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Subchronic oral toxicity (six months) of carboxyethylgermanium sesquioxide $[(HOOCCH_2CH_2Ge)_2O_3]_n$ in rats

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After briefly renewing toxicological data on germanium compounds, the authors report on the subchronic oral toxicity of carboxyethylgermanium sesquioxide in rats. During six months, male and female animals received 1 g kg^{-1} day^{-1}. No particular toxic symptoms, and no behaviour problems except a small decrease of body weight in male rats, at the end of the six-month experimentation period, were observed. A significant decrease of erythropoiesis and some significant changes in leucocyte ratios were demonstrated. The main marked effect was a moderate renal dysfunction characterized by a tubular disease with the presence of cylinders, swelling of tubulus cells and flocculus deposits. Germanium urinary excretion was constant and linked to the received dose. Six months later, no preferential accumulation in organs was evident.

Keywords: Carboxyethylgermanium sesquioxide, subchronic oral toxicity, Rat

1 INTRODUCTION

In spite of its rarity, germanium and some of its salts do have some industrial applications, mainly in the optical and electronic fields.

Coal seems to be one of the major natural sources of germanium and its combustion releases important amounts into the atmosphere. As there exists no pure ore, germanium is co-extracted industrially with zinc and silver.[1] The chemical properties of germanium are similar to those of tin but its physical properties are closer to those of silicon.[2]

Germanium has been considered for a long time as a negligible contaminant for environmental quality and human health assessment; therefore bibliographic data on its toxicity are relatively limited.

The main studies devoted to the toxicity of germanium salts in mammals were conducted 20–30 years ago. Table 1 gives, for some compounds, the lethal dose values found in the literature for different animal species. It can be concluded that germanium presents a low acute experimental toxicity. In the rat several studies using ^{14}C-labelled carboxyethylgermanium sesquioxide, $[(HOOCCH_2CH_2Ge)_2O_3]_n$, Compound **A**, show, after an oral ingestion (100 mg kg^{-1}), a blood peak at 3 h and a nearly total elimination within 24 h, mainly by renal clearance.[3] Intravenous (i.v.) injections (50 mg kg^{-1}) in the rabbit result in an elimination of about 70% within 1 h and 90% within 3 h. Germanium does not accumulate in mammals.[4] Similar results had been obtained in the rat and the dog, after i.v. administration of germanium oxide ($^{71}GeO_2$).[1]

As regards its delayed toxicity, sodium germanate (Na_2GeO_3) seems more harmful in the rat when it is mixed with drinking water than with food. Incorporation of GeO_2 (10 ppm) in food stimulates the growth of rats and chickens.[5] Preliminary studies carried out in rats (oral administration: 30–300 and 3000 mg kg^{-1}) or in dogs (i.v. administration: 125–250 and 500 mg kg^{-1}), during six months, have revealed no apparent toxic effects.[3,4]

Germanium(**IV**) oxide (GeO_2) has proved to have an antimutagenic effect on *Salmonella typhimurium* TA 98 and TA 1538.[6] Compound **A** has an antitumor effect in methylcholanthrene-induced tumorigenesis in mice.[7]

Although embryotoxic effects of dimethylgermanium oxide (Me_2GeO) in chicks were found by

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Table 1 Acute experimental toxicity of some germanium compounds

Substance	Animal species	Route	DL_x [a]	Dose (mg kg^{-1})	Reference
Ge powder	Rabbit	Subcutaneous	DL_{100}	586	2
Ge oxide	Rat	Subcutaneous	DL_{100}	>180	2
		Intraperitoneal	DL_{50}	750	2
		Oral	DL_{50}	3 700	28
	Mouse	Oral	DL_{50}	6 300	28
	Rabbit	Subcutaneous	DL_{50}	845	2
	Guinea pig	Intraperitoneal	DL_{50}	400	2
Ge hydride	Mouse	Pulmonary	CL_{100} (4 h)	610 mg m^{-3}	2
Ge chloride	Mouse	Pulmonary	CL_{50} (2 h)	44 mg dm^{-3}	2
Et_3Ge acetate	Rat	Oral	DL_{50}	250	2
		Intraveinous	DL_{50}	50	2
Me_3Ge sulfide	Mouse	Intraperitoneal	DL_{100}	250	2
Ge sesquioxide	Rat	Oral	DL_{50}	>10 000	3
		Subcutaneous	DL_{50}	>10 000	26
		Intraperitoneal	DL_{50}	>3 000	27
	Mouse	Oral	DL_{50}	>10 000	3
		Subcutaneous	DL_{50}	>7 500	3
		Intraperitoneal	DL_{50}	>2 000	3

[a] DL_x = lethal dose for x% of animals tested.

Caujolle,[8] no teratogenic effect of compound **A** was demonstrated.[9]

In professional occupations, a few references concern possible exposure of workers to germanium compounds. The only damage observed was irritation of ocular, pulmonary and cutaneous mucous membranes due to tetrachloride ($GeCl_4$) and hydride (GeH_4).[10, 11]

GeO_2 and compound **A** present some interesting pharmacological and therapeutic properties[12–14] and have already been used on humans in clinics, mainly in Japan.[9] However, a few years ago chronic utilization in man caused some side effects such as renal dysfunction.[15] Later this pathological adverse effect was also described after repeated oral ingestion of GeO_2 in the rat but it did not occur with compound **A**.[16]

In this paper we report the results of studies on the subchronic oral toxicity of compound **A** [1 g (kg body weight)$^{-1}$ in rats over a period of six months.

2 MATERIAL AND METHODS

2.1 Animals

Two groups (control and assay) of 30 male and female Wistar rats weighing 200–220 g were obtained from R. Janvier (Le Genest, Saint Isle, France). They had free access to a commercial diet (UAR A 04) and tap-water throughout the experiment.

2.2 Reagents

Carboxyethylgermanium sesquioxide was suspended in an aqueous solution of carboxymethylcellulose (0.5%). This suspension was prepared each day and given to the animals orally [1 g kg bw)$^{-1}$, five days a week, for six months. The control group was given only an aqueous solution of carboxymethylcellulose.

2.3 Experimental design

Behavioural reactions and food and water consumption were noted daily. Average body weight was determined twice a week. At the end of the experiment all the survivors in each group were sacrificed. Their blood was sampled (retro-orbital sinus) for haematocrit, haemoglobin, RBC and WBC determinations (Coulter counter). Serum analysis (urea, glucose, ASAT and ALAT transaminases, cholesterol, albumin, total proteins and electrolytes) was performed (Hitachi 705) to investigate possible changes in renal and liver functions. The significance of the differences between results for controls and treated animals was determined by student's t test. A difference was considered to be significant when $P<0.05$.

Pathological examinations and autopsies were performed. Each animal's main organs were removed and preserved in a 10% formalin solution for macroscopic investigations.

2.4 Toxicokinetic studies

Six treated male rats were kept in three metabolic cages (R. Pajon, Semoy, France) in order to collect urine and faeces separately. They all had free access to both food and water. Germanium concentrations in the urine was determined during months 1, 3 and 6. At the end of the experiment, all the animals were sacrificed. Their main organs (heart, liver, lungs, spleen, brain, kidneys and testes) were removed, accurately weighed and analysed for their germanium concentration. Germanium concentrations in biological media were determined by AA with a graphite furnace (Varian, SpectrAA 300 Zeeman) at 265.1 nm, by the method of Shinogi *et al.*[17]

3 RESULTS

Throughout the experiment, no particular sign of intoxication or behavioural reaction could be seen in either the control or the treated group. No death occurred in the controls. Three deaths occurred in the treated group but pathological examination showed that they were due to 'false route' administration.

There was no significant difference in food and water consumption between the control and treated groups.

Growth rate was similar in both groups. However, a slightly lower growth rate was noted after the 20 week in the treated male group.

Haematological analysis (Table 2) carried out at the end of the assay showed a significant decrease of the number of RBCs in the male treated group and some significant changes ($P<0.001$) in leucocyte ratios.

With regard to serum analysis (Table 3), interpretation of the results obtained in each group is rather difficult because of inter-individual variations. It has been mainly noted that there occurred:

(a) a significant decrease of total proteins, albumin and some cellular enzymic activities in the male treated group;
(b) a slight renal dysfunction, characterized by an increase of creatine, always in the male treated group.

Histological examination confirmed a renal dysfunction (60% of treated animals). It concerned mainly the tubuli, where a widening of the cylinders and some flocculus deposits were noted. These moderate lesions were not observed in the treated female group. None of the other organs showed any macroscopic or microscopic alterations either in the treated or in the control group.

Toxicokinetic studies revealed that urinary excretion increases progressively from the beginning to the end of the assay, when it amounts to nearly 15 mg day^{-1} per animal and remains proportional to the administered doses in a roughly constant ratio (Fig. 1). The germanium absorbed does not remain in the body and does not accumulate in preferential tissues since residual

Table 2 Haematological analysis in rats after six months

Haematological parameter	Male control	Male treated	S[a]	Female control	Female treated	S[a]
WBC (10 per mm^3)	15.32 ± 4.03	14.92 ± 2.55		11.00 ± 3.04	10.44 ± 3.84	
RBC (10 per mm^3)	8.47 ± 0.29	8.13 ± 0.34	*	8.03 ± 0.37	7.69 ± 0.49	
Haemoglobin (g per 100 cm^3)	16.43 ± 0.46	16.07 ± 0.58		15.70 ± 0.86	15.26 ± 1.05	
Haematocrit (%)	44.68 ± 2.06	46.73 ± 2.15		44.49 ± 2.63	42.71 ± 3.47	
VGM (μm^3)	52.7 ± 1.50	53.72 ± 1.33		55.33 ± 1.08	55.47 ± 1.58	
Globular haemoglobin (g)	19.34 ± 0.28	19.75 ± 0.38	**	19.53 ± 0.20	19.84 ± 0.37	**
Haemoglobin globular concentration (g%)	36.77 ± 1.04	36.78 ± 0.98		35.30 ± 0.39	35.79 ± 0.83	
Blood platelets	1130.5 ± 13.39	1166.6 ± 111.8		1085.3 ± 70.3	1182 ± 170.7	
Blood formula (%):						
Granulocytes	14.1 ± 6.40	16 ± 4.9		10.90 ± 5.44	27.80 ± 15.56	***
Lymphocytes	84.1 ± 6.7	71.55 ± 6.52	**	85.90 ± 5.95	68.30 ± 17.44	***
Monocytes	1.8 ± 2.5	7.33 ± 2.87	***	2.70 ± 2.11	3.90 ± 2.60	

[a] Significance: * $P<0.05$; ** $P<0.01$; *** $P<0.001$.

Table 3 Serum analysis in rats after six months

Serum parameter	Male control	Male treated	S[a]	Female control	Female treated	S[a]
Glucose (mmol dm^{-3})	3.44 ± 0.54	2.96 ± 0.62	*	3.71 ± 0.50	2.538 ± 0.700	***
Urea (mmol dm^{-3})	5.17 ± 1.28	4.88 ± 0.45		5.27 ± 1.06	5.17 ± 0.66	
Phosphorus (mmol dm^{-3})		2.17 ± 2.10		1.45 ± 1.07	0.939 ± 0.321	
Total proteins (g dm^{-3})	71.9 ± 5.38	59.5 ± 4.7	***	67.3 ± 6.48	60.1 ± 6.8	**
Total bilirubin (μmol dm^{-3})	13.2 ± 3.91	12.9 ± 12.3		11.4 ± 4.76	25.1 ± 17.2	**
Creatinine (μmol dm^{-3})	37.87 ± 12.60	42.1 ± 5.10		36.6 ± 5.27	38.7 ± 6.9	
Transaminases						
GOT (mUI dm^{-3})	108 ± 32.9	56.77 ± 4.68	***	100.8 ± 61.3	62.5 ± 24.4	
GPT (mUI dm^{-3})	66 ± 36.57	46.66 ± 6.32		59.4 ± 42.9	41.3 ± 17.4	
Total LDH (mUI cm^{-3})	1896 ± 504	293.1 ± 88.6	***	733.8 ± 941.3	240.3 ± 81.6	
CPK (mUI cm^{-3})	228 ± 170	214.3 ± 123.4		125.4 ± 70.6	205 ± 172.5	
Amylase (mUI cm^{-3})	3740 ± 584.9	3756 ± 508.4		3194 ± 731	3205 ± 1124	
Albumin (g dm^{-3})	34.3 ± 1.42	21.1 ± 4.7	***	30.1 ± 8.45	24.8 ± 3.45	
Cholesterol (mmol dm^{-3})	3.09 ± 0.53	2.78 ± 0.46		3.4 ± 0.5	3.04 ± 0.61	
Triglycerides (mmol dm^{-3})	1.91 ± 0.20	2.143 ± 0.44		2.33 ± 0.81	2.61 ± 1.12	
Calcium (mmol dm^{-3})	2.30 ± 0.13	2.22 ± 0.14		2.29 ± 0.17	2.13 ± 0.24	
Sodium (mmol dm^{-3})	121.6 ± 3.37	143.1 ± 2.18		133.2 ± 14.8	142.7 ± 2.26	
Potassium (mmol dm^{-3})	10.10 ± 0.79	9.84 ± 0.81		7.93 ± 1.38	8.48 ± 0.59	
Chlorides (mmol dm^{-3})	91.7 ± 2.21	98.3 ± 2.83		92.3 ± 5.73	92.2 ± 4.04	

[a] Significance: * $P<0.05$; ** $P<0.01$; *** $P<0.001$.
GOT: Glutamate oxaloacetate transaminase; GPT: Glutamate pyruvate transaminase; LDH: Lactate dehydrogenase; CPK: Creatinine phosphokinase. All these parameters are expressed in international milli unities (mUI/cm^3).

amounts are about 1.5 and 2.5 μg g tissue)$^{-1}$ (Table 4). This general and low impregnation seems to be due to visceral irrigation.

4 DISCUSSION

For several years, some organic germanium compounds have proved to be pharmacologically active against a wide range of serious diseases including cancer, malaria and arthritis. Clinical animal and *in vitro* trials are now being conducted in the United States, Japan and Europe to prove their interest and to establish levels of effectiveness and proper dosages. These germanium compounds may become useful drugs in the future. Thus carboxyethylgermanium sesquioxide (compound **A**), synthesized by Asai in 1968,[22] exhibits experimentally various pharmacological properties, especially the following.

(1) An antitumour effect against induced tumours in rats and mice.[7, 18–20]
(2) An antiviral effect against the influenza virus, in infected mice.[13, 21]

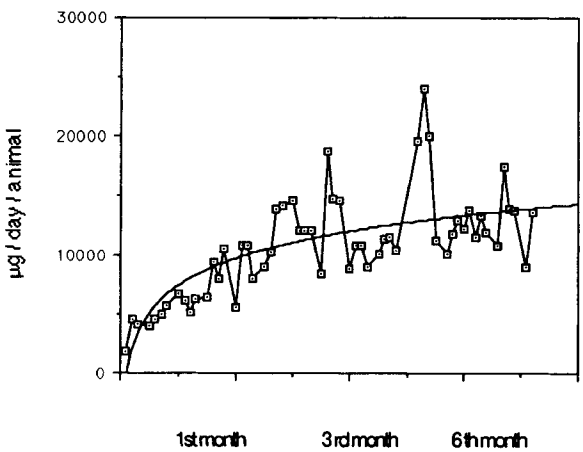

Figure 1 Germanium urinary elimination in the rat during six months.

Table 4 Germanium tissue contents (μg g^{-1}) in the rats after six months

Organs	Controls ($n=5$)[a]	6 months experimental ($n=5$)
Spleen	ND	1.48 ± 0.59
Brain	ND	0.08 ± 0.02
Liver	ND	1.51 ± 0.24
Kidney	ND	1.86 ± 0.63
Heart	ND	0.57 ± 0.05
Lung	ND	0.56 ± 0.42
Testis	ND	0.26 ± 0.09

[a] ND, not detected.

These two actions seem to be based on interferon-inducing activity and its associated biological response-modifying activities such as activation of macrophage increase of NK activity and increase of cell-mediated and humoral immunity.[22]

(3) A regulating effect on calcium metabolism resulting from an osteoblastic action which was observed *in vitro* and demonstrated *in vivo* in a clinical study by a significant decrease of the para-thor-mone (parathyroid hormone) level, which usually increases in many cases of senile osteoporosis.[23]

(4) An enkephalinase inhibitory activity, and an increased pain-relieving effect, demonstrated by a 0.5 mg kg^{-1} morphine analgesia enhancement in the tail-flick test in rats.[14]

(5) Finally, a protective effect against free radicals produced in rat kidneys after a 45-minute warm ischemia.[24]

On account of these therapeutic properties associated with low experimental toxicity, several germanium compounds have been used in clinical investigations, mainly in Japan, for treating numerous diseases such as lung cancer, respiratory failure, leukaemias, lymphatic cancers, rheumatoid arthritis, hepatitis and senile osteoporosis, with doses ranging between 20 and 40 mg (kg body weight)$^{-1}$.[9]

However, chronic utilization of germanium in man has allowed us to discover some adverse effects that have invalidated or confirmed several experimental data. Thus a myopathy not reproducible experimentally in rats and monkeys was observed in several adult patients[25] and also a renal dysfunction linked to degeneration of the tubulus epithelial cells without a haematuria or proteinuria.[15] This renal disease was described in the rat after repeated oral intake of GeO_2 during six months, but a similar pathology did not appear with compound **A**.[16]

Our results confirm the experimental data of Asai *et al.* and lead to the conclusion of a relative low toxicity for compound **A** after repeated oral intake in rats. Biochemical and histological data demonstrated renal disease after six months' uptake, as for GeO_2. However, Sanai *et al.*[17] did not observe any renal dysfunction but their experimentation lasted for only 10 weeks. The authors suggest that this difference was linked to the low residual amount of germanium found in the kidneys in the case of chronic ingestion of compound **A**.

It is also true that a pharmacokinetic study carried out on rabbits, the results of which will be published soon has demonstrated that GeO_2 presents a better biodisposability (10%) by oral intake than compound **A** (2.6%). This poor biodisposability explains the low tissue contents in all viscera (Table 4) in so far as germanium diffuses in the blood but quickly passes out with urine.

If the pharmacological efficiency of carboxyethylgermanium sesquioxide (**A**) and its low toxicity seem to have been experimentally proved, further investigations on its biodisposability and medicinal virtues in man will probably be undertaken.

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REFERENCES

1. Haguenoer, J M and Furon, D *Toxicologie et Hygiène Industrielles*, 1st edn, Technique et Documentation, Paris, 1982, pp 7–15.
2. Furst, A *Toxicol. Ind. Health*, 1987, 3: 167
3. Asai, K *Report of Asai Germanium Research Institute*, 1972
4. Asai, K *Miracle Cure Organic Germanium*, Japan Publications, Tokyo, 1980.
5. Venugopal, B and Luckey, T D In *Chemical Toxicity of Metals and Metalloids*, Vol 2, *Metals Toxicity in Mammals*, Plenum Press, New York, 1978
6. Kada, T, Mochizuki, H and Miyao, K *Mutat. Res.*, 1984, 125: 145
7. Kumano, N, Nakai, Y, Ishikawa, T *et al* Sci. Rep. Res. Inst. Tohoku Univ. Med., 1978, 25 (3–4): 89
8. Caujolle, F, Huron, R and Moulas, F *Ann. Pharm. Fr.*, 1966, 24: 23
9. Kidd, P M *Int. Clin. Nutr. Rev.*, 1987, 7(1): 11
10. Gus'kova, E I *Gig. Tr. Prof. Zabol.*, 1974, 18: 56
11. Tesarik, B *Pracovni Lekarstvi*, 1971, 23: 370
12. Asai, K *J. Appl. Nutr.*, 1977, 29: 53
13. Aso, H, Suzuki, F, Yamaguchi, T, *et al J. Biol. Response Mod.*, 1989, 8 (2): 180
14. Hachisu, M., Takahashi, H and Koeda, T *J. Pharmacobiol. Dyn.*, 1983, 6: 814
15. Okuda, S, Kiyama, S, Oh, Y, *et al Curr. Ther. Res.*, 1987, 41(2): 265
16. Sanai, T, Okuda, S, Onayam, K *et al Nephron.*, 1990, 54(1): 53
17. Shinogi, M, Masaki, T and Mori, I *J. Trace Elem. Electrolytes Health Dis.*, 1989, 3: 25
18. Jao, S W, Lee, W and Ho, Y S *Dis. Colon. Rectum* 1990, 33(2): 99

19. Kumano, N *Tohoku J. Exp. Med.*, 1985, 146: 97
20. Sato, I, Yuan, B D and Nishimura, T *J. Biol. Response Modif.*, 1985(4): 159
21. Aso, H, Suzuki, F and Yamaguchi, T *Microbiol. Immunol.*, 1985, 29: 65
22. Miyao, K and Tanaka, N *Drugs in the Future* 1988, 12 (5): 441
23. Orimo, H and Agakuchi, I *J. Med. Pharm. Sci.*, 1983, 9 (5): 1507
24. Masaki, Y, Kumano, K, Iwamura, M, *et al Transplant. Proc.*, 1989, 21: 1250
25. Higuchi, I, Izumo, S, Kuriyama, M, *et al Acta Neuropathol.*, 1989, 79(3): 300
26. Tomizawa, S, Suguro, N and Kagoshima, M, *Oyo Yakuri*, 1978, 16: 671–682
27. Nakayama, S, Tsuji, Y and Usami, K *Showa Igaku Zasshi*, 1986, 46: 227
28. Hatano, M, Ishimura, K, Fuchigami, K *et al Oyo Yakuri*, 1981, 21: 773–796