A toxicological assessment of bis (2-carboxyethylgermanium sesquioxide) (organic germanium)

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Abstract/Poster ID
3542/P338

2-carboxyethylgermanium sesquioxide

Abstract

Bis (2-carboxyethylgermanium sesquioxide) is an organic compound containing the trace element germanium. While inorganic germanium (e.g. germanium dioxide) has been shown to cause renal toxicity and accumulation in the body, the toxicity and accumulation of this organic form of germanium appears to be low. Of concern has been contamination/adulteration of this organic germanium product with inorganic germanium (such as germanium dioxide). We investigated the toxicological potential of a pure form of this compound (bis (2-carboxyethylgermanium sesquioxide), manufactured by Designed Nutritional Products, Inc. in the U.S.) according to internationally accepted guidelines, in (1) a bacterial reverse mutation test, (2) an in vitro mammalian chromosomal aberration test, (3) an in vivo mammalian micronucleus test, and (4) a 90-day repeated-dose oral toxicity study in rats. The in vitro studies revealed no mutagenic or clastogenic activity of the test article, both in the absence and presence of metabolic activation and up to the maximum OECD recommended test concentrations. There was also no genotoxicity noted in the mouse micronucleus study up to the highest dose tested of 2000 mg/kg bw (given twice at a 24-hour interval). No mortality, general toxicity, or toxic effects on organs or tissues was observed in Han:WIST rats in the 90-day gavage study at doses of 500, 1000 and 2000 mg/kg bw/day, and the NOAEL was considered the highest dose level tested in both male and female rats.

Test Item

- Name: organic germanium, bis (2-carboxyethylgermanium sesquioxide), Mu-trioxo-bis [betacarboxyethyl] germanic anhydride, germanium sesquioxide, Ge-132, SK818, propagermanium.
- CAS [12758-40-6]
- Molecular formula $C_6H_{10}Ge_2O_7$, molecular weight 339.399 g/mol.
- Bis (2-carboxyethylgermanium sesquioxide), is an organic form of the naturally occurring, non-essential trace element germanium and was supplied by the sponsor, Designed Nutritional Products (West Orem, Utah).
- White crystalline powder, >99% pure.
- Synthesis involves transformation of germanium dioxide followed by pH adjustment, filtration, washing, and drying.
- Batches contain 42.5–43.1% germanium, and <0.005% germanium dioxide.

Conclusions

- Bis (2-carboxyethylgermanium sesquioxide) lacked mutagenic potential in a bacterial reverse mutation test, an in vitro chromosomal aberration test, and an in vivo mammalian micronucleus test.
- The bis (2-carboxyethylgermanium sesquioxide) NOAELs in the OECD compliant 14- and 90-day repeated dose oral toxicity studies were both 2000 mg/kg bw/day, the highest doses tested.

Bacterial Reverse Mutation Test

OECD Test Guideline 471 and GLP compliant.
Test System: *Salmonella typhimurium* tester strains TA98, TA100, TA1535, and TA1537 and *Escherichia coli* strain WP2 *uvrA*.
Test Concentrations: 5000, 1600, 500, 160, 50, and 16 µg/plate.
Positive Controls: 4-Nitro-1,2-phenylenediamine (NPD), sodium azide (SAZ), 9-aminoacridine (9AA), methyl methanesulfonate (MMS), 2-aminoanthracene (2AA). 2AA was the positive control with metabolic activation for all tester strains. Without metabolic activation positive controls were: NPD (TA98), SAZ (TA100 & TA1535), 9AA (TA1537), and MMS (WP2 *uvrA).* DMSO was the vehicle/negative control for NPD, 9AA, and 2AA. Ultrapure water was the vehicle/negative control for SAZ and MMS.
Metabolic activation system: post mitochondrial supernatant (S9) prepared from livers of Phenobarbital/β-naphthoflavone-induced rats.
Initial (plate incorporation method) and **confirmatory** (pre-incubation method) tests were conducted.

- **Outcome:** The test article did not induce gene mutations up to the highest concentration tested with or without metabolic activation in any of the five bacterium tester strains.
- **Conclusion:** Bis (2-carboxyethylgermanium sesquioxide) was not mutagenic under the applied conditions up to the maximum recommended concentration for soluble non-cytotoxic substances.

In Vitro Chromosomal Aberration Test

OECD Test Guideline 473 and GLP compliant.
Test System: V79 (male Chinese hamster lung) cells.
Test Concentrations: 500, 1000, & 2000 µg/mL for all experiments
Positive Controls: Ethyl methanesulfonate without metabolic activation, cyclophosphamide monohydrate with metabolic activation.
Metabolic activation system: post mitochondrial supernatant (S9) prepared from livers of Phenobarbital/β-naphthoflavone-induced rats.
Two independent experiments were conducted at treatment/sampling intervals of 3/20 hours (Experiment A without and with S9-mix), 20/20 and 20/28 hours (Experiment B without S9-mix), and 3/28 hours (Experiment B with S9-mix).

- **Outcome:** The test article did not induce structural chromosomal aberrations in Chinese Hamster lung V79 cells, with or without metabolic activation, up to the highest concentrations tested.
- **Conclusion:** Bis (2-carboxyethylgermanium sesquioxide) was not clastogenic under the applied conditions.

In Vivo Mammalian Micronucleus Test

OECD Test Guideline 474 and GLP compliant.
Animals: Male Crl:NMRI BR mice, five animals per dose group.
Dose Levels: 0, 500, 1000, and 2000 mg/kg bw (two gavage treatments at a 24-hour interval).
Positive/Negative-Vehicle Control: Cyclophosphamide (one treatment, 60 mg/kg bw IP) / 1% aqueous methylcellulose.
Bone Marrow Sampling: Once, 24 hours following the final treatment. 4000 polychromatic erythrocytes (PCEs) were scored per animal.

- **Outcomes:**
  - No deaths or abnormal clinical signs occurred.
  - No biologically or statistically significant increases in micronucleated PCEs were observed in bone marrow of treated mice compared to concurrent and historical negative controls up to the highest dose tested.
- **Conclusion:** Bis (2-carboxyethylgermanium sesquioxide) did not induce in vivo chromosomal damage in the bone marrow of mice under the tested conditions.

Fourteen-Day Dose-Range Finding Study

OECD Test Guideline 407 and GLP compliant.
Animals: Male and Female Hsd.Han:WIST rats (5/sex/group).
Dose Levels: 0, 500, 1000, and 2000 mg/kg bw/day via gavage.
Objectives: Obtain information on the toxic potential of the test article and for dose-selection for the 90-day repeated-dose study.

- **Conclusion:** The no observed adverse effect level (NOAEL) for bis (2-carboxyethylgermanium sesquioxide) was 2000 mg/kg bw/day in the 14-day study, the highest dose tested.

Ninety-Day Repeated Dose Oral Toxicity Study

OECD Test Guideline 408 and GLP compliant.
Animals: Male and Female Hsd.Han:WIST rats (10/sex/group).
Dose Levels: 0, 500, 1000, and 2000 mg/kg bw/day via gavage (Lot # 4895).
Objectives: Obtain information on the toxic potential of Organic germanium (bis (2-carboxyethylgermanium sesquioxide).
Negative (Vehicle) Control: 1% methylcellulose

- **Outcomes:**
  - There was no mortality and no clinical signs of toxicity or functional defects were noted.
  - Paler than normal stool in the high dose group and softer than normal stool in the mid- and high-dose groups in both sexes were considered test article-related; however they were not accompanied by adverse findings, and were not considered toxicologically relevant.
  - Slightly decreased body weight gain in males of the high dose group was < 9% of the control group and was judged not to be toxicologically meaningful. Daily mean food consumption and feed efficiency were not different between the test groups and controls.
  - No eye alterations were observed in control or high-dose animals.
  - No toxicologically relevant effects on hematological and clinical chemistry parameters were observed. Decreases in CREA, Na+, Cl- and increased Pi compared to controls occurred in females of the mid- and high-dose groups, and high-dose females additionally had decreased bilirubin, albumin and total protein. In males, ALP and total protein decreased in the mid- and high-dose groups, and high-dose males additionally had decreased UREA, Cl, Alb, and increased A/G ratio. Although the findings were statistically significant, they were of minor degree, and values were well within historical control ranges or were marginal (e.g. control values were also outside ranges), and there were no related histopathological or other findings.
  - Macroscopic and organ weight examinations did not show test article-related alterations or differences.
  - There were no histopathological findings that were considered of toxicological relevance.
- **Conclusion:** Based on the findings, the no observed adverse effect level (NOAEL) for bis (2-carboxyethylgermanium sesquioxide) was 2000 mg/kg bw/day in the 90-day study, the highest dose tested.

Males and Females: Histopathology Findings from 90-Day Study

Organs	Observations	Incidence of observations per group: Males and Females: Control	500 mg/kg bw/day	1000 mg/kg bw/day	2000 mg/kg bw/day
Adrenal glands	No lesion	10/10 (10/10)	/ (/)	/ (/)	10/10 (10/10)
Aorta	No lesion	10/10 (10/10)	/ (/)	/ (/)	10/10 (10/10)
Bone marrow	No lesion	10/10 (10/10)	/ (/)	/ (/)	10/10 (10/10)
Brain	No lesion	10/10 (10/10)	/ (/)	/ (/)	10/10 (10/10)
Caecum	**Dilatation**	**0/10 (0/10)**	/ (/)	/ (/)	**1/10 (0/10)**
Colon	No lesion	10/10 (10/10)	/ (/)	/ (/)	10/10 (10/10)
Duodenum	No lesion	10/10 (10/10)	/ (/)	/ (/)	10/10 (10/10)
Eyes + optic nerve	No lesion	10/10 (10/10)	/ (/)	/ (/)	10/10 (10/10)
Epididymides	**Sperm granulomas**	**0/10 (n/a)**	**1/1 (n/a)**	/ (n/a)	**0/10 (n/a)**
Esophagus	No lesion	10/10 (10/10)	/ (/)	/ (/)	10/10 (10/10)
Harderian glands	No lesion	10/10 (10/10)	/ (/)	/ (/)	10/10 (10/10)
Heart	No lesion	10/10 (10/10)	/ (/)	/ (/)	10/10 (10/10)
Ileum	No lesion	10/10 (10/10)	/ (/)	/ (/)	10/10 (10/10)
Jejunum	No lesion	10/10 (10/10)	/ (/)	/ (/)	10/10 (10/10)
Kidneys	**Pyelectasis**	**0/10 (0/10)**	**1/1 (1/1)**	**1/1 (1/1)**	**2/10 (0/10)**
	Cyst	**1/10 (0/10)**	**0/1 (/)**	**0/1 (/)**	**0/10 (0/10)**
	Hydronephrosis	**0/10 (1/10)**	/ **(0/1)**	/ **(/)**	**0/10 (0/10)**
Lachrymal glands	No lesion	10/10 (10/10)	/ (/)	/ (/)	10/10 (10/10)
Liver	**Congestion**	**0/10 (0/10)**	/ (/)	/ (/)	**1/10 (0/10)**
Lungs	**Alveolar emphysema**	**1/10 (1/10)**	/ (/)	/ (/)	**1/10 (0/10)**
	Hyperplasia of BALT	**1/10 (0/10)**	/ (/)	/ (/)	**0/10 (0/10)**
	Acute hemorrhage	**0/10 (1/10)**	/ (/)	/ (/)	**0/10 (0/10)**
Mammary gland	No lesion	n/a (10/10)	n/a (/)	n/a (/)	n/a (10/10)
Mesenteric lymph nodes	No lesion	10/10 (10/10)	/ (/)	/ (/)	10/10 (10/10)
Muscle (quadriceps)	No lesion	10/10 (10/10)	/ (/)	/ (/)	10/10 (10/10)
Ovaries	No lesion	n/a (10/10)	/ (/)	/ (/)	n/a (10/10)
Pancreas	No lesion	10/10 (10/10)	/ (/)	/ (/)	10/10 (10/10)
Nasal turbinates	No lesion	10/10 (10/10)	/ (/)	/ (/)	10/10 (10/10)
Pituitary	No lesion	10/10 (10/10)	/ (/)	/ (/)	10/10 (10/10)
Prostate	No lesion	10/10 (n/a)	/ (n/a)	/ (n/a)	10/10 (n/a)
Rectum	No lesion	10/10 (10/10)	/ (/)	/ (/)	10/10 (10/10)
Salivary glands (submx.) †	No lesion	10/10 (10/10)	/ (/)	/ (/)	10/10 (10/10)
Sciatic nerve	No lesion	10/10 (10/10)	/ (/)	/ (/)	10/10 (10/10)
Seminal vesicle ††	No lesion	10/10 (n/a)	/ (n/a)	/ (n/a)	10/10 (n/a)
Skin	**Atrophy of hair follicles**	**0/10 (1/10)**	**1/1 (/)**	/ (/)	**0/10 (0/10)**
Spinal cord	No lesion	10/10 (10/10)	/ (/)	/ (/)	10/10 (10/10)
Spleen	**Hyperplasia**	**0/10 (0/10)**	/ (/)	/ (/)	**1/10 (0/10)**
Sternum	No lesion	10/10 (10/10)	/ (/)	/ (/)	10/10 (10/10)
Stomach	No lesion	10/10 (10/10)	/ (/)	/ (/)	10/10 (10/10)
Sublumb.lymph nodes†	No lesion	10/10 (10/10)	/ (/)	/ (/)	10/10 (10/10)
Thymus	**Acute hemorrhage**	**0/10 (1/10)**	**1/1 (/)**	/ **(1/1)**	**0/10 (2/10)**
Thyroid + parathyroid	No lesion	10/10 (10/10)	/ (/)	/ (/)	10/10 (10/10)
Testes	No lesion	10/10 (n/a)	/ (n/a)	/ (n/a)	10/10 (n/a)
Trachea	No lesion	10/10 (10/10)	/ (/)	/ (/)	10/10 (10/10)
Urinary bladder	No lesion	10/10 (10/10)	/ (/)	/ (/)	10/10 (10/10)
Uterus	**Dilatation**	**n/a (0/10)**	**n/a (/)**	**n/a (1/1)**	**n/a (0/10)**
Vagina	No lesion	n/a (10/10)	n/a (/)	n/a (/)	n/a (10/10)

Abbreviations: Incidence = Number of animals with observations / number of animals examined
/ = Not examined.
† = Sublus = Submandibular
†† = Seminal vesicles with coagulating gland

Males: Body Weight Gain (g) Between Days in 90-Day Study

Group mg/kg bw/day		0-3	3-7	7-10	10-14	14-17	17-21	21-24	24-28	28-35	35-42	42-49	49-56	56-63	63-70	70-77	77-84	84-89	SUM. 0-89
Control	Mean	12.4	23.9	6.4	22.3	7.5	19.6	5.2	17.0	20.2	16.4	14.7	13.2	9.2	7.3	10.5	2.8	5.6	214.2
	SD	4.0	3.6	3.2	3.4	4.1	3.6	2.0	2.7	3.5	4.8	4.4	3.9	5.3	3.9	5.0	4.0	4.5	28.5
	n	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
500	Mean	14.6	25.7	7.5	23.1	3.9	20.4	7.9	14.3	18.4	14.2	14.0	14.0	9.7	9.9	6.4	1.3	5.6	210.4
	SD	3.1	3.0	3.4	3.5	4.6	3.5	2.2	3.7	4.1	3.0	4.1	4.6	4.7	3.1	3.6	4.3	4.2	16.3
	n	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
						*		*											
1000	Mean	18.1	24.2	8.4	20.7	7.4	16.3	7.2	10.6	20.3	13.8	12.9	11.8	11.3	6.5	5.5	2.2	7.6	204.8
	SD	4.4	7.1	2.8	4.9	3.7	5.5	2.8	4.5	4.9	5.8	4.0	2.5	6.8	4.4	4.7	6.1	3.9	47.7
	n	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
		**							**	**									
2000	Mean	15.9	22.4	6.9	19.6	2.0	17.7	4.5	10.3	16.9	10.2	8.6	10.9	10.0	11.6	5.7	5.6	-2.0	176.8
	SD	3.4	3.7	2.6	4.8	4.3	3.9	3.2	4.5	5.8	5.8	7.7	4.2	9.4	9.2	12.6	6.1	3.7	23.4
	n	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
						*		**		*	*					*		**	**
		DN	NS	NS	NS	DN	NS	DN	DN	NS	DN	DN	NS	NS	NS	U	NS	DN	U

±% =, Percent Deviation Versus Control; NS =, Not Significant; * =, p < 0.05; ** =, p < 0.01;
U =, Mann-Whitney U-test Versus Control; DN =, Duncan's multiple range test; - =, no data.

Males and Females: Hematology Results from 90-Day Study

Group mg/kg bw/day		WBC [x10^3/L]	NEU [%]	LYM [%]	MONO [%]	EOS [%]	BASO [%]	RBC [x10^6/L]	HGB [g/L]	HCT [L/L]	MCV [fL]	MCH [pg]	MCHC [g/L]	PLT [x10^3/L]	RET [%]	PT [sec]	APTT [sec]
MALES																	
Control	Mean	6.88	17.87	77.89	1.79	2.07	0.09	8.76	154.9	0.48	54.28	17.75	326.6	679.0	1.80	10.74	12.96
	SD	1.51	4.79	5.21	0.42	0.56	0.03	0.49	6.7	0.02	2.32	1.14	8.1	150.0	0.20	0.29	1.64
	n	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
500	Mean	5.90	19.90	75.29	1.97	2.48	0.06	8.94	154.0	0.47	52.44	17.22	328.7	739.1	1.60	10.71	13.39
	SD	1.17	11.26	14.96	0.98	3.12	0.05	0.22	5.2	0.01	1.50	0.61	6.7	154.1	0.15	0.24	1.67
	n	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
	±%	-14	11	-3	10	20	-33	2	-1	-1	-3	-3	1	9	-11	0	3
											*						
1000	Mean	6.42	18.53	77.25	1.93	1.79	0.06	8.62	153.6	0.47	53.98	17.83	330.3	741.3	1.54	10.72	13.54
	SD	1.20	1.92	1.98	0.61	1.02	0.05	0.41	4.6	0.02	1.51	0.88	8.9	90.4	0.24	0.35	1.57
	n	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
	±%	-7	4	-1	8	-14	-33	-2	-1	-2	-1	0	1	9	-14	0	4
															*		
2000	Mean	6.15	18.10	78.52	1.74	1.21	0.08	8.76	154.8	0.47	53.02	17.70	334.0	818.4	1.55	10.79	13.75
	SD	1.39	5.04	5.47	0.58	0.28	0.04	0.50	5.7	0.02	1.72	0.83	9.0	111.4	0.34	0.24	1.04
	n	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
	±%	-11	1	1	-3	-42	-11	0	0	-2	-2	-2	2	21	-14	0	6
						**									*		
		NS	NS	NS	NS	U	NS	NS	NS	NS	DN	NS	NS	NS	DN	NS	NS
Historical		4.95	9.7	56.5	1.8	0.3	0.0	7.52	141	0.404	45.4	16.6	349	595	2.05	16.5	15.2
		-11.12	-38.7	-87.8	-5.1	-9.0	-0.2	-10.21	-180	-0.488	-53.7	-18.8	-379	-957	-4.65	-27.3	-31.8
FEMALES																	
Control	Mean	3.84	22.12	73.29	1.85	2.23	0.10	8.20	148.9	0.46	55.63	18.2	327.2	707.2	2.18	10.04	13.33
	SD	1.00	7.51	7.38	0.65	0.84	0.05	0.39	5.1	0.02	1.56	0.7	6.9	151.8	0.34	0.15	1.39
	n	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
500	Mean	3.89	18.03	78.19	1.71	1.64	0.08	8.09	147.0	0.45	55.32	18.2	329.0	786.3	1.93	10.11	13.09
	SD	0.88	7.17	7.18	0.46	0.81	0.08	0.30	2.6	0.01	1.36	0.5	7.0	136.1	0.36	0.26	1.12
	n	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
	±%	1	-18	7	-8	-26	-20	-1	-1	-2	-1	0	1	11	-12	1	-2
1000	Mean	4.43	24.98	70.86	2.16	1.49	0.10	8.35	149.4	0.45	53.97	17.9	331.9	892.6	1.80	10.01	13.88
	SD	1.12	18.66	19.03	0.83	0.37	0.08	0.38	5.6	0.02	1.43	0.7	4.7	108.1	0.40	0.10	1.80
	n	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
	±%	15	13	-3	17	-33	0	2	0	-1	-3	-2	1	26	-17	0	4
														**			
2000	Mean	4.36	23.06	72.97	1.99	1.52	0.05	8.21	148.7	0.45	54.69	18.1	331.2	849.4	1.90	10.03	13.51
	SD	0.92	13.15	14.61	0.92	0.81	0.05	0.25	5.1	0.01	1.97	0.8	6.5	109.3	0.34	0.13	1.42
	n	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
	±%	14	4	0	8	-32	-50	0	0	-1	-2	0	1	20	-13	0	1
		NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	DN	NS	NS	NS
Historical		2.81	5.8	63.4	1.1	0.4	0.0	7.71	148	0.415	47.0	17.2	346	549	2.77	16.4	17.4
		-10.66	-33.3	-91.1	-3.9	-2.1	-0.2	-9.17	-168	-0.467	-60.1	-20.8	-372	-1103	-5.63	-26.7	-27.9

±% =, Percent Deviation Versus Control; NS =, Not Significant; * =, p < 0.05; ** =, p < 0.01;
U =, Mann-Whitney U-test Versus Control; DN =, Duncan's multiple range test; - =, no data.

Males and Females: Clinical Chemistry Results from 90-Day Study

Group mg/kg bw/day		ALT [U/L]	AST [U/L]	ALP [U/L]	TBIL [µmol/L]	CREA [µmol/L]	UREA [mmol/L]	GLUC [mmol/L]	CHOL [mmol/L]	Pi [mmol/L]	Ca^{++} [mmol/L]	Na^+ [mmol/L]	K^+ [mmol/L]	CT [mmol/L]	ALB [g/L]	TPROT [g/L]	A/G
MALES																	
Control	Mean	51.5	86.4	142.0	1.02	25.4	7.90	7.56	2.35	1.91	2.68	142.94	4.59	98.85	44.29	65.93	2.08
	SD	8.8	11.0	41.2	0.41	2.5	0.51	0.89	0.41	0.13	0.06	0.97	0.33	1.48	1.78	2.24	0.30
	n	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
500	Mean	49.5	80.3	125.4	1.00	25.7	7.21	7.35	2.56	1.90	2.66	142.28	4.35	98.55	43.54	64.80	2.06
	SD	6.7	10.0	27.7	0.32	2.5	0.51	0.34	0.40	0.19	0.05	0.50	0.23	0.78	1.37	2.30	0.19
	n	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
	±%	-4	-7	-12	-2	1	-9	-3	9	0	-1	0	-5	0	-2	-2	-1
1000	Mean	54.6	84.6	107.1	0.85	26.4	7.18	7.47	2.37	1.98	2.65	142.28	4.33	98.13	43.54	63.74	2.16
	SD	9.5	18.1	24.7	0.35	3.5	0.92	0.67	0.38	0.17	0.06	1.49	0.31	2.00	1.10	2.09	0.16
	n	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
	±%	6	-2	-25	-17	4	-9	-1	1	4	-1	0	-6	-1	-2	-3	4
				*												*	
2000	Mean	46.0	79.9	110.1	1.18	24.5	6.93	6.85	2.09	1.87	2.64	142.37	4.50	97.36	42.63	60.98	2.34
	SD	5.2	12.6	21.7	0.36	2.3	1.06	0.37	0.26	0.21	0.05	0.68	0.30	0.99	0.74	1.12	0.19
	n	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
	±%	-11	-8	-22	16	-4	-12	-9	-11	-2	-2	0	-2	-2	-4	-7	13
				*			*							*	**	*	*
		NS	NS	DN	NS	NS	DN	NS	NS	NS	NS	NS	NS	U	U	DN	DN
Historical		28.0	67.9	56	0.71	18.3	3.60	4.58	1.27	1.40	2.40	140	3.84	102.7	33.0	56.8	1.0
		-86.2	-135.7	-184	-2.79	-37.2	-9.26	-8.24	-2.44	-2.41	-2.80	-146	-5.04	-106.9	-37.4	-68.0	-1.4
FEMALES																	
Control	Mean	86.1	141.4	65.6	1.46	31.20	6.91	6.54	2.11	1.18	2.67	143.50	4.04	101.44	50.88	69.11	2.80
	SD	32.1	51.1	38.0	0.48	2.49	1.26	0.62	0.32	0.30	0.09	2.01	0.37	2.22	2.50	2.98	0.22
	n	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
500	Mean	59.9	101.0	62.7	1.12	29.30	6.83	7.11	1.81	1.36	2.65	142.72	4.09	100.38	50.42	67.53	2.96
	SD	16.9	13.6	16.1	0.20	3.71	0.87	0.42	0.40	0.27	0.08	1.49	0.39	1.67	2.69	3.42	0.30
	n	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
	±%	-30	-29	-4	-23	-6	-1	9	-14	15	-1	-1	1	-1	-1	-2	6
		*						*									
1000	Mean	73.5	106.3	48.7	1.34	27.00	7.08	6.59	2.10	1.66	2.67	140.93	3.99	98.41	49.77	67.47	2.85
	SD	22.2	22.2	11.7	0.36	2.62	1.16	0.56	0.38	0.31	0.11	1.14	0.21	1.85	3.20	3.84	0.40
	n	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
	±%	-15	-25	-26	-8	-13	2	1	0	41	0	-2	-1	-3	-2	-2	2
						**				**		**		**			
2000	Mean	61.3	97.1	58.2	1.08	27.20	6.90	6.78	2.08	1.71	2.67	140.92	3.95	97.43	47.83	64.62	2.87
	SD	20.1	20.4	26.6	0.36	2.90	1.12	0.51	0.34	0.27	0.05	1.42	0.11	1.27	2.23	1.76	0.41
	n	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
	±%	-29	-31	-11	-26	-13	0	4	-1	46	0	-2	-2	-4	-6	-6	3
					*	**				**		**		**	*	**	
		DN	NS	NS	DN	DN	NS	DN	NS	DN	NS	DN	NS	DN	DN	DN	NS
Historical		23.4	71.0	25	1.23	25.6	4.25	4.44	1.35	0.93	2.51	135	3.42	103.3	33.2	57.1	1.1
		-87.7	-141.8	-126	-3.30	-40.8	-8.50	-7.55	-3.39	-2.01	-2.77	-148	-4.35	-109.6	-41.0	-74.5	-1.5

±% =, Percent Deviation Versus Control; NS =, Not Significant; * =, p < 0.05; ** =, p < 0.01;
U =, Mann-Whitney U-test Versus Control; DN =, Duncan's multiple range test; - =, no data.