258
9/4/79
LA. 3083

ORNL/TM-6955

MASTER

**Program for the Development of Physical
and Biological Concepts as a Basis for
Health Protection Criteria for the
Exposure of Humans to Metals**

J. E. Turner
M. W. Williams
K. B. Jacobson
S. Mitra
S. K. Niyogi
H. Witschi

OAK RIDGE NATIONAL LABORATORY
OPERATED BY UNION CARBIDE CORPORATION · FOR THE DEPARTMENT OF ENERGY

REPRODUCTION OF THIS DOCUMENT IS UNLIMITED

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.

Printed in the United States of America. Available from
National Technical Information Service
U.S. Department of Commerce
5285 Port Royal Road, Springfield, Virginia 22161
Price: Printed Copy \$4.00; Microfiche \$3.00

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, contractors, subcontractors, or their employees, makes any warranty, express or implied, nor assumes any legal liability or responsibility for any third party's use or the results of such use of any information, apparatus, product or process disclosed in this report, nor represents that its use by such third party would not infringe privately owned rights.

Contract No. W-7405-eng-26

PROGRAM FOR THE DEVELOPMENT OF PHYSICAL AND BIOLOGICAL CONCEPTS
AS A BASIS FOR HEALTH PROTECTION CRITERIA
FOR THE EXPOSURE OF HUMANS TO METALS

J. E. Turner and M. W. Williams
Health and Safety Research Division

and

K. B. Jacobson, S. Mitra, S. K. Niyogi,
and H. Witschi
Biology Division

NOTICE

This report was prepared as an account of work sponsored by the United States Government. Neither the United States nor the United States Department of Energy, nor any of their employees, nor any of their contractors, subcontractors, or 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.

Date Published - August 1979

OAK RIDGE NATIONAL LABORATORY
Oak Ridge, Tennessee 37830
operated by
UNION CARBIDE CORPORATION
for the
DEPARTMENT OF ENERGY

THIS PAGE
WAS INTENTIONALLY
LEFT BLANK

CONTENTS

	<u>Page</u>
Highlights	v
Abstract	1
1. The Choice of Metals as a Class for Investigation	1
2. Some Specific Studies of Effects of Metals on Biological Systems	3
3. Physical-Chemical Approach to Interaction of Metals with Nucleic Acids and Proteins	6
4. Research Program	11
References	12

THIS PAGE
WAS INTENTIONALLY
LEFT BLANK

HIGHLIGHTS

This is the first report from a program recently established at ORNL. The aim in this program is to discover physical and/or biological concepts upon which to base health protection criteria for exposure to chemical pollutants. Emphasis is being placed on approaches which could yield an understanding of the basic interactions associated with toxicity so that predictions of toxicity may be made, at least for certain classes of pollutants.

Metals have been selected as the first class of toxic chemicals for study. Experiments on the chain initiation of RNA synthesis in the presence of various metal ions are being performed in the Biology Division, ORNL. A correlation has already been found between the carcinogenic or mutagenic action of a metal and the degree of stimulation of chain initiation of RNA synthesis. The relative amounts of the isoacceptors for the tRNAs extracted from *Drosophila* raised on diets containing Zn or Cd are also being examined. In yet another study, Zn and Cd are being compared for their effects on the chromatographic behavior of *Drosophila* tyrosine and leucine tRNAs. In all three studies being conducted in the Biology Division, the congeners Zn and Cd show different effects. Studies have been suggested in the Health and Safety Research Division that might help elucidate the experimental results obtained. Calculations are proposed whereby the physical association of metals with biological macromolecules will be characterized by accommodation of metal complexes or hydrated metal complexes of the "right" size, shape, and electronic charge distribution to "fit" into a molecular structure. In so doing, the metal complex might alter the molecular conformation to create the stereochemical conditions it needs for attachment. X-ray studies of tRNA crystallized in the presence of metals, for example, show specific binding sites where a metal attaches to the molecule. Electronic states of the metal complex must match available sites on the macromolecule, with respect to both charge and three-dimensional stereochemical positioning. In this respect, the splitting of the available atomic electronic states of a metal ion in the presence of other charge distributions is of importance.

Although details of such interactions will be very complicated, qualitative predictions might be made.

If successful, the interpretation of metal toxicity data in such physical-chemical terms should provide some insight into the different effects of Zn and Cd as manifested in the experiments considered. It should also lead to predictions of the relative toxicities of a large number of metals, predictions which could then be tested experimentally.

PROGRAM FOR THE DEVELOPMENT OF PHYSICAL AND BIOLOGICAL CONCEPTS
AS A BASIS FOR HEALTH PROTECTION CRITERIA
FOR THE EXPOSURE OF HUMANS TO METALS

J. E. Turner, M. W. Williams, K. B. Jacobson, S. Mitra,
S. K. Niyogi, and H. Witschi

ABSTRACT

This report outlines some preliminary ideas for a new program to develop physical and biological principles for possible use in formulating health protection criteria for exposure to metals.

1. THE CHOICE OF METALS AS A CLASS FOR INVESTIGATION

The task is to discover, develop, or to invent general physical and biological concepts and principles upon which to base health protection criteria for exposure to chemical pollutants. The number of different chemicals and the complexity of living organisms provide an almost endless variety of "exposure-response" systems that could be studied. Initially, a limited class of toxic substances will be considered which, depending on the experimental results, may enable expansion to additional substances and, possibly, invention of new pertinent assays. Emphasis will be placed on finding fundamental properties of a toxic substance that will allow understanding of the reason for its toxic behavior, so that predictions can be made for all substances that possess the same toxic tendencies rather than having to depend on an endless array of biological tests.

Metals have been selected as the first class of toxic chemicals for study. Metals are used widely in our industrial society and can cause various health problems (e.g., cadmium causes pulmonary disorders, kidney damage, and testicular destruction; lead causes anemia; a number of metals are mutagenic or carcinogenic; mercury causes neurological damage). Metals cause their effects through a variety of mechanisms, most of which are poorly delineated; some metals are accumulated while others are readily excreted. Each metal seems to have one or more target organs. In short, a great deal of descriptive information has been accumulated on the toxic effects of metals. The reasons for selecting metals as the

first class of chemicals for study may be summarized as follows: First, a considerable amount of biological data is available for metals. Second, unlike many organic compounds that lose their identity after being metabolized, metal ions can be followed to their ultimate biological end point. Third, metals therefore represent a relatively distinct and simple class of important toxic pollutants for which one might construct general concepts for determining the permissible human exposure. Fourth, and perhaps crucial for this new effort, a number of programs and facilities already exist at ORNL, and significant work can begin immediately. The lead time for obtaining answers to many questions about toxic metals is short, so a rapid response to this challenge is possible.

The effects of metals are known to include reactions with several functional groups on proteins and nucleic acids. How the reaction between a metal and one or more of these macromolecules causes damage to specific cells, tissues, and organs is one of the key questions to understanding their toxic effects. For those metals known to be carcinogenic, the question arises as to what nucleic acids or proteins are altered, and how, to result in carcinogenesis. For those metals that cause specific and characteristic damage such as testicular deterioration, kidney malfunction, or pulmonary incapacitation, it may be useful to know what components of the cell were altered that result in the malfunctions. Since nucleic acids and proteins are the fundamental substances that determine normal reproduction and growth, a significant effort should be made to achieve an understanding of the manner in which metals and these macromolecules interact. In parallel with these kinds of studies, the integrated activities of cells and organisms should be examined. The integrated activities are represented by processes such as (1) transport of substances into cells, (2) protein synthesis, (3) nucleic acid synthesis, (4) the living state as exemplified by cells and organisms, and (5) inheritance of genetic traits. Further amplification on these integrated activities will be the topic of a subsequent report.

2. SOME SPECIFIC STUDIES OF EFFECTS OF METALS ON BIOLOGICAL SYSTEMS

The observations of correlations between the properties of several metals and their biological action could offer valuable guidance in setting exposure guidelines, especially if they were of predictive value. However, correlations, *per se*, do not necessarily imply a cause-effect relationship. Studies at the level of nucleic acid synthesis may involve a fuller understanding of possible interactions of metal cations with biological molecules--in effect, biochemical characterization of reactions taking place at certain sites on the biological macromolecules. Observed correlations between biochemical changes and effect at this basic molecular level would suggest specific cause-effect hypotheses that could be tested experimentally. Data obtained for one metal could be applied with some degree of understanding to another toxicologically significant metal for which data are lacking.

Two facets of a program to develop health protection concepts for metals (as well as other chemicals) can thus be recognized. First, general correlations can be sought to find empirically any quantities that might appear to be related to toxicity. Second, and of much more significance, basic studies can be carried out at the molecular level to understand fundamental processes that characterize biochemical changes brought about by metals. Such studies would lead naturally to the formulation of relevant concepts based on actual mechanisms; they should also yield contributions of basic scientific importance. Of course, ideally, both of these approaches should be basic to all toxicological studies.

In vitro assays have been proposed as a method for screening chemicals as potential carcinogens and mutagens. For example, in the well known Ames test,¹ suspected mutagenic chemicals are evaluated by their ability to revert mutations included previously in special strains of *S. typhimurium*. In a similar vein, other kinds of *in vitro* assays may be developed for metals. In fact, several examples of *in vitro* assays can be identified as possible starting points for developing the needed concepts for exposure to metals. Sirover and Loeb² tested 31 salts of several metals for their effect on the fidelity of DNA synthesis. Hoffman and Niyogi³ investigated chain initiation of RNA synthesis in the presence of chloride salts of 10

metals. In both sets of experiments a rather striking correlation was found between the carcinogenic or mutagenic action of a metal and its detrimental influence relative to controls in the experimental system, viz., the decreased fidelity of DNA synthesis or stimulation of chain initiation of RNA synthesis.

The metals studied in these two experiments are indicated in Fig. 1 with symbols enclosed by a square (little or no effect on system) or a triangle (adverse effect on system). The monovalent alkali metals and divalent alkaline earths do not appear to disrupt the processes studied, even in relatively large concentrations. Since beryllium is uniquely situated among the elements, it could probably be argued that this metal is a special case--a very small divalent ion not necessarily to be included in a generalized dosimetric framework for the toxicity of most metals. Trivalent aluminum in aqueous solution may also exhibit special properties. Beryllium has a number of industrial applications and, if workers are unprotected, causes severe toxic effects. Aluminum is widely used in food preparation and has little toxic effect as the metal or the metal ion. The transition metals, however, represent a group of elements of successive atomic number whose physics and chemistry can be systematically studied. From among the six metals used from the first transition series in these *in vitro* studies, Fe shows contrasting behavior to the others (a square instead of a triangle in Fig. 1). The congeners Zn and Cd have different effects. Niyogi and co-workers are continuing the studies on RNA synthesis³ in the Biology Division at ORNL. They are presently exploring the effects of several of the metals in greater detail than before.

Other experiments involving metals are being carried out in the Biology Division of ORNL using *Drosophila*. *Drosophila* offers several advantages for toxicological studies because it has been more thoroughly characterized genetically than mammals, it has a short life span (~60-100 days), and it can be grown very inexpensively. When tRNA is extracted from a cell or organism, the relative amounts of each tRNA isoacceptor are found to be quite rigidly controlled. These relative amounts of the isoacceptors for tyrosine, histidine, and aspartic acid tRNAs are altered in proportion to the Cd concentration in the diet on which *Drosophila* are

ORNL-DWG 79-11487

H																		He
Li	Be											B	C	N	O	F	Ne	
Na	Mg											Al	Si	P	S	Cl	Ar	
K	Ca	Sc	Ti	V	Cr	Mn	Fe	Co	Ni	Cu	Zn	Ga	Ge	As	Se	Br	Kr	
Rb	Sr	Y	Zr	Nb	Mo	Tc	Ru	Rh	Pd	Ag	Cd	In	Sn	Sb	Te	I	Xe	
Cs	Ba	La	Hf	Ta	W	Re	Os	Ir	Pt	Au	Hg	Tl	Pb	Bi	Po	At	Rn	

RARE
EARTHS

△ METALS AFFECTING FIDELITY OF DNA SYNTHESIS OR STIMULATION OF
CHAIN INITIATION OF RNA SYNTHESIS IN VITRO.

□ METALS SHOWING LITTLE OR NO EFFECT ON THESE PROCESSES.
AT THE LEVELS STUDIED.

raised.⁴ On the other hand, if comparable or much larger amounts of Zn are included in the diet, the tRNA isoacceptors remain in the normal ratios. Again, Cd and Zn have different effects. The consistently predictable patterns of tRNA isoacceptors are examples of integrated systems functioning in the organism; it will be of interest to learn how the balance of such integrated systems is upset by Cd, but not by Zn. *Drosophila* has many metabolic functions in common with mammals. There are many similarities in the existence of isoacceptors for the tRNAs. Leucine tRNA has six to eight isoacceptors in both *Drosophila* and the rat; tyrosine tRNA has two in *Drosophila* and four in mammalian cells, but in each case one-half of the number present contain a specific nucleoside queuosine and the other half do not; and aspartic acid tRNA, histidine tRNA, and asparagine tRNA are distributed similarly to tyrosine tRNA due to the presence or absence of queuosine. No clear metabolic role has been assigned to the many isoacceptors of tRNA, but their relative amounts are rigidly controlled in all animals. The information obtained from *Drosophila*, therefore, may be directly applicable to higher animals, including man.

In another series of experiments being conducted in the Biology Division, Zn and Cd are compared for their effects on the chromatographic behavior of *Drosophila* tyrosine and leucine tRNAs.⁵ For example, it has been found that as the concentration of Cd^{++} is increased, isoacceptors for each of these amino acids appear to merge and to elute earlier. There is no corresponding merging of isoacceptors with Zn^{++} , and they elute later relative to zero Zn^{++} concentration. Again, the congeners Zn and Cd have different effects.

This program will attempt to explain metal toxicity data, such as those described in this section, in terms of physical and chemical interactions between the metal ions and biological macromolecules.

3. PHYSICAL-CHEMICAL APPROACH TO INTERACTION OF METALS WITH NUCLEIC ACIDS AND PROTEINS

No obvious atomic periodicity can be discerned in Fig. 1 in the biological toxicity of metals as expressed in the nucleic acid experiments. Nevertheless, atomic parameters may be useful for distinguishing general characteristics of different metals. For example, the ratio of charge and ionic radius often gives an indication of the tendency of an ion to

form complexes or to become hydrated in aqueous solution. This ratio is smallest (~ 0.6 - 1.0 electronic charges/ $\text{\AA}$) for the alkali metals, which show less tendency to form complexes than for Al^{3+} or Pt^{4+} , for which the ratio of ~ 5.3 - 6.0 is the largest. The transition metals have intermediate values (e.g., 4.5 for Co^{3+}). As another example, a rule of thumb holds that soft metals tend to bind to nucleic acid bases and hard metals to phosphates.

Coordination chemistry will undoubtedly play an essential role in the search for a general characterization of hazardous metals, since it appears unlikely that specific atomic properties of individual metals are reflected in any direct way. In fact, biological macromolecules may not be exposed to bare metal ions to any appreciable extent *in vivo* or during *in vitro* experiments. The ions become hydrated in aqueous solution and can form other complexes in media where other chemical species are present. Ions of the same metal in different oxidation states can have entirely different chemical properties.

The physical association of metals with biological macromolecules through various types of bonds can probably be characterized by accommodation of metal complexes or hydrated metal complexes of the "right" size, shape, and electronic charge distribution to "fit" into a molecular structure. In so doing, the metal complex might alter the molecular conformation to create the stereochemical conditions it needs for attachment. X-ray studies of tRNA crystallized in the presence of metals, for example, show specific binding sites where a metal attaches to the molecule. Electronic states of the metal complex must match available sites on the macromolecule, with respect to both charge and three-dimensional stereochemical positioning. Figure 2 shows an example from crystallographic studies of the binding of an hydrated manganese ion to guanosine monophosphate at the N-7 position.⁶ Five coordinated water molecules complete the octahedral complex of the metal. Two of the phosphate oxygens are hydrogen-bonded to coordinated water molecules.

The first transition series of metals (^{21}Sc through ^{29}Cu) offers an especially interesting group because of the availability of 3d states for electrons to form bonds. The five spatial 3d states, which can be occupied by 10 electrons, are degenerate in a pure Coulombic field, which is

ORNL- DWG 79-11488

Fig. 2. Schematic structure of the $[\text{Mn}(5'\text{-GMP})(\text{H}_2\text{O})_5]$ molecule from de Meester et al., *Biochem. J.* 139, 791 (1974).

approximately that of an isolated metal atom or ion. In the presence of other charge distributions (such as might occur in a complex or in the vicinity of a macromolecule) the d states of the perturbed atom are split. The nature of the splitting will depend on the charge distribution presented to the metal by the complexing atoms and by the macromolecule. Under some circumstances bonding will occur between a metal or metal ligand and a macromolecule. It is interesting to consider the general conditions under which the perturbation of available states in response to charge sites on biological macromolecules would occur in such a way as to encourage the formation of bonds. Certain proteins and enzymes can function normally only when the proper metal ion is present. Studies on metalloproteins would provide a useful orientation for some of these considerations.

The splitting of available atomic electronic states is of recognized importance in understanding the structure of crystals of metal salts and hydrated metal salts, the coordination chemistry of the transition metals, and ligand field theory.⁷ The existence of many stereochemical structures, such as square-planar, tetrahedral, and octahedral complexes, can be accounted for simply by considering the number and angular dependence of orbitals available for bonding. For example, Fig. 3 shows the way in which the energies of the five independent 3d orbital states change in response to several different distributions of negative charge about the free ion. Although details of the interactions are very complicated, the change in the energies of the five states can be predicted qualitatively from the knowledge of where the other charges are, coupled with the known spatial properties of the 3d wave functions. The actual states of electrons in such chemical bonding do not generally correspond to integral occupation numbers of these orbitals. However, knowledge of the number and nature of possible ligands can be obtained from quite general arguments on the known number and spatial characteristics of the available quantum states.

We propose to apply this kind of analysis to understanding and predicting certain features of biological metal-ion structures, such as the one shown in Fig. 2. A proper characterization of macromolecules is needed in terms of their charge distributions (static and induced) at locations available for bonding, such as the distributions presented by

Fig. 3. Characteristic splitting of the five degenerate 3d orbitals of a free ion in tetrahedral and octahedral fields. Tetragonal distortion of the octahedral field is also shown.

phosphate bonds and by the purine N7 and pyrimidine N3 sites. These characteristics could then be analyzed in terms of their stereochemical availability to hydrated metal-ion complexes. Arguments of this nature have been used by Jack *et al.*⁸ in analyzing strong Mg binding sites on yeast phenylalanine tRNA: "Magnesium is normally hexa-hydrated in solution, and may be expected to bind to nucleic acids *via* hydrogen bonds between these water molecules and suitable acceptor atoms, such as phosphate oxygens. An alternative mode of binding would involve loss of one or more water molecules from the hydration shell, and the formation of a direct bond to the phosphate oxygen atom. This is the binding scheme proposed at the cooperative sites..., and we have taken our criterion of a strongly bound magnesium to be that it should be bonded directly to one or more phosphate oxygens."

The characterization of hydrated metal ion complexes in aqueous solution in ways that might suggest their tendency to exchange one or more water molecules is also of general relevance and will be investigated. Metal ions and the charge sites on biological macromolecules in aqueous solution compete for the positioning of water dipoles about themselves. The tetrahedrally structured liquid water itself shows a preference for the formation of certain complexes to the exclusion of others. We plan to explore the extent to which the biological activity of metals might be directly related to the hydration structures of metal ions in solution. Such structures can be calculated and/or determined experimentally by x-ray and neutron scattering from the surfaces of ionic solutions.

4. RESEARCH PROGRAM

The new program to develop physical and biological concepts for characterizing potential hazards of metals is based on detailed theoretical and experimental investigations of microscopic interactions of metal ions and complexes with biological macromolecules. The program includes the study of the relation of the hydration structure of metal ions in solution to their reactivity with biological macromolecules.

The attachment of metals and metal complexes to nucleotides, nucleic acids, proteins, and other structures results in modification of normal

cellular functions. Thus, it appears reasonable to study and to characterize systematically the three-dimensional sites available for binding on macromolecules--sites such as those presented by phosphodiester bonds and by specific atoms in the purine and pyrimidine structures of nucleic acids. It should be possible to characterize the states of the metal complexes made available for bonding, such as those derived from perturbation of the d states in the transition metals. General conditions favoring the bonding of different metal complexes can, hopefully, be identified and considered as a basis for interference with normal biochemical processes. If successful, the interpretation of metal toxicity data in such physical-chemical terms should lead to predictions of the relative toxicity of metals. Such predictions could then be tested by experiments such as those discussed in Sect. 2.

REFERENCES

1. B. N. Ames, P. Sims and P. L. Grover, *Science* 176, 47 (1972); J. McCann and B. N. Ames, *Proc. Natl. Acad. Sci. USA* 72, 4135 (1975) and 73, 950 (1976).
2. M. A. Sirover and L. A. Loeb, *Science* 194, 1434 (1976).
3. D. J. Hoffman and S. K. Niyogi, *Science* 198, 513 (1977).
4. Lee Opresko, K. B. Jacobson, and R. K. Owenby, in preparation.
5. Vivian S. Hiatt and K. B. Jacobson, in preparation.
6. P. de Meester, D. M. L. Goodgame, T. J. Jones, and A. C. Skopski, *Biochem. J.* 139, 791 (1974).
7. Cf., e.g., F. A. Cotton and G. Wilkinson, *Advanced Inorganic Chemistry*, Interscience Division of John Wiley and Sons (New York, 1972) 3rd Ed., Chap. 20.
8. A. Jack, J. E. Ladner, D. Rhodes, R. S. Brown, and A. Klug, *J. Mol. Biol.* 111, 315 (1977).

INTERNAL DISTRIBUTION

1. Biology Library	35. R. F. Kimball
2-3. Central Research Library	36. F. F. Knapp
4. Document Reference Section	37-42. S. Mitra
5-6. Laboratory Records Department	43. A. H. Narten
7. Laboratory Records, ORNL R.C.	44-49. S. K. Niyogi
8. ORNL Patent Office	50. D. C. Parzyck
9. R. D. Birkhoff	51. M. L. Randolph
10. L. G. Christophorou	52. C. R. Richmond
11. J. L. Epler	53. P. S. Rohwer
12-17. J. D. Hoeschele	54. J. B. Storer
18. A. W. Hsie	55. R. Triolo
19-31. K. B. Jacobson	56-68. J. E. Turner
32. S. V. Kaye	69. T. Vo-Dinh
33. O. L. Keller	70-81. M. W. Williams
34. G. G. Killough	82-87. H. Witschi

EXTERNAL DISTRIBUTION

88. R. S. Berry, Department of Chemistry, University of Chicago, Chicago, IL 60637
89. G. G. Eichholz, Department of Nuclear Engineering, Georgia Institute of Technology, Atlanta, GA 30332
90. P. S. Furcinitti, Safety and Environmental Protection Division, Brookhaven National Laboratory, Upton, NY 11973
91. S. Georghiou, Physics Department, University of Tennessee, Knoxville, TN 37916
92. C. E. Hildebrand, Los Alamos Scientific Laboratory, University of California, Los Alamos, NM 87545
93. F. Hudson, Department of Energy, Washington, DC 20545
94. D. G. Jacobs, Evaluation Research Corporation, 25 E. Tennessee Avenue, Oak Ridge, TN 37830
95. P. H. M. Lohman, Secretary, ICPEMC, Medical Biological Laboratory TNO, P. O. Box 45, 2280 AA Riiswiik, The Netherlands
96. V. A. Newill, Environmental Health Research Division, Medical Department, Exxon Corporation, P. O. Box 45, Linden, NJ 07036
97. C. M. Shy, Institute for Environmental Studies, Department of Epidemiology, University of North Carolina, Chapel Hill, NC 27514
98. Paul W. Todd, 403 Althouse Laboratory, University Park, PA 16802
99. R. W. Wood, Department of Energy, Washington, DC 20545
100. McDonald E. Wrenn, Building 522, The University of Utah College of Medicine, Salt Lake City, UT 84112
101. Office of Assistant Manager, Energy Research and Development, Department of Energy, Oak Ridge Operations Office, Oak Ridge, TN 37830
- 102-359. Given Distribution as shown in TID-4500, distribution category UC-41