MASTER

C00-4369-3

MIXED LIGAND CHELATE THERAPY
FOR PLUTONIUM AND TOXIC METALS
FROM ENERGY POWER PRODUCTION

Final Report

For Period April 15, 1977 - October 14, 1980

Jack Schubert, Ph.D.
Hope College
Division of Natural Sciences
Holland, Michigan 49423

DISCLAIMER

This book was prepared as an account of work sponsored by an agency of the United States Government. Neither the United States Government nor any agency thereof, nor any of their employees, makes any warranty, express or implied, or assumes any legal liability or responsibility for the accuracy, completeness, or usefulness of any information, apparatus, product, or process disclosed, or represents that its use would not infringe privately owned rights. Reference herein to any specific commercial product, process, or service by trade name, trademark, manufacturer, or otherwise, does not necessarily constitute or imply its endorsement, recommendation, or favoring by the United States Government or any agency thereof. The views and opinions of authors expressed herein do not necessarily state or reflect those of the United States Government or any agency thereof.

October 14, 1980

Prepared For
The Department of Energy
Under Contract EE-77-S-02-4369.A000

DISTRIBUTION OF THIS DOCUMENT IS UNLIMITED

34B

DISCLAIMER

This report was prepared as an account of work sponsored by an agency of the United States Government. Neither the United States Government nor any agency Thereof, nor any of their employees, makes any warranty, express or implied, or assumes any legal liability or responsibility for the accuracy, completeness, or usefulness of any information, apparatus, product, or process disclosed, or represents that its use would not infringe privately owned rights. Reference herein to any specific commercial product, process, or service by trade name, trademark, manufacturer, or otherwise does not necessarily constitute or imply its endorsement, recommendation, or favoring by the United States Government or any agency thereof. The views and opinions of authors expressed herein do not necessarily state or reflect those of the United States Government or any agency thereof.

DISCLAIMER

Portions of this document may be illegible in electronic image products. Images are produced from the best available original document.

CONTENTS

TITLE PAGE

ABSTRACT

I. INTRODUCTION

II. SUMMARY OF ACTIVITIES FOR PROJECT PERIOD

A. List of Publications

B. General Observations

Pu

Cd

III. PROGRESS REPORT FOR PROJECT PERIOD 10/15/79 - 10/14/80

A. Treatment of Acute Cd Poisoning

B. Conditional Constants and Therapeutic Effectiveness

C. Potentiometric Titrations and Screening

D. Genotoxicity of Chelants and Chromium

1. Mutagenicity of chelants

2. Chromium mutagenicity

IV. FUTURE INVESTIGATIONS

V. COMPLIANCE WITH CONTRACT REQUIREMENTS

VI. EFFORT OF PRINCIPAL INVESTIGATOR

NOTICE

This report was prepared as an account of work sponsored by the United States Government. Neither the United States nor the United States Energy Research and Development Administration, nor any of their employees, nor any of their contractors, subcontractors, or their employees, makes any warrant, express or implied, or assumes any legal liability or responsibility for the accuracy, completeness, or usefulness of any information, apparatus, product or process disclosed or represents that its use would not infringe privately owned rights.

ABSTRACT

The results of experiments are summarized on the ability of combinations of chelating agents to modify the genotoxicity or tissue distributions. The mutagenicities of Cr and of chelating agents* were determined. The metals described in the report are Pu(IV), Cd(II), Cr(III) and Cr(VI). Accurate measurements were made of the ability of CaNa_2EDTA , CaNa_3DTPA , and DMPS to reduce mortality in mice given doses (i.p.) of CdCl_2 well above the 100% lethal level. The efficacy in terms of the mmoles/kg needed to reduce the mortality was: $\text{DTPA} > \text{EDTA} > \text{DMPS}$. The combination of DTPA + DMPS proved most promising though little evidence for mixed complex formation was noted. Potentiometric titration studies as a means for screening chelants useful for biological testing are given. In the case of Pu(IV) a few combinations proved effective, but only when given shortly after Pu administration and then only in the liver but not the skeleton. It is recommended that metabolically stable chelating agents be used in combinations, especially for those combinations which may form very stable mixed ligand chelates.

*Abbreviations for various chelants mentioned in this report are BAL-2,3-dimercaptopropanol; CAT-catechol; DCTA-1,2-diaminocyclohexanetetraacetic acid; DFOA-desferrioxamine- β -methane sulfonate; 2,3-DHB-2,3-dihydroxybenzoic acid; dithiocarb-sodium diethyldithiocarbamate; DMPS-2,3-dimercaptopropanol-1-sulfonate; DTPA-diethylenetriaminepentaacetic acid; EDTA-ethylenediaminetetraacetic acid; HEDTA-hydroxyethylenediaminetetraacetic acid; NTA-nitrilotriacetic acid; PA-penicillamine; PCA-protocatechuic acid; RES-resorcylic acid; SA-salicylic acid; SHA-salicylhydroxyamic acid; Tiron-1,2-dihydroxybenzene-3,5-disulfonate.

I. INTRODUCTION

The present project, begun April 15, 1977, and now terminating, was initiated by the Principal Investigator because it was universally recognized that the efficacy of chelation therapy for toxic metals (i.e., metallic salts) had reached a plateau. In particular, the usefulness of single chelating agents for therapy diminishes rapidly the longer treatment is delayed following exposure. This is especially the case for the decorporation of plutonium and other transuranic elements. Consequently, the Principal Investigator decided to test the effects of combinations of chelants. The rationale for this approach was two-fold: (1) Differences in the pharmacokinetics of the individual chelants could result in an overall greater removal of a toxic metal from target organs; and/or (2) Suitable combinations of chelants would form mixed ligand chelates having substantially higher stabilities than any 1:1 metal chelate, and thus be more effective for therapy. (For references to the literature see the Initiating and Progress Reports for this Project.)

II. SUMMARY OF ACTIVITIES FOR PROJECT PERIOD

A. List of Publications

During the course of the program seven papers were completed as shown below. Of these, two have been published, three are in press, one has been submitted, and one (#7) is still in preparation, though most of its experimental work has been completed.

1. Schubert, J., and Derr, S. K., 1978-79. "Mixed Ligand Chelate Therapy for Plutonium and Cadmium Poisoning," Nature, 275, 311-313; 278, 581-582.
2. Schubert, J., 1979. "Mixed Ligand Chelate Therapy for Plutonium and Cadmium Poisoning," Nature 281, 406.

3. Schubert, J., 1980. "Mixed Complex Formation--New Therapeutic Approaches," Trends in Pharmacological Sciences. Part I.
4. Schubert, J., 1980. "Mixed Complex Formation--New Therapeutic Approaches," Trends in Pharmacological Sciences. Part II.
5. Gentile, J. M., Hyde, K., and Schubert, J., 1980. "Chromium Genotoxicity as Influenced by Complexation and Rate Effects," Naturwissenschaften (submitted for publication 9 July, 1980).
6. Schubert, J., 1980. "Chelating Agents in Biological Systems," Proceedings of Workshop/Conference on the Role of Metals in Carcinogenesis, Atlanta, Georgia, 1980, Environmental Health Perspectives (in press).
7. Schubert, J., "Acute Toxicity of Cadmium in Mice as Affected by Chelant Combinations," (in preparation).

B. General Observations

Pu -- Metabolic factors are very important, so even though a given chelant combination is capable of mixed ligand chelate formation, the secondary chelants such as catechol and salicylate derivatives are too rapidly metabolized for decorporation of Pu(IV) under delayed conditions or from skeletal deposits. Synergistic effects, however, were observed for decorporation from the liver of female mice when treatment was begun 2 hours post intraperitoneal injection of monomeric Pu(IV) citrate. The following combinations involving DTPA or EDTA with bidentate secondary chelants were synergistic in their ability to reduce the level of Pu(IV) in the liver: DTPA+PAS, DTPA+CAT, EDTA+PAS, EDTA+SHA while the ineffective combinations included DTPA+SHA, DTPA+DHB, DTPA,PCA, DTPA+DNS, DTPA+RES, and DTPA+ASC. No synergistic effects on the decorporation of Pu(IV) in the skeleton were observed.

Cd -- The ability of single and mixed chelants to reduce the mortality of Cadmium poisoned mice (acute toxicity) was investigated in some detail. The LD1, LD50, and LD99 of mice given a single intraperitoneal injection of cadmium chloride was calculated means of a computer program (Schubert et al, J. Toxicol. Environ. Health 4, 763-776, 1978). The effects of different doses of chelants, singly and in combination, given at different times post-Cd were evaluated. The Cd doses used were 100% lethal. DTPA was the most effective. In terms of the dose, in millimoles/kg needed to reduce the mortality by half, the most effective single chelant followed the order: DTPA > EDTA > DMPS. Details are given later in this report. The most effective combination was DTPA+DMPS.

Potentiometric titration studies suggested that while DTPA+DMPS may form a mixed ligand chelate, no significant mixed ligand chelate is formal at neutral pH. Hence the effect of the combination appeared to be additive with each chelant acting independently. Combinations involving PA were ineffective, if anything, PA appeared to reduce the efficacy of other chelants.

III. PROGRESS REPORT FOR PROJECT PERIOD 10/15/79 - 10/14/80

The data reported here were largely accumulated since the last Progress Report, No. C00-4369-2. The potentiometric experiments were done in collaboration with Prof. M. Seymour of the Chemistry Department and Mr. Paul Knoll, as part of his undergraduate research training. The biological data were obtained with the assistance of Mr. Gordon Van Woerkom. Statistical evaluation of the data utilized the computer program described elsewhere and was carried out with the collaboration of Mr. Sylvanus Tyler at the Argonne National Laboratory.

A. Treatment of Acute Cd Poisoning

Statistically reliable data on the reduction of mortality in female mice given an i.p. supralethal dose of $\text{CdCl}_2 \cdot \text{H}_2\text{O}$ and then treated 5 minutes later with varying doses of single chelants, namely Na_2CaEDTA , Na_3CaDTPA , and DMPS. The results and further experimental details are given in Figure 1. In Table 1 the LD1, LD50, and LD99 values are calculated.

FIGURE 1

Treatment of female mice (Spartan Strain, Swiss-Jebster, 20-25 gm).

Treatment given 5 minutes post $\text{CdCl}_2 \cdot \text{H}_2\text{O}$ (0.0837 mmole/kg).

Survival at least ten days post Cd. All injections i.p.

	EDTA(mmole/kg)	DTPA(mmole/kg)	DMPS(mmole/kg)
LD1 :	0.24±0.02	0.089±0.004	0.424±0.008
LD50:	0.10±0.01	0.066±0.003	0.353±0.006
LD99:	0.045±0.003	0.049±0.002	0.294±0.005

Table 1 -- Mmol/kg chelant needed to reduce the lethal dose. (see Figure 1)

The therapeutic effectiveness decreases, as mentioned, as the treatment post-Cd injection is delayed. However, when the dosages of the chelants are increased, or multiple treatments are given, the therapeutic effect can be extended to later times post-Cd administration. We also investigated the effectiveness of combining DTPA with DMPS. The procedure utilized was as follows: the LD1 dose of DMPS was kept fixed and the dose of DTPA was varied until a complete dose-response was obtained. These data are being analyzed statistically but they show that the combination is more effective than either of the single chelants.

B. Conditional Constants and Therapeutic Effectiveness

In order to correlate or predict the in vivo effectiveness of chelants, it is necessary that the conditional constants be utilized so as to take into account at least part of the reactions which compete with the injected chelant for metal binding (see Schubert, in "Iron Metabolism", F. Gross, Ed., Springer-Verlag, Berlin, pp. 466-494, 1964). Two of the most important interfering ions in the case of Cd^{2+} are H^+ and Ca^{2+} , both of which compete for the chelant; EDTA and DTPA, but Ca^{2+} does not compete for DMPS. At pH7 and taking plasma Ca^{2+} to be 10^{-3}M , we find that $\log K'_{\text{CdL}} = 18.9$ for CdEDTA, 11.5 for CdDTPA and 9.6 for Cd-DMPS.

From the data of Figure 1 and Table 1 we see that DTPA is, indeed, more effective than EDTA as the log K' values indicate. On a molar basis, however, DMPS is less effective than its log K'_{CdL} would lead one to expect. However, this appears to be a special case since it is known that the Cd levels above the controls in the kidneys of rodents treated with sulfur-containing ligands increase in the acutely poisoned animals (Niemeir, Int. Arch. Gewerbepath. Gewerbehyg. 24, 160-168, 1967). In combination, however, with DTPA which prevents or reduce the Cd kidney deposits, great improvement in therapeutic effectiveness ensues. In fact, in clinical practice, the related combination of EDTA+BAL has saved many lives of children suffering from acute lead poisoning who would have otherwise died.

C. Potentiometric Titrations and Screening

We have completed the titration of DTPA, EDTA, and DMPS with and without the presence of Cd^{2+} in order to evaluate the binding affinities and to assess the degree, if any, of mixed ligand chelate formation. At the outset, it must be noted that the aforementioned chelants all form strong 1:1 chelates with Cd^{2+} of approximately similar strengths. This means that it is unlikely that combinations of these particular chelants will form strong mixed ligand chelates. However, as mentioned earlier, the pharmacokinetic advantages are also important. The last two protons of the three chelants are tightly bound, with pK 's of ~ 8.5 for one and ~ 11 for the other. This means that a metal ion must form a strong 1:1 chelate in order to displace the protons at pH7. The species fraction for DMPS is shown in Figure 2. Titration data for EDTA, DTPA, DMPS, singly and in combination, are shown in Figures 3 and 4. The proton formation constants as calculated from these data using the Gran analysis

(Gran Analyst 77, 661-671, 1952; and Seymour, Doctoral Thesis, University of Arizona, 1978) were in reasonably good agreement with literature values.

In the presence of Cd^{2+} , all of the protons from EDTA, DTPA, and DMPS are displaced at a pH of about 4.0, demonstrating the very strong chelating ability. The log of the 1:1 formation constants (not the conditional constants) are $\text{CdEDTA} = 16.5$; $\text{CdDTPA} = 19.1$; $\text{CdDMPS} = 16.7$; and $\text{Cd}(\text{DMPS})_2 = 26.9$. Our calculations, as given in the literature from the data in the Figures, are qualitatively in agreement, but as discussed later, additional data on pCd^{2+} are needed to complete our calculations.

When equimolar combinations of Cd and two chelants are titrated, mixed ligand chelates may be formed, but at pH's above the physiological (Figures 3-6). The formation of a 1:2 CdDMPS is clearly shown in Figure 7 by the release of four protons ($a=4$) beginning at pH 7:

Figure 2. The species fraction of DMPS (H_3L), shown below, is given as a function of pH at ionic strength 0.1. The first proton, being that of a sulfonate group, is loosely bound. Those protons associated with sulfhydryl groups are lightly bound.

The $pK_2 = 8.84$ and $pK_3 = 11.20$ as given by Pilipento et al, Ukm. Khim Zhurn 36, 278-284 (1970), were used to construct the figure.

Figure 6

D. Genotoxicity of Chelants and Chromium

The first phase of investigations was completed on the genotoxicity of chromium compounds with a variety of complexing and chelating agents using tests with Bacillus subtilus and Salmonella typhimurium rec-assay procedures (Gentile, Hyde and Schubert, #7 in list of publications). At the same time we determined whether the individual and combinations of chelating agents were mutagenic. These studies were done in collaboration with Prof. J. Gentile of the Department of Biology.

1. Mutagenicity of chelants

More of the chelating agents--citrate, CaNa_2EDTA , Na_4EDTA , NaSA, Tiron, $\text{CaNa}_2\text{EDTA/NaSA}$, $\text{CaNa}_2\text{EDTA/Tiron}$, or $\text{CaNa}_2\text{EDTA/DTPA}$ gave a positive response except CaNa_2EDTA (0.8M) and NaSA (1.2M) at the highest concentrations tested. In the absence of calcium, i.e., $\text{Na}_2\text{H}_2\text{EDTA}$, a positive response was noted even at 0.1 - 0.4M.

2. Chromium mutagenicity

As has been shown by numerous investigators, we also found that Cr(VI) salts are mutagenic but not Cr(III) compounds, namely CrCl_3 , $\text{CrK}(\text{SO}_4)_2$, and $\text{Cr}_2(\text{SO}_4)_3$. The positive responses of $\text{Cr}_2\text{O}_7^{2-}$ in the B. subtilus rec-assay decreases with increasing concentration of NaSA, CaNa_2EDTA , or Tiron. Tiron was the most effective because it acted by reductive chelation, i.e., it immediately reduced Cr(VI) to Cr(III) prior to chelation. No synergistic effects were observed with chelant combinations.

Since the lack of mutagenicity by Cr(III), the ultimate mutagen, is due to its inability to be transported within the

cell, unlike Cr(VI), we induced a positive but small response when we complexed Cr(III) with SA or citrate.

The mutagenicity of $\text{Cr}_2\text{O}_7^{2-}$ was time dependent, decreasing with a half-time of 30 minutes measured from the time of solution of the crystals in water.

IV. FUTURE INVESTIGATIONS

From the results and experience gained during the course of the Project, we feel that combinations of chelating agents offer the best hope for improvement of therapy for toxic metal poisoning. The use of metabolically stable chelants which form mixed ligand chelants of extraordinary high formation constants offer even more interesting possibilities. Here is a summary of recommended investigations:

- (1) Screen possible combinations of chelating agents for treatment of toxic metal poisoning by potentiometric titration.
- (2) To obtain precise formation constants it will be necessary, depending on the chelating agent, to use, for example, metal ion specific electrodes in order to obtain pM's in the low pH region.
- (3) Biological testing for metal poisoning and decorporation of the most promising chelating agent combinations as revealed by chemical screening.
- (4) Evaluation of the toxicity and efficacy of treatment when combinations of toxic metals are administered to experimental animals.

Finally, in order to complete an evaluation of the enhanced effectiveness of the DTPA + DMPS combination in Cd poisoning we must carry out studies on the tissue distribution of Cd following treatment with different doses of single and combined chelants.

V. COMPLIANCE WITH CONTRACT REQUIREMENTS

We carried out systematic investigations. (1) The effectiveness in mice of single and combinations of chelating agents for the decorporation of monomeric Pu(IV); (2) Treatment of metal poisoning by chelation therapy, especially of cadmium by chelant combinations; (3) Potentiometric titration of Cd^{2+} with various single and mixed chelants in order to correlate their binding affinities with Cd^{2+} ; (4) The influence of complexation on the mutagenicity of Cr(III) and Cr(VI); (5) The mutagenicity of single and mixed chelants; and (6) The effect of chelants on the elimination of endogenous trace metals. All of these studies were devoted to the improvement of therapy of toxic metal poisoning and the decorporation of transuranium radioactive metals.

VI. EFFORT OF PRINCIPAL INVESTIGATOR

The Principal Investigator has devoted about 65% of his time to the project compared to the 50% budgeted time.