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Toxicological evaluation of the flavour ingredient 4-amino-5-(3-(isopropylamino)-2,2-dimethyl-3-oxopropoxy)-2-methylquinoline-3-carboxylic acid

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ABSTRACT

A toxicological evaluation of 4-amino-5-(3-(isopropylamino)-2,2-dimethyl-3-oxopropoxy)-2-methylquinoline-3-carboxylic acid(S9632; CAS 1359963-68-0), a flavour with modifying properties,was completed for the purpose of assessing its safety for use in food and beverage applications. No Phase I biotransformations of S9632 were observed in rat or human microsomes *in vitro*, and in rat pharmacokinetic studies, the compound was poorly orally bioavailable and rapidly eliminated. S9632 was not found to be mutagenic or clastogenic *in vitro*, and did not induce micronuclei or indicate interactions with the mitotic spindle in an *in vivo* mouse micronucleus assay at oral doses up to 2000 mg/kg. In subchronic oral toxicity studies in rats, the NOEL was 100 mg/kg/day (highest dose tested) for S9632 when administered as a food ad-mix for 90 consecutive days. Furthermore, S9632 demonstrated a lack of maternal toxicity, as well as adverse effects on fetal morphology at the highest dose tested, providing a NOEL of 1000 mg/kg/day for both maternal toxicity and embryo/fetal development when administered orally during gestation to pregnant rats.

1. Introduction

4-Amino-5-(3-(isopropylamino)-2,2-dimethyl-3-oxopropoxy)-2-methylquinoline-3-carboxylic acid (S9632; CAS 1359963-68-0) is a flavour with modifying properties (FMP). FMP is a term used by the flavour industry to describe ingredients that function as part of a flavour system, also known as compounded flavours [7], to modify or enhance the flavour profile of a variety of food and beverages. Similar to the FMPs S6973 and S617 previously reported [1], S9632 is a positive allosteric modulator of the human sweet receptor which, in addition to modifying certain aspects of the flavour profile, allow for a reduction of carbohydrate sweeteners in food and beverage products while maintaining the desired sweet taste of natural sugars [19,20,22]. FMPs may not necessarily have a taste on their own, but work in concert with other flavour ingredients in a flavour system to change the flavour profile of a food product, such as by decreasing or increasing the intensity of specific flavour characteristics [8]. For example, S9632 has been shown to function in food and beverage products with reduced sweetener to restore sweetness, as well as modify other aspects of the flavour profile, such as caramel, butterscotch and molasses attributes when present in a butterscotch pudding, or citrus and cherry attributes when used in a tropical punch beverage (Senomyx, unpublished data). The structure of S9632 is shown in Fig. 1.

This substance was reviewed by the Expert Panel of the Flavour and Extract Manufacturers Association of the United States (FEMA) and determined to be generally recognized as safe (GRAS) under the conditions of intended use as a flavour ingredient [7,13] and therefore is available for use in human food in the United States as a "FEMA GRAS" flavour ingredient. S9632 was assigned the FEMA GRAS Number 4774 in 2012 [13]. S9632 was also determined to be safe at the current levels of intake by the Joint FAO/WHO Expert

Abbreviations: AUC, area under the curve; C_{max}, peak plasma concentration; FDA, Food and Drug Administration; FEMA, Flavour and Extract Manufacturers Association of the United States; FMP, flavour with modifying properties; GLP, Good Laboratory Practices; GMP, Good Manufacturing Practices; HPBL, human peripheral blood lymphocytes; LC/MS, liquid chromatography with mass spectrometry; MC, methylcellulose; NOAEL, no-observed-adverse-effect-level; NOEL, no-observed-effect-level; OECD, Organization for Economic Cooperation and Development; PCE, polychromatic erythrocytes; PK, pharmacokinetics; RCG, Relative Cell Growth; RMI, Relative Mitotic Index; T_{max}, time to reach C_{max}; TK, toxicokinetics.

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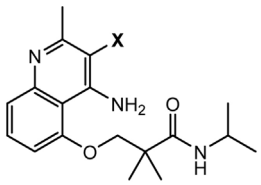

S9632 (X = CO_2H), Chemical Formula $C_{19}H_{25}N_3O_4$, Molecular Weight 359.42
S9379 (X = H), Chemical Formula $C_{18}H_{25}N_3O_2$, Molecular Weight 315.41

Fig. 1. Structures of S9632 and Impurity S9379.

Committee on Food Additives [11] (assigned JECFA No. 2204 and 2204.1 for the S9632-sulfate salt) and has recently been submitted to the European Union for review. Other jurisdictions permit the use of S9632 including Japan, Korea, and Mexico (CO-6.12.2013).

The purpose of this publication is to summarize the results obtained from *in vitro* metabolism and *in vivo* pharmacokinetic (PK) studies, general toxicology studies in rodents, genotoxicity studies, and developmental toxicity studies conducted with S9632. Additional supporting data obtained in these studies is included in a Supplementary Data section in the online publication.

2. Materials and methods

The batches of S9632 used for the *in vitro* metabolism, *in vivo* PK, genotoxicity, and 28-day range-finding toxicity studies (Batch ID no. 51837,343 and 52185,558, purity >98.5%), were synthesized at Senomyx (San Diego, CA) using the procedure described in US Patent No. 8815956 [21]. The batch of S9632 used for the 90-day subchronic and developmental toxicity studies (Batch ID no. 57341902, purity 98.8%), was synthesized at Cambridge Major Laboratories (Germantown, WI) using the same synthetic method but prepared in conformance with Good Manufacturing Practices (GMPs) as described in the ICH GMP Guidelines for APIs [9]. The sodium (S3333), phosphate (S3337) and hemisulfate (S1638) salts of S9632 were also prepared at Senomyx for solubility and bioequivalence studies (purity of each >98%). A minor impurity (>0.1%), 4-amino-5-(3-(isopropylamino)-2,2-dimethyl-3-oxopropoxy)-2-methylquinoline (S9379, see Fig. 1) has been observed in various batches of S9632, including the GMP batch used for the 90-day and developmental toxicity studies; an authentic standard (synthesized at Senomyx) confirmed the structure of the impurity by liquid chromatography with mass spectrometry (LC/MS) and was used for the abbreviated Ames (2-strains) assay. The internal standard used for the pharmacokinetic studies, 4-amino-5-cyclopentyloxy-2-methylquinoline-3-carboxylic acid (S0176), was also synthesized at Senomyx by the procedure described in US Patent No. 8815,956 [21].

With the exception of the abbreviated Ames assays, all genetic toxicology studies were conducted in compliance with the FDA Good Laboratory Practices (GLP) regulations 21CFR Part 58 [5] and OECD guidelines [17]. The experimental design for these studies followed the OECD Guidelines for the Testing of Chemicals - 471, 473, and 474 [14–16]. The 28-day dose-range finding studies and 90-day toxicology studies in rats were conducted in compliance with the United States Food and Drug Administration (FDA) Guidelines [6] Toxicological Principles for the Safety of Food Ingredients; the 90-day subchronic toxicology study was also conducted in compliance with the FDA Good Laboratory Practice (GLP) Regulations, 21CFR Part 58. The developmental toxicity range-finder and definitive studies were conducted in accordance with the OECD Guidelines for Testing of Chemicals Guideline 414, Prenatal Developmental Toxicity Study [18] and the United States FDA Redbook 2000: IV.C.9.b Guidelines for Developmental Toxicity Studies [4] and the FDA GLP regulations 21CFR Part 58 and OECD guidelines OECD, 1998.

The microsomal metabolism studies on S9632 were conducted at PharmOptima (Portage, MI). Pharmacokinetic studies and excretion studies on S9632 in rats were conducted at Senomyx (San Diego, CA). The abbreviated Ames (2-strains) tests on S9632 and the decarboxylated impurity S9379 were conducted at BioReliance (Rockville, MD). The other genotoxicity studies, *i.e.* bacterial reverse mutation (5-strain Ames), *in vitro* chromosome aberration, and *in vivo* mouse micronucleus assays, were conducted at Nucro-Technics (Scarborough, Ontario, Canada). The 28-day and 90-day subchronic toxicity studies were conducted at MPI Research (Mattawan, WI). The developmental toxicity study was conducted at WIL Research (Ashland, OH). A description of the study designs is included in the individual study sections below. Detailed data tables for the genotoxicity, subchronic and developmental toxicity studies can be found in the Supplementary Data files published online.

3. Absorption, distribution, metabolism, excretion

The *in vitro* metabolism of S9632 was studied using both rat and human liver microsomes. The *in vivo* metabolism and PK of S9632 was studied in rats, including bioequivalence studies of the S9632 sodium, phosphate, and hemisulfate salts.

3.1. In Vitro metabolism

The potential of S9632 to undergo oxidative metabolism was investigated using Sprague-Dawley rat and human liver microsomes (XenoTech, Lenexa, KS). The compound was incubated with mixed gender, pooled liver microsomes from rat and human in the presence of NADPH for 60 min at 37 °C, at which point microsomal activity was quenched by the addition of acetonitrile. Control samples included time zero and 60 min incubates without NADPH as well as testosterone incubation samples in order to verify microsome functionality. Samples were analyzed by LC/MS along with multi-wavelength UV detection (214, 240 and 254 nm) in order to evaluate the metabolism of S9632.

Based on concerted and detailed analysis of the full scan MS of study samples, S9632 was not metabolized by the rat or human microsomes during the 60 min incubation period. Mass chromatograms were generated for the common Phase I transformations of M + 16, M + 32, M – 16, M – 32, M + 18, M – 18, M – 42 and M – 44 (decarboxylation), and M – 141 (O-dealkylation of the 2,2-dimethyl-*N*-(propan-2-yl)-propanamide moiety). Full scan and mass chromatograms were examined in detail to support that no metabolism was observed above the level of 0.1% of the parent drug. No Phase I biotransformations of S9632 were observed in either the rat or human microsomal incubation samples, to the level of 0.1% of the parent compound; statements of scale (quantitative) assume that the relative response factor for all metabolites is equivalent for the mass spectrometry data.

3.2. Pharmacokinetics in Rats

The PK parameters and oral bioavailability of S9632 were evaluated after single intravenous administration or up to seven days of oral administration to Sprague-Dawley rats (CD® [Crl:CD®(SD); Charles River Laboratories, Hollister, CA]).

For single intravenous administration, male and female Sprague-Dawley rats (n = 4/sex/group) were given an intravenous dose (1 mg/kg in a 1% ethanol solution) of S9632 and blood samples (0.2 mL) were collected from implanted jugular cannulae of each rat at pre-dose and at approximately 2, 5, 10, 30 min, 1, 2, 4, and 8 h after the intravenous dose. For oral administration, male and

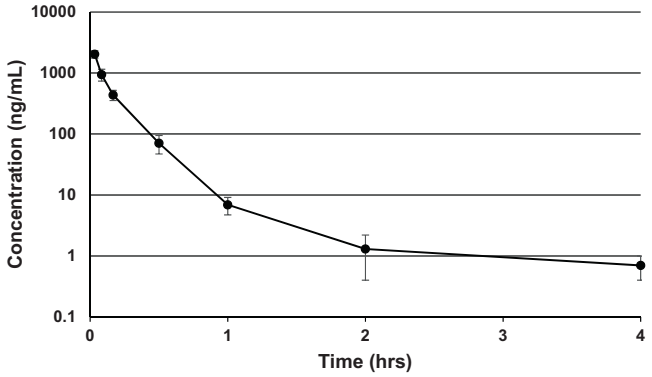

Fig. 2. Mean plasma concentrations of S9632 on Day 1 after intravenous administration to male Sprague-Dawley rats ($n = 4$).

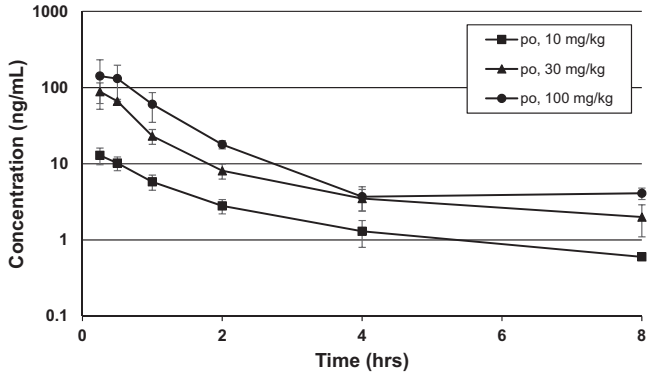

Fig. 3. Mean plasma concentrations of S9632 on Day 1 after oral administration to male Sprague-Dawley rats ($n = 3$/group).

female Sprague-Dawley rats ($n = 3$/sex/dose group) were administered daily doses (10, 30, or 100 mg/kg S9632 in 1% methylcelluose, MC) by oral gavage for seven consecutive days. Blood samples were collected from the jugular catheter at pre-dose and approximately 15, 30 min, 1, 2, 4, 8, and 24 h on Days 1 and 7.

S9632 was poorly orally bioavailable (%F = 0.53–1.19%) and rapidly eliminated after either intravenous ($t_{1/2} < 0.27$ h) or oral administration ($t_{1/2} < 1.39$ h). As expected on the basis of its poor oral bioavailability, systemic exposure to S9632 was relatively low. For example, at 100 mg/kg/day, the combined mean C_{max} on Day 7 was 153.8 ng/mL (0.428 μM), and the combined mean AUC_{0-24} was 232.1 ng·hr/mL. Based on AUC_{last} and C_{max}, the exposure to S9632 in plasma was roughly proportional with dose. Exposure (AUC_{last}) to S9632 was not significantly different in either male or female rats on Day 7 *vs* Day 1 of dosing. No significant accumulation of S9632 was found in plasma after repeated dosing for 7 consecutive days. See Table 1 and Figs. 2, 3, and 4.

3.3. Relative oral bioavailability of sodium, phosphate, and hemisulfate salts of S9632

The objective of this study was to determine whether the systemic exposure following an oral dose of either the sodium (S3333), phosphate (S3337), or hemisulfate (S1638) salt forms of S9632 differs significantly from that of the parent compound. Each group of 4 male Sprague-Dawley rats was treated with a single dose of 30 mg/kg of either S9632, S3333, S3337, or S1638 in 1% MC by oral gavage. Blood samples were taken from a jugular catheter at approximately 15, 30 min, 1, 2, 4, 8, and 24 h post dose. Plasma samples were analyzed for S9632 by LC/MS/MS with an internal standard (S0176).

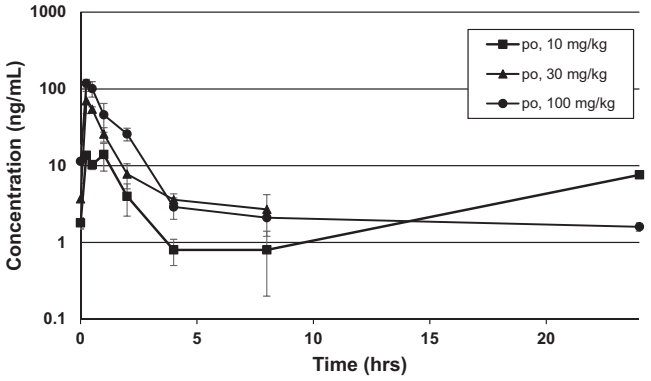

Fig. 4. Mean plasma concentrations of S9632 on Day 7 after oral administration to male Sprague-Dawley rats ($n = 3$/group).

The plasma AUC_{last} and C_{max} of sodium salt S3333 relative to that of the parent compound S9632 was 89.5% and 99.4%, respectively (after correcting for differences in molecular weight). Similarly, the plasma AUC_{last} and C_{max} of phosphate salt S3337 relative to that of the parent compound S9632 was 111.9% and 99.9%, respectively. Finally, the plasma AUC_{last} and C_{max} of sulfate salt S1638 relative to that of the parent compound S9632 was 127.1% and 106.7%, respectively. Based on AUC_{last}, C_{max} and T_{max} data, all three salt forms of S9632 are not significantly different and considered to be bioequivalent to S9632 in terms of systemic exposure. See Table 2.

3.4. Excretion in Rats

Male and female Sprague-Dawley rats ($n = 4$/sex/group) were administered 10 mg/kg of S9632 as a suspension in 1% MC (10 mL/kg) by oral gavage and urine and feces were collected at four time intervals, 0–8, 8–24, 24–48, and 48–72 h, after dosing. Fecal homogenate and urine samples were analyzed by LC/MS/MS with an internal standard (S0176).

An average of 86.2% from male rats and 91.4% from female rats, of S9632 was recovered from feces and urine over the combined 72 h collection period. The vast majority of the compound was excreted during the 8–24 h time interval, the majority of which was recovered from the feces (an average of 88.4% in feces *versus* 0.4% in urine, males and females combined). The trace amounts of S9632 oxidative metabolites (M + 16) were seen in the urine samples but were not detectable in fecal samples. These results are consistent with the hypothesis that S9632 is poorly absorbed and mainly excreted unchanged.

4. Genotoxicity and mutagenicity studies

S9632 was evaluated for its genotoxic potential through abbreviated Ames (2-strains), standard (5-strain) Ames, chromosome aberration, and micronucleus tests (see Table 3). Due to higher solubility in DMSO, the sodium salt of S9632 (S3333) was utilized in the 5-strain Ames and chromosome aberration assays. The decarboxylated impurity, S9379, was also evaluated in an abbreviated Ames (2-strains) assay. The data tables for the genotoxicity studies can be found in the Supplemental material.

4.1. Abbreviated Ames assay

S9632 and impurity S9379 were evaluated for the potential to induce point mutations or frame shifts in *S. typhimurium* strains TA98 and TA100 in a plate incorporation assay in the presence or absence of metabolic activation (rat liver S9). The assay was designed to meet the current OECD Guideline for Testing of

Table 1
Pharmacokinetics of S9632 in Male and Female Sprague-Dawley Rats Following Repeated Administration for 7 Days.

Route	Day	Dose (mg/kg)	Sex	C_{max} (ng/mL) ± SD	T_{max} (hr)	$t_{1/2}$ (hr)	$AUC_{0\text{-last}}$ (ng·hr/mL) ± SD	$AUC_{0\text{-last}}$/dose (ng·hr/mL/mg/kg)	%F
iv	1	1.0	M	2036.7 ± 281.9	0.03	0.19	330.6 ± 58	330.6	–
			F	2625.8 ± 679.9	0.03	0.27	411.9 ± 116.3	411.9	–
Oral gavage	1	10	M	12.9 ± 3.2	0.25	1.21	17.9 ± 2.3	1.79	0.54
			F	34.0 ± 3.9	0.25	1.17	49.2 ± 7.8	4.92	1.19
		30	M	90.0 ± 23.9	0.33	0.88	90.8 ± 11.0	3.03	0.92
			F	62.4 ± 25.9	0.42	1.06	95.0 ± 20.4	3.17	0.77
		100	M	147.2 ± 86.3	0.33	0.71	176.2 ± 73.8	1.76	0.53
			F	268.5 ± 90.7	0.25	0.99	341.1 ± 81.0	3.41	0.83
Oral gavage	7	10	M	16.2 ± 3.6	0.50	0.84	48.1 ± 33.2	4.81	–
			F	32.7 ± 9.7	0.33	1.17	58.9 ± 25.4	5.89	–
		30	M	74.5 ± 16.2	0.33	0.94	86.0 ± 8.7	2.87	–
			F	77.1 ± 41.7	0.25	1.39	106.8 ± 8.7	3.56	–
		100	M	117.9 ± 15.3	0.25	0.72	185.3 ± 15.3	1.85	–
			F	189.7 ± 79.5	0.25	0.89	278.9 ± 79.0	2.79	–

Male rat: CL = 51.5 mL/min/kg; V_{ss} = 440.3 mL/kg; Female rat: CL = 42.5 mL/min/kg; V_{ss} = 320.9 mL/kg.
SD = standard deviation, CL = clearance, V_{ss} = steady-state volume of distribution, %F = bioavailability.

Table 2
Comparison of the Pharmacokinetics of S9632 and its Sodium (S3333), Phosphate (S3337), and Sulfate (S1638) Salts after a Single Oral Administration of 30 mg/kg in Male Sprague-Dawley Rats.

Compound	MW	S9632 Equiv. Dose (mg/kg)	AUC_{last} (ng·hr/mL)	AUC_{last}/Equiv. Dose (ng·hr /mL/mg/kg)	%AUC_{last} Relative to S9632	$C_{max\ (ng/mL)}$	C_{max}/Equiv. dose (ng/mL/mg/kg)	%C_{max} Relative to S9632
S9632	359.42	30.00	59.57 ± 26.08	1.986	100.0%	38.73 ± 24.50	1.291	100.0%
S3333	381.40	28.27	50.20 ± 6.35	1.776	89.5%	36.28 ± 6.66	1.283	99.4%
S3337	457.41	23.57	52.39 ± 4.85	2.223	111.9%	30.40 ± 3.81	1.290	99.9%
S1638	426.47	25.28	63.83 ± 15.72	2.525	127.1%	34.80 ± 7.55	1.377	106.7%

Table 3
Summary of genotoxicity studies conducted on S9632.

End-Point	Test System	Concentration/dose	Result
Reverse mutation (*in vitro*)	*S. typhimurium* strains TA98, TA100, TA1535, TA1537 and *E. coli* strain WP2 *uvrA*	51–5000 ug/plate, plate incorporation and pre-incubation, ±S9[a]	Negative
Chromosome aberration (*in vitro*)	HPBL	125–500 ug/mL, 3 h exposure ±S9[b], 20 h exposure -S9	Negative
Micronucleus formation (*in vivo*)	Male Swiss albino mice (CD-1)	500–2000 mg/kg bw (oral)	Negative

[a] S9 from rat liver homogenate from male Sprague-Dawley rats treated with Aroclor-1254.
[b] S9 from rat liver homogenate from male Sprague-Dawley rats treated with phenobarbital/5,6-benzoflavone.

Chemicals—471, Bacterial Reverse Mutation Test [14]. Both compounds were tested over a range of concentrations up to and including 5000 μg/plate. For S9632, neither bacterial toxicity nor an increase in the number of revertant colonies with or without metabolic activation was observed. For S9379, cytotoxicity (reduction in the background lawn) was observed at 5000 μg/plate in TA98 and TA100 without metabolic activation. With S9379, there was no increase in the number of revertant colonies as compared with the vehicle control in either strain with or without metabolic activation. Positive and vehicle controls yielded the expected results. It was concluded that S9632 and S9379 were not mutagenic to *S. typhimurium* strains TA98 and TA100 under the test conditions.

4.2. Bacterial reverse mutation test (5-strain Ames)

The sodium salt of S9632 (S3333) was evaluated for the potential to induce point mutations in *S. typhimurium* strains, TA98, TA100, TA1535, TA1537 and *Escherichia coli* strain WP2 *uvrA* in the presence and absence of metabolic activation (rat S9). The assay was designed to meet the current OECD Guideline for Testing of Chemicals—471, Bacterial Reverse Mutation Test [14]. The bacteria were treated with each test article at doses ranging from 0 to 5000 μg/plate, in the presence and absence of metabolic activation.

No visible precipitate was formed and there were no obvious toxicity observed at any concentration. For the plate incorporation test, with or without metabolic activation, S3333 did not produce any increases in revertants over the concurrent negative controls. The preincubation test confirmed the negative results. The negative controls for each tester strain were within the historical negative control data. All concurrent positive controls induced at least 3.1-fold increase in colony counts per plate when compared to the corresponding negative controls and were at levels similar to the historical positive control data. Thus, it was concluded that S3333 was not mutagenic to *S. typhimurium* strains TA98, TA100, TA1535, TA1537 and *E. coli* strain, WP2 *uvrA* at concentrations up to 5000 μg/plate, both in the presence and absence of metabolic activation with rat liver S9.

4.3. Chromosome aberration test

S3333 was investigated for the potential to induce structural and numerical chromosome aberrations in human peripheral blood lymphocytes (HPBL) in both the presence and absence of a metabolic activation (rat S9). The experimental design followed the

OECD Guideline for the Testing of Chemicals—473, *In Vitro* Mammalian Chromosome Aberration Test [15].

HPBL were treated with S3333 at concentrations ranging from 0 to 500 μg/mL for 3 and 20 h in the non-activated test system, and for 3 h in the presence of S9-activated test system. Solvent and positive control (mitomycin C, -S9; cyclophosphamide, +S9) cultures were also included. Only the highest exposure concentration of 500 μg/mL produced precipitates in the treatment medium at the beginning of the treatment period. With all three test conditions, Relative Cell Growth (RCG) of ≥72% and Relative Mitotic Index of ≥75% were seen at the highest concentration of S3333. Based on these findings, the three highest concentrations (125, 250 and 500 μg/mL) of S3333 were chosen for chromosomal aberration analysis for each condition. Therefore, the test article was tested at the limit of solubility.

The percentage of cells with structural or numerical aberrations in the test article-treated groups was not significantly increased relative to solvent control at any dose level. The positive and solvent controls fulfilled the requirements for a valid test. Based on the findings of this study, S3333 was concluded to be negative for the induction of structural and numerical chromosome aberrations in both non-activated and S9-activated test systems in the *in vitro* mammalian chromosome aberration test using human peripheral blood lymphocytes.

4.4. Micronucleus assay

S9632 was evaluated for potential clastogenic activity and/or interference with the spindle apparatus as measured by its ability to increase the incidence of micronucleated polychromatic erythrocytes (mnPCEs) in the bone marrow of male and female mice. The study was designed to meet the current OECD Guideline for the Testing of Chemicals—474, Mammalian Erythrocyte Micronucleus Test [16]. Male and female Swiss albino (CD-1) mice (Charles River, Canada) were treated with S9632 as suspension in 1% MC/purified water (20 mL/kg body weight dose volume) by oral gavage for both the dose range finding and definitive phases of the study. In the definitive phase of the study, 1% MC was used as the vehicle (negative) control and cyclophosphamide, at a dose of 70 mg/kg, was used as the positive control article. Animals were observed for signs of toxicity during the course of the study.

In a preliminary dose range finding study, mice (2 animals/sex/group) were dosed at 500, 1000 and 2000 mg/kg. There was no toxicity noted up to 2000 mg/kg, and no difference in toxicity between genders. In the definitive phase, male mice (21 animals/group) were treated at 500, 1000 or 2000 mg/kg, and animals from each group were sacrificed at 24, 36 or 48 h after dosing (7 animals per time point). For each sacrificed animal, bone marrow was recovered and pooled from both femora. Bone marrow smears were prepared, fixed and stained for evaluation. Two thousand polychromatic erythrocytes (PCEs) per animal were scored for the presence of micronuclei. In addition, the number of normochromatic erythrocytes with micronuclei were scored. The polychromatic/normochromatic ratio was established (per 200 cells). The presence/absence of micronuclei was also confirmed by applying a DNA-specific stain to slides from the positive control group, and the test high dose group, for the samples collected at 24 h.

There was no S9632 dose-related increase in micronucleated PCEs. There were no statistically significant differences in the number of PCEs with micronuclei between the test article (all three dose levels) and the negative control group. There was no statistically significant change in the ratio of PCEs to normochromatic erythrocytes in the test article-treated groups compared to the negative control group, suggesting that the test article did not inhibit erythropoiesis. In the positive control group, this ratio was decreased at the 48-h time point, indicating bone marrow suppression. There were statistically significant differences ($p \leq 0.05$) at 24, 36 and 48 h in the number of PCEs with micronuclei in the positive control group when compared to both the negative control group and the test article at all three dose levels. Based on these results, the test article, S9632, did not induce micronuclei at dose levels up to 2000 mg/kg, administered by as a single dose by oral gavage to mice. The test article was not clastogenic and did not interact with the mitotic spindle.

5. *In Vivo* toxicological studies

S9632 was evaluated in 28-day dose-range finding and 90-day toxicology studies in rats in compliance with the United States Food and Drug Administration (FDA) Guidelines [6] Toxicological Principles for the Safety of Food Ingredients. S9632 was also evaluated for potential embryo/fetal toxicity in a gestational developmental toxicity study in rats. The developmental toxicity study consisted of two phases, a range-finding study and a definitive study in which the test animals were evaluated for both maternal toxicity and effects on embryo/fetal development (see Table 4). Summary data tables for 90-day toxicology and definitive developmental toxicity studies for S9632 can be found in the Supplemental material.

5.1. Subchronic toxicology studies

5.1.1. 28-Day dose-range finding toxicity study

The purpose of this study was to evaluate the potential systemic toxicity of S9632 in rats after dietary administration for 28 days in order to select doses for 90-day subchronic toxicity studies in rats. Three treatment groups of male and female CD® [Crl:CD®(SD)] rats ($n = 5$/sex/group; Charles River, Portage, Michigan) were administered S9632 in the diet at dose levels of 10, 30, or 100 mg/kg/day. One additional group of five animals/sex served as the control and received the vehicle diet. The vehicle or test article diet was available *ad libitum* for 28 consecutive days.

Survival, clinical observations, body weight, food consumption, clinical chemistry, ophthalmic examination, organ weights, and macroscopic evaluations of all animals were used to assess potential toxicity. The livers (0 and 100 mg/kg/day dose groups only) and any gross lesions of all animals were subjected to histopathological examination.

No test article-related effects were noted for any parameter examined. One female at 100 mg/kg/day exhibited mildly increased lymphocytes but being an isolated incidence this was not considered related to test article administration. One male at 100 mg/kg/day exhibited markedly increased bile acids and mild to moderate increases of aspartate aminotransferase, alanine aminotransferase, γ-glutamyltransferase, and sorbitol dehydrogenase. These are all related to liver function and injury, but because this was an isolated occurrence they were believed to be incidental in this animal and not test article related. Group mean spleen weights were increased in males of the 100 mg/kg/day group which was the result of one animal having a spleen weight approximately twice that of the other animals of the group. This is the same animal noted above with elevated bile acid and liver enzyme levels; the increased spleen weight in this animal was not considered to be test article related. As a result, the no-observed-effect-level (NOEL) following 28 days of dietary administration was 100 mg/kg/day, the highest dose level tested, in male and female rats.

5.1.2. 90-Day subchronic toxicity study

The purpose of this study was to evaluate the potential subchronic toxicity and toxicokinetic profile of S9632, in rats after dietary administration for 90 consecutive days. Test article was administered in the diet to four groups of twenty male and twenty

Table 4
Summary of subchronic and developmental toxicity studies conducted on S9632.

Study	Species/Gender (N value)	Dose	Findings
28-Day dose range-finding toxicity study	Male & Female Sprague-Dawley Rats –5 animals/sex/group	10, 30, 100 mg/kg/day (food ad-mix)	No test-article related findings; NOEL = 100 mg/kg/day
90-Day sub-chronic toxicity study	Male & Female Sprague-Dawley Rats Main study: –20 animals/sex/group TK satellite group: –6 animals/sex/group	30, 60, 100 mg/kg/day (food ad-mix)	No test-article related findings; NOEL = 100 mg/kg/day
Dose range-finding developmental toxicity study	Bred Female Sprague-Dawley Rats –8 animals/group	125, 250, 500, 1000 mg/kg/day (oral gavage)	No maternal toxicity or effects on intrauterine growth and survival up to 1000 mg/kg/day
Definitive developmental toxicity study	Bred Female Sprague-Dawley Rats –25 animals/group	250, 500, 1000 mg/kg/day (oral gavage)	NOEL for both maternal toxicity and embryo/fetal develop-ment = 1000 mg/kg/day

female Sprague-Dawley [Crl:CD®(SD)] rats at dose levels of 0 (basal diet), 30, 60, or 100 mg/kg/day for a 90-day period. Additionally, one control group of three animals/sex and three treated groups of six animals/sex/group served as toxicokinetic (TK) animals and received the vehicle or test article diet in the same manner as the main study groups at respective dose levels of 0 (control), 30, 60, or 100 mg/kg/day.

Survival, clinical observations, a functional observational battery (including, but not limited to, evaluation of activity, arousal, autonomic and physical function, neuromuscular function, salvation, and respiration), ophthalmic examination, body weight gain, food consumption, hematology, clinical chemistry, urinalysis, organ weights, macroscopic examination, and histopathologic evaluation were performed to assess potential toxicity.

In the TK study, there were no consistent gender differences in the TK parameters calculated for male and female rats on Day 7 or on Day 90 (Table 5). On Day 90, systemic exposure, as estimated by AUC_{0-24} and C_{max}, increased in approximate proportion to the increase in dose between 30 and 100 mg/kg/day, but tended to be greater than dose proportional at the 100 mg/kg/day dose on Day 7. Combined mean T_{max} ranged from 1.50 to 3.00 h on Day 7 and from 1.50 to 7.50 h on Day 90. Combined mean T_{max} was generally longer at 60 and 100 mg/kg/day than at 30 mg/kg/day on Day 90. Consistent with results from PK studies in rats, systemic exposure to S9632 was relatively low. For example, at 100 mg/kg/day, the combined mean C_{max} on Day 7 was 114 ng/mL (0.317 μM), and the combined mean AUC_{0-24} was 1040 ng·hr/mL. Systemic exposure to S9632 was higher on Day 90 than on Day 7. Combined mean accumulation ratios ranged from 1.15 to 2.79 for AUC_{0-24}, and ranged from 1.77 to 5.21 for C_{max}.

There were no test article-related effects for any parameter examined, including clinical signs, body weights (see Figs. 5 and 6), ophthalmic examinations, hematology parameters, coagulation times, clinical chemistry parameters, urinalysis parameters, organ weight, macroscopic or microscopic changes, or in the functional observation battery in either sex at any dose level. As a result, the NOEL following 13-weeks of dietary administration was 100 mg/kg/day, the highest dose level tested, in male and female rats.

5.2. Developmental toxicity studies

5.2.1. Dose range-finding developmental toxicity study

The objective of the study was to determine dosage levels of S9632 to be evaluated in a definitive developmental toxicity study conducted in rats. The test article, S9632, in the vehicle (1% MC) was administered orally by gavage to 4 groups of 8 bred female Crl:CD(SD) rats once daily from gestation Days 6 through 20, with dosage levels of 125, 250, 500, or 1000 mg/kg/day. A concurrent control group composed of 8 bred females received the vehicle on a comparable regimen. The females were approximately 13 weeks of age when paired for breeding. Positive evidence of mating was confirmed by the presence of a vaginal copulatory plug or the presence of sperm in a vaginal lavage. The day on which evidence of mating was identified was termed gestation Day 0. All animals were observed for mortality, moribundity, clinical

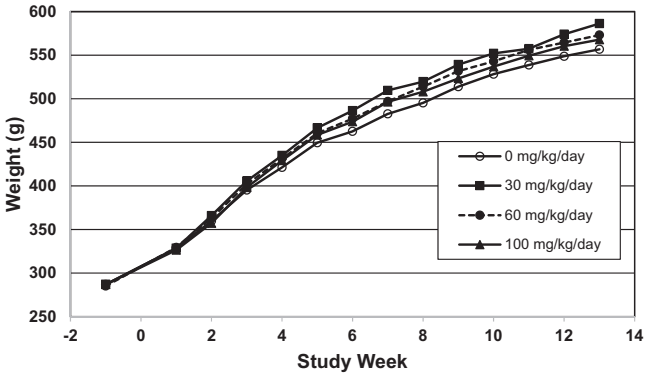

Fig. 5. Mean body weights of male Sprague-Dawley rats receiving S9632 in diet for 13 weeks (n = 20/group).

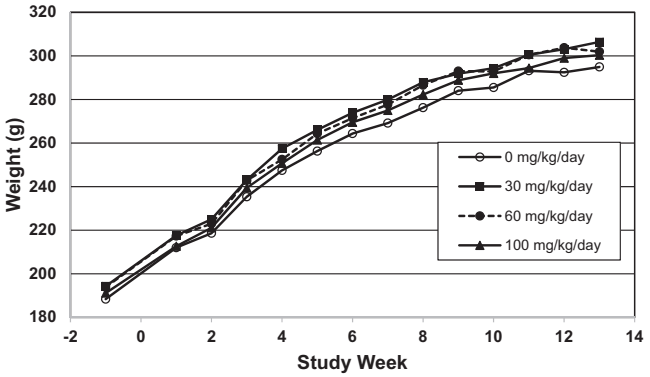

Fig. 6. Mean body weights of female Sprague-Dawley rats receiving S9632 in diet for 13 weeks (n = 20/group).

Table 5
Toxicokinetics of S9632 in male and female Sprague-Dawley rats (food ad-mix).

Day	Dose Level (mg/kg/day)	Sex	C_{max} (ng/mL)	T_{max} (hr)	$AUC_{(0-24)}$ (ng·hr/mL)	AUC_{0-24}/dose (ng·hr/mL/mg/kg)
7	30	Male	18.4	3.00	250	8.34
		Female	7.47	0	105	3.5
		Combined	12.9	1.5	178	5.92
	60	Male	15.9	0	228	3.79
		Female	38.5	6.00	566	9.43
		Combined	27.2	3.00	397	6.61
	100	Male	79.2	3.00	915	9.15
		Female	149	0	1170	11.7
		Combined	114	1.50	1040	10.4
90	30	Male	46.8	0	293	9.75
		Female	31.2	3.00	389	13.0
		Combined	39.0	1.50	341	11.4
	60	Male	146	6.00	1050	17.5
		Female	47.5	3.00	549	9.14
		Combined	97.0	4.50	798	13.3
	100	Male	231	3.00	1000	10.0
		Female	92.0	12.0	1400	14.0
		Combined	162	7.50	1200	12.0

observations, body weights, and food consumption. On gestation Day 21, a laparohysterectomy was performed on each female. The uteri, placentae, and ovaries were examined, and the numbers of fetuses, early and late resorptions, total implantations, and *corpora lutea* were recorded. Gravid uterine weights were recorded, and net body weights and net body weight changes were calculated. The fetuses were weighed, sexed, and examined for external malformations and developmental variations.

All females survived to the scheduled necropsy on gestation Day 21. No remarkable clinical findings were observed, and mean body weights, body weight gains, net body weights, net body weight gains, gravid uterine weights, and food consumption in the 125, 250, 500 and 1000 mg/kg/day groups were unaffected by test article administration. No macroscopic findings were noted at the scheduled necropsy on gestation Day 21. Intrauterine growth and survival were unaffected by test article administration at all dosage levels. A single low-weight fetus in the 1000 mg/kg/day group was noted with craniorachischisis, microphthalmia, gastroschisis, tarsal flexure, bent tail, and anal atresia. There were no other external malformations or external developmental variations noted in this study.

There were no remarkable maternal clinical or macroscopic findings and mean maternal body weight, body weight gain, and food consumption were unaffected by test article administration at all dosage levels evaluated. Additionally, intrauterine growth and survival, and fetal morphology were unaffected by test article administration at all dosage levels tested. Based on the results of this study, dosage levels of 250, 500 and 1000 mg/kg/day were selected for a definitive embryo/fetal development study of S9632 administered orally by gavage to bred Crl:CD(SD) rats.

5.2.2. Definitive developmental toxicity study

The objective of the study was to determine the potential of S9632 to induce developmental toxicity after maternal exposure from implantation to one day prior to expected parturition, to characterize maternal toxicity at the exposure levels tested, and to determine a no-observed-adverse-effect-level (NOAEL) for maternal and developmental toxicity. This study was conducted in general accordance with the OECD guidelines [18] and the FDA Redbook [4], and in compliance with the FDA GLP regulations 21CFR Part 58 and OECD guidelines [17].

S9632, was administered orally by gavage (in vehicle 1% MC) to 3 groups of 25 bred female Crl:CD(SD) rats twice daily (approximately 4 h apart at 10 mL/kg/dose) from gestation days 6 through 20, at dosage levels 250, 500, or 1000 mg/kg/day. A concurrent control group composed of 25 bred females received the vehicle on a comparable regimen. The females were approximately 13 weeks of age when paired for breeding. Positive evidence of mating was confirmed by the presence of a vaginal copulatory plug or the presence of sperm in a vaginal lavage. The day on which evidence of mating was identified was termed gestation Day 0. All animals were observed for mortality, moribundity, clinical observations, body weights, and food consumption. On gestation Day 21, a laparohysterectomy was performed on each female. The uteri, placentae, and ovaries were examined, and the numbers of fetuses, early and late resorptions, total implantations, and *corpora lutea* were recorded. Gravid uterine weights were recorded, and net body weights and net body weight changes were calculated. The fetuses were weighed, sexed, and examined for external, visceral, and skeletal malformations and developmental variations.

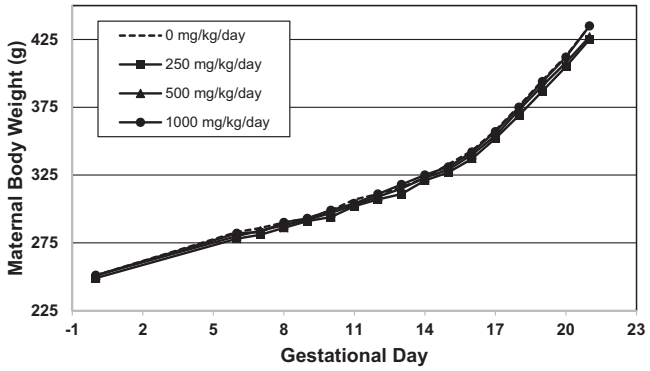

Fig. 7. Oral (Gavage) Developmental Toxicity Study of S9632 in Rats: Mean maternal body weights during gestation (n = 25/group).

All females survived to the scheduled necropsy on gestation Day 21. No test article-related clinical or macroscopic findings were noted at any dosage level. Mean maternal body weights (Fig. 7), body weight gains, gravid uterine weights, and food consumption were unaffected by test article administration at all dosage levels. No test article-related findings were noted on intrauterine growth and survival and fetal morphology at any dosage level. Parameters evaluated included post-implantation loss, live litter size, mean fetal body weights, and fetal sex ratios (Table 6 and Fig. 7).

The mean litter proportion of late resorptions in the 500 mg/kg/day group (2.8% per litter) was above the maximum mean value in the laboratories historical control data (0.5% per litter); however, this did not occur in a dose-related manner, the value was primarily due to 1 litter with 64.3% late resorptions, and the

Table 6
Developmental Toxicity Study of S9632 in Rats: summary of fetal data.

Dose group (mg/kg/d)	Fetuses	Sex		Viable fetuses	Dead fetuses	Resorptions		Post-implant. loss	Implant. sites	Corpora Lutea	Pre-implant. loss	Fetal Wt. (g)	No. of gravid females
		M	F			Early	Late						
0	Total	192	182	374	0	9	2	11	385	418	33	NA	25
	Mean	7.7	7.3	15.0	0	0.4	0.1	0.4	15.4	16.7	1.3	5.6	
	S.D.	2.10	2.49	2.39	0	0.49	0.40	0.71	2.22	2.70	1.77	0.30	
	S.E.	0.42	0.50	0.48	0	0.10	0.08	0.14	0.44	0.54	0.35	0.06	
250	Total	189	169	358	0	24	0	24	382	407	25	NA	25
	Mean	7.6	6.8	14.3	0	1.0	0	1.0	15.3	16.3	1.0	5.5	
	S.D.	2.10	2.11	2.21	0	0.98	0	0.98	2.13	2.26	1.35	0.32	
	S.E.	0.42	0.42	0.44	0	0.20	0	0.20	0.43	0.45	0.27	0.06	
500	Total	177	184	361	0	12	10	22	383	421	38	NA	25
	Mean	7.1	7.4	14.4	0	0.5	0.4	0.9	15.3	16.8	1.5	5.4	
	S.D.	2.25	2.20	2.48	0	0.71	1.80	1.83	1.44	2.79	2.14	0.50	
	S.E.	0.45	0.44	0.50	0	0.14	0.36	0.37	0.29	0.56	0.43	0.10	
1000	Total	181	194	375	0	18	0	18	393	413	20	NA	25
	Mean	7.2	7.8	15.0	0	0.7	0	0.7	15.7	16.5	0.80	5.6	
	S.D.	1.85	2.77	1.83	0	0.98	0	0.98	1.77	1.81	1.08	0.33	
	S.E.	0.37	0.55	0.37	0	0.20	0	0.20	0.35	0.36	0.22	0.07	

NA = not applicable.

difference from the concurrent control group was not statistically significant.

The numbers of fetuses (litters) available for morphological evaluation were 374(25), 358(25), 361(25) and 375(25) in the control, 250, 500 and 1000 mg/kg/day groups, respectively. Malformations were observed in 0(0), 1(1), 1(1), and 3(3) fetuses (litters) in these same respective dose groups and were considered spontaneous in origin. In the 250 mg/kg/day group, malformation of anophthalmia (right) was observed in one fetus. However, this finding was not considered to be test-article related because it occurred in single fetus in a manner that was not dose-related, and mean litter proportions were within the laboratories historical control data ranges. No other external malformations were observed at any dosage level. No external developmental variations were noted in this study.

There were no test article-related soft tissue malformations or variations noted for fetuses at any dosage level. One fetus in the 1000 mg/kg/day group had an interrupted aortic arch in which the brachiocephalic trunk and left carotid arteries arose from the ascending aorta, the left subclavian arose from the descending aorta, and the ductus arteriosus communicated with the descending aorta. In addition, one fetus in the 500 mg/kg/day group had a stenotic pulmonary trunk. These findings occurred in single fetuses and the mean litter proportions of these findings were not statistically significantly different from the concurrent control group. Therefore, these findings were not attributed to test article administration. No other visceral malformations were noted at any dosage level. Visceral developmental variations noted in the test article-treated groups, including renal papilla(e) not developed and/or distended ureter(s), accessory lobule(s) of the liver, and major blood vessel variation, were noted infrequently, in similar frequencies in the concurrent control group, and/or in a manner that was not dose-related. The mean litter proportion of renal papilla(e) not developed and/or distended ureter(s) in the 250 mg/kg/day group (4.3% per litter) was above the maximum mean value in the laboratory's historical control data (3.5% per litter). However, this result did not occur in a dose-related manner and the mean litter proportion was not statistically significantly different from the concurrent control group. Therefore, no visceral developmental variations were attributed to test article administration.

There were no test article-related skeletal malformations or variations noted for fetuses at any dosage level. One fetus in the 1000 mg/kg/day group had sternoschisis (sternal band nos. 1 through 6 not joined) and another fetus in the same dose group had a bent scapula (bilateral) and bent limb bones (left femur and left and right radius, ulna, and humerus). Skeletal variations were observed with similar frequency in all groups, including the control group, and consisted mainly 14th rudimentary rib(s), 7th cervical rib(s), and sternebra(e) malaligned (slight or moderate). The aforementioned visceral and skeletal malformations and variations were not considered test article-related because they were noted in single fetuses, not in a dose-related manner, and/or the mean litter proportions were within the laboratory's historical control data ranges. A detailed summary of all of the external, visceral, and skeletal malformations and variations seen in this study can be found in the Supplemental Data section.

Based on the lack of test article-related effects at any dosage level, a dosage level of 1000 mg/kg/day, the highest dosage level evaluated, was considered to be the NOEL for maternal toxicity and embryo/fetal development when S9632 was administered orally by gavage to bred Crl:CD(SD) rats.

6. Discussion

Based on *in vitro* studies with rat and human liver microsomes, S9632 is a poor substrate for cytochrome P450 oxidative enzymes with no metabolism observed above the level of 0.1% after a 60 min incubation period. After intravenous administration to male and female rats, the mean plasma clearance of S9632 is 93.6% (males) and 77.3% (females) of hepatic blood flow [2] resulting in an apparent plasma half-life ($t_{1/2}$) of only 0.19–0.27 h. The volume of distribution at steady state (V_{ss}) was 440.3 mL/kg in male rats and 320.9 mL/kg in female rats, 0.48–0.66 times total body water [2]. After oral administration to the rat, the plasma elimination half-life ($t_{1/2}$) for S9632 ranged from 0.71–1.39 h and the $AUC_{0\text{-last}}$ and C_{max} increased roughly in proportion to dose after oral administration in the rat. Bioavailability (%F) remained relatively fixed with increasing oral dose, ranging from 0.53% to 1.19%.

In order to exclude the possibility that S9632 is undergoing presystemic metabolism by the gut microflora, the amount of S9632 recovered in the feces and urine following a single oral dose was investigated in male and female rats. At an oral dose of 10 mg/kg, an average of 88.4% of the dose was recovered in feces, and 0.4% recovered in urine in the first 24 h. The trace amounts of S9632 oxidative metabolites (M + 16) were seen in the urine samples but were not detectable in fecal samples. These results suggest that

S9632 does not undergo significant metabolism in the gut and is largely excreted unchanged. Taken together with the compound's rather low systemic bioavailability after oral administration, it is likely that S9632 is poorly absorbed in the intestinal tract.

S9632 was evaluated for its genotoxic potential through a standard battery of *in vitro* and *in vivo* genotoxicity assays which included an abbreviated Ames (*S. typhimurium* strains TA98 and TA100), a bacterial reverse mutation assay (*S. typhimurium* strains, TA98, TA100, TA1535, TA1537 and *E. coli* strain WP2 *uvrA*), a chromosome aberration test in HPBL, and an *in vivo* micronucleus test in mice. S9632 did not exhibit any genotoxic concerns, *i.e.* the compound was not found to be mutagenic or clastogenic *in vitro*, and did not induce micronuclei or interact with the mitotic spindle in bone marrow erythrocytes after oral administration of S9632 at a dose of 2000 mg/kg to male CD-1 mice.

The doses of S9632 selected for the 28- and 90-day toxicology studies were designed to provide a high margin of safety rather than define a maximum tolerated dose (MTD) in rats. Applying a 1000-fold margin of exposure in extrapolating animal data to humans to account for species differences in susceptibility, numerical differences in population ranges between the test animals and the human population, the greater variety of complicating disease processes in the human population, and the possibility of synergistic action among food additives, is believed to be an adequate margin of safety for most substances proposed for use in food [12,3].

The Joint FAO/WHO Expert Committee on Food Additives (JECFA) employs both the maximized survey-derived intake (MSDI) method and single portion exposure technique (SPET) as measures of dietary exposure to flavouring agents for use in their safety evaluation of these compounds [10]. The MSDI is based on the reported amount of a flavouring agent introduced into the food supply per year in specific regions of the world and provides a *per capita* estimate of the exposure to the flavouring agent, assuming that 10% of the relevant population would consume foods containing the flavouring agent. However, in many cases the MSDI is believed to underestimate the dietary exposure to some flavouring agents. The SPET was developed to account for specific consumer patterns of behaviour with respect to food consumption and possible uneven distribution of dietary exposure for consumers of foods containing flavouring agents [10]. The SPET provides an estimate of the dietary exposure for an individual who consumes a specific food product containing the flavouring agent every day and combines an average added use level with the standard portion size for a particular food category. The dietary exposure from the single food category leading to the highest dietary exposure from one portion is taken as the SPET estimate. Based on the SPET value obtained from non-alcoholic beverages, JECFA estimated the dietary exposure of S9632 (JECFA No. 2204) as 2400 μg/day [11]. Therefore, based on this calculation, a NOAEL of 100 mg/kg bw/day in a 90 day sub-chronic toxicology study would be 2500 times the estimated dietary exposure to S9632 when used as a flavouring agent.

In the 28-day range-finding toxicity study of S9632 in male and female Sprague-Dawley rats, the test article was administered as a food ad-mix at dose levels of 10, 30 and 100 mg/kg/day. There were no treatment-related changes in mortality, clinical observations, body weights, body weight gains, food consumption, urinalysis parameters, hematology parameters, or clinical chemistry parameters in the 28-day dose-range finding study. There were also no test article-related organ weight or macroscopic changes noted at any dose level, and no abnormalities observed after gross necropsy and the histopathological examination of the liver of all animals was unremarkable. The NOEL for S9632 in the 28-day range-finding study was 100 mg/kg/day.

Guided by the findings of the 28-day range-finding toxicity study, S9632 was evaluated in a 90-day subchronic toxicology study in both male and female Sprague-Dawley rats (20 animals/sex/group) at 30, 60, and 100 mg/kg/day, administered as a food ad-mix. The study included a functional observation battery and motor activity assessments, in addition to the evaluation of body weight gain, food consumption, hematology, clinical chemistry, urinalysis, organ weights, macroscopic and histopathological examination of at least 53 tissues (control and high dose animals only; see Supplementary Data for list of tissues examined histopathologically). The study also included a TK assessment of compound exposure on a group of satellite animals on Days 7 and 90.

On Day 7, the systemic exposure (AUC) to S9632 observed in the TK study conducted as part of the 90-day subchronic toxicology study where the compound was administered as a food ad-mix was significantly higher (2.9–5.3 fold) than in the repeat dose PK study performed by oral gavage, suggesting that the absorption of S9632 may be increased by the presence of food. Alternatively, the higher exposure in the dietary study may be due to the slow absorption over a longer period when being consumed throughout the day rather than received as a bolus dose. There were no consistent gender differences seen in either the repeat PK or TK studies. In both studies, systemic exposure to S9632 (estimated by AUC_{0-24} or AUC_{last} and C_{max}) was relatively low.

In the 90-day study, there were no test article-related effects among clinical signs, body weights, ophthalmic examinations, hematology parameters, coagulation times, clinical chemistry parameters, or urinalysis parameters, or in the functional observation battery in either sex at any dose level. There were no test article-related organ weight, macroscopic or microscopic changes in the tissues examined noted at any dose level. Therefore, the results of the 90-day subchronic toxicity study established a NOEL for S9632 of 100 mg/kg/day (the highest dose evaluated), for both male and female Sprague-Dawley rats.

S9632 was also evaluated for its potential to induce developmental toxicity when administered orally to bred female rats from gestation Days 6 through 20, at dosage levels 250, 500, or 1000 mg/kg/day. No test article-related clinical or macroscopic findings were noted at any dosage level. Mean maternal body weights, body weight gains, gravid uterine weights, and food consumption were unaffected by test article administration at all dosage levels. No test article-related findings were noted on intrauterine growth and survival and fetal morphology at any dosage level. There were no test article-related external malformations, soft tissue, or skeletal malformations noted for fetuses at any dosage level. Based on the lack of adverse maternal toxicity or effects on intrauterine growth and survival and fetal morphology at any dosage level, a dosage level of 1000 mg/kg/day (the highest dosage level evaluated) was considered to be the NOEL for S9632 for both maternal toxicity and embryo/fetal development.

7. Conclusions

S9632 demonstrated a lack of genotoxicity with or without metabolic activation *in vitro* at concentrations that greatly exceed those observed in rat plasma following oral administration of S9632 at doses up to 100 mg/kg. In addition, S9632 demonstrated a lack of genotoxicity in mice at an oral dose of 2000 mg/kg, and had a NOEL of 1000 mg/kg/day for both maternal toxicity and embryo/fetal development in rats. The results of the 90-day subchronic toxicity study established a NOEL of 100 mg/kg/day (the highest dose evaluated), for male and female rats. Assuming that the systemic exposure to S9632 after oral administration to humans is

comparable to that observed at an equivalent dose in the rat, these NOELs are orders of magnitude higher than the anticipated human exposure for S9632 [13] under the conditions of intended use.

Conflict of Interest

All the studies described herein were funded by Senomyx, Inc.

Acknowledgements

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.toxrep.2015.08.012.

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