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Safety studies conducted on a proprietary high-purity aloe vera inner leaf fillet preparation, Qmatrix®

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ABSTRACT

The aloe vera plant has a long history of safe use for oral and topical applications. This publication describes safety studies conducted on a proprietary high-purity aloe vera inner leaf fillet preparation, Qmatrix®. In a 13-week study in rats, Qmatrix® was administered *via* gavage at 0, 500, 1000 and 2000 mg/kg body weight (bw)/day. There were no significant changes in food or water consumption, body weight, serum biochemistry or hematology at any of the doses tested. Sporadic, significant increases were observed in some of the measured urinalysis parameters; however, these variations were not treatment-related, as most were observed only in one sex, not dose-dependent and within historical control values. Organ weights were unaffected, except for a statistically significant, though not dose-dependent, increase in absolute and relative weights of the right kidney in males at 500 and 2000 mg/kg bw/day, respectively. Histopathological analysis revealed no abnormal signs. Qmatrix® was non-mutagenic in an Ames test and a chromosomal aberration test at concentrations up to 10,000 μg/plate, and in an *in vivo* bone marrow micronucleus test at doses up to 5000 mg/kg bw/day. Based on these results, Qmatrix® is not genotoxic *in vitro* or *in vivo* and; has an oral NOAEL greater than 2000 mg/kg bw/day following 90 days of oral exposure.

1. Introduction

The *Aloe vera* (L.) Burm. f., plant (synonym = *Aloe barbadensis* Miller) is commonly referred to as aloe vera and belongs to the lily family (family Liliaceae, tribe Aloinae). This species is one of approximately 420 species of aloe (Burdock, 1997). The aloe plant is native to South Africa, but can also be found in dry sub-tropical and tropical climates, including the southern United States (Boudreau and Beland, 2006). The plant is a perennial succulent that is characterized by its capacity to store large volumes of water in its tissues (Burdock, 1997). The plant has green fleshy leaves covered by a thick cuticle or rind and an inner clear pulp (Boudreau and Beland, 2006).

Just below the surface of the aloe vera leaf skin are pericyclic cells that produce a bitter yellow latex often referred to as aloe sap or simply latex (Andersen, 2007). The latex contains approximately 80 phenolic anthraquinones, some of which, such as aloe-emodin and aloin, have been shown to be phototoxic and gastrointestinal irritants with cathartic properties (Andersen, 2007). The inner central area of the aloe vera leaf contains thin walled parenchymal cells that produce a clear slightly viscous (mucilaginous) fluid known as aloe gel or inner gel (Andersen, 2007) and is synonymous with more recent descriptives, inner leaf, inner leaf fillet or simply fillet. Aloe vera inner leaf fillet is not to be confused with aloe sap, which as previously stated, refer to the bitter yellow exudate found in the pericyclic cells of the latex. The inner leaf functions primarily in the storage of water, carbohydrates and minerals that the plant uses for energy. The primary constituents of aloe vera inner leaf are water and non-starch polysaccharides such as pectins, hemicelluloses, glucomannan, acemannan (*beta*-(1,4)-acetylated mannan) and mannose derivatives (WHO, 1999). It also contains amino acids, lipids, sterols, tannins and enzymes (WHO, 1999).

There is a long history of aloe vera plant use in folk medicine and it is still extensively used in traditional medicine in China, India, the West Indies and Japan (Boudreau and Beland, 2006). Aloe vera inner leaf fillet is the product most frequently used in the cosmetic and health food industries and to a lesser extent in the pharmaceutical industry. The gel has been used topically as a healing

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agent for abrasions and burns and to promote healing of dermal ulcers, wounds and frostbite (Boudreau et al., 2005). Aloe vera has been suggested to possess anti-cancer, anti-viral, anti-inflammatory, anti-diabetic and anti-bacterial properties; however, the mechanism(s) of these proposed effects is not fully understood (Leung and Foster, 1996; Evans, 2002). Aloe extracts have also been used for many years as an ingredient in a variety of personal care and cosmetic products, such as face and hand creams, cleansers, soaps, suntan lotions, shampoos and hair tonics, shaving preparations, bath aids, makeup and fragrance preparations, skin moisturizers, baby lotions and wipes (Boudreau and Beland, 2006; Andersen, 2007). In addition to these uses, several species of aloe (*A. barbadensis*, *A. perryi*, *A. ferox* and hybrids of *A. ferox* with *A. Africana* and *A. spicata*) are approved by the US Food and Drug Administration (FDA) for use in human food as natural flavoring agents and in over-the-counter (OTC) drugs, dietary supplements and cosmetic products (FDA, 2008).

Despite aloe vera's long history of use, several toxicity studies in the scientific literature have reported contrasting results, with some suggesting that aloe vera possesses toxic potential and others reporting no adverse effects. In these studies, various aloe preparations were utilized, including crude, poorly characterized extracts of the *A. vera* and *A. ferox* plants, or constituents of the aloe vera plant of varying purity. These studies included single and repeated dose studies in which the test materials were administered orally or *via* intraperitoneal or intravenous injections, as well as *in vitro* and *in vivo* genotoxicity studies (Andersen, 2007). Boudreau and Beland (2006) indicated that one possible reason for these conflicting results is the difficulty in clearly differentiating the plant part and the species utilized in these studies. Also, the method of production may result in the inclusion of products of the outer pulp (just inside the cuticle and containing the pericyclic cells), which contains a variety of anthraquinones (including, but not limited to aloins A and B, aloe-emodin and diacetylrhein). The sum of the two aloins (A and B), called barbaloin, is the predominant anthraquinone(s) and, aloin content is therefore the marker indicating the presence of anthraquinones (Andersen, 2007).

The disparate and incongruous results of previous studies led the authors to believe the problem may lie with test substances that included these pharmacologically potent anthraquinones. Therefore, we conducted a 90-day repeated dose study, as well as *in vitro* and *in vivo* genotoxicity studies, to investigate the potential toxicity of a well-characterized and highly-purified inner leaf fillet preparation derived from *A. vera* (L.), derived from the mucilaginous fluid of parenchymal cells found in the inner central area of the aloe vera leaf (commonly referred to as aloe vera gel, inner leaf gel or inner leaf fillet), which has a very low level of anthraquinones. These studies were conducted in compliance with GLP and OECD guidelines.

2. Materials and methods

2.1. Test material

The test material, Qmatrix® aloe vera inner leaf fillet (hereinafter referred to as Qmatrix®) that was used in these subchronic and genotoxicity studies, is a white to light tan powder derived from mucilaginous parenchymal cells found in the inner central area of the *A. vera* (L.) leaf (Aloecorp, Inc., Lacey, WA). Qmatrix® is manufactured using a proprietary extraction process in which the aloe latex contaminant (containing anthraquinones) is substantially reduced (including aloin, aloeresin A), as determined by the method of Zahn et al. (2007). A single lot of Qmatrix® (No. G7304B15) was used in all studies and conforms to Qmatrix® specifications (Table 1).

Table 1
Qmatrix® aloe vera inner leaf fillet powder specifications.

Description	Specification	Reference
Botanical source	*Aloe vera* (L.) Burm. f. (syn. *Aloe barbadensis*)	NA
Appearance	White to light tan	NA
Absorbance @ 400 nm	<0.500[a]	Spectrophotometer
Gardner color	<4[a]	Orbeco-Hellige Gardner Varnish Comparator
Turbidity	<0.200[a]	Spectrophotometer
pH value	3.5–5.0[a]	USP 30/NF 25 <791>
Moisture content	<5.0%	AOAC 934.06
Ash content	<40.0%	AOAC 930.30
Heavy metals	<10 ppm	EPA 6020/ EPA 7471A
Aloin	<10 ppm	Zahn et al. (2007)
IASC certification	Certification Program	http://www.iasc.org/certify.html
ACTIValoe®	>10% total polysaccharides by dry weight	60-QCWI-017
Total aerobic plate count	<10,000 CFU/g	USP 30/NF 25 <61>
Yeast and mold	<1,000 CFU/g	USP 30/NF 25 <61>
Coliforms/ pathogens	Negative/25 g	USP 30/NF 25 <61>

[a] Specification and result of 1:1 reconstituted product.

2.2. Bacterial reverse mutation test (Ames test)

Qmatrix® was evaluated for mutagenic activity in the bacterial reverse mutation test using standard *Salmonella typhimurium* direct plate incorporation method in accordance with OECD guideline number 471 "Bacterial Reverse Mutation Test". The vehicle (distilled water) was used as the negative control and 2-aminoanthracene, sodium azide, 4-nitroquinoline-N-oxide, 9-aminoaridine and acridine mutagens were used as positive controls. The potential for mutagenicity was assessed in *S. typhimurium* tester strains TA98, TA100, TA1535, TA1537 and *Escherichia coli* tester strain WP2 uvrA. The tester strains were incubated with Qmatrix® (dissolved in sterile distilled water) at concentrations of 185, 556, 1667, 5000 and 10,000 μg/plate in the presence and absence of the post-mitochondrial fraction of liver homogenates (S9) from rats pre-treated with Aroclor® 1254. Toxicity was evaluated based on a decrease in the number of revertant colonies and/or thinning of the bacterial lawn.

2.3. In vitro mammalian chromosome aberration test

Qmatrix® was evaluated for mutagenic potential in Chinese Hamster Lung (CHL) cells, in accordance with OECD guideline number 473 "Standard Chromosome Aberration Test." The vehicle (complete culture media) was used as the negative control and cyclophosphamide and ethylmethanesulfonate were used as positive controls. Genotoxicity was assessed in the presence and absence of metabolic activation (S9). The potential mutagenicity of Qmatrix® was evaluated in two experiments in which the cells were exposed to the test material at short-term (6 h) and long-term (24 h) exposure periods at concentrations of 2500, 5000 and 10,000 μg/ml.

2.4. Mouse micronucleus test

Qmatrix® was evaluated for mutagenic potential in mouse bone marrow cells and in accordance with the OECD guideline 474 "Mammalian Erythrocyte Micronucleus Test."

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2.4.1. Animals

Adult ICR mice (7-weeks-old) were obtained from Koatec Co. (Gyeonggi, Korea). Animals were housed in polycarbonate cages with Aspen chips bedding (ABEDD Baltic SIA, Atlantijas iela 23, Riga 1015, Latvia; analyzed prior to use for pesticides, heavy metals and microbiological load)." A standardized diet was provided (Harlan Co., Ltd, USA. Teklad Certified Global 18% Protein Rodent Diet, 2918C), and the animals were held in a controlled 12-h light/dark cycle. Temperature was maintained at 20–26 °C with a relative humidity of 40–70%. Potable water and standardized diet were provided *ad libitum*.

2.4.2. Experimental procedure

In a preliminary range-finding mouse micronucleus study, male and female ICR mice (2/sex/group) were administered Qmatrix® *via* gavage in sterile distilled water at doses of 0, 2500 and 5000 mg/kg bw/day for two consecutive days; dosing volume was 20 ml/kg bw/day for all groups. The animals were observed daily for mortality and clinical signs of toxicity and were sacrificed 24 h following the second test article administration. There was no mortality or clinical signs of toxicity in any of the dose groups in either sex; therefore, in the main study only male mice were utilized. In the main study, groups of male ICR mice (six *per* group) were administered the Qmatrix® in sterile distilled water *via* gavage at doses of 1250, 2500 and 5000 mg/kg/day once daily for two consecutive days. A positive control group was administered cyclophosphamide once *via* intraperitoneal injection on the second day of the Qmatrix® dosing schedule. A negative control group was administered sterile distilled water *via* gavage. The animals were observed daily for clinical signs and mortality and were euthanized 24 h following the second test article administration. Body weight was measured prior to study initiation, at dosing and necropsy. Bone marrow samples were collected from the femur of each animal and two bone marrow smears *per* animal were prepared for analysis. For each animal, the number of micronucleated polychromatic erythrocytes (MNPCE) was counted in 2000 polychromatic erythrocytes (PCE). The PCE/(PCE + NCE) ratio was determined after scoring 500 erythrocytes.

2.5. 13-Week study

The potential toxicity of Qmatrix® was evaluated in a subchronic toxicity study in rats conducted in accordance with OECD Guideline 408 "Repeated Dose 90 Day Oral Toxicity Study in Rodents."

2.5.1. Animals

Sprague–Dawley rats were supplied from Koatec Co. (Gyeonggido, Korea), and were 6–7 weeks of age at the beginning of the study. The animals were housed singly in an animal room with a 12-h light/dark cycle and temperature was maintained at 20–26 °C with a relative humidity of 40–70%. The animals were acclimated for 7 days prior to testing. The same bedding and diet was used as in the previously described mouse study. Potable water and diet were provided *ad libitum*.

2.5.2. Experimental procedure

Four groups of 20 rats (10/sex/group) were administered Qmatrix® in sterile distilled water orally *via* gavage at doses of 0 (control), 500, 1000, or 2000 mg/kg bw/day for 13 weeks; dosing volume was 10 ml/kg bw/day for all groups. The animals were observed daily for mortality and signs of gross toxicity. Food and water consumption was recorded weekly. Body weights were recorded prior to test initiation, weekly during the study, and just prior to terminal sacrifice. Ophthalmic examinations using an ophthalmoscope and a fundus camera were conducted before the beginning of treatment and during week 13 on animals in the control and high-dose group.

Urine samples were collected from each animal at week 13, the measured volumes were then analyzed for pH, ketone, color, glucose, bilirubin, specific gravity, blood, protein, urobilinogen, nitrate and leukocytes on a Clinitek® Automated Urine Chemistry analyzer (Siemens Medical Solutions Diagnostics, Tarrytown, NY).

Blood samples were collected from each animal at necropsy for hematology and clinical chemistry. Blood samples were collected from the posterior vena cava and EDTA was used as an anticoagulant. Animals were fasted overnight prior to collection of samples. Hematological parameters measured included white blood cell count, hemoglobin concentration, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, hemoglobin concentration distribution width, red cell count, red blood cell distribution width, platelet count, mean platelet volume, reticulocyte, differential white blood cell count.

Clinical biochemistry values analyzed were: alanine aminotransferase, albumin, albumin/globulin ratio, alkaline phosphatase, aspartate aminotransferase, bilirubin, blood creatinine, blood urea nitrogen, calcium, chloride, creatine phosphokinase, fasting glucose, globulin, inorganic phosphorus, potassium, sodium, total cholesterol, total serum protein and triglycerides. Prothrombin time and active partial thromboplastin time were also measured to evaluate blood clotting time.

Animals were euthanized with ether and underwent a detailed necropsy. All animals were subjected to a full macroscopic examination of tissues. All external features and orifices were visually examined. The thoracic, abdominal and pelvic cavities and their viscera were also examined. The adrenal glands, brain, epididymis, heart, kidneys, liver, lungs, ovaries, pituitary gland, prostate, spleen, testes and thymus were weighed and examined microscopically. Testes and epididymides were fixed in Bouin's solution and the eyes were fixed in Davidson's fluid. The seminal vesicles, prostate, ovaries, uterus, vagina, urinary bladder, spleen, stomach, pancreas, duodenum, jejunum, ileum, cecum, colon, rectum, mesenteric lymph node, adrenal gland, kidney, liver, skeletal muscle, sciatic nerve, femur, mandibular lymph node, salivary gland, sternum, thymus, heart, lung, aorta, thoracic spinal cord, tongue, trachea, esophagus, thyroid gland, brain, pituitary gland and mammary glands were preserved in 10% neutral buffered formalin. The fixed organs and tissues from the control and high-dose groups were evaluated for histological changes following staining with hematoxylin and eosin and examination by light microscopy.

2.5.3. Statistical analysis

Statistical analysis of the in-life and organ weight data (*i.e.*, means and standard deviations of body weight, daily food and consumption, absolute and relative organ weights, hematology and serum biochemistry parameters) were initially subjected to a One-Way Analysis of Variance (ANOVA). If the results were significant, the Levene test was performed to evaluate the variance homogeneity. When the variances were homogeneous, treated and control groups were compared using the Duncan's multiple range test. When the variances were not homogeneous, treated and control groups were compared using the Dunnett's *T*-test.

For the urinalysis results, data which expressed the severity of the observed signs were ranked, as shown in Table 2, and the groups were compared using a non-parametric method (Kruskal–Wallis non-parametric analysis of variance). If significant, the Mann–Whitney *U*-test was conducted for comparison with the values from the vehicle control group. Fisher's exact test was applied to the urine color test.

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Table 2
Urinalysis grades.

Grades	GLU, KET, PRO, OB, WBC	SG	pH	URO	NITBIL
0	–	⩽1.005	7.0	0.1	–
1	+/–	1.010	7.5		
2	1+	1.015	8.0		
3	2+	1.020	8.5		
4	3+	1.025	9.0		
5		⩾1.030			

GLU, glucose; KET, ketone body; PRO, total protein; OB, occult blood; WBC, white blood cell count; SG, specific gravity; URO, urobilinogen; NIT, nitrite; BIL, bilirubin.

3. Results

3.1. Bacterial reverse mutation test (Ames test)

There were no statistically significant increases in the number of revertant colonies or cytotoxicity in any *S. typhimurium* or *E. coli* strain exposed to Qmatrix®, with or without metabolic activation, at concentrations up to 10,000 μg/plate (Table 3). Under the conditions of this study, Qmatrix® was non-mutagenic in *S. typhimurium* strains TA98, TA100, TA1535, TA1537 and *E. coli* strain WP2uvrA.

3.2. In vitro mammalian chromosome aberration test

Qmatrix® did not induce a statistically significant increase in the incidence of chromosomal aberrations or polyploidy in cultured Chinese Hamster lung cells after 6- and 24-h treatments at any of the doses tested, with or without S9 metabolic activation (Table 4). Under the conditions of this study, Qmatrix® does not produce chromosomal aberrations or polyploidy.

3.3. Mouse micronucleus test

There was no mortality or statistically significant differences in body weight between the control and Qmatrix®-treated animals in any of the dose groups. Also, Qmatrix® did not induce any statistically significant increases in the incidence of polychromatic immature erythrocytes or micronucleated immature erythrocytes and was not cytotoxic in ICR mice administered Qmatrix® at doses of 1250, 2500 or 5000 mg/kg bw, compared to the vehicle control (Table 5). Under the conditions of this study, Qmatrix® is not genotoxic in the micronucleus assay.

Table 4
Chromosomal aberrations test with Qmatrix® in the absence (A) or presence (B) of S9 mix.

Treatment group	Mean total metaphases		Mean aberrant metaphases		Mean of PP + ER	RCC (%)
	Total (+gap)	Total (−gap)	Mean % (+gap)	Mean % (−gap)		
Group A: −S9 mix						
6-h treatment-recovery time; dose (μg/ml)[a]						
0	0.0	0.0	0.0	0.0	0.0 + 0.0	100
2500	0.0	0.0	0.0	0.0	0.0 + 0.0	102
5000	0.0	0.0	0.0	0.0	0.0 + 0.0	98
10,000	0.5	0.5	0.5	0.5	0.0 + 0.0	97
800 EMS	23.0	19.0	20.0	16.5[b,*]	0.0 + 0.0	73
24-h treatment-recovery time						
0	0.5	0.5	0.5	0.5	0.0 + 0.0	100
2500	0.0	0.0	0.0	0.0	0.0 + 0.0	99
5000	0.0	0.0	0.0	0.0	0.0 + 0.0	95
10,000	0.0	0.0	0.0	0.0	0.0 + 1.0	94
600 EMS	14.5	12.0	13.0	11.0[b,*]	0.0 + 0.0	74
Group B:+S9 mix						
6-h treatment-recovery time						
0	0.0	0.0	0.0	0.0	0.0 + 0.0	100
2500	0.5	0.0	0.5	0.0	0.0 + 0.0	103
5000	0.5	0.5	0.5	0.5	0.0 + 0.0	104
10,000	0.0	0.0	0.0	0.0	0.0 + 0.0	85
12 CPA	57.0	53.5	36.0	35.0[b,*]	0.0 + 0.0	68

CPA, cyclophosphamide (positive control); EMS, ethylmethanesulfonate (positive control); RCC, relative cell counts = cell counts of treated flask/cell counts of control flask × 100 (%).
[a] Nominal concentration of Qmatrix®.
[b] Fisher's exact test.
[*] Significantly different from the control at $p < 0.01$.

3.4. 13-Week study

There were no treatment-related deaths or clinical signs of toxicity in any of the treatment groups. Ophthalmological examinations revealed no differences between the high-dose animals and the controls. There were no statistically significant dose-related changes in body weight, body weight gain or food consumption in the Qmatrix®-treated animals as compared to the controls (Table 6). Water consumption was significantly increased on weeks

Table 3
Reverse mutation assay with Qmatrix® in the absence or presence of S9 mix.

Concentration (μg/plate)	TA98		TA100		TA1535		TA1537		WP2uvrA	
	−S9	+S9	−S9	+S9	−S9	+S9	−S9	+S9	−S9	+S9
0	20	25	116	118	11	9	7	10	15	19
185	20	28	112	118	11	7	5	7	15	20
556	19	29	120	114	12	11	5	9	15	20
1667	21	30	104	117	12	10	9	7	17	20
5000	23	26	108	104	14	11	5	9	16	17
10,000	27	27	111	113	11	10	8	9	15	13
Positive control										
0.5	20[a] 445[c]	309[a]	444[b]	434[a]	234[b]		211[e]		174[c]	
1.0								89[a]		
2.0						95[a]				58[a]
50.0							754[d]			

−S9/+S9 = Colonies/plate [factor] – No. of colonies of treated plate/No. of colonies of negative control plate.
[a] 2-Aminoanthracene.
[b] Sodium azide.
[c] 4-Nitroquinoline N-oxide.
[d] 9-Aminoacridine.
[e] Acridine mutagen ICR 191.

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Table 5
Micronucleus test of Qmatrix® in male ICR mice.

Test material	Dose[b] (mg/kg/day)	Animals *per* dose	MNPCE/2000 PCE (Mean ± SD)	PCE/ (PCE + NCE)
Vehicle[a]	0	6	0.67 ± 0.82	0.42 ± 0.03
Qmatrix®	1250	6	1.83 ± 1.47	0.43 ± 0.05
Qmatrix®	2500	6	0.50 ± 0.55	0.43 ± 0.04
Qmatrix®	5000	6	0.83 ± 0.75	0.39 ± 0.03
CPA	70	6	92.17 ± 10.23**	0.28 ± 0.01**

PCE, polychromatic erythrocyte; NCE, normochromatic erythrocyte; MNPCE, micronucleated polychromatic erythrocyte.
[a] Vehicle: sterilized distilled water.
[b] Vehicle and test article were administered for two consecutive days.
** Significantly different from the vehicle control group at $p < 0.01$; CPA (cyclophosphamide; positive control) administered once on the day of second day of Qmatrix® dosing.

1, 4 and 5 in all male dose groups ($p < 0.05$ or $p < 0.01$), on week 3 at 500 mg/kg bw/day ($p < 0.05$) and on week 6 at 2000 mg/kg bw/day ($p < 0.01$) in male rats, as compared to the controls. In female rats, water consumption was significantly increased at 1000 ($p < 0.05$) and 2000 mg/kg bw/day ($p < 0.01$) on week 1. However, over the entire duration the 13-week study, total water consumption in the Qmatrix® treatment groups was not significantly different from the controls. There were no statistically significant differences between the control and treated animals in the measured serum biochemistry (Table 7), and hematological parameters were comparable between control and Qmatrix®-treated animals, except for a significant increase in monocytes which was only observed in males at 2000 mg/kg bw/day. The increase in monocytes in males was only observed in one sex and was therefore not considered to be treatment related.

Evaluation of urinalysis parameters revealed a tendency in both males and females for protein, leukocytes, specific gravity and pH results to be slightly higher in the Qmatrix®-treated animals as compared to the controls. In females at all dose levels, specific gravity and pH values were significantly higher than control values and protein was significantly higher at 1000 and 2000 mg/kg bw/day as compared to the controls (Table 8), although the increased specific gravity is likely explained in part by the low urine volume

Table 6
Body weight changes of male and female rats administered Qmatrix® for 13 weeks.

Weeks	Males				Females			
	0 mg/kg/day[b]	500 mg/kg/day	1000 mg/kg/day	2000 mg/kg/day	0 mg/kg/day	500 mg/kg/day	1000 mg/kg/day	2000 mg/kg/day
0	181.67 ± 5.05[c]	182.49 ± 5.54	182.03 ± 5.59	182.10 ± 5.60	143.14 ± 3.43	143.51 ± 2.95	143.16 ± 2.98	143.69 ± 1.80
1	234.70 ± 6.96	236.47 ± 6.24	235.58 ± 9.32	234.60 ± 6.76	163.64 ± 7.76	162.50 ± 7.12	163.08 ± 6.79	161.20 ± 4.92
2	284.53 ± 8.57	289.40 ± 7.52	281.77 ± 11.27	282.61 ± 10.60	183.06 ± 13.22	183.18 ± 8.34	186.67 ± 8.96	184.03 ± 5.65
3	319.84 ± 7.27	324.68 ± 9.76	315.14 ± 12.46	311.72 ± 12.72	201.04 ± 21.19	199.38 ± 11.57	201.20 ± 8.66	197.50 ± 7.50
4	347.72 ± 10.66	354.77 ± 11.49	344.65 ± 17.57	341.62 ± 17.77	212.67 ± 18.13	212.64 ± 12.64	213.54 ± 9.83	210.76 ± 8.97
5	372.27 ± 14.48	381.91 ± 15.34	370.04 ± 18.70	364.40 ± 21.68	226.34 ± 18.73	223.68 ± 11.67	228.13 ± 13.25	224.47 ± 9.53
6	392.11 ± 14.33	404.29 ± 16.78	391.59 ± 20.75	384.43 ± 23.87	236.78 ± 20.81	234.60 ± 14.76	238.40 ± 12.39	231.91 ± 11.05
7	415.09 ± 16.96	427.98 ± 18.21	413.56 ± 20.70	406.97 ± 24.40	250.34 ± 23.50	244.57 ± 14.43	250.19 ± 14.62	242.66 ± 9.40
8	430.31 ± 17.10	443.00 ± 18.52	425.25 ± 21.81	421.04 ± 23.97	252.94 ± 23.91	246.07 ± 13.70	252.66 ± 12.17	246.28 ± 9.14
9	438.78 ± 22.60	455.64 ± 17.89	438.35 ± 20.26	431.26 ± 26.09	260.42 ± 22.54	252.90 ± 14.79	257.53 ± 15.96	250.70 ± 9.10
10	448.51 ± 24.51	466.92 ± 18.28	449.31 ± 20.25	440.89 ± 27.58	264.11 ± 24.26	255.62 ± 13.87	260.29 ± 10.95	256.61 ± 10.21
11	462.78 ± 25.49	481.78 ± 19.97	463.13 ± 20.52	454.65 ± 28.33	270.97 ± 23.84	262.99 ± 14.50	266.86 ± 13.78	259.97 ± 9.65
12	470.83 ± 28.82	491.32 ± 18.86	470.22 ± 19.52	465.95 ± 27.24	275.74 ± 25.85	268.28 ± 11.54	271.71 ± 16.00	263.58 ± 10.09
13	476.60 ± 28.98	498.30 ± 19.28	478.62 ± 19.94	473.82 ± 28.64	276.93 ± 29.61	268.18 ± 10.86	272.47 ± 16.56	266.29 ± 9.84
Weight gains[a]	294.93 ± 28.71	315.81 ± 19.36	296.59 ± 18.96	291.72 ± 27.43	133.79 ± 26.81	124.67 ± 8.48	129.31 ± 16.75	122.59 ± 9.32

[a] Weight gains are body weight difference between week 13 and the 1st dosed day.
[b] $N = 10$/sex/group.
[c] Average weight in grams ± standard deviation.

Table 7
Serum biochemical values of male and female rats administered Qmatrix® for 13 weeks.

Parameter	Males				Females			
	0 mg/kg/day[a]	500 mg/kg/day	1000 mg/kg/day	2000 mg/kg/day	0 mg/kg/day	500 mg/kg/day	1000 mg/kg/day	2000 mg/kg/day
AST (U/L)	90.9 ± 10.3	85.9 ± 15.5	90.5 ± 12.7	90.0 ± 14.3	87.0 ± 12.1	98.3 ± 24.4	96.8 ± 21.7	93.0 ± 18.0
ALT (U/L)	42.1 ± 5.8	38.7 ± 6.2	41.3 ± 6.0	42.9 ± 7.4	32.8 ± 5.8	32.4 ± 2.5	34.2 ± 4.9	30.9 ± 5.2
ALP (U/L)	92.7 ± 18.8	90.4 ± 16.1	80.7 ± 14.1	91.5 ± 24.7	69.8 ± 19.3	67.3 ± 14.9	69.4 ± 18.1	67.8 ± 15.3
BUN (mg/dl)	15.1 ± 1.2	14.5 ± 1.4	15.5 ± 2.7	16.3 ± 2.9	15.8 ± 3.0	15.5 ± 1.7	16.3 ± 2.2	16.4 ± 15.3
CRE (mg/dl)	0.55 ± 0.03	0.54 ± 0.06	0.55 ± 0.05	0.56 ± 0.10	0.59 ± 0.07	0.58 ± 0.05	0.60 ± 0.06	0.57 ± 0.04
GLU (mg/dl)	126.5 ± 6.9	127.8 ± 10.2	127.5 ± 13.0	129.4 ± 9.3	117.8 ± 11.5	115.2 ± 13.1	114.1 ± 10.8	114.4 ± 7.2
CHO (mg/dl)	99.0 ± 14.6	116.7 ± 10.0	110.9 ± 21.4	110.2 ± 26.4	122.1 ± 22.8	120.4 ± 21.7	119.0 ± 27.0	109.0 ± 9.6
PRO (g/dl)	6.64 ± 0.25	6.90 ± 0.21	6.70 ± 0.14	6.73 ± 0.27	6.43 ± 0.21	6.39 ± 0.27	6.34 ± 0.22	6.34 ± 0.23
CPK (U/L)	165.7 ± 56.3	216.3 ± 180.1	227.9 ± 117.1	182.3 ± 76.3	253.9 ± 125.0	305.7 ± 177.6	298.6 ± 190.5	278.6 ± 149.5
ALB (g/dl)	3.32 ± 0.11	3.43 ± 0.10	3.34 ± 0.06	3.37 ± 0.15	3.38 ± 0.16	3.40 ± 0.19	3.34 ± 0.15	3.34 ± 0.11
BIL (mg/dl)	0.20 ± 0.02	0.19 ± 0.01	0.19 ± 0.01	0.18 ± 0.02	0.22 ± 0.02	0.22 ± 0.03	0.22 ± 0.03	0.22 ± 0.02
TG (mg/dl)	42.9 ± 11.9	55.6 ± 21.7	44.2 ± 11.2	48.8 ± 18.2	39.2 ± 7.5	41.3 ± 5.3	36.4 ± 6.8	34.3 ± 3.8
IP (mg/dl)	7.04 ± 0.33	6.88 ± 0.50	7.43 ± 0.47	7.34 ± 0.33	6.49 ± 0.67	6.50 ± 1.04	6.45 ± 1.14	6.98 ± 0.72
Ca^{2+} (mg/dl)	10.19 ± 0.22	10.25 ± 0.14	10.02 ± 0.20	10.11 ± 0.18	9.86 ± 0.24	9.77 ± 0.26	9.74 ± 0.17	9.72 ± 0.32
A/G (ratio)	1.00 ± 0.06	0.99 ± 0.04	1.00 ± 0.04	1.00 ± 0.03	1.11 ± 0.06	1.14 ± 0.05	1.11 ± 0.06	1.12 ± 0.03
Na^+ (mmol/L)	146.9 ± 4.3	146.8 ± 4.6	146.8 ± 4.1	145.8 ± 4.0	141.8 ± 0.6	141.5 ± 0.5	141.6 ± 1.4	141.5 ± 1.2
K^+ (mmol/L)	4.42 ± 0.18	4.4 ± 0.2	4.5 ± 0.3	4.4 ± 0.2	4.1 ± 0.3	4.1 ± 0.2	4.3 ± 0.3	4.3 ± 0.2
Cl^- (mmol/L)	108.1 ± 0.6	107.3 ± 1.2	107.9 ± 1.2	107.6 ± 1.1	107.8 ± 1.1	107.5 ± 1.3	107.8 ± 1.8	108.3 ± 1.2

AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatases; BUN, blood urea nitrogen; CRE, creatinine; GLU, glucose; CHO, total cholesterol; PRO, total protein; CPK, creatine phosphokinase; ALB, albumin; BIL, total bilirubin; TG, triglycerides; IP, inorganic phosphorus; Ca^{2+}, calcium; A/G, albumin/globulin ratio; Na^+, sodium; K^+, potassium; Cl^-, chlorine.
[a] $N = 10$/sex/group.

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in the mid and high dose females. In males, ketone bodies and protein at 500 and 2000 mg/kg bw/day and leukocytes at 1000 mg/kg bw/day were significantly higher than control values. Urine volume was significantly decreased in females at 2000 mg/kg bw/day as compared to the controls. In contrast, urine volume was significantly increased in the 2000 mg/kg bw/day male dose group, as compared to the controls.

With the exception of the increased protein value mentioned above, there is no comparable finding in the opposite sex and for many (most) of the differences in treated animal urine values (compared to control) likewise, there is no dose–response relationship, nor is there a corroborative clinical chemistry or histopathological change for the difference(s) reported. In addition, all of these values are within the range of historical control values for this strain of rat. Lastly, at least some of the findings of statistical significance between the same values (zero in most cases), may be an artifact of the analysis. Therefore, the authors do not believe these findings are relevant to the determination of safety for Qmatirx.

There were no statistically significant differences in organ weights between the control and treated animals except for statistically significant increases in absolute weight of the right kidney in males at 500 mg/kg bw/day and relative weight of the right kidney in males at 2000 mg/kg bw/day (Table 9). The left and right kidneys of the females rats in all dose groups were unaffected by treatment with Qmatrix®. At necropsy, one control male animal exhibited a soft and discolored liver and one low-dose male was noted to have a small, discolored liver, but there were no effects on low-dose group liver weights. One high dose male was reported as having an enlarged, soft kidney that was milky white in color and partially protruded. In females, necropsy revealed one control animal with a dark spot on the thymus and two females in the 500 mg/kg bw/day group with retention of clear fluid in the uterus. These changes observed in males and females were not considered treatment-related because of their low frequency, their observation in only one sex and the lack of a dose-dependent pattern. Histopathological analysis found that all microscopic changes observed in the animals euthanized at study termination were of a type commonly observed in control laboratory rats, and were of comparable incidence in controls and high-dose groups in this study.

4. Discussion

Aloe vera has a long history of topical and oral use in the United States as well as around the world. Several studies in the scientific literature have investigated the potential toxicity of various prep-

Table 8
Urinalysis of male and female rats administered Qmatrix® for 13 weeks.

Urinalysis										
Test items	Result	Grade	Groups (mg/kg/day)[a]							
			Male				Female			
			0	500	1000	2000	0	500	1000	2000
GLU	–	0	4	4	5	5	5	4	5	4
	±	1	1	1	0	0	0	1	0	1
BIL	–	0	5	5	5	5	5	5	5	5
KET	–	0	4	0	1	0	5	5	5	5
	±	1	1	5	3	4	0	0	0	0
	1+	2	0	0 *	1	1 *	0	0	0	0
SG	⩽1.005	0	1	0	0	0	5	0	0	0
	1.010	1	2	0	0	0	0	2	0	0
	1.015	2	1	4	0	0	0	2	1	0
	1.020	3	0	1	4	0	0	1	2	1
	1.025	4	0	0	0	4	0	0	2	1
	⩾1.030	5	1	0	1	1	0	0 **	0 **	3 **
pH	7.0	0	0	0	0	0	2	0	0	0
	7.5	1	0	0	0	0	2	0	0	0
	8.0	2	2	0	1	0	1	4	1	2
	8.5	3	2	5	4	5	0	1	4	3
	9.0	4	1	0	0	0	0	0 *	0 **	0 *
PRO	–	0	0	0	0	0	3	2	0	0
	±	1	0	0	0	0	2	2	1	1
	1+	2	1	0	0	0	0	0	1	2
	2+	3	4	2	3	0	0	1	2	1
	3+	4	0	3 *	2	5 **	0	0	1 *	1 *
URO	0.1	0	5	5	5	5	5	5	5	5
NIT	–	0	5	5	5	5	5	5	5	5
OB	–	0	0	1	5	5	5	5	4	5
	±	1	3	3	0	0	0	0	0	0
	1+	2	2	2	0 **	0	0	0	1	0
WBC	–	0	0	0	0	2	2	1	1	0
	±	1	2	0	0	0	0	2	1	1
	1+	2	2	3	0	3	3	2	2	3
	2+	3	1	2	5 *	0	0	0	1	1
Volume (ml)			14.6 ± 2.6	12.2 ± 1.8	16 ± 3.6	16.4 ± 6.7	24 ± 8.2	24.4±10	17.8±6.3	11.4±2.1

GLU, glucose; BIL, bilirubin; KET, ketone body; SG, specific gravity; PRO, protein; URO, urobilinogen; NIT, nitrite; OB, occult blood; WBC, leukocyte.

[a] $N = 5$/sex/group.

* Significant difference in the cumulative grade for the dose group at $p < 0.05$ level compared with the vehicle control group.

** Significant difference in the cumulative grade for the dose group at $p < 0.01$ level compared with the vehicle control group.

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Table 9
Absolute and relative organ weights of male and female rats administered Qmatrix® for 13 weeks.

Organ	Males				Females			
	0 mg/kg/day[b]	500 mg/kg/day	1000 mg/kg/day	2000 mg/kg/day	0 mg/kg/day	500 mg/kg/day	1000 mg/kg/day	2000 mg/kg/day
Terminal body weight[a] (g)	452.14 ± 27.46	474.21 ± 19.20	453.92 ± 18.68	447.69 ± 27.90	263.87 ± 27.95	253.56 ± 11.32	257.52 ± 15.49	250.23 ± 9.12
Adrenal gland Lt. (g)	0.0279 ± 0.0050	0.0283 ± 0.0044	0.0277 ± 0.0030	0.0281 ± 0.0043	0.0325 ± 0.0053	0.0311 ± 0.0030	0.0301 ± 0.0021	0.0306 ± 0.0020
% of body weight	0.0062 ± 0.0012	0.0069 ± 0.0010	0.0061 ± 0.0005	0.0063 ± 0.0009	0.0124 ± 0.0022	0.0123 ± 0.0012	0.0117 ± 0.0012	0.0123 ± 0.0009
Adrenal gland Rt. (g)	0.0276 ± 0.0036	0.0279 ± 0.0033	0.0276 ± 0.0038	0.0272 ± 0.0042	0.0295 ± 0.0049	0.0299 ± 0.0032	0.0299 ± 0.0035	0.0300 ± 0.0034
% of body weight	0.0061 ± 0.0010	0.0059 ± 0.0007	0.0061 ± 0.0008	0.0061 ± 0.0009	0.0112 ± 0.0020	0.0118 ± 0.0012	0.0117 ± 0.0016	0.0120 ± 0.0015
Pituitary gland (g)	0.0118 ± 0.0014	0.0121 ± 0.0013	0.0121 ± 0.0014	0.0119 ± 0.0010	0.0129 ± 0.0012	0.0124 ± 0.0013	0.0127 ± 0.0013	0.0126 ± 0.0014
% of body weight	0.0026 ± 0.0003	0.0026 ± 0.0003	0.0027 ± 0.0003	0.0027 ± 0.0001	0.0049 ± 0.0005	0.0049 ± 0.0004	0.0050 ± 0.0006	0.0051 ± 0.0006
Thymus (g)	0.2653 ± 0.0348	0.3062 ± 0.0657	0.2793 ± 0.0530	0.2912 ± 0.0503	0.2565 ± 0.0613	0.2330 ± 0.0275	0.2106 ± 0.0369	0.2390 ± 0.0614
% of body weight	0.0588 ± 0.0079	0.0645 ± 0.0125	0.0615 ± 0.0113	0.0649 ± 0.0099	0.0965 ± 0.0151	0.0919 ± 0.0104	0.0819 ± 0.0141	0.0954 ± 0.0243
Prostate (g)	0.6862 ± 0.1610	0.6752 ± 0.1564	0.5614 ± 0.1207	0.6186 ± 0.1699	–	–	–	–
% of body weight	0.1516 ± 0.0354	0.1424 ± 0.0324	0.1235 ± 0.0255	0.1389 ± 0.0402	–	–	–	–
Testis Lt. (g)	2.0140 ± 0.0961	2.0847 ± 0.1641	2.0374 ± 0.0934	2.0794 ± 0.1479	–	–	–	–
% of body weight	0.4466 ± 0.0302	0.4393 ± 0.0234	0.4495 ± 0.0279	0.4654 ± 0.0342				
Testis Rt.	2.0392 ± 0.1014	2.0740 ± 0.1584	2.0335 ± 0.0980	2.0622 ± 0.1606	–	–	–	–
% of body weight	0.4524 ± 0.0331	0.4371 ± 0.0238	0.4486 ± 0.0272	0.4611 ± 0.0293				
Ovary Lt. (g)	–	–	–	–	0.0469 ± 0.0118	0.0407 ± 0.0106	0.0421 ± 0.0096	0.0444 ± 0.0057
% of body weight					0.0177 ± 0.0033	0.0160 ± 0.0040	0.0164 ± 0.0039	0.0177 ± 0.0019
Ovary Rt. (g)	–	–	–	–	0.0450 ± 0.0075	0.0381 ± 0.0135	0.0445 ± 0.0079	0.0428 ± 0.0050
% of body weight					0.0171 ± 0.0029	0.0150 ± 0.0052	0.0173 ± 0.0030	0.0171 ± 0.0017
Epididymis Lt. (g)	0.6929 ± 0.0272	0.7123 ± 0.0840	0.6785 ± 0.0549	0.6785 ± 0.0396	–	–	–	–
% of body weight	0.1536 ± 0.0092	0.1500 ± 0.0138	0.1496 ± 0.0129	0.1520 ± 0.0116	–	–	–	–
Epididymis Rt. (g)	0.6958 ± 0.0371	0.7031 ± 0.0661	0.6597 ± 0.0450	0.7040 ± 0.0385	–	–	–	–
% of body weight	0.1544 ± 0.0128	0.1482 ± 0.0118	0.1456 ± 0.0116	0.1576 ± 0.0103	–	–	–	–
Spleen (g)	0.8301 ± 0.0740	0.8605 ± 0.1179	0.8410 ± 0.0796	0.8435 ± 0.0754	0.5680 ± 0.0614	0.5683 ± 0.0563	0.5327 ± 0.0651	0.5693 ± 0.0811
% of body weight	0.1840 ± 0.0179	0.1812 ± 0.0210	0.1856 ± 0.0194	0.1887 ± 0.0173	0.2158 ± 0.0182	0.2242 ± 0.0216	0.2068 ± 0.0224	0.2278 ± 0.0334
Kidney Lt. (g)	1.3099 ± 0.0999	1.4345 ± 0.0903	1.3175 ± 0.0991	2.0592 ± 2.2036	0.7778 ± 0.0541	0.7630 ± 0.0579	0.7671 ± 0.0423	0.7676 ± 0.0500
% of body weight	0.2899 ± 0.0172	0.3029 ± 0.0224	0.2901 ± 0.0161	0.4656 ± 0.5133	0.2961 ± 0.0196	0.3010 ± 0.0208	0.2986 ± 0.0210	0.3066 ± 0.0125
Kidney Rt. (g)	1.3267 ± 0.1154	1.4603 ± 0.0654**	1.3177 ± 0.0771	1.4162 ± 0.1402	0.7736 ± 0.0521	0.7757 ± 0.0534	0.7682 ± 0.0352	0.7562 ± 0.0827
% of body weight	0.2935 ± 0.0185	0.3081 ± 0.0111	0.2902 ± 0.0105	0.3168 ± 0.0314*	0.2949 ± 0.0249	0.3059 ± 0.0168	0.2989 ± 0.0173	0.3017 ± 0.0266
Heart (g)	1.4344 ± 0.0697	1.5100 ± 0.0862	1.3996 ± 0.1070	1.4377 ± 0.0926	0.9211 ± 0.0851	0.9502 ± 0.0665	0.9054 ± 0.0829	0.8955 ± 0.0634
% of body weight	0.3180 ± 0.0199	0.3184 ± 0.0115	0.3085 ± 0.0227	0.3214 ± 0.0153	0.3500 ± 0.0211	0.3748 ± 0.0228	0.3517 ± 0.0259	0.3578 ± 0.0204
Lung (g)	1.8434 ± 0.1008	1.8795 ± 0.1544	1.8131 ± 0.1291	1.8003 ± 0.1456	1.3824 ± 0.1372	1.3457 ± 0.1057	1.3939 ± 0.1488	1.3547 ± 0.1017
% of body weight	0.4085 ± 0.0232	0.3961 ± 0.0246	0.4000 ± 0.0315	0.4021 ± 0.0188	0.5251 ± 0.0345	0.5305 ± 0.0304	0.5402 ± 0.0288	0.5411 ± 0.0294
Brain (g)	1.9910 ± 0.0555	2.0308 ± 0.0544	2.0014 ± 0.0582	1.9902 ± 0.0750	1.7785 ± 0.0958	1.7535 ± 0.0879	1.7770 ± 0.0708	1.7981 ± 0.0798
% of body weight	0.4416 ± 0.0266	0.4286 ± 0.0143	0.4417 ± 0.0240	0.4457 ± 0.0262	0.6788 ± 0.0595	0.6925 ± 0.0417	0.6918 ± 0.0420	0.7194 ± 0.0402
Liver (g)	11.2395 ± 0.8659	12.2369 ± 0.7849	11.2629 ± 0.9273	11.2441 ± 1.1662	6.1702 ± 0.6942	6.3243 ± 0.4771	6.0588 ± 0.5365	5.9796 ± 0.2701
% of body weight	2.4859 ± 0.1170	2.5813 ± 0.1494	2.4790 ± 0.1327	2.5079 ± 0.1496	2.3418 ± 0.1704	2.4953 ± 0.1670	2.3509 ± 0.1175	2.3899 ± 0.0753

[a] Body weights right before necropsy, after fasting.
[b] $N = 10$/sex/group.
* Significant difference at $p < 0.05$ level compared with the vehicle control.
** Significant difference at $p < 0.01$ level compared with the vehicle control.

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arations of the aloe vera plant and its constituents. In a 90 day subchronic toxicity study, male Swiss albino mice were orally administered a crude, ethanol extract of the aerial portion of the aloe vera plant (species not indicated) in drinking water at a dose of 100 mg/kg bw/day for 90 days (Shah et al., 1989). It was reported that six of the 20 animals dosed with aloe vera extract died during the study and that 20% of the treated animals experienced alopecia of the genital area and degeneration and putrification of the sex organs. Also, 10% of the treated animals had inflammation of the hind limb and there was a statistically significant decrease in red blood cell counts in the aloe vera-treated animals as compared to the controls. The overall relevance of the results of this study is questionable, because chemical composition (including purity and potential contaminants) of the extract were not specified and, that these test results are inconsistent with other studies.

Herlihy et al. (1998) administered two aloe vera (*Aloe barbidensis*) preparations at 1% and 10% in the diet (approximately equivalent to 500 and 5000 mg/kg bw/day, respectively) to male Fischer 344 rats (five *per* group) for 1.5 or 5.5 months. The first preparation (A1 and A10) was prepared from homogenized aloe filets from the inner leaf of the plant, which were then lyophilized and frozen prior to mixing with the diets. The second preparation (B1 and B10) was prepared in an identical manner as preparation A, except that the aloe filet homogenate was charcoal filtered prior to lyophilization to more closely resemble commercial products. Due to reported diarrhea and a reduction in growth rate, the concentration of preparation A10 was reduced from 10% to 5% and 3% in the diet however, despite these successive reductions in dose, the reduced growth rate and diarrhea persisted therefore the A10 group was eliminated from the study. In the groups fed preparations A1 and B1 and B10, there were no reported adverse effects on body weight, food consumption or organ weights. Also, there were no changes in serum chemistry, except for statistically significant decreases in aspartate aminotransferase at all dosages and alanine aminotransferase in preparations A1 and B10 as compared to the control group ($p < 0.05$). Also, serum cholesterol and high-density lipoproteins were significantly decreased in preparation B10 as compared to the control group ($p < 0.05$). Gross and histopathological analysis of organs revealed no treatment-related adverse effects in the animals fed either preparation. The authors stated that the effects observed in preparation A10 may have been due to the presence of the anthraquinone, barbaloin, which has been shown to have cathartic effects. This suggestion was supported by the lack of diarrhea or reduced growth rates in animals fed preparation B in which anthraquinones were removed by charcoal filtration. The authors concluded that long-term ingestion of the aloe vera preparations A and B are safe at concentrations up to 1% in the diet (500 mg/kg bw/day).

In a 90-day study conducted with acemannan, the primary polysaccharide constituent of aloe vera inner leaf, Beagle dogs were fed acemannan in the diet at doses of 0, 100, 400 and 1500 mg/kg/day (Fogleman et al., 1992). Food consumption and body weight was measured throughout the study period and ophthalmic examinations and hematology, serum chemistry and urinalysis determinations were performed. The results revealed no mortality or significant signs of systemic toxicity at any of the doses tested.

Fogleman et al. (1992) also administered acemannan to Sprague–Dawley rats in the diet at doses of 0, 200, 650 and 2000 mg/kg/day for 6 months. Evaluation of the same toxicological endpoints as in the dog study was evaluated and there was no reported mortality or adverse effects in any of the measured parameters at any of the doses tested.

In addition, several studies have evaluated the potential genotoxicity of various preparations of aloe vera and its constituents. In a gene mutation (rec assay) test conducted with *Bacillus subtilis*, an aqueous extract of *A. ferox*, positive results were observed with and without metabolic activation at concentrations of 5, 10, 20, 50 and 100 mg/ml (Morimoto et al., 1982). Conversely, in the same publication, the aqueous *A. ferox* extract was negative for genotoxic effects in *S. typhimurium* strains TA98 and TA100 with and without metabolic activation. In addition, the genotoxicity of an ethanolic extract of *A. ferox* was evaluated in a gene mutation assay conducted with *B. subtilis* at the previously listed concentrations, and the test material did not exhibit any genotoxic effects at any of the concentrations tested (Morimoto et al., 1982). In another gene mutation assay conducted with *S. typhimurium* strains TA98 and TA100, an extract of the crushed leaves of *A. barbadensis* (concentrations not specified) did not exhibit genotoxic effects with or without metabolic activation (Badria, 1994). In a genotoxicity study that utilized gel electrophoresis, a sodium chloride extract of the leaf pulp of *A. barbadensis* was reported to induce dose-dependent single-strand breaks in the plasmid DNA incubated with the test material at concentrations of 3, 30 and 300 μg/ml (Paes-Leme et al., 2005). However, this assay is not a standard genotoxicity assay and is not listed in the OECD or FDA Redbook guidelines for genotoxicity studies. In addition, the purity and chemical composition (including potential contaminants) of the aloe vera extract could not be determined.

There have also been a series of *in vitro* and *in vivo* genotoxicity studies conducted with anthraquinones found in the latex segment of the aloe vera plant including aloin and aloe-emodin. In the *in vitro* studies, aloe-emodin exhibited positive effects in bacterial and mammalian cell assays at concentrations ranging from 10–5000 μg/plate (Brown et al., 1977; Brown and Dietrich, 1979; Westendorf et al., 1990; Heidemaan et al., 1993, 1996; Müller et al., 1996). In an Ames test conducted with aloin, there was no reported genotoxicity at concentrations ranging from 50 to 250 μg/plate in *S. typhimurium* strains TA98, TA100, TA1535, TA1537 and TA1538 with and without metabolic activation (Brown and Dietrich, 1979; Paes-Leme et al., 2005). In contrast to the positive *in vitro* results, aloe-emodin was shown to be non-genotoxic in a variety of *in vivo* assays that included the mouse micronucleus test, chromosomal aberration test in rats, unscheduled DNA synthesis test in rats and a mouse spot test at doses up to 2000 mg/kg (Heidemaan et al., 1993, 1996). Conflicting *in vivo* and *in vitro* genotoxicity test data are not uncommon and to the extent that this conflict does occur, the US Environmental Protection Agency (EPA) indicates in its Guidelines for Mutagenicity Risk Assessment (EPA, 1986) that more weight should be placed on *in vivo* mutagenicity studies as compared to *in vitro* mutagenicity studies. Further, in the FDA Toxicological Principles for the Safety Assessment of Food Ingredients (FDA, 1982), it is noted that positive genotoxicity results that may not be relevant *in vivo* may arise *in vitro* due to changes in pH, osmolality or high levels of cytotoxicity. Therefore, the negative results observed *in vivo* studies should outweigh the positive results obtained in the *in vitro* studies.

We believed the inconsistent results of previous studies performed with various types of aloe vera extracts was due, in large part, to the amount of anthraquinones present in the test material. The specifications for Qmatrix® require an anthraquinone content of <10 ppm (as aloin, aloeresin A and other anthraquinones). In our studies, the potential toxicity of the reduced anthraquinone product Qmatrix®, was investigated *in vitro* and *in vivo*. *In vivo*, male and female Sprague–Dawley rats were administered Qmatrix® at doses up to 2000 mg/kg bw/day for 90 days. There was no mortality and no treatment-related adverse effects in any of the measured parameters in male or female rats. In addition, the potential genotoxicity of Qmatrix® was investigated *in vitro* and *in vivo* in a series of studies, which included an Ames test, chromosomal aberration test and a bone marrow micronucleus test. In the *in vitro* studies, *S. typhimurium*, *E. coli* and Chinese Hamster lung

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cells were exposed to Qmatrix® at concentrations up to 10,000 μg/ml for 4–48 h. Qmatrix® did not exhibit any mutagenic activity in any of the strains utilized at any of the concentrations tested. In an *in vivo* mouse micronucleus test in rats at doses up to 5000 mg/kg bw, Qmatrix® did not cause any genotoxic effects at any of the doses tested.

Therefore, based on the totality of evidence, including our empirical observations presented herein and reports in the scientific literature, we have concluded that Qmatrix® is non-genotoxic *in vitro* or *in vivo* and the NOAEL in the subchronic toxicity study is greater than 2000 mg/kg bw/day.

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