CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

21-749

21-751

PHARMACOLOGY REVIEW

Review and Evaluation of Pharmacology and Toxicology Data*

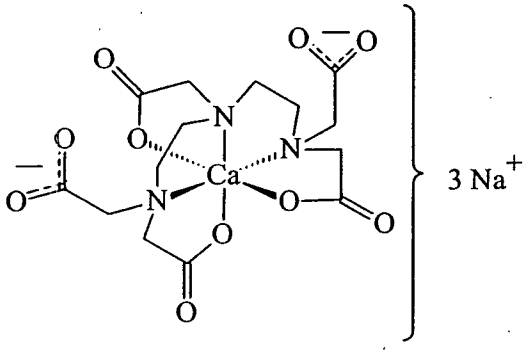
Division of Medical Imaging and Radiopharmaceutical Drug Products
HFD-160

Reviewer: Adebayo, A. Laniyonu, Ph.D.

Chemical Name: Trisodium calcium diethylenetriaminepentaacetate

CAS Number:

Structure:



Molecular Weight: 497.40

Relevant IND's: 4041;

Drug Class: Chelating agents

Indication: Enhancement of the excretion of trans-uranium elements (plutonium, americium, curium,) from the body

Clinical Formulation (and components):
1g/vial, total volume 5.0 mL.

Route of Administration/dose: 1g, intravenous, Inhalation

* This review of Ca-DTPA is a companion review for Zn-DTPA. Both are recommended for approval with Ca-DTPA as initial dose followed by Zn-DTPA for maintenance. Please refer to my review of Zn-DTPA dated 01/24/2003.

Executive Summary

Recommendations

Ca-DTPA is recommended for approval from preclinical pharmacology and toxicology perspective. It is indicated for enhancement of the excretion of actinide trans-uranium elements (plutonium, americium, curium, [redacted]) in patients internally contaminated with the isotopes.

Recommendation for post-approval non-clinical studies:

None.

Recommendation on labeling:

Please refer to label as written by the FDA review team.

Summary of Nonclinical Findings

Trans-uranium (TU) elements are elements with atomic number higher than uranium. These elements are products of nuclear reactors or particle accelerators and do not exit naturally. TU include americium, [redacted] curium, [redacted] and plutonium. TU enters the body principally through inhalation or contamination of punctured wounds or skin abrasions. Following a TU element exposure, the initial treatment objective is to stop the transfer from the site of deposition to internal organs especially bone and liver and other body tissues.

To this end, chelation therapy with trisodium calcium diethylenetriaminepentaacetate (Ca-DTPA), which is the primary focus of this review, is one of the modalities proposed to be effective in reducing TU body burden. Chelating agents are compounds that react with metallic ions to form stable complexes called metal chelates. Chelate is derived from the Greek word for great claw, "chela" conceptualizing that a chelator resembles the great claws of crustaceans grasping the metal ion. Thus as an example, Ca-DTPA effectively exchanging calcium for another metal of greater binding power such as americium or plutonium. As chelates, the characteristic chemical and eventually biological properties of the TU metal ions are masked. The metal-DTPA complexes are excreted in the urine thereby reducing the biological half-life. Ideally, a decorporation agent should be metabolically stable, reach effective concentration in critical organs, bind the specific toxic metal selectively and with high affinity, and excreted rapidly.

Pharmacology:

The main pharmacological effect of Ca-DTPA is an enhancement of the excretion of the TU elements (Plutonium, americium, curium, [redacted]) from the body. Most of the pharmacology studies reviewed for the chelation experiment focussed on either immediate or time-delayed administration of Ca-DTPA to reduce nuclide burden resulting from i.v. or ip injection of mostly soluble actinides. Chelation effectiveness was dependent on chelate dose and the time interval between nuclide contamination and chelator administration, being more effective when given immediately following exposure. This is due to the fact that TU are subject to translocation to different body organs and become less available with time. Moreover with immediate administration,

Ca-DTPA is able to chelate circulating TU thereby reducing net organ exposure to radiation (see representative tables 1 and 2).

Time of treatment	*Treatment*	*Number of rats*	*Percentage of injected ^{242}Cm[b]*		
			Skeleton[a]	*Liver*	*Kidneys*
1.5 min	NaCl	10	23.1 ± 0.7	38.2 ± 1.5	0.65 ± 0.04
	CaDTPA	5	4.9 ± 0.3	4.7 ± 0.7	0.16 ± 0.02
	ZnDTPA	4	8.8 ± 0.8	10.2 ± 1.5	0.21 ± 0.003
1.5 hr[d]	CaDTPA	4	14.1 ± 0.6	12.5 ± 1.0	0.33 ± 0.03
	ZnDTPA	4	17.2 ± 0.8	16.9 ± 0.8	0.38 ± 0.03
1 day	NaCl	10	22.1 ± 0.8	33.3 ± 1.7	0.59 ± 0.03
	CaDTPA	4	18.9 ± 0.8	17.4 ± 0.9	0.51 ± 0.03
	ZnDTPA	5	18.8 ± 1.1	15.3 ± 1.9	0.38 ± 0.02
2 days	NaCl	5	20.8 ± 0.6	22.2 ± 1.6	0.48 ± 0.04
	CaDTPA	5	14.4 ± 0.7	9.4 ± 1.1	0.35 ± 0.03
	ZnDTPA	5	15.8 ± 0.4	10.4 ± 0.6	0.36 ± 0.02
3 days	NaCl	5	18.0 ± 0.2	21.9 ± 1.9	0.51 ± 0.03
	CaDTPA	5	16.0 ± 0.8	10.6 ± 0.8	0.39 ± 0.03
	ZnDTPA	5	15.8 ± 0.7	11.7 ± 0.7	0.38 ± 0.03
4 days	NaCl	5	22.0 ± 0.6	26.9 ± 0.5	0.59 ± 0.02
	CaDTPA	5	18.8 + 0.6	12.5 ± 0.5	0.40 ± 0.01
	ZnDTPA	5	21.2 ± 0.8	16.3 ± 1.5	0.38 ± 0.01

Table 1: Influence of time interval between ^{242}Cm-citrate injection and a single injection of DTPA in reducing ^{242}Cm organ content. The table showed the percentage of injected dose remaining in skeleton, liver and kidneys seven days after chelator administration.

Treatment	*Chelate amount (μmole/kg)*	*Percentage of injected ^{242}Cm[a]*		
		Skeleton[b]	*Liver*	*Kidneys*
NaCl	—	23.3 ± 1.2	38.9 ± 2.7	0.71 ± 0.05
CaDTPA	10	6.64 ± 0.24	8.92 ± 0.51	0.19 ± 0.01
ZnDTPA	10	10.9 ± 0.7	15.5 ± 1.6	0.23 ± 0.02
CaDTPA	100	3.81 ± 0.18	1.45 ± 0.44	0.14 ± 0.01
ZnDTPA	100	5.71 ± 0.37	3.22 ± 0.34	0.20 ± 0.05
NaCl	—	22.9 ± 0.8	37.5 ± 1.5	0.59 ± 0.06
CaDTPA	30	4.90 ± 0.27	4.71 ± 0.66	0.16 ± 0.02
ZnDTPA	30	8.80 ± 0.76	10.2 ± 1.5	0.21 ± 0.01
CaDTPA	1000	2.34 ± 0.19	0.22 + 0.02	0.12 ± 0.01
ZnDTPA	1000	4.83 ± 0.79	0.37 ± 0.06	0.14 ± 0.01

Table 2: Dependence of DTPA effectiveness on dosage (single treatment 1.5 minute after ^{242}Cm-citrate administration). The table showed the percentage of injected dose remaining in skeleton, liver and kidneys seven days after chelator administration.

Results from several studies demonstrate that when therapy is initiated promptly, both Ca- and Zn-DTPA are effective, with Ca-DTPA being more effective than Zn-DTPA.

With delayed treatment, both chelators were not as effective, moreover success with delayed treatment is organ selectivity, with Ca-DTPA able to remove more plutonium from the liver compared to the bone. In general, the order is, Liver > kidneys > skeleton.

These results illustrated the importance of early intervention following exposure to a TU compound and support the clinical practice of preferring Ca-DTPA to Zn-DTPA as the first dose in case of chronic chelation therapy. For long term therapy Zn-DTPA can be substituted for CA-DTPA since on an equimolar basis, the efficacy of Ca-DTPA was not greater than that of Zn-DTPA with delayed treatment. Moreover, Zn-DTPA is less toxic compared to Ca-DTPA (please see toxicology summary).

Study results also emphasized the importance of not fractionating Ca-DTPA dose. Dose fractionating (divided doses administered on the same day even though the total amount injected corresponded to a single dose) in beagle dogs and rats led to increase toxicity (See paper by Lloyds et. al. 1976, Health Physics 31, 281-284). The toxicity is believed to reflect increase excretion of zinc and manganese from the body. Similar toxicity was not observed when the dose of Zn-DTPA was fractionated. The same publication also showed that efficacy depended on the treatment duration. Zn-DTPA completely removed ^{241}Am from the liver and substantially reduces skeletal burden when treatment was continued for two years.

The study by Cohen and colleagues (Cohen et.al. 1976, enhancement of ^{241}Am excretion by intravenous administration of Ca-DTPA in man and baboon) compared the responses to Ca-DTPA therapy of humans with a non-human primate. Age appears to play a role in the response to Ca-DTPA therapy in both humans and baboons. In a situation where treatment was delayed (13 months in baboons and 12 years in humans), Ca-DTPA was more effective in increasing americium excretion in both juvenile baboon and adolescent human compared to adults. By this time, most of the body americium burden is located in the skeleton. It is reasonable to infer that the difference in response might be due in part to active bone development in young baboon and juvenile humans. The miniscule amount of americium excreted in adult human in response to therapy makes one to wonder about the utility of treatment after a delay of 12 years. Whether similar age difference in response to decorporation of other TU element or in case of immediate treatment will occur is not clear. Similar study was not conducted for Zn-DTPA.

TABLE III. TOTAL BODY CONTENT OF ^{241}Am IN MAN[a]

Age	Before Most Recent Chelation Therapy[b] (nCi)	After Most Recent Chelation Therapy (nCi)
Adult	69.6±2.7[c]	67.2±2.8
Adolescent	20.1±1.6	12.7±2.7

[a] Measured by external *in vivo* gamma scintillation techniques using meter arc geometry and NaI(Tl)-CsI(Tl) scintillation detectors.

[b] Most recent chelation therapy was in 1975. Two previous periods of DTPA therapy occurred in 1970 and 1973.

TABLE IV. EFFECT OF SUBJECT AGE ON THE EXCRETION OF ^{241}Am AS PROVOKED BY Na$_3$(Ca-DTPA) CHELATION THERAPY[a] IN BABOONS

Animal Number	Age	Therapy Initiation (Months after Exposure)	Percent of Body Burden[b] Excreted During Chelation Period		
			Urine	Feces	Total
B-400	Juvenile (DTPA-Treated)	1.6	28.7	5.1	33.8
B-406	Juvenile (Control)	1.6	0.7	3.5	4.2
(B-400)-(B-406)	Juvenile (Net)	-	28.0	1.6	29.6
B-520	Adult (DTPA-Treated)	1.5	12.0	9.3	20.3
B-352	Adult (Control)	1.5	0.8	5.4	6.2
(B-520)-(B-352)	Adult (Net)	-	11.2	3.9	15.1

[a] After 10-12 chelation treatments in about one month.
[b] Body burden determined immediately prior to chelation.

The baboon study provided an opportunity to evaluate the variation in body deposition of americium with time. The next day after baboon contamination, 60% of the total americium body burden was associated with soft tissues, at 1 ½ months after nuclide exposure, 21 % was associated with soft tissues, by 13 months only 5 % was associated with soft tissues with the remaining being associated with the skeleton. The diminishing effectiveness of Ca-DTPA with time clearly demonstrates the need to initiate therapy as soon as practicable following exposure to a trans-uranium element. Ca-DTPA treatment led to an increase in urinary excretion of zinc, and to a concomitant decrease in erythrocytic aminoevulinic acid dehydratase (ALAD) activity. Zinc is an essential requirement of many metalloenzymes including DNA and RNA polymerase. The teratological effects of Ca-DTPA have been linked to its reduction of body zinc level.

The study by Morin and colleagues (Morin et.al. 1973, Health Physics 24, 311-315) showed that Ca-DTPA was not effective in the treatment of neptunium contamination. The authors ascribed the ineffectiveness to the instability of the ^{237}Np-DTPA complex in vivo. Zn-DTPA is not expected to be effective either, since ^{237}Np-DTPA complex is unstable in vivo.

Most of the studies reviewed used Ca- or Zn-DTPA enhancement of the excretion of TU elements as a proxy for efficacy, with a tacit assumption that a reduction in total body burden of TU element will lead to a reduction in risk from cancer. The question of whether lowering nuclide burden could prolong survival, eliminate or diminish the risk of induction or at least substantially increase its latent period has been addressed only sparingly. In this regard, the study by Bruenger and colleagues (Bruenger et.al. 1991. Int. J. Radiation Biol 60, 803-818) readily came to mind.

In the study, dog survival and the latent period for bone tumor formation was evaluated. Both Ca- and Zn-DTPA were used for the study, however because of toxicity associated with protracted treatment with Ca-DTPA, the animals received more Zn-DTPA treatment

compared to Ca-DTPA. By day 138 of treatment, only 19 Ca-DTPA treatment were given compared to 131 for Zn-DTPA.

In the study, mean skeletal dose to death with bone marrow was about 2.5 Gy for both Ca- and Zn-DTPA-treated dogs compared to 7.1 Gy in the non-chelated controls. However, the Zn-DTPA group received the dose over a longer period of time 3520 days vs 1636 days for Ca-DTPA group, resulting in a lower dose rate for the Zn-DTPA group. The lower dose rate resulted in a substantial increase in latent period between plutonium exposure and death with bone marrow cancer. Translated into beagle life span, without chelation or following Ca-DTPA treatment, the dogs died at about six years of age, while those treated with Zn-DTPA survived to age 11 (normal life expectancy of beagles is about 14 years). Ca-DTPA reduced organ plutonium burden. However, all 3 dogs in the long-term study died from bone cancer with average time to death of 1636±162 days. Time to death was not statistically different from controls.

Pharmacokinetics:

A comprehensive review by Volf (1978, treatment of incorporated trans-uranium elements; IAEA Technical Report) indicated that DTPA is poorly absorbed from the gastrointestinal tract (3-5%), about 20-30% is absorbed from the lungs while intraperitoneal and intramuscular absorption is complete and rapid. The distribution volume is identical with extra-cellular water. The $t_{1/2}$ in rats is about 20-40 minutes. A small fraction is eliminated more slowly from the blood. DTPA is excreted via the kidney mainly by glomerular filtration, and to a lesser extent in feces. According to Volf, DTPA is not subject to metabolic degradation.

Toxicology

The toxicity of Ca-DTPA is highly dependent on the dose, and whether the dose is fractionated or not. Fractionating doses increased lethality in both dogs and rats. Increased lethality was also seen with Ca-DTPA infusion. Cause of death has been ascribed to rapid depletion of zinc from the body. No toxicity was associated with fractionating or infusing Zn-DTPA. Study by — and colleagues showed that in rat, there was no significant toxicity when X0.5 MHD (based on body surface area) of the human dose was injected intraperitoneally twice a week for 44 weeks.

Ca-DTPA inhalation produced transitory vesicular emphysema and transitory epitheleal atypia in rats that resolved with cessation of treatment.

Ca-DTPA is embryo toxic in both dogs and mice. When pregnant dogs were administered Ca-DTPA at 14mg/kg (x 0.5MHD) beginning day 15 after first mating until day of parturition. The pups birth weights were lower compared with colony controls. All pubs were born with abnormal silver–gray hair coloration that was never observed in any of the 2000 pups born in the colony. The pigmentation was temporary as normal coloration returned after weaning. Lissencephally was noted in two of the dead pups. There was no maternal toxicity. Zn-DTPA was not studied.

TABLE 1

Ca-DTPA injection schedule and fetal mortality

Injection days	Daily dose Ca-DTPA	No. litters	Fetuses (resorbed + dead)/ total	Live fetal wt. (g)
	μmol/kg			*(x̄ ± SD)*
2-6	1,440	5	(8+0)/43 = 19%	1.02 ± 0.12
	720	6	(12+2)/62 = 23%	1.13 ± 0.18
	0	5	(3+0)/43 = 7%	1.14 ± 0.10
7-11	1,440	2	(3+2)/16 = 31%	1.11 ± 0.07
	720	7	(6+3)/61 = 15%	1.01 ± 0.13
	0	7	(2+1)/39 = 8%	1.18 ± 0.15
12-16	2,880	8	(13+5)/79 = 23%	1.07 ± 0.11
	1,440	6	(3+1)/45 = 9%	1.14 ± 0.14
	720	4	(1+1)/28 = 7%	1.25 ± 0.12
	0	7	(3+0)/47 = 6%	1.11 ± 0.12

In mice injected with very high doses of Ca-DTPA or Zn-DTPA (X2-7.8 MHD), Ca-DTPA increased fetal mortality with greater sensitivity during early and mid gestation. Resorption occurred more frequently than dead formed fetuses. The frequency of gross malformation increased with dose, with highest susceptibility in early and mid gestation. Zn-DTPA did not produce similar effects.

Gross malformations among fetuses not removed for alizarin staining

Injection days	Daily dose Ca-DTPA	Fetuses malformed/ examined	Malformations observed
	μmol/kg		
2-6	1,440	2/25 = 8%	exencephaly with ablepharia, ablepharia
	720	1/38 = 3%	exencephaly, spina bifida aperta, ablepharia, cleft palate
	0	0/30 = 0%	
7-11	1,440	2/9 = 22%	exencephaly, exencephaly with ablepharia
	720	0/38 = 0%	
	0	0/27 = 0%	
12-16	2,880	2/46 = 4%	polydactyly
	1,440	1/24 = 4%	polydactyly
	720	0/20 = 0%	
	0	0/31 = 0%	

The authors concluded that Ca-DTPA is teratogenic and ascribed the toxic effects of Ca-DTPA to zinc depletion.

Overall Conclusion:

In conclusion, the issue of whether pharmacology/toxicology studies typically conducted and considered critical to support the safety of an NDA have been reported in the literature was examined for Ca-DTPA. Overall data from the literature provided substantial support for the effectiveness of Ca-DTPA in enhancing the excretion of TU elements from the body.

Introduction/Drug History:

Trans-uranium (TU) elements are elements with atomic number higher than uranium. These elements are products of nuclear reactors or particle accelerators and do not exit naturally. TU include americium, curium, neptunium and plutonium. These heavy elements are all actinides, occupying the bottom of the periodic table. In terms of amount available, projected usage, extent of anticipated accidental human exposure and radiotoxicity, plutonium is the most formidable TU element.

Trans-uranium (TU) elements enter the body principally through inhalation or contamination of punctured wounds or skin abrasions. Ingestion is usually of minor importance because of poor absorption from the gastrointestinal tract. However, a considerable amount of inhaled radioactivity is transferred from the pulmonary tree to the gastrointestinal tract by normal respiratory system clearing mechanisms. The kinetics of absorption, distribution and excretion of a given TU depend on its chemical and physical properties, for example, metal and high fired oxides are less soluble than nitrates and perchlorates hence less well absorbed from the site of deposition. Insoluble or sparingly soluble particulate will remain at the entry site for long period of time or migrate within the body as particulate matter resulting in the formation of local hot spots. From the primary site of entry, a fraction of TU is translocated (absorbed) into the blood stream and deposited in body organs. The limiting step for redistribution is the rate of dissolution from the primary deposition pool. These elements are subject to continued translocation within various body compartments especially the bone leading to protracted influx into the organ and increasing difficulty to remove as time passes thus reducing treatment efficacy.

Following a TU element exposure, the initial treatment objective is to stop the transfer from the site of deposition to internal organs especially bone and liver and other body tissues. Therefore prevention of initial entry into the blood stream is of immediate essence. This can be achieved by decontamination procedures including chemically or mechanically cleansing of the local deposit site. In view of the fact that TU elements are not readily absorbed from the gastrointestinal tract, treatment of ingested TU should be aimed at shortening intestinal transit time for these elements in order to reduce local radiation toxicity. After absorption of TU into the systemic circulation, the focus of treatment should be to minimize their translocation and deposition in tissues and to enhance their elimination from the body. To this end, chelation therapy with trisodium calcium diethylenetriaminepentaacetate (Ca-DTPA), which is the primary focus of this review, is one of the modalities proposed to be effective in reducing TU body burden.

Chelating agents are compounds that react with metallic ions to form stable complexes called metal chelates. Chelate is derived from the Greek word for great claw, "chela" conceptualizing that a chelator resembles the great claws of crustaceans grasping the metal ion. Thus as an example, Ca-DTPA effectively exchanges calcium for another metal of greater binding power such as americium or plutonium. As chelates, the characteristic chemical and eventually biological properties of the TU metal ions are masked. The metal-DTPA complexes are excreted in the urine thereby reducing the biological half-life. It is essential that both chelating agent and the complex formed be readily excreted so as to avoid their redistribution or precipitation within the kidneys. The effectiveness of a chelating agent can be evaluated in terms of its Influence on the metabolic behavior of the metal, especially enhancement of its excretion and diminution

of its toxicity. The toxic side effects of the chelating agent at therapeutic doses are also of important consideration.

While there are many published preclinical pharmacology studies concerning the utility of Ca-DTPA to enhance the excretion of various TU elements from animals, there is paucity of human data. However, Ca-DTPA is a common formulation component in FDA-approved nuclear medicine products. Oak Ridge Associated Universities (ORAU) currently manages IND 4,041 for Ca-DTPA on behalf of U.S. Department of Energy (DOE) that provided case reports of industrial decontamination responses.

As with any other radionuclide, the treatment objective is rapid removal of radioactivity from the body and reduction of effective half-life. The purpose of this survey is to review the available scientific literature in an attempt to examine the scientific basis of use and, to determine whether Pharmacology/Toxicology studies typically conducted and considered critical to support the safety of an NDA application have been reported in the literature. The experiments upon which the scientific evidences are based were not conducted with an NDA format in mind. Nevertheless, most of the articles were published in peer reviewed journals and most of the findings were reported from multiple laboratories. This review aims to (1) identify the concepts that are scientifically valid and that appear to have general support within the scientific community and (2) Identify the areas where more information is required.

Previous clinical experience:

The search did not reveal any article that solely addresses the safety or included adequate safety monitoring. No serious adverse reaction to Ca-DTPA has been documented at clinically relevant doses (see medical officer review).

Pharmacology:

Pharmacological Studies:

Takeda, K. & Volf, V. (1977): Comparison of the effectiveness of Ca-DTPA and Zn-DTPA in removing ^{242}Cm from the rat. Radiation Research 70, 164-172

The study evaluated the effectiveness of Ca-DTPA and Zn-DTPA in removing curium-242, (^{242}Cm) from the rat as a function of the amount of chelate and the time interval between ^{242}Cm injection and treatment by a single injection of DTPA.

Female albino rats (180-210g) were used for the study. ^{242}Cm (~ 1.5 µCi/kg in 0.25 ml) as the citrate was injected into the tail vein of each animal. Single ip injection of 30 µmole/kg (X 0.162 MHD based on body surface area (BSA)) of Ca-DTPA or Zn-DTPA were administered at 1.5 min, 1.5 hr, or 1, 2, 3, or 4 days after iv injection of ^{242}Cm citrate. The objective was to evaluate chelate effectiveness as a function of time interval between ^{242}Cm and DTPA. In another experiment, single injection of 10, 30, 100, or 1000 µmole of Ca-DTPA or Zn-DTPA/kg (X 0.05 - 5 MHD based on BSA) were administered 1.5 min or 1 day after iv injection of ^{242}Cm. The experiment evaluated chelate effectiveness as a function of the amount of DTPA administered at two different time intervals after ^{242}Cm. For all experiments, the animals were sacrificed 7 days after administration of DTPA. Tissue radioactivity was measured by scintillation counting.

Results and conclusions:

As shown in table 1, the effectiveness of both Ca-DTPA and Zn-DTPA in reducing ^{242}Cm-organ content was highest when administered 1.5 min (almost simultaneously) with ^{242}Cm. Efficacy was reduced when there was a delay (as short as 1.5 hr) between ^{242}Cm injection and the administration of DTPAs. Under conditions of early treatment, the effect of Ca-DTPA was significantly greater than that of Zn-DTPA. After 1 day, the effectiveness of both chelates was very similar.

Time of treatment	*Treatment*	*Number of rats*	*Percentage of injected ^{242}Cm[b]*		
			Skeleton[a]	*Liver*	*Kidneys*
1.5 min	NaCl	10	23.1 ± 0.7	38.2 ± 1.5	0.65 ± 0.04
	CaDTPA	5	4.9 ± 0.3	4.7 ± 0.7	0.16 ± 0.02
	ZnDTPA	4	8.8 ± 0.8	10.2 ± 1.5	0.21 ± 0.003
1.5 hr[d]	CaDTPA	4	14.1 ± 0.6	12.5 ± 1.0	0.33 ± 0.03
	ZnDTPA	4	17.2 ± 0.8	16.9 ± 0.8	0.38 ± 0.03
1 day	NaCl	10	22.1 ± 0.8	33.3 ± 1.7	0.59 ± 0.03
	CaDTPA	4	18.9 ± 0.8	17.4 ± 0.9	0.51 ± 0.03
	ZnDTPA	5	18.8 ± 1.1	15.3 ± 1.9	0.38 ± 0.02
2 days	NaCl	5	20.8 ± 0.6	22.2 ± 1.6	0.48 ± 0.04
	CaDTPA	5	14.4 ± 0.7	9.4 ± 1.1	0.35 ± 0.03
	ZnDTPA	5	15.8 ± 0.4	10.4 ± 0.6	0.36 ± 0.02
3 days	NaCl	5	18.0 ± 0.2	21.9 ± 1.9	0.51 ± 0.03
	CaDTPA	5	16.0 ± 0.8	10.6 ± 0.8	0.39 ± 0.03
	ZnDTPA	5	15.8 ± 0.7	11.7 ± 0.7	0.38 ± 0.03
4 days	NaCl	5	22.0 ± 0.6	26.9 ± 0.5	0.59 ± 0.02
	CaDTPA	5	18.8 + 0.6	12.5 ± 0.5	0.40 ± 0.01
	ZnDTPA	5	21.2 ± 0.8	16.3 ± 1.5	0.38 ± 0.01

Table 1: Influence of time interval between ^{242}Cm-citrate injection and DTPA administration in reducing ^{242}Cm organ content. The percentage of injected dose remaining in skeleton, liver and kidneys seven days after chelator administration. Same saline control used for both 1.5 min and 1.5hr time of treatment.

Table 2: Dependence of DTPA effectiveness on its amount (single treatment 1.5 minute after ^{242}Cm-citrate).

Treatment	*Chelate amount (μmole/kg)*	*Percentage of injected ^{242}Cm[a]*		
		Skeleton[b]	*Liver*	*Kidneys*
NaCl	—	23.3 ± 1.2	38.9 ± 2.7	0.71 ± 0.05
CaDTPA	10	6.64 ± 0.24	8.92 ± 0.51	0.19 ± 0.01
ZnDTPA	10	10.9 ± 0.7	15.5 ± 1.6	0.23 ± 0.02
CaDTPA	100	3.81 ± 0.18	1.45 ± 0.44	0.14 ± 0.01
ZnDTPA	100	5.71 ± 0.37	3.22 ± 0.34	0.20 ± 0.05
NaCl	—	22.9 ± 0.8	37.5 ± 1.5	0.59 ± 0.06
CaDTPA	30	4.90 ± 0.27	4.71 ± 0.66	0.16 ± 0.02
ZnDTPA	30	8.80 ± 0.76	10.2 ± 1.5	0.21 ± 0.01
CaDTPA	1000	2.34 ± 0.19	0.22 + 0.02	0.12 ± 0.01
ZnDTPA	1000	4.83 ± 0.79	0.37 ± 0.06	0.14 ± 0.01

Treatment	Chelate amount (μmole/kg)	Percentage of injected ^{242}Cm		
		Skeleton	Liver	Kidneys
NaCl	—	23.0 ± 1.3	32.9 ± 3.3	0.54 ± 0.05
CaDTPA	10	20.8 ± 1.0	19.7 ± 1.0	0.41 ± 0.01
ZnDTPA	10	18.6 ± 0.3	20.4 ± 0.8	0.43 ± 0.01
CaDTPA	100	17.6 ± 0.7	10.4 ± 1.2	0.40 ± 0.01
ZnDTPA	100	16.7 ± 0.9	9.0 ± 0.6	0.39 ± 0.03
NaCl	—	21.3 ± 0.6	33.7 ± 1.7	0.63 ± 0.04
CaDTPA	30	18.9 ± 0.8	17.4 ± 0.9	0.51 ± 0.03
ZnDTPA	30	18.8 ± 1.1	15.3 ± 1.9	0.38 ± 0.02
CaDTPA	1000	13.2 ± 0.6	4.0 ± 0.4	0.34 ± 0.03
ZnDTPA	1000	13.9 ± 0.9	4.4 ± 0.3	0.32 ± 0.02

Table 3:Dependence of DTPA effectiveness on its amount (single treatment 1 day after ^{242}Cm-citrate

The effect of the amount of chelate on the retention of ^{242}Cm in the organs of rat is shown in tables 2 and 3. Ca- or Zn-DTPA was injected 1.5 minute or 24 hours after ^{242}Cm. The content of ^{242}Cm in all the organs decreased as the amount of DTPA increased. Amount reduced was organ dependent.

	Skeleton	Liver	Kidneys
ZnDTPA/CaDTPA*	9.5 (8.5–10.5)	2.3 (1.7–3.3)	11.9 (10.8–13.2)

Table4: Ratio of equally effective ZnDTPA and CaDTPA molar doses in removing ^{242}Cm from the rat. Single DTPA injections were administered 1.5 minutes after ^{242}Cm

Reviewer's comments:

I agree with the study conclusion that Ca- and Zn-DTPA were more effective in reducing organ burden of curium when administered almost simultaneously with ^{242}Cm, effectiveness diminished considerably with longer time interval separating ^{242}Cm and chelate administration. This study illustrated the importance of early intervention following exposure to a trans-uranium compound. Chelator effectiveness was dose dependent. When treatment was started early, (less than 1 day), the effect of equimolar amount of Ca-DTPA was significantly greater than that of Zn-DTPA at all doses, suggestive that Ca-DTPA should be preferred over Zn-DTPA for the initial dose, provided that there are no contra-indicating factor for the use of Ca-DTPA. At time period greater that 24 hours, the effects of both chelates were practically the same. However single dose treatment did not completely eliminate organ burden for this element.

The ability of both Ca- and Zn-DTPA to reduce the organ burden for ^{242}Cm appears to be highest for the liver followed by the kidney, with the skeleton being the least susceptible. This imply that both Ca- and ZnDTPA were still able to mobilize ^{242}Cm from the liver even after translocation from the blood to the organs might have been completed.

These results support the concept that Ca-DTPA is preferred over Zn-DTPA in early phase of treatment because of its effectiveness at early time points. However, it is the view of this reviewer that the practicality of such early treatment intervention of

approximately 2 hours or so is questionable under wide spread disaster scenario. Thus if treatment with Zn-DTPA possess other advantages over Ca-DTPA that might compensate for its lower efficacy at early time points its use is more appropriate. The choice of Ca-DTPA over Zn-DTPA immediately following a nuclear incident remains a valid concept in situations of contained accidents such that might occur in accidental contamination at nuclear weapon facilities or at research laboratories where prompt treatment is possible. Other disaster scenarios should be considered on a case by case basis.

Lloyd, R.D., Mays, C.W., McFarland, S.S., Taylor, G.N. & Atherton D.R. (1976): A comparison of Ca-DTPA and Zn-DTPA for chelating ^{241}Am in Beagles. Health Physics, 31, 281-284.

The study evaluated the ability of Ca-DTPA and Zn-DTPA to remove firmly fixed body burden of Americium-241 (^{241}Am) burden in beagles.

Adult beagle dogs (n=7), were injected intravenously with 0.3μCi/kg of ^{241}Am (III) in a solution of citrate-citric acid buffer. Subcutaneous daily injection of Ca-DTPA or Zn-DTPA treatments either as single 30 μmole DTPA/kg/day (X 0.5 MHD BSA) or fractionated (up to five daily doses; ~30 μmole total dose) started two weeks after ^{241}Am injection. Treatment in two of the dogs was discontinued because of extreme toxicity (type not stated) encountered with frequent administration of Ca-DTPA. ^{241}Am content was determined by total body/excreta counting.

Results and conclusions:

1): ^{241}Am removal efficiency was not significantly different (table 1) for dogs that received a single daily injection of Zn-DTPA, 30 μmole /kg (dogs T108W3, T109W3) or Ca-DTPA, 30 μmole /kg (dog T103W3).

2): According to the author, the arm of the study administering five fractionated daily injections of Ca-DTPA was not completed due to toxicity complications (dogs T104W3 and T106W3) were discontinued after the third day while Zn-DTPA arm of the study (dogs T110W3 and T111W3) was completed. Comparison of the cumulative excretion of ^{241}Am for the first 3 days of treatment showed that decorporation efficiency for Zn-DTPA was significantly greater than that of Ca-DTPA (14.84% vs 6.58%).

3): Continued daily treatments with Zn-DTPA resulted in the removal of essentially all the ^{241}Am in liver by 1 year and about 80% of skeletal ^{241}Am by 2 years (table 2).

4): ^{241}Am excreted during the first week of treatment by both Ca-DTPA and Zn-DTPA was removed from the liver (table 2).

5): Untreated beagles excreted far less ^{241}Am compared to treated beagles.

Calcium-DTPA				
Days after ^{241}Am injection	Days of DTPA treatment	Dog T103W3	Dog T104W3	Dog T106W3
13-14	-1 to 0	0.07 (0)	0.070	0.09 (0)
14-15	0-1	1.38 (1)	1.883	0.90 (3)
15-16	1-2	3.10 (1)	2.945	3.14 (5)
16-17	2-3	2.16 (1)	2.515	1.72 (2)
17-18	3-4	2.40 (1)	1.600	6.16 (0)
18-19	4-5	1.34 (1)	0.540	1.21 (0)
19-20	5-6	1.47 (1)	0.710	1.57 (0)
20-21	6-7	1.01 (1)	0.190	0.78 (0)
Subtotal	0-3	6.64	7.33	5.7
Total	0-7	12.86	10.37	15.48

Zinc-DTPA					
Days after ^{241}Am injection	Days of DTPA treatment	Dog T108W3	Dog T109W3	Dog T110W3	Dog T111W3
13-14	-1 to 0	0.07 (0)	0.04 (0)	0.06 (0)	0.02 (0)
14-15	0-1	3.20 (1)	2.17 (1)	1.69 (5)	1.95 (5)
15-16	1-2	4.59 (1)	7.74 (1)	6.51 (5)	1.54 (5)
16-17	2-3	9.10 (1)	4.47 (1)	7.86 (5)	3.25 (5)
17-18	3-4	4.68 (1)	7.76 (1)	7.08 (5)	1.66 (5)
18-19	4-5	2.75 (1)	3.19 (1)	3.10 (5)	5.6 (6)
19-20	5-6	2.29 (1)	4.29 (1)	3.79(5)	1.50 (5)
20-21	6-7	1.33 (1)	2.18 (1)	2.31 (5)	2.10 (5)
Subtotal	0-3	16.89	14.65	16.06	11.74
Total	0-7	27.94	32.07	32.34	22.66

Table 1: ^{241}Am excretion by beagles before and during the first week of DTPA therapy (% of injected ^{241}Am excreted per day). Values in parentheses indicate the number of DTPA injections received in the 24-hr period preceding the collection of urine and feces. (0)= no DTPA; 1 = single injection of about 0.03 mmole DTPA/kg; (2), (3) or (5) = that number of injections each of about 0.006 mmole DTPA/kg. Between 100 and 200 ml of blood were removed from T104W3 on alternate days during this period. Another dog given no DTPA was subjected to the same schedule of blood removal.

Days after ^{241}Am Injection	Days after 1st Ca-DTPA treatment	Dog T103W3		Dog T104W3		Dog T106 W3		Control dog No DTPA	
		liver	Non liver	Liver	Non liver	Liver	Non liver	Liver	Non liver
14	0	51.6	37.6	47.7	33.7	48.2	38.6	50	40
21	7	37.7	38.7	40.3	30.9	34.6	38.2	49	39
~700								42	35

Table 2: ^{241}Am content in seven beagles just before and at various times after the beginning of Ca-DTPA treatment (% of injected ^{241}Am)

Days after ^{241}Am Injection	Days after 1st Zn-DTPA treatment	Dog T108W3		Dog T109W3		Dog T110 W3		Dog T111W3	
		liver	Non liver	Liver	Non liver	Liver	Non liver	Liver	Non liver
14	0	43.8	49.4	43.2	44.6	48.9	40.3	41.4	46.8
21	7	17.6	49.3	13.8	44.1	16.9	39.9	19.4	45
415	401	0	11.6	0	12.4	0	8.9	0	12.2
496	482	0	11.1	0	11.5	0	9.0	0	11.6
623	609	0	10.8	0	11.4	0	8.3	0	10.8
797	783	0	10.3	0	10.4	0	7.7	0	10.6

Table 2: ^{241}Am content in seven beagles just before and at various times after the beginning of Zn-DTPA treatment (% of injected ^{241}Am)

Reviewer's comments:

For this study, treatment was started two weeks after ^{241}Am treatment by which time ^{241}Am is already translocated into body organs and became fixed. Both Ca- and Zn-DTPA, were still effective in decorporation therapy. However, on an equimolar basis the efficacy of Ca-DTPA was not greater than that of Zn-DTPA. The study also indicated that Zn-DTPA was able to completely remove ^{241}Am from the liver, and substantially reduce (~ 80%) the ^{241}Am skeletal burden when treatment was continued for up to two years. Whether complete removal from bones was possible had treatment continued for longer than 2 years was not established. The toxicity of Ca-DTPA did not allow for comparative effectiveness of long term therapy to be made. Fractionating Ca-DTPA doses increased the toxic responses that culminated in early termination of scheduled dosage regimen. Although the reason for this increased toxicity was not explored in this study, it is widely believed to be due to increased excretion of zinc from the body. Fractionating the dose of Zn-DTPA did not lead to increase in toxicity. Comparing dog T103W3 that received Ca-DTPA with dog T108W3 that received Zn-DTPA showed that cumulative excretion of americium was significantly greater with Zn-DTPA treatment.

Seidel, A. (1976): Removal of ^{252}Cf and ^{241}Am from the rat by means of Ca-DTPA and Zn-DTPA. In diagnosis and treatment of incorporated radionuclide (Proc. Seminar Vienna, 1975) IAEA-SR-6/2

The study evaluated the effect of time between radionuclide injection and DTPA administration on the effectiveness of single or multiple doses of Ca- or Zn-DTPA as decorporating agent for californium (^{252}Cf) or americium (^{241}Am) in rats.

Female albino rats (175-205g) were used for the study. Monomeric ^{252}Cf citrate (~ 0.3-3 µCi/kg, depending on study design) was injected intravenously.

For the study evaluating chelate effectiveness as a function of DTPA dosage, Ca-DTPA or Zn-DTPA (10-1000 µmole/kg, X0.05-5 MHD BSA) was administered as single intraperitoneal injection at 1.5 min or 1 day after iv injection of ^{252}Cf.

For the study evaluating chelate effectiveness as a function of time interval between ^{252}Cf injection and DTPA treatment, single ip injection of 30 µmole (X .16 MHD BSA) of

Ca-DTPA or Zn-DTPA/kg was administered with time interval between 1.5 min and 64 days after iv injection of ^{252}Cf citrate.

Experiments with repeated DTPA administration were initiated either 1.5 min (early treatment) or on day 4 (late treatment) after radionuclide injection. For early treatment animals received 30 µmole of Ca- or Zn-DTPA/kg at 1.5 min, 24 hr, thereafter at weekly interval ending on day 64 after radionuclide injection. An additional group was treated with 30 µmole/kg Ca-DTPA after 1.5 min and 30 µmole/kg Zn-DTPA at 24 h, thereafter at weekly interval ending on day 71. Late treatment consisted of injecting 30 µmole/kg of Ca- or Zn-DTPA on days 4 followed by weekly injection until day 165.

For all experiments, the animals were sacrificed 7 days after the last administration of DTPA. Tissue radioactivity was measured by liquid scintillation counting.

Results and conclusions:

When administered immediately after ^{252}Cf administration, (1.5 min), Ca-DTPA was more effective than Zn-DTPA over the whole dose range. Although dose-dependent, effectiveness as a function of dose deviated from linearity especially for kidney and liver (Ca-DTPA) and for skeleton (Zn-DTPA) (Fig 1). For both Ca- and Zn-DTPA ^{252}Cf removal was less effective if treatment was delayed up to 24 hours after radionuclide injection.

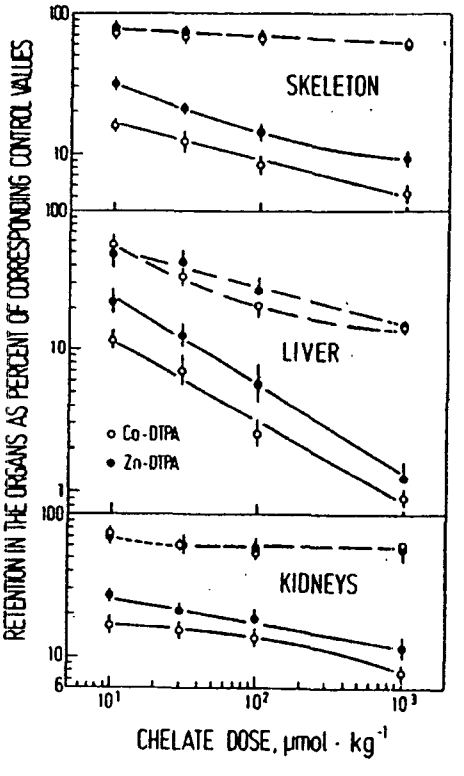


Fig 1: Effect of chelate dosage on the retention of ^{252}Cf in the organ of the rat. Chelates injected either at 1.5 min (solid lines) or 24 h (broken lines) after ^{252}Cf. Geometric means and fiducial limits 9P=0.05, n=4-6)

Time of treatment with DTPA	Skeleton		Liver		Kidneys	
	^{241}Am	^{252}Cf	^{241}Am	^{252}Cf	^{241}Am	^{252}Cf
1.5 min	9 5.7-14	3.5-6.8	3.4 2.7-4.4	2.8 2.4-3.3	8.2 3.5-24.3	4.5-12
24 hour	1.2 0.2 – 3.9	2.5 2.4 – 2.6	2.4 1.6 – 3.6	0.8-3.3	2.4	1.0

Table1: Relative potency defined as the ratio of equally effective Zn-DTPA: Ca-DTPA doses.

Chelate effectiveness decreases rapidly with increasing time interval between ^{252}Cf- and DTPA-injection (table 2). Up to the fourth day, DTPA efficacy diminishes in the order: Liver > Kidneys > Skeleton. When treatment was delayed for 64 days, 30% of the ^{252}Cf-kidney burden was removed by DTPA treatment. Whereas removal from the liver was less and that from the skeleton was virtually nil.

Time of Treatment	Treatment	Skeleton[a]	Liver	Kidneys	n
1.5 min	NaCl	44.40 ± 1.21	12.93 ± 1.06	1.30 ± 0.07	5
	Ca-DTPA	5.52 ± 0.37	0.93 ± 0.08	0.20 ± 0.01	6
	Zn-DTPA	9.47 ± 0.31	1.66 ± 0.13	0.27 ± 0.02	6
90 min	NaCl	38.00 ± 2.03	11.84 ± 0.75	1.41 ± 0.06	6
	Ca-DTPA	21.99 ± 0.72	2.68 ± 0.14	0.59 ± 0.03	6
	Zn-DTPA	25.53 ± 0.91	4.04 ± 0.29	0.65 ± 0.03	6
6 h	NaCl	45.53 ± 1.10	11.35 ± 0.68	1.21 ± 0.07	6
	Ca-DTPA	27.64 ± 1.17	3.40 ± 0.11	0.70 ± 0.04	6
	Zn-DTPA	33.14 ± 1.48	5.00 ± 0.49	0.80 ± 0.04	6
24 h	NaCl	47.93 ± 1.43	10.00 ± 0.64	1.12 ± 0.04	5
	Ca-DTPA	33.29 ± 1.28	3.38 ± 0.20	0.68 ± 0.02	6
	Zn-DTPA	35.75 ± 0.80	4.38 ± 0.30	0.70 ± 0.04	6
4 d	NaCl	42.31 ± 0.43	7.61 ± 0.80	1.24 ± 0.07	5
	Ca-DTPA	33.33 ± 0.86	3.96 ± 0.22	0.79 ± 0.04	5
	Zn-DTPA	36.02 ± 1.31	4.05 ± 0.49	0.82 ± 0.03	4
64 d	NaCl	38.57 ± 0.99	1.29 ± 0.09	0.44 ± 0.03[b]	8
	Ca-DTPA	37.27 ± 0.62	1.06 ± 0.11	0.31 ± 0.03	8
	Zn-DTPA	37.44 ± 1.07	1.08 ± 0.06	0.29 ± 0.01	8

Arithmetic means ± S.E.; n = number of animals per group. Animals sacrificed 7 d after DTPA administration.

a ^{252}Cf-activity of one femur x 20. b n = 7.

Table 2: Influence of time interval between i.v. ^{252}Cf-citrate injection and DTPA administration (30µmol/kg, i.p.) on the ^{252}Cf content of rat organs.

The results of the series with repeated DTPA injection are shown in table 3. When treatment started after 1.5 minutes, the fraction removed from the liver after 12 chelate treatment was markedly higher than from skeleton or kidneys. Zn-DTPA schedule II was less effective than Ca-DTPA, schedule I.

When Ca-DTPA was given as the first dose, followed by Zn-DTPA (schedule III) the results were identical with those of Ca-DTPA alone in all organs.

Compared to 6 doses, 12 doses produced statistically significant results in the skeleton and liver. 6 doses produced maximum effect in the kidney. For all treatment schedules, efficacy of repeated chelate injection is considerably reduced if treatment did not start until day 4 (not shown).

			Ca-DTPA		Zn-DTPA		Ca- followed by Zn-DTPA	
No. of dose	NaCl	n	I	n	II	n	III	n
			Skeleton[a]					
1	44.40 ± 1.21	5	5.52 ± 0.37 (12)	6	9.47 ± 0.31 (21)	6	-	
6	43.85 ± 1.45	6	3.71 ± 0.17 (8)	6	7.19 ± 0.53 (16)	6	3.67 ± 0.24 (8)	6
12	41.35 ± 1.20	5	2.67 ± 0.15 (6)	6	4.82 ± 0.17 (12)	6	2.71 ± 0.11 (7)	6
			Liver					
1	12.93 ± 1.06	5	0.93 ± 0.08 (7)	6	1.66 ± 0.13 (12)	6	-	
6	2.32 ± 0.15	6	0.074 ± 0.008 (3)	6	0.17 ± 0.02 (7)	6	0.088 ± 0.005 (4)	6
12	1.76 ± 0.08	5	0.038 ± 0.004 (2)	6	0.076 ± 0.006 (4)	6	0.048 ± 0.005 (3)	6
			Kidneys					
1	1.30 ± 0.07	5	0.20 ± 0.009 (15)	6	0.27 ± 0.02 (21)	6	-	
6	0.53 ± 0.02	6	0.032 ± 0.004 (6)	6	0.063 ± 0.006 (12)	6	0.034 ± 0.003 (6)	6
12	0.37 ± 0.02	5	0.017 ± 0.003 (5)	6	0.041 ± 0.004 (11)	6	0.022 ± 0.003 (6)	6

Arithmetic means ± S.E., n = number of animals. Values in brackets indicate percentage of control values. For explanation of treatment schedules I - III see methods; all animals sacrificed 7 d after last DTPA injection. [a] ^{252}Cf-activity of one femur x 20.

Table 3: Influence of repeated 1.9 injections of Ca-DTPA or Zn-DTPA on the retention of i.v. administered monomeric ^{252}Cf-citrate in the rat organs (1st injection 1.5 min after ^{252}Cf, % of injected ^{252}Cf dose)

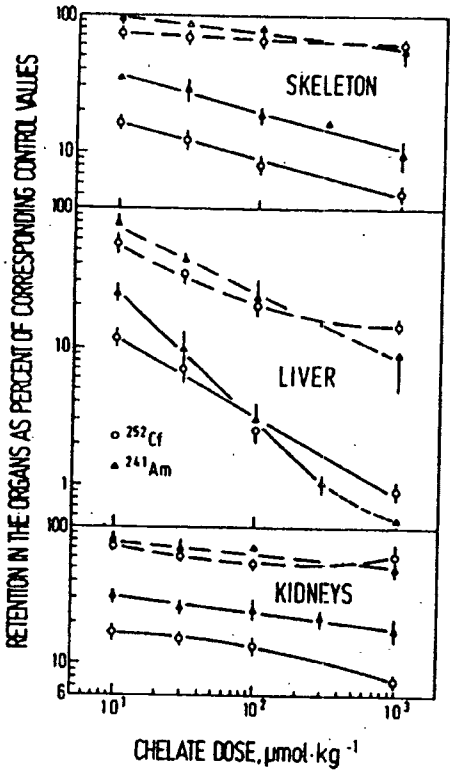


Fig 2: Comparison of the effect of chelate dosage on the retention of ^{241}Am and ^{252}Cf in the organs of the rat. Ca-DTPA was injected either 1.5 min (solid lines) or 24 hours (broken lines) after radionuclides.

Reviewer's Comments:

I agree with the study conclusions that the effectiveness of chelate therapy is dose dependent, and diminishes with increasing time interval between radionuclide injection and onset of DTPA administration. That the efficacy of Zn-DTPA injected 1.5 minutes after radionuclide administration was less than that of Ca-DTPA. When a time period of at least 24 hours separated nuclide and chelate injection, there was no difference in effectiveness. In long term administration of DTPA, If first treatment was Ca-DTPA and the remaining doses were Zn-DTPA, the total removal was the same as for Ca-DTPA alone through out treatment. Overall efficacy was dependent on the deposition site of the contaminant in the body.

Interestingly, there was no report of toxic effect of Ca-DTPA despite the long treatment period. This is in contrast to the study of Lloyds et al. in beagle dogs where treatment was stopped when beagles were given fractionated doses of up to five times daily compared with once a week in this treatment schedule for long term therapy.

Cohen, N. Wrenn, McD . E. Guilmette, R.A. Lo Sasso T. (1976): Enhancement of ^{421}Am excretion by intravenous administration of Na_3(Ca-DTPA) in man and baboon. In Seminar on the diagnosis and treatment of incorporated radionuclids Proceedings of an International seminar organized by the Atomic Energy Agency.

The study compared the responses of humans with that of a non-human primate to Ca-DTPA therapy of well-established burden of ^{241}Am.

Table 1: Summary of experimental parameters

Order	Primate	Primate
Genus & Species	Man (*Homo sapiens*)	Baboon (*Papio "kenya"*)[a]
Sex	Male	Female
Number & Age[b]	a) 1 adult (62 yrs) b) 1 adolescent (15 yrs)	a) 6 adults (11-14 yrs) b) 2 juveniles (4-5 yrs)
Internal Contaminant	^{241}Am	^{241}Am
Chemical Form	Hydroxide, "apparent"[c]	Citrate
Exposure Route	Inhalation (Ingestion)	Intravenous
Duration of Exposure	Chronic	Acute (instantaneous)
Body Burden[b]	a) Adult (70 nCi) b) Adolescent (20 nCi)	a) Adult (2-3 μCi) b) Juvenile (0.6-0.8 μCi)
Age of ^{241}Am Burden[b]	12 yrs	1 day to 2 yrs
Chelating Agent Employed	Na_3(Ca-DTPA)	Na_3(Ca-DTPA)
Mode of Administration	IV-infusion	IV-infusion
Therapy Regimen[b]	Single injection per week for 4 weeks	Triweekly injections for 4 weeks
Dose of DTPA	a) Adult-23.3 μmoles/kg/treatment b) Adolescent-41.8 μmoles/kg/treatment	28.7 μmoles/kg/treatment
Measurement Techniques & Samples Collected	*In vivo* whole body (external) and excreta bioassay	*In vivo* whole body (external), excreta bioassay, tissue biopsy and sacrifice

In addition, zinc was measured in urine by standard atomic absorption spectrophotometry and x-ray fluorescence techniques. Erythrocytic aminoevulinic acid dehydratase (ALAD) was also determined.

Results and conclusions:

1): The efficacy of chelation treatment depends on the deposition site of actinides in the body with soft tissues being more susceptible than bones, and on how soon treatment is initiated following exposure (table II).

TABLE II. EFFECT OF ^{241}Am DISTRIBUTION ON EFFICACY OF Na_3(Ca-DTPA) CHELATION[a] THERAPY IN THE ADULT BABOON

Animal Number	Gross Distribution of ^{241}Am as % Body Burden	Therapy Initiation (Time After ^{241}Am Injection)	Percent Body Burden Excreted (net)[b] Due to Chelation Therapy in			
			Urine	Feces	Total	Total Measured by External Counting
B-164	60% soft tissues 40% skeleton	1 day	36.6	11.4	48.0	52.3±5.2
B-520	21% soft tissues (mostly liver) 79% skeleton	1.5 months	11.2	3.9	15.1	32.4±5.1
B-460	5% soft tissues 95% skeleton	13 months	7.8	0.05	7.9	9.3±1.6

[a] After 10-12 chelation treatments in about one month.
[b] Net excretion was determined by subtracting values obtained from untreated controls.

2) Ca-DTPA was more effective in removing ^{241}Am from both the adolescent human and juvenile baboon compared to adults similarly exposed (tables III and IV).

TABLE III. TOTAL BODY CONTENT OF ^{241}Am IN MAN[a]

Age	Before Most Recent Chelation Therapy[b] (nCi)	After Most Recent Chelation Therapy (nCi)
Adult	69.6±2.7[c]	67.2±2.8
Adolescent	20.1±1.6	12.7±2.7

[a] Measured by external *in vivo* gamma scintillation techniques using meter arc geometry and NaI(Tl)-CsI(Tl) scintillation detectors.

[b] Most recent chelation therapy was in 1975. Two previous periods of DTPA therapy occurred in 1970 and 1973.

using meter arc geometry and NaI(Tl)-CsI(Tl) scintillation detectors.

[b] Most recent chelation therapy was in 1975. Two previous periods of DTPA therapy occurred in 1970 and 1973.

[c] 1 σ propagated counting errors only.

TABLE IV. EFFECT OF SUBJECT AGE ON THE EXCRETION OF ^{241}Am AS PROVOKED BY Na_3(Ca-DTPA) CHELATION THERAPY[a] IN BABOONS

Animal Number	Age	Therapy Initiation (Months after Exposure)	Percent of Body Burden[b] Excreted During Chelation Period		
			Urine	Feces	Total
B-400	Juvenile (DTPA-Treated)	1.6	28.7	5.1	33.8
B-406	Juvenile (Control)	1.6	0.7	3.5	4.2
(B-400)-(B-406)	Juvenile (Net)	-	28.0	1.6	29.6
B-520	Adult (DTPA-Treated)	1.5	12.0	9.3	20.3
B-352	Adult (Control)	1.5	0.8	5.4	6.2
(B-520)-(B-352)	Adult (Net)	-	11.2	3.9	15.1

[a] After 10-12 chelation treatments in about one month.
[b] Body burden determined immediately prior to chelation.

3): Ca-DTPA administration led to increased excretion of zinc in the urine (10-60-fold increase), and to inhibition of metalloenzyme ALAD.

4): Primary route of ^{241}Am enhanced excretion by Ca-DTPA was the urine. Ca-DTPA also increased fecal elimination.

Reviewer's Comments: Agreed with study results and conclusions. There are many notable features in this study. The human subjects have been contaminated for 12 years from occupational exposure apparently with no symptomatic manifestations. By this time, most of the americium is deposited in hard tissues like the bone. The baboon study was an acute subcutaneous study, however, it provided an opportunity to evaluate the variation in body deposition of americium with time. 24 hours after baboon contamination, 60% of the total americium body burden was associated with soft tissues, at 1 ½ months after nuclide exposure, 21 % was associated with soft tissues, by 13 months only 5 % was associated with soft tissues with the remaining being associated with the skeleton. Since it is more difficult to remove skeletal bound actinides, it is not surprising that the percentage total burden excreted diminishes with time (table II). The diminishing effectiveness of Ca-DTPA with time clearly demonstrates the need to initiate therapy as soon as practicable following exposure to a trans-uranium element.

Age appears to play a role in the response to Ca-DTPA therapy in both humans and baboons. In a situation where treatment was delayed (13 months in baboons and 12 years in humans), Ca-DTPA was more effective in increasing americium excretion in both juvenile baboon and adolescent human compared to adults. By this time, most of the body americium burden is located in the skeleton. It is reasonable to infer that the difference in response might be due in part to active bone development in young baboon and juvenile humans. The miniscule amount of americium excreted (table III) in adult human in response to therapy makes one to wonder about the utility of treatment after a delay of 12 years. Whether similar age difference in response to decorporation of other TU element or in case of immediate treatment will occur is not clear. Similar study was not conducted for Zn-DTPA.

Ca-DTPA treatment led to an increase in urinary excretion of zinc, and to a concomitant decrease in ALAD activity. Zinc is an essential requirement of many metalloenzymes including DNA and RNA polymerase. The teratological effects of Ca-DTPA have been linked to its diminution of body zinc level.

Smith, V. H., & Smith, M. L. (1971): The effect of DTPA dose on plutonium removal from rats. Rep. BNWL-1550 (pt 1) 96-97.

The study examined the efficacy of various doses of Ca- or Zn-DTPA (10-1,000 µmol/kg, 0.54 - 54 MHD, BSA) in reducing the body level of plutonium in rats. The rats were either treated 1 hour after intravenous administration of plutonium citrate (single dose) or received twelve doses of the chelators over a 6-week period starting on day 6 following plutonium administration.

Results and conclusions:

The results of the study indicated that when therapy is initiated promptly after exposure to plutonium, both Ca- and Zn-DTPA were effective in removing plutonium from bone and liver with the liver being more susceptible (fig 1).

With delayed treatment, (Fig 2) both chelators were not as effective in removing plutonium from the bone, compared with when treatment was not delayed. Higher doses of Ca- or Zn-DTPA were effective in removing plutonium from the liver.

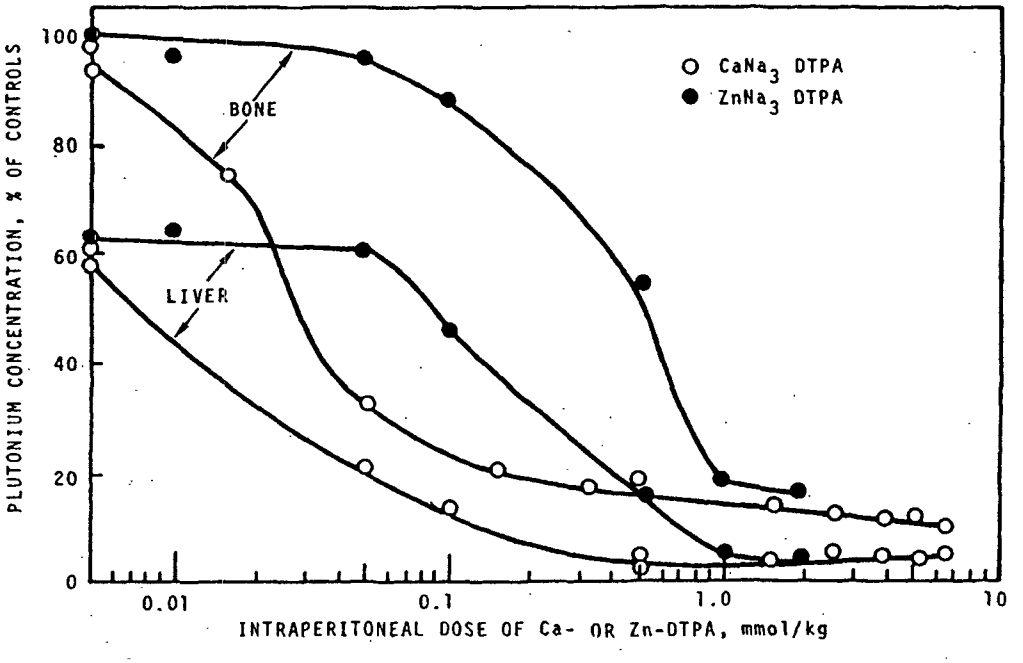


FIGURE 1. Removal of Intravenously Injected Plutonium Citrate from Rats as a Function of Prompt Treatment with Various Levels of Chelating Agent (Each point represents the average of data from ten rats.)

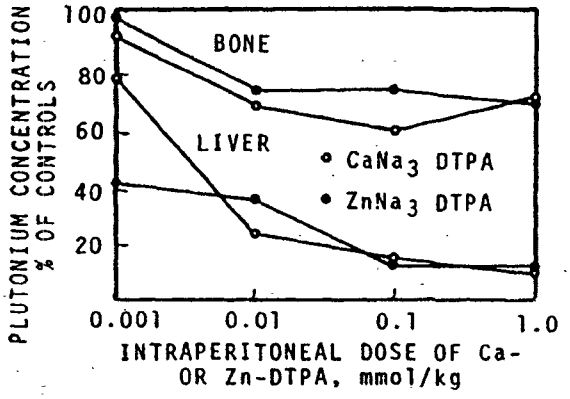


FIGURE 2. Removal of Intravenously Injected Plutonium Citrate from Rats as a Function of Delayed Treatment with Various Levels of Chelating Agent (Each point represents the average of data from ten rats.)

Reviewer's comments: Agreed with study conclusion. With prompt treatment, Ca-DTPA was more effective compared with Zn-DTPA. The study again emphasize the need to begin treatment promptly with the chelators following exposure to trans-uranium elements and the difficulty associated with removing plutonium from the bone. Both Ca- and Zn-DTPA were effective in the delayed decorporation of plutonium from the liver.

Bruenger, F.W., Taylor, D.M., Taylor, G.N., & Lloyds, R.D. (1991): Effectiveness of DTPA treatments following the injection of particulate plutonium. Int. J. Radiat. Biol. 60 803-818

The study evaluated the effects of prolonged chelation treatment on:

(1): The distribution pattern of an internal source of plutonium particles.

(2): Dog survival and the latent period for bone tumor formation and other radiation-induced lesion.

Young adult beagles (sex not specified, n=12) were grouped as follows:

1): Control (no treatment) free translocation of solubilized plutonium particles to critical organs for secondary deposition on bone surfaces and hepatocytes in the liver parenchyma.

2): Weekly subcutaneous injections of Ca-DTPA 30 µmole/kg (X0.5 MHD, BSA) beginning 2 hours following injection of Plutonium (interception of translocating solubilized plutonium in the blood stream). The reason for administering Ca-DTPA

weekly instead of daily was because of the reported toxicity and fatalities of multiple daily injection of Ca-DTPA.

3): Daily subcutaneous injections of Zn-DTPA 30 µmole/kg beginning 2 hours following injection of Plutonium (interception of translocating solubilized plutonium in the blood stream).

All animals received intravenous injection of a suspension of polydisperse particles of hydrolysed plutonium (31.4 Kbq ^{239}Pu/kg). Treatment began 2 hours later. The time point was chosen for practicality in case of human exposure and to allow the particles to become firmly fixed at their primary deposition sites. Dogs were sacrificed at pre-determined times or when death was imminent or for humane reasons. A detailed examination of all tissues was performed during necropsy followed by histopathological and radiographic examination.

Results and Conclusions:

It should be noted that the animals received more injections with Zn-DTPA compared to CA-DTPA. (weekly treatment for Ca-DTPA vs daily treatment for Zn-DTPA). By day 138, 19 Ca-DTPA treatments were given compared to 131 for Zn-DTPA within 132 days.

Distributions studies:

1): Under condition of treatment, Zn-DTPA was more effective in reducing the percentage of plutonium retained in organs following treatment (Table 1). Numerical dosimetry data is provided in Table 2 in which quantitative information on local doses at several skeletal locations is provided. Redeposition of Pu in the DTPA treated groups was effectively reduced.

Table 1. Percentage of injected Pu retained as a function of treatment†

Group	Days after injection	Percentage in skeleton	Percentage in liver	Percentage in spleen
No treatment (control)	124 (sacrificed)	10·8	53·9	6·5
	1429±176	35·9±5·4	17·3±5·6	0·45±0·20
30 µmol/kg Ca-DTPA weekly	138 (sacrificed)	7·8	58·6	1·96
	1636±126	6·7±1·6	2·9±1·0	0·09±0·04
30 µmol/kg Zn-DTPA daily	132 (sacrificed)	4·4	36·1	0·7
	1097‡	4·5	2·0	0·1
	3508–3533	1·8	0·12	0·002

† Standard deviations were calculated whenever possible.
‡ Dog died of a pulmonary embolism unrelated to radiation.

Table 2. Average terminal local dose-rates in trabecular bone resulting from various treatments (mGy/day $\pm \sigma/\bar{x}$)

Skeletal location	No treatment 1267–1568 days	Ca-DTPA weekly 1660–1783 days	Zn-DTPA daily 1079 days	Zn-DTPA daily 3508–3533 days
Bone mineral				
Distal femur metaphysis	23·5±0·3	16·6±0·3	5·8±0·3	1·4±0·3
Body lumbar vertebrae	22·2±0·4	9·6±0·6	3·6±0·3	1·7±0·4
Proximal ulna†	4·7±0·4	2·0±0·4	1·6±0·6	0·9±0·4
Pelvis†	21·6±0·3	7·1±0·5		1·2±0·5
Bone marrow				
Distal femur metaphysis	2·0±0·7	1·0±0·5	0·2±0·7	0·02±0·4
Body lumbar vertebrae	8·6±0·5	1·4±0·7	0·3±0·7	0·04±0·4
Proximal ulna†	1·5±0·5	0·2±1·0	0·1±0·9	0·02±0·8
Pelvis†	4·8±0·6	2·2±0·6		0·03±0·5
Endosteal surface				
Distal femur metaphysis	92·4±0·2	37·4±0·2	1·9±0·6	0·4±0·3
Body lumbar vertebrae	97·5±0·3	8·8±0·5	1·3±0·6	0·2±0·8
Proximal ulna†	30·7±0·2	8 6±0·4	1·7±0·6	0·3±0·5
Pelvis†	104·6±0·3	13·8±0·4		0·2±0·8

† Single dog only.

2): Survival Studies:

A): The four untreated dogs died of osteosarcoma at 1429 ± 176 days.

B): Ca-DTPA reduced organ plutonium burden. However, all 3 dogs in the long-term study died from bone cancer (two with osteosarcoma and one with adamantino carcinoma). Average time to death was 1636 ± 162 days. Time to death was not statistically different from controls (table 3).

C): Zn-DTPA reduced organ plutonium burden. However, Zn-DTPA treatment did not prevent the formation of osteosarcoma or benign liver lesions, as these were the cause of death in both long term-treated animals (table 3).

D): Zn-DTPA prolonged survival time by a factor of 2.1 when compared with the Ca-DTPA group.

E): Non-malignant liver lesions and fibrosis observed with both Ca- and Zn-DTPA groups were less severe than those observed in the non-chelated groups.

Table 3. Effect of protracted DTPA treatment on beagles injected with 31·45 ± 1·11 kBq of particulate Pu/kg

Number of dogs in group	Treatment	Time, d, injection to death	Number of dogs with			
			Bone tumours	Additional bone lesions	Liver lesions	Persistent leucopenia
4	Controls	1429 ± 176	4	3	4	4
3	30 μmol Ca-DTPA/kg weekly	1636 ± 126	3	3	2	2
2*	30 μmol Zn-DPTA/kg daily	3508–3533 (range)	2	2	2	—

†A third beagle of this group died at 1097 days from a pulmonary embolism not related to radiation.

Table 4. Significance of group survival differences

Group	One-way ANOVA	Mann–Whitney	Kruskal–Wallis	Cox–Mantel	Δσ†
No-treatment + Ca-DTPA vs. Zn-DTPA	0·0001	0·040		0·03	9·7
No-treatment vs. Ca-DTPA	>0·1	>0·1	>0·1	>0·1	none
No-treatment vs. Zn-DTPA	0·0001	0·06	0·05	<0·05	10·8
Ca-DTPA vs. Zn-DTPA	0·0006	0·08	<0·1	0·06	10·5

† Number of combined standard deviations separating the means.

3): Latent Periods:

Mean skeletal dose to death with bone marrow was about 2.5 Gy for both Ca- and Zn-DTPA-treated dogs compared to 7.1 Gy in the non-chelated controls. However, the Zn-DTPA group received the dose over a longer period of time 3520 days vs 1636 days for Ca-DTPA group, resulting in a lower dose rate for the Zn-DTPA group. The lower dose rate resulted in a substantial increase in latent period between plutonium exposure and death with bone marrow. Translated into beagle life span, without chelation or following Ca-DTPA treatment,the dogs died at about six years of age, while those treated with Zn-DTPA survived to age 11 (normal life expectancy of beagles is about 14 years).

The authors concluded that Zn-DTPA increased the latent period between plutonium exposure and death with a bone tumor by a factor of 2.1 as compared to non-chelated or Ca-DTPA-treated dogs.

Reviewer's Comments: A direct comparison of the efficacy of Ca-DTPA with Zn-DTPA is impossible in view of the significant difference in the total number of injection that the animals received depending on whether they were receiving Ca-DTPA or Zn-DTPA. Despite this limitation, I agree with the conclusions by the authors that both chelators reduced the percentage of plutonium retained in the organs. In addition, Zn-DTPA reduced the incidence of osteosarcomas, and increased the latent period between plutonium exposure and death with a bone tumor by a factor of 2.1 as compared to non-chelated dogs.

Morin, M., Nenot J.C. Lafuma, J. (1973): The behavior of ^{237}Np in the rat. Health physics 24, 311-315

Study summary

The study showed that Ca-DTPA was not effective in the treatment of neptunium contamination. The author ascribed the ineffectiveness to the instability of the ^{237}Np-DTPA complex in vivo. This may lead to increase in neptunium bone deposition if the broken complex is transported to the bone from the primary deposit site for example on the skin.

Reviewer's comments: Agreed. Zn-DTPA is not expected to be effective either, since ^{237}Np-DTPA complex is unstable in vivo.

Seidel, A. & Volf, V.(1972): Removal of internally deposited transuranium elements by Zn-DTPA. Health Physics; 22 779-783

The paper evaluated the effectiveness of Ca-DTPA and Zn-DTPA in removing internally deposited ^{239}Pu, ^{241}Am, ^{242}Cm in rats.

Female albino rats (180-200) were used for the study. The radionuclide were administered intravenously (0.1 – 0.3 µCi) as the citrate form (soluble). Control rats receiving no treatment were sacrificed up to the 10th week after administration of the isotopes. Ca- or Zn-DTPA (1 mmole/kg/day, X5 MHD, BSA) was administered intraperitoneally on the 6th, 8th, and 11th, day after isotope injection. The animals were killed by exsanguination on day 13 or day 19 (for a plutonium group). Radioactivity was assayed by alpha liquid scintillation counting.

Results & Conclusions:

In view of the fact that other articles have addressed ^{241}Am and ^{242}Cm in some details, the result section will concentrate on ^{239}Pu.

1): Organ retention is shown in figure 1 for untreated animals. Skeletal burden remains fairly constant for all radionuclides while there is a rapid decline of the liver burden. The initial ^{239}Pu retention was highest for the bone, exceeding that of ^{241}Am and ^{242}Cm by a factor of 2-3. Initial ^{239}Pu burden for the liver is about a third of the value for ^{241}Am and ^{242}Cm.

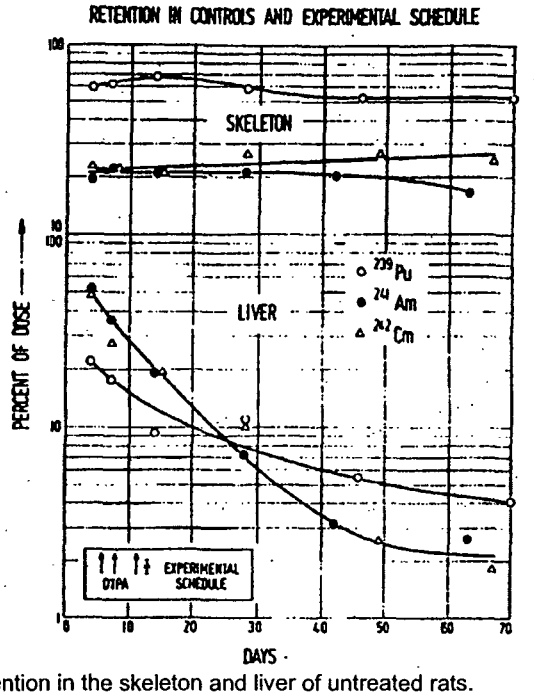


Fig 1: radionuclide retention in the skeleton and liver of untreated rats.

2): For ^{239}Pu, chelate administration resulted in a statistically significant reduction of the organ content of isotopes as compared to control animals. For all organs except the kidneys, where Zn-DTPA was more effective than Ca-DTPA, there were no statistically significant differences between Ca-DTPA and Zn-DTPA (Table 1).

Isotope	(% of control) ^{239}Pu		^{241}Am		^{242}Cm	
Treatment	Ca-DTPA	Zn-DTPA	Ca-DTPA	Zn-DTPA	Ca-DTPA	Zn-DTPA
Skeleton	73 ± 4	72 ± 2	72 ± 6	77 ± 7	62 ± 2	67 ± 2
Liver	20 ± 2	23 ± 3	8 ± 1	9 ± 1	8 ± 2	9 ± 1
Spleen	50 ± 5	56 ± 5	67 ± 6	60 ± 7	52 ± 4	52 ± 4
Kidneys	61 ± 6	49 ± 4	66 ± 6	68 ± 8	69 ± 8	66 ± 8
Lung	39 ± 3	45 ± 5	78 ± 11	89 ± 11	67 ± 9	74 ± 10
Thyroid	56 ± 4	50 ± 4	50 ± 9	71 ± 13	68 ± 5	50 ± 5
Adrenals	46 ± 5	48 ± 4	60 ± 14	60 ± 23	50 ± 11	50 ± 7
Ovaries	63 ± 9	56 ± 9	64 ± 15	75 ± 16	55 ± 10	55 ± 10

Table 1: Influence of Ca-DTPA and Zn-DTPA on the removal of ^{239}Pu, ^{241}Am and ^{242}Cm from the rat; day 13

The authors concluded that both Ca-DTPA and Zn-DTPA were effective in reducing organ burdens of injected monomeric ^{239}Pu even when substantial skeletal deposition has occurred (Day 6).

Reviewer's comments: Agreed, however it is noted that treatment was for a short duration, and that a longer treatment period is required to substantially remove most of the trans-uranium elements from the organs.

Smith, V. H., Ballou, J. E., Lund, J E., Dagle, G. E., Ragan, H. A., Busch, R. H. Hackett, P. L. Willard, D. W. (1976): Aspects of inhaled DTPA toxicity in the rat, hamster and beagle dog and treatment effectiveness for excorporation of plutonium from the rat. In: Diagnosis and treatment of incorporated radionuclides (Proc. Seminar Vienna, 1975, IAEA)

The paper examined the effectiveness and potential toxicity of inhaled Ca-DTPA in plutonium decorporation.

Efficacy of inhaled Ca-DTPA:

Adult female rats were injected with 1.2 µCi ^{238}Pu nitrate intramuscularly. The animals were treated as follow:

Prompt Treatment: Rats were treated with 0.5 or 0.035 mmoles/kg Ca or Zn-DTPA intraperitoneally. The lower dose was also given by inhalation over a 30-minute period. Plutonium-injected but untreated rats were exposed to aerosols or injected with saline. Animals were sacrificed after 4 days.

Delayed treatment: 8 months following i.m. injection of 1.2 µCi ^{238}Pu nitrate, rats were treated 8 times over a 21/2 week period by injection or inhalation of 0.02 mmol of Zn- or Ca-DTPA.

Results & Conclusions:

For prompt treatment regimen, control rats retained an average of 23% of the injected plutonium in the liver, 41% in the skeleton while 22% remained at the injection site. Treatment effectiveness is summarized below:

	Percent of Control Retention[a]					
Treatment Route→	Intraperitoneal				Inhaled	
Treatment Level→	0.5 mmol/kg		0.035 mmol/kg		0.035 mmol/kg	
Agent→	Ca-DTPA	Zn-DTPA	Ca-DTPA	Zn-DTPA	Ca-DTPA	Zn-DTPA
Tissue						
Liver	9	20a	30b	57c	22ab	45c
Femur	17	57	40a	88b	31a	73b
Injection Site	55a	82b	79b	92c	64a	84bc

[a] Numbers in the same row with the same letter (a, b or c) are not statistically different at 5% significance level according to Duncan's multiple range test.

Table 1: Retention of intramuscularly deposited ^{238}Pu nitrate in the rat four days after treatment with inhaled or injected Ca- or Zn-DTPA.

0.5 mmols/kg dose level was more effective than 0.035 mmols/kg with the Ca salt being superior to Zn salt under prompt treatment conditions. At 0.035 mmol/kg, inhalation was equivalent to intraperitoneal administration.

According to the authors, for the delayed treatments, the amount of Pu measured in the urine of DTPA treated rats was 240 times greater than that excreted by control rats although this amounted to only 0.3% of the injected radionuclide.

The authors concluded that the pulmonary route was as effective as intraperitoneal route in excorporating radionuclides.

Toxicity of inhaled Ca-DTPA

The study examined early pathology associated with Ca-DTPA inhalation in adult rats and Golden Syrian hamster. Ca-DTPA was delivered as aerosols by a Retec nebulizer. Several experiments were performed. In one, rats received 14 or 28 mg/kg while the hamsters received 28 or 56 mg/kg. Absorbed doses were estimated from chemical analysis of DTPA in urine. Animals were exposed for a total of 3 times within one week spaced 1 day apart and were sacrificed either after the first or third exposure or at weekly intervals for the following five weeks. There were 3 control groups, saline, sham exposure or no exposure with a total of 45 animals in each group. The lungs, liver, kidneys, spleen, adrenals, trachea, esophagus, stomach, duodenum, jejenum, ileum, eyes, and large intestine were examined histologically.

The authors reported that transitory vesicular emphysema as the only pathology. This was not found in animals sacrificed 3 weeks after the last exposure.

In another study, dogs were anesthetized and administered Ca-DTPA aerosols via an intratracheal catheter for 30 min/day for 5 days. The average exposure was 56 mg/kg (X .64 MHD, BSA). One week after the last exposure, ¾ treated dog and 0/2 dogs exposed to saline control showed enlargement and submucosal lymphoid follicles in the pyloric region of the stomach that was not present in dogs sacrificed at 4, 8, or 18 weeks post exposure. Epithelial atypia in the alveolar lining was noted in 5/16 dogs inhaling Ca-DTPA and in 1/8 dogs exposed to saline aerosol. Urinary Zinc was increased on the days of treatment with Ca-DTPA up to 30-40 folds.

Reviewer's comments: Agreed with study conclusion. The inhalation route is a viable route of administration for Ca-DTPA. Reported pathological findings appear to be transient. A common difficulty with most of these published reports is that the basis of dose selection or duration of treatment is never reported. Hence their appropriateness to clinical setting remains difficult to establish.

Pharmacokinetics:

Stevens, W., Bruenger, F.W., Atherton, D.R., Buster, D.S. and Howerton, G.: (1978): The retention and distribution of ^{241}Am and ^{65}Zn, given as DTPA chelates in rats and of [^{14}C]DTPA in rats and Beagles.

Study Summary

I will only provide a summary of the study, in view of the fat that a rather small number of animals n=2 were utilized for each time point.

The retention and distribution of ^{241}Am and ^{65}Zn given as the DTPA chelates were studied in rats for 48 hours following intravenous injection. The retention and distribution of [^{14}C] DTPA injected, as the zinc chelate was determined in rats and two beagles. At 24 hr post injection, rats retained 5% of the ^{241}Am, 35% of ^{65}Zn and 4% of the [^{14}C]DTPA. The loss of these nuclides from plasma could be described by the sum of two exponential. In rats, the respective biological half-lives $t_{1/2}$ for early times post-injection are $t_{1/2}$ ^{65}Zn =1.4 hr, $t_{1/2}$ ^{241}Am =0.75 hr and $t_{1/2}$ [^{14}C]DTPA =0.65 hr respectively. The second component half lives for ^{241}Am, ^{65}Zn and [^{14}C]DTPA are 0.2, 6, and 1.6 days respectively. In beagles, the initial $t_{1/2}$ for loss of [^{14}C]DTPA from the blood was 0.77 hr and the second component was 4.4 days. The urine was the primary route of excretion in both species. In both rats and dogs, the liver contained the highest concentrations of ^{241}Am, ^{65}Zn and [^{14}C]DTPA respectively. The concentration of ^{65}Zn in the liver reached a plateau after ~ 4hr. Significant amounts of ^{65}Zn were found in the lungs, spleen and femur.

Toxicology:

Study Title: Planas-Bohne, F., Lohbreier, J. (1976): Toxicological studies of DTPA. In Diagnosis and treatment of incorporated radionuclides (Proc. Seminar Vienna , 1975, IAEA)
Volume #, and page #: Not applicable.
Conducting laboratory and location: Not stated.
Date of study initiation: Not provided.
GLP compliance: Not stated but unlikely.
QA report: yes ( ) no (x)
Drug, lot #, radiolabel, and % purity: Not stated
Formulation: Not stated

The study had two principal objectives; to determine the toxicological effects of prolonged treatment with Ca- or Zn-DTPA in rats and to evaluate the dependency of chelator toxicity on treatment schedule.

Methods:
Three groups of male and female rats (n=10, 5 weeks old) were injected intraperitoneally twice a week with either 100 µmol/kg (~X.5 MHD, BSA) Ca- or Zn-DTPA or were injected with 0.9% saline. Treatment was continued for 44 weeks.

Observations and Times:
Clinical signs: daily
Body weights: weekly
Food consumption: Not stated
Hematology/clinical chemistry: every six weeks; in addition at necropsy, Zn and Mn concentrations in organs were determined.
Organ weight: Not done
Histopathology: at necropsy

Results:
Clinical signs: Not reported

Body weight: No effect

Food intake: Not reported
Hematology and blood chemistry: No significant findings
Mortality: No animal died during the study
Organ weight: Not evaluated
Gross and histological findings: No significant findings

In a second study reported in the same publication, Ca-DTPA was administered to female rats (~180g) either once daily or in five fractions per day with an interval of 2 hours between administration or the chelate was administered as a continuos infusion. Dose schedule is shown in table 1. Surviving animals were sacrificed at the end of study and hematological parameters evaluated.

Group No.	Chelate	Mmole/kg/day	Duration of treatment	Schedule	Lethality (%)
1	Ca-DTPA	1 (~X 5MHD)	5	1daily	0/7 = 0
2	Ca-DTPA	1 (~X 5MHD)	5	5 fractionated injections/day	20/27 =74
3	Ca-DTPA	.75 (~4 MHD)	5	5 fractionated injections/day	11/24 = 46
4	Ca-DTPA	.526 (~3 MHD	3	infusion	6/27 =22
5	Ca-DTPA	.1 (~ .5MHD)	5	infusion	2/4 =50
6	Ca-DTPA	.08 (~.4MHD)	5	infusion	0/5
7	Ca-DTPA	.063 (~ .3 MHD)	5	infusion	0/5
8	Ca-DTPA	.050 (.25 MHD	5	infusion	0/6
9	Ca-DTPA	.037 (.18MHD	10	infusion	0/7
10	Zn-DTPA	5 (X25 MHD)	5	infusion	0/6
11	Zn-DTPA	5 (X25 MHD)	9	infusion	0/8

Results:
Dose fractionation resulted in a drastic increase in lethality (group 1 compared with group 2).

Continuous infusion of Ca-DTPA increased lethality, cause of death was not stated.

Toxicity was highly dependent on daily dose as no lethality occurred with doses ≤ 0.08mmol/kg per day.

The animals tolerated extremely high cumulative doses of Zn-DTPA.

Other toxic effects of Ca-DTPA included severe diarrhea and exsiccosis. Autopsy revealed only slight hemorrhage in the intestinal mucosa.

Conclusion: The study concluded that repeated or continuous infusion of CA-DTPA led to an increase in lethality that was not observed when Zn-DTPA was infused. The study also concluded that repeated administration of Ca-DTPA at approximately 3 times MHD did not produce adverse effects.

Reviewer's comments: Agreed with study conclusions. The study findings illustrate the importance of not fractionating Ca-DTPA dose or even administering Ca-DTPA as a continuous infusion. Unfortunately, there was no Zn-DTPA with fractionated doses although none of the animals in the Zn-DTPA infusion group died from the treatment and a previous study in beagle dogs where Ca-DTPA was fractionated did result in increase mortality. No significant histopathological finding was reported. The low dose multiples of MHD is also noted.

Gabard, B. (1974): The influence of diethylenetrianimepentaacetate on the synthesis of DNA, RNA and proteins in the regenerating rat liver. Biochemical Pharmacology 23 901-909

Summary:

Administration of high doses of Ca-DTPA (4-8 mmole/kg; 3.6-7.2 MHD, BSA) after partial hepatectomy inhibited the synthesis of DNA, RNA and proteins in the regenerating rats liver. Zn-DTPA was ineffective. Impairment by Ca-DTPA of DNA synthesis can be completely restored by subsequent joint administration of Zn^{2+} and Mn^{2+}. The dependency of the inhibitory action on dosage and time of administration is consistent with the assumption that the inhibition of DNA synthesis is not the primary but the consequence of impaired synthesis of protein ascribed to a disturbed conformation of RNA due to removal of Zn^{2+} and Mn^{2+}.

Carcinogenicity:

Study addressing the carcinogenicity of Ca-DTPA was not identified.

Immunotoxicology:

Study addressing the immunotoxicity of Ca-DTPA was not identified

Genotoxicity:

Study addressing the genotoxicity of Ca-DTPA was not identified

Reproductive Toxicology:

Study title: Taylor, G. L., & Mays, C.W. (1978): Fetal injury induced by Ca-DTPA in dogs. Health Physics 35, 858-860

Study No: and number: N/A
Site and testing facility: N/A
GLP compliance: Not stated
QA- Reports Yes ( ) No ( ): Not Stated

The study evaluated the effects of Ca-DTPA on beagle dog fetuses.

Methods:

Two female Beagle dogs (8.6 and 10.4 kg) were mated three times with intervals between mating of 1-3 days. Ca-DTPA (30μm/kg, X 0.5 MHD, BSA) was administered intravenously once daily begining15 days after the first mating and continuing until the day of parturition. Both dogs were fed once daily with commercial dog food plus meat. No further experimental detail was provided.

Results:

Maternal:

No detectable symptoms were produced in the dam. Appetite was said to be normal. Biochemical and hematological parameters examined twice during treatment period were within normal values (time of examination not provided). Urinalysis was normal. Duration of gestation (65-67) was within normal for the colony. Maternal macroscopic examination was not done.

Fetal:

Dam #	Average birth weight	# of pups with abnormal hair color	Pups surviving beyond one week	# of living pups at 6 months with neutropenia	# of dead pups with lissencephally
1	202	6/6	4/6	3 /4	0/2
2	194	5/5	1/5	0/1	2/4

Birth weight:
The average birth weight of 194-202 g was much lower than the colony average of 300g. However, only one of the surviving 5 pups showed permanent growth stunting, with the remaining four reaching normal weight by approximately 20 weeks after birth.

Hair color:
All newborn of both litters showed abnormal silver-gray hair coloration involving all body regions. This coloration has never been observed in any of the 2000 pups born in the colony. The pigmentation was temporary as normal coloration began to return at 3-4 weeks of age, when supplemental feeding begun.

Macroscopic examination:
Lissencephally (smooth brain surface without convolution) was noted in two of the dead pups from dam 2.

Offspring development:
The only surviving pup from dam 2 was slow in learning normal feeding habits during weaning.

Clinical Chemistry:
Clinical chemistry performed in the five surviving pups at age six months showed normal biochemical profiles. However, neutropenia was observed in 3 of the pups.

Conclusions: The authors ascribed the abnormal skin pigmentation to loss of copper; an essential element in the formation of melanin. They also speculated that pregnancy

may have provided some protective benefit to the mother since they were able to tolerate repeated dosing with Ca-DTPA unlike non pregnant beagle dogs.

Reviewer's comment: The dose employed for this study 14 mg/kg (X0.5 MHD, BSA) did not produce any noticeable maternal toxicity. One might infer that the dose employed was not high enough for conduct of reproductive toxicity study. However, similar study in non-pregnant beagles produced signs of overt toxicity and I am intrigued by the authors' suggestion that pregnancy may provide some protective benefits to the dam. The protective effect is rather mute, in view of the serious teratogenic effects produced in the litters. The study also lacks concurrent control for comparison especially since the numbers of surviving pubs at six months 3 /12 seem low to this reviewer. Moreover, detailed histopathological examination was not conducted on the dead pups. However despite these limitations, fetal abnormalities were observed including abnormal hair pigmentation that was resolved when the pups were weaned and presumably had access to supplemental minerals in their diet. More importantly was the finding of lissencephally in two of the dead pups. One can conclude that in beagle dogs, use of ca-DTPA during pregnancy caused fetal malformation and decreased survival for the pups.

Study title: Fisher, D .R., Mays, C. W., Taylor, G .N. (1975): Ca-DTPA toxicity in the mouse fetus. Health Physics 29, 782-785

Study No: and number: N/A
Site and testing facility: N/A
GLP compliance: Not stated

The main study objective was evaluation of the effect of Ca-DTPA on developing fetus.

Methods:

Group	Chelator	Dose mmole/kg	# Dams	% with litter	Litter size	Average pup weight	Average litter weight
1	Ca-DTPA	2.9 (X 7.8MHD)	6	0	0	0	0
2	Ca-DTPA	0.36 (X1 MHD	12	67	5.6	1.42	7.95
3	Zn-DTPA	2.9 (X 7.8MHD)	6	100	8.0	1.28	10.24
4	Zn-DTPA	0.36 (X 1MHD)	6	83	5.5	14.3	7.87
5	Saline		12	83	5.7	1.40	7.98

Virgin female C57 mice were used for the study. The animals were grouped as shown in the table and placed with male mice for 18 days. After the first 4 days, the female received repeated subcutaneous injection ca-DTPA or Zn-DTPA or saline, the males were not treated. The injections of the dam continued until the pups had reached age 13 days.

Results:

Group 1 mice did not produce any viable offspring, only one dead fetus at birth. Fecundi ty was near normal for group 2 as were fetal development and growth rates during the lactation period. Zn-DTPA at doses employed did not affect the dams or the pups. According to the authors, all the group 1 dams were able to subsequently produce normal litters when mated following discontinuation of Ca-DTPA therapy.

Conclusions:

The authors concluded that CA-DTPA should not be given to pregnant women in need of chelation therapy. They recommended Zn-DTPA.

Reviewer's comments:

The study was not detailed enough for thorough analysis. However, one can surmise that at the doses employed, Zn-DTPA did not produce any overt toxicity in the mouse fetus. A high NOEL (X7.8 MHD, BSA) was established. Although this NOEL had to be tempered with the fact that no histological evaluation of the dam or fetus was performed. Nevertheless, a head to head comparison of CA-DTPA and Zn-DTPA indicated that Zn-DTPA is preferred over Ca-DTPA.

Study title: Fisher, D .R., Calder, R. F., Mays, C. W., Taylor, G .N. (1976): Ca-DTPA-induced fetal death and malformation toxicity in the mouse fetus. Teratology, 14, 123-127

The main study objective was evaluation of the effect of Ca-DTPA at various stages of fetal development.

Methods:
C57/B1 Mice were grouped as shown in table 1, and placed 3/cage with a male mouse of the same strain. They were allowed to mate overnight. Vaginal plug was used as evidence of successful mating. Pregnant females received five daily subcutaneous injection of 720-2880 µmole/kg Ca-DTPA (~X2-7.8 MHD) as scheduled in table 1. All animals were sacrificed on day18 followed by necropsy. The abdominal wall was opened and the uterine horn examined for resorption sites, dead and live fetuses. ¼ of the fetuses alive were stained with alizarin red. The remaining were fixed in buffered 10% formalin and examined for gross defects microscopically.

Results:

Table 1 showed that CA-DTPA increased fetal mortality with greater sensitivity during early and mid gestation. Resorption occurred more frequently than dead formed fetuses.

The frequency of gross malformation increased with dose, with highest susceptibility in early and mid gestation (table 2). Ca-DTPA was not lethal to the pregnant mice. However, according to the authors hemorrhage sites were seen in the histological sections of the uterine walls of some female mated that failed to produce litters (dose level and numbers of such females were not provided).

Gross malformations among fetuses not removed for alizarin staining

Injection days	Daily dose Ca-DTPA	Fetuses malformed/ examined	Malformations observed

TABLE 1

Ca-DTPA injection schedule and fetal mortality

Injection days	Daily dose Ca-DTPA	No. litters	Fetuses (resorbed + dead)/ total	Live fetal wt. (g)
	μmol/kg			*(x̄ ± SD)*
2-6	1,440	5	(8+0)/43 = 19%	1.02 ± 0.12
	720	6	(12+2)/62 = 23%	1.13 ± 0.18
	0	5	(3+0)/43 = 7%	1.14 ± 0.10
7-11	1,440	2	(3+2)/16 = 31%	1.11 ± 0.07
	720	7	(6+3)/61 = 15%	1.01 ± 0.13
	0	7	(2+1)/39 = 8%	1.18 ± 0.15
12-16	2,880	8	(13+5)/79 = 23%	1.07 ± 0.11
	1,440	6	(3+1)/45 = 9%	1.14 ± 0.14
	720	4	(1+1)/28 = 7%	1.25 ± 0.12
	0	7	(3+0)/47 = 6%	1.11 ± 0.12

Conclusions: The authors concluded that Ca-DTPA is teratogenic and ascribed the toxic effects of Ca-DTPA to zinc depletion.

Reviewer's Comments: Agreed

Overall Conclusions and Recommendations:

Ca-DTPA is approvable from preclinical pharmacology and toxicology perspective. Please refer to the overall executive summary and individual study evaluation. Please see the executive summary.