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Sucralose administered in feed, beginning prenatally through lifespan, induces hematopoietic neoplasias in male swiss mice

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Background: Sucralose is an organochlorine artificial sweetener approximately 600 times sweeter than sucrose and used in over 4,500 products. Long-term carcinogenicity bioassays on rats and mice conducted on behalf of the manufacturer have failed to show the evidence of carcinogenic effects.

Objective: The aim of this study was to evaluate the carcinogenic effect of sucralose in mice, using a sensitive experimental design.

Methods: Five groups of male (total $n = 457$) and five groups female (total $n = 396$) Swiss mice were treated from 12 days of gestation through the lifespan with sucralose in their feed at concentrations of 0, 500, 2,000, 8,000, and 16,000 ppm.

Results: We found a significant dose-related increased incidence of males bearing malignant tumors ($p < 0.05$) and a significant dose-related increased incidence ($p < 0.01$) of hematopoietic neoplasias in males, in particular at the dose levels of 2,000 ppm ($p < 0.01$) and 16,000 ppm ($p < 0.01$).

Conclusions: These findings do not support previous data that sucralose is biologically inert. More studies are necessary to show the safety of sucralose, including new and more adequate carcinogenic bioassay on rats. Considering that millions of people are likely exposed, follow-up studies are urgent.

Keywords: Sucralose, Prenatal, Carcinogenicity bioassays, Mice, Hematopoietic neoplasias

Introduction

Sucralose (4, 1, 6-trichlorogalactosucrose) is an intense organochlorine artificial sweetener approximately 600 times sweeter than sucrose and manufactured substituting chlorine for three of the hydroxyl groups in sucrose.^{1,2} The structural formula is presented in Fig. 1.

Sucralose was originally approved for use as a food ingredient in Canada in 1991. In 1998, the United States (US) Food and Drug Administration (FDA) permitted the use of sucralose in 15 food and beverage categories.³ In 1999, the FDA expanded the use of sucralose to all categories of food and beverage.⁴ The acceptable daily intake level of sucralose in the US was established at 5 mg/kg of body weight (b.w.)³ and in Europe at 15 mg/kg b.w.⁵ In 2004, the European Union approved the use of sucralose in a variety of products.⁶ Sucralose accounts for 27.9% of the \$1.146 billion global sweetener market⁷ and is

utilized in over 4,500 products, including foods, beverages, and drugs.⁸ For sucralose to be approved as a food additive, over 100 studies were included in the Sucralose Food Additive Petition (compiled by McNeil company) to demonstrate product safety.⁹ This is consistent with the 1958 Food Additive Amendment¹⁰ that holds the manufacturer responsible for demonstrating product safety using well-conducted research and producing reliable results. Critical safety studies are available in the published literature and are briefly summarized below.

Sucralose is claimed stable at elevated temperatures and in low-pH products and does not have the bitter aftertaste attributed to some other artificial sweeteners.¹¹ Hydrolysis of sucralose can occur during prolonged storage at high temperatures in highly acidic aqueous food products (e.g. sodas). The hydrolysis products, which may be ingested by consumers in limited situations, are 4-chloro-4-deoxy-galactose (4-CG) and 1,6-dichloro-1,6-dideoxyfructose (1,6-DCF).¹ The absorption, distribution and metabolism

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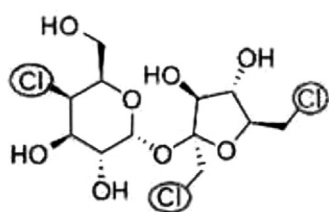


Figure 1 Sucralose Chemical Structure.

pattern in five species, including humans, seems to be similar.^{12–15}

Sucralose is minimally absorbed after oral administration, with 5–15% excreted in the urine and 85–90% excreted unchanged in the feces.¹ Of the approximately 15% absorbed, most is excreted unchanged and about 2–3% undergoes common phase II metabolism, specifically, glucuronidation.¹³ Unchanged sucralose and its glucuronide conjugates are excreted in the urine without bioaccumulation.^{12–15} Thus, an oral intake of sucralose is claimed to be largely unabsorbed, excreted largely unchanged, not metabolized as a source of energy or broken down to yield smaller chlorinated compounds, and therefore essentially inert in the body. However, the same authors detected the presence of metabolites of sucralose in the feces and urine of rats and humans by thin-layer chromatographic methods indicating that sucralose is metabolized in the gastrointestinal tract and not secreted unchanged.

A recent report summarizing the results from a number of unpublished *in vitro* and *in vivo* genotoxicity studies, conducted as part of the sucralose safety investigation, reported no evidence of genotoxicity of sucralose.¹⁶ However, in other studies, the hydrolyzed product 1,6-DCF was weakly mutagenic in both the Ames test and the L5178Y TK +/- assay and sucralose was weakly mutagenic in the mouse lymphoma mutation assay.^{3,17} A previous study also concluded that sucralose damaged the gastrointestinal organ's DNA of mice in a comet test.¹⁸

In a short-term *in vivo* study, male Sprague-Dawley rats were orally treated for 12 weeks with Splenda, a commercial intense artificial sweetener containing 1.1% sucralose and 93.6% maltodextrine. The main aim of the study was to determine the effects of orally administered Splenda on the composition of the variously enhanced population of fecal microflora. At the end of the treatment, half of the animals were sacrificed to determine the intestinal expression of the membrane efflux transporter P-glycoprotein (P-gp) and the cytochrome P-450 (CYP) metabolism system. The remaining animals were allowed to recover for 12 weeks and then sacrificed. The study found several adverse effects on the intestines including reduction of beneficial fecal microflora, increased fecal pH, increased body weight, enhanced intestinal expression of P-gp, CYP3A4, and CYP2D1; several of these changes differed from controls even after discontinuation of the treatment with Splenda.¹⁹

Acute toxicity exposure of mice and rats to sucralose administered by gavage did not show any adverse effect on the highest tested doses 10–16 g/kg b.w.²⁰ To test the carcinogenic potential of sucralose, long-term carcinogenicity bioassays on Sprague-Dawley rats and on CD-1 mice were performed.^{21,22} The results of both studies failed to show any evidence of carcinogenic effects.

As part of a project started in 1997 by the Ramazzini Institute in Bologna (Italy) to test the carcinogenic potential of widely used intense artificial sweeteners primarily in aspartame,^{23–28} a study on the carcinogenic potential of sucralose administered with feed to Swiss mice was undertaken. Because human exposure to sucralose might occur during *in utero* development and during adolescence and in order to increase the sensitivity of the study, the exposure began prenatally and lasted until death.

Material and methods

Nutraceutica (Monterenzio, Bo, Italy) supplied the sucralose for this experiment. The certified grade of purity was 99.4% and the impurities were as follows: methanol 0.05%; heavy metals < 0.1 ppm. Sucralose was pulverized in a standard pelleted diet at concentrations 0, 500, 2,000, 8,000, and 16,000 ppm and was administered to groups of male (n = 117, 114, 90, 66, and 70, respectively) and female (n = 102, 105, 60, 65, and 64, respectively) Swiss mice from the 12th day of fetal life until death. The 16,000 ppm dose level of sucralose was selected based on available data reported in the literature and to avoid dose levels producing poor palatability. Laboratorio Dottori Piccioni (Milan, Italy) provided the standard “Corticella diet.” This same diet has been used for more than 30 years in the laboratory. The diet was analyzed for nutritional components, micro-organisms, and potential contaminants (pesticides, heavy metals, estrogen activity, nitrosamines) every six months. The main constituents of the diet were: 12% water; 24% raw protein; 3.5% raw fat; 5.5% raw fibers; 10.5% ashes; and 56.5% non-nitrogen extracts. The pulverized sucralose was stable in the pelleted feed before the start of the study and was periodically checked during the experiment. Feed and water were supplied *ad libitum*. Charles River Laboratories (Milan, Italy) supplied pathogen-free Swiss mice used to generate the experimental animals. After a 40-day quarantine period the breeders were randomly distributed by weight into three groups of 40 and two groups of 60, encompassing the same number of males and females (n = 240). Due to the external origin of the animals, this matching is considered outbred. At 13 weeks of age, one male and one female breeder were placed in each cage for five days, after which males were removed from the cages. Dietary exposure of the experimental animals started on day 12 of fetal life with the administration of sucralose in feed to female breeders. All male and female pups of each litter were used in the experiment to reach the programmed number per sex per group and to allow the evaluation of a potential family

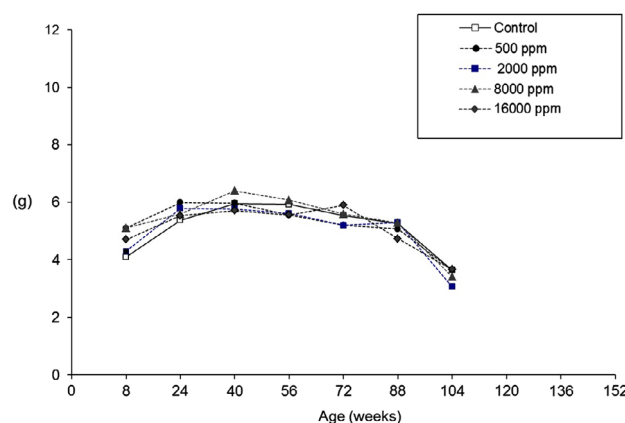


Figure 2 Mean daily feed consumption in males.

effect in the carcinogenic process. This results in an unequal distribution of animals among groups, with an “odd” number per group. The pups were weaned between four and five weeks of age, identified by ear punch and assigned to the respective dose groups. The offspring were housed 10 per cage in polycarbonate cages (41 x 25 x 15 cm) with stainless steel wire tops and a shallow layer of white wood shavings as bedding. All cages were housed in the same room, with a daily light cycle of 12 h, at a temperature of 23 ± 2 °C and relative humidity of 50–60%. The experiment was conducted in accordance with Italian law regulating the use and humane treatment of animals for scientific purposes.²⁹ Drinking water and feed consumption by cage were measured weekly for 13 weeks, and every two weeks thereafter, from six weeks of age until 110 weeks of age. The body weight of each animal was measured weekly for 13 weeks and thereafter every two weeks until 110 weeks of age, and after that, every eight weeks until the end of the experiment. Clinical checks on the animals to monitor and record healthy signs and symptoms and gross pathological lesions were performed concurrently with the measurement of body weight from 6 to 110 weeks of age and, after that, every two weeks until the end of the experiment. To avoid *postmortem* modifications as much as possible, a patrol was performed two to three times daily. During the health control, unhealthy or moribund animals were isolated from the others to avoid cannibalization. Moribund animals were euthanized to minimize suffering and autolysis. Deceased animals were stored in a refrigerator at 4 °C and necropsies were performed within 16–19 h following detection.

At 130 weeks of age, 57 mice were still alive (6.7%), equally distributed per group in females but less homogeneous among males (0.0% at the highest dose and 4.5, 2.5, 2.6% for the other decreasing doses, compared to 7.7% in controls). Since the number of animal alive was < 10%, a final sacrifice was planned and the remaining mice were euthanized with CO₂ overexposure over the following 9 days, equally distributing the animals per group and sex each day. Liver, femur, and neoplastic lesions (> 1 cm

of diameter) samples were frozen in liquid nitrogen and stored at -70 °C for further biomolecular analysis.

During necropsy, all tissues and organs were examined to detect visible alterations. The following organs and tissues were collected: skin and subcutaneous tissue, mammary gland (four sites: axillary and inguinal, right and left), brain (three sagittal sections), pituitary gland, Zymbal glands, salivary glands, Harderian glands, cranium (five sections, encompassing oral and nasal cavity, external and internal ear ducts), tongue, thyroid, and parathyroid, pharynx, larynx, thymus and mediastinal lymph nodes, trachea, lung and mainstream bronchi, heart, diaphragm, liver, cholecyst, spleen, pancreas, kidneys, adrenal glands, esophagus, stomach (fore and glandular), intestine (four levels), urinary bladder, prostate, testes, uterus, vagina, subcutaneous, and mesenteric lymph nodes, sternum with bone marrow, and other organs/tissues with pathological changes. All organs and tissues were preserved in solvanol 70% (a mixture of ethyl and isopropyl alcohol, respectively, approx. 60% and 40%, obtained from VITAL SRL, (Bologna, Italy), apart from bone tissues, which were preserved in 10% formalin and then decalcified with 10% formaldehyde and 20% formic acid in water solution. All lesions were trimmed including a portion of normal adjacent tissue. The tissues were embedded in paraffin blocks and sections of 3–5 µm were cut for each specimen and stained with hematoxylin and eosin. Microscopical evaluation was performed for all tissues and organs of all animals in each group. A single pathologist evaluated all slides and two pathologists reviewed all tumors and lesions of oncologic interest. The statistical analyses of survival and of the malignant neoplastic lesions were based on the Cox proportional hazard regression model,³⁰ which adjusts for possible differential survival among experimental groups.

Results

The breeding proceeded well without any setbacks and was not affected by the treatment with sucralose. Indeed, the percentage of pregnant females varied between 90% and 96% among the groups, apart from the group exposed

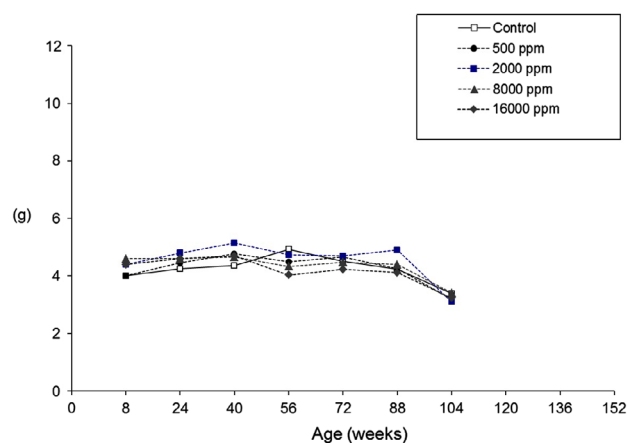


Figure 3 Mean daily feed consumption in females.

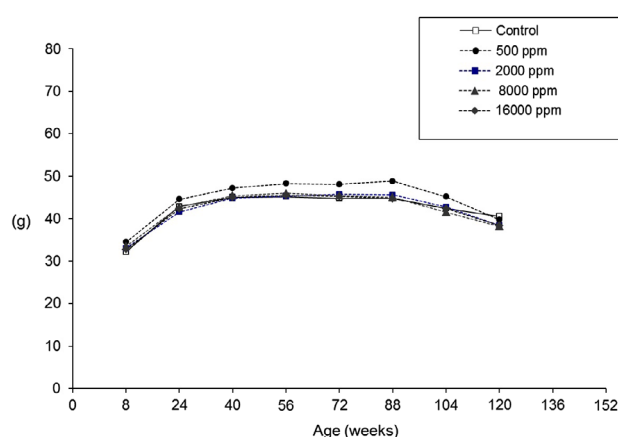


Figure 4 Mean body weight in males.

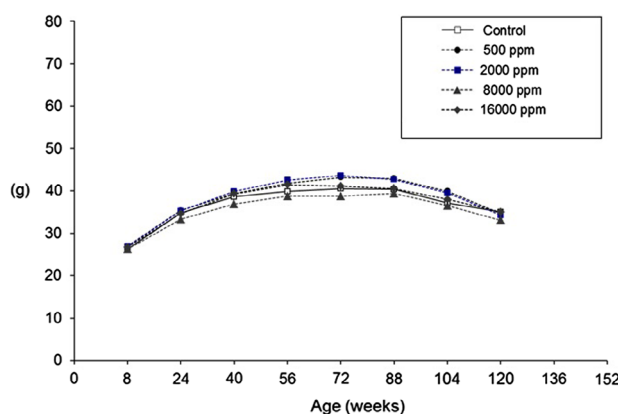


Figure 5 Mean body weight in females.

at the highest dose in which the percentage was slightly lower (83%). The average number of pups per litter was between 12 and 13 and the body weight of each pup measured one week after delivery was between 4.4 and 4.5 g in the treated and control groups.

The feed consumption showed that right from the beginning both males and females were unaffected by any reduced palatability of the diet containing sucralose. As shown in Figs. 2 and 3, no substantial differences in food

consumption were observed in treated males and females compared to controls.

Concerning body weight, a slight increase was observed in males treated at the lowest dose (500 ppm) compared to controls (Fig. 4). This increase was evident from 40 weeks until 104 weeks of age. No differences were observed among other male treated groups. In females treated at 500 and 2,000 ppm, a slight increase in body weight was evident from 72 to 104 weeks of age (Fig. 5). No clinical

Table 1 Incidence of various type of benign and malignant tumors in male Swiss mice in a transplacental lifespan feed carcinogenicity study of sucralose^{a,b}

Site	Histotype	Treatments (ppm)									
		0		500		2,000		8,000		16,000	
		No.	(%)	No.	(%)	No.	(%)	No.	(%)	No.	(%)
Skin	Dermatofibroma	4	(3.4)	2(3)	(1.8)	5	(6.3)	0	-	2	(2.9)
	Papilloacanthoma	2	(1.7)	1	(0.9)	0	-	0	-	0	-
	Squamous cell carcinoma	0	-	0	-	0	-	1	(1.5)	0	-
	Basal cell carcinoma	1	(0.9)	0	-	0	-	0	-	0	-
Subcutaneous tissue	Fibroma and fibroadenoma	2	(1.7)	1	(0.9)	0	-	0	-	0	-
	Lipoma and fibrolipoma	0	-	0	-	0	-	0	-	1	(1.4)
	Fibrosarcoma	42(58)	(35.9)	46(66)	(40.4)	22(29)	(27.5)	16(22)	(24.2)	22(25)	(31.4)
	Liposarcoma	1	(0.9)	0	-	0	-	0	-	1	(1.4)
	Hemangiosarcoma	0	-	1	(0.9)	0	-	0	-	1	(1.4)
Mammary glands	Adenoma, fibroma and fibroadenoma	0	-	0	-	0	-	0	-	1	(1.4)
Harderian glands	Adenoma	4	(3.4)	4	(3.5)	0	-	3(4)	(4.5)	0	-
	Carcinoma	1	(0.9)	0	-	0	-	1	(1.5)	0	-
Ear ducts	Papilloacanthoma	1	(0.9)	0	-	0	-	0	-	0	-
	Squamous cell carcinoma	1	(0.9)	0	-	1	(1.3)	0	-	0	-
Lung	Adenoma	8	(6.8)	19	(16.7)	4	(5.0)	13	(19.7)	7	(10.0)
	Carcinoma	7	(6.0)	8	(7.0)	3	(3.8)	6	(9.1)	2	(2.9)
	Fibrosarcoma	0	-	1	(0.9)	0	-	0	-	0	-
Stomach											
- Forestomach	Acanthoma	0	-	1	(0.9)	0	-	0	-	0	-
- Glandular stomach	Adenoma (polyp)	1	(0.9)	0	-	0	-	0	-	1	(1.4)
	Adenocarcinoma	0	-	0	-	2	(2.5)	0	-	0	-
Liver	Hepatocellular adenoma	9	(7.7)	8	(7.0)	11	(13.8)	3	(4.5)	7	(10.0)
	Haemangioma and fibroangioma	2	(1.7)	0	-	0	-	0	-	1	(1.4)
	Hepatocellular carcinoma	6	(5.1)	4	(3.5)	1	(1.3)	4	(6.1)	4	(5.7)
	Hemangiosarcoma	5	(4.3)	1	(0.9)	2	(2.5)	2	(3.0)	1	(1.4)
Pancreas	Islet cell adenoma	1	(0.9)	2	(1.8)	4	(5.0)	1	(1.5)	0	-
Kidneys	Tubular cell adenoma	1	(0.9)	0	-	0	-	0	-	2	(2.9)
Urinary bladder	Hemangioma	0	-	0	-	0	-	0	-	1	(1.4)
Testes	Interstitial cell adenoma	6	(5.1)	3	(2.6)	2	(2.5)	4(5)	(6.1)	3	(4.3)
	Benign cystoadenoma	1	(0.9)	2	(1.8)	3	(3.8)	1(2)	(1.5)	0	-
	Hemangioma	0	-	0	-	0	-	1	(1.5)	0	-
Epididymis	Interstitial cell adenoma	0	-	1	(0.9)	0	-	0	-	0	-
	Carcinoma	1	(0.9)	0	-	0	-	0	-	0	-
Seminal vesicles	Fibroma	0	-	0	-	1	(1.3)	0	-	0	-
Peritoneum	Mesothelioma	0	-	0	-	1	(1.3)	1	(1.5)	0	-
Thyroid gland	Follicular adenoma	2	(1.7)	1	(0.9)	2	(2.5)	0	-	0	-
	C-cell adenoma	1	(0.9)	0	-	0	-	0	-	0	-
	Follicular cell carcinoma	0	-	0	-	0	-	1	(1.5)	1	(1.4)
Parathyroid glands	Adenoma	1	(0.9)	0	-	0	-	0	-	0	-

differences were observed during the study in both treated and untreated males and females.

The survival function for Kaplan–Meier estimation showed significant differences in male survival among the groups (Fig. 6). Compared to controls, a significant

dose-related decrease ($p \leq 0.02$) in survival was observed among treated male mice and, in particular, in males treated at 16,000 ($p \leq 0.009$) and 2,000 ppm ($p \leq 0.04$). A non-significant decrease in survival was observed in females treated at 2,000 ppm.

Table 2 Incidence of various type of benign and malignant tumors in female Swiss mice in a transplacental lifespan feed carcinogenicity study of sucralose^{a,b}

Site	Histotype	Treatments (ppm)									
		0		500		2.000		8.000		16.000	
		No.	(%)	No.	(%)	No.	(%)	No.	(%)	No.	(%)
Skin	Dermatofibroma	1	(1.0)	0	-	0	-	0	-	0	-
	Trichoepithelioma malignant	1	(1.0)	0	-	0	-	0	-	0	-
	Squamous cell carcinoma	2	(2.0)	1	(1.0)	0	-	0	-	0	-
Subcutaneous tissue	Fibroma and fibroadenoma	0	-	0	-	0	-	0	-	0	-
	Fibrosarcoma	8(9)	(7.8)	2	(1.9)	2	(3.3)	1(2)	(1.5)	0	-
	Liposarcoma	1	(1.0)	3(4)	(2.9)	0	-	1	(1.5)	0	-
Mammary glands	Adenoma , fibroma and fibroadenoma	10(12)	(9.8)	5	(4.8)	3(4)	(5.0)	4	(6.2)	1(3)	(1.6)
	Adenocarcinoma	14(16)	(13.7)	22(27)	(21.0)	8(10)	(13.3)	7	(10.8)	7	(10.9)
	Fibrosarcoma	0	-	0	-	0	-	0	-	1	(1.6)
Harderian glands	Adenoma	0	-	5	(4.8)	3	(5.0)	1	(1.5)	3	(4.9)
	Carcinoma	0	-	0	-	1	(1.7)	0	-	0	-
Oral cavity, lips and tongue	Squamous cell carcinoma	0	-	1	(1.0)	0	-	1	(1.5)	0	-
Nasal cavities	Adenoma	0	-	0	-	0	-	0	-	1	(1.6)
	Olfactory neuroblastoma	0	-	0	-	0	-	0	-	1	(1.6)
Lung	Adenoma	4	(3.9)	9	(8.6)	3	(5.0)	4	(6.2)	1	(1.6)
	Carcinoma	7	(6.9)	7	(6.7)	5	(8.3)	4	(6.2)	5	(7.8)
Stomach - Forestomach	Acanthoma	0	-	1	(1.0)	1	(1.7)	1	(1.5)	0	-
	Adenoma (polyp)	0	-	4	(3.8)	1	(1.7)	2	(3.1)	4	(6.3)
Liver	Hepatocellular adenoma	1	(1.0)	0	-	0	-	0	-	2	(3.1)
	Hemangiosarcoma	4	(3.9)	2	(1.9)	3	(5.0)	0	-	1	(1.6)
Pancreas	Islet cell adenoma	3	(2.9)	4	(3.8)	0	-	0	-	0	-
Ovaries	Adenoma and cystadenoma	5	(4.9)	6	(5.7)	4	(6.7)	5	(7.7)	7	(10.9)
	Benign Granulosa and Theca cell tumour	1(2)	(1.0)	0	-	0	-	2	(3.1)	0	-
	Fibroangioma	0	-	0	-	1	(1.7)	0	-	1	(1.6)
	Luteoma	1	(1.0)	0	-	0	-	1	(1.5)	1	(1.6)
	Leiomyoma	0	-	0	-	0	-	1	(1.5)	0	-
	Benign Sertoli cell tumor	0	-	0	-	0	-	2	(3.1)	0	-
	Malignant granulosa cell tumor	3(4)	(3.9)	1	(1.0)	0	-	0	-	0	-
	Adenocarcinoma	1	(1.0)	1	(1.0)	1	(1.7)	1	(1.5)	0	-
	Leiomyosarcoma	0	-	0	-	0	-	1	(1.5)	0	-
	Hemangiosarcoma	0	-	1	(1.0)	0	-	1	(1.5)	0	-
Uterus	Polyp	10	(9.8)	10	(9.5)	6	(10.0)	9	(13.8)	12	(19.7)
	Fibroma	0	-	0	-	0	-	1	(1.5)	0	-
	Fibroangioma	1	(1.0)	1	(1.0)	0	-	0	-	1	(1.6)
	Leiomyoma	3	(2.9)	3	(2.9)	0	-	0	-	4	(6.3)
	Benign Schwannoma	0	-	0	-	0	-	1	(1.5)	0	-
	Ganglioneuroma	0	-	0	-	1	(1.7)	0	-	0	-
	Adenocarcinoma	1	(1.0)	0	-	1	(1.7)	0	-	0	-
	Leiomyosarcoma	0	-	1	(1.0)	0	-	1	(1.5)	1	(1.6)
	Haemangiosarcoma	1	(1.0)	1	(1.0)	0	-	0	-	0	-

The oncological results are reported in Tables 1–3. Multiple tumors of different types and sites, of different types at the same site, of the same type in bilateral organs, of the same type in the skin, subcutaneous tissue, mammary

glands, or at distant sites of diffuse tissue (i.e. bones and skeletal muscle) were counted as single/independent tumors. Multiple tumors of the same type in the same tissue and organ, apart from those listed above, were counted only once.

Table 3 Incidence of neoplastic lesions in male (M) and female (F) Swiss mice in the lifespan feed carcinogenicity study on sucralose

Dose (ppm)	Animals at start	Total animals bearing neoplasias ^a				Animal bearing hematopoietic neoplasias ^a								
		Benign neoplasias		Malignant neoplasias	Total	Lymphomas		Leukaemias		Histiocytic sarcoma				
		No.	%			No.	%	No.	%					
0 (control)	M	117	42	35.9	66	56.4 [#]	10	8.5 ^{##}	7	6.0	2	1.7 ^{##}	1	0.9 [#]
	F	102	39	38.2	69	67.6	39	38.2	18	17.6	14	13.7	7	6.9
	M+F	219	81	37.0	135	61.6	49	22.4	25	11.4	16	7.3	8	3.7
500	M	114	42	36.8	67	58.8	11	9.6	4	3.5	6	5.3	1	0.9
	F	105	41	39.0	68	64.8	31	29.5	16	15.2	9	8.6	6	5.7
	M+F	219	83	37.9	135	61.6	42	19.2	20	9.1	15	6.8	7	3.2
2,000	M	80	28	35.0	47	58.8	16	20.0 ^{**}	2	2.5	14	17.5 ^{**}	0	-
	F	60	21	35.0	38	63.3	20	33.3	8	3.3	8	13.3	4	6.7
	M+F	140	49	35.0	85	60.7	36	25.7	10	7.1	22	15.7	4	2.9
8,000	M	66	25	37.9	35	53.0	10	15.2	2	3.0	7	10.6 [*]	1	1.5
	F	65	29	44.6	36	55.4	24	36.9	5	7.7	17	26.2	2	3.1
	M+F	131	54	41.2	71	54.2	34	26.0	7	5.3	24	18.3	3	2.3
16,000	M	70	25	35.7	44	62.9 [*]	18	25.7 ^{**}	4	5.7	11	15.7 ^{**}	3	4.3
	F	64	29	45.3	38	59.4	21	32.8	8	12.5	11	17.2	2	3.1
	M+F	134	54	40.3	82	61.2	39	29.1	12	9.0	22	16.4	5	3.7

^a Tumor rates are based on the number of animals at start;

^{*} Statistically significant ($p \leq 0.05$) or ^{**} ($p \leq 0.01$) using Cox Proportional Hazard Model;

[#] Near the control incidence are the p-values ($p \leq 0.05$) or ^{##} ($p \leq 0.01$) associated with Cox Proportional Hazard Model for the analysis of the trend.

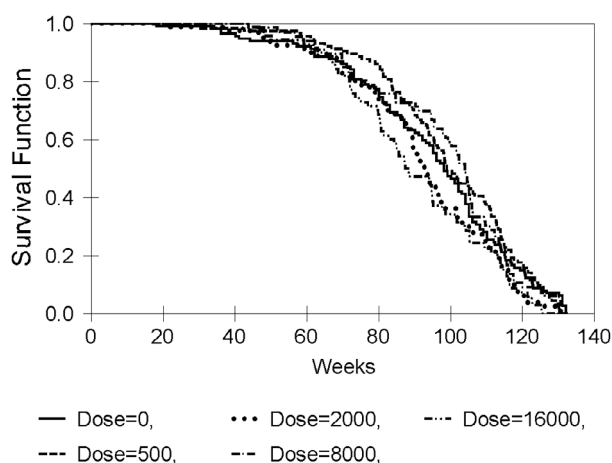


Figure 6 Survival Function for Kaplan–Meier estimation in male mice.

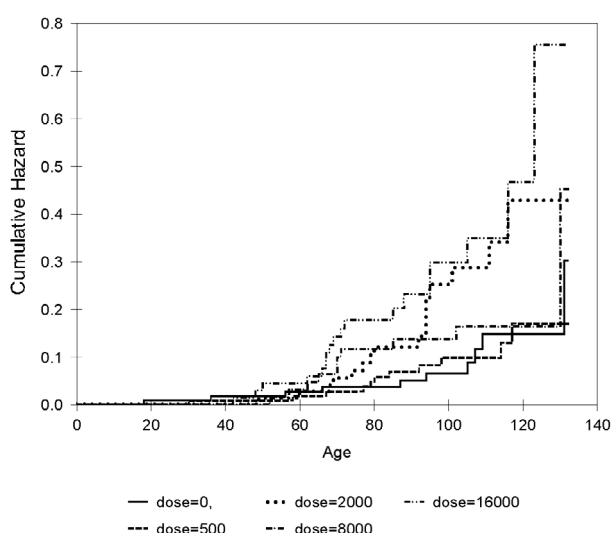


Figure 7 Cumulative hazard for Kaplan–Meier estimation: lymphomas and leukemias in male mice.

Total benign and malignant tumors

The descriptive occurrence of benign and malignant tumors of various organs and tissues in males and females is reported in Tables 1 and 2, respectively. The overall incidences of benign and malignant tumors are reported in Table 3. The data show: 1) in males, an increased incidence of cortical adenoma in the various treated groups; a significant increased incidence of malignant tumor-bearing animals exposed at 16,000 ppm ($p \leq 0.05$) with a significant dose-related trend ($p \leq 0.05$). The tumor type that contributed most to this increased incidence was hematopoietic neoplasias; 2) in females, an increased incidence benign tumors and total tumors per 100 animals treated at various doses of sucralose, in particular harderian adenoma, polyp of the glandular stomach, adenoma, and cystadenoma of the ovaries and polyp of the uterus.

Hematopoietic neoplasias

The occurrence of hematopoietic neoplasias in males and females is reported in Table 3. The data show that sucralose induces significant increased incidence of all hematopoietic neoplasias in males exposed to 2,000 ($p \leq 0.01$), and 16,000 ppm ($p \leq 0.01$), with a significant dose-related relationship ($p \leq 0.01$), as shown also by the cumulative hazard by Kaplan–Meier estimation (Fig. 7). The majority of these neoplasias were grossly visible during necropsy and involved multiple organs such as thymus, spleen, liver, and lymph nodes.

Microscopically, most of the neoplasias among males treated with sucralose at 2,000–16,000 ppm were leukemias involving lungs, liver, spleen, lymph nodes, and bone marrow with diffuse permeation of vessels (Fig. 8) and extensive infiltration of adjacent tissues (Figs. 9 and 10). Sparse cases of histiocytic sarcomas in males were observed among the groups. At an advanced stage of the

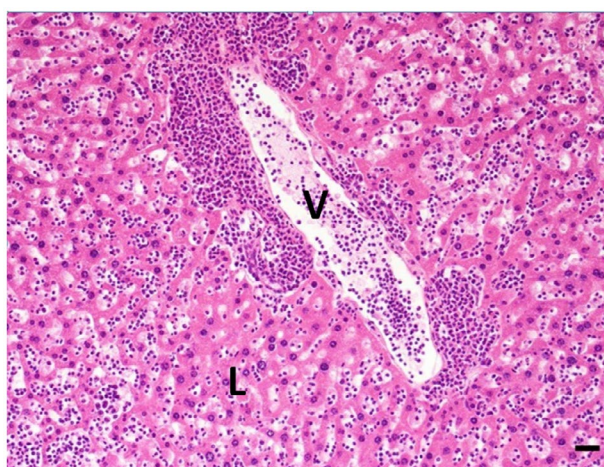


Figure 8 Leukemia in liver: lymphoblastic cells spread throughout the liver parenchyma (L) with evident vascular permeation (V). HE, magnification 200X; bar = 50 μ m.

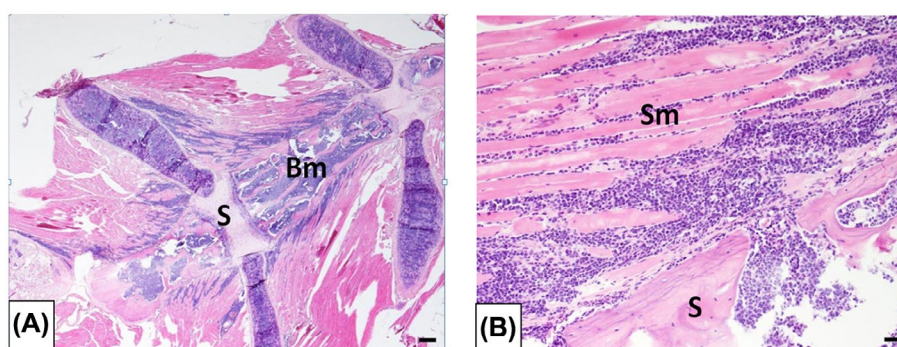


Figure 9 A: leukemia in bone marrow (Bm) present in the sternum (S). HE, magnification 25X; bar = 500 μ m. B: the neoplastic cells, leaked from bone marrow of the sternum (S), invade the surrounding skeletal muscle (Sm). HE, magnification 200X; bar = 50 μ m.

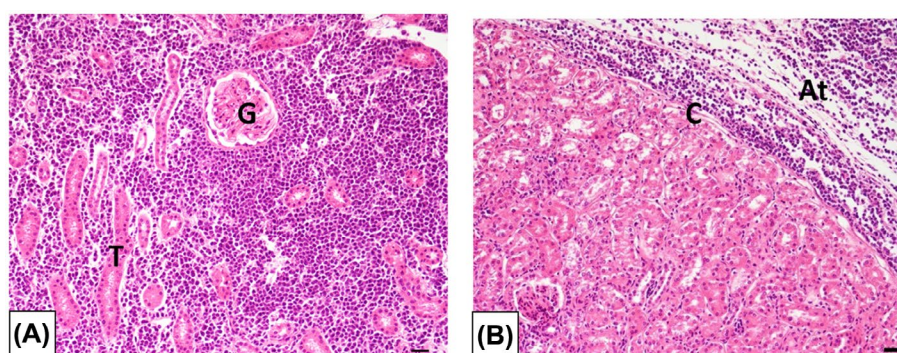


Figure 10 A: leukemia in kidney infiltrating the glomeruli (G) and renal tubules (T). HE, magnification 200X; bar = 50 μ m. B: the neoplastic cells leaked from renal capsule (C) invade the surrounding pararenal adipose tissue (At). HE, magnification 200X; bar = 50 μ m.

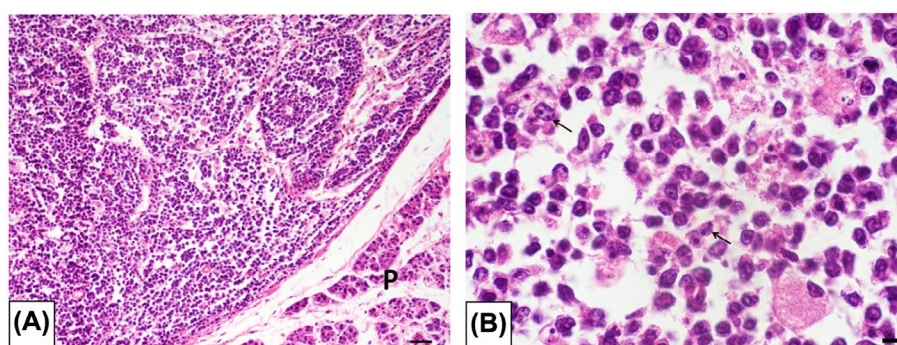


Figure 11 A: leukemia in mesenteric lymph-node near the pancreas (P). HE, magnification 200X; bar = 50 μ m. In B an higher magnification of the previous figure: the cytoplasm of neoplastic cells is moderate in amount and basophilic; nuclear to cytoplasmic ratio was high and mitotic figures are numerous (arrows). HE, magnification 1000X oil; bar = 10 μ m.

neoplasias, the spleen was markedly enlarged with loss of the normal architecture. The liver was infiltrated by large neoplastic cells sometimes associated with degenerative changes, which were accompanied by nodular regenerative hyperplastic lesions. Other organs such as brain and adrenal glands were sometimes also involved. The cells appeared medium–large sized with a non-cohesive pattern of homogeneous sheets, while nuclei were round, oval, and pleomorphic, with varying degrees of differentiation. Cytoplasm was moderate in amount, basophilic, and at

times vacuolated. The nuclear to cytoplasmic ratio was high and mitotic figures were numerous (Fig. 11).

Discussion

This study showed that sucralose administered in feed to Swiss mice at dose levels of 0, 500, 2,000, 8,000, and 16,000 ppm from prenatal life until natural death induces a significant dose-related increased incidence of malignant tumors ($p \leq 0.01$) in male mice. Moreover, a significant dose-related increased incidence ($p \leq 0.01$) of

hematopoietic neoplasias was observed in males, in particular at the exposure levels of 2,000 ppm ($p \leq 0.01$) and 16,000 ppm ($p \leq 0.01$). Since the survival among males showed significant differences among the groups, the Cox proportional hazard regression model was used to evaluate the results of the neoplasias.³⁰

Concerning the plausibility of a causal correlation between exposure to sucralose and significant increase in the incidence of hematopoietic neoplasias in male mice, it is unlikely that this finding is due to chance if we consider that: 1) the incidence of the concurrent control group (8.5%) falls in the range of the overall historical control incidence (5.7%; range: 0.0–12.5%) and the incidence of hematopoietic neoplasias in the males exposed to 16,000, 8,000, and 2,000 ppm significantly exceeded the higher range of the historical controls; 2) if among the males exposed to the highest dose we exclude the animals bearing hematopoietic neoplasms, the survival is almost the same as among controls; 3) the cumulative hazard is much higher among males treated at 16,000, 8,000, and 2,000 ppm than in males exposed to 500 or 0 ppm (Fig. 7). Moreover in our study, we observed: 1) an anticipated onset of hematopoietic neoplasias associated with early increased mortality; and 2) an increased incidence of such lesions at the end of the study. This is in agreement with previous literature concerning induced hematopoietic neoplasias in mice.^{31,32}

The evidence of the carcinogenic effects of sucralose in our mouse study is not in agreement with the negative conclusions obtained with the CD-1 mouse study performed by Mann *et al.*²¹

Indeed the Mann *et al.* results showed: 1) no statistically significant differences in feed consumption among the groups, apart from a slightly lower feed intake among females of the high-dose group as compared to controls. Water consumption in male mice treated at the highest dose was moderately increased as compared to controls; 2) the mean body weight gain in both males and females receiving the 30,000 ppm concentration of sucralose was significantly lower than 20% ($p < 0.01$) compared to controls during the 104 weeks of treatment; 3) differences in survival were not observed among the groups at the end of treatment. At 110–111 weeks of age (end of the experiment), the percentages of survival were 35% or greater among the groups; 4) mice treated at the highest dose had erythrocyte counts that were 7–9% lower than those of the controls and in females the difference was statistically significant ($p < 0.05$). Other hematological parameters were unaffected and no difference in the incidence of hematopoietic neoplasia was observed among the groups; 5) no statistically significant increase in the incidence of male and female mice bearing malignant tumors were observed in treated groups compared to controls. No significant increase in the incidence of any type of tumors related to exposure to sucralose was observed; and 6) concerning the

difference in the incidences of animals bearing malignant tumors, it must be noted that among males treated at the highest dose, the incidence was lower than 14% compared to controls (38% vs. 44%) and in females the incidence at the highest dose was 25% lower than in controls (29% vs. 39%).

These results may be affected by the 20% decrease in the mean body weight in both males and females treated at the highest dose as compared to controls. This observation is reinforced by the negative results obtained in the sucralose rat study performed by Mann *et al.*²² in which the male and female rats, at all doses, had significant ($p < 0.01$) decreased body weight compared to controls and the incidence of benign and malignant tumors was lower in all treated groups than in controls. It has long been known, of course, how body weight may influence the incidence of malignant tumors in humans³³ and in mice and rats.^{34,35}

Moreover, the negative results of Mann *et al.*²¹ may be due to the different experimental conditions in which the compound was tested in mice, and particularly to the fact that in that bioassay the number of animals per sex/group was lower, while the experiment started at adolescent age, and terminated at 110 weeks of age. These factors, are considered to be critical points for identification and assessment of carcinogenic risks,^{28,36–40} make the Mann *et al.* study on mice less sensitive than ours.

Finally, one cannot disregard the fact that sucralose itself and the hydrolysis product 1,6-DCF were found to be weakly mutagenic and that sucralose is able to alter the bacterial composition of the gastrointestinal tract. In fact, accumulating data suggest that the microbiota has a role in the causes of several types of cancer by influencing inflammation, DNA damage, and apoptosis.⁴¹

In conclusions, our data do not support the previous findings of industry authors that sucralose is a biologically inert compound. More studies are necessary to determine the safety of this food additive, including a new more adequate long-term transplacental carcinogenic bioassay on rats. We believe these studies are urgent, considering that millions of people may be exposed, including women in child-bearing age and children.

Conflict of interest

All authors declare no conflict of interest in relation to this work. We also declare that our funding sources had no direct role in the study design, data collection, analysis, and interpretation of the data in the writing of the manuscript, or in decision to publish the work.

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References

- Grice HC, Goldsmith LA. Sucralose—an overview of the toxicity data. *Food Chem Toxicol.* 2000;38(Suppl 2):1–6.
- Merck. Sucralose. In: O'Neil, M.J. (ed.), *The merck index*. 14th ed. Merck & Co: Whitehouse station, NJ; 2006: 1523.
- US Food and Drug Administration. Food additives permitted for direct addition to food for human consumption; sucralose.. Federal Register: US FDA p 63 (64) 16417-16433. 1998;63(4):16417-33
- US Food and Drug Administration. Food additives permitted for direct addition to food for human consumption; sucralose.. Federal Register: US FDA 64 (155) p 43908-43909. 1999;64(155) p 43908-9
- Scientific Committee On Food. Opinion of the scientific committee on food on sucralose. Scf/cs/adds/edul/190 final. In: Health and Consumer Directorate-General EC, editor Brussels: European Commission; 2000. 1–25.
- European Union. Directive 2003/115/ec of the european parliament and of the council of 22 December 2003 amending directive 94/35/ec on sweeteners for use in foodstuffs. *Off J Eur Union.* 2004;47:65–71.
- Leatherhead Food Research. The global food additives market, UK: Surrey; 2011.
- Davies E. Sweet for my sweet. *Chem world.* 2010;7:46–9.
- Brusick D, Borzelleca JF, Gallo M, Williams G, Kille J, Wallace Hayes A, Xavier Pi-Sunyer F, Williams C, Burks W. Expert Panel report on a study of Splenda in male rats. *Regul Toxicol Pharmacol.* 2009;55:6–12.
- US Food and Drug Administration. Food additives amendment in federal food, drug, and cosmetic act. Public Law 85-929 72 Stat 1784; 1958.
- Wiet SG, Beyts PK. Sensory characteristics of sucralose and other high intensity sweeteners. *J Food Sci.* 1992;57:1014–9.
- Sims J, Roberts A, Daniel JW, Renwick AG. The metabolic fate of sucralose in rats. *Food Chem Toxicol.* 2000;38(Suppl 2):115–21.
- Roberts A, Renwick AG, Sims J, Snodin DJ. Sucralose metabolism and pharmacokinetics in man. *Food Chem Toxicol.* 2000;38(Suppl 2):31–41.
- Grotz VL, Munro IC. An overview of the safety of sucralose. *Regul Toxicol Pharmacol.* 2009;55:1–5.
- John BA, Wood SG, Hawkins DR. The pharmacokinetics and metabolism of sucralose in the rabbit. *Food Chem Toxicol.* 2000a;38(Suppl 2):111–3.
- Brusick D, Grotz VL, Slesinski R, Kruger CL, Hayes AW. The absence of genotoxicity of sucralose. *Food Chem Toxicol.* 2010;48:3067–72.
- World Health Organization. Toxicological evaluation of certain food additives and contaminants. Trichlorogalactosucrose. In: Cambridge University Press, editor. WHO Food Additives Series 24, nos 651-654 on INCHEM, 1989.
- Sasaki YF, Kawaguchi S, Kamaya A, Ohshita M, Kabasawa K, Iwama K, Taniguchi K, Tsuda S. The comet assay with 8 mouse organs: results with 39 currently used food additives. *Mutat Res.* 2002;519:103–19.
- Abou-Donia MB, El-Masry EM, Abdel-Rahman AA, McLendon RE, Schiffman SS. Splenda Alters Gut Microflora and Increases Intestinal P-Glycoprotein and Cytochrome P-450 in Male Rats. *J Toxicol Environ Health, Part A.* 2008;71:1415–29.
- Goldsmith LA. Acute and subchronic toxicity of sucralose. *Food Chem Toxicol.* 2000;38(Suppl 2):53–69.
- Mann SW, Yuschak MM, Amyes SJ, Aughton P, Finn JP. A carcinogenicity study of sucralose in the CD-1 mouse. *Food Chem Toxicol.* 2000b;38(Suppl 2):91–7.
- Mann SW, Yuschak MM, Amyes SJ, Aughton P, Finn JP. A combined chronic toxicity/carcinogenicity study of sucralose in Sprague–Dawley rats. *Food Chem Toxicol.* 2000a;38(Suppl 2):71–89.
- Soffritti M, Belpoggi F, Degli Esposti D, Lambertini L. Aspartame induces lymphomas and leukaemias in rats. *Eur J Oncol.* 2005;10:107–16.
- Soffritti M, Belpoggi F, Degli Esposti D, Lambertini L, Tibaldi E, Rigano A. First experimental demonstration of the multipotential carcinogenic effects of aspartame administered in the feed to sprague-dawley rats. *Environ Health Perspect.* 2006;114:379–85.
- Soffritti M, Belpoggi F, Manservigi M, Tibaldi E, Lauriola M, Falcioni L, Bua L. Aspartame administered in feed, beginning prenatally through life span, induces cancers of the liver and lung in male Swiss mice. *Am J Ind Med.* 2010;53:1197–206.
- Soffritti M, Belpoggi F, Tibaldi E, Degli Esposti D, Lauriola M. Life-span exposure to low doses of aspartame beginning during prenatal life increases cancer effects in rats. *Environ Health Perspect.* 2007;115:1293–7.
- Belpoggi F, Soffritti M, Padovani M, Degli Esposti D, Lauriola M, Minardi F. Results of long-term carcinogenicity bioassay on sprague-dawley rats exposed to aspartame administered in feed. *Ann N Y Acad Sci.* 2006;1076:559–77.
- Soffritti M, Padovani M, Tibaldi E, Falcioni L, Manservigi F, Belpoggi F. The carcinogenic effects of aspartame: The urgent need for regulatory re-evaluation. *Am J Ind Med.* 2014;57:383–97.
- Decreto Legislativo. Decreto legislativo 116, 1992. Attuazione della direttiva. 86/609/cee in materia di protezione degli animali utilizzati a fini sperimentali o ad altri fini scientifici [in italian]. *Gazzetta Ufficiale* 1992;Supplemento ordinario:5–25.
- Cox DR. Regression models and life tables (with discussion). *J R Stat Soc* 1972;Series B, 34:187–220.
- Prejean JD, Peckham JC, Casey AE, Cox DR, Griswold DP, Weisburger EK, Weisburger JH. Spontaneous tumors in sprague-dawley rats and swiss mice. *Cancer Res.* 1973;33:2768–73.
- Ward JM. Lymphomas and leukemias in mice. *Exp Toxicol Pathol.* 2006;57:377–81.
- Doll R, Peto R. The causes of cancer: quantitative estimates of avoidable risks of cancer in the united states today. *J Natl Cancer Inst.* 1981;66:1191–308.
- Tannenbaum A. The initiation and growth of tumors: Introduction. Effects of undernutrition. *Am J Cancer.* 1940;38:335–50.
- Tucker MJ. The effect of long-term food restriction on tumours in rodents. *Int J Cancer.* 1979;23:803–7.
- Huff J, Jacobson MF, Davis DL. The limits of two-year bioassay exposure regimens for identifying chemical carcinogens. *Environ Health Perspect.* 2008;116:1439–42.
- Haseman J, Melnick R, Tomatis L, Huff J. Carcinogenesis bioassays: study duration and biological relevance. *Food Chem Toxicol.* 2001;39:739–44.
- Soffritti M, Belpoggi F, Minardi F, Bua L, Maltoni C. Mega-experiments to identify and assess diffuse carcinogenic risks. *Ann N Y Acad Sci.* 1999;895:34–55.
- Soffritti M, Belpoggi F, Degli Esposti D, Falcioni L, Bua L. Consequences of exposure to carcinogens beginning during developmental life. *Basic Clin Pharmacol Toxicol.* 2008;102:118–24.
- Newbold RR, McLachlan JA. Vaginal adenosis and adenocarcinoma in mice exposed prenatally or neonatally to diethylstilbestrol. *Cancer Res.* 1982;42:2003–11.
- Louis P, Hold GL, Flint HJ. The gut microbiota, bacterial metabolites and colorectal cancer. *Nat Rev Microbiol.* 2014;12:661–72.