance and Enforcement, Office of Nutritional Products, Labeling and Dietary Supplements, Center for Food Safety and Applied Nutrition informing K. LaSasso, Solgar Vitamin and Herb, that "new dietary ingredient should be placed on public display ... after December 12, 2000."

Sato, M., G. Maulik, D. Bagchi, and D.K. Das. 2000. Myocardial protection by Protykin™, a novel extract of *trans*-resveratrol and emodin. *Free Radical Res.* 32(2):135-144. Internet address: <http://www.ncbi.nlm.nih.gov>. Abstract from PubMed 10653484.

Seidelin, M., and O. Vang. 2001 abstr. Resveratrol causes decreased growth and S-phase accumulation of human colon cancer cells. In: *Food and Nutrition for Better Health: A European Conference: Highlights from EC research programmes (HEALFO Conference)*. Santa

Maria Imbaro and Lanciano, Italy, June 13-15, 2001. Abstract available at Internet address: <http://www.cmns.mnagri.it/en/congres/healfo/selected/files62.htm>.

Sgambato, A., R. Ardito, B. Faraglia, A. Boninsegna, F.I. Wolf, and A. Cittadini. 2001. Resveratrol, a natural phenolic compound, inhibits cell proliferation and prevents oxidative DNA damage. *Mutat. Res.* 496(1-2):171-180.

Sherman, D. 1999. Mom's advice goes public. The Southern Region Small Fruit Consortium: Special Reports. October 25, 1999. Internet address: [http://www.smallfruits.org/SFC\\_News/health.htm](http://www.smallfruits.org/SFC_News/health.htm).

Stahl, S., T.-Y. Chun, and W.G. Gray. 1998. Phytoestrogens act as estrogen agonists in an estrogen-responsive pituitary cell line. *Toxicol. Appl. Pharmacol.* 152(1):41-48.

Stanley, D. 1997. America's first grape: The muscadine. *Agric. Res. Mag.* November 20, 1997. Internet addresses: <http://www.ars.usda.gov/is/pr/1997/971120.htm> and <http://www.ars.usda.gov/is/AR/archive/nov97/musc1197.htm>. Last accessed on July 17, 2001.

Steele, V., M. Wargovich, K. McKee, S. Sharma, B. Wilkinson, G. Wyatt, P. Gao, and G. Kelloff. 1998. Cancer chemoprevention drug development strategies for resveratrol. *Pharm. Biol.* 36 (Suppl.):62-68. Abstract at Internet address: <http://www.swets.nl/sps/journals/pb360000s.html>. Last accessed on July 17, 2001.

Surh Y.J., Y.J. Hurh, J.Y. Kang, E.Y. Lee, G. Kong, and S.J. Lee. 1999. Resveratrol, an antioxidant present in red wine, induces apoptosis in human promyelocytic leukemia (HL-60) cells. *Cancer Lett.* 140(1/2):1-10. Abstract from CAB International, 00-01 20001406416.

Svegliati-Baroni, G., A. Di Sario, A. Casini, G. Ferretti, L. D'Ambrosio, F. Ridolfi, L. Bolognini, R. Salzano, F. Orlandi, and A. Benedetti. 1999. The Na<sup>+</sup>/H<sup>+</sup> exchanger modulates the fibrogenic effect of oxidative stress in rat hepatic stellate cells. *J. Hepatol.* 30:868-875.

United Medical and Dental Schools of Guy's and St. Thomas' Hospitals (UMDS). 1998. Grant program for research on resveratrol-Resverin<sup>®</sup>, the pure wine antioxidant. June 2, 1998. *Research News* 8(11). Internet address: <http://www.umds.ac.uk/admin/extrel-local/researchnews/jun2.htm>.

University of Arizona, Tucson. 1998. Research on *trans*-resveratrol: *Pharmascience*. July 1, 1998. *Research Review* 27(1):14. Internet address: <http://vpr2.admin.Arizona.edu/rso/u27-1.pdf>.

University of Texas, Smithville Center for Environmental Research. 2001. Pilot projects. Internet address: <http://www.niehs.nih.gov/centers/pilot/uts-pp.htm>. Last accessed on July 18, 2001.

University of Texas, Smithville Center for Research on Environmental Disease. 2001. Mechanisms of disease prevention research core. Internet address: <http://www.niehs.nih.gov/centers/res-core/uts-res5htm>. Last accessed on July 18, 2001.

Uptown Health Foods. Undated. Greens+ Nutritional Supplement. Internet address: <http://www.getset.com/uptown/greens+>. Last accessed on April 9, 2001.

Virgili, M., and A. Contestabile. 2000. Partial neuroprotection of *in vivo* excitotoxic brain damage by chronic administration of the red wine oxidant agent, *trans*-resveratrol, in rats. Abstract from Internet address: <http://www.alcohol-aware.com/pages/arch/general/excit.htm>. Last accessed July 17, 2001.

Vitacost.com. Undated. Encyclopedia of health concerns and individual nutrients: Resveratrol. Internet address: <http://www.vitacost.com/science/nutrients/resveratrol.html>. Last accessed on April 9, 2001.

Vrhovsek, U., S. Wendelin, and R. Eder. 1997. Effects of various vinification techniques on the concentration of *cis*- and *trans*-resveratrol and resveratrol glucoside isomers in wine. *Am. J. Enol. Vitac.* 48(2):214-219. Abstract from Food Science and Technology Abstracts 98-11 1998-11-h2171-FSTA.

Warner, T.D., F. Giuliano, I. Vojnovic, A. Bukasa, J.A. Mitchell, and J.R. Vane. 1999. Nonsteroid drug selectivities for cyclo-oxygenase-1 rather than cyclo-oxygenase-2 are associated with human gastrointestinal toxicity: A full *in vitro* analysis. *Proc. Natl. Acad. Sci.* 96:7563-7568.

Watkins, T., and M. Bierenbaum. Grape juice accentuates cardiovascular risk factors in the hyperlipidemic subject. *Pharm. Biol.* 36 (Suppl.):75-80.

Wine Institute. 1997. Antioxidants & phenolic compounds. Internet address: [http://www.wineinstitute.org/res\\_ed/wine\\_spec\\_rsched/antioxid.htm](http://www.wineinstitute.org/res_ed/wine_spec_rsched/antioxid.htm). Last accessed on December 12, 2001.

Yoon, K., L. Pellaroni, K. Ramamoorthy, K. Gaido, and S. Safe. 2000. Ligand structure-dependent differences in activation of estrogen receptor  $\alpha$  in human HepG2 liver and U2 osteogenic cancer cell lines. *Molec. Cell. Endocrinol.* 162(1-2):211-220.

Zhu, Y.X., L.A. Coury, H. Long, C.T. Duda, C.B. Kissinger, and P.T. Kissinger. 2000. Liquid chromatography with multichannel electrochemical detection for the determination of resveratrol in wine, grape juice, and grape seed capsules with automated solid phase extraction. *J. Liq. Chromatogr. Rel. Technol.* 23(10):1555-1564.

**Acknowledgements**

Support to the National Toxicology Program for the preparation of *trans*-Resveratrol [501-36-0] —Review of Toxicological Literature was provided by Integrated Laboratory Systems, Inc., through NIEHS Contract Number N01-ES-65402. Contributors included: Karen E. Haneke, M.S. (Principal Investigator); Bonnie L. Carson, M.S. (Co-Principal Investigator); Claudine A. Gregorio, M.A.; Rachel Hardy, M.A.; and Nathan S. Belue, B.S. (library retrieval support).

**Appendix: Units and Abbreviations**

°C = degrees Celsius

µg/L = microgram(s) per liter

µg/mL = microgram(s) per milliliter

µM = micromolar

BCE = bovine capillary endothelial

bw = body weight

CA = chromosome aberration

DNA = 2'-deoxy-5'-ribonucleic acid

ER = estrogen receptor

EROD = 7-ethoxyresorufin-*o*-dealkylation

F = female(s)

FDA = Food and Drug Administration

FDCA = Federal Drug and Cosmetics Act

FGF-2 = fibroblast growth factor-2

g = gram(s)

g/mL = gram(s) per milliliter

h = hour(s)

IC<sub>50</sub> = inhibitory concentration for 50% of cells

i.p. = intraperitoneal(ly)

IHV = Institute for Human Virology

kg = kilogram(s)

L = liter(s)

LC = liquid chromatography

LC<sub>50</sub> = lethal concentration for 50% of test animals

LD<sub>50</sub> = lethal dose for 50% of test animals

LOD = limit of detection

M = male(s)

mg/kg = milligram(s) per kilogram

mg/m<sup>3</sup> = milligram(s) per cubic meter

mg/mL = milligram(s) per milliliter

min = minute(s)

mL/kg = milliliter(s) per kilogram

mM = millimolar

mmol = millimole(s)

mmol/kg = millimoles per kilogram

mo = month(s)

mol = mole(s)

mol. wt. = molecular weight

NIEHS = National Institute of Environmental Health Sciences

NOAEL = no observed adverse effect level

n.p. = not provided

NRU = neutral red uptake

NRU<sub>50</sub> = midpoint cytotoxicity, NRU assay

PhIP = 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine

ppb = parts per billion

ppm = parts per million

p.o. = peroral(ly), *per os*

PR = progesterone receptor

RNA = ribonucleic acid

s = second(s)

s.c. = subcutaneous(ly)

SCE = sister chromatid exchange

S-G = Smulow-Glickman

VEGF = vascular endothelial growth factor

wk = week(s)

yr = year(s)