NTP
National Toxicology Program
U.S. Department of Health and Human Services

NTP Technical Report on the Toxicity Studies of *Trans*-resveratrol (CASRN 501-36-0) Administered by Gavage for Two Weeks or Three Months to F344/NTac Rats, Wistar Han [Crl:WI(Han)] Rats, and B6C3F1/N Mice

NTP TOX 102

DECEMBER 2021

NTP Technical Report on the Toxicity Studies of *Trans*-resveratrol (CASRN 501-36-0) Administered by Gavage for Two Weeks or Three Months to F344/NTac Rats, Wistar Han [Crl:WI(Han)] Rats, and B6C3F1/N Mice

Toxicity Report 102

December 2021

National Toxicology Program
Public Health Service
U.S. Department of Health and Human Services
ISSN: 2378-8992

Research Triangle Park, North Carolina, USA

Foreword

The National Toxicology Program (NTP), established in 1978, is an interagency program within the Public Health Service of the U.S. Department of Health and Human Services. Its activities are executed through a partnership of the National Institute for Occupational Safety and Health (part of the Centers for Disease Control and Prevention), the Food and Drug Administration (primarily at the National Center for Toxicological Research), and the National Institute of Environmental Health Sciences (part of the National Institutes of Health), where the program is administratively located. NTP offers a unique venue for the testing, research, and analysis of agents of concern to identify toxic and biological effects, provide information that strengthens the science base, and inform decisions by health regulatory and research agencies to safeguard public health. NTP also works to develop and apply new and improved methods and approaches that advance toxicology and better assess health effects from environmental exposures.

The Toxicity Report series began in 1991. The studies described in the NTP Toxicity Report series are designed and conducted to characterize and evaluate the toxicological potential of selected substances in laboratory animals (usually two species, rats and mice). Substances (e.g., chemicals, physical agents, and mixtures) selected for NTP toxicity studies are chosen primarily on the basis of human exposure, level of commercial production, and chemical structure. The interpretive conclusions presented in the Toxicity Reports are derived solely from the results of these NTP studies, and extrapolation of these results to other species, including characterization of hazards and risks to humans, requires analyses beyond the intent of these reports. Selection for study per se is not an indicator of a substance's toxic potential.

NTP conducts its studies in compliance with its laboratory health and safety guidelines and Food and Drug Administration Good Laboratory Practice Regulations and meets or exceeds all applicable federal, state, and local health and safety regulations. Animal care and use are in accordance with the Public Health Service Policy on Humane Care and Use of Laboratory Animals. Studies are subjected to retrospective quality assurance audits before they are presented for public review. Draft reports undergo external peer review before they are finalized and published.

The NTP Toxicity Reports are available free of charge on the NTP website and cataloged in PubMed, a free resource developed and maintained by the National Library of Medicine (part of the National Institutes of Health). Data for these studies are included in NTP's Chemical Effects in Biological Systems database.

For questions about the reports and studies, please email NTP or call 984-287-3211.

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Peer Review

The National Toxicology Program (NTP) conducted a peer review of the draft *NTP Technical Report on the Toxicity Studies of Trans-resveratrol (CASRN 501-36-0) Administered by Gavage for Two Weeks or Three Months to F344/NTac Rats, Wistar Han [Crl:WI(Han)] Rats, and B6C3F1/N Mice* by letter in March and April 2021 by the experts listed below. Reviewer selection and document review followed established NTP practices. The reviewers were charged to:

(1) Peer review the draft *NTP Technical Report on the Toxicity Studies of Trans-resveratrol*.

(2) Comment on NTP's interpretations of the data.

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Publication Details

Publisher: National Toxicology Program

Publishing Location: Research Triangle Park, NC

ISSN: 2378-8992

DOI: https://doi.org/10.22427/NTP-TOX-102

Report Series: NTP Toxicity Report Series

Report Series Number: 102

Official citation: National Toxicology Program (NTP). 2021. NTP technical report on the toxicity studies of *trans*-resveratrol (CASRN 501-36-0) administered by gavage for two weeks or three months to F344/NTac rats, Wister Han [Crl:WI(Han)] rats, and B6C3F1/N mice. Research Triangle Park, NC: National Toxicology Program. Toxicity Report 102.

Acknowledgments

This work was supported by the Intramural Research Program (ES103316, ES103318, and ES103319) at the National Institute of Environmental Health Sciences, National Institutes of Health and performed for the National Toxicology Program, Public Health Service, U.S. Department of Health and Human Services under contracts HHSN273201800006C, HHSN273201600011C, GS00Q14OADU417 (Order No. HHSN273201600015U), HHSN273201600020C, HHSN273201500006C, HHSN273201500012C, HHSN273201500014C, HHSN273201400022C, HHSN273201400004C, HHSN273201300009C, HHSN316201200054W, HHSN273201100003C, N01-ES-65554, N01-ES-55541, N01-ES-05455, N01-ES-45517, N01-ES-65557, and N01-ES-75408-21-0-1.

Abstract

Trans-resveratrol (RES) is a polyphenol found in various fruits and plants. Numerous in vitro studies have shown its clear antioxidant and anti-inflammatory effects, which has led to additional in vivo and clinical studies evaluating the use of RES to treat diseases such as cancer, cardiometabolic disease, and neurodegenerative disease. Despite growing interest in and use of RES, limited studies have assessed the safety of RES exposure, especially perinatally. The National Toxicology Program conducted toxicity studies to provide these data.

In the 3-month studies, RES (in 0.5% aqueous methylcellulose) was administered via gavage to time-mated Wistar Han rats from gestation day (GD) 6 through lactation day (LD) 21 at doses of 0, 78, 156, 312.5, 625, or 1,250 mg RES/kg body weight/day (mg/kg/day). Doses were selected based on the lack of observed toxicity in 2-week studies in Fischer 344 (F344/NTac) rats. Offspring were administered the same dose as respective dams from postnatal day (PND) 12 through PND 21 and then for 3 months after weaning. In addition, male and female B6C3F1/N mice at 5–6 weeks of age were administered 0, 156, 312, 625, 1,250, or 2,500 mg/kg/day of RES for 3 months.

In Wistar Han rats, no dose-related effects of RES on dam survival, gestation length, litter size, or pup weight on PND 1 were identified. Maternal mean body weights and body weight gains of RES-dosed dams were significantly decreased (4%–10% and 19%–35%, respectively) relative to the vehicle control group, especially during the later period of gestation (GD 15–21). The presence of RES and its metabolites in fetal tissue suggested low maternal transfer, and the presence of RES and its metabolites in PND 4 whole pups suggested lactational transfer. Pup mean body weights of RES-dosed groups (≥312.5 mg/kg/day) were lower starting on PND 4 through weaning. During the postweaning period, there was no dose-related effect on survival. Interim mean body weights of male and female rats in the 1,250 mg/kg/day groups during lactation were approximately 20% lower than those of the vehicle control groups. By study termination, mean body weights of all RES-dosed Wistar Han rats were within 10% of the vehicle control groups. There were no dose-related changes in sperm count or estrous cycling. Dose-related histological findings in the Wistar Han rat included nephropathy and renal pelvis and renal tubule dilatation in the kidney and lymphatic ectasia in the small intestine.

In B6C3F1/N mice, mean body weights were similar between RES-dosed and vehicle control groups throughout the study. After 3 months, there were no dose-related effects on survival. Minimal indications of decreased sperm count and impaired estrous cycling were observed, but these findings were not considered indicative of reproductive toxicity. The absolute and relative liver weights of 2,500 mg/kg/day male mice were significantly increased. Relative liver weights of ≥625 mg/kg/day female mice and relative kidney weights of ≥1,250 mg/kg/day female mice were significantly increased. These increased organ weights were not associated with microscopic findings. Respiratory metaplasia in the olfactory epithelium of the nose was observed in female mice. Increased incidences of this lesion were significant only at the highest dose (2,500 mg/kg/day).

No changes in the frequency of micronucleated reticulocytes and erythrocytes were considered biologically relevant in either species. RES was not mutagenic in the *Salmonella typhimurium* strains tested.

Under the conditions of this study, the lowest-observed-effect level (LOEL) was 312.5 mg/kg/day in rats as indicated by significantly decreased pup mean body weights of Wistar Han rats exposed perinatally. These body weight differences were resolved in the rat pups by the end of the 3-month study. In B6C3F1/N mice, the LOEL was 625 mg/kg/day as indicated by significantly increased relative liver weights in females; however, these changes in liver weight were not associated with microscopic lesions. The no-observed-effect levels were 156 mg/kg/day in rats and 312 mg/kg/day in mice. Target organs included the kidney and small intestine in rats and the nose in female mice. There was no evidence of genetic toxicity in the micronucleus assay of RES at oral gavage doses up to 1,250 mg/kg/day in Wistar Han rats or up to 2,500 mg/kg/day in B6C3F1/N mice. No clear effects on reproductive parameters were observed. The presence of RES and its metabolites in fetal tissue suggested low maternal transfer, and the presence of RES and its metabolites in PND 4 whole pups suggested lactational transfer.

Synonyms: resveratrol; 3,4',5-stilbenetriol; 3,4',5-trihydroxystilbene; 3,5,4'-trihydroxystilbene

Summary of Findings Considered Toxicologically Relevant in Male and Female Rats and Mice Administered *Trans*-resveratrol by Gavage for Three Months

	Male Wistar Han Rats	Female Wistar Han Rats	Male B6C3F1/N Mice	Female B6C3F1/N Mice
Doses in Aqueous Methylcellulose	0, 78, 156, 312.5, 625, or 1,250 mg/kg/day	0, 78, 156, 312.5, 625, or 1,250 mg/kg/day	0, 156, 312, 625, 1,250, or 2,500 mg/kg/day	0, 156, 312, 625, 1,250, or 2,500 mg/kg/day
Survival Rates	10/10, 10/10, 10/10, 10/10, 10/10, 10/10	10/10, 10/10, 10/10, 10/10, 10/10, 10/10	10/10, 10/10, 10/10, 10/10, 10/10, 10/10	10/10, 10/10, 10/10, 10/10, 10/10, 10/10
Body Weights	↓ Pup mean body weight during lactation (up to 22% less than the vehicle control group; 312.5, 625, 1,250 mg/kg/day dosed groups) Final mean body weights of dosed groups within 10% of the vehicle control group	↓ Dam mean body weight from GD 15 through 21 (up to 10% less than the vehicle control group; 156, 312.5, 625, 1,250 mg/kg/day dosed groups) ↓ Pup mean body weight during lactation (up to 21% less than the vehicle control group; 625, 1,250 mg/kg/day dosed groups) Final mean body weights of dosed groups within 10% of the vehicle control group	Dosed groups within 10% of the vehicle control group	Dosed groups within 10% of the vehicle control group
Clinical Findings	None[a]	None	None	None
Organ Weights	None	None	↑ Absolute and relative liver weight	↓ Absolute heart weight ↑ Relative kidney weight ↑ Relative liver weight

	Male Wistar Han Rats	Female Wistar Han Rats	Male B6C3F1/N Mice	Female B6C3F1/N Mice
Nonneoplastic Effects	Kidney: nephropathy (0/10, 3/10, 1/10, 4/10, 2/10, 7/10); renal tubule, dilatation (0/10, 0/10, 0/10, 0/10, 1/10, 7/10) Small intestine: jejunum, lymphatic ectasia (0/10, 0/10, 0/10, 0/10, 2/10, 2/10); Peyer's patch, lymphatic ectasia (0/10, 0/10, 1/10, 0/10, 2/10, 1/10)	Kidney: nephropathy (0/10, 0/10, 1/10, 2/10, 3/10, 6/10); renal pelvis, dilatation (0/10, 1/10, 0/10, 1/10, 1/10, 4/10); renal tubule, dilatation (0/10, 0/10, 1/10, 0/10, 0/10, 7/10) Small intestine: jejunum, lymphatic ectasia (0/10, 0/10, 0/10, 0/10, 0/10, 2/10); Peyer's patch, lymphatic ectasia (0/10, 2/10, 0/10, 1/10, 0/10, 1/10)	None	Nose: olfactory epithelium, metaplasia, respiratory (0/10, 0/10, 0/10, 2/10, 2/10, 4/10)
Clinical Pathology	None	None	None	None
Reproductive Findings	None	None	None	None
Genetic Toxicology				
Bacterial Gene Mutations		Negative in *Salmonella typhimurium* strains TA98, TA100, and TA102, with or without S9		
Micronucleated Erythrocytes (In Vivo)				
Rat peripheral blood:		Negative in males and females		
Mouse peripheral blood:		Negative in males and females		

[a]None = no toxicologically relevant effects for this endpoint.

Introduction

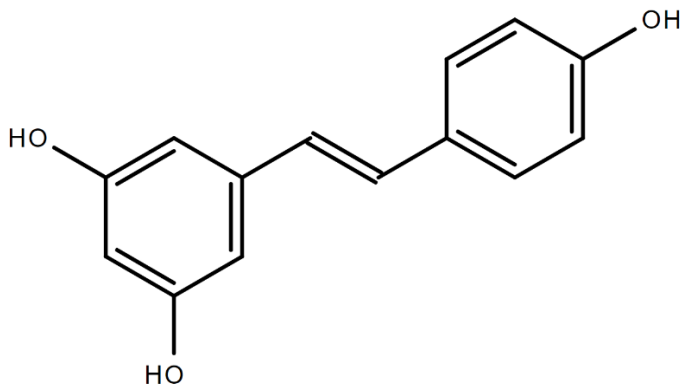


Figure 1. *Trans*-resveratrol (CASRN 501-36-0; Chemical Formula: $C_{14}H_{12}O_3$; Molecular Weight: 228.25)

Image generated with ChemSpider[1]
Synonyms: resveratrol; 3,4',5-stilbenetriol; 3,4',5-trihydroxystilbene; 3,5,4'-trihydroxystilbene.

Chemical and Physical Properties

Trans-resveratrol (RES) is a polyphenolic phytoalexin produced by some plants in response to ultraviolet (UV) light, injury, or infection. Stilbene synthase is the final enzyme in the RES synthesis pathway and is stimulated by UV light or fungal infections.[2; 3] Due to its double bond, resveratrol can exist in the *trans*- or *cis*-form; the *trans*-isomer is more sterically stable.[4] Conversion between the *trans*- and *cis*-forms can occur with exposure to UV light and is dependent on concentration and pH.[5] RES has low water solubility (<0.05 mg/mL) and is more soluble in organic solvents (log K_{ow} of 3.10).[6; 7] It is an off-white powder with a melting point of 253–255ºC.[8] UV absorbance of *trans*- and *cis*-resveratrol in ethanol is 306–320 nm and 286–288 nm, respectively.[4]

Production, Use, and Human Exposure

RES can be isolated from the leaves of white hellebore (*Veratrum grandiflorum*) and roots of Japanese knotweed (*Polygonum cuspidatum*), plants used in traditional medicine.[9; 10] In addition, RES is found in several foods, including grapes, peanuts, pistachios, itadori tea, and various berries.[11-13] RES is also present in wine and is found at higher concentrations in red wine.[10; 14-17]

RES can be commercially produced by isolating it from grape plants, which can be induced to produce greater quantities by applying aluminum chloride or aluminum sulfate to grape shoots and vines.[18; 19] Production of RES in harvested grapes increased twofold with irradiation by UVB light and threefold with irradiation by UVC light.[20] RES can also be produced by treating cell suspension cultures of grapes with Onozuka R-10, a cellulase derived from the fungus *Trichoderma viride*.[21] In addition, stilbene synthase genes have been isolated and inserted into plants, creating transgenic varieties of tobacco, grape, tomatoes, potatoes, rice, and alfalfa with higher RES concentrations.[22-25] Lastly, RES can be synthesized from chemical precursors, as is the case with DSM Nutritional Products' resVida®.[26]

Due to its low water solubility, RES bioavailability is low to moderate. Thus, formulations for increasing RES bioavailability have been developed, involving micro- and nanoparticle excipients, liposomes, and complexation with cyclic oligosaccharides.[7] Research is ongoing to improve the bioavailability and bioefficacy (e.g., increased concentration at tissue-specific sites) of prodrugs for RES.[27]

Traditional Asian medicine has long used RES from the root of *P. cuspidatum* as a tonic to treat hypertension and cardiovascular disease, among other uses.[10; 28; 29] Primarily due to its antioxidant and anti-inflammatory effects observed in laboratory studies, RES has been marketed to treat and prevent a variety of diseases, improve glucose metabolism (e.g., beneficial effects on insulin sensitivity),[30] provide neuro- and cardio-protection,[31-33] enhance the immune system,[34; 35] protect against cancer,[36] and prolong life.[37]

Human exposure to RES is mainly through ingestion, particularly of peanuts, grapes, and related products.[38; 39] In 2018, per capita wine consumption in the United States was 11.2 L or 2.95 gallons.[40] The content of RES in wine depends on the variety of grape used and on the geographic region in which the grapes were grown. The average red wine contains 1.9 ± 1.7 mg/L RES,[41] whereas the average white wine contains ≤0.02 mg/L.[42]

RES levels in peanuts and peanut products are lower than those in grape products and are summarized in a review by Sales and Resurreccion.[38] In one study, RES concentrations were 0.06 μg/g (0.24 nmol/g) for roasted peanuts, 0.32 μg/g (1.42 nmol/g) for peanut butter, and 5.14 μg/g (22.51 nmol/g) for boiled peanuts.[43] Hendler and Rorvik[28] reported the levels of RES in peanuts to be 0.02–1.79 μg/g (0.09–7.84 nmol/g). Estimates of U.S. peanut consumption per capita annually from 2012 to 2018 range from 6.5 to 7.5 pounds, with peanut butter the primary product consumed.[44]

Although dietary intake of total polyphenols has been documented in several studies, comprehensive estimations of RES consumption through diet are limited. One study involving the European Prospective Investigation into Cancer and Nutrition (Spain) cohort evaluated dietary intake of RES and its derivative, piceid.[45] Using a compilation of studies on the amounts of *trans*- and *cis*-resveratrol in various foods, researchers estimated RES intake in the Spanish cohort to be 100 μg/day. Intake was highest in older men. The primary sources of RES were wine and grape juice (98.4% and 1.6%, respectively); peanuts, pistachios, and berries contributed <0.01%.[45] Studies in other European cohorts have estimated RES intake through wine and grapes to be in the range of 0–200 μg/day.[46-48] Estimates of RES intake via diet vary between populations and countries due to different food availability and food consumption patterns.

Oral exposure to RES also occurs via dietary supplements. For dietary supplements, amounts found in products and dosage recommendations vary. RES supplements can contain from 1 to 500 mg per tablet or capsule. Although the amount individuals in the general population are taking is unclear, up to 5 g per day has been observed to be safe in clinical trials[49] and is a recommended upper limit reported on health websites.[50; 51] Due to its antioxidant and potential antiaging properties, RES is being formulated for dermal application through which more direct application to the target tissue is possible, potentially resulting in higher bioavailability.[52; 53] Thus, some dermal exposure to RES is possible.

Regulatory Status

RES available in dietary supplements is regulated under the U.S. Food, Drug, and Cosmetic Act. Manufacturers and distributors must notify the Food and Drug Administration when they plan to market dietary supplements that contain "new dietary ingredients" (Section 413b of the Act, 21 U.S.C. 350b).[54]

Absorption, Distribution, Metabolism, and Excretion

A number of animal studies and small clinical trials evaluating kinetics and metabolism of RES are available. They have been reviewed elsewhere,[49; 55; 56] but overall findings are summarized here. Generally, RES is rapidly absorbed, highly metabolized in the liver and small intestine, and excreted through urine.

Experimental Animals

The disposition of RES has been studied primarily in rodents; one limited study was conducted in beagle dogs. In both cases, RES was rapidly absorbed following oral administration.

In male CD or Sprague Dawley rats given a single gavage dose of 50 or 150 mg RES/kg body weight (mg/kg), the time at which maximal plasma concentrations were reached (T_{max}) was ≤1 hour.[57; 58] After repeated dosing (daily for 14 days) in the CD rats, the T_{max} decreased to 0.25 hours in the 50 mg/kg/day group but increased to 2 hours in the 150 mg/kg/day group.[57] The National Toxicology Program (NTP) previously investigated the toxicokinetic (TK) behavior of RES in male and female Sprague Dawley (Hsd:Sprague Dawley® SD®) rats (312.5, 625, or 1,250 mg/kg) and B6C3F1/N mice (625, 1,250, or 2,500 mg/kg) following a single intravenous or gavage administration.[59] In both male and female rats and mice, T_{max} was predicted by modeling to be ≤4.4 hours, with observed values ranging from 1.5 to 8 hours. Secondary peaks in the plasma concentration-time curves were observed, suggesting enterohepatic recirculation.[59] The plasma elimination half-life in male and female rats for the two lower doses were 5.6–8.4 hours but increased to 17.7–22.1 hours in the 1,250 mg/kg/day dose group. In male and female mice, the elimination half-life ranged between 0.3 and 10.7 hours without any clear dose-related effect. Systemic exposure parameters maximum concentration (C_{max}) and area under the concentration-time curve (AUC) increased linearly with the dose in both rats and mice. In general, the TK behavior of RES was similar between sexes.

The plasma elimination half-life found in previous NTP studies is similar to what has been reported in other studies. In male Wistar rats given red wine containing 6.5 mg/L of RES, the plasma elimination half-life was estimated to be 0.5 hours.[60] Other studies in rats following oral administration of RES (50–150 mg/kg/day) report plasma elimination half-lives in the range of 1.8–11.8 hours.[57; 58] Unlike previous NTP studies in which the highest dose (i.e., 1,250 mg/kg) yielded longer half-lives in both mice and rats,[59] Kapetanovic et al.[57] reported that higher and repeated doses resulted in shorter half-lives. This discrepancy may be due to differences in doses used, 50–150 mg/kg/day versus 312.5–1,250 mg/kg/day.

Although RES is rapidly absorbed by the gastrointestinal tract in rodents, oral bioavailability of RES is low to moderate. In previous NTP studies, the oral bioavailability estimated was higher in rats (~12%–31%) compared to mice (~3%–6%).[59] Other studies also report low oral bioavailability ranging from 18% to 38%,[57; 58] due to extensive first pass metabolism in the

intestine and liver. Gestational transfer of RES has been observed in both Japanese macaques exposed to 0.37% RES via the diet during pregnancy[61] and rats following exposure to 4 g/kg RES via the diet from gestation day (GD) 7 to GD 21.[62]

RES undergoes extensive phase II metabolism in the liver of rodents, producing sulfate and glucuronide conjugates such as *trans*-resveratrol-3-O-B-D-glucuronide (*trans*-R3G), *trans*-resveratrol-4-O-glucuronide, *trans*-resveratrol-diglucuronide, *trans*-resveratrol-3-O-sulfate (*trans*-R3S), and *cis*-resveratrol-3,4′-disulfate.[57; 59; 63-66] Concentrations of these metabolites, particularly *trans*-R3G and *trans*-R3S, are generally higher than the concentration of the parent compound. Resveratrol metabolites tend to have similar half-lives to the parent compound in rodents.[57; 59] Some studies reviewed in Detampel et al.[67] suggest RES inhibits phase I metabolism and induces phase II metabolism, indicating the potential for RES-drug interactions.

RES and its metabolites are present in the systemic circulation and are distributed widely to a variety of tissues in rodents including heart, lung, liver, kidney, brain, and spleen.[59; 60; 63; 64; 66; 68; 69] Dermal administration of RES yielded a similar distribution profile of RES in tissues to that seen with oral administration.[70] Tissue concentrations of RES are highly dependent on the expression of glucuronidating and sulfating enzymes, which can be tissue-specific, but also on how much is transported into cells.[71] Resveratrol sulfates may be hydrolyzed to regenerate the parent compound by sulfatases.[71] Thus, the amount of RES in tissues, and likewise RES elimination and metabolism, is a function of a complex interplay between metabolism and transport mechanisms. Excretion of RES and its metabolites is primarily through renal elimination. Concentrations of RES-derived radioactivity 18 hours postgavage was highest in the urine (3.3%) followed by feces (1.6%).[69]

In dogs following oral administration of 200, 600, or 1,200 mg/kg/day for 13 weeks, T_{max} of RES and metabolites ranged from 1 to 7 hours, with no apparent differences by dose.[72] As observed with rats,[58; 59] secondary peaks in the plasma concentration-time curves were observed in some dogs, suggesting enterohepatic recirculation.[72] In dogs, the half-life of RES was 2–4 hours.[72] RES sulfate had a longer half-life than the parent compound in dogs, although the study was limited.[72]

Humans

Studies in humans have found rapid absorption of orally administered RES. Absorption of 25 mg radiolabeled RES was 70%, with a plasma T_{max} reached in about 1 hour.[73] Studies of single or repeated administration of 0.5–5 mg RES also reported rapid absorption of RES, with T_{max} in the range of 1–1.5 hours.[74; 75] As in rodents, enterohepatic recirculation has been observed 5–6 hours after dosing.[73; 74] In a study evaluating single and repeat dosing of 200 mg RES, T_{max} did not change with repeated dosing and the degree of accumulation was moderate with accumulation ratios (AUC of repeated dose to AUC of single dose) ranging from 1.44 to 2.09.[76] In both studies, as in rats, bioavailability was low (<1%), likely due to the extensive metabolism of RES in the liver and intestine.[77] Some studies have investigated how the bioavailability of RES changes depending on how it is consumed: as a pure compound, in grape juice or wine, or with certain foods.[78; 79] For instance, high lipid-content foods were found to decrease the bioavailability of a 2 g dose of RES,[80] although this finding was not confirmed in another study evaluating RES consumed through red wine.[81]

Metabolism of RES in humans also is extensive, producing sulfate and glucuronic acid conjugates similar to those found in rodents, although at different proportions.[74; 75; 79; 82; 83] In humans, sulfate conjugates are more prominent,[55] while in rodents glucuronide conjugates are more common.[55; 59] Additionally, resveratrol-4′-O-glucuronide has been measured in human plasma after dosing, but much less is found in rodent plasma and tissues. A comparative study of glucuronidation in human, dog, rat, and mouse liver microsomes found higher rates of glucuronidation and higher production of resveratrol-3-O-glucuronide and insignificant formation of resveratraol-4-O-glucuronide in rodent microsomes compared to human and dog microsomes after incubation with 100 µM RES.[71] Lastly, differences in microbiota or anatomical differences in the intestine may lead to variations in microbially derived RES metabolites.[63]

Data on the tissue distribution of RES and its metabolites in humans are limited. One study reported RES and metabolites (resveratrol-3-O-glucuronide, resveratrol-4′-O-glucuronide, resveratrol-3-O-sulfate, resveratrol-4′-O-sulfate, resveratrol disulfate, and resveratrol sulfate glucuronide) in samples of normal and neoplastic colon tissue in colorectal cancer patients who consumed 500–1,000 mg RES per day for 8 days.[83] While mean RES concentrations in neoplastic colon tissues from patients given 1,000 mg for 8 days were 94 nmol per gram,[83] mean concentrations of RES in hepatic metastases from colorectal cancer patients on a similar RES treatment regimen (5 g per day for 10–21 days) were much lower (4.8 nmol per gram).[84]

Similar to what has been observed in animals, the plasma half-life of RES in humans ranges from 1 to 11 hours with minimal dose dependence.[74-76; 84] In a small study of elderly and young male and female participants (n = 6 per age group) given 200 mg RES once on the first day and then three times daily for 3 more days, the half-life of RES ranged from 2.5 to 4.7 hours.[76] No significant differences in half-life were observed due to age, sex, or dosing frequency.[76] Other human studies found the half-life of RES to be 2–9 hours, with differences varying with increasing dose (0.5 to 5 mg RES) or dose frequency (single versus repeated dose for 28 days).[74; 75] In those studies, RES metabolites in plasma tended to be similar to those of the parent compound.[74; 75]

As in experimental animals, RES and its metabolites are rapidly excreted in humans, primarily through the urine. Following a single oral dose of 0.5–5 g to healthy volunteers, 77% of the urinary metabolites of RES were recovered within 4 hours after dosing.[74] In that study, fecal concentrations of RES metabolites were less than that of RES, which is consistent with enterohepatic recirculation.[74]

Toxicity

Experimental Animals

Animal studies show minimal toxicity associated with oral RES administration. A 28-day study in Wistar Han rats given RES via diet (0, 50, 150, 500 mg/kg/day) showed no exposure-related effects on body weight, clinical signs, hematology, clinical chemistry, or histopathology at any exposure concentration.[85] Another 28-day study in CD® Virus Antibody Free (VAF) rats given ≤3,000 mg/kg/day found slight hemolytic anemia in male rats, with no histological correlate, which resolved after a 4-week recovery period.[86; 87] A 90-day study in Wistar Han rats established a no-observed-adverse-effect level (NOAEL) of 750 mg/kg/day.[85] In CD (Crl:CD®[SD]IGS) rats given 200, 400, or 1,000 mg/kg/day via gavage for 90 days, the middle

and high female dose groups had significantly decreased mean body weight gains relative to the vehicle control group; no body weight changes were observed in males.[88] Additionally, there were significant increases in bilirubin at the high dose and hepatomegaly at the middle and high doses in both sexes. No dose-related changes in hematology, clinical chemistry, coagulation parameters, other organ weights, or histopathology were observed. Oral dosing in rats for 6 months resulted in decreased body weights and discolored feces at the highest dose (2,000 mg/kg/day); the NOAEL in this and other studies was 300 mg/kg/day.[86; 87] Additional studies have reported even higher NOAELs (e.g., 1,000 mg/kg/day).[89] At higher doses (>2,000 mg/kg/day), nephrotoxicity has been observed in male and female CD® VAF rats.[86]

In male and female dogs, RES-related effects involved only abnormally colored feces during dosing; there were no dose-related changes in body weight, organ weight, or histopathological evaluations or any cardiotoxic effects.[87] The NOAEL in dogs was 300 mg/kg/day. A separate 90-day oral toxicity study in dogs demonstrated no dose-related mortality, clinical signs of toxicity, or gross pathology at 200, 600, or 1,200 mg/kg/day.[88] However, body weights for both male and female dogs were significantly decreased at the highest dose, which was associated with decreased feed consumption. For this reason, the NOAEL for RES in dogs was 600 mg/kg/day.[88] RES did not cause skin or eye irritation in male and female New Zealand white rabbits.[85]

Humans

In humans, RES appears to be generally well tolerated. Clinical trials with low doses of RES show no adverse effects. At higher doses (≥0.5 g/day), the most common adverse effects were gastrointestinal (abdominal pain, nausea, flatulence, diarrhea).[75; 90; 91]

Reproductive and Developmental Toxicity

Experimental Animals

A limited number of studies have evaluated the effects of RES exposure in utero and its potential reproductive toxicity. In developing chick embryos of white Leghorns, RES (1, 10, 25, 50, or 100 µg/disk [0.004, 0.044, 0.11, 0.22, or 0.438 µmol/disk] incubated for 48–72 hours) induced avascular zones in the developing chorioallantoic membrane.[92] RES did not elicit any effects on implantation number, resorptions, live young, or pre- and postimplantation losses in pregnant Sprague Dawley rats consuming 0, 120, 300, or 750 mg RES/kg/day in the diet from gestation day (GD) 5 through 20.[85] Furthermore, uterine, placental, litter, and fetal weights were similar and there were no fetal abnormalities. Thus, the NOAEL for maternal toxicity and embryofetal development in Sprague Dawley rats in this study was the highest dose tested, 750 mg/kg/day. Another reproductive toxicity study was conducted in rats administered 300, 1,000, or 3,000 mg/kg/day via oral gavage from GD 7 through GD 17. While some (5 out of 25) dams were euthanized due to adverse clinical conditions, there were no effects on the fetuses at any dose. The maternal NOAEL was 300 mg/kg/day and the developmental NOAEL was 1,000 mg/kg/day.[87] A study in CD-1 (ICR) mice exposed to concentrations of RES similar to concentrations found in wine (3 mg/L in water) also found no effect on litter size or sex ratio.[93] RES has been reported, however, to impair morphogenesis in the P19C5 embryoid body murine morphogenesis assay via changes in *Wnt3a*, *Tbx6*, and *Cyp26a1* gene expression.[94]

The effects of RES on reproductive organs are varied. In one study, Sprague Dawley rats exposed to 120, 300, or 750 mg/kg/day via the diet for 90 days did not show changes in sperm count and quality, ovarian morphology, estrous cycling, or reproductive organ histopathology.[85] In CD-1 (ICR) mice, there was no effect of exposure to drinking water containing 3 mg/L RES for 90 days on body weight, sperm count, sperm morphology, or ovary morphology but there was a decrease in seminal vesicle and ovary weight compared to control animals.[93] On the other hand, administration of 20 mg/kg/day via gavage for 90 days in Sprague Dawley rats resulted in reduced diameter of seminiferous tubules, increased density of seminiferous tubules, and increased sperm count and plasma concentrations of luteinizing hormone, follicle stimulating hormone, and testosterone.[95] RES has been reported to bind to estrogen receptor (ER)α and ERβ with similar affinity, and is mitogenic in ERα and β transfected CHO-K1 cells, and the ER positive (ER+) cell lines MCF-7 and T47T.[96; 97]

RES has been studied as a protective agent against chemical-, disease-, or age-related impairments in reproductive organs. Due in part to its antioxidant properties, RES has been shown to be protective against chemical-induced damage to the ovaries and spermatogonia.[98-102] RES improved estrus cyclicity in rat models of polycystic ovary syndrome (PCOS), increased the ovarian follicle reserve, and extended ovarian life span in rats (reviewed in Ortega and Duleba[103]). In vitro incubation with RES induced maturation and blastocyst formation in oocytes from aged mice.[104] Additionally, RES modulated the insulin signaling pathways in theca-interstitial cells that may prevent the theca-interstitial cell hyperplasia seen in PCOS.[105]

Humans

The literature contains no studies on the reproductive and developmental toxicity of RES in humans. In a study evaluating RES as a protective agent for reproductive function, hyperandrogenic PCOS patients took 1,500 mg/day of RES for 3 months and exhibited decreased testosterone levels and increased insulin sensitivity.[106] Incubation of oocytes from women aged 38–45 years in RES-containing media improved oocyte quality.[104] These improvements were attributed to increased mitochondrial function and improved spindle and chromosomal formation in the oocytes.

Immunotoxicity

A previous NTP study found no significant toxicity on innate or adaptive immune system function after oral gavage exposure of male B6C3F1/N mice to ≤2,500 mg/kg/day resveratrol for 28 days.[107] An independent safety study found no evidence of sensitization induced by RES in the local lymph node assay.[85] Other studies have described immunomodulatory effects of RES, both experimentally and in humans,[35; 108-110] primarily for its use as a preventive agent against chronic, immune-related diseases rather than in the context of evaluating toxicity.

Carcinogenicity

The literature contains no studies on the carcinogenicity of RES in animals or humans. One study assessed the carcinogenicity of RES in TSG-p53$^{+/-}$ (heterozygous p53 knockout; p53$^{+/-}$) mice. There were no increases in the incidence of benign or malignant neoplasms in TSG-p53$^{+/-}$ mice after 6 months of exposure via oral gavage at 1,000, 2,000, or 4,000 mg/kg/day, but urothelial hyperplasia was observed at 2,000 and 4,000 mg/kg/day.[89] In this study, there was high mortality

in the 2,000 and 4,000 dose groups (40% and 70%, respectively), likely due to gastrointestinal test article impaction.[89]

Genetic Toxicity

Although RES has been investigated for antioxidant and antigenotoxic effects, several studies (described below) indicate it has genotoxic activity in vitro, which may be partially dependent on forming a complex with copper and oxygen. However, the few in vivo studies reported for this compound suggest that RES does not appear to be genotoxic.

RES was reported as negative in *Salmonella typhimurium* tester strains TA98 and TA100, and in *Escherichia coli* WP2 *uvrA*, when tested at several concentrations ranging from 0.02 to 5,000 μg/plate in the absence or presence of rat metabolic liver enzymes (S9 mix).[111] A highly purified preparation of RES, called resVida®, was also reported as negative in *S. typhimurium* tester strains TA98, TA100, TA1535, TA1537, and *E. coli* WP2 *uvrA* (pKM101), when tested at ≤5,000 μg/plate in the absence or presence of rat S9 mix.[85]

In cell culture studies conducted by Matsuoka et al.,[111] exposure to 20 μg/mL resveratrol for 24–48 hours increased the frequency of micronuclei in Chinese hamster lung (CHL) fibroblasts. Chromosomal aberrations, primarily chromatid breaks and exchanges, increased when CHL fibroblasts were exposed to 10 μg/mL resveratrol for 48 hours or to 20 μg/mL resveratrol for 54 hours. Exposure to 10 μg/mL resveratrol for 48 or 72 hours also changed the modal number of chromosomes in metaphase spreads from these cells, suggesting that RES may have an aneugenic effect.

The genotoxicity of RES was also evaluated in Chinese hamster V79 lung fibroblasts and L5178Y $Tk^{+/-}$ mouse lymphoma cells by Schmitt et al.[112] V79 cells were exposed to 100 μM RES for 6 hours, washed, and then evaluated for micronuclei every hour from 2 to 18 hours after exposure. Micronuclei were increased by approximately 10-fold at time points ranging from 14 to 18 hours. L5178Y $Tk^{+/-}$ cells were exposed to several concentrations of RES, ranging from 1 to 60 μM, for 4 hours. Cells were washed and allowed to grow for another 20 hours before harvest. Dose-dependent induction of micronuclei was observed, with a fivefold induction at 60 μM. Cells from this experiment were also evaluated for kinetochore-positive micronuclei, aberrant spindle morphology, and displacement of chromosomes from metaphase rings, which increased by 2.3-, 2.9-, and 1.7-fold, respectively, at 60 μM, suggesting that RES might induce micronuclei by an aneugenic mode-of-action. RES (≤200 μM) did not affect microtubule assembly in a cell-free tubulin polymerization assay, however.

Human colonic epithelial NCM460 cells were exposed to 0, 0.01, 0.1, 1, 10, or 100 μM RES for 24 hours and evaluated in a cytokinesis-block micronucleus assay.[113] A U-shaped curve was observed for micronuclei at concentrations from 0 to 10 μM, with levels of micronuclei dropping below control levels from 0.01 to 0.1 μM and increasing to control levels from 1 to 10 μM, with a modest induction of micronuclei above control levels at 100 μM.

Several studies have investigated the mechanisms by which RES causes genotoxicity in vitro. Using a set of six analogues of RES that differed in the number and placement of hydroxy groups, Matsuoka et al.[114] showed that the 4'-hydroxy group was necessary for the genotoxic effects of RES in the micronucleus assay and chromosomal aberration assay when conducted using CHL fibroblasts. For each experimental endpoint, CHL fibroblasts were exposed to

concentrations of RES analogues ranging from 2.5 to 20 µg/mL for 24 or 48 hours, or to 10 µg/mL of RES for 48 hours for comparison. In a study by Liu et al.,[115] a panel of isogenic DT40 chicken B-lymphoma cells deficient for genes involved in different DNA repair pathways were compared to wildtype DT40 cells when exposed to concentrations of RES ranging from 5 to 20 µM for 72 hours. This panel indicated that genes related to DNA single strand break repair (*Parp1*), base excision repair (*Polβ*), homologous recombination (*Brca1* and *Brca2*), and translesion synthesis (*Rev3* and *Rad18*) were required to survive exposure to RES, whereas cells did not depend on nucleotide excision repair (*Xpa*) and nonhomologous end joining repair (*Ku70*) for survival. Furthermore, deficiency for *Parp1* or *Polβ* modestly increased sensitivity to RES-dependent induction of γ-H2AX (a biomarker of double strand DNA breaks) and chromosomal aberrations compared to WT DT40 cells. Biochemical studies indicate that RES forms a copper-peroxide complex that may target reactive oxygen to DNA, resulting in DNA damage.[116] In the presence of cupric ions (Cu^{2+}) and oxygen, the dihydroxylated benzene ring of RES is converted to a trihydroxylated benzene ring complexed with Cu^{2+}, oxygen, and water. This complex promoted cleavage of DNA in a plasmid-based DNA cleavage assay when present at concentrations as low as 1 µM. This effect was not observed with other metal ions, such as Mg^{2+}, Mn^{2+}, Zn^{2+}, Ni^{2+}, Co^{2+}, or Fe^{3+}.

Although RES has been shown to cause DNA damage in vitro, it was not genotoxic in the few in vivo studies that have been reported for this compound. Micronuclei were not induced in polychromatic erythrocytes (PCEs) obtained from the bone marrow of male Swiss albino mice when evaluated 24 hours after exposure to 0, 6.25, 12.5, 25, 50, or 100 mg/kg RES by gavage.[117] No significant increases in micronucleated PCEs, and no apparent toxicity to PCEs, were observed in bone marrow from male Sprague Dawley rats administered 0, 500, 1,000, or 2,000 mg/kg/day resVida® by gavage on 2 consecutive days (24 hours apart).[85] resVida® was present in plasma and widely distributed among various organs in male Sprague Dawley rats, although bone marrow was not specifically assessed for the presence of resVida®.[85]

Study Rationale

RES was nominated by the National Institute of Environmental Health Sciences for toxicological evaluation by NTP due to its widespread use as a dietary supplement and insufficient information on its potential toxicity and carcinogenicity. In particular, data were lacking on effects associated with perinatal exposure and effects at the doses being investigated for therapeutic purposes. Because exposure to RES occurs primarily through ingestion, oral gavage was the selected route of administration.

Materials and Methods

Procurement and Characterization

Trans-resveratrol

Trans-resveratrol (RES) was obtained from Bayville Chemical Supply Co. Inc. (Deer Park, NY) in a single lot (156AB). Identity, purity, and stability analyses were conducted by the analytical chemistry lab at RTI International (Research Triangle Park, NC) (Appendix A). Reports on analyses performed in support of the RES study are on file at the National Institute of Environmental Health Sciences (NIEHS).

Lot 156AB, a fine off-white powder with a melting point of 253.5°C, was identified as RES using Fourier transform infrared (FT-IR) spectroscopy and ^{1}H nuclear magnetic resonance (NMR) spectroscopy. Low- and high-resolution mass spectrometry were conducted by the analytical chemistry laboratory at RTI International (Table A-1) and the University of South Carolina (Columbia, SC), respectively. No reference spectra were available for comparison, but all spectra were consistent with the mass and structure of RES.

Purity evaluation using high-performance liquid chromatography (HPLC) with ultraviolet (UV) detection did not identify any impurities. Lot 156AB was found to contain the *trans*-isomer of RES exclusively in an analysis using HPLC with a photodiode array (PDA) detector (Table A-1). Karl Fisher titration performed at Galbraith Laboratories (Knoxville, TN) indicated a water content of 0.24%. The overall purity of lot 156AB was determined to be >99%.

Accelerated stability studies conducted using HPLC/UV confirmed the bulk chemical is stable when protected from light and stored for 2 weeks at refrigerated (5°C), room (25°C), elevated (60°C), and frozen (–20°C) temperatures. The bulk chemical was stored at –20°C and protected from light, per the manufacturer's recommendations.

Methylcellulose

Methylcellulose used to make the 0.5% aqueous vehicle for gavage formulations was obtained from Spectrum (Gardena, CA) in three lots (UR1026, WL0069, and XB1050). Lot UR1026 was used in the 2-week studies and lots WL0069 and XB1050 were used in the 3-month studies.

The identity of the methylcellulose was confirmed by the study laboratory using FT-IR spectroscopy. Periodic purity analyses were performed by Galbraith Laboratories, Inc. (Knoxville, TN) during the 3-month studies to determine the methoxy group content. The August 27, 2008, sample of lot WL0069, used to prepare the first dose formulation, had methoxy group content (32.2%) outside of the acceptance criteria of 27.5%–31.5%. A replacement lot (XB1050) was procured for the remaining formulations and was within the acceptance criteria for methoxy group content (29.3%).

Deionized water was used to make the 0.5% aqueous methylcellulose vehicle for gavage formulations.

Preparation and Analysis of Dose Formulations

Dose formulations of RES in 0.5% methylcellulose were prepared at concentrations of 15.6, 31.2, 62.5, 125, or 250 mg/mL for the 2-week study in Fischer 344 (F344/NTac) rats, the 3-month study in Wister Han rats, and the 2-week and 3-month studies in B6C3F1/N mice (Table A-2). The method of preparation was validated for concentration ranges of 15–500 mg/mL. The formulations were found to be resuspended and homogeneous after blending.

Stability of the 15.6 mg/mL and 500 mg/mL formulations was confirmed for 42 days when protected from light and stored at room (~25°C), refrigerated (5°C), and freezer (–20°C) temperatures. A dosing simulation study on the 15.6 mg/mL formulation found that it was stable when stored at room temperature in an open amber vessel for 3 hours.

Analysis of preadministration and postadministration dose formulations were conducted throughout the study by the study laboratory (Appendix A). Postadministration samples were collected from the bottles used to dose the animals. All preadministration samples from the 2-week and 3-month studies administered to animals were within 10% of the target concentrations, except for a 125 mg/mL dose formulation prepared for the mouse study in September 2008. Postadministration samples of the 62.5 mg/mL dose formulation in the 2-week studies for mice and F344/NTac rats were 17.5% and 19.5% above the target concentration, respectively. Postadministration samples of the 125 and 250 mg/mL dose formulations prepared in September 2008 for the 3-month mouse studies were 10.9% and 14.3% above the target concentrations, respectively. All other samples were within 10% of the target concentration.

Animal Source

Male and female F344/NTac rats and B6C3F1/N mice were obtained from Taconic Biosciences, Inc. (Germantown, NY) for the 2-week studies. For the 3-month studies, time-mated (F_0) female Wistar Han [Crl:WI(Han)] rats were obtained from Charles River Laboratories (Raleigh, NC); male and female B6C3F1/N mice were obtained from Taconic Biosciences, Inc. (Germantown, NY). The rationale for change of rat strain from F344/NTac to Wistar Han [Crl:WI(Han)] was a programmatic decision. For many years, the National Toxicology Program (NTP) used the inbred F344/N rat for its toxicity and carcinogenicity studies. Over time, the F344/N rat exhibited sporadic seizures and idiopathic chylothorax, and consistently high rates of mononuclear cell leukemia and testicular neoplasia. Because of these issues in the F344/N rat and NTP's desire to find a more fecund rat model that could be used in both reproductive and carcinogenesis studies for comparative purposes, a change in the rat model was explored. After a workshop held by NTP in 2005, use of the F344/N rat was discontinued and NTP switched to the F344/NTac rat[118] while other rat models were being investigated. The Wistar Han rat, an outbred stock, [Crl:WI(Han)] was eventually selected[119] because it was projected to have a long lifespan, resistance to disease, large litter size, and low neonatal mortality.[120]

Animal Welfare

Animal care and use are in accordance with the Public Health Service Policy on Humane Care and Use of Animals. All animal studies were conducted in an animal facility accredited by AAALAC International. Studies were approved by the Battelle (Columbus, OH) Animal Care and Use Committee and conducted in accordance with all relevant National Institutes of Health

(NIH) and NTP animal care and use policies and applicable federal, state, and local regulations and guidelines.

Two-week Studies

F344/NTac rats and B6C3F1/N mice were approximately 3 to 4 (rats) or 4 to 5 (mice) weeks old on receipt. Animals were quarantined for 11 days, and both F344/NTac rats and B6C3F1/N mice were approximately 5 to 6 weeks old on the first day of the studies. F344/NTac rats and B6C3F1/N mice were randomly assigned to one of six dose groups before the start of the study. Randomization was stratified by body weight to produce similar group mean weights using PATH/TOX SYSTEM software (Xybion Medical Systems Corporation, Lawrenceville, NJ).

Before the studies began, five male and five female F344/NTac rats and B6C3F1/N mice were randomly selected for parasite evaluation and gross observation for evidence of disease. Additionally, the health of the animals was monitored during the studies according to the protocols of the NTP Sentinel Animal Program (Appendix C). All test results were negative.

Groups of five male and five female F344/NTac rats and five male and five female B6C3F1/N mice were administered 0, 78, 156, 312.5, 625, or 1,250 mg RES/kg body weight/day (mg/kg/day) (F344/NTac rats) and 0, 156, 312, 625, 1,250, or 2,500 mg/kg/day (B6C3F1/N mice) in 0.5% aqueous methylcellulose by gavage 5 days per week for 2 weeks. These doses were selected based on available literature at the time of study design[86] and limitations in chemical formulation and administration. Although a previous 28-day study in CD® Virus Antibody Free (VAF) rats reported nephrotoxicity and limited lethality at 3,000 mg/kg/day,[86] challenges with formulating a gavage-able dose in methylcellulose to achieve that concentration necessitated lowering the high dose for both rats and mice. Because no data were available on the toxicity of higher doses in mice, the high dose given to mice was the highest possible given chemistry limitations and was greater than the high dose administered in rats.

Vehicle control animals were administered the 0.5% aqueous methylcellulose vehicle alone; dosing volumes were 5 mL/kg body weight (mL/kg) for F344/NTac rats and 10 mL/kg for B6C3F1/N mice. Animals were administered the dose for at least 2 consecutive days prior to necropsy. Feed and water were available ad libitum. F344/NTac rats and B6C3F1/N mice were housed individually (male B6C3F1/N mice) or five per cage by sex (male and female F344/NTac rats, female B6C3F1/N mice). F344/NTac rats and B6C3F1/N mice were observed twice daily for signs of mortality or moribundity. Clinical observations were recorded daily, and body weights were recorded initially, on day 8, and at study termination. Details of the study design and animal maintenance are summarized in Table 1. Information on feed composition and contaminants is provided in Appendix B.

Necropsies were performed on all F344/NTac rats and B6C3F1/N mice. Organ weights were determined for the heart, right kidney, liver, lung, right testis, and thymus. Tissues for microscopic examination were fixed and preserved in 10% neutral buffered formalin (except eyes, which were first fixed in Davidson's solution), processed and trimmed, embedded in paraffin, sectioned to a thickness of approximately 5 μm, and stained with hematoxylin and eosin (H&E). Gross lesions were examined microscopically. As no dose-related changes were found at gross necropsy in F344/NTac rats, no further microscopic examinations were performed. While no dose-related changes were found at gross necropsy in B6C3F1/N mice, there were possible

dose-related changes in the weights of the liver and thymus; the liver and thymus were examined microscopically on all vehicle control and 2,500 mg/kg/day group mice. Table 1 lists the tissues and organs examined.

Three-month Studies

Study Design for Wistar Han Rats

F_0 female Wistar Han rats were 11 to 12 weeks old upon receipt. Gestation day (GD) 1 was defined as the first day with evidence of mating; F_0 females were received from Charles River Laboratories (Raleigh, NC) on GD 2 and held for 4 days. F_0 females were randomly assigned to one of six dose groups on GD 5. To ensure at least 16 females in the 0, 78, 312.5, and 1,250 mg/kg/day groups, 33 females were assigned to each dose group to allow sufficient animals for biological sampling. Randomization was stratified by body weight to produce similar group mean weights using PATH/TOX SYSTEM software (Xybion Medical Systems Corporation, Lawrenceville, NJ).

F_0 Wistar Han females were quarantined for 37 days after receipt. Ten nonmated females received in the same shipment as the time-mated dams were designated for disease monitoring and were used for gross necropsies 2 days after arrival; samples were collected for parasite evaluation and gross observation of disease. The health of the F_1 animals was monitored during the studies according to the protocols of the NTP Sentinel Animal Program (Appendix C). All test results were negative.

Beginning on GD 6, F_0 female Wistar Han rats were administered RES in 0.5% aqueous methylcellulose by gavage throughout gestation and lactation at one of five dose levels (78, 156, 312.5, 625, or 1,250 mg/kg/day) or the vehicle control (0.5% aqueous methylcellulose). Formulations were administered daily, except for the day of delivery if the dam was in the process of delivering; dosing volumes were 5 mL/kg. F_0 females were weighed on GD 5 (for randomization) and daily (except for the day of delivery) throughout the perinatal period; the dosing volume was calculated using the dam's most recent body weight. Feed and water were available ad libitum. F_0 females were housed individually during gestation and with their respective litters during lactation. Cages were changed weekly for pregnant dams before delivery and twice weekly for dams and their litters after postnatal day (PND) 4.

The day of parturition was considered lactation day (LD) 0 for dams and PND 0 for pups. F_0 female Wister Han rats that did not deliver were euthanized on GD 27, and the uteri were examined for evidence of implantation. On PND 1, clinical observations and litter weights by sex were recorded. From PND 2 through PND 12 (when the F_1 pups began receiving the formulations by gavage), the number of pups for each litter was recorded twice daily. F_1 pups were individually weighed on PNDs 4, 7, 12, 15, 18, and 21.

On PND 4, the number of litters was reduced to seven litters per dose group, and litters were standardized to eight Wistar Han pups per litter (four males and four females when possible); litters used had a minimum of at least three pups per sex. On the day the last litter reached PND 18, five litters per group were randomly selected and two pups per sex from each litter were randomly selected for use in the 3-month study. On the day the last litter reached PND 21, the

pups were weaned, and dams were removed from the cages and humanely euthanized with carbon dioxide. Weaning marked the beginning of the 3-month study.

After weaning, F_1 pups were housed five per cage. Groups of 10 male and 10 female Wistar Han F_1 pups were administered 0, 78, 156, 312.5, 625, or 1,250 mg/kg/day in 0.5% aqueous methylcellulose by gavage 5 days per week for 3 months. Pups were administered the same dose their respective dam received during gestation and lactation, and F_1 pups began receiving these doses on PND 12 via gavage. Vehicle control animals were administered the 0.5% aqueous methylcellulose vehicle alone. Dosing volumes were 5 mL/kg, using the animal's most recent body weight, and dosing was completed by noon each day. Feed and water were available ad libitum. Two diets were utilized in the rat studies: (1) NIH-07 during the perinatal phase and (2) NTP-2000 during the postweaning phase. The NIH-07 diet is a higher protein diet that supports reproduction and lactation in rodents, whereas the NTP-2000 diet is a lower protein diet that decreases the incidence of chronic nephropathy in adult rats. Cages were changed twice weekly and rotated every 2 weeks. Details of the study design and animal maintenance are summarized in Table 1. Information on feed composition and contaminants is provided in Appendix B.

Throughout the perinatal phase and at study termination, biological samples were collected from the 0, 78, 312.5, and 1,250 mg/kg/day groups and stored at approximately −20°C before shipment on dry ice to RTI International (Research Triangle Park, NC) for analysis to confirm internal dose (Appendix D). On GD 18, blood was collected from the retroorbital sinus of three randomly selected dams from each dose group at 30 minutes, 60 minutes, or 90 minutes after RES administration (nine dams per dose group in total). Animals were anesthetized with a carbon dioxide/oxygen mixture and blood was collected into tubes containing sodium heparin, centrifuged, and the plasma harvested. The dams were then humanely euthanized with carbon dioxide, and the fetuses were removed and individually flash frozen in liquid nitrogen. On PND 4, following dose administration to dams, 10 male and 10 female randomly selected standardized pups for each dose group were humanely euthanized by decapitation by noon, placed into individual vials (one pup per vial), and flash frozen in liquid nitrogen. On PND 21, following the last dose administration to pups, blood was collected via cardiac puncture from five male and five female Wistar Han rat pups from each dose group. Animals were first anesthetized with a carbon dioxide/oxygen mixture and blood was then collected into tubes containing lithium heparin, centrifuged, and the plasma harvested. After blood collection, the pups were humanely euthanized by carbon dioxide inhalation overdose and were disposed of without further evaluation. At study termination (24 hours after the last dose), blood was collected from the retroorbital sinus of five randomly selected animals per sex for each dose group for biological sampling. Animals were anesthetized with a carbon dioxide/oxygen mixture and blood was collected into tubes containing sodium heparin, centrifuged, and the plasma harvested.

Study Design for B6C3F1/N Mice

Male and female B6C3F1/N mice were 4–5 weeks old on receipt. Animals were quarantined for 11 (females) or 12 (male) days and mice were approximately 5 to 6 weeks old on the first day of the study. Mice were randomly assigned to one of six dose groups before the start of the study. Randomization was stratified by body weight to produce similar group mean weights using PATH/TOX SYSTEM software (Xybion Medical Systems Corporation, Lawrenceville, NJ).

Before the studies began, five male and five female mice were randomly selected for parasite evaluation and gross observation for evidence of disease. In addition, 10 male and 10 female mice were selected for 4-week and end-of-study serologies. The health of the animals was monitored during the studies according to the protocols of the NTP Sentinel Animal Program (Appendix C). All test results were negative.

Groups of 10 male and 10 female mice were administered RES at doses of 0, 156, 312, 625, 1,250, or 2,500 mg/kg/day in 0.5% aqueous methylcellulose by gavage 5 days per week for 3 months. Vehicle control animals were administered the 0.5% aqueous methylcellulose vehicle alone. Dosing volumes were 10 mL/kg, using the animal's most recent body weight, and dosing was completed by noon each day. Animals were administered the dose for at least 2 consecutive days prior to necropsy. Feed and water were available ad libitum. Male mice were housed individually, whereas female mice were housed five per cage. Cages were changed weekly (males) or twice weekly (females) and rotated every 2 weeks. Details of the study design and animal maintenance are summarized in Table 1. Information on feed composition and contaminants is provided in Appendix B.

Clinical Examinations and Pathology

During the 3-month studies, Wistar Han rats and B6C3F1/N mice were observed twice daily for signs of morbidity or moribundity and weighed prior to dosing on day 1, weekly thereafter, and at study termination. Clinical observations were recorded after dosing on day 1, weekly thereafter, and at study termination.

At the end of the 3-month studies, animals were anesthetized with a carbon dioxide/oxygen mixture and bled in random order. Blood was collected from the retroorbital site of all animals for hematology, clinical chemistry (rats only), and erythrocyte micronuclei analyses. Blood for hematology and micronuclei determinations was collected into tubes containing ethylenediaminetetraacetic acid (EDTA). Blood for clinical chemistry measurements was collected into serum separator tubes, centrifuged, and the serum harvested. Hematology parameters were analyzed using an Advia 120 hematology analyzer (Bayer Diagnostics Division, Tarrytown, NY). Clinical chemistry parameters were analyzed using a Roche cobas c501 chemistry analyzer (Roche, Indianapolis, IN). The parameters measured are listed in Table 1. Samples for erythrocyte micronuclei determination were stored at 2°C–8°C immediately after collection and shipped to Integrated Laboratory Systems, LLC (ILS, Durham, NC) for analysis.

At the end of the 3-month studies, samples were collected for sperm motility and vaginal cytology evaluations from F_1 male and female Wistar Han rats in the 0, 312.5, 625, and 1,250 mg/kg/day groups and from male and female B6C3F1/N mice in the 0, 625, 1,250, and 2,500 mg/kg/day groups. The parameters evaluated are listed in Table 1. For 16 consecutive days before scheduled study termination, the vaginal vaults of the females were moistened with saline, if necessary, and samples of vaginal fluid and cells were collected and subsequently stained. Relative numbers of leukocytes, nucleated epithelial cells, and large squamous epithelial cells were determined and used to ascertain estrous cycle stage (i.e., diestrus, proestrus, estrus, and metestrus). Measured parameters of cycle length, number of cycles, and time spent in any specific stage of the estrous cycle of female rats and mice are presented in Appendix F. Male animals were evaluated for sperm count and motility. The left testis and left epididymis were

isolated and weighed. The tail of the epididymis (cauda epididymis) was then removed from the epididymal body (corpus epididymis) and weighed. Test yolk (rats) or modified Tyrode's buffer (mice) was applied to slides and a small incision was made at the distal border of the cauda epididymis. The sperm effluxing from the incision were dispersed in the buffer on the slides, and the numbers of motile and nonmotile spermatozoa were counted for five fields per slide by two observers. After completion of sperm motility estimates, each left cauda epididymis was placed in buffered saline solution. Caudae were finely minced, and the tissue was incubated in the saline solution at 34°C–38°C. Sperm density was determined microscopically with the aid of a hemocytometer. To quantify spermatogenesis, the testicular spermatid head count was determined by removing the tunica albuginea and homogenizing the left testis in phosphate-buffered saline containing 10% dimethyl sulfoxide. Homogenization-resistant spermatid nuclei were counted with a hemocytometer.

Necropsies were performed on all animals. Organ weights were determined for the heart, right kidney, liver, lung, right testis, and thymus. Tissues for microscopic examination were fixed and preserved in 10% neutral buffered formalin (except eyes, which were first fixed in Davidson's solution, and testes, vaginal tunics, and epididymides, which were first fixed in modified Davidson's solution), processed and trimmed, embedded in paraffin, sectioned to a thickness of 5 μm, and stained with H&E. Complete histopathological examinations were performed by the study laboratory pathologist on all vehicle control and 1,250 mg/kg/day Wistar Han rats and all vehicle control and 2,500 mg/kg/day B6C3F1/N mice. The kidney, intestine (jejunum; rats only), and nose (mice only) were identified as target organs and examined to a no-effect level. Table 1 lists the tissues and organs examined.

After a review of the laboratory reports and selected histopathological slides by a quality assessment (QA) pathologist, the findings and reviewed slides were submitted to an NTP Pathology Working Group (PWG) coordinator for a second independent review. Any inconsistencies in the diagnoses made by the study laboratory and QA pathologists were resolved by the NTP pathology peer-review process. Final diagnoses for reviewed lesions represent a consensus of the PWG or a consensus between the study laboratory pathologist, NTP pathologist, QA pathologist, and the PWG coordinator. Details of these review procedures have been described, in part, by Maronpot and Boorman[121] and Boorman et al.[122]

Table 1. Experimental Design and Materials and Methods in the Two-week and Three-month Gavage Studies of *Trans*-resveratrol

Two-week Studies	Three-month Studies
Study Laboratory	
Battelle (Columbus, OH)	Same as 2-week studies
Strain and Species	
Rats: Fischer 344 (F344/NTac)	Rats: Wistar Han [Crl:WI(Han)]
Mice: B6C3F1/N	Mice: Same as 2-week studies
Animal Source	
Rats: Taconic Biosciences, Inc. (Germantown, NY)	Rats: Charles River Laboratories (Raleigh, NC)
Mice: Taconic Biosciences, Inc. (Germantown, NY)	Mice: Same as 2-week studies

Two-week Studies	Three-month Studies
Time Held Before Studies	
Rats: 11 days	F_0 female rats: 4 days
Mice: 11 days	Mice: 11 (females) or 12 (males) days
Average Age When Studies Began	
Rats: 5 to 6 weeks	F_0 female rats: 12 to 13 weeks
Mice: 5 to 6 weeks	Mice: 5 to 6 weeks
Date of First Dose	
Rats: October 16, 2006	F_0 female rats: October 17, 2008 F_1 rats: PND 12 (November 14 [males] or 15 [females], 2008)
Mice: October 16, 2006	Mice: September 22 (females) or 23 (males), 2008
Duration of Dosing	
Rats: 5 days a week for 2 weeks	F_0 female rats: GD 6 through LD 21 F_1 rats: daily PND 12 to 21 then 5 days a week for 3 months
Mice: 5 days a week for 2 weeks	Mice: 5 days a week for 3 months
Date of Last Dose	
Rats: October 30, 2006	F_0 female rats: November 24, 2008 F_1 rats: February 25 (males) or 26 (females), 2009
Mice: October 31, 2006	Mice: December 21 (females) or 22 (males), 2008
Necropsy Dates	
Rats: October 31, 2006	F_1 rats: February 26 (males) or 27 (females), 2009
Mice: November 1, 2006	Mice: December 22 (females) or 23 (males), 2008
Average Age at Necropsy	
Rats: 7 to 8 weeks	F_1 rats: 17 weeks
Mice: 8 to 9 weeks	Mice: 18 to 19 weeks
Size of Study Groups	
Rats: 5 males and 5 females	F_0 female rats: 33 (0, 78, 312.5, and 1,250 mg/kg/day) or 16 (156 and 625 mg/kg/day) F_1 rats: 10 males and 10 females
Mice: 5 males and 5 females	Mice: 10 males and 10 females

Two-week Studies	Three-month Studies
Method of Distribution	
Rats: Animals were distributed randomly into groups of approximately equal initial mean body weights.	Rats: Dams were distributed randomly into groups of approximately equal initial mean body weights. Pups were standardized on each litter's respective PND 4 to a maximum of eight pups/litter. On the day the last litter reached PND 18, five litters/group were randomly selected and two pups/sex from each litter were randomly selected for use in the 3-month study.
Mice: Animals were distributed randomly into groups of approximately equal initial mean body weights.	Mice: Same as 2-week studies
Animals/Cage	
Rats: 5 (males) or 5 (females)	F_0 female rats: 1 (with litter) F_1 rats: 5 (males) or 5 (females)
Mice: 1 (male) or 5 (females)	Mice: 1 (male) or 5 (females)
Method of Animal Identification	
Tail tattoo	F_0 female rats: Cage card and tail mark F_1 rats (pups): Limb tattoo F_1 rats (3-month): Cage card and tail tattoo Mice: Cage card and tail tattoo
Diet	
Rats: Irradiated NTP-2000 wafer feed (Zeigler Brothers, Gardners, PA), available ad libitum, changed at least weekly	F_0 female rats: Irradiated NIH-07 wafer feed (Zeigler Brothers, Gardners, PA), available ad libitum, changed at least weekly F_1 rats: Same as 2-week studies
Mice: Irradiated NTP-2000 wafer feed (Zeigler Brothers, Gardners, PA), available ad libitum, changed at least weekly	Mice: Same as 2-week studies
Water	
Tap water (Columbus municipal supply) via automatic watering system (Edstrom Industries, Waterford, WI), available ad libitum, changed at least every 2 weeks	Same as 2-week studies
Cages	
Solid polycarbonate (Lab Products, Inc., Seaford, DE)	Same as 2-week studies
Rats: Changed at least twice weekly, rotated every 2 weeks	Rats: Changed at least weekly for pregnant dams, at least twice weekly for dams and their litters after PND 4, and at least twice weekly for F_1 rats; rotated every 2 weeks
Mice: Changed at least weekly (males) or twice weekly (females), rotated every 2 weeks	Mice: Same as 2-week studies
Bedding	
Irradiated Sani-Chips® (P.J. Murphy Forest Products Corporation, Montville, NJ), changed with cage changes	Same as 2-week studies

Two-week Studies	Three-month Studies
Racks	
Stainless steel (Lab Products, Inc., Seaford, DE), changed and rotated every 2 weeks	Same as 2-week studies
Rack Filters	
Spun-bonded polyester (Snow Filtration Company, Cincinnati, OH), changed every 2 weeks	Same as 2-week studies
Animal Room Environment	
Temperature: 72°F ± 3°F Relative humidity: 50% ± 15% Room fluorescent light: 12 hours/day Room air changes: at least 10/hour	Same as 2-week studies
Doses	
Rats: 0, 78, 156, 312.5, 625, and 1,250 mg/kg/day in 0.5% aqueous methylcellulose; 5 mL/kg dosing volume	Rats: Same as 2-week studies
Mice: 0, 156, 312, 625, 1,250, and 2,500 mg/kg/day in 0.5% aqueous methylcellulose; 10 mL/kg dosing volume	Mice: Same as 2-week studies
Type and Frequency of Observation	
Observed twice daily. Weighed initially, on day 8, and at study termination. Clinical observations were recorded daily throughout the study.	F_0 female rats: Observed twice daily. Weighed on GD 5 and daily throughout the perinatal period (except possibly the day of delivery). F_1 rats: Observed twice daily. Litter data (litter weights by sex and litter observations) were recorded on PND 1. Pups/litter were recorded daily from PND 2 through PND 21. Pups were weighed on PNDs 4, 7, 12, 15, 18, and 21, weekly thereafter, and at study termination. Clinical observations were recorded initially, weekly thereafter, and at study termination. Mice: Observed twice daily. Weighed and clinical observations recorded initially, weekly thereafter, and at the study termination.
Method of Euthanasia	
100% carbon dioxide	Same as 2-week studies
Necropsy	
Necropsies were performed on all animals. Organs weighed at study termination were: liver, thymus, right kidney, right testis, heart, and lungs.	Same as 2-week studies

Two-week Studies	Three-month Studies
Clinical Pathology	
None	At study termination, blood was collected from the retroorbital site for hematology, clinical chemistry (rats only), and erythrocyte micronuclei determinations. *Hematology*: hematocrit; manual hematocrit; hemoglobin concentration; erythrocyte, reticulocyte, and platelet counts; mean cell volume; mean cell hemoglobin; mean cell hemoglobin concentration; white blood cell count and differential; blood smear morphological evaluation *Clinical chemistry (rats)*: urea nitrogen, creatinine, glucose, total protein, albumin, globulin, A:G ratio, cholesterol, triglycerides, alanine aminotransferase, alkaline phosphatase, creatine kinase, sorbitol dehydrogenase, bile acids
Histopathology	
Rats: Gross lesions were examined microscopically, and, as no dose-related changes were found at gross necropsy, no further microscopic examinations were performed. Mice: Gross lesions in all dose groups were examined microscopically; the liver and thymus were also examined in the vehicle control and 2,500 mg/kg/day groups.	Complete histopathology was performed on all F_1 rats and all mice in the vehicle control and 1,250 (rats) and 2,500 mg/kg/day (mice) groups. The kidney, intestine (jejunum) (rats), and nose (mice) were identified as target organs and examined to a no-effect level. In addition to gross lesions and tissue masses, the following tissues were examined: adrenal gland, bone with marrow, brain, clitoral gland, esophagus, eyes, gallbladder (mice), Harderian gland, heart and aorta, kidneys, large intestine (cecum, colon, and rectum), small intestine (duodenum, jejunum, and ileum), liver, lung, lymph nodes (mandibular and mesenteric), mammary gland, nose, ovary, pancreas, parathyroid gland, pituitary gland, preputial gland, prostate gland, salivary gland, seminal vesicle, spleen, stomach (forestomach and glandular), testis with epididymis, thymus, thyroid gland, trachea, urinary bladder, and uterus.
Sperm Motility and Vaginal Cytology	
None	At study termination, sperm samples were collected from F_1 male rats in the vehicle control, 312.5, 625, and 1,250 mg/kg/day groups and from male mice in the vehicle control, 625, 1,250, and 2,500 mg/kg/day groups for sperm motility evaluations. The following parameters were evaluated: spermatid heads per testis and per gram testis and epididymal spermatozoal motility and concentration. The left cauda, left epididymis, and left testis were weighed. Vaginal samples were collected for up to 16 consecutive days before study termination from F_1 female rats in the vehicle control, 312.5, 625, and 1,250 mg/kg/day groups and from female mice in the vehicle control, 625, 1,250, and 2,500 mg/kg/day groups for vaginal cytology evaluations.

Two-week Studies	Three-month Studies
Internal Dose Assessment	
None	Rats: *Trans*-resveratrol, *trans*-resveratrol-3-O-B-D-glucuronide, and *trans*-resveratrol-3-sulfate concentrations were measured in the vehicle control, 78, 312.5, and 1,250 mg/kg/day groups in the following Wistar Han rat samples: F_0 maternal plasma on GD 18; F_1 fetuses at 30, 60, and 90 minutes following dose administration on GD 18; whole pups on PND 4; F_1 pup plasma on PND 21; and F_1 offspring plasma at necropsy. Mice: None

PND = postnatal day; GD = gestation day; LD = lactation day.

Statistical Methods

Calculation and Analysis of Nonneoplastic Lesion Incidences

Incidences of nonneoplastic lesions are presented as numbers of animals bearing such lesions at a specific anatomical site and the numbers of animals with that site examined microscopically. For the 3-month study in mice, Fisher's exact test,[123] a procedure that uses the overall proportion of affected animals, was used to identify statistically significant differences between animals administered RES and vehicle control animals, and the Cochran-Armitage trend test was used to test for significant trends.[124]

Because up to two pups/sex/litter were present in the perinatal and 3-month study in rats, the Cochran-Armitage test was modified to accommodate litter effects using the Rao-Scott approach.[125] The Rao-Scott approach accounts for litter effects by estimating the ratio of the variance in the presence of litter effects to the variance in the absence of litter effects. This ratio is then used to adjust the sample size downward to yield the estimated variance in the presence of litter effects. The Rao-Scott approach was implemented in the Cochran-Armitage test as recommended by Fung et al.[126] formula $\overline{T}_{RS2}$.

Analysis of Continuous Variables

Two approaches were employed to assess the significance of pairwise comparisons between dosed and vehicle control groups in the analysis of continuous variables. Organ and body weight data, which historically have approximately normal distributions, were analyzed with the parametric multiple comparison procedures of Dunnett[127] and Williams.[128; 129] Hematology, dam gestation length, litter size and survival, internal dose assessment, spermatid, and epididymal spermatozoal data, which typically have skewed distributions, were analyzed using the nonparametric multiple comparison methods of Shirley[130] (as modified by Williams[131] and Dunn[132]). The Jonckheere test[133] was used to assess the significance of dose-related trends and to determine whether a trend-sensitive test (the Williams or Shirley test) was more appropriate for pairwise comparisons than a test that does not assume a monotonic dose-related trend (the Dunnett or Dunn test).

For the perinatal and 3-month study in rats, postweaning body weights were measured on two pups/sex/litter; more than two pups/sex/litter were possible in preweaning body weight

measurements. The analyses of preweaning pup body weights took litter effects into account by use of mixed-effects regression wherein litters were the random effects.

For some endpoints in the perinatal and 3-month study in rats, such as hematology, clinical chemistry, and internal dose, data were collected for 10 animals across five different source litters. In these cases, the trend across dose groups was analyzed by a permutation test based on the Jonckheere trend test, implemented by randomly permuting whole litters across dose groups and bootstrapping within the litters.[134] Pairwise comparisons were made using a modified Wilcoxon test that incorporated litter effects.[135] The Hommel procedure was used to adjust for multiple comparisons.

Before statistical analysis, extreme values identified by the outlier test of Dixon and Massey[136] were examined by NTP personnel, and implausible values were eliminated from the analysis.

Analysis of Vaginal Cytology Data

Vaginal cytology data consist of daily observations of estrous cycle stages over a 16-day period. Differences from the vehicle control group for cycle length and number of cycles were analyzed using a Datta-Satten modified Wilcoxon test with a Hommel adjustment for multiple comparisons.

To identify disruptions in estrous cyclicity, a continuous-time Markov chain model (multi-state model) was fit using a maximum likelihood approach,[137] producing estimates of stage lengths for each dose group. Confidence intervals for these estimates were obtained based on bootstrap sampling of the individual animal cycle sequences. Stage lengths that were significantly different from the vehicle control group were identified using permutation testing with a Hommel adjustment for multiple comparisons.

Analysis of Reproductive Performance Data

Reproductive performance data for the perinatal and 3-month study in rats were analyzed using the Cochran-Armitage trend test and the Fisher's exact pairwise test.

Quality Assurance Methods

The 2-week and 3-month studies were conducted in compliance with the U.S. Food and Drug Administration Good Laboratory Practice Regulations.[138] In addition, the 2-week and 3-month study reports were audited retrospectively by an independent QA contractor against study records submitted to the NTP Archives. Separate audits covered completeness and accuracy of the pathology data, pathology specimens, final pathology tables, and a draft of this NTP Toxicity Report. Audit procedures and findings are presented in reports on file at NIEHS. The audit findings were reviewed and assessed by NTP staff, and all comments were resolved or otherwise addressed during the preparation of this toxicity report.

Genetic Toxicology

The genetic toxicity of RES was assessed by testing the ability of the chemical to induce mutations in various strains of *Salmonella typhimurium* and to increase the frequency of micronucleated erythrocytes in rat and mouse peripheral blood. The protocol for these studies and the results are given in Appendix D. The RES test article for the bacterial mutagenicity

studies was obtained from a different manufacturer (Chromadex; Los Angeles, CA) than that used for the in vivo studies.

The genetic toxicity studies have evolved from an earlier effort by NTP to develop a comprehensive database permitting a critical anticipation of a chemical's carcinogenicity in experimental animals based on numerous considerations, including the molecular structure of the chemical and its observed effects in short-term in vitro and in vivo genetic toxicity tests (structure-activity relationships). The short-term tests were originally developed to clarify proposed mechanisms of chemical-induced DNA damage based on the relationship between electrophilicity and mutagenicity[139] and the somatic mutation theory of cancer.[140; 141] Of note, however, not all cancers arise through genotoxic mechanisms.

Bacterial Mutagenicity

DNA reactivity combined with *Salmonella* mutagenicity is highly correlated with induction of carcinogenicity in multiple species/sexes of rodents and at multiple tissue sites.[142] A positive response in the *Salmonella* test was shown to be the most predictive in vitro indicator for rodent carcinogenicity (89% of the *Salmonella* mutagens are rodent carcinogens).[143; 144] Additionally, no battery of tests that included the *Salmonella* test improved the predictivity of the *Salmonella* test alone. However, these other tests can provide useful information on the types of DNA and chromosomal damage induced by the chemical under investigation.

Peripheral Blood Micronucleus Test

Micronuclei (literally "small nuclei" or Howell-Jolly bodies) are biomarkers of induced structural or numerical chromosomal alterations and are formed when acentric fragments or whole chromosomes fail to incorporate into either of two daughter nuclei during cell division.[145; 146] The predictivity for carcinogenicity of a positive response in acute in vivo bone marrow chromosome aberration or micronucleus tests appears to be less than that in the *Salmonella* test.[147; 148] However, clearly positive results in long-term peripheral blood micronucleus tests have high predictivity for rodent carcinogenicity; a weak response in one sex only or negative results in both sexes in this assay do not correlate well with either negative or positive results in rodent carcinogenicity studies.[149] Because of the theoretical and observed associations between induced genetic damage and adverse effects in somatic and germ cells, the determination of in vivo genetic effects is important to the overall understanding of the risks associated with exposure to a particular chemical.

Results

Data Availability

The National Toxicology Program (NTP) evaluated all study data. Data relevant for evaluating toxicological findings are presented here. All study data are available in the NTP Chemical Effects in Biological Systems (CEBS) database: https://doi.org/10.22427/NTP-DATA-TOX-102.[150]

Rats

Two-week Study in F344/NTac Rats

All Fischer 344 (F344/NTac) rats survived until study termination following administration of *trans*-resveratrol (RES) (Table 2). Mean body weights of all dosed groups were within 5% of the vehicle control groups at all measured time points (Table 2; Figure 2). No dose-related clinical observations were noted (Appendix F). There were no significant dose-related differences in organ weights and no dose-related gross or microscopic findings (Appendix F).

Table 2. Summary of Survival and Mean Body Weights of Male and Female F344/NTac Rats in the Two-week Gavage Study of *Trans*-resveratrol

Concentration (mg/kg/day)	Survival[a]	Initial Body Weight (g)	Final Body Weight (g)	Final Weight Relative to Control Group (%)[b]
Male				
0	5/5	88.0	155.8	–
78	5/5	88.3	161.5	103.7
156	5/5	87.0	155.1	99.6
312.5	5/5	87.6	157.8	101.3
625	5/5	87.4	157.7	101.3
1,250	5/5	87.3	149.3	95.8
Female				
0	5/5	77.7	117.0	–
78	5/5	77.4	118.3	101.1
156	5/5	78.0	121.7	104.0
312.5	5/5	77.7	116.0	99.1
625	5/5	78.0	117.7	100.6
1,250	5/5	79.0	119.4	102.0

[a]Number of animals surviving at 2 weeks/number initially in group.
[b]100 × [(dose group mean − vehicle control group mean)/vehicle control group mean].

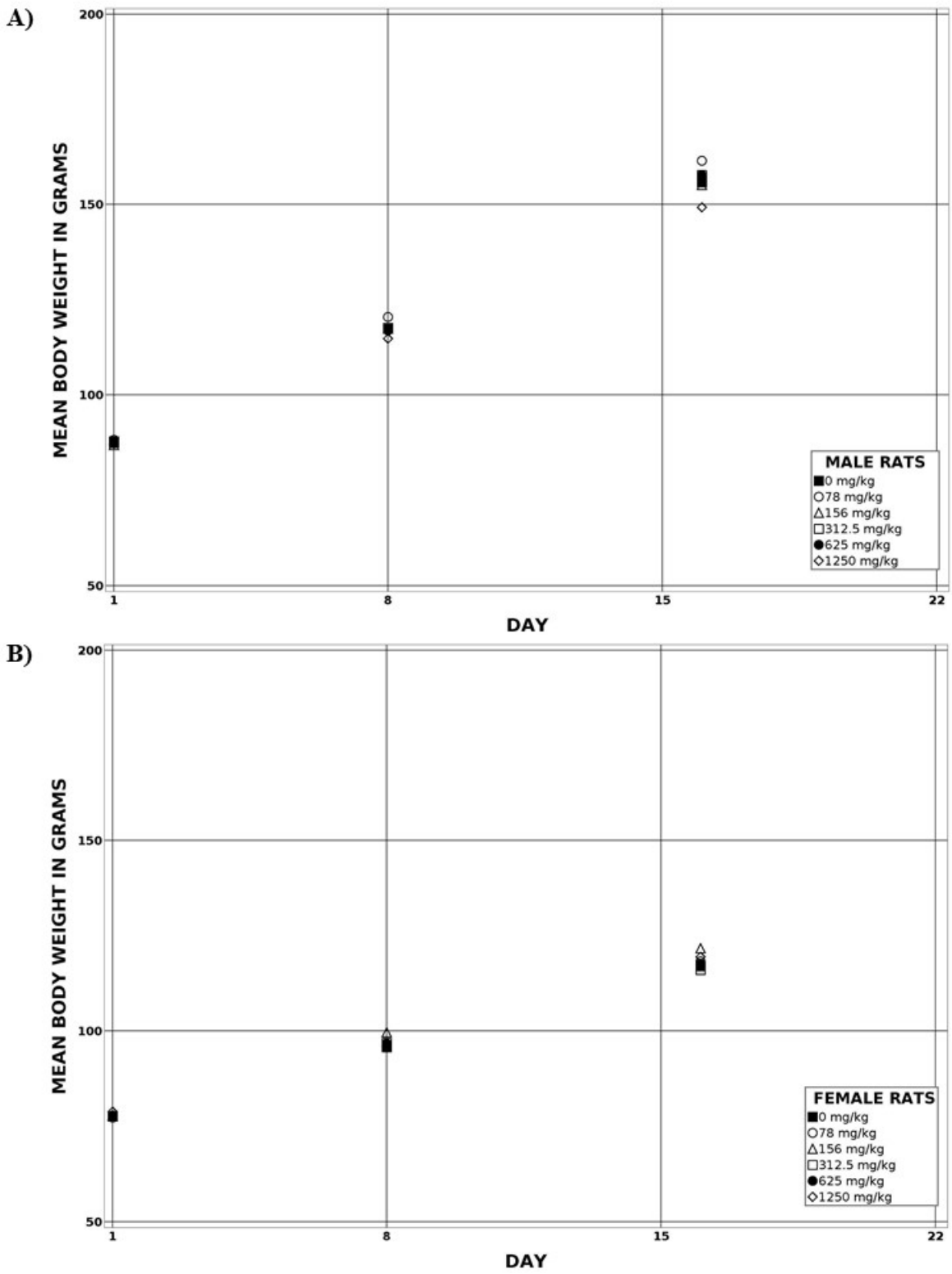


Figure 2. Growth Curves for Male and Female F344/NTac Rats in the Two-week Gavage Study of *Trans*-resveratrol

Growth curves for rats administered *trans*-resveratrol by gavage in (A) males and (B) females.

Dose Selection Rationale for Three-month Studies in Wister Han Rats

The same doses were used in the 3-month study in Wistar Han rats as were used in the 2-week studies in F344/NTac rats due to the absence of observed toxicity at the 2-week study doses. Due to programmatic changes in the NTP testing program, the F344/NTac strain of rat was changed to Wistar Han.[119] There was no evidence that there would be differential toxicity in this strain, thus a 2-week study in Wistar Han rats was not conducted.

Three-month Study in Wistar Han Rats (Perinatal Phase)

No significant dose-related differences were observed in pregnancy status, maternal survival, gestation length, or number of Wistar Han dams that littered (Table 3). Maternal mean body weights and body weight gains were lower in all dosed animals except for those in the lowest dose group, 78 mg RES/kg body weight/day (mg/kg/day). Maternal mean body weights were significantly decreased (4%–10% lower) relative to the vehicle control group starting on gestation day (GD) 15 in the 625 and 1,250 mg/kg/day groups, on GD 17 in the 312.5 mg/kg/day group, and on GD 18 in the 156 mg/kg/day group. Body weight gains were significantly decreased relative to the vehicle control group by 19%–35% in all but the 78 mg/kg/day group throughout gestation (GD 6–21). During lactation, there were no significant effects of RES on maternal mean body weights or body weight gains (Table 4). No dose-related clinical observations in pups or dams were noted (Appendix F). No significant differences in total or live litter size, sex ratio, or pup survival at PND 1 or 4 were observed (Table 5).

Table 3. Summary of the Disposition of F_0 Female Wistar Han Rats during Perinatal Exposure in the Perinatal and Three-month Gavage Study of *Trans*-resveratrol

Reproductive Performance	0 mg/kg/day	78 mg/kg/day	156 mg/kg/day	312.5 mg/kg/day	625 mg/kg/day	1,250 mg/kg/day
Time-mated Females (GD 6)	33	33	16	33	16	33
Females Pregnant (%)[a]	28 (84.8)	31 (93.9)	15 (93.8)	29 (87.9)	13 (81.3)	27 (81.8)
Females Not Pregnant (%)	5 (15.2)	2 (6.1)	1 (6.2)	4 (12.1)	3 (18.8)	6 (18.2)
Dams Not Delivering with Evidence of Pregnancy (%)	0 (0.0)	0 (0.0)	1 (6.7)	1 (5.0)	2 (15.4)	0 (0.0)
Dams with Litters on LD 0 (%)[b]	19 (100.0)	22 (100.0)	14 (93.3)	19 (95.0)	11 (84.6)	18 (100.0)
Gestation Length (Days)[c,d]	22.6 ± 0.1 (19)	22.7 ± 0.1 (22)	22.9 ± 0.1 (14)	22.9 ± 0.1 (19)	22.7 ± 0.2 (11)	22.7 ± 0.1 (18)
Litters Poststandardization (LD 4)[e]	7	7	7	7	7	7
Weaned Males/Females (LD 21)	28/28	28/24	28/27	29/27	29/27	28/21

GD = gestation day; LD = lactation day.
[a]Statistical analysis performed by the Cochran-Armitage (trend) and Fisher's exact (pairwise) tests.
[b]Animals (9 dams/group) from the 0, 78, 312.5, and 1,250 mg/kg/day groups were removed on GD 18 for biological sampling.
[c]Gestation length calculated for sperm-positive females that delivered a litter. Data are presented as mean ± standard error (number of dams).
[d]Statistical analysis performed by the Jonckheere (trend) and Shirley or Dunn (pairwise) tests.
[e]Standardization to eight pups/litter (four pups/sex when possible). Only litters with at least three pups/sex were used.

Table 4. Summary of Mean Body Weights and Body Weight Gains of F_0 Female Wistar Han Rats during Gestation and Lactation in the Perinatal and Three-month Gavage Study of *Trans*-resveratrol

Parameter[a,b]	0 mg/kg/day	78 mg/kg/day	156 mg/kg/day	312.5 mg/kg/day	625 mg/kg/day	1,250 mg/kg/day
Gestation Day						
6	210.4 ± 3.2 (28)	210.3 ± 2.8 (31)	210.7 ± 4.7 (15)	211.5 ± 3.1 (29)	211.8 ± 4.7 (13)	211.8 ± 3.1 (27)
9	220.0 ± 3.2 (28)	217.2 ± 3.0 (31)	218.3 ± 4.7 (15)	218.2 ± 3.0 (29)	217.0 ± 4.5 (13)	217.5 ± 3.2 (27)
12	234.1 ± 3.4 (28)	231.1 ± 2.9 (31)	229.2 ± 4.7 (15)	230.1 ± 3.0 (29)	227.1 ± 4.6 (13)	228.6 ± 3.3 (27)
15	248.8 ± 3.6** (28)	243.2 ± 3.0 (31)	240.3 ± 4.8 (15)	239.3 ± 3.2 (29)	231.8 ± 4.6* (13)	236.1 ± 3.1** (27)
18[c]	273.3 ± 5.4** (19)	263.6 ± 4.3 (22)	256.6 ± 5.8* (15)	260.0 ± 4.4* (20)	250.2 ± 6.0* (13)	258.4 ± 2.6** (18)
21	305.0 ± 6.4** (19)	298.7 ± 5.1 (22)	287.5 ± 7.6 (15)	286.7 ± 6.1* (20)	273.7 ± 8.3* (13)	288.8 ± 3.2* (18)
Gestation Body Weight Change						
Gestation Day Interval						
6–9	9.6 ± 0.8** (28)	6.9 ± 0.6* (31)	7.6 ± 0.6* (15)	6.6 ± 0.6** (29)	5.1 ± 1.0** (13)	5.6 ± 0.9** (27)
9–12	14.1 ± 0.6** (28)	13.9 ± 0.6 (31)	10.9 ± 0.8 (15)	12.0 ± 0.8* (29)	10.1 ± 1.0* (13)	11.2 ± 1.1** (27)
12–15	14.7 ± 0.6** (28)	12.1 ± 0.9* (31)	11.0 ± 1.0* (15)	9.1 ± 1.0** (29)	4.7 ± 1.9** (13)	7.4 ± 0.9** (27)
15–18[c]	24.2 ± 1.8 (19)	20.5 ± 1.3 (22)	16.3 ± 2.1** (15)	20.3 ± 1.7 (20)	18.4 ± 2.3 (13)	21.2 ± 1.3 (18)
18–21	31.7 ± 1.9 (19)	35.1 ± 1.4 (22)	30.9 ± 2.7 (15)	26.7 ± 2.4 (20)	23.5 ± 2.8* (13)	30.4 ± 1.6 (18)
6–21	95.0 ± 4.5** (19)	88.5 ± 3.4 (22)	76.8 ± 5.4** (15)	71.9 ± 5.4** (20)	61.8 ± 6.3** (13)	75.6 ± 3.1** (18)
Lactation Day						
1	245.6 ± 4.7* (19)	242.5 ± 3.9 (22)	239.2 ± 6.1 (14)	241.2 ± 3.7 (19)	234.5 ± 6.3 (11)	236.3 ± 2.9 (18)
4	256.3 ± 1.9 (7)[d]	250.3 ± 7.6 (7)	253.7 ± 8.5 (7)	251.9 ± 6.6 (7)	242.8 ± 7.9 (7)	244.9 ± 5.0 (7)
7	270.1 ± 3.2 (7)	260.5 ± 6.8 (7)	260.9 ± 7.9 (7)	266.2 ± 7.3 (7)	253.3 ± 7.6 (7)	258.7 ± 6.0 (7)
10	279.5 ± 4.0 (7)	267.1 ± 8.4 (7)	271.5 ± 8.0 (7)	271.1 ± 6.5 (7)	261.4 ± 7.8 (7)	267.6 ± 6.4 (7)
14	293.1 ± 5.5 (7)	275.8 ± 8.4 (7)	280.3 ± 7.5 (7)	285.1 ± 8.9 (7)	268.8 ± 7.9 (7)	277.0 ± 8.9 (7)

Parameter[a,b]	0 mg/kg/day	78 mg/kg/day	156 mg/kg/day	312.5 mg/kg/day	625 mg/kg/day	1,250 mg/kg/day
19	288.3 ± 4.2 (7)	279.2 ± 8.1 (7)	285.6 ± 9.1 (7)	284.5 ± 10.3 (7)	276.3 ± 9.3 (7)	282.2 ± 11.1 (7)
Lactation Body Weight Change						
Lactation Day Interval						
1–4	11.6 ± 2.9* (7)	14.8 ± 2.7 (7)	16.4 ± 3.0 (7)	11.8 ± 1.3 (7)	7.8 ± 1.5 (7)	7.5 ± 3.4 (7)
4–7	13.8 ± 3.1 (7)	10.2 ± 1.5 (7)	7.3 ± 2.9 (7)	14.3 ± 2.1 (7)	10.6 ± 3.2 (7)	13.8 ± 3.3 (7)
7–10	9.4 ± 2.9 (7)	6.6 ± 2.2 (7)	10.6 ± 2.8 (7)	4.9 ± 2.0 (7)	8.1 ± 2.5 (7)	8.9 ± 4.9 (7)
10–14	13.6 ± 3.0 (7)	8.7 ± 2.6 (7)	8.7 ± 2.6 (7)	13.9 ± 2.7 (7)	7.4 ± 2.3 (7)	9.5 ± 5.0 (7)
14-19	−4.8 ± 3.0 (7)	3.4 ± 2.2 (7)	5.4 ± 3.1 (7)	–0.5 ± 2.1 (7)	7.4 ± 4.3 (7)	5.2 ± 5.4 (7)
1–21	43.6 ± 3.7 (7)	43.8 ± 3.9 (7)	48.4 ± 4.0 (7)	44.5 ± 4.5 (7)	41.3 ± 6.4 (7)	44.8 ± 9.9 (7)

Statistical significance for a dose group indicates a significant pairwise test compared to the vehicle control group. Statistical significance for the vehicle control group indicates a significant trend test.
*Statistically significant at $p \leq 0.05$; **$p \leq 0.01$.
[a]Each dose group was compared to the vehicle control group with the Williams test when a trend was present ($p \leq 0.01$ from the Jonckheere trend test) or with the Dunnett test when no trend was present.
[b]Data are presented as mean ± standard error (number of dams). Body weight data are presented in grams.
[c]Animals (9 dams/group) from the 0, 78, 312.5, and 1,250 mg/kg/day groups were removed on gestation day 18 for biological sampling.
[d]Groups were standardized to seven litters/group on lactation day 4.

Table 5. Summary of Mean Litter Size and Survival Ratio of F_1 Male and Female Wistar Han Rats during Lactation in the Perinatal and Three-month Gavage Study of *Trans*-resveratrol

Parameter	0 mg/kg/day	78 mg/kg/day	156 mg/kg/day	312.5 mg/kg/day	625 mg/kg/day	1,250 mg/kg/day
PND 1						
Total[a,b]	8.58 ± 0.56 (19)	8.91 ± 0.50 (22)	8.00 ± 0.78 (13)	7.53 ± 0.76 (19)	7.64 ± 0.99 (11)	8.67 ± 0.54 (18)
Live[a,b]	8.58 ± 0.56 (19)	8.86 ± 0.49 (22)	8.00 ± 0.78 (13)	7.53 ± 0.76 (19)	7.64 ± 0.99 (11)	8.67 ± 0.54 (18)
% Male[c,d]	51.0 (149)	53.0 (185)	56.4 (94)	54.5 (143)	52.4 (84)	58.0 (150)
% Male/Litter[a,b]	51.3 ± 2.9 (18)	53.0 ± 5.2 (21)	58.4 ± 6.0 (12)	54.4 ± 4.9 (19)	43.4 ± 7.5 (11)	59.6 ± 4.5 (17)
Male[a,b]						
PND 1	4.22 ± 0.37 (18)	4.67 ± 0.52 (21)	4.42 ± 0.62 (12)	4.11 ± 0.57 (19)	4.00 ± 0.76 (11)	5.12 ± 0.44 (17)
PND 4 Prestandardization	4.17 ± 0.35 (18)	4.67 ± 0.52 (21)	4.42 ± 0.62 (12)	4.05 ± 0.55 (19)	4.00 ± 0.76 (11)	5.06 ± 0.46 (17)
PND 4 Poststandardization	4.00 ± 0.00 (7)	4.00 ± 0.00 (7)	4.00 ± 0.22 (7)	4.14 ± 0.14 (7)	4.14 ± 0.14 (7)	4.00 ± 0.22 (7)

Parameter	0 mg/kg/day	78 mg/kg/day	156 mg/kg/day	312.5 mg/kg/day	625 mg/kg/day	1,250 mg/kg/day
Female[a,b]						
PND 1	4.06 ± 0.36 (18)	4.14 ± 0.48 (21)	3.42 ± 0.57 (12)	3.42 ± 0.45 (19)	3.64 ± 0.36 (11)	3.71 ± 0.53 (17)
PND 4 Prestandardization	4.06 ± 0.36 (18)	4.14 ± 0.48 (21)	3.42 ± 0.57 (12)	3.37 ± 0.46 (19)	3.64 ± 0.36 (11)	3.71 ± 0.53 (17)
PND 4 Poststandardization	4.00 ± 0.00 (7)	4.00 ± 0.00 (7)	4.00 ± 0.22 (7)	3.86 ± 0.14 (7)	3.86 ± 0.14 (7)	4.00 ± 0.22 (7)
Male and Female[a,b]						
PND 4 Prestandardization	8.53 ± 0.56 (19)	8.86 ± 0.49 (22)	8.00 ± 0.78 (13)	7.42 ± 0.77 (19)	7.64 ± 0.99 (11)	8.61 ± 0.53 (18)
PND 4 Poststandardization	8.00 ± 0.00 (7)	8.00 ± 0.00 (7)	8.00 ± 0.00 (7)	8.00 ± 0.00 (7)	8.00 ± 0.00 (7)	8.00 ± 0.00 (7)
PND 21	8.00 ± 0.00 (7)	8.00 ± 0.00 (7)	7.86 ± 0.14 (7)	8.00 ± 0.00 (7)	8.00 ± 0.00 (7)	8.00 ± 0.00 (7)
Survival/Litter						
Total Dead: PND 1–4[d,e]	1 (1)	1 (1)	0 (0)	2 (2)	0 (0)	1 (1)
Total Dead: PND 4–21[d,e]	0 (0)	0 (0)	1 (1)	0 (0)	0 (0)	0 (0)
Dead: PND 1–4[a,b,f]	0.05 ± 0.05 (19)	0.05 ± 0.05 (22)	0.00 ± 0.00 (13)	0.11 ± 0.07 (19)	0.00 ± 0.00 (11)	0.06 ± 0.06 (18)
Dead: PND 4–21[a,b,f]	0.00 ± 0.00 (7)	0.00 ± 0.00 (7)	0.14 ± 0.14 (7)	0.00 ± 0.00 (7)	0.00 ± 0.00 (7)	0.00 ± 0.00 (7)
Survival Ratio: PND 1–4[a,b,g]	1.00 ± 0.01 (19)	1.00 ± 0.00 (22)	1.00 ± 0.00 (13)	0.98 ± 0.01 (19)	1.00 ± 0.00 (11)	0.99 ± 0.01 (18)
Survival Ratio: PND 4–21[a,b,h]	1.00 ± 0.00 (7)	1.00 ± 0.00 (7)	0.98 ± 0.02 (7)	1.00 ± 0.00 (7)	1.00 ± 0.00 (7)	1.00 ± 0.00 (7)

PND = postnatal day.
[a]Each dose group was compared to the vehicle control group with the Shirley test when a trend was present ($p \leq 0.01$ from the Jonckheere trend test) or with the Dunn test when no trend was present.
[b]Data are presented as mean ± standard error (number of litters).
[c]100 × [number of live males in dose group]/[number of live males and females in dose group] (number of pups).
[d]No statistical analysis performed on this endpoint.
[e]Total number of dead pups in dose group (number of litters with dead pups).
[f]Number dead/litter (number of litters).
[g]Survival/litter: Number of live pups on PND 4/number of live pups on PND 1.
[h]Survival/litter: Number of live pups on PND 21/number of live pups poststandardization on PND 4.

From PND 4 through weaning, male and female pup mean body weights of RES-dosed groups tended to be lower than those of the vehicle control groups, with a negative trend with dose and pairwise significance in one or more dosed groups (312.5, 625, and 1,250 mg/kg/day). At weaning (PND 21), mean body weights of males and females in the highest dosed group, 1,250 mg/kg/day, were significantly decreased (20%–21%) relative to the vehicle control groups. In other male and female dosed groups on PND 21, the difference from the vehicle control groups ranged from 4% to 12% (Table 6). There were no significant dose-related differences in pup survival from PND 4 through weaning.

Table 6. Summary of Preweaning F_1 Male and Female Wistar Han Rat Pup Mean Body Weights Following Perinatal Exposure to *Trans*-resveratrol

Postnatal Day	0 mg/kg/day	78 mg/kg/day	156 mg/kg/day	312.5 mg/kg/day	625 mg/kg/day	1,250 mg/kg/day
Male (g)						
1[a,b,c]	7.33 ± 0.18 (18)	6.83 ± 0.15 (21)	7.29 ± 0.18 (12)	7.10 ± 0.19 (19)	6.84 ± 0.20 (9)	6.78 ± 0.21 (17)
4[d,e,f,g]	11.35 ± 0.26** (28/7)	10.27 ± 0.25 (28/7)	10.60 ± 0.23 (29/7)	9.90 ± 0.32* (29/7)	9.91 ± 0.53* (29/7)	9.33 ± 0.31** (28/7)
7[d,e,h]	17.25 ± 0.20** (28/7)	15.58 ± 0.41 (28/7)	15.71 ± 0.29 (29/7)	15.44 ± 0.46* (29/7)	14.85 ± 0.67** (29/7)	13.84 ± 0.60** (28/7)
15[d,e,h]	35.61 ± 0.55** (28/7)	31.96 ± 0.91* (28/7)	32.34 ± 0.91 (29/7)	32.32 ± 0.82 (29/7)	30.53 ± 1.08** (29/7)	28.02 ± 1.08** (28/7)
21[d,e,h]	52.87 ± 1.08** (28/7)	48.50 ± 1.47 (28/7)	49.90 ± 1.23 (28/7)[i]	49.45 ± 1.34 (29/7)	46.21 ± 1.77** (29/7)	41.96 ± 1.44** (28/7)
Female (g)						
1	6.93 ± 0.17 (18)	6.30 ± 0.17 (20)	6.91 ± 0.19 (11)	6.71 ± 0.19 (18)	6.62 ± 0.29 (11)	6.33 ± 0.24 (16)
4	10.71 ± 0.28** (28/7)	10.15 ± 0.34 (28/7)	10.28 ± 0.20 (27/7)	9.52 ± 0.38 (27/7)	9.68 ± 0.50 (27/7)	8.84 ± 0.40** (28/7)
7	16.41 ± 0.35** (28/7)	15.60 ± 0.32 (28/7)	15.36 ± 0.29 (27/7)	14.80 ± 0.46 (27/7)	14.62 ± 0.62* (27/7)	13.30 ± 0.53** (28/7)
15	33.85 ± 0.83** (28/7)	31.91 ± 0.73 (28/7)	32.10 ± 0.96 (27/7)	31.38 ± 0.52 (27/7)	29.93 ± 0.88** (27/7)	26.99 ± 0.88** (28/7)
21	50.10 ± 1.50** (28/7)	47.73 ± 1.30 (24/6)[j]	49.01 ± 1.19 (27/7)	47.18 ± 0.99 (27/7)	44.24 ± 1.58* (27/7)	39.52 ± 1.72** (21/5)[j]
Male and Female (g)						
1	7.12 ± 0.17 (19)	6.61 ± 0.13 (22)	7.09 ± 0.17 (13)	6.95 ± 0.18 (19)	6.77 ± 0.27 (11)	6.71 ± 0.23 (18)
4	11.03 ± 0.25** (56/7)	10.21 ± 0.29 (56/7)	10.43 ± 0.20 (56/7)	9.70 ± 0.34* (56/7)	9.80 ± 0.51 (56/7)	9.08 ± 0.34** (56/7)
7	16.83 ± 0.25** (56/7)	15.59 ± 0.34 (56/7)	15.54 ± 0.28 (56/7)	15.11 ± 0.42* (56/7)	14.74 ± 0.63** (56/7)	13.58 ± 0.53** (56/7)
15	34.73 ± 0.60** (56/7)	31.94 ± 0.78 (56/7)	32.21 ± 0.92 (56/7)	31.84 ± 0.64 (56/7)	30.25 ± 0.97** (56/7)	27.54 ± 0.92** (56/7)
21	51.49 ± 1.20** (56/7)	48.23 ± 1.25 (52/7)	49.39 ± 1.14 (55/7)[i]	48.32 ± 1.12 (56/7)	45.29 ± 1.62** (56/7)	41.23 ± 1.34** (49/7)

Statistical significance for a dose group indicates a significant pairwise test compared to the vehicle control group. Statistical significance for the vehicle control group indicates a significant trend test.

*Statistically significant at $p \leq 0.05$; **$p \leq 0.01$.

[a]Data are presented as mean ± standard error (number of litters). Body weight data are presented in grams.

[b]Each dose group was compared to the vehicle control group with the Williams test when a trend was present ($p \leq 0.01$ from the Jonckheere trend test) or with the Dunnett test when no trend was present.

[c]Total pup weight at postnatal day (PND) 1 divided by number of live pups at PND 1.

[d]Data are presented as the mean of litter means ± standard error (number of pups/number of litters). Body weight data are presented in grams.

[e]Statistical analysis performed using mixed models with random litter effect for both trend and pairwise tests, using the Dunnett-Hsu adjustment for multiple comparisons.

[f]PND 4 poststandardization.

[g]Individual pup weights first adjusted for live litter size on PND 1.

[h]Individual pup weights first adjusted for live litter size on PND 4 poststandardization.

[i]One male pup from the 156 mg/kg dose group was euthanized on PND 18 due to moribundity.

[j]Body weights were not measured for 11 females on PND 21.

Internal Dose Assessment (Preweaning)

RES and two metabolites, *trans*-resveratrol-3-O-B-D-glucuronide (*trans*-R3G) and *trans*-resveratrol-3-sulfate (*trans*-R3S), were quantified using validated analytical methods (Appendix D) in the following samples: Wistar Han maternal plasma and whole fetuses at 30, 60, and 90 minutes following dose administration on GD 18; male and female whole pups following dose administration to dams on PND 4; and male and female pup plasma following the last dose administration to pups on PND 21. Corresponding data are presented in Table 7 and Table 8.

In GD 18 maternal plasma, RES, *trans*-R3G and *trans*-R3S concentrations increased proportionately with dose at all three time points with some exceptions (Table 7). For RES and both metabolites, the highest dam plasma concentrations were generally seen 90 minutes postdose. Concentrations of *trans*-R3G and *trans*-R3S were much higher than those of RES demonstrating significant first pass metabolism of RES following oral administration (Table 7). For example, at the 90-minute time point, *trans*-R3G and *trans*-R3S concentrations were 33- to 109-fold and 25- to 39-fold higher, respectively, than RES concentrations at all doses and time points (Table 7). RES and metabolites were detected in pooled fetuses on GD 18 at all doses and time points and, as in maternal plasma, increased with dose and time; the highest concentrations were measured at 90 minutes. Concentrations of RES and metabolites in whole fetuses were approximately 1%–11% of dam plasma concentrations demonstrating that the transfer of RES and metabolites from dams to fetuses is low (Table 7).

Table 7. Summary of Internal Dose Data for Wistar Han Rats in the Perinatal and Three-month Gavage Study of *Trans*-resveratrol[a,b]

	0 mg/kg/day	78 mg/kg/day	312.5 mg/kg/day	1,250 mg/kg/day
GD 18, Dam Plasma Concentrations (ng/mL)[c]				
n	3	3	3	3
Trans-resveratrol (RES)				
30 Minutes Postdose	BD[d]	150 ± 61.1	508 ± 129	1,640 ± 288
60 Minutes Postdose	BD	268 ± 52.0	687 ± 276	984 ± 691
90 Minutes Postdose	BD	274 ± 87.4	1,150 ± 449	3,480 ± 832
Trans-resveratrol-3-O-B-D-glucuronide (*trans*-R3G)				
30 Minutes Postdose	BD	14,900 ± 2,600	31,100 ± 2,190	56,000 ± 5,400
60 Minutes Postdose	BD	21,900 ± 814	21,700 ± 2,630	59,100 ± 23,700
90 Minutes Postdose	BD	30,000 ± 2,580	38,100 ± 6,470	151,000 ± 7,220
Trans-resveratrol-3-sulfate (*trans*-R3S)				
30 Minutes Postdose	BD	3,660 ± 736	9,530 ± 2,510	44,400 ± 1,180
60 Minutes Postdose	BD	6,410 ± 893	10,300 ± 2,690	36,700 ± 26,800
90 Minutes Postdose	2.46 ± 1.72**	10,700 ± 1,660*	32,300 ± 13,300*	87,300 ± 3,690**

	0 mg/kg/day	78 mg/kg/day	312.5 mg/kg/day	1,250 mg/kg/day
GD 18, Pooled Whole Fetus Concentrations (ng/g)[c]				
n	3	3	3	3
Trans-resveratrol (RES)				
30 Minutes Postdose	BD	8.52 ± 3.36	22.0 ± 4.34	107 ± 27.6
60 Minutes Postdose	BD	11.2 ± 1.51	26.8 ± 8.74	91.5 ± 40.6
90 Minutes Postdose	BD	13.4 ± 1.93	66.3 ± 28.8	369 ± 64.7
Trans-resveratrol-3-O-B-D-glucuronide (*trans*-R3G)				
30 Minutes Postdose	BD	137 ± 24.6	476 ± 88.0	1,730 ± 235
60 Minutes Postdose	BD	156 ± 16.3	495 ± 176	1,890 ± 541
90 Minutes Postdose	BD	250 ± 25.6	729 ± 210	4,150 ± 250
Trans-resveratrol-3-sulfate (*trans*-R3S)				
30 Minutes Postdose	BD	93.3 ± 11.2	336 ± 87.2	902 ± 89.7
60 Minutes Postdose	BD	150 ± 15.2	402 ± 156	1,200 ± 378
90 Minutes Postdose	BD	275 ± 11.6	823 ± 279	2,340 ± 114

Statistical significance for a dose group indicates a significant pairwise test compared to the vehicle control group. Statistical significance for the vehicle control group indicates a significant trend test.
*Statistically significant at $p \leq 0.05$; **$p \leq 0.01$.
GD = gestation day; BD = below detection; group did not have >20% of its values above the limit of detection (LOD).
[a]Statistical analysis performed by the Jonckheere (trend) and Shirley or Dunn (pairwise) tests.
[b]Data are presented as mean ± standard error.
[c]If >20% of the animals in a group were above the LOD, one-half of the LOD value was substituted for values below the LOD.
[d]If ≥80% of the values in the vehicle control group were below the LOD, no mean or standard error were calculated, and no statistical analysis performed.

Male and female PND 4 pups showed dose-dependent increases in RES, *trans*-R3G and *trans*-R3S concentrations (Table 8). Similar to GD 18, the metabolite concentrations in whole pups at PND 4 were higher than RES concentrations. *Trans*-R3G and *trans*-R3S concentrations in PND 4 pups were 5- to 12-fold and 4- to 7-fold higher than RES concentrations at all doses, respectively. The detection of RES and its metabolites in pups indicated transfer of RES and conjugated RES forms from the dam to pups during lactation.

RES concentrations in PND 21 male and female pup plasma were lower than concentrations in PND 4 whole pups in all dosed groups except the highest dosed group (1,250 mg/kg/day), whereas *trans*-R3G and *trans*-R3S concentrations were higher in PND 21 plasma (Table 8). There was no apparent sex difference in the concentration of RES or metabolites in PND 21 pups.

Table 8. Summary of Internal Dose Data for Preweaning F_1 Male and Female Wistar Han Rats in the Perinatal and Three-month Gavage Study of *Trans*-resveratrol[a,b]

	0 mg/kg/day	78 mg/kg/day	312.5 mg/kg/day	1,250 mg/kg/day
F_1 Male				
Trans-resveratrol (RES)				
PND 4, Whole Pup Concentrations (ng/g)[c]	2.66 ± 1.36** (4)	53.7 ± 15.8* (3)	341 ± 44.4* (4)	1,170 ± 137* (3)
PND 21, Plasma Concentrations (ng/mL)[c]	BD[d]	12.4 ± 9.20 (2)	137 ± 81.8 (2)	1,750 ± 201 (2)
Trans-resveratrol-3-O-B-D-glucuronide (*trans*-R3G)				
PND 4, Whole Pup Concentrations (ng/g)	BD	560 ± 109 (3)	3,440 ± 388 (4)	10,200 ± 253 (3)
PND 21, Plasma Concentrations (ng/mL)	BD	13,200 ± 5,050 (2)	54,700 ± 11,100 (2)	416,000 ± 4,000 (2)
Trans-resveratrol-3-sulfate (*trans*-R3S)				
PND 4, Whole Pup Concentrations (ng/g)	BD	275 ± 134 (3)	2,470 ± 693 (4)	6,820 ± 365 (3)
PND 21, Plasma Concentrations (ng/mL)	BD	314 ± 187 (2)	4,100 ± 2,640 (2)	28,600 ± 1,870 (2)
F_1 Female				
Trans-resveratrol (RES)				
PND 4, Whole Pup Concentrations (ng/g)	BD	67.5 ± 14.2 (3)	403 ± 106 (5)	1,200 ± 137 (3)
PND 21, Plasma Concentrations (ng/mL)	BD	23.6 ± 18.9 (2)	73.4 ± 24.5 (2)	1,290 ± 390 (2)
Trans-resveratrol-3-O-B-D-glucuronide (*trans*-R3G)				
PND 4, Whole Pup Concentrations (ng/g)	BD	823 ± 203 (3)	2,060 ± 542 (5)	10,300 ± 469 (3)
PND 21, Plasma Concentrations (ng/mL)	BD	18,200 ± 5,010 (2)	44,500 ± 550 (2)	321,000 ± 122,000 (2)
Trans-resveratrol-3-sulfate (*trans*-R3S)				
PND 4, Whole Pup Concentrations (ng/g)	BD	305 ± 116 (3)	1,680 ± 629 (5)	6,960 ± 351 (3)
PND 21, Plasma Concentrations (ng/mL)	BD	713 ± 486 (2)	2,510 ± 1,290 (2)	34,500 ± 11,000 (2)

Statistical significance for a dose group indicates a significant pairwise test compared to the vehicle control group.
Statistical significance for the vehicle control group indicates a significant trend test.
*Statistically significant at $p \leq 0.05$; **$p \leq 0.01$.
PND = postnatal day; BD = below detection; group did not have > 20% of its values above the limit of detection (LOD).
[a]Statistical analysis performed using a bootstrapped Jonckheere test for trend, and a Datta-Satten modified Wilcoxon test with Hommel adjustment for pairwise comparisons.
[b]Data are presented as mean of litter means ± standard error (number of litters).
[c]If >20% of the animals in a group were above the LOD, one-half of the LOD value was substituted for values below the LOD.
[d]If ≥80% of the values in the vehicle control group were below the LOD, no mean or standard error were calculated, and no statistical analysis performed.

Three-month Study in Wistar Han Rats (Postweaning Phase)

All animals survived to study termination (Table 9). There were no dose-related clinical observations (Appendix F). Mean body weights of RES-dosed animals in all but the 1,250 mg/kg/day group were within 2%–9% of the vehicle control groups at study termination (Table 9; Figure 3). In the 1,250 mg/kg/day group, mean body weights of male and female Wistar Han rats were 21% and 16% lower, respectively, relative to the vehicle control groups 8 days after weaning (study day [SD] 8) (Appendix F). From SD 15 onward, mean body weights of male and female Wistar Han rats in the 1,250 mg/kg/day group were within 20% of the vehicle control groups. By study termination (~SD 95), female Wistar Han rats in the 1,250 mg/kg/day group had mean body weights similar to those of the vehicle control group; male Wistar Han rats weighed 9% less than the vehicle control group (Table 9).

Table 9. Summary of Survival and Mean Body Weights of Male and Female Wistar Han Rats in the Perinatal and Three-month Gavage Study of *Trans*-resveratrol

Concentration (mg/kg/day)	Survival[a]	Initial Body Weight (g)	Final Body Weight (g)	Final Weight Relative to Control Group (%)[b]
Male				
0	5/5 (10)	53.5	391.9	–
78	5/5 (10)	50.4	374.3	95.5
156	5/5 (10)	49.6	409.7	104.5
312.5	5/5 (10)	52.2	383.0	97.7
625	5/5 (10)	49.1	363.6	92.8
1,250	5/5 (10)	41.8	356.5	91.0
Female				
0	5/5 (10)	56.9	226.0	–
78	5/5 (10)	54.1	242.9	107.5
156	5/5 (10)	52.3	230.0	101.8
312.5	5/5 (10)	51.8	227.3	100.6
625	5/5 (10)	50.8	219.2	97.0
1,250	5/5 (10)	45.3	225.0	99.6

[a]Number of litters surviving at 3 months/number of litters initially in group (number of individual animals).
[b]100 × [(dose group mean − vehicle control group mean)/vehicle control group mean].

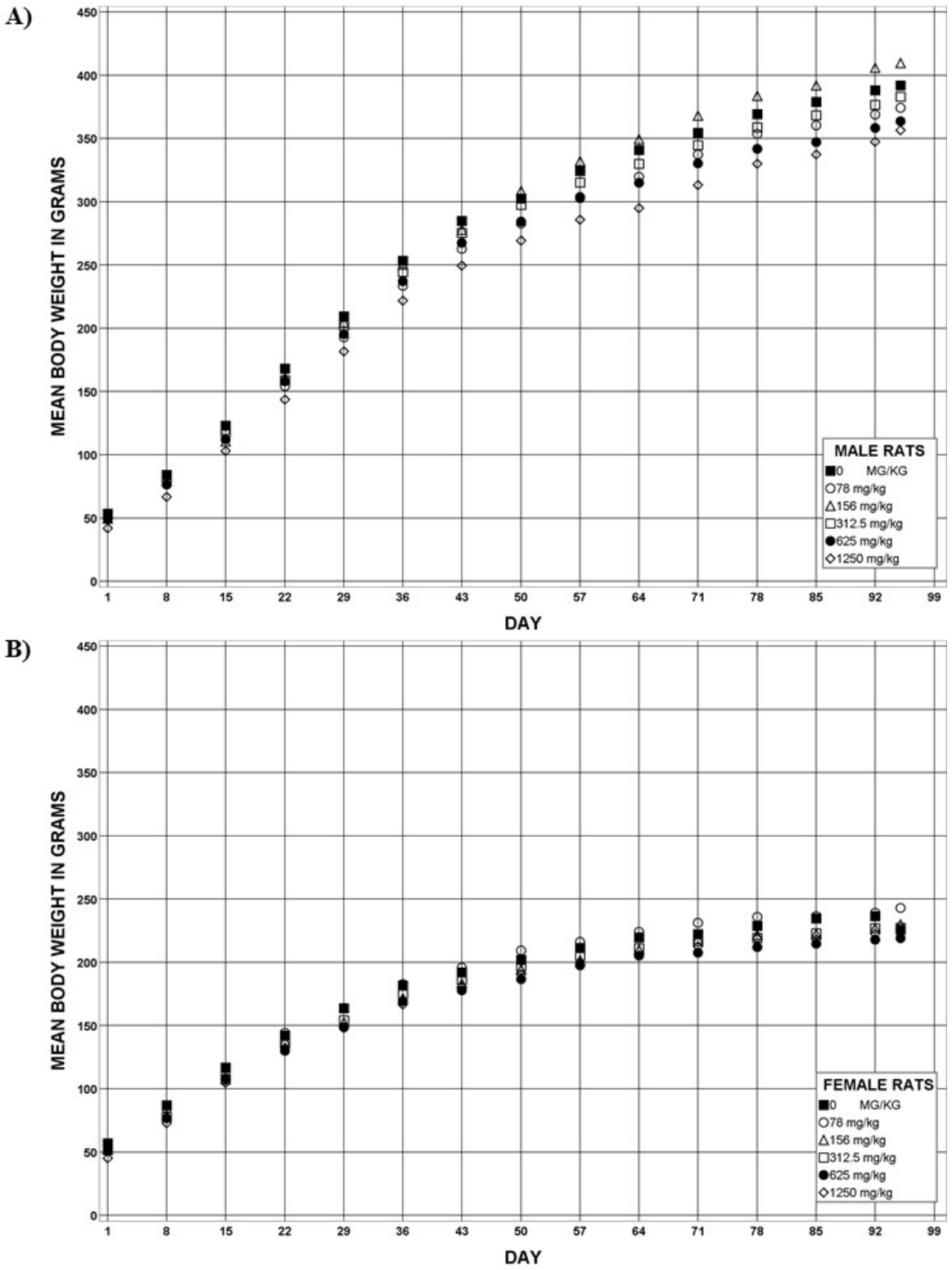


Figure 3. Growth Curves for Male and Female Wistar Han Rats in the Perinatal and Three-month Gavage Study of *Trans*-resveratrol

Growth curves for rats administered *trans*-resveratrol by gavage in (A) males and (B) females.

At study termination, there were no dose-related effects on clinical chemistry measures (Appendix F). Several significant differences were observed in the female hematology. These differences were small in magnitude, not observed in the males, or within biological variability, and thus were not considered related to dose. Organ weights were not affected by RES administration (Appendix F).

Vaginal cytology and sperm counts were assessed in the 0, 312.5, 625, and 1,250 mg/kg/day groups after 3 months of exposure. Cycle length, number of cycles, and time in each respective stage in RES-dosed Wistar Han rats were similar to the vehicle control group (Appendix F).

Wistar Han rats administered RES did not display any biologically significant changes in testis or epididymis weights, spermatid or spermatozoa counts, or sperm motility (Appendix F). RES did not exhibit the potential to be a male or female reproductive toxicant in the Wistar Han rat.

Internal Dose Assessment (Postweaning)

RES and two metabolites, *trans*-R3G and *trans*-R3S, were quantified using a validated analytical method (Appendix D) in male and female Wistar Han rat plasma collected 24 hours after the last dose administration on SD 95. Plasma concentrations of RES and *trans*-R3S were much lower in offspring at necropsy compared to those in dams on GD 18 or in offspring on PND 21 (Table 7), likely due to the extended time between the last dose and when samples were collected (24 hours) (Table 10).

Table 10. Summary of Internal Dose Data for Postweaning F_1 Male and Female Wistar Han Rats in the Perinatal and Three-month Gavage Study of *Trans*-resveratrol[a,b]

	0 mg/kg/day	78 mg/kg/day	312.5 mg/kg/day	1,250 mg/kg/day
n	5	5	5	5
F_1 Male				
Trans-resveratrol (RES)				
SD 95, Plasma Concentrations (ng/mL)[c,d]	BD[e]	BD	8.2 ± 7.35	32.9 ± 27.0
Trans-resveratrol-3-O-B-D-glucuronide (*trans*-R3G)				
SD 95, Plasma Concentrations (ng/mL)	BD	21.8 ± 5.37	638 ± 473	6,170 ± 5,040
Trans-resveratrol-3-sulfate (*trans*-R3S)				
SD 95, Plasma Concentrations (ng/mL)	BD	BD	51.3 ± 45.9	763 ± 527
F_1 Female				
Trans-resveratrol (RES)				
SD 95, Plasma Concentrations (ng/mL)	BD	BD	1.68 ± 0.832	21.6 ± 10.5
Trans-resveratrol-3-O-B-D-glucuronide (*trans*-R3G)				
SD 95, Plasma Concentrations (ng/mL)	BD	642 ± 384	1,770 ± 1,100	20,200 ± 12,300

	0 mg/kg/day	78 mg/kg/day	312.5 mg/kg/day	1,250 mg/kg/day
Trans-resveratrol-3-sulfate (*trans*-R3S)				
SD 95, Plasma Concentrations (ng/mL)	BD	22.8 ± 15.6	153 ± 115	1,760 ± 1,250

SD = study day for postweaning exposure; BD = below detection; group did not have >20% of its values above the limit of detection (LOD).
[a]Statistical analysis performed by the Jonckheere (trend) and Shirley or Dunn (pairwise) tests.
[b]Data are presented as mean ± standard error.
[c]SD 95 plasma samples were isolated from blood collected 24 hours after the last dose.
[d]If >20% of the animals in a group were above the LOD, one-half of the LOD value was substituted for values below the LOD.
[e]If ≥80% of the values in the vehicle control group were below the LOD, no mean or standard error were calculated, and no statistical analysis performed.

Histopathology

This section describes the statistically significant or biologically noteworthy changes in the incidences of nonneoplastic lesions of the kidney, jejunum, and Peyer's patch in male and female Wistar Han rats. No dose-related neoplasms were present. Summaries of the incidences of nonneoplastic lesions mentioned in this section are presented in Appendix F.

Kidney: Nephropathy occurred in all groups of dosed male Wistar Han rats with a positive trend with dose and a significantly increased incidence in the 1,250 mg/kg/day group compared to the vehicle control group (Table 11). This lesion, also known as chronic progressive nephropathy, also occurred in female Wistar Han rats administered ≥156 mg/kg/day, with a positive dose-related trend and a significantly increased incidence in the 1,250 mg/kg/day group compared to the vehicle control group (Table 11). Histologically, this lesion was characterized by focal to multifocal small clusters of tubules within the renal cortex that displayed cytoplasmic basophilia (regeneration), thickened basement membranes, and peritubular mononuclear cell infiltrates.

Renal tubule dilatation occurred in male and female Wistar Han rats with a positive trend with dose and a significantly increased incidence in the 1,250 mg/kg/day groups compared to the vehicle control groups (Table 11). This diagnosis was reserved for those cases with one or two large ectatic tubules within the renal medulla that frequently contained eosinophilic intraluminal material (Figure 4) without histological evidence of lower urinary tract obstruction in the tissue sections evaluated. When the lesion was adjacent to a focus of nephropathy, tubular dilatation was deemed to be part of nephropathy and not diagnosed separately.

The lesion of renal pelvis dilatation occurred with a positive trend for incidence in female Wistar Han rats (Table 11). This lesion was characterized by distention and dilatation of the renal pelvis and occurred without histological evidence of lower urinary tract obstruction in the tissue sections evaluated.

Table 11. Incidences of Select Nonneoplastic Lesions of the Kidney in Male and Female Wistar Han Rats in the Perinatal and Three-month Gavage Study of *Trans*-resveratrol

	0 mg/kg/day	78 mg/kg/day	156 mg/kg/day	312.5 mg/kg/day	625 mg/kg/day	1,250 mg/kg/day
n[a]	10 (5)	10 (5)	10 (5)	10 (5)	10 (5)	10 (5)
Male						
Nephropathy[b]	0**	3 (1.0)[c]	1 (1.0)	4 (1.0)	2 (1.0)	7** (1.0)
Renal Tubule, Dilatation	0**	0	0	0	1 (1.0)	7** (1.0)
Female						
Nephropathy	0**	0	1 (1.0)	2 (1.0)	3 (1.0)	6* (1.0)
Renal Pelvis, Dilatation	0*	1 (1.0)	0	1 (1.0)	1 (1.0)	4 (1.5)
Renal Tubule, Dilatation	0**	0	1 (1.0)	0	0	7** (1.0)

Statistical significance for a dose group indicates a significant pairwise test compared to the vehicle control group. Statistical significance for the vehicle control group indicates a significant trend test.
*Statistically significant at $p \leq 0.05$ by the Rao-Scott-adjusted Cochran-Armitage (trend and pairwise) tests; **$p \leq 0.01$.
[a]Number of animals examined microscopically (number of litters).
[b]Number of animals with lesion.
[c]Average severity grade of observed lesion in affected animals: 1 = minimal; 2 = mild; 3 = moderate; 4 = marked.

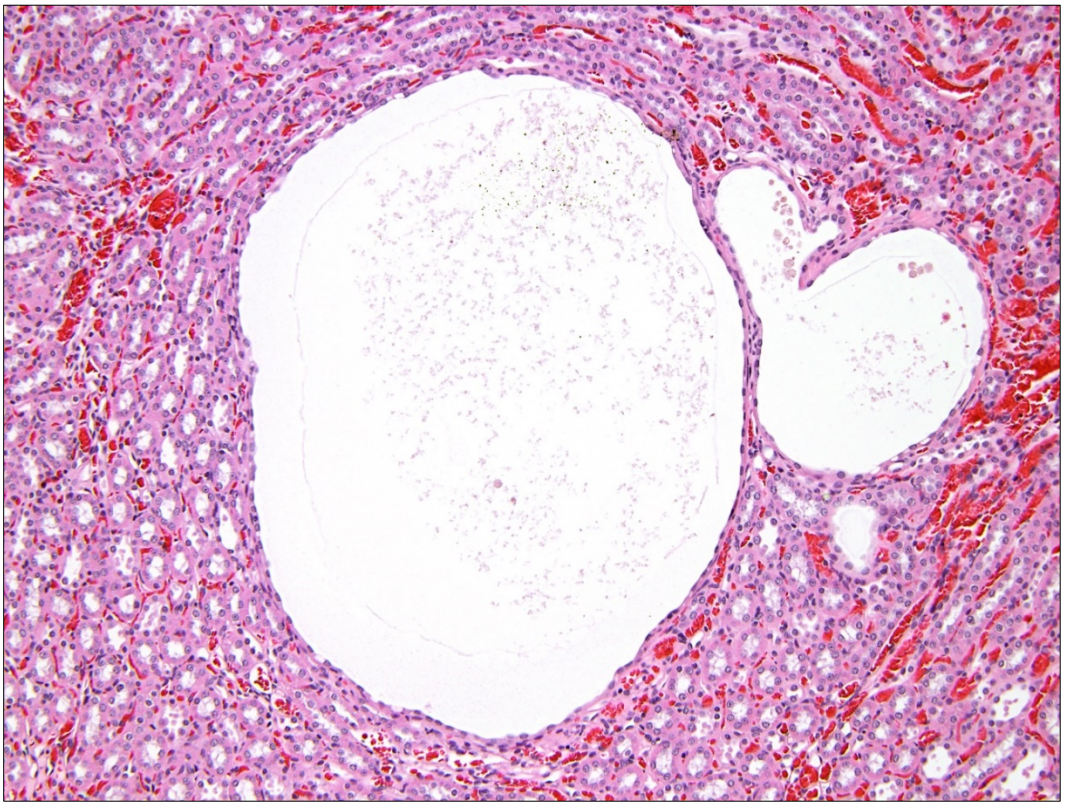


Figure 4. Representative Image of Renal Tubule Dilatation in the Kidney of a Female Wistar Han Rat in the Perinatal and Three-month Gavage Study of *Trans*-resveratrol (H&E)

Note the two large dilated renal tubules lined by flattened epithelial cells within the renal medullary region in a female Wistar Han rat administered 1,250 mg/kg/day *trans*-resveratrol (16x). H&E = hematoxylin and eosin stain.

Jejunum: Lymphatic ectasia occurred in the jejunum in the 625 and 1,250 mg/kg/day male groups and in the 1,250 mg/kg/day female group (Table 12). A positive trend with dose was found for the incidence in males. This unique lesion was characterized as central lymphatic dilatation of the villous tips within the jejunum (Figure 5).

Peyer's patch: Lymphatic ectasia occurred in the Peyer's patches in the 156, 625, and 1,250 mg/kg/day groups of male Wistar Han rats, and in the 78, 312.5, and 1,250 mg/kg/day groups of female Wistar Han rats (Table 12). This lesion was characterized by a few discrete clear dilated lymphatics within the lymphoid tissue, usually located in the subepithelial dome region (Figure 5).

Table 12. Incidences of Select Nonneoplastic Lesions of the Small Intestine in Male and Female Wistar Han Rats in the Perinatal and Three-month Gavage Study of *Trans*-resveratrol

	0 mg/kg/day	**78 mg/kg/day**	**156 mg/kg/day**	**312.5 mg/kg/day**	**625 mg/kg/day**	**1,250 mg/kg/day**
n[a]	10 (5)	10 (5)	10 (5)	10 (5)	10 (5)	10 (5)
Male						
Jejunum						
Lymphatic ectasia[b]	0*	0	0	0	2 (1.5)[c]	2 (2.0)
Peyer's Patch						
Lymphatic ectasia	0	0	1 (1.0)	0	2 (2.0)	1 (2.0)
Female						
Jejunum						
Lymphatic ectasia	0	0	0	0	0	2 (2.0)
Peyer's Patch						
Lymphatic ectasia	0	2 (1.5)	0	1 (2.0)	0	1 (2.0)

Statistical significance for the vehicle control group indicates a significant trend test.
*Statistically significant at $p \leq 0.05$ by the Rao-Scott-adjusted Cochran-Armitage (trend) test.
[a]Number of animals examined microscopically (number of litters).
[b]Number of animals with lesion.
[c]Average severity grade of observed lesion in affected animals: 1 = minimal; 2 = mild; 3 = moderate; 4 = marked.

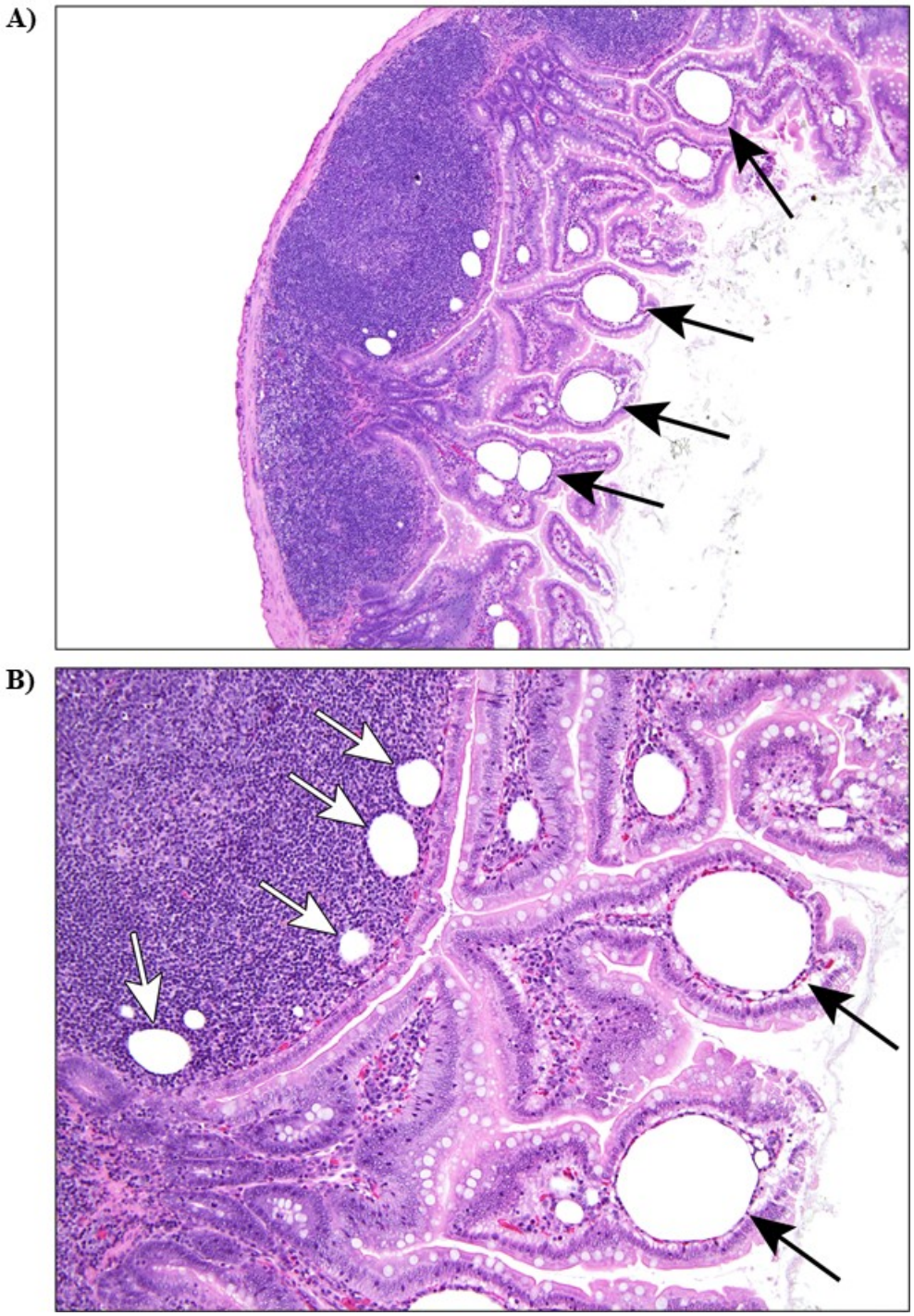


Figure 5. Representative Images of Lymphatic Ectasia in the Jejunum of a Male Wistar Han Rat in the Perinatal and Three-month Gavage Study of *Trans*-resveratrol (H&E)

(A) Mild lymphatic ectasia in the jejunum showing clear lymphatic dilatation within the villous tips (arrows) in a male Wistar Han rat administered 1,250 mg/kg/day *trans*-resveratrol (6.4x). (B) Higher magnification (16x) of panel A showing lymphatic ectasia within the Peyer's patch. Note the discrete clear dilated lymphatics within the subepithelial dome region (white arrows). This image also shows a higher magnification of the villous tip lymphatic dilatation (black arrows). H&E = hematoxylin and eosin stain.

Mice

Two-week Study in B6C3F1/N Mice

All B6C3F1/N mice survived until study termination, and mean body weights of all dosed groups were within 7% of the vehicle control groups at measured time points (Table 13; Figure 6). No dose-related clinical observations were noted (Appendix F).

Table 13. Summary of Survival and Mean Body Weights of Male and Female B6C3F1/N Mice in the Two-week Gavage Study of *Trans*-resveratrol

Dose (mg/kg/day)	Survival[a]	Initial Body Weight (g)	Final Body Weight (g)	Final Weight Relative to Control Group (%)[b]
Male				
0	5/5	22.1	24.7	–
156	5/5	22.0	25.0	101.1
312	5/5	22.5	26.4	107.1
625	5/5	22.4	25.5	103.2
1,250	5/5	21.9	24.3	98.3
2,500	5/5	22.1	24.4	98.9
Female				
0	5/5	17.1	19.9	–
156	5/5	17.3	19.5	98.1
312	5/5	17.3	20.1	101.2
625	5/5	17.3	20.3	101.8
1,250	5/5	17.1	19.6	98.4
2,500	5/5	17.4	19.6	98.4

[a]Number of animals surviving at 2 weeks/number initially in group.
[b]100 × [(dose group mean − vehicle control group mean)/vehicle control group mean].

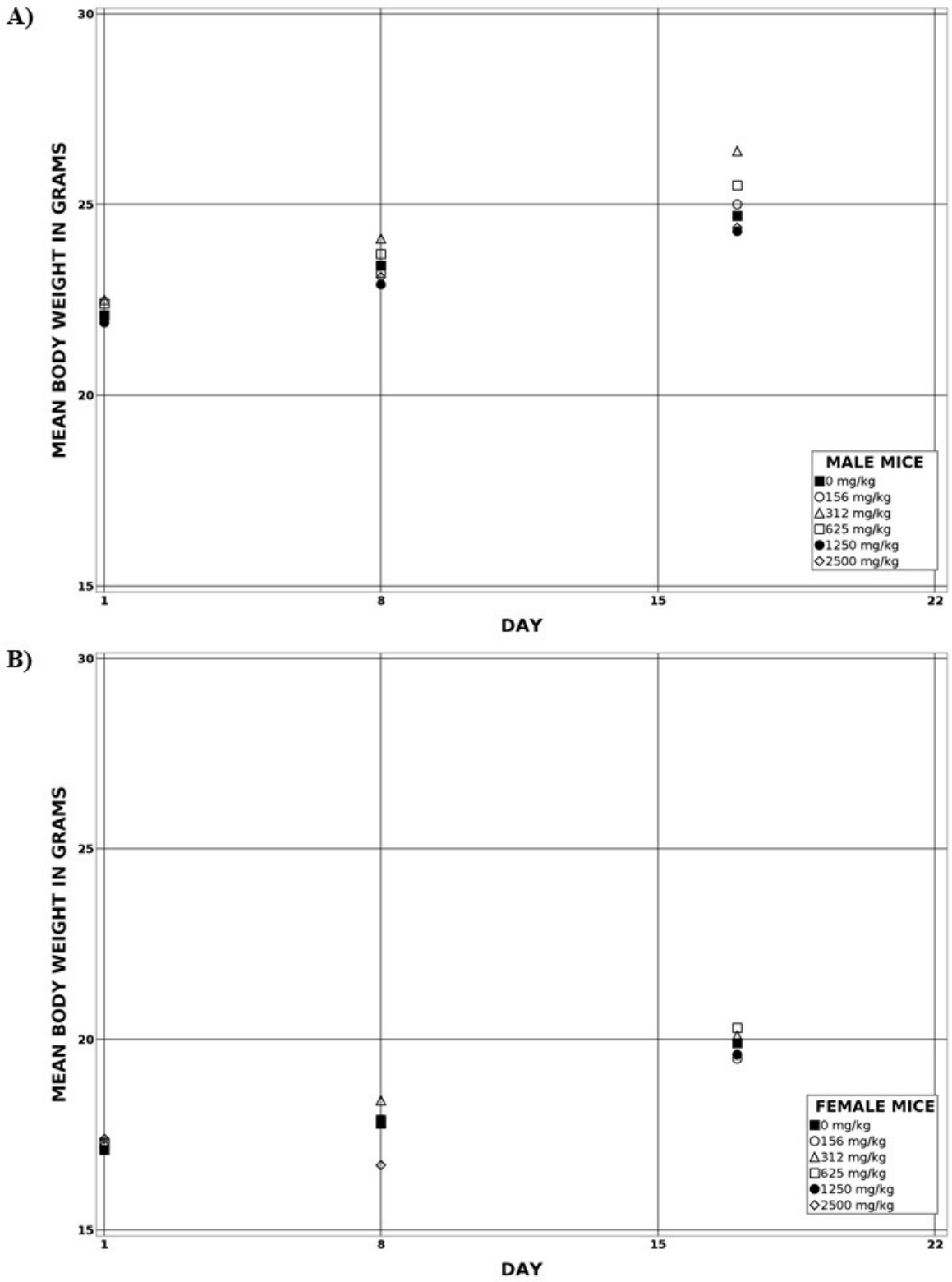


Figure 6. Growth Curves for Male and Female B6C3F1/N Mice in the Two-week Gavage Study of *Trans*-resveratrol

Growth curves for mice administered *trans*-resveratrol by gavage in (A) males and (B) females.

A significant increase in relative liver weight was observed in the 2,500 mg/kg/day group of female mice, as well as a positive trend (Table 14). There were no associated dose-related gross or microscopic findings (Appendix F).

Table 14. Summary of Liver Weights and Liver-Weight-to-Body-Weight Ratios for Female B6C3F1/N Mice in the Two-week Gavage Study of *Trans*-resveratrol[a,b]

	0 mg/kg/day	156 mg/kg/day	312 mg/kg/day	625 mg/kg/day	1,250 mg/kg/day	2,500 mg/kg/day
n	5	5	5	5	5	5
Female						
Necropsy Body Wt. (g)	19.9 ± 0.2	19.5 ± 0.4	20.1 ± 0.5	20.3 ± 0.4	19.6 ± 0.5	19.6 ± 0.4
Liver						
Absolute (g)	1.19 ± 0.05	1.16 ± 0.04	1.18 ± 0.04	1.22 ± 0.03	1.19 ± 0.04	1.30 ± 0.05
Relative (mg/g)[c]	59.95 ± 2.07**	59.39 ± 0.65	58.76 ± 1.42	60.42 ± 0.90	60.64 ± 1.16	66.21 ± 1.78**

Statistical significance for a dose group indicates a significant pairwise test compared to the vehicle control group. Statistical significance for the vehicle control group indicates a significant trend test.
**Statistically significant at $p \leq 0.01$.
[a]Data are presented as mean ± standard error.
[b]Statistical analysis performed by the Jonckheere (trend) and Williams or Dunnett (pairwise) tests.
[c]Relative organ weights (organ-weight-to-body-weight ratios) are given as mg organ weight/g body weight.

Dose Selection Rationale for Three-month Studies in B6C3F1/N Mice

The same doses were used in the 3-month study in B6C3F1/N mice as were used in the 2-week study due to the lack of observed toxicity at the doses used in the 2-week study.

Three-month Study in B6C3F1/N Mice

All animals survived to study termination (Table 15). Mean body weights of RES-dosed male and female B6C3F1/N mice ranged from within 1%–10% of vehicle control animals and were not significantly different from vehicle control animals at any point during the study (Table 15; Figure 7). No dose-related clinical observations were noted (Appendix F).

Table 15. Summary of Survival and Mean Body Weights of Male and Female B6C3F1/N Mice in the Three-month Gavage Study of *Trans*-resveratrol

Dose (mg/kg/day)	Survival[a]	Initial Body Weight (g)	Final Body Weight (g)	Final Weight Relative to Control Group (%)[b]
Male				
0	10/10	22.2	35.3	–
156	10/10	22.6	37.8	107.3
312	10/10	22.3	37.4	106.1
625	10/10	22.5	37.2	105.5
1,250	10/10	22.0	36.1	102.3
2,500	10/10	22.1	35.4	100.3
Female				
0	10/10	17.3	29.1	–
156	10/10	17.0	28.4	97.6
312	10/10	17.1	28.2	96.9
625	10/10	17.6	29.6	101.6
1,250	10/10	17.2	27.6	94.5
2,500	10/10	17.2	26.5	90.9

[a]Number of animals surviving at 3 months/number initially in group.
[b]100 × [(dose group mean − vehicle control group mean)/vehicle control group mean].

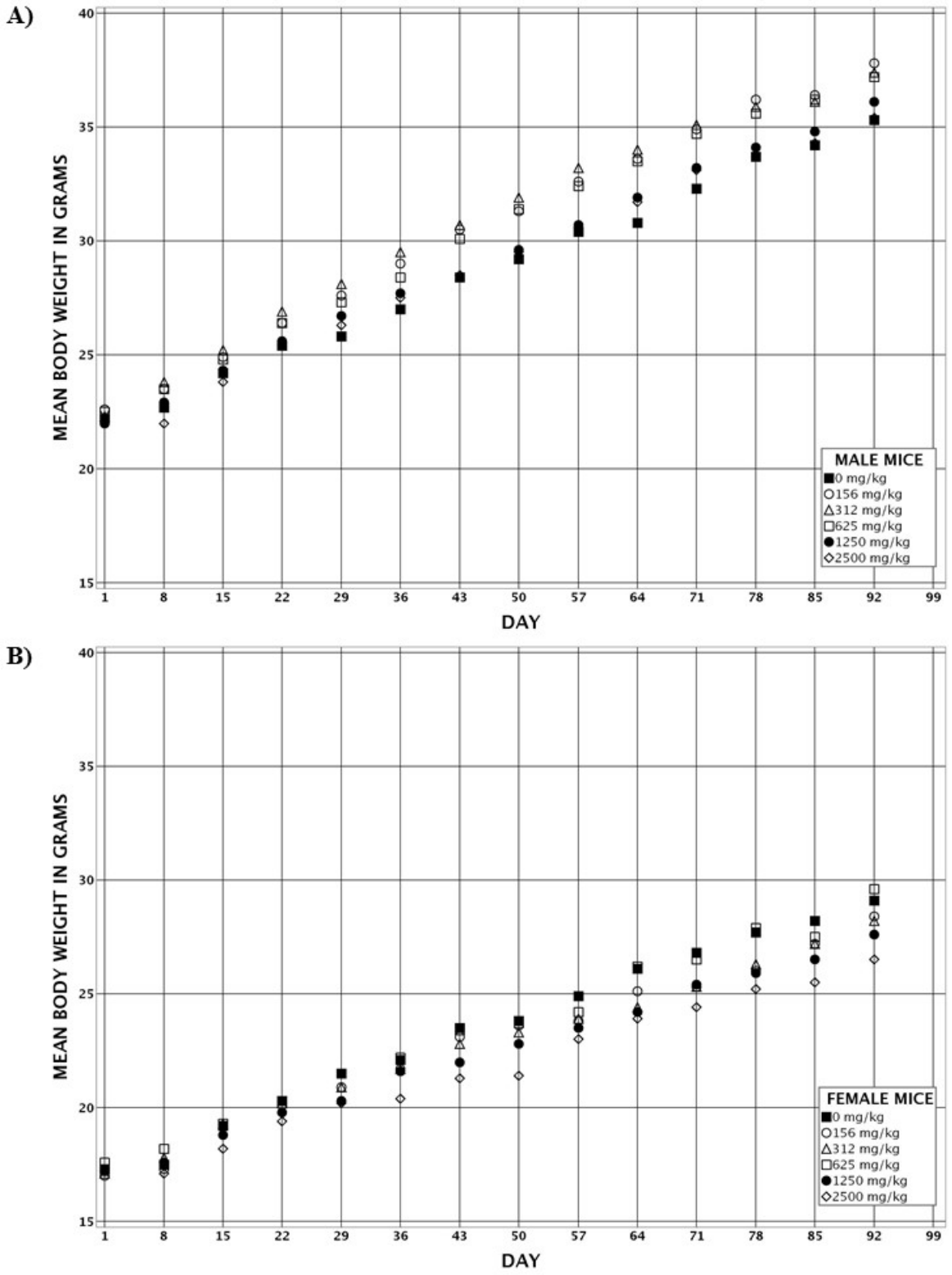


Figure 7. Growth Curves for Male and Female B6C3F1/N Mice in the Three-month Gavage Study of *Trans*-resveratrol

Growth curves for mice administered *trans*-resveratrol by gavage in (A) males and (B) females.

No effects on hematological parameters were observed with RES exposure (Appendix F).

In male mice, absolute and relative liver weights were significantly increased in the highest dose group (2,500 mg/kg/day) relative to the vehicle control group (Table 16). In female mice, absolute heart weights were significantly decreased in the 1,250 and 2,500 mg/kg/day groups compared to the vehicle control group. Additionally, there were significant increases in relative kidney weights (1,250 and 2,500 mg/kg/day groups) and relative liver weights (625, 1,250, and 2,500 mg/kg/day groups) compared to the vehicle control group (Table 16). No other effects on organ weights were observed.

Table 16. Summary of Select Organ Weights and Organ-Weight-to-Body-Weight Ratios for Male and Female B6C3F1/N Mice in the Three-month Gavage Study of *Trans*-resveratrol[a,b]

	0 mg/kg/day	156 mg/kg/day	312 mg/kg/day	625 mg/kg/day	1,250 mg/kg/day	2,500 mg/kg/day
n	10	10	10	10	10	10
Male						
Necropsy Body Wt. (g)	35.3 ± 1.1	37.8 ± 0.9	37.4 ± 1.0	37.2 ± 1.2	36.1 ± 1.0	35.4 ± 0.8
Liver						
Absolute (g)	1.56 ± 0.05**	1.68 ± 0.05	1.70 ± 0.03	1.71 ± 0.07	1.69 ± 0.06	1.80 ± 0.06**
Relative (mg/g)[c]	44.27 ± 0.59**	44.24 ± 0.57	45.55 ± 1.01	45.86 ± 1.02	46.95 ± 0.99	50.82 ± 1.15**
Female						
Necropsy Body Wt. (g)	29.1 ± 1.1*	28.4 ± 0.6	28.2 ± 0.6	29.6 ± 1.1	27.6 ± 0.9	26.5 ± 0.7
Heart						
Absolute (g)	0.19 ± 0.01**	0.17 ± 0.01	0.17 ± 0.00	0.18 ± 0.00	0.17 ± 0.00*	0.16 ± 0.01**
Relative (mg/g)	6.38 ± 0.16	6.00 ± 0.22	6.10 ± 0.26	6.12 ± 0.19	6.22 ± 0.24	5.86 ± 0.20
Right Kidney						
Absolute (g)	0.16 ± 0.00	0.17 ± 0.00	0.17 ± 0.00	0.17 ± 0.00	0.17 ± 0.00	0.16 ± 0.00
Relative (mg/g)	5.57 ± 0.18**	5.82 ± 0.13	5.89 ± 0.10	5.85 ± 0.15	6.08 ± 0.11**	6.20 ± 0.11**
Liver						
Absolute (g)	1.30 ± 0.04	1.32 ± 0.03	1.35 ± 0.03	1.44 ± 0.06	1.40 ± 0.06	1.41 ± 0.05
Relative (mg/g)	44.79 ± 0.84**	46.33 ± 1.18	47.79 ± 0.88	48.64 ± 1.10*	50.71 ± 1.23**	52.96 ± 0.93**

Statistical significance for a dose group indicates a significant pairwise test compared to the vehicle control group. Statistical significance for the vehicle control group indicates a significant trend test.
*Statistically significant at $p \leq 0.05$; **$p \leq 0.01$.
[a]Data are presented as mean ± standard error.
[b]Statistical analysis performed by the Jonckheere (trend) and Williams or Dunnett (pairwise) tests.
[c]Relative organ weights (organ-weight-to-body-weight ratios) are given as mg organ weight per g body weight.

Reproductive parameters were evaluated in male mice in the 0, 625, 1,250, and 2,500 mg/kg/day groups. There were no significant differences in testis or epididymis weights, testicular sperm count, or percent motile sperm (Table 17). Cauda epididymis sperm counts were 24%, 14%, and 13% lower than the vehicle control group in the 625, 1,250, and 2,500 mg/kg/day groups, respectively, with only the 625 mg/kg/day group attaining statistical significance (Table 17). Histopathological lesions were not observed in either the epididymis or testis.

Table 17. Summary of Reproductive Tissue Evaluations for Male B6C3F1/N Mice in the Three-month Gavage Study of *Trans*-resveratrol[a]

	0 mg/kg/day	625 mg/kg/day	1,250 mg/kg/day	2,500 mg/kg/day
n	10	10	10	10
Weights (g)[b]				
Left cauda epididymis	0.014 ± 0.000	0.015 ± 0.000	0.014 ± 0.001	0.015 ± 0.001
Left epididymis	0.040 ± 0.001	0.041 ± 0.001	0.041 ± 0.001	0.042 ± 0.001
Left testis	0.112 ± 0.002	0.113 ± 0.001	0.112 ± 0.002	0.114 ± 0.001
Spermatid Measurements[c]				
Spermatid heads (10^6/g testis)	214.1 ± 18.0	198.4 ± 16.3	195.2 ± 18.3	193.2 ± 15.4
Spermatid heads (10^6/testis)	23.8 ± 1.9	22.3 ± 1.8	21.9 ± 2.2	22.1 ± 1.8
Epididymal Spermatozoal Measurements[c]				
Sperm motility (%)	85.7 ± 0.3	86.6 ± 0.6	86.2 ± 0.9	86.2 ± 0.6
Sperm (10^3/mg cauda epididymis)	1,609.6 ± 72.7	1,189.8 ± 63.2**	1,403.9 ± 129.7	1,331.5 ± 96.3
Cauda epididymis sperm count (millions)	22.8 ± 1.5	17.4 ± 1.1*	19.6 ± 1.6	19.9 ± 1.4

Statistical significance for a dose group indicates a significant pairwise test compared to the vehicle control group.
*Statistically significant at $p \leq 0.05$; **$p \leq 0.01$.
[a]Data are presented as mean ± standard error.
[b]Statistical analysis performed by the Jonckheere (trend) and Williams or Dunnett (pairwise) tests.
[c]Statistical analysis performed by the Jonckheere (trend) and Shirley or Dunn (pairwise) tests.

Vaginal cytology evaluations were conducted in female mice in the 0, 625, 1,250, and 2,500 mg/kg/day groups. Cycle length and number of cycles in RES-dosed mice were not significantly different from vehicle control mice (Appendix F). The 625 and 2,500 mg/kg/day groups spent less time in estrus and more time in metestrus than the vehicle control group (Appendix F). Extended diestrus was more prevalent in all RES-dosed groups relative to the vehicle control group. Although there were some apparent changes in estrus and metestrus in RES-dosed mice, they were minimal in magnitude and not dose dependent (Appendix F).

Histopathology

This section describes the statistically significant or biologically noteworthy changes in the incidence of nonneoplastic lesions of the nose in female B6C3F1/N mice. No dose-related neoplasms were present. Summaries of the nonneoplastic lesions mentioned in this section are presented in Appendix F.

Nose: Respiratory metaplasia of the olfactory epithelium occurred with a positive dose-related trend and a significantly increased incidence in the 2,500 mg/kg/day female dosed group relative to the vehicle control group (Table 18). This lesion is characterized by focally extensive areas of replacement of olfactory epithelium with columnar ciliated respiratory epithelium. This change was most often present in the dorsal aspect of the nasal cavity at Level II (Figure 8).

Table 18. Incidences of Select Nonneoplastic Lesions of the Nose in Female B6C3F1/N Mice in the Three-month Gavage Study of *Trans*-resveratrol

	0 mg/kg/day	156 mg/kg/day	312 mg/kg/day	625 mg/kg/day	1,250 mg/kg/day	2,500 mg/kg/day
n[a]	10	10	10	10	10	10
Olfactory Epithelium, Metaplasia, Respiratory[b]	0**	0	0	2 (1.0)[c]	2 (1.0)	4* (1.0)

Statistical significance for a dose group indicates a significant pairwise test compared to the vehicle control group. Statistical significance for the vehicle control group indicates a significant trend test.
*Statistically significant at $p \leq 0.05$ by the Cochran-Armitage (trend) or Fisher's exact (pairwise) tests; **$p \leq 0.01$.
[a]Number of animals examined microscopically.
[b]Number of animals with lesion.
[c]Average severity grade of observed lesion in affected animals: 1 = minimal; 2 = mild; 3 = moderate; 4 = marked.

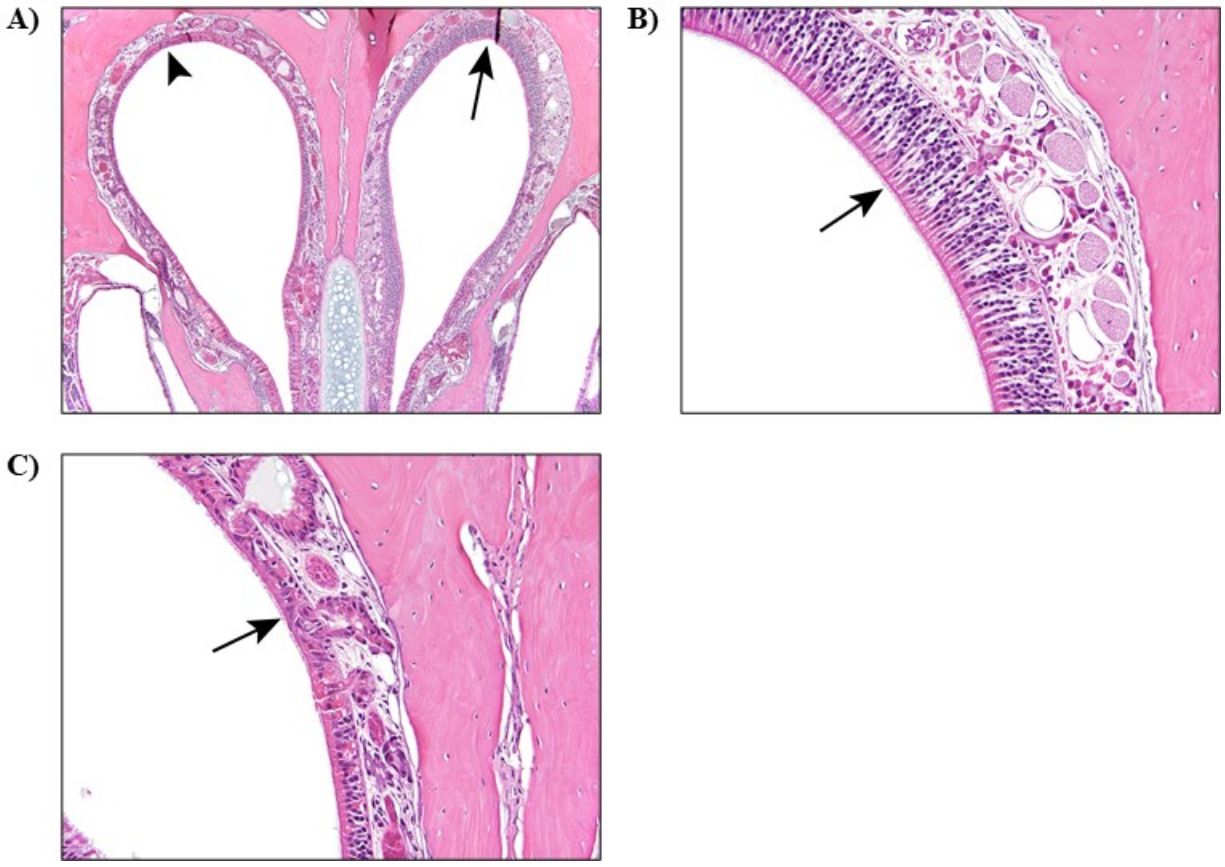


Figure 8. Representative Images of Respiratory Metaplasia of Olfactory Epithelium in the Nose of a Female B6C3F1/N Mouse in the Three-month Gavage Study of *Trans*-resveratrol (H&E)

(A) Low magnification (10x) that shows respiratory metaplasia of the olfactory epithelium in a Level II section of the nose in a female B6C3F1/N mouse administered 2,500 mg/kg/day *trans*-resveratrol. Compared to the normal olfactory epithelium on the right (arrow), the olfactory epithelium on the left has been replaced by respiratory epithelium (arrowhead). (B) Higher magnification (40x) of panel A showing normal olfactory epithelium. This tissue is characterized by pseudo-stratified columnar epithelium that is composed of supporting cells, basal cells, neurons, and specialized cilia extensions (arrow). (C) Higher magnification (40x) of panel A showing respiratory metaplasia. This lesion is characterized by replacement of the olfactory epithelium with columnar, and sometimes disorganized, ciliated epithelium (arrow). H&E = hematoxylin and eosin stain.

Other lesions: There were positive trends with dose in the occurrence of kidney nephropathy in male mice, kidney mineralization in female mice, and mixed cell infiltration in the livers of male and female mice (Appendix F), but the biological significance of these lesions is unknown.

Genetic Toxicology

RES (33 to 3,333 μg/plate) was not mutagenic in *Salmonella typhimurium* strains TA98, TA100, or TA102 when tested with or without exogenous metabolic activation provided by Aroclor 1254-induced rat liver S9 and cofactors (Table E-1).

In rats, the reticulocyte population is the only red blood cell population that can be accurately assessed for micronucleus frequency in peripheral blood due to efficient splenic scavenging of damaged erythrocytes soon after they emerge from the bone marrow. In both sexes of Wistar Han rats in the 3-month study, there were no significant increases in the frequencies of micronucleated reticulocytes (polychromatic erythrocytes; PCEs) (Table E-2). A positive trend in the percentage of PCEs was observed in female Wistar Han rats; however, the absolute increase (0.38%) in the 1,250 mg/kg/day group compared to the vehicle control group was small and was not considered to be biologically relevant.

No increases in the frequency of micronucleated erythrocytes (either immature or mature) were seen in the peripheral blood of female B6C3F1/N mice in the 3-month study (Table E-3). Significant increases in micronucleated mature erythrocytes (NCEs) were observed for every dosed group in male B6C3F1/N mice. However, the absolute difference in micronucleated NCEs in the dosed groups relative to the vehicle control group ranged from 0.06% to 0.16%. These small increases were not considered biologically relevant.

Discussion

Trans-resveratrol (RES) has captured the attention of researchers and clinicians for years as a potential treatment or preventive natural product for various conditions, including cancer, cardiometabolic diseases, and neurodegenerative diseases. The development of novel techniques and strategies to improve its bioavailability and bioefficacy suggests continued prevalence and use of RES both therapeutically and in consumer products. Due to its low bioavailability, RES used in dietary supplements and in clinical trials can be found at high concentrations, with doses up to 5 g per day. The National Toxicology Program (NTP) conducted the present studies to address key knowledge gaps in resveratrol safety, especially those for perinatal RES exposure.

The doses used in the present studies approximate human exposure. Oral doses of 2.5 and 5 g RES in humans, as used in some clinical studies,[59] are similar to an oral gavage dose of 312.5 and 625 mg/kg/day in rats (625 and 1,250 mg/kg/day in mice), after adjusting for body surface area.[151] For example, 625 mg/kg exposure in rats corresponds to a human equivalent dose (HED) of 101.4 mg/kg, or a single oral dose of 6.1 to 9.1 g, accounting for human body weight ranging from 60 to 90 kg. These HEDs are 1.2- to 1.8-fold higher than the doses used in clinical studies. Furthermore, a toxicokinetic study of RES in the same strains of rodents showed that systemic exposures following a single gavage dose at the same dose levels used in this study were within an order of magnitude of those observed in a clinical study.[59] Rat-to-human exposure multiples calculated using internal exposure measures, maximum concentration (C_{max}), and area under the concentration-time curve (AUC), were 2.2–3.4-fold and 9.4–10.4-fold, respectively.[59] Thus, the internal doses in the present study in animals are similar to what could be expected with therapeutic use of RES in humans. The 5-day exposure paradigm in these rodent studies likely affected the amount of time animals maintained a steady-state internal dose, given the rapid metabolism of RES, but may not have affected the findings substantially given the length of the study. Overall, however, these internal concentrations are higher than would be expected after moderate RES supplementation or from RES obtained from foods.

Given the potential use of RES in women of child-bearing age, perinatal exposure was included in the current Wistar Han rat study. In this study, there were no significant effects of RES on reproductive parameters. Lower mean body weights and body weight gains of dams in dosed groups ≥156 mg RES/kg body weight/day (mg/kg/day) during late gestation were observed. There were no dose-related effects on total or live litter size, pup survival, or pup weight on postnatal day (PND) 1. These findings corroborate previous studies in rodents that show no significant effects on litter size or reproduction after exposure to RES during gestation.[85; 87; 93; 152] Throughout lactation, male and female Wistar Han rat pups exposed to RES (≥312.5 mg/kg/day) had lower mean body weights than the vehicle control groups, which potentially could be due to maternal effects and/or postnatal effects on the pups. These animals were exposed to RES in utero and during lactation, both via milk and (starting on PND 12) via direct oral gavage. However, by the end of the 3-month study, RES-dosed Wistar Han rat offspring had similar mean body weights compared to vehicle control animals. A similar growth pattern was observed in a study of spontaneously hypertensive rats given RES in the diet from the beginning of gestation through PND 21 (4 g/kg diet).[152] Assuming feed consumption of approximately 20 g/animal/day and an average body weight of 300 g during gestation, the maternal exposure would be equivalent to 270 mg/kg/day in that study. Although there was no effect of gestational RES exposure on maternal or fetal body weight, mean body weights of offspring from RES-

dosed dams were lower (17%) than vehicle control animals by PND 21; dams continued to consume RES in the diet during lactation.[152] By 5 weeks of age, there was no difference in body weights among the offspring.[152] The authors of that study proposed RES may affect offspring growth by altering quantity or quality of milk production via altered prolactin synthesis, as shown in Chao et al.[153] Taken together, the findings suggested a developmental effect of RES resulting in growth retardation, potentially due to effects on the dam during gestation or lactation.[152]

In the current Wistar Han rat studies, RES and its metabolites were found in dam plasma and in whole fetuses and pups, with no clear sex difference. Internal concentrations of RES in this study were lower than doses commonly used for in vitro studies, such as those that evaluate genetic toxicity. The presence of RES and its metabolites in fetal tissue suggested low maternal transfer, and the presence of RES and its metabolites in PND 4 whole pups suggested lactational transfer. Data regarding maternal RES concentrations during pregnancy or RES in breast milk or amniotic fluid is limited and only include RES concentrations. In a study of Japanese macaques exposed to 0.37% RES via the diet during pregnancy, gestational transfer of RES was reported with slightly higher RES concentrations measured in the plasma of the fetus than the dam.[61] Another study in rats described placental transfer of RES following exposure to 4 g/kg RES via the diet from gestation day (GD) 7 to GD 21.[62] RES metabolites were detected in the fetuses and pups in the current study, generally with more *trans*-resveratrol-3-O-B-D-glucuronide (*trans*-R3G) than *trans*-resveratrol-3-sulfate (*trans*-R3S) and a low percentage of transfer from the dams. Because some sulfotransferases, but very few uridine 5'-diphospho-glucuronosyl transferases, are found in fetal livers,[154] some metabolism (i.e., sulfonation) might occur in the fetus; however, it is more likely that most of the RES metabolites detected in the fetus have crossed the placenta. Because RES and its metabolites were detected in fetuses and pups, developmental effects of RES could be related to direct effects of RES or metabolite exposure in addition to indirect effects through the dam.

RES exposure has been associated with decreased body weights of similar magnitude in other rat studies. In a 13-week exposure study in adult Wistar rats, mean body weights and body weight gains were approximately 10% lower in the highest exposure group (750 mg/kg/day via the diet) compared to control animals starting at week 4 and throughout the remainder of the study.[85] In CD® Virus Antibody Free (VAF) rats administered RES via oral gavage for four weeks, terminal mean body weights of male rats were 12% lower than vehicle control animals; however, in females, mean body weights were slightly higher than vehicle control animals (2%) in the high dose group (3,000 mg/kg/day).[86] Another 90-day oral gavage study in CD (Crl:CD®[SD]IGS) rats showed no effect on body weights in males and slight reductions (8%–9%) in females during weeks 10–13 at the 1,000 mg/kg/day dose.[88] In the current study, B6C3F1/N mice administered RES did not have significant differences in body weight. Similarly, in a multigenerational drinking water study in CD-1 (ICR) mice, male and female RES-exposed mice in the parental generation had 6%–9% lower mean body weights than control animals, which was not significant; there was no appreciable difference in the F_1 generation.[93] Decreased body weight is a common endpoint for establishing the no-observed-effect level (NOEL) in other rodent studies of RES. The NOELs in the present study were 156 mg/kg/day in rats and 312 mg/kg/day in mice, which are consistent with findings in other oral administration studies that report NOELs in rats of 200,[88] 300,[86] and 750[85] mg/kg/day.

Dose-related histopathological findings in the current studies consisted of renal and intestinal lesions in Wistar Han rats and nasal lesions in B6C3F1/N mice. The renal lesions in Wistar Han rats consisted of nephropathy and renal tubule dilatation in male and female rats and renal pelvis dilatation in female rats. The nephropathy lesions were of minimal severity and characterized by focal to multifocal small clusters of proximal tubules within the renal cortex that displayed cytoplasmic basophilia (regeneration), thickened basement membranes, and peritubular mononuclear cell infiltrates. This lesion is also known as chronic progressive nephropathy (CPN) and is a spontaneous background lesion in rodents whose incidence/severity may be exacerbated by various chemicals.[155] The exact etiology of CPN is unknown, but it is influenced by various physiological factors such as caloric intake, dietary protein content, and male hormones. In young adult rats, the early lesion consists of foci of basophilic proximal tubules with crowded nuclei and/or a thickened basement membrane. With age, the lesion progresses and may present prominent hyaline casts in the medullary region, tubule atrophy, tubule dilatation, focal glomerular sclerosis, glomerular atrophy, mononuclear cell infiltration and transitional cell hyperplasia of the renal pelvis lining. Renal tubule dilatation was diagnosed when it occurred in the renal medullary region and was not associated with any histological evidence of nephropathy or lower urinary tract obstruction. Typically, only one or a few tubules were affected, and the dilated tubules frequently contained an eosinophilic intraluminal material, consistent with protein. The renal pelvis dilatation occurred with a positive trend in female Wistar Han rats only and was characterized by distention of the renal pelvis without histological evidence of lower urinary tract obstruction and without renal papilla atrophy. Although there are many etiologies associated with this lesion, most are due to lower urinary tract obstruction. In this study, the cause was undetermined. Some of the same renal lesions were also reported in a 28-day rat study.[86] Lesions observed included tubule dilatation, papillary necrosis, hyperplasia of the pelvic epithelium, and nephropathy, among others. Most lesions occurred in the male and female 3,000 mg/kg/day dosed groups.[86]

The intestinal lesions in male and female Wistar Han rats consisted of minimal to mild lymphatic ectasia of the lacteals within the jejunum villous tips and the lymphatic vessels in the subepithelial dome region of the Peyer's patches. This lesion is also known as lymphangiectasis.[156] The cause of the lymphatic ectasia in these tissues was not determined. The jejunum contains lacteals that are lymphatic capillaries in the villous tips. These function to absorb products from the breakdown of dietary fats (fatty acids and glycerol). The lacteals can merge to form larger lymphatic vessels that transport chyle via the lymphatic system to the thoracic duct where it is emptied into the blood stream. Peyer's patches are a part of this lymphatic system, presenting as prominent masses of lymphatic tissue located in the submucosa and lamina propria throughout the rodent small intestine. Lymphatics within the Peyer's patches are involved in the lymphatic drainage of the intestines and function in immune surveillance. A similar histological finding was described in an NTP study of Fischer 344 (F344/N) rats administered indole-3-carbinol via oral gavage.[157] These animals demonstrated a dose-related dilatation of lymphatics (lymphangiectasis) of the duodenum, jejunum, and mesenteric lymph nodes. Material within dilated lacteals stained with Oil Red O and Sudan black, consistent with lipid accumulation. Electron microscopic evaluation confirmed extracellular lipid accumulation within the villar lamina propria, lacteals, and within villar macrophages. It was suggested that the accumulation of large lipid droplets was possibly due to an impairment of lipid transport in the lacteals. For a more detailed description of lipid transport, see Friedman and Nylund.[158]

The dose-related nasal lesion in female B6C3F1/N mice consisted of respiratory metaplasia of the olfactory epithelium. Respiratory metaplasia in this study was of minimal severity and defined as transformation or replacement of olfactory epithelium with a more resistant, ciliated, columnar epithelium that resembles respiratory epithelium. The presence of this metaplasia implies that loss of olfactory epithelium has occurred through necrosis or atrophy and is considered an adaptive change. The lesions were focally extensive and occurred most often in the dorsal aspect (dorsal medial meatus) of the nasal cavity at Level II. Mechanisms of exposure of nasal tissues to toxicants in noninhalation studies can be via the bloodstream or via regurgitation/reflux, inhalation of volatiles from the stomach, or by exhaling the parent or a toxic metabolite.[159] Metabolism of a parent compound or metabolites that arrive from the blood stream may occur in respiratory tissues and studies have shown that significant enzymatic activity occurs in the cytoplasm of olfactory epithelium; the resulting metabolism may detoxify a material or result in a more toxic metabolite.[159; 160]

RES-dosed male and female mice had higher liver weights than vehicle control animals, with significantly increased relative weights among the ≥625 mg/kg/day female dosed groups and significantly increased absolute and relative weights in the 2,500 mg/kg/day male dosed group. These organ weight changes did not correlate with any microscopic observations. Hepatomegaly was also reported in CD male and female rats given 1,000 mg/kg/day for 13 weeks, along with increases in bilirubin.[88] In that study, there was no microscopic evidence of hepatotoxicity. The increase in liver weight observed may be due to induction of hepatic enzymes involved in metabolism and/or antioxidant pathways.[33]

Data from Wistar Han rats and B6C3F1/N mice presented here provide little indication of reproductive toxicity in males and females. There were no significant differences in testes weights and no microscopic findings in the male or female reproductive tracts of rats or mice. Differences in cauda epididymis sperm count and alterations in vaginal cytology in B6C3F1/N mice were not considered related to dose as they were small in magnitude and not consistently dose dependent. Similarly, other studies in rodents report no change in reproductive organ weight or histopathology at doses up to 1,000 mg/kg/day[85; 88] or no significant differences in sperm count/quality, ovarian morphology, or estrous cycling at doses up to 750 mg/kg/day.[85; 93] There have been indications, however, of an effect of lower doses of RES on reproductive biology. In Sprague Dawley rats administered 20 mg/kg/day via gavage for 90 days, sperm counts were higher in RES-dosed rats, accompanied by increases in seminiferous tubule density, but without changes in testes weight or sperm quality.[95] In addition, RES consumption was linked to increased ovary weight and disrupted estrous cycling in Sprague Dawley rats exposed to 100 μM RES in drinking water.[161] While the differences in findings could be attributed to hormetic effects of RES, evidence for such is limited to in vitro and a few in vivo studies.[162] Additional studies evaluating the dose response of the reproductive effects would help clarify these disparate findings and the underlying mechanisms.

Under the conditions of this study, the lowest-observed-effect level (LOEL) was 312.5 mg/kg/day in rats as indicated by significantly decreased pup mean body weights of Wistar Han rats exposed perinatally. These body weight differences were resolved in the rat pups by the end of the 3-month study. In B6C3F1/N mice, the LOEL was 625 mg/kg/day as indicated by significantly increased relative liver weights in females; however, these changes in liver weight were not associated with microscopic lesions. The no-observed-effect levels were 156 mg/kg/day in rats and 312 mg/kg/day in mice. Target organs included the kidney and small intestine in rats

and the nose in female mice. There was no evidence of genetic toxicity in the micronucleus assay of RES at oral gavage doses up to 1,250 mg/kg/day in Wistar Han rats or up to 2,500 mg/kg/day in B6C3F1/N mice. No clear effects on reproductive parameters were observed. The presence of RES and its metabolites in fetal tissue suggested low maternal transfer, and the presence of RES and its metabolites in PND 4 whole pups suggested lactational transfer.

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Appendix A. Chemical Characterization and Dose Formulation Studies

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A.1. Procurement and Characterization

A.1.1. *Trans*-resveratrol

Trans-resveratrol (RES) was obtained from Bayville Chemical Supply Co. Inc. (Deer Park, NY) in a single lot (156AB). Identity, purity, and stability analyses were conducted by the analytical chemistry lab at RTI International (Research Triangle Park, NC). Reports on analyses performed in support of the RES study are on file at the National Institute of Environmental Health Sciences.

RES lot 156AB is a fine off-white powder with a melting point of 253.5°C. The identity of lot 156AB was evaluated using Fourier transform infrared (FT-IR) spectroscopy and ^{1}H nuclear magnetic resonance (NMR) spectroscopy. Low-resolution mass spectrometry and high-resolution mass spectrometry were conducted by the analytical chemistry laboratory at RTI International (Research Triangle Park, NC) and the University of South Carolina (Columbia, SC), respectively. The FT-IR and ^{1}H NMR spectra (Figure A-1 and Figure A-2) were consistent with the structure of RES, although no reference spectra were available. The low- and high-resolution mass spectra were consistent with the mass and structure of resveratrol.

Purity evaluation using high-performance liquid chromatography (HPLC) with ultraviolet (UV) detection was conducted with two different columns (Table A-1, Systems A and B). No impurities were identified using HPLC/UV, which could indicate that the minor diethyl ether and hexane impurities were eluted in the solvent front. Additionally, the presence of the *cis*-isomer of RES within the lot was evaluated using HPLC with a photodiode array (PDA) detector (Table A-1, System C). The *cis*-isomer was not detected with a limit of detection of 0.018% weight basis. Karl Fisher titration performed at Galbraith Laboratories (Knoxville, TN) indicated a water content of 0.24%. The overall purity of lot 156AB was determined to be >99%.

Accelerated stability studies were conducted using the HPLC/UV protocol outlined above (Table A-1, System A). Stability was confirmed when protected from light and stored for 2 weeks at refrigerated (5°C), room (25°C), elevated (60°C), and frozen (−20°C) temperatures. Short-term storage (up to 2 weeks) of RES at room temperature when protected from light was deemed acceptable. The bulk chemical was stored at −20°C and protected from light, per the manufacturer's recommendations.

A.1.2. Methylcellulose

Methylcellulose used to make the 0.5% aqueous vehicle for gavage formulations was obtained from Spectrum (Gardena, CA) in three lots (UR1026, WL0069, and XB1050). Lot UR1026 was used in the 2-week studies and lots WL0069 and XB1050 were used in the 3-month studies.

The identity of the methylcellulose was confirmed by the study laboratory using FT-IR spectroscopy. Periodic purity analyses were performed by Galbraith Laboratories, Inc (Knoxville, TN) during the 3-month studies to determine the methoxy group content. The August 27, 2008 sample of lot WL0069, used to prepare the first dose formulation, had methoxy group content (32.2%) outside of the acceptance criteria of 27.5%–31.5%. A replacement lot (XB1050) was procured for the remaining formulations and was within the acceptance criteria for methoxy group content (29.3%).

Deionized water was used to make the 0.5% aqueous methylcellulose vehicle for gavage formulations.

A.2. Preparation and Analysis of Dose Formulations

Dose formulations of RES in 0.5% methylcellulose were prepared following the protocols outlined in Table A-2. Formulations of 15.6, 31.2, 62.5, 125, and 250 mg/mL were used in the 2-week study in Fischer 344 (F344/NTac) rats, the 3-month study in Wistar Han rats, and the 2-week and 3-month studies in B6C3F1/N mice (Table A-2). Formulation concentrations and homogeneity were evaluated using HPLC/UV (Table A-1, System A). The method of preparation was validated for concentration ranges of 15−500 mg/mL. The RES formulations were confirmed to be resuspended after overnight storage by using a Polytron blender. Homogeneity was confirmed in 15.6 mg/mL preparations of dose formulation.

Stability of 15.6 mg/mL and 500 mg/mL formulations was confirmed for 42 days when protected from light and stored at room (~25°C), refrigerated (5°C), and freezer (−20°C) temperatures. A dosing simulation study on the 15.6 mg/mL formulation found that it was stable when stored at room temperature in an open amber vessel for 3 hours.

Analyses of preadministration and postadministration dose formulations were conducted throughout the study by the study laboratory (Table A-3, Table A-4, Table A-5, Table A-6). Postadministration samples were collected from the bottles used to dose the animals. Two dose formulations (31.2 and 125 mg/mL) prepared for the 2-week studies were >10% above the target concentrations and were replaced by freshly prepared formulations that met criteria prior to administration. Postadministration samples of the 62.5 mg/mL dose formulation in the 2-week studies for mice and F344/NTac rats were 17.7% and 19.5% above the target concentration, respectively (Table A-3, Table A-4). All preadministration samples from the 3-month studies were within 10% of the target concentrations, except for a 125 mg/mL formulation prepared for the mouse study in September 2008 which was replaced by a freshly prepared formulation that met criteria prior to administration. Postadministration samples of the 125 and 250 mg/mL dose formulations prepared in September 2008 for the 3-month mice studies were 10.9% and 14.3% above the target concentrations, respectively. All other samples were within 10% of the target concentration (Table A-3, Table A-4, Table A-5, Table A-6).

Table A-1. Chromatography Systems Used in the Two-week and Three-month Gavage Studies of *Trans*-resveratrol

Chromatography	Detection System	Column	Mobile Phase
System A			
High-performance Liquid Chromatography	Ultraviolet (300 nm)	Zorbax Rx-C8 (250 mm × 4.6 mm ID)	A: water:methanol, pH ~4 (95:5) B: methanol:water, pH ~4 (90:10) Gradient program: A:B 90:10 for 5 minutes; 90:10 to 10:90 in 25 minutes; 10:90 to 90:10 in 20 minutes; hold at 90:10 for 10 minutes 1.0 mL/minute flow rate

Chromatography	Detection System	Column	Mobile Phase
System B			
High-performance Liquid Chromatography	Ultraviolet (300 nm)	Waters Nova-Pak Phenyl 60Å (150 mm × 3.9 mm ID)	A: water:methanol, pH ~4 (95:5) B: methanol:water, pH ~4 (90:10) Gradient program: A:B 90:10 for 5 minutes; 90:10 to 10:90 in 25 minutes; 10:90 to 90:10 in 20 minutes; hold at 90:10 for 20 minutes 1.0 mL/minute flow rate
System C			
High-performance Liquid Chromatography	Photodiode array (220–500 nm, extracted at 305 nm)	Waters Atlantis T3 C18 (100 mm × 2.1 mm ID, 3 µm particle size) with Waters Atlantis HILIC C18 guard (10 mm × 2.1 mm ID, 3 µm particle size)	A: 5 mM ammonium acetate in DI water:2-propanol (98:2, v/v) B: methanol:2-propanol (98:2, v/v) Gradient program: A:B 100:0 to 40:60 in 15 minutes; 40:60 to 0:100 in 0.5 minutes; hold at 0:100 for 2 minutes; 0:100 to 100:0 in 0.5 minutes; hold at 100:0 for 2 minutes 0.3 mL/minute flow rate

ID = internal diameter; DI = deionized.

Table A-2. Preparation and Storage of Dose Formulations in the Two-week and Three-month Gavage Studies of *Trans*-resveratrol

Preparation
Formulations of *trans*-resveratrol (lot 156AB) in 0.5% methylcellulose were prepared by transferring an appropriate amount of *trans*-resveratrol (determined by weight) into a graduated centrifuge tube containing ~4 mL of 0.5% methylcellulose. The contents were mixed by vortex action and sonification, then stored in a refrigerator overnight. The following day, the tube was removed from the refrigerator and sonicated for 20 minutes. The formulations were brought to volume with 0.5% methylcellulose and liquified by mixing with a Polytron blender.
Chemical Lot Number
Lot 156AB (Bayville Chemical Supply Co. Inc.)
Maximum Storage Time
42 days
Storage Conditions
Clear glass bottled placed in amber bags stored at ~2°C−8°C (refrigerated)
Study Laboratory
RTI International (Research Triangle Park, NC)

Table A-3. Results of Analyses of Dose Formulations Administered to Male and Female F344/NTac Rats in the Two-week Gavage Study of *Trans*-resveratrol

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration (mg/mL)[a]	Difference from Target (%)
October 2, 2006	October 5–11, 2006	0	BLOQ	NA
		15.6	15.25 ± 0.03	−2.3
		31.2[b]	37.00 ± 0.07	18.6
		62.5	68.58 ± 0.49	9.7
		125[b]	143.0 ± 1.0	14.4
		250	270.4 ± 1.2	8.2
October 12, 2006	October 12–13, 2006	31.2	30.53 ± 0.37	−2.1
		125	121.7 ± 2.1	−2.6
Animal Room Samples				
October 2, 2006	November 1–3, 2006	0	BLOQ	NA
		15.6	15.95 ± 0.18	2.2
		62.5	74.70 ± 0.39	19.5
		250	276.1 ± 1.4	10.5
October 12, 2006	November 1–3, 2006	31.2	30.83 ± 0.17	−1.2
		125	119.2 ± 0.7	−4.6

BLOQ = below the limit of quantification; NA = not applicable.
[a]Average of triplicate analysis.
[b]The formulation was not used in the study and was replaced by the formulation prepared on October 12, 2006.

Table A-4. Results of Analyses of Dose Formulations Administered to Male and Female B6C3F1/N Mice in the Two-week Gavage Study of *Trans*-resveratrol

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration (mg/mL)[a]	Difference from Target (%)
October 2, 2006	October 5–11, 2006	0	BLOQ	NA
		15.6	15.25 ± 0.03	−2.3
		31.2[b]	37.00 ± 0.07	18.6
		62.5	68.58 ± 0.49	9.7
		125[b]	143.0 ± 1.0	14.4
		250	270.4 ± 1.2	8.2
October 12, 2006	October 12–13, 2006	31.2	30.53 ± 0.37	−2.1
		125	121.7 ± 2.1	−2.6
Animal Room Samples				
October 2, 2006	November 1–3, 2006	0	BLOQ	NA
		15.6	15.71 ± 0.07	0.7

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration (mg/mL)[a]	Difference from Target (%)
		62.5	73.58 ± 0.61	17.7
		250	272.2 ± 2.0	8.9
October 12, 2006	November 1–3, 2006	31.2	30.61 ± 0.11	−1.9
		125	119.5 ± 3.8	−4.4

BLOQ = below the limit of quantification; NA = not applicable.
[a]Average of triplicate analysis.
[b]The formulation was not used in the study and was replaced by the formulation prepared on October 12, 2006.

Table A-5. Results of Analyses of Dose Formulations Administered to Male and Female Wistar Han Rats in the Three-month Gavage Study of *Trans*-resveratrol

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration (mg/mL)[a]	Difference from Target (%)
October 1, 2008	October 3, 2008	0	BLOQ	NA
		15.6	16.3 ± 0.1	4.5
		15.6	16.3 ± 0.1	4.5
		15.6	16.1 ± 0.1	3.0
		31.2	32.6 ± 0.1	4.4
		31.2	32.1 ± 0.2	2.8
		31.2	31.6 ± 0.3	1.4
		62.5	65.0 ± 0.8	4.0
		62.5	64.5 ± 0.2	3.2
		62.5	65.4 ± 0.8	4.7
		125	126 ± 1	1.1
		125	130 ± 1	4.3
		125	128 ± 1	2.7
		250	257 ± 3	2.9
		250	257 ± 2	2.8
		250	260 ± 1	4.0
October 22, 2008	October 29, 2008	0	BLOQ	NA
		15.6	15.8 ± 0.1	1.5
		15.6	15.7 ± 0.1	0.8
		15.6	15.6 ± 0.1	0.2
		31.2	31.6 ± 0.3	1.4
		31.2	31.5 ± 0.1	1.0
		31.2	31.1 ± 0.1	−0.4
		62.5	63.3 ± 0.5	1.3

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration (mg/mL)[a]	Difference from Target (%)
		62.5	62.2 ± 0.2	−0.5
		62.5	62.9 ± 0.2	0.7
		125	126 ± 0	0.8
		125	125 ± 0	0.0
		125	121 ± 2	−3.2
		250	247 ± 5	−1.3
		250	252 ± 1	0.7
		250	250 ± NA[b]	−0.2
November 21, 2008	November 24, 2008	0	BLOQ	NA
		15.6	15.8 ± 0.1	1.1
		15.6	15.8 ± 0.2	1.1
		15.6	15.5 ± 0.1	−0.8
		31.2	31.5 ± 0.1	1.0
		31.2	31.1 ± 0.3	−0.3
		31.2	30.9 ± 0.1	−1.0
		62.5	62.6 ± 0.5	0.1
		62.5	63.1 ± 0.2	1.0
		62.5	62.8 ± 0.2	0.4
		125	124 ± 1	−1.1
		125	124 ± 1	−1.1
		125	126 ± 1	0.5
		250	248 ± 1	−0.8
		250	247 ± 1	−1.2
		250	247 ± 1	−1.2
January 20, 2009	January 21, 2009	0	BLOQ	NA
		15.6	15.8 ± 0.2	1.5
		15.6	15.8 ± 0.1	1.1
		31.2	31.6 ± 0.2	1.3
		31.2	31.6 ± 0.2	1.3
		62.5	63.6 ± 0.2	1.8
		62.5	60.5 ± 0.2	−3.2
		125	127 ± 0	1.6
		125	126 ± 1	0.5
		250	249 ± 1	−0.3
		250	251 ± 1	0.5

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration (mg/mL)[a]	Difference from Target (%)
Animal Room Samples				
October 1, 2008	November 10, 2008	0	BLOQ	NA
		15.6	15.2 ± 0.3	−2.3
		31.2	30.5 ± 0.8	−2.2
		62.5	63.2 ± 0.1	1.1
		125	126 ± 1	0.8
		250	248 ± 2	−0.7
October 22, 2008	December 4, 2008	0	BLOQ	NA
		15.6	15.8 ± 0.2	1.5
		31.2	31.8 ± 0.2	1.8
		62.5	62.6 ± 0.5	0.1
		125	124 ± 1	−1.1
		250	244 ± 6	−2.5
November 21, 2008	January 5, 2009	0	BLOQ	NA
		15.6	15.7 ± 0.0	0.6
		31.2	30.8 ± 0.4	−1.3
		62.5	62.4 ± 0.2	−0.2
		125	124 ± 1	−0.5
		250	249 ± 1	−0.4
January 20, 2009	March 2, 2009	0	BLOQ	NA
		15.6	15.9 ± 0.1	2.1
		31.2	31.8 ± 0.1	1.8
		62.5	61.6 ± 0.1	−1.5
		125	127 ± 0	1.6
		250	254 ± 1	1.7

BLOQ = below the limit of quantification; NA = not applicable.
[a]Average of triplicate analysis.
[b]Average of duplicate analysis.

Table A-6. Results of Analyses of Dose Formulations Administered to Male and Female B6C3F1/N Mice in the Three-month Gavage Study of *Trans*-resveratrol

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration (mg/mL)[a]	Difference from Target (%)
September 10, 2008	September 12, 2008	0	BLOQ	NA
		15.6	15.3 ± 0.1	−1.9
		31.2	30.5 ± 1.2	−2.1
		62.5	62.8 ± 0.4	0.5
		125	140 ± 1	11.7
		125[b]	128 ± 3	2.4
		250	266 ± 1	6.4
October 1, 2008	October 3, 2008	0	BLOQ	NA
		15.6	16.3 ± 0.1	4.5
		15.6	16.3 ± 0.1	4.5
		15.6	16.1 ± 0.1	3.0
		31.2	32.6 ± 0.1	4.4
		31.2	32.1 ± 0.2	2.8
		31.2	31.6 ± 0.3	1.4
		62.5	65.0 ± 0.8	4.0
		62.5	64.5 ± 0.2	3.2
		62.5	65.4 ± 0.8	4.7
		125	126 ± 1	1.1
		125	130 ± 1	4.3
		125	128 ± 1	2.7
		250	257 ± 3	2.9
		250	257 ± 2	2.8
		250	260 ± 1	4.0
October 22, 2008	October 29, 2008	0	BLOQ	NA
		15.6	15.8 ± 0.1	1.5
		15.6	15.7 ± 0.1	0.8
		15.6	15.6 ± 0.1	0.2
		31.2	31.6 ± 0.3	1.4
		31.2	31.5 ± 0.1	1.0
		31.2	31.1 ± 0.1	−0.4
		62.5	63.3 ± 0.5	1.3
		62.5	62.2 ± 0.2	−0.5
		62.5	62.9 ± 0.2	0.7

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration (mg/mL)[a]	Difference from Target (%)
		125	126 ± 0	0.8
		125	125 ± 0	0.0
		125	121 ± 2	−3.2
		250	247 ± 5	−1.3
		250	252 ± 1	0.7
		250	250 ± NA[c]	−0.2
November 21, 2008	November 24, 2008	0	BLOQ	NA
		15.6	15.8 ± 0.1	1.1
		15.6	15.8 ± 0.2	1.1
		15.6	15.5 ± 0.1	−0.8
		31.2	31.5 ± 0.1	1.0
		31.2	31.1 ± 0.3	−0.3
		31.2	30.9 ± 0.1	−1.0
		62.5	62.6 ± 0.5	0.1
		62.5	63.1 ± 0.2	1.0
		62.5	62.8 ± 0.2	0.4
		125	124 ± 1	−1.1
		125	124 ± 1	−1.1
		125	126 ± 1	0.5
		250	248 ± 1	−0.8
		250	247 ± 1	−1.2
		250	247 ± 1	−1.2
Animal Room Samples				
September 10, 2008	October 22, 2008	0	BLOQ	NA
		15.6	16.1 ± 0.0	3.2
		31.2	32.2 ± 0.3	3.3
		62.5	67.0 ± 0.6	7.2
		125[b]	139 ± 1	10.9
		250	286 ± 3	14.3
October 22, 2008	December 4, 2008	0	BLOQ	NA
		15.6	15.8 ± 0.1	1.3
		31.2	31.8 ± 0.2	1.9
		62.5	64.6 ± 0.4	3.4
		125	125 ± 1	0
		250	245 ± 1	−2.0

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration (mg/mL)[a]	Difference from Target (%)
November 21, 2008	January 5, 2009	0	BLOQ	NA
		15.6	15.6 ± 0.1	−0.2
		31.2	31.1 ± 0.1	−0.4
		62.5	62.9 ± 0.2	0.6
		125	126 ± 1	1.1
		250	247 ± 2	−1.3

BLOQ = below the limit of quantification; NA = not applicable.
[a]Average of triplicate analysis.
[b]Formulation prepared in a separate batch on September 15, 2008.
[c]Average of duplicate analysis.

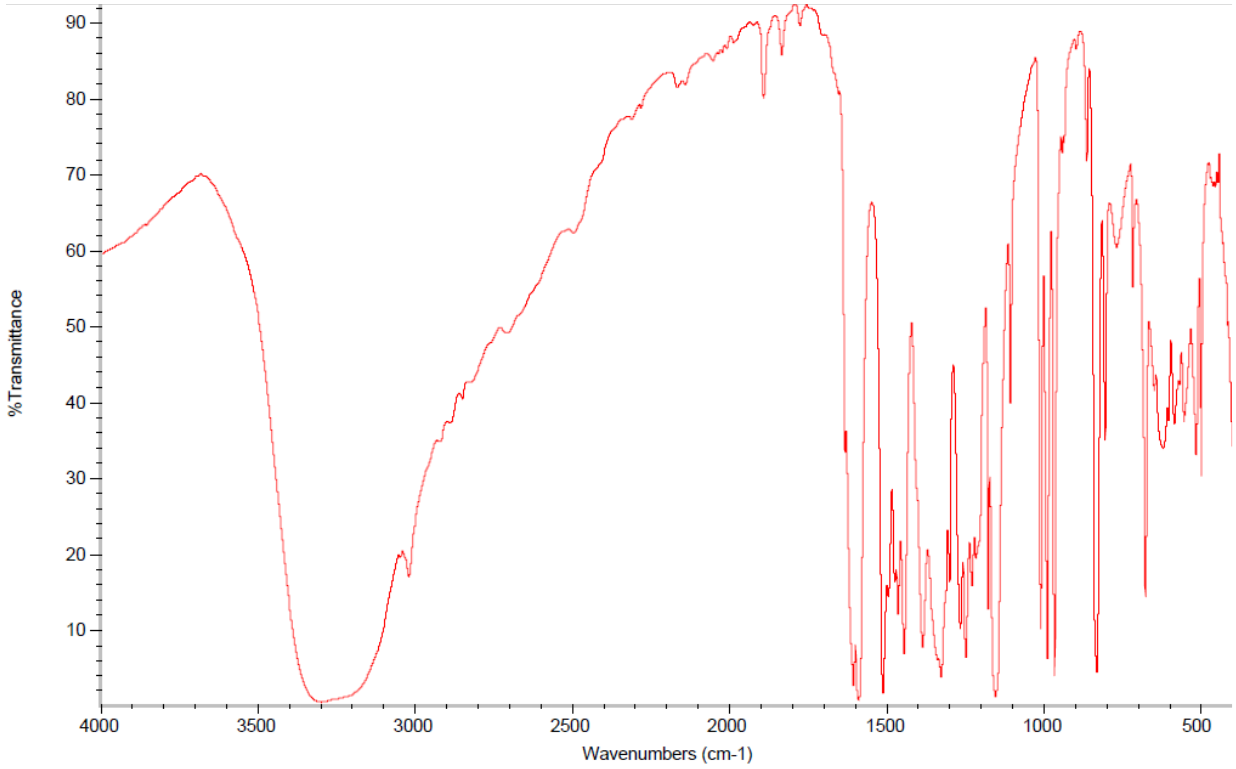


Figure A-1. Infrared Absorption Spectrum of *Trans*-resveratrol

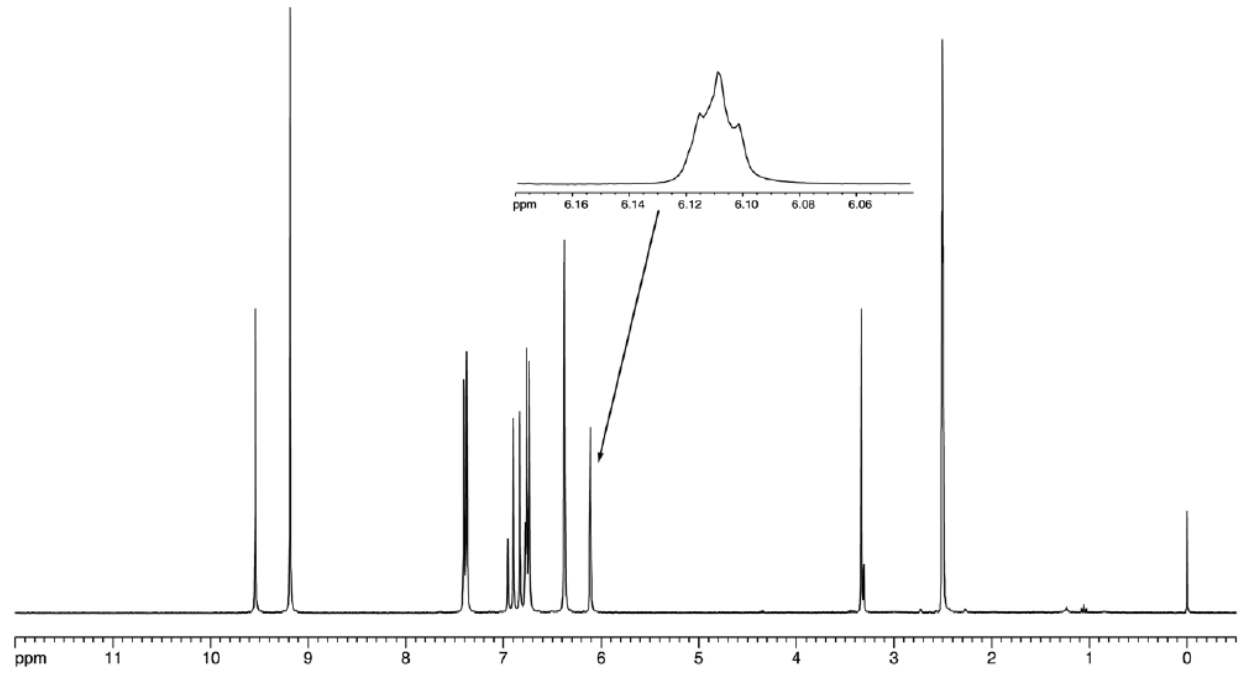


Figure A-2. ^{1}H Nuclear Magnetic Resonance Spectrum of Reference Sample of *Trans*-resveratrol

Appendix B. Ingredients, Nutrient Composition, and Contaminant Levels in NIH-07 Rat and NTP-2000 Rat and Mouse Ration

Tables

Table B-1. Ingredients of NIH-07 Rat Ration B-2
Table B-2. Vitamins and Minerals in NIH-07 Rat Ration B-2
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Table B-1. Ingredients of NIH-07 Rat Ration

Ingredients	Percent by Weight
Ground Hard Winter Wheat	23.00
Ground #2 Yellow Shelled Corn	24.25
Wheat Middlings	10.0
Oat Hulls	0.0
Alfalfa Meal (Dehydrated, 17% Protein)	4.0
Purified Cellulose	0.0
Soybean Meal (47% Protein)	12.0
Fish Meal (62% Protein)	10.0
Corn Oil (Without Preservatives)	0.0
Soy Oil (Without Preservatives)	2.5
Dried Brewer's Yeast	2.0
Calcium Carbonate (USP)	0.5
Vitamin Premix[a]	0.25
Mineral Premix[b]	0.15
Calcium Phosphate, Dibasic (USP)	1.25
Sodium Chloride	0.5
Choline Chloride (70% Choline)	0.10
Dried Skim Milk	5.00
Dried Molasses	1.50
Corn Gluten Meal (60% Protein)	3.00
Methionine	0.0

USP = United States Pharmacopeia.
[a]Wheat middling as carrier.
[b]Calcium carbonate as carrier.

Table B-2. Vitamins and Minerals in NIH-07 Rat Ration

	Amount[a]	Source
Vitamins		
Vitamin A	6,062 IU	Stabilized vitamin A palmitate or acetate
Vitamin D	5,070 IU	D-activated animal sterol
Vitamin K	3.1 mg	Menadione sodium bisulfite complex
Vitamin E	22 IU	α-Tocopheryl acetate
Niacin	33 mg	–
Folic Acid	2.4 mg	–
d-Pantothenic Acid	19.8 mg	d-Calcium pantothenate
Riboflavin	3.8 mg	–

	Amount[a]	Source
Thiamine	11 mg	Thiamine mononitrate
B_{12}	50 µg	–
Pyridoxine	6.5 mg	Pyridoxine hydrochloride
Biotin	0.15 mg	d-Biotin
Minerals		
Iron	132 mg	Iron sulfate
Zinc	18 mg	Zinc oxide
Manganese	66 mg	Manganese oxide
Copper	4.4 mg	Copper sulfate
Iodine	2.0 mg	Calcium iodate
Cobalt	0.44 mg	Cobalt carbonate

[a]Per kg of finished product.

Table B-3. Nutrient Composition of NIH-07 Rat Ration

Nutrient	Mean ± Standard Deviation	Range	Number of Samples
Protein (% by Weight)	24	–	1
Crude Fat (% by Weight)	5	–	1
Crude Fiber (% by Weight)	3.6	–	1
Ash (% by Weight)	6.77	–	1
Amino Acids (% of Total Diet)			
Arginine	1.380 ± 0.06	1.3–1.49	10
Cystine	0.322 ± 0.031	0.274–0.372	10
Glycine	1.150 ± 0.070	1.06–1.31	10
Histidine	0.518 ± 0.024	0.497–0.553	10
Isoleucine	0.984 ± 0.024	0.952–1.03	10
Leucine	2.018 ± 0.067	1.93–2.13	10
Lysine	1.243 ± 0.051	1.13–1.32	10
Methionine	0.488 ± 0.016	0.468–0.515	10
Phenylalanine	1.097 ± 0.022	1.07–1.12	10
Threonine	0.918 ± 0.031	0.883–0.961	10
Tryptophan	0.277 ± 0.020	0.265–0.326	10
Tyrosine	0.860 ± 0.037	0.785–0.894	10
Valine	1.134 ± 0.025	1.11–1.17	10
Essential Fatty Acids (% of Total Diet)			
Linoleic	2.30 ± 0.219	1.99–2.59	10
Linolenic	0.25 ± 0.275	0.217–0.296	10

Nutrient	Mean ± Standard Deviation	Range	Number of Samples
Vitamins			
Vitamin A (IU/kg)	4,640	–	1
α-Tocopherol (ppm)	6,704 ± 21,045	40.3–66,600	10
Thiamine (ppm)[a]	10.6	–	1
Riboflavin (ppm)	14.47 ± 3.352	10.0–19.8	10
Niacin (ppm)	99.33 ± 8.235	87.0–112.0	10
Pantothenic Acid (ppm)	44.38 ± 3.806	38.2–51.1	10
Pyridoxine (ppm)[a]	12.876 ± 3.171	9.63–19.7	10
Folic Acid (ppm)	2.482 ± 0.487	1.68–3.09	10
Biotin (ppm)	0.3283 ± 0.172	0.0–0.638	10
B_{12} (ppb)	49.4 ± 6.83	41.8–61.6	10
Choline (as chloride) (ppm)	1,821.0 ± 197.5	1,570–2,200	10
Minerals			
Calcium (%)	1.150	–	1
Phosphorus (%)	0.930	–	1
Potassium (%)	0.830 ± 0.036	0.769–0.88	10
Chloride (%)	0.652 ± 0.106	0.441–0.8	10
Sodium (%)	0.378 ± 0.46	0.318–0.469	10
Magnesium (%)	0.187 ± 0.014	0.17–0.218	10
Iron (ppm)	385.1 ± 54.9	276.0–469.0	10
Manganese (ppm)	90.81 ± 7.566	80.7–104.0	10
Zinc (ppm)	64.15 ± 10.07	52.4–89.2	10
Copper (ppm)	14.13 ± 2.57	11.9–21.1	10
Iodine (ppm)	1.811 ± 0.992	0.54–3.45	10
Chromium (ppm)	1.422 ± 0.934	0.277–3.89	10
Cobalt (ppm)	0.5155 ± 0.267	0.01–0.963	10

[a]As hydrochloride.

Table B-4. Contaminant Levels in NIH-07 Rat Ration

	Level	Number of Samples
Contaminants		
Arsenic (ppm)	0.46	1
Cadmium (ppm)	0.11	1
Lead (ppm)	0.11	1
Mercury (ppm)	0.01	1

	Level	Number of Samples
Selenium (ppm)	0.46	1
Aflatoxins (ppb)[a]	5	1
Nitrate Nitrogen (ppm)[b]	14.2	1
Nitrite Nitrogen (ppm)[a,b]	<0.61	1
BHA (ppm)[a,c]	<1.0	1
BHT (ppm)[a,c]	<1.0	1
Aerobic Plate Count (CFU/gm)	<10	1
Coliform (MPN/gm)	<3	1
Escherichia coli (MPN/gm)	<10	1
Salmonella sp. (MPN/gm)	negative	1
Total Nitrosamines (ppb)[d]	8.7	1
N-Ndimethylamine (ppb)[d]	4.4	1
N-Npyrrolidine (ppb)[d]	4.3	1
Pesticides (ppm)		
α-BHC[a]	<0.01	1
β-BHC[a]	<0.02	1
γ-BHC[a]	<0.01	1
δ-BHC[a]	<0.01	1
Heptachlor[a]	<0.01	1
Aldrin[a]	<0.01	1
Heptachlor Epoxide[a]	<0.01	1
DDE[a]	<0.01	1
DDD[a]	<0.01	1
DDT[a]	<0.01	1
HCB[a]	<0.01	1
Mirex[a]	<0.01	1
Methoxychlor[a]	<0.05	1
Dieldrin[a]	<0.01	1
Endrin[a]	<0.01	1
Telodrin[a]	<0.01	1
Chlordane[a]	<0.05	1
Toxaphene[a]	<0.10	1
Estimated PCBs[a]	<0.20	1
Ronnel[a]	<0.01	1
Ethion[a]	<0.02	1
Trithion[a]	<0.05	1

	Level	Number of Samples
Diazinon[a]	<0.10	1
Methyl Chlorpyrifos	0.0335	1
Methyl Parathion[a]	<0.02	1
Ethyl Parathion[a]	<0.02	1
Malathion	0.043	1
Endosulfan I[a]	<0.01	1
Endosulfan II[a]	<0.01	1
Endosulfane Sulfate[a]	<0.03	1

All samples were irradiated.
BHA = butylated hydroxyanisole; BHT = butylated hydroxytoluene; CFU = colony-forming units; MPN = most probable number; BHC = hexachlorocyclohexane or benzene hexachloride; DDE = dichlorodiphenyldichloroethylene; DDD = dichlorodiphenyldichloroethane; DDT = dichlorodiphenyltrichloroethane; HCB = hexachlorobenzene; PCB = polychlorinated biphenyl.
[a]All values were below the detection limit. The detection limit is given as the mean.
[b]Sources of contamination include alfalfa, grains, and fish meal.
[c]Sources of contamination include soy oil and fish meal.
[d]All values were corrected for percent recovery.

Table B-5. Ingredients of NTP-2000 Rat and Mouse Ration

Ingredients	Percent by Weight
Ground Hard Winter Wheat	23.00
Ground #2 Yellow Shelled Corn	22.44
Wheat Middlings	15.0
Oat Hulls	8.5
Alfalfa Meal (Dehydrated, 17% Protein)	7.5
Purified Cellulose	5.5
Soybean Meal (49% Protein)	4.0
Fish Meal (60% Protein)	4.0
Corn Oil (without Preservatives)	3.0
Soy Oil (without Preservatives)	3.0
Dried Brewer's Yeast	1.0
Calcium Carbonate (USP)	0.9
Vitamin Premix[a]	0.5
Mineral Premix[b]	0.5
Calcium Phosphate, Dibasic (USP)	0.4
Sodium Chloride	0.3
Choline Chloride (70% Choline)	0.26
Methionine	0.2

USP = United States Pharmacopeia.
[a]Wheat middling as carrier.
[b]Calcium carbonate as carrier.

Table B-6. Vitamins and Minerals in NTP-2000 Rat and Mouse Ration

	Amount[a]	Source
Vitamins		
Vitamin A	4,000 IU	Stabilized vitamin A palmitate or acetate
Vitamin D	1,000 IU	D-activated animal sterol
Vitamin K	1.0 mg	Menadione sodium bisulfite complex
α-Tocopheryl Acetate	100 IU	–
Niacin	23 mg	–
Folic Acid	1.1 mg	–
d-Pantothenic Acid	10 mg	d-Calcium pantothenate
Riboflavin	3.3 mg	–
Thiamine	4 mg	Thiamine mononitrate
B_{12}	52 µg	–
Pyridoxine	6.3 mg	Pyridoxine hydrochloride
Biotin	0.2 mg	d-Biotin
Minerals		
Magnesium	514 mg	Magnesium oxide
Iron	35 mg	Iron sulfate
Zinc	12 mg	Zinc oxide
Manganese	10 mg	Manganese oxide
Copper	2.0 mg	Copper sulfate
Iodine	0.2 mg	Calcium iodate
Chromium	0.2 mg	Chromium acetate

[a]Per kg of finished product.

Table B-7. Nutrient Composition of NTP-2000 Rat and Mouse Ration

Nutrient	Mean ± Standard Deviation	Range	Number of Samples
Protein (% by Weight)	15.05 ± 0.794	13.9–15.9	5
Crude Fat (% by Weight)	8.32 ± 0.172	8.0–8.5	5
Crude Fiber (% by Weight)	9.05 ± 0.400	8.64–9.73	5
Ash (% by Weight)	5.24 ± 0.170	5.0–5.43	5
Amino Acids (% of Total Diet)			
Arginine	0.805 ± 0.075	0.67–0.97	29
Cystine	0.220 ± 0.021	0.15–0.25	29
Glycine	0.702 ± 0.038	0.62–0.8	29
Histidine	0.342 ± 0.070	0.27–0.68	29
Isoleucine	0.549 ± 0.040	0.43–0.66	29

Nutrient	Mean ± Standard Deviation	Range	Number of Samples
Leucine	1.100 ± 0.063	0.96–1.24	29
Lysine	0.700 ± 0.104	0.31–0.86	29
Methionine	0.409 ± 0.042	0.26–0.49	29
Phenylalanine	0.623 ± 0.047	0.471–0.72	29
Threonine	0.513 ± 0.041	0.43–0.61	29
Tryptophan	0.155 ± 0.027	0.11–0.2	29
Tyrosine	0.422 ± 0.066	0.28–0.54	29
Valine	0.666 ± 0.040	0.55–0.73	29
Essential Fatty Acids (% of Total Diet)			
Linoleic	3.94 ± 0.235	3.49–4.55	29
Linolenic	0.30 ± 0.064	0.005–0.368	29
Vitamins			
Vitamin A (IU/kg)	2,965 ± 40	2,350–3,510	5
Vitamin D (IU/kg)[a]	1,000	–	–
α-Tocopherol (ppm)	2,456 ± 12,817	13.6–69,100	29
Thiamine (ppm)[b]	6.83 ± 1.329	5.3–9.0	5
Riboflavin (ppm)	8.17 ± 2.841	4.2–17.5	29
Niacin (ppm)	78.66 ± 8.11	66.4–98.2	29
Pantothenic Acid (ppm)	26.42 ± 11.05	17.4–81.0	29
Pyridoxine (ppm)[b]	9.75 ± 2.045	6.44–14.3	29
Folic Acid (ppm)	1.58 ± 0.43	1.15–3.27	29
Biotin (ppm)	0.323 ± 0.093	0.2–0.704	29
B_{12} (ppb)	50.41 ± 34.89	18.3–174	29
Choline (as chloride) (ppm)	2,593 ± 633.8	1,160–3,790	29
Minerals			
Calcium (%)	0.926 ± 0.024	0.898–0.969	5
Phosphorus (%)	0.570 ± 0.026	0.504–0.606	5
Potassium (%)	0.668 ± 0.029	0.626–0.733	29
Chloride (%)	0.392 ± 0.044	0.3–0.517	29
Sodium (%)	0.195 ± 0.027	0.16–0.283	29
Magnesium (%)	0.217 ± 0.054	0.185–0.49	29
Iron (ppm)	191.6 ± 36.18	135–311	29
Manganese (ppm)	50.11 ± 9.42	21–73.1	29
Zinc (ppm)	57.3 ± 25.54	43.3–184	29
Copper (ppm)	7.57 ± 2.49	3.21–16.3	29

Nutrient	Mean ± Standard Deviation	Range	Number of Samples
Iodine (ppm)	0.513 ± 0.221	0–0.972	29
Chromium (ppm)	1.02 ± 1.04	0.33–3.97	28
Cobalt (ppm)	0.222 ± 0.152	0.0857–0.864	27

[a]From formulation.
[b]As hydrochloride.

Table B-8. Contaminant Levels in NTP-2000 Rat and Mouse Ration

	Mean ± Standard Deviation	Range	Number of Samples
Contaminants			
Arsenic (ppm)	0.251 ± 0.020	0.220–0.277	5
Cadmium (ppm)	0.064 ± 0.016	0.055–0.096	5
Lead (ppm)	0.084 ± 0.006	0.075–0.090	5
Mercury (ppm)	0.014 ± 0.005	0.01–0.02	5
Selenium (ppm)	0.181 ± 0.014	0.165–0.198	5
Aflatoxins (ppb)[a]	<5.0	–	5
Nitrate Nitrogen (ppm)[b]	19.13 ± 5.641	11.2–26.5	5
Nitrite Nitrogen (ppm)[a,b]	<0.61	–	5
BHA (ppm)[a,c]	<1.00	–	5
BHT (ppm)[a,c]	<1.00	–	5
Aerobic Plate Count (CFU/gm)	<10.00	–	5
Coliform (MPN/gm)	<3.00	–	5
Escherichia coli (MPN/gm)	<10.0	–	5
Salmonella sp. (MPN/gm)	Negative	–	5
Total Nitrosamines (ppb)[d]	6.9 ± 4.72	2.0–13.9	5
N-Ndimethylamine (ppb)[d]	1.7 ± 1.7	1.0–5.1	5
N-Npyrrolidine (ppb)[d]	5.4 ± 3.7	1.0–9.3	5
Pesticides (ppm)			
α-BHC[a]	<0.01	–	5
β-BHC[a]	<0.02	–	5
γ-BHC[a]	<0.01	–	5
δ-BHC[a]	<0.01	–	5
Heptachlor[a]	<0.01	–	5
Aldrin[a]	<0.01	–	5
Heptachlor Epoxide[a]	<0.01	–	5
DDE[a]	<0.01	–	5
DDD[a]	<0.01	–	5
DDT[a]	<0.01	–	5

	Mean ± Standard Deviation	Range	Number of Samples
HCB[a]	<0.01	–	5
Mirex[a]	<0.01	–	5
Methoxychlor[a]	<0.05	–	5
Dieldrin[a]	<0.01	–	5
Endrin[a]	<0.01	–	5
Telodrin[a]	<0.01	–	5
Chlordane[a]	<0.05	–	5
Toxaphene[a]	<0.10	–	5
Estimated PCBs[a]	<0.20	–	5
Ronnel[a]	<0.01	–	5
Ethion[a]	<0.02	–	5
Trithion[a]	<0.05	–	5
Diazinon[a]	<0.10	–	5
Methyl Chlorpyrifos	0.074 ± 0.054	0.020–0.170	5
Methyl Parathion[a]	<0.02	–	5
Ethyl Parathion[a]	<0.02	–	5
Malathion	0.065 ± 0.025	0.020–0.094	5
Endosulfan I[a]	<0.01	–	5
Endosulfan II[a]	<0.01	–	5
Endosulfane Sulfate[a]	<0.03	–	5

All samples were irradiated.
BHA = butylated hydroxyanisole; BHC = hexachlorocyclohexane or benzene hexachloride; BHT = butylated hydroxytoluene; CFU = colony-forming units; DDE = dichlorodiphenyldichloroethylene; DDD = dichlorodiphenyldichloroethane; DDT = dichlorodiphenyltrichloroethane; HCB = hexachlorobenzene; MPN = most probable number; PCB = polychlorinated biphenyl.
[a]All values were below the detection limit. The detection limit is given as the mean.
[b]Sources of contamination include alfalfa, grains, and fish meal.
[c]Sources of contamination include soy oil and fish meal.
[d]All values were corrected for percent recovery.

Appendix C. Sentinel Animal Program

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C.1. Methods

Rodents used in the National Toxicology Program are produced in optimally clean facilities to eliminate potential pathogens that might affect study results. The Sentinel Animal Program is part of the periodic monitoring of animal health that occurs during the toxicological evaluation of test compounds. Under this program, the disease state of the rodents is monitored via sera or feces from extra (sentinel) or dosed animals in the study rooms. The sentinel animals and the study animals are subject to identical environmental conditions. Furthermore, the sentinel animals are from the same production source and weanling groups as the animals used for the studies of test compounds.

In these toxicity studies, blood samples were collected from each sentinel animal, allowed to clot and the serum was separated. All samples were processed appropriately with serology testing performed by BioReliance Corp., Rockville, MD for the 2-week studies and IDEXX BioResearch (formerly Rodent Animal Diagnostic Laboratory (RADIL), University of Missouri), Columbia, MO, for the 3-month studies, for determination of the presence of pathogens. Evaluation for endo- and ectoparasites was performed in-house by the testing laboratory.

The laboratory methods and agents for which testing was performed are tabulated below; the times at which samples were collected during the studies are also listed (Table C-1, Table C-2).

Table C-1. Methods and Results for Sentinel Animal Testing in Male and Female Rats

Collection Time Points	Two-week Study (F344/NTac Rats)	Three-month Study (Wistar Han Rats)		
	End of Quarantine	Quarantine[a]	4 Weeks[b]	End of Study
Number Examined (Males/Females)	5/5	0/10	0/10	5/5
Method/Test				
Enzyme-linked Immunosorbent Assay (ELISA)				
Pneumonia virus of mice (PVM)	–	NT	NT	NT
Rat coronavirus/sialodacryoadenitis virus (RCV/SDA)	–	NT	NT	NT
Sendai	–	NT	NT	NT
Multiplex Fluorescent Immunoassay (MFI)				
Kilham rat virus (KRV)	NT	–	–	–
Mycoplasma pulmonis	NT	–	–	–
Parvo NS-1	NT	–	–	–
Pneumonia virus of mice (PVM)	NT	–	–	–
Rat coronavirus/sialodacryoadenitis virus (RCV/SDA)	NT	–	–	–
Rat minute virus (RMV)	NT	–	–	–
Rat parvo virus (RPV)	NT	–	–	–

Collection Time Points	Two-week Study (F344/NTac Rats)	Three-month Study (Wistar Han Rats)		
	End of Quarantine	Quarantine[a]	4 Weeks[b]	End of Study
Rat theilovirus (RTV)	NT	–	NT	–
Sendai	NT	–	–	–
Theiler's murine encephalomyelitis virus (TMEV)	NT	–	–	–
Toolan's H-1	NT	–	–	–
Immunofluorescence Assay (IFA)				
Parvovirus	–	NT	NT	NT

– = negative; + = positive; NT = not tested.
[a]Age-matched nonpregnant females.
[b]Time-mated females that did not have a litter; 3.5 weeks after arrival.

Table C-2. Methods and Results for Sentinel Animal Testing in Male and Female B6C3F1/N Mice

Collection Time Points	Two-week Study	Three-month Study	
	Quarantine	1 Month	End of Study
Number Examined (Males/Females)	5/5	5/5	5/5
Method/Test			
Enzyme-linked Immunosorbent Assay (ELISA)			
Ectromelia virus	–	NT	NT
Epizootic diarrhea of infant mice (EDIM)	–	NT	NT
Lymphocytic choriomeningitis virus (LCMV)	–	NT	NT
Mouse adenovirus (MAd-1)	–	NT	NT
Mouse hepatitis virus (MHV)	–	NT	NT
Mouse parvovirus (MPV)	–	NT	NT
Mouse minute virus (MMV)	–	NT	NT
Pneumonia virus of mice (PVM)	–	NT	NT
Reovirus type 3 (Reo-3)	–	NT	NT
Sendai	–	NT	NT
Theiler's encephalomyelitis virus (TMEV GD VII)	–	NT	NT
Multiplex Fluorescent Immunoassay (MFI)			
Ectromelia virus	NT	–	–
Epizootic diarrhea of infant mice (EDIM)	NT	–	–
Lymphocytic choriomeningitis virus	NT	–	–

Collection Time Points	Two-week Study	Three-month Study	
	Quarantine	1 Month	End of Study
Mycoplasma pulmonis	NT	–	–
Mouse hepatitis virus (MHV)	NT	–	–
Mouse norovirus (MNV)	NT	–	–
Parvo NS-1	NT	–	–
Mouse parvovirus (MPV)	NT	–	–
Minute virus of mice (MVM)	NT	–	–
Pneumonia virus of mice (PVM)	NT	–	–
Reovirus (REO3)	NT	–	–
Sendai	NT	–	–
Theiler's murine encephalomyelitis virus (TMEV) GDVII	NT	–	–

– = negative; + = positive; NT = not tested.

C.2. Results

F344/NTac Rats: All test results were negative.

Wistar Han Rats: All test results were negative.

B6C3F1/N Mice: All test results were negative.

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D.1. Sample Collection

Biological samples were collected from Wistar Han rats in the 0, 78, 312.5, and 1,250 mg/kg/day *trans*-resveratrol (RES) dose groups and stored at approximately −20°C before shipment on dry ice to RTI International (Research Triangle Park, NC) for analysis to confirm internal concentration. On gestation day (GD) 18, blood was collected from the retroorbital sinus of three randomly selected dams from each dose group at 30 minutes, 60 minutes, or 90 minutes after RES administration (nine dams per dose group in total). Animals were anesthetized with a carbon dioxide/oxygen mixture and blood was collected into tubes containing sodium heparin, centrifuged, and the plasma harvested. The dams were then humanely euthanized with carbon dioxide, and the fetuses were removed and individually flash frozen in liquid nitrogen. On postnatal day (PND) 4, 10 male and 10 female randomly selected standardized pups for each dose group were humanely euthanized by decapitation, placed into individual vials (one pup per vial), and flash frozen in liquid nitrogen. On PND 21, blood was collected via cardiac puncture from five male and five female randomly selected, standardized pups for each dose group. Animals were first anesthetized with a carbon dioxide/oxygen mixture and blood was then collected into tubes containing lithium heparin, centrifuged, and the plasma harvested. After blood collection, the pups were humanely euthanized by carbon dioxide inhalation overdose and were disposed of without further evaluation. At study termination, blood was collected from the retroorbital site of five randomly selected animals per sex for each dose group. Animals were anesthetized with a carbon dioxide/oxygen mixture and blood was collected into tubes containing sodium heparin, centrifuged, and the plasma harvested.

D.2. Sample Analysis

Concentrations of RES in rat blood plasma and homogenized fetal tissues were quantified using a validated analytical method; method validation data are given in Table D-1. All sample handling and analysis was performed under yellow light to minimize the conversion of *trans*-resveratrol to the *cis*- form.

Plasma samples were allowed to equilibrate to room temperature and were mixed well prior to removing sample aliquots for analysis. A 50 µL aliquot was transferred from each sample to a 1.5 mL microcentrifuge tube to which 50 µL of methanol was added, followed by 20 µL of internal standard (IS) solution (100 ng 2′,4′-dihydroxypropiophenone/mL methanol) and 200 µL of acetonitrile. All tubes were mixed, then centrifuged for approximately 20 minutes at 0°C. Aliquots of supernatant were transferred to glass autosampler vials for analysis.

Fetuses were removed from freezer storage, and a weight was recorded for a single representative fetus sample in each litter. The samples were pooled by litter into wide-mouth glass bottles, allowed to equilibrate to room temperature, and then homogenized. From each pooled sample an approximately 0.5 g aliquot was transferred to a 20 mL scintillation vial to which a 20 µL aliquot of methanol was added followed by 20 µL of IS solution and 300 µL of deionized water. A 1 mL aliquot of methanol was added to each vial. The vials were mixed, sonicated for approximately 5 minutes, and then centrifuged for approximately 30 minutes. Each supernatant was decanted and filtered into 1.5-mL microcentrifuge tubes, equilibrated in liquid nitrogen, and then centrifuged for approximately 20 minutes at approximately −9°C. Aliquots of supernatant were transferred to glass autosampler vials for analysis.

Each pup sample was removed from freezer storage and the weights of individual samples were recorded. Each sample was then equilibrated in liquid nitrogen, placed into double plastic bags, shattered, transferred to an individual wide-mouth glass bottle, allowed to thaw to room temperature, and then homogenized. For each pup sample, an approximately 0.5 g aliquot of homogenate was transferred to a 20 mL scintillation vial to which was added a 20 µL aliquot of methanol followed by 20 µL of IS solution and 300 µL of deionized water. A 1 mL aliquot of methanol was added to each vial. The vials were mixed, sonicated for approximately 5 minutes, and then centrifuged for approximately 30 minutes. Each supernatant was decanted and filtered into 1.5 mL microcentrifuge tubes, equilibrated in liquid nitrogen, then centrifuged for approximately 20 minutes at approximately −9°C. Aliquots of supernatant were transferred to glass autosampler vials for analysis.

D.3. Instrumentation and Quantitation

Samples were analyzed using a Waters Acquity (Milford, MA) ultra-performance liquid chromatograph (UPLC) coupled with a 4000 QTRAP hybrid triple quadrupole/linear ion trap mass spectrometer with a TurboSpray source (Sciex, Framingham, MA). Chromatographic analysis was performed using a Waters Acquity UPLC HSS T3 column (100 mm × 2.1 mm internal diameter [ID], 1.8 µm particle size) with guard column (5 mm × 2.1 mm ID, 1.8 µm particle size). Two µL of sample were injected onto the column and elution was achieved at ambient temperature using a binary gradient and a flow rate of 0.3 mL/minute. The mobile phases consisted of: (A) 5 mM ammonium acetate in water with 2% (v/v) isopropyl alcohol (pH unadjusted) and (B) methanol with 2% (v/v) isopropyl alcohol. The gradient was 15% B for 2 minutes, 15% to 65% B from 2 to 15 minutes, then to 95% B for 0.5 minutes. The electrospray ion source was operated in negative ion mode with a voltage of −3,700 V and source temperature of 600°C. The collision gas was high, the curtain gas was 15 psi, the nebulizer gas was 60 psi, the auxiliary gas was 40 psi, and the interface heater was on. The selected multiple reaction monitoring (MRM) transitions were m/z 227 → 185 for RES, and m/z 165 → 109 for 2′,4′-dihydroxypropiophenone (IS). The optimized compound-dependent parameters for RES were: declustering potential (DP) = −65 V; entrance potential (EP) = −10 V; collision energy (CE) = −26 V; and collision cell exit potential (CXP) = −15 V.

The performance of the calibration curve was evaluated prior to the analysis of each sample set. A successful calibration was indicated by the following: correlation coefficient (r) ≥ 0.99; relative standard deviation (RSD) less than or equal to ±15% (except at the limit of quantitation [LOQ] where RSD is less than or equal to ±20%); relative error (RE) less than or equal to ±15% (except at the LOQ where RE is less than or equal to ±20%).

Analytical method validation data for resveratrol-3-O-B-D-glucuronide (*trans*-R3G) and resveratrol-3-O-sulfate (*trans*-R3S) are given in Table D-2 and Table D-3, respectively. Samples were prepared and analyzed as above for RES except that the selected MRM transitions used for *trans*-R3G was m/z 403 → 227 and for *trans*-R3S was m/z 307 → 227. The optimized compound-dependent mass spectrometer parameters for *trans*-R3G and *trans*-R3S were: DP = −55 V; EP = −10 V; CE = −34 V; and CXP = −11 V.

Table D-1. Analytical Method Validation and Stability Data for *Trans*-resveratrol in Wistar Han Plasma, Pup Homogenate, and Fetuses

Validation Parameter	Rat Plasma[a]	Rat Pup[a]
Matrix Concentration Range (ng/mL or ng/g)	5–5,000	10–2,500
LOQ (ng/mL or ng/g)	5.06	10.0
LOD (ng/mL or ng/g)	1.70	2.25
Correlation Coefficient (r)	≥0.99	≥0.99
Recovery (%)[b]	105	70.4
Precision and Accuracy[c,d]		
Intra-day % RSD	≤7.5	≤2.0
Intra-day % RE	≤ ± 6.2	≤ ± 7.3
Inter-day % RSD	≤7.9	≤8.4
Inter-day % RE	≤ ± 5.8	≤ ± 5.2
Dilution Verification[e]		
% RSD	NA	NA
% RE	NA	NA
Extract Stability (Average % RE)[c,d]		
Ambient storage (RT, ~7 days)	−10.0 to −4.5	−2.8 to 0.0
Refrigerated storage (4°C, ~7 days)	−4.9 to 4.9	−8.6 to −1.9
Matrix Stability (Average % RE)[c,f]		
Freeze-thaw (3 cycles, over 5–7 days)	−8.7 to −0.1	−12 to −4.6
Frozen matrix (−80°C for up to ~60 days)	1.0	0.7
Secondary Matrix Evaluation	Male Rat Plasma	Fetus Homogenate
Precision (% RSD)[c]	≤7.3	≤3.0
Accuracy (% RE)[c]	≤ ± 14.5	≤ ± 9.6

LOQ = lower limit of quantitation; LOD = limit of detection; RSD = relative standard deviation; RE = relative error; NA = not applicable; RT = room temperature.
[a]Method was fully validated in female Wistar Han rat plasma and pup homogenate using matrix standard curves and cross-validated in rat fetuses using 3 concentrations (100, 200, and 1,000 ng/g) of quality control (QC) samples prepared in rat fetus homogenate and analyzed using the pup matrix curve.
[b]Estimated by comparing the response of the matrix sample to the response of the solvent sample.
[c]Precision was estimated as % RSD. Accuracy was estimated as average % RE.
[d]Determined for four replicate QC samples at three concentrations: 20, 102, and 2,040 ng/mL for rat plasma and for three replicate QC samples at three concentrations: 100, 200, and 1,000 ng/g in pup homogenate.
[e]NA = validation test not performed.
[f]Study sample matrices were assessed using four replicate QC samples at three concentrations: 20, 102, and 2,040 ng/mL for rat plasma and for three replicate QC samples at three concentrations: 100, 200, and 1,000 ng/g in pup homogenate evaluated using freshly extracted standards and stored standard extracts.

Table D-2. Analytical Method Validation and Stability Data for *Trans*-resveratrol-3-O-B-D-glucuronide in Wistar Han Plasma, Pup Homogenate, and Fetuses

Validation Parameter	Rat Plasma[a]	Rat Pup[a]
Matrix Concentration Range (ng/mL or ng/g)	96.5–11,600	96.0–12,000
LOQ (ng/mL or ng/g)	96.5	96.0
LOD (ng/mL or ng/g)	30.7	27.3
Correlation Coefficient (r)	≥0.99	≥0.99
Recovery (%)[b]	111	76.8
Precision and Accuracy[c,d]		
Intra-day % RSD	≤5.7	≤8.6
Intra-day % RE	≤ ± 5.0	≤ ± 3.3
Inter-day % RSD	≤8.9	≤5.7
Inter-day % RE	≤ ± 3.9	≤ ± 3.0
Dilution Verification		
% RSD	6.8	10
% RE	−12.3	−20
Extract Stability (Average % RE)[c,d,e]		
Ambient storage (RT, ~7 days)	NA	NA
Refrigerated storage (4°C, ~7 days)	NA	NA
Matrix Stability (Average % RE)[c,f]		
Freeze-thaw (3 cycles, over 5–7 days)	−18.2 to −3.3	−12 to 12
Frozen matrix (−80°C for up to ~60 days)	−10.2	NA[e]
Secondary Matrix Evaluation	Male Rat Plasma[e]	Fetus Homogenate
Precision (% RSD)[c]	NA	≤4.1
Accuracy (% RE)[c]	NA	≤ ± 13

LOQ = lower limit of quantitation; LOD = limit of detection; RSD = relative standard deviation; RE = relative error; NA = not applicable; RT = room temperature.
[a]Method was fully validated in female Wistar Han rat plasma and pup homogenate using matrix standard curves and cross-validated in rat fetuses using 3 concentrations (240, 2,400, and 6,000 ng/g) of quality control (QC) samples prepared in rat fetus homogenate and analyzed using the pup matrix curve.
[b]Estimated by comparing the response of the matrix sample to the response of the solvent sample.
[c]Precision was estimated as % RSD. Accuracy was estimated as average % RE.
[d]Determined for four replicate QC samples at three concentrations: 241, 1,930, and 4,830 ng/mL for rat plasma and for three replicate QC samples at three concentrations: 240, 2,400, and 6,000 ng/g in pup homogenate.
[e]NA = validation test not performed.
[f]Study sample matrices were assessed using four replicate QC samples at three concentrations: 241, 1,930, and 4,830 ng/mL for rat plasma and for three replicate QC samples at three concentrations: 240, 2,400, and 6,000 ng/g in pup homogenate evaluated using freshly extracted standards and stored standard extracts.

Table D-3. Analytical Method Validation and Stability Data for *Trans*-resveratrol-3-O-sulfate in Wistar Han Plasma, Pup Homogenate, and Fetuses

Validation Parameter	Rat Plasma[a]	Rat Pup[a]
Matrix Concentration Range (ng/mL or ng/g)	5.03–12,100	99.2–12,400
LOQ (ng/mL or ng/g)	5.03	99.2
LOD (ng/mL or ng/g)	1.48	17.5
Correlation Coefficient (r)	≥0.99	≥0.99
Recovery (%)[b]	115	109
Precision and Accuracy[c,d]		
Intra-day % RSD	≤12	≤4.3
Intra-day % RE	≤ ± 11.1	≤ ± 5.2
Inter-day % RSD	≤12	≤9.4
Inter-day % RE	≤ ± 10.3	≤ ± 4.3
Dilution Verification		
% RSD	11	NA[e]
% RE	−3.0	NA[e]
Extract Stability (Average % RE)[c,d,e]		
Ambient storage (RT, ~7 days)	NA	NA
Refrigerated storage (4°C, ~7 days)	NA	NA
Matrix Stability (Average % RE)[c,f]		
Freeze-thaw (3 cycles, over 5–7 days)	−5.7 to 4.1	−6.0 to 2.3
Frozen matrix (−80°C for up to ~60 days)	0.7	−10
Secondary Matrix Evaluation	Male Rat Plasma[e]	Fetus Homogenate
Precision (% RSD)[c]	NA	≤3.4
Accuracy (% RE)[c]	NA	≤ ± 17

LOQ = lower limit of quantitation; LOD = limit of detection; RSD = relative standard deviation; RE = relative error; NA = not applicable; RT = room temperature.
[a]Method was fully validated in female Wistar Han rat plasma and pup homogenate using matrix standard curves and cross-validated in rat fetuses using 3 concentrations (240, 2,400, 6,000 ng/g) of quality control (QC) samples prepared in rat fetus homogenate and analyzed using the pup matrix curve.
[b]Estimated by comparing the response of the matrix sample to the response of the solvent sample.
[c]Precision was estimated as % RSD. Accuracy was estimated as average % RE.
[d]Determined for four replicate QC samples at three concentrations: 20, 503, and 3,020 ng/mL for rat plasma and for three replicate QC samples at three concentrations: 240, 2,400, and 6,000 ng/g in pup homogenate.
[e]NA = validation test not performed.
[f]Study sample matrices were assessed using four replicate QC samples at three concentrations: 20, 503, and 3,020 ng/mL for rat plasma and for three replicate QC samples at three concentrations: 240, 2,400, and 6,000 ng/g in pup homogenate evaluated using freshly extracted standards and stored standard extracts.

Appendix E. Genetic Toxicology

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E.1. Bacterial Mutagenicity

E.1.1. Bacterial Mutagenicity Test Protocol

Testing procedures were those reported by Zeiger et al.[163] Briefly, a commercially obtained sample of *trans*-resveratrol (RES) (lot number 02-18090-601 from ChromaDex) was sent to the laboratory under code. It was incubated with each of the *Salmonella typhimurium* tester strains (TA98, TA100, and TA102) either in buffer or S9 mix (metabolic activation enzymes and cofactors from Aroclor 1254-induced male Sprague Dawley rat liver) for 20 minutes at 37°C. Top agar supplemented with *L*-histidine and *d*-biotin was added, and the contents of the tubes were mixed and poured onto the surfaces of minimal glucose agar plates. Histidine-independent mutant colonies arising on these plates were counted after incubation for 2 days at 37°C.

Each trial consisted of triplicate plates of concurrent positive and negative controls and at least five doses of RES. RES was tested up to 3,333 µg per plate for each tester strain in the presence or absence of S9 mix.

In this assay, a positive response is defined as a reproducible, dose-related increase in histidine-independent (revertant) colonies in any one strain/activation combination. An equivocal response is defined as an increase in revertants that is not related to dose, is not reproducible, or is not of sufficient magnitude to support a determination of mutagenicity. A negative response is obtained when no increase in revertant colonies is observed after chemical treatment. No minimum percentage or fold increase is required for a chemical to be judged positive or weakly positive, although positive calls are typically reserved for increases in mutant colonies that are at least twofold over background.

E.1.2. Results

RES (33 to 3,333 µg/plate) was not mutagenic in *S. typhimurium* strains TA98, TA100, or TA102 when tested with or without exogenous metabolic activation provided by Aroclor 1254-induced rat liver S9 and cofactors (Table E-1).

Table E-1. Mutagenicity of *Trans*-resveratrol in Bacterial Tester Strains[a]

Strain	Concentration (µg/plate)	Without S9	With 10% Rat S9
TA98			
	0	15 ± 1	43 ± 3
	33	13 ± 2	25 ± 2
	100	19 ± 1	33 ± 2
	333	19 ± 1	38 ± 1
	1,000	16 ± 2	31 ± 3
	3,333	14 ± 1	34 ± 3
Trial Summary		Negative	Negative
Positive Control[b]		97 ± 4	594 ± 29

Strain	Concentration (µg/plate)	Without S9	With 10% Rat S9
TA100			
	0	126 ± 9	147 ± 2
	33	149 ± 3	139 ± 5
	100	141 ± 6	148 ± 5
	333	137 ± 1	136 ± 5
	1,000	133 ± 5	108 ± 4
	3,333	51 ± 14	74 ± 21
Trial Summary		Negative	Negative
Positive Control[c]		434 ± 21	929 ± 43
TA102			
	0	286 ± 14	384 ± 15
	34	288 ± 4	389 ± 18
	102	299 ± 8	386 ± 12
	340	304 ± 11	397 ± 19
	1,019	272 ± 29	365 ± 43
	3,333	132 ± 17	180 ± 21
Trial Summary		Negative	Negative
Positive Control[d]		930 ± 64	1,310 ± 22

[a]Studies performed at BioReliance. Data are presented as revertants/plate (mean ± standard error) from three plates; 0 µg/plate served as the solvent control.
[b]The positive control in the absence of metabolic activation was 4-nitro-o-phenylenediamine (1.0 µg/plate); the positive control for metabolic activation was 2-aminoanthracene (0.4 µg/plate).
[c]The positive control in the absence of metabolic activation was sodium azide (0.5 µg/plate); the positive control for metabolic activation was 2-aminoanthracene (0.75 µg/plate).
[d]The positive control in the absence of metabolic activation was mitomycin C (75.0 µg/plate); the positive control for metabolic activation was sterigmatocystin (10.0 µg/plate).

E.2. Micronucleus Assay

E.2.1. Peripheral Blood Micronucleus Test Protocol

At termination of the 3-month toxicity studies of RES, blood samples were collected from male and female Wistar Han rats and B6C3F1/N mice, placed in ethylenediaminetetraacetic acid (EDTA)-coated tubes, fixed in ultracold methanol, and frozen at −80°C until analysis. Thawed blood samples were analyzed for frequency of micronucleated immature erythrocytes (polychromatic erythrocytes [PCEs], reticulocytes) and mature erythrocytes (normochromatic erythrocytes [NCEs]) using a flow cytometer;[164] both the mature and immature reticulocyte population can be analyzed separately by employing special cell surface markers to differentiate the two cell types. Because the very young reticulocyte subpopulation (CD71+ cells) can be targeted using this technique, rat blood samples can be analyzed for damage in the bone marrow that occurred within the past 24 to 48 hours, before the rat spleen appreciably alters the percentage of PCEs in circulation.[165] In mice, both the mature and immature erythrocyte populations can be evaluated for micronucleus frequency because the mouse spleen does not

sequester and eliminate damaged erythrocytes. Damaged erythrocytes achieve steady state in the peripheral blood of mice after four weeks of continuous exposure. Approximately 20,000 PCEs and 1×10^6 NCEs were analyzed per animal for frequency of micronucleated cells, and the percentage of immature erythrocytes (% PCE) was calculated as a measure of bone marrow toxicity resulting from chemical exposure.

Prior experience with the large number of cells scored using flow cytometric scoring techniques[166] suggests it is reasonable to assume the proportion of micronucleated reticulocytes is approximately normally distributed. The statistical tests selected for trend and for pairwise comparisons with the vehicle control group depend on whether the variances among the groups are equal. The Levene test at $\alpha = 0.05$ is used to test for equal variances. In the case of equal variances, linear regression is used to test for a linear trend with dose and the Williams test is used to test for pairwise differences between each dosed group and the vehicle control group. In the case of unequal variances, the Jonckheere test is used to test for linear trend and the Dunn test is used for pairwise comparisons of each dosed group with the vehicle control group. To correct for multiple pairwise comparisons, the p value for each comparison with the vehicle control group is multiplied by the number of comparisons made. If this product is >1.00, it is replaced with 1.00. Trend tests and pairwise comparisons with the vehicle control groups are considered statistically significant at $p \leq 0.025$.

In the micronucleus test, it is preferable to base a positive result on the presence of both a positive trend as well as at least one significantly elevated dosed group compared with the corresponding vehicle control group. The presence of either a positive trend or a single significant dosed group generally results in an equivocal call. The absence of both a trend and a significant dosed group results in a negative call. Ultimately, the scientific staff determines the final call after considering the results of statistical analyses, reproducibility of any effects observed (in acute studies), and the magnitudes of those effects.

E.2.2. Evaluation Protocol

These are the basic guidelines for arriving at an overall result for assays performed by the National Toxicology Program. Statistical as well as biological factors are considered. For an individual assay, the statistical procedures for data analysis are described in the preceding protocols. There have been instances, however, in which multiple samples of a chemical were tested in the same assay, and different results were obtained among the samples and/or among laboratories. Results from more than one aliquot or from more than one laboratory are not simply combined into an overall result. Rather, all the data are critically evaluated, particularly those concerning pertinent protocol variations, in determining the weight of evidence for an overall conclusion of chemical activity in an assay. In addition to multiple aliquots, the in vitro assays have another variable that must be considered in arriving at an overall test result. In vitro assays are conducted with and without exogenous metabolic activation. Results obtained in the absence of activation are not combined with results obtained in the presence of activation; each testing condition is evaluated separately. The summary table in the abstract of this toxicity report presents a scientific judgment of the overall evidence for activity of the chemical in an assay.

E.2.3. Results

In rats, the reticulocyte population is the only red blood cell population that can be accurately assessed for micronucleus frequency in peripheral blood due to efficient splenic scavenging of

damaged erythrocytes soon after they emerge from the bone marrow. In both sexes of Wistar Han rats in the 3-month study, there were no significant increases in the frequencies of micronucleated PCEs (Table E-2). A positive trend in the percentage of PCEs was observed in female rats; however, the absolute increase (0.38%) in the 1,250 mg RES/kg body weight/day (mg/kg/day) group compared to the vehicle control group was very small and was not considered to be biologically relevant. No increases in the frequency of micronucleated erythrocytes (either immature or mature) were seen in the peripheral blood of female mice in the 3-month study (Table E-3). Significant increases in micronucleated NCEs were observed for every dosed group in male mice; however, the absolute difference in micronucleated NCEs in the dosed groups relative to the vehicle control group ranged from 0.06% to 0.16%. These very small increases were not considered to be biologically relevant.

Table E-2. Frequency of Micronuclei in Peripheral Blood Erythrocytes of Male and Female Wistar Han Rats in the Perinatal and Three-month Gavage Study of *Trans*-resveratrol[a]

	Micronucleated PCEs/1,000 PCEs[b]	P Value[c]	Micronucleated NCEs/1,000 NCEs[b]	P Value[c]	PCEs (%)[b]	P Value[c]
n	5		5		5	
Male						
Dose (mg/kg/day)						
0	0.890 ± 0.108		0.138 ± 0.018		0.937 ± 0.129	
78	0.760 ± 0.144	1.000	0.106 ± 0.022	1.000	0.982 ± 0.062	0.616
156	1.610 ± 0.360	0.503	0.281 ± 0.053	0.555	1.506 ± 0.115	0.115
312.5	0.820 ± 0.041	1.000	0.173 ± 0.073	1.000	1.134 ± 0.118	0.122
625	0.795 ± 0.104	1.000	0.082 ± 0.011	1.000	0.997 ± 0.142	0.125
1,250	0.720 ± 0.075	1.000	0.134 ± 0.039	1.000	1.126 ± 0.085	0.125
Trend[d]	p = 0.878		p = 0.907		p = 0.872	
Female						
Dose (mg/kg/day)						
0	0.708 ± 0.092		0.073 ± 0.007		0.814 ± 0.024	
78	0.680 ± 0.150	0.531	0.063 ± 0.008	1.000	0.867 ± 0.095	0.761
156	0.750 ± 0.175	0.595	0.074 ± 0.010	1.000	1.027 ± 0.126	0.284
312.5	0.780 ± 0.102	0.629	0.081 ± 0.015	1.000	0.971 ± 0.088	0.305
625	0.670 ± 0.115	0.649	0.077 ± 0.010	1.000	0.936 ± 0.063	0.314
1,250	0.610 ± 0.151	0.661	0.091 ± 0.018	1.000	1.198 ± 0.132	0.018
Trend[d]	p = 0.770		p = 0.168		p = 0.016	

PCE = polychromatic erythrocyte; NCE = normochromatic erythrocyte.
[a]Study was performed at Integrated Laboratory Systems, LLC.
[b]Data are presented as mean ± standard error.
[c]Pairwise comparisons with the vehicle control group performed using the Williams or Dunn test ($p \leq 0.025$).
[d]Dose-related trends evaluated by linear regression or the Jonckheere test ($p \leq 0.025$).

Table E-3. Frequency of Micronuclei in Peripheral Blood Erythrocytes of Male and Female B6C3F1/N Mice in the Three-month Gavage Study of *Trans*-resveratrol[a]

	Micronucleated PCEs/1,000 PCEs[b]	P Value[c]	Micronucleated NCEs/1,000 NCEs[b]	P Value[c]	PCEs (%)[b]	P Value[c]
n	5		5		5	
Male						
Dose (mg/kg/day)						
0	2.610 ± 0.185		1.429 ± 0.035		1.367 ± 0.046	
156	2.839 ± 0.142	0.416	1.576 ± 0.029	0.014	1.458 ± 0.087	0.546
312	2.570 ± 0.213	0.489	1.584 ± 0.035	0.016	1.417 ± 0.066	0.652
625	2.600 ± 0.168	0.520	1.488 ± 0.023	0.016	1.387 ± 0.053	0.695
1,250	2.780 ± 0.244	0.356	1.546 ± 0.051	0.016	1.487 ± 0.029	0.202
2,500	2.790 ± 0.185	0.349	1.577 ± 0.036	0.005	1.518 ± 0.066	0.114
Trend[d]	p = 0.259		p = 0.090		p = 0.073	
Female						
Dose (mg/kg/day)						
0	2.730 ± 0.362		1.209 ± 0.061		1.699 ± 0.246	
156	2.389 ± 0.193	0.869	1.181 ± 0.061	1.000	2.004 ± 0.208	0.463
312	2.630 ± 0.295	0.927	1.132 ± 0.043	1.000	1.799 ± 0.206	0.555
625	2.518 ± 0.102	0.944	1.120 ± 0.046	1.000	1.762 ± 0.109	0.594
1,250	1.900 ± 0.208	0.952	1.085 ± 0.015	1.000	1.905 ± 0.171	0.467
2,500	1.930 ± 0.146	0.958	1.170 ± 0.020	1.000	2.059 ± 0.146	0.175
Trend[d]	p = 0.996		p = 0.813		p = 0.197	

PCE = polychromatic erythrocyte; NCE = normochromatic erythrocyte
[a]Study was performed at Integrated Laboratory Systems, LLC.
[b]Data are presented as mean ± standard error.
[c]Pairwise comparisons with the vehicle control group performed using the Williams or Dunn test ($p \leq 0.025$).
[d]Dose-related trends evaluated by linear regression or the Jonckheere test ($p \leq 0.025$).

Appendix F. Supplemental Data

Tables with supplemental data can be found here: https://doi.org/10.22427/NTP-DATA-TOX-102.

F.1. Two-week Study in F344/NTac Rats

E01 – Animal Removal Summary by Treatment Group
2032301_E01_Animal_Removal_Summary_By_Treatment_Group.pdf

E02 – Animals Removed from Experiment
2032301_E02_Animals_Removed_from_Experiment.pdf

E03 – Growth Curves
2032301_E03_Growth_Curves.pdf

E04 – Mean Body Weights and Survival Table
2032301_E04_Mean_Body_Weights_and_Survival_Table.pdf

E05 – Clinical Observations Summary
2032301_E05_Clinical_Observations_Summary.pdf

P03 – Incidence Rates of Non-Neoplastic Lesions by Anatomic Site
2032301_P03_Incidence_Rates_of_Non-Neoplastic_Lesions_by_Anatomic_Site.pdf

P04 – Neoplasms by Individual Animal
2032301_P04_Neoplasms_by_Individual_Animal.pdf

P08 – Statistical Analysis of Primary Tumors
2032301_P08_Statistical_Analysis_of_Primary_Tumors.pdf

P09 – Non-Neoplastic Lesions by Individual Animal
2032301_P09_Non-Neoplastic_Lesions_by_Individual_Animal.pdf

P10 – Statistical Analysis of Non-Neoplastic Lesions
2032301_P10_Statistical_Analysis_of_Non-Neoplastic_Lesions.pdf

P14 – Individual Animal Pathology Data
2032301_P14_Individual_Animal_2032301_PAthology_Data.pdf

P18 – Incidence Rates of Non-Neoplastic Lesions by Anatomic Site with Average Severity Grades
2032301_P18_Incidence_Rates_of_Non-Neoplastic_Lesions_by_Anatomic_Site_with_Average_Severity_Grades.pdf

P22 – Cause of Death Summary
2032301_P22_Cause_of_Death_Summary.pdf

P40 – Survival Curves
2032301_P40_Survival_Curves.pdf

PA06 – Organ Weights Summary
2032301_PA06_Organ_Weights_Summary.pdf

F.2. Two-week Study in F344/NTac Rats – Individual Animal Data

Female Individual Animal Body Weight Data All Animals
2032301_Female_Individual_Animal_Body_Weight_Data_All_Animals.xls

Female Individual Animal Non-Neoplastic Pathology Data
2032301_Female_Individual_Animal_Non_Neoplastic_Pathology_Data.xls

Female Individual Animal Survival Data
2032301_Female_Individual_Animal_Survival_Data.xls

Female Individual Animal Terminal Body Weight Data
2032301_Female_Individual_Animal_Terminal_Body_Weight_Data.xls

Male Individual Animal Body Weight Data All Animals
2032301_Male_Individual_Animal_Body_Weight_Data_All_Animals.xls

Male Individual Animal Non-Neoplastic Pathology Data
2032301_Male_Individual_Animal_Non_Neoplastic_Pathology_Data.xls

Male Individual Animal Survival Data
2032301_Male_Individual_Animal_Survival_Data.xls

Male Individual Animal Terminal Body Weight Data
2032301_Male_Individual_Animal_Terminal_Body_Weight_Data.xls

F.3. Two-week Study in B6C3F1/N Mice

E01 – Animal Removal Summary by Treatment Group
2032302_E01_Animal_Removal_Summary_By_Treatment_Group.pdf

E02 – Animals Removed from Experiment
2032302_E02_Animals_Removed_from_Experiment.pdf

E03 – Growth Curves
2032302_E03_Growth_Curves.pdf

E04 – Mean Body Weights and Survival Table
2032302_E04_Mean_Body_Weights_and_Survival_Table.pdf

E05 – Clinical Observations Summary
2032302_E05_Clinical_Observations_Summary.pdf

P03 – Incidence Rates of Non-Neoplastic Lesions by Anatomic Site
2032302_P03_Incidence_Rates_of_Non-Neoplastic_Lesions_by_Anatomic_Site.pdf

P04 – Neoplasms by Individual Animal
2032302_P04_Neoplasms_by_Individual_Animal.pdf

P08 – Statistical Analysis of Primary Tumors
2032302_P08_Statistical_Analysis_of_Primary_Tumors.pdf

P09 – Non-Neoplastic Lesions by Individual Animal
2032302_P09_Non-Neoplastic_Lesions_by_Individual_Animal.pdf

P10 – Statistical Analysis of Non-Neoplastic Lesions
2032302_P10_Statistical_Analysis_of_Non-Neoplastic_Lesions.pdf

P14 – Individual Animal Pathology Data
2032302_P14_Individual_Animal_Pathology_Data.pdf

P18 – Incidence Rates of Non-Neoplastic Lesions by Anatomic Site with Average Severity Grades
2032302_P18_Incidence_Rates_of_Non-Neoplastic_Lesions_by_Anatomic_Site_with_Average_Severity_Grades.pdf

P22 – Cause of Death Summary
2032302_P22_Cause_of_Death_Summary.pdf

P40 – Survival Curves
2032302_P40_Survival_Curves.pdf

PA06 – Organ Weights Summary
2032302_PA06_-_Organ_Weights_Summary.pdf

F.4. Two-week Study in B6C3F1N Mice – Individual Animal Data

Female Individual Animal Body Weight Data All Animals
2032302_Female_Individual_Animal_Body_Weight_Data_All_Animals.xls

Female Individual Animal Non-Neoplastic Pathology Data
2032302_Female_Individual_Animal_Non_Neoplastic_Pathology_Data.xls

Female Individual Animal Survival Data
2032302_Female_Individual_Animal_Survival_Data.xls

Female Individual Animal Terminal Body Weight Data
2032302_Female_Individual_Animal_Terminal_Body_Weight_Data.xls

Male Individual Animal Body Weight Data All Animals
2032302_Male_Individual_Animal_Body_Weight_Data_All_Animals.xls

Male Individual Animal Non-Neoplastic Pathology Data
2032302_Male_Individual_Animal_Non_Neoplastic_Pathology_Data.xls

Male Individual Animal Survival Data
2032302_Male_Individual_Animal_Survival_Data.xls

Male Individual Animal Terminal Body Weight Data
2032302_Male_Individual_Animal_Terminal_Body_Weight_Data.xls

F.5. Perinatal and Three-month Study – Wistar Han Rats

E01 – Animal Removal Summary by Treatment Group
2032303_E01_Animal_Removal_Summary_By_Treatment_Group.pdf

E02 – Animals Removed from Experiment
2032303_E02_Animals_Removed_from_Experiment.pdf

E03 – Growth Curves
2032303_E03_Growth_Curves.pdf

E04 – Mean Body Weights and Survival Table
2032303_E04_Mean_Body_Weights_and_Survival_Table.pdf

E05 – Clinical Observations Summary
2032303_E05_Clinical_Observations_Summary.pdf

E12 – Animal History
2032303_E12_Animal_History.pdf

Gestational Body Weight Changes (g)
Gestational_Body_Weight_Changes.pdf

Gestational Body Weights (g)
2032303_Gestational_Body_Weights.pdf

Lactational Body Weight Changes (g)
2032303_Lactational_Body_Weight_Changes.pdf

Lactational Body Weights (g)
2032303_Lactational_Body_Weights.pdf

Live Litter Size and Survival
2032303_Litter_Size_and_Survival.pdf

P03 – Incidence Rates of Non-Neoplastic Lesions by Anatomic Site
2032303_P03_Incidence Rates of Non-Neoplastic Lesions by Anatomic Site.pdf

P04 – Neoplasms by Individual Animal
2032303_P04_Neoplasms by Individual Animal.pdf

P05 – Incidence Rates of Neoplasms by Anatomic Site (Systemic Lesions Abridged)
2032303_P05_Incidence Rates of Neoplasms by Anatomic Sites (Systemic Lesions Abridged).pdf

P09 – Non-Neoplastic Lesions by Individual Animal
2032303_P09_Non-Neoplastic_Lesions_by_Individual_Animal.pdf

P10 – Statistical Analysis of Non-Neoplastic Lesions
2032303_P10_Statistical_Analysis_of_Non-Neoplastic_Lesions.pdf

P10 – Statistical Analysis of Non-Neoplastic Lesions – Litter based
2032303_P10_Statistical_Analysis_of_Non-Neoplastic_Lesions.pdf

P14 – Individual Animal Pathology Data
2032303_P14_Individual_Animal_Pathology_Data.pdf

P18 – Incidence Rates of Non-Neoplastic Lesions by Anatomic Site with Average Severity Grades
2032303_P18_Incidence_Rates_of_Non-Neoplastic_Lesions_by_Anatomic_Site_with_Average_Severity_Grades.pdf

P40 – Survival Curves
2032303_P40_Survival_Curves.pdf

PA06 – Organ Weights Summary
2032303_PA06_-_Organ_Weights_Summary.pdf

PA41 – Clinical Chemistry Summary
2032303_PA41_-_Clinical_Chemistry_Summary.pdf

PA43 – Hematology Summary
2032303_PA43_-_Hematology_Summary.pdf

PA48 – Summary of Tissue Concentration
2032303_PA48_-_Summary_of_Tissue_Concentration.pdf

PND 1 Data
2032303_PND_1_Data.pdf

Pup Body Weights (g)
Pup_Body_Weights.pdf

R02 – Reproductive Performance Summary
2032303_R02_-_Reproductive_Performance_Summary.pdf

R06 – Andrology Summary
2032303_R06_-_Andrology_Summary.pdf

Vaginal Cytology Markov Model
2032303_Vaginal_Cytology_Markov_Model.pdf

Vaginal Cytology Plots
2032303_Vaginal_Cytology_Plots.pdf

Vaginal Cytology Summary
2032303_Vaginal_Cytology_Summary.pdf

F.6. Perinatal and Three-month Study in Wistar Han Rats – Individual Animal Data

Female Individual Animal Body Weight Data All Animals
2032303_Female_Individual_Animal_Body_Weight_Data_All_Animals.xls

Female Individual Animal Non-Neoplastic Pathology Data
2032303_Female_Individual_Animal_Non_Neoplastic_Pathology_Data.xls

Female Individual Animal Survival Data
2032303_Female_Individual_Animal_Survival_Data.xls

Female Individual Animal Terminal Body Weight Data
2032303_Female_Individual_Animal_Terminal_Body_Weight_Data.xls

Male Individual Animal Body Weight Data All Animals
2032303_Male_Individual_Animal_Body_Weight_Data_All_Animals.xls

Male Individual Animal Non-Neoplastic Pathology Data
2032303_Male_Individual_Animal_Non_Neoplastic_Pathology_Data.xls

Male Individual Animal Survival Data
2032303_Male_Individual_Animal_Survival_Data.xls

Male Individual Animal Terminal Body Weight Data
2032303_Male_Individual_Animal_Terminal_Body_Weight_Data.xls

Individual Animal Andrology Data
2032303_Individual_Animal_Andrology_Data.xlsx

Individual Animal Clinical Chemistry Data
2032303_Individual_Animal_Clinical_Chemistry_Data.xlsx

Individual Animal Dam ID and Pup ID Data
2032303_Individual_Animal_DamID_and_PupID_Data.xlsx

Individual Animal Hematology Data
2032303_Individual_Animal_Hematology_Data.xlsx

Individual Animal Organ Weight Data
2032303_Individual_Animal_Organ_Weight_Data.xlsx

Individual Animal Reproductive Performance Data
2032303_Individual_Animal_Reproductive_Performance_Data.xlsx

Individual Animal Tissue Concentration Data
2032303_Individual_Animal_Tissue_Concentration_Data.xlsx

F.7. Three-month Study – B6C3F1/N Mice

E03 – Growth Curves
2032304_E03_Growth_Curves.pdf

E04 – Mean Body Weights and Survival Table
2032304_E04_Mean_Body_Weights_and_Survival_Table.pdf

E05 – Clinical Observations Summary
2032304_E05_Clinical_Observations_Summary.pdf

P03 – Incidence Rates of Non-Neoplastic Lesions by Anatomic Site
2032304_P03_Incidence_Rates_of_Non-Neoplastic_Lesions_by_Anatomic_Site.pdf

P04 – Neoplasms by Individual Animal
2032304_P04_Neoplasms_by_Individual_Animal.pdf

P05 – Incidence Rates of Neoplasms by Anatomic Site (Systemic Lesions Abridged)
2032304_P05_Incidence_Rates_of_Neoplasms_by_Anatomic_Site_(Systemic_Lesions_Abridged).pdf

P09 – Non-Neoplastic Lesions by Individual Animal
2032304_P09_Non-Neoplastic_Lesions_by_Individual_Animal.pdf

P10 – Statistical Analysis of Non-Neoplastic Lesions
2032304_P10_Statistical_Analysis_of_Non-Neoplastic_Lesions.pdf

P14 – Individual Animal Pathology Data
2032304_P14_Individual_Animal_Pathology_Data.pdf

P18 – Incidence Rates of Non-Neoplastic Lesions by Anatomic Site with Average Severity Grades
2032304_P18_Incidence_Rates_of_Non-Neoplastic_Lesions_by_Anatomic_Site_with_Average_Severity_Grades.pdf

P40 – Survival Curves
2032304_P40_Survival_Curves.pdf

PA06 – Organ Weights Summary
2032304_PA06_-_Organ_Weights_Summary.pdf

PA43 – Hematology Summary
2032304_PA43_-_Hematology_Summary.pdf

R06 – Andrology Summary
2032304_R06_-_Andrology_Summary.pdf

Vaginal Cytology Markov Model
2032304_Vaginal_Cytology_Markov_Model.pdf

Vaginal Cytology Plots
2032304_Vaginal_Cytology_Plots.pdf

Vaginal Cytology Summary
2032304_Vaginal_Cytology_Summary.pdf

F.8. Three-month Study in B6C3F1/N Mice – Individual Animal Data

Female Individual Animal Body Weight Data All Animals
2032304_Female_Individual_Animal_Body_Weight_Data_All_Animals.xls

Female Individual Animal Non-Neoplastic Pathology Data
2032304_Female_Individual_Animal_Non_Neoplastic_Pathology_Data.xls

Female Individual Animal Survival Data
2032304_Female_Individual_Animal_Survival_Data.xls

Female Individual Animal Terminal Body Weight Data
2032304_Female_Individual_Animal_Terminal_Body_Weight_Data.xls

Male Individual Animal Body Weight Data All Animals
2032304_Male_Individual_Animal_Body_Weight_Data_All_Animals.xls

Male Individual Animal Non-Neoplastic Pathology Data
2032304_Male_Individual_Animal_Non_Neoplastic_Pathology_Data.xls

Male Individual Animal Survival Data
2032304_Male_Individual_Animal_Survival_Data.xls

Male Individual Animal Terminal Body Weight Data
2032304_Male_Individual_Animal_Terminal_Body_Weight_Data.xls

Individual Animal Andrology Data
2032304_Individual_Animal_Andrology_Data.xlsx

Individual Animal Hematology Data
2032304_Individual_Animal_Hematology_Data.xlsx

Individual Animal Organ Weight Data
2032304_Individual_Animal_Organ_Weight_Data.xlsx

F.9. Genetic Toxicology

F.9.1. Wistar Han Rats

G04 – In Vivo Micronucleus Summary Data
G01090_G04_-_In_Vivo_Micronucleus_Summary_Data.pdf

Individual Animal In Vivo Micronucleus Data
G01090_Individual_Animal_In_Vivo_Micronucleus_Data.xlsx

F.9.2. B6C3F1/N Mice

G04 – In Vivo Micronucleus Summary Data
G01090B_G04_In_Vivo_Micronucleus_Summary_Data.pdf

Individual Animal In Vivo Micronucleus Data
G01090B_Individual_Animal_In_Vivo_Micronucleus_Data.xlsx

F.9.3. Bacterial Mutagenicity

G06 – Ames Summary Data
A12955_G06_Ames_Summary_Data.pdf



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ISSN 2378-8992