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US ARMY MEDICAL RESEARCH INSTITUTE OF CHEMICAL DEFENSE
ABERDEEN PROVING GROUND, MARYLAND 21010-5425

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DECISION TREE NETWORK FOR THE IDENTIFICATION OF ANTICYANIDE COMPOUNDS

Edited by

David E. Lenz
Thomas Brewer

July 1989

US ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
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An Anticyanide Decision Tree Network (DTN) has been designed to rapidly identify drugs which, when used on a short-term basis before expected agent exposure or immediately after exposure, will be effective in the prevention or treatment of cyanide poisoning. Three basic guidelines were used in the design of the DTN: 1) that both animal and test drug requirements be the minimum needed to provide valid comparisons of proposed therapies; 2) that proposed compounds showing no promise be eliminated from consideration as rapidly as possible; and 3) that the testing strategy provide the maximum amount of useful information possible for quantitative structure activity relationship (QSAR) studies. Although pretreatment compounds are considered more important, all compounds are scored equally depending on their activity. A series of in vitro tests will be conducted first to quickly screen for a test compound's activity and to help determine its mechanism of action before in vivo safety and efficacy tests are carried out on different animal models.

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Preface

In August 1985, the Commander, U.S. Army Medical Research Institute of Chemical Defense (USAMRICD) appointed an Ad Hoc Tech Base Drug Development Task Area Working Group composed of members from USAMRICD and Walter Reed Army Institute of Research (WRAIR). The working group was given a number of objectives, the overall scope of which was to recommend improvements in the Medical Chemical Defense Tech Base Drug Development Program. The working group met on a number of occasions. At the 15 January 1986 meeting, it recommended the establishment of technical evaluation committees (TECs) to evaluate and make recommendations relating to drug screening. Since, at that time, formal decision networks for tech base drug development did not exist, the working group recommended that the TECs develop these networks as an item of high priority. The concept of the TEC was implemented at USAMRICD in June 1986 by the formation of the Drug Assessment Technical Evaluation Committee (DATEC). Soon after the first DATEC meeting, subcommittees were appointed to develop specific drug decision networks. Because the networks had a tree-like branching structure, they soon became known as Decision Tree Networks (DTNs). It was recognized that different networks were needed for different classes of drugs being screened and for the kind of chemical threat agent being countered. The subcommittee's work addressed the following DTNs:

Anticonvulsant - to identify drugs having anticonvulsant properties which might be effective in either the pretreatment or treatment modes against organophosphorus agents.

Anticyanide - to identify compounds having efficacy (by any mechanism) in the prevention of therapy of cyanide poisoning.

Antivesicant - to identify compounds having efficacy (by any mechanism) in the prevention or therapy of the vesicant injury.

Cholinolytic - to identify compounds clearly superior to atropine with respect to efficacy and safety.

Pretreatment - to identify drugs which, when used on a short-term basis before expected nerve agent exposure, might be effective (in combination with therapy) in the amelioration of organophosphorus agent injury.

Reactivator - to identify compounds that are thought to have efficacy based on a potential for reactivating organophosphorus-inhibited acetylcholinesterase (AChE).

The objective of the DTN development project was to provide a management concept and program strategy that would facilitate the rapid identification of the best candidate compounds. Proponents believed that DTNs would provide the following advantages:

- Rapid selection of the most promising candidates,

- Quick elimination of poor candidates and conservation of resources,
- Assurance of comparability of data,
- Provision of quantitative data for new drug design, and
- Ease of prioritization of resources.

Contributors

The testing strategy reflected in the Anticyanide DTN represents the scientific consensus of the Anticyanide DTN Subcommittee. The subcommittee met on a weekly basis, in 2- to 4-hour sessions, over the course of a 6-month period. The subcommittee received final DATEC approval of the Anticyanide DTN in September 1988. The DTN presented here was recommended to the Commander, USAMRICD, and represents the best effort of the scientists who staffed the committee. The subcommittee members were as follows:

David E. Lenz, Chairman
Pharmacology Division
USAMRICD

Steven Baskin
Pharmacology Division
USAMRICD

Thomas Brewer
Department of Pharmacology
WRAIR

Larry Brown
Department of Pharmacology
WRAIR

David Davidson
Department of Pharmacology
WRAIR

Philip Hammond
Drug Assessment Division
USAMRICD

Walter E. Sultan
Drug Assessment Division
USAMRICD

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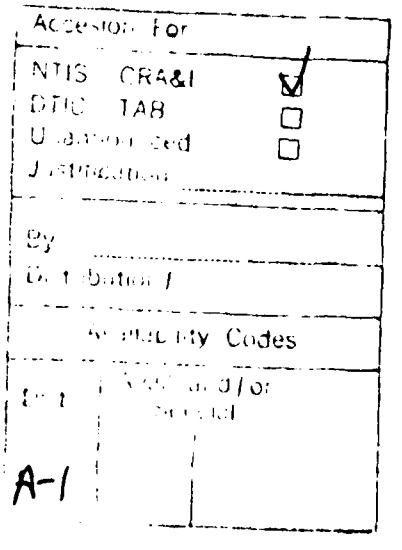

U.S. Army Medical Research Institute of Chemical Defense
Aberdeen Proving Ground, MD 21010-5425

DECISION TREE NETWORK

for the identification of

ANTICYANIDE COMPOUNDS

Edited by:

David E. Lenz
and
Thomas Brewer

FIRST EDITION

May 1989

Introduction

The Anticyanide DTN (Figure 1) is designed to rapidly identify drugs which, when used on a short-term basis before expected agent exposure or immediately after exposure, will be effective in the prevention or treatment of cyanide poisoning.

Anticyanide drugs are being considered for both pretreatment and treatment modalities to protect the exposed soldier from the effects of cyanide or to treat the afflicted soldier once poisoning has occurred. Although pretreatment compounds are considered more important, all compounds are being scored equally depending on their activity. Compounds that show efficacy as both pretreatment and treatment will achieve the highest scores based on the design of the network. A series of *in vitro* tests will be conducted first to quickly screen for a test compound's activity and to help determine its mechanism of action before *in vivo* safety and efficacy tests are done on different animal models.

Pretreatment compounds should be void of undesirable side effects that would cause its consumption prior to an actual threat to be objectionable. They will also be tested for oral bioavailability, since the oral route of administration is presently the most convenient method of pretreatment dosing. A higher incidence of side effects may be tolerated in effective treatment compounds where the life saving benefits of treatment outweigh the risk of side effects in an already compromised casualty.

The Anticyanide DTN was designed to capture information that will help evaluate the above characteristics and allow the best compounds to be identified. It is intended that these preliminary tests be followed by more complete safety and efficacy evaluations as part of the predevelopment testing phase.

Three basic guidelines were used in the design of the DTN:

- That both animal and test drug requirements be the minimum needed to provide valid comparisons of proposed therapies,
- That proposed compounds showing no promise be eliminated from consideration as rapidly as possible, and
- That the testing strategy provide the maximum amount of useful information possible for quantitative structure activity relationship (QSAR) studies.

Compound prioritization will be a dynamic process in which only the best candidate(s) available at any given time will be advanced. At all modules beyond Decision Point 2, only the "best of the best" available at that time will proceed.

Although highly structured to provide maximum definition to the drug assessment program, it is envisioned that all DTNs are flexible working documents which may be altered to reflect changes in program objectives or availability of resources. While tests included in this DTN have been developed previously and used by a number of different investigators and organizations, they have not been evaluated in the sequence or for the purpose that is proposed. The subcommittee members, therefore, expect that a validation study using several members of each proposed anticyanide class will be conducted to ensure the validity of the screen up to the final selection stage.

The subcommittee recognizes that, while there are many alternatives to the proposed sequence of the testing modules, the one defined in this document is considered to be a reasonable approach that should meet the objectives of the Medical Chemical Defense Research Program.

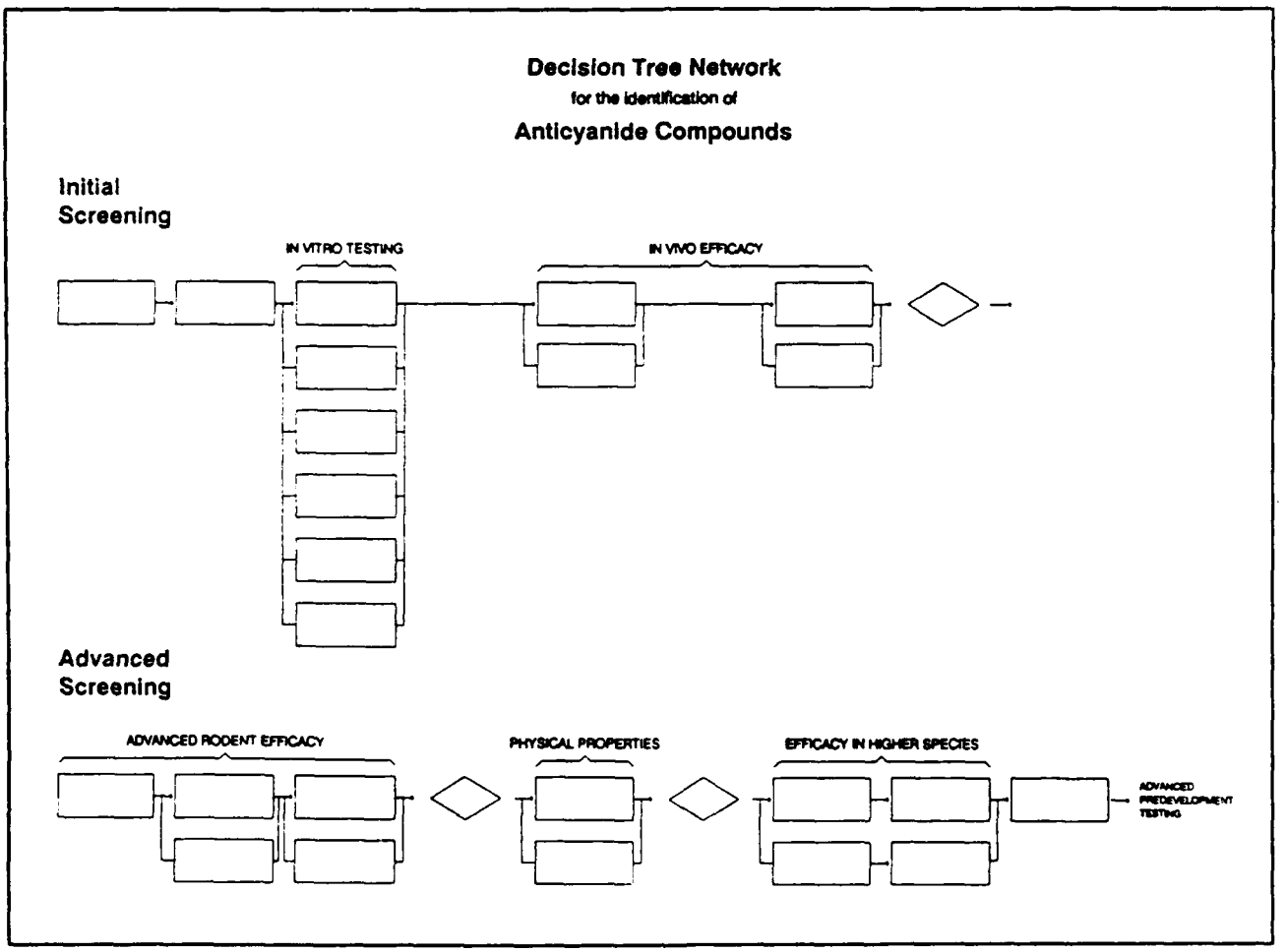

Figure 1

Overview of the Anticyanide Decision Tree Network

The major segments of the Anticyanide DTN are composed of a series of testing modules arranged in a logical sequence. The DTN has four decision points designed to ensure that only the most promising compounds undergo further testing. At each point, the results of the test compound are compared against scores of other experimental compounds; only the best are selected to proceed.

In the initial screening phase, the first segment is composed of *in vitro* evaluations from which testing priority of the available compounds is assigned. Even though it is now acknowledged that there is often poor correlation between activity and *in vivo* efficacy, the *in vitro* tests are useful to assess compound potency for well-characterized, desirable effects and to provide information on mechanism of action.

The second segment consists of *in vivo* screening. The initial *in vivo* screens evaluate compound side effects, therapeutic efficacy against cyanide lethality, and agent-induced incapacitation of survivors. Oral efficacy is assessed early in the DTN and compared to the results of the intramuscular testing. Anticyanide agents which show good efficacy intramuscularly, but are not active orally, will be reserved for treatment modality.

Since soldiers must perform their missions while being protected by pretreatment anticyanide agents, behavioral testing is evaluated, and efficacy at the no observable effect level (NOEL) is determined. Protective ratios for candidate compounds will be determined for intravenous and inhaled cyanide. To ensure that the results to this point are not the effect of a species-dependent process, efficacy in a second species is also determined.

Chemical stability and ease of laboratory scale-up of compound synthesis will be determined to ensure that detailed studies are not begun with unstable materials or compounds too costly for consideration in large-scale use.

At this point, a formal DATEC review is conducted to ensure that only the most promising compounds proceed to advanced predevelopment testing. Numerical scores will be computed for each compound and those with the highest scores will be given priority throughout the DTN.

It is important to note that much of the data generated during the initial and advanced screening phases is reported back to synthesis contractors in order to facilitate QSAR research. In addition, summary information for each test module is entered into the Drug Assessment Compound Tracking System (DACTS), a computerized program information system. This system tracks each compound through the screening process.

A thorough review of all data derived from the testing modules will be conducted. A clear recommendation for either selection or rejection of the candidate pretreatment will emerge from this

formal review. All data generated from these studies will be made accessible to all interested parties in the U.S. Army Medical Research and Development Command. This review and data access will provide guidance for both program management and QSAR, and is critical to the success of the testing program.

Detailed Description

INITIAL SCREENING

Initial Screening is designed to efficiently identify those compounds having the greatest efficacy against cyanide, those having the ability to speed recovery from cyanide-induced incapacitation, and those having a good margin of safety. This segment consists of compound accession, solubility determination, and *in vitro* and *in vivo* tests. The animal model of choice is the mouse.

Understanding the mechanism involved in cyanide poisoning and the treatment required will help explain what is being done during the *in vitro* testing phase of the initial screening. The extreme toxicity of cyanide is postulated to be the result of its rapid reaction with the trivalent iron of the intracellular enzyme cytochrome oxidase. The role of this enzyme in cellular oxygen utilization is inhibited by the formation of the cytochrome oxidase-cyanide complex. The resultant cytotoxic hypoxia leads to cellular dysfunction and death. One of the treatment objectives is to stimulate the production of methemoglobin which is normally present in the cells in a ratio of 99 parts of its predecessor hemoglobin to 1 part methemoglobin. The trivalent iron of methemoglobin competes with cytochrome oxidase for the cyanide ion forming a nontoxic cyanmethemoglobin compound. Various compounds will stimulate the oxidation of hemoglobin to methemoglobin, those that increase the ratio of methemoglobin to 10 percent of available hemoglobin will pass the screen. Compounds that protect cytochrome oxidase from the effects of cyanide will also be identified.

The major mechanism for removing cyanide from the body is its enzymatic conversion, by the mitochondrial enzyme rhodanese to thiocyanate, which is relatively nontoxic. To accelerate detoxification, thiosulfate is administered and the thiocyanate formed is readily excreted in the urine. Compounds that stimulate rhodanese activity will be identified during the *in vitro* testing. Because cyanide toxicity takes place within the cell, compound candidates will be screened for activity in a whole cell system. The assumption being, if a compound does not enter the cell, it can do little to counter the toxic effects of cyanide.

In vivo testing will be conducted for toxicity of both methemoglobin and nonmethemoglobin forming compounds will be followed by tests for efficacy.

All candidate compounds will be evaluated up to Decision Point 1. This will account for compounds that may have a mechanism of action that were not identified in the *in vitro* screen.

The Title, Purpose/Rationale, Description and Reference for each testing module of the Anticyanide DTN are presented below. Module numbers correspond to the numbering of the network modules illustrated in Figures 2 and 3.

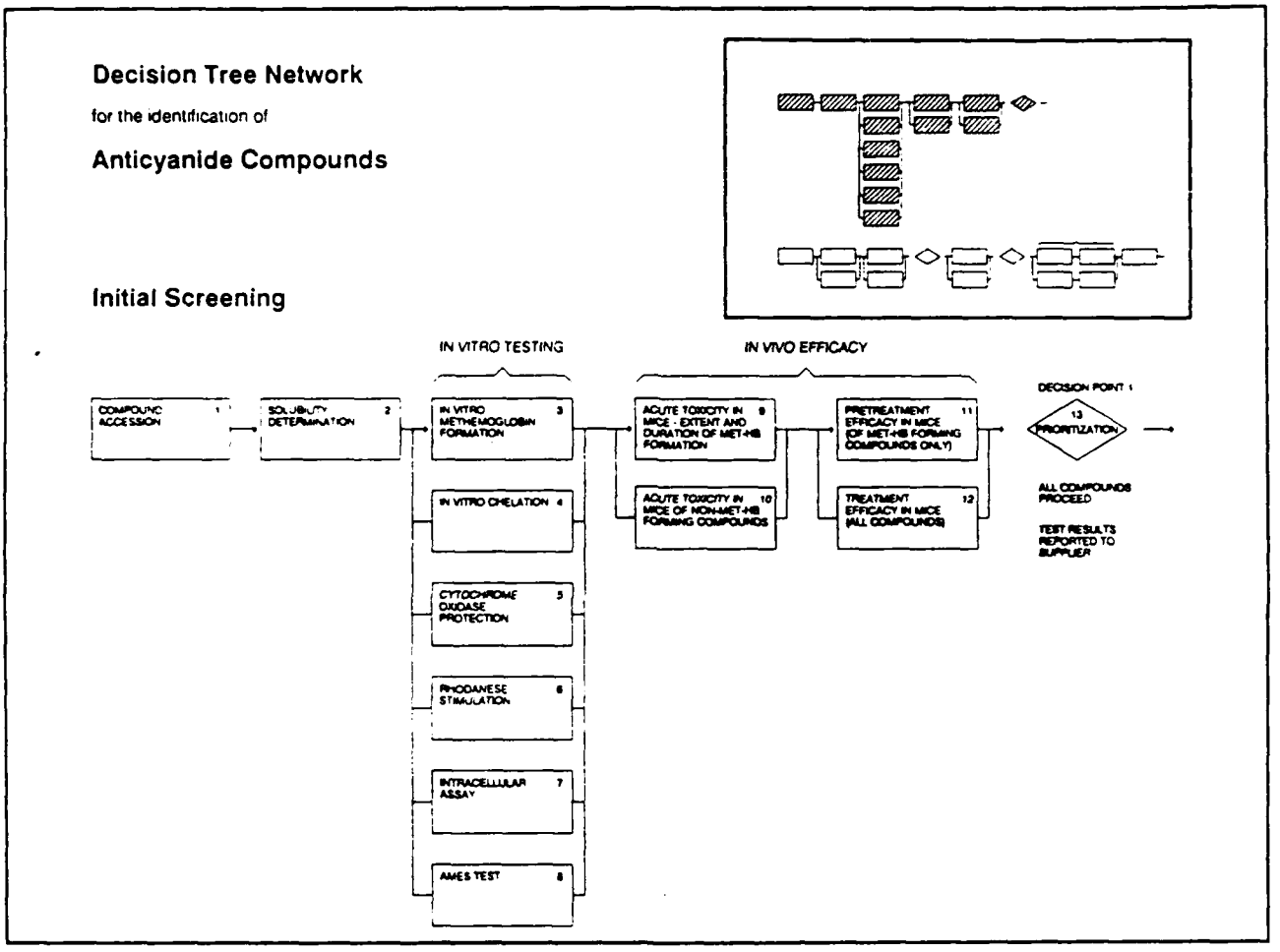

Figure 2

1. Compound Accession

Purpose/Rationale: To provide storage, audit, and tracking procedures which document disposition and testing of candidate compounds in contractor and government laboratories.

Description: Compounds submitted for testing will be processed through WRAIR, which will serve as the chief repository for the chemicals. WRAIR will ensure that the materials are properly identified, stored, and shipped and will maintain or obtain sufficient quantities for testing. All compounds received at USAMRICD will be logged into the inventory tracking system of the DACTS. Entries into the data base will be keyed to bottle#, ICD#, WRAIR#, quantity received, and quantity used during testing.

Reference:USAMRICD SOP# SGRD-UV-DB-6-87.

2. Solubility Determination

Purpose/Rationale: To identify the solvent in which the compound is most soluble, and to standardize the solubility testing procedures using a carefully selected and prioritized group of physiologically compatible solvents.

Description: The solubility of each compound will be determined in a number of solvents suitable for human use. Maximum solubility is determined in mg/ml, and the optimum solvent is selected.

Reference: USAMRICD SOP# SGRD-UV-DB-1-87.

3. In Vitro Methemoglobin Formation

Purpose/Rationale: To identify compounds which stimulate the formation of methemoglobin by oxidizing hemoglobin.

Description: Those compounds that increase the ratio of methemoglobin from 1 percent to at least 10 percent of available hemoglobin will be identified using a spectrophotometric test. Two points will be awarded to those compounds showing positive results. The potential of allowing increased amounts of methemoglobin to be available prior to attack by cyanide will allow these compounds to be evaluated as pretreatment agents.

Reference: SOP in preparation.

4. In Vitro Chelation

Purpose/Rationale: To identify anticyanide compounds which are capable of chelating with cyanide.

Description: Cyanide's affinity to form complexes with heavy metals will be measured spectrophotometrically. Compounds that successfully chelate cyanide will be awarded two points. These scavengers will be tested for both pretreatment and treatment activity.

Reference: SOP in preparation.

5. Cytochrome Oxidase Protection

Purpose/Rationale: To identify candidate compounds that will protect the target enzyme cytochrome oxidase from complexing with cyanide.

Description: This test will measure cytochrome oxidase activity after it has been combined with a candidate compound and challenged with cyanide. If successful the candidate compound will be awarded two points and evaluated as a pretreatment compound.

Reference: SOP in preparation.

6. Rhodanese Stimulation

Purpose/Rationale: To identify those compounds that 'stimulate' rhodanese activity. Rhodanese is the primary enzyme that detoxifies cyanide by sulfur conversion to thiocyanate.

Description: Increases in cyanide's conversion to thiocyanate will be measured after rhodanese is exposed to candidate compound. The successful candidate compounds will be evaluated as both pretreatment and treatment agents and will be awarded two points.

Reference: SOP in preparation.

7. Intracellular Assay

Purpose/Rationale: To determine a candidate compounds ability to function on an intracellular level.

Description: A candidate compound's intracellular activity is measured using a cell culture test. It is felt that if a compound does not get into the cell, where cyanide's toxic action occurs, it might not be a useful anticyanide drug. Activity will be scored as follows:

Points	Action on Cell and Ability to Penetrate
4	nontoxic and successful
2	toxic and successful
0	nontoxic but unsuccessful
0	toxic but unsuccessful

Reference: SOP in preparation.

8. Ames Test

Purpose/Rationale: Candidate compounds are tested for mutagenicity. It is known that a large series of carcinogenic compounds give positive mutagenic signs in this test, which would indicate the need for more extensive mutagenicity testing. A negative response does not mean a compound will be free of mutagenic or carcinogenic effects.

Description: Candidate compounds are tested on special strains of *Salmonella typhimurium* for a mutagenic response. Candidate compounds causing a mutagenic response would not be eliminated immediately, but would continue to Decision Point 1, when all previous test results could be summarized and sent to the synthesis contractor as QSAR information. One point will be awarded to those compounds passing the Ames Test and zero points for those causing a mutagenic response.

Reference: SOP in preparation.

9. Acute Toxicity in Mice - Extent and Duration of Methemoglobin Formation

Purpose/Rationale: To obtain an initial toxicity assessment of methemoglobin forming compounds. This test will give some indication of the methemoglobin formation abilities of pretreatment compounds.

Description: Using a maximum dose of 600 mg/kg, an intramuscular LD50 will be determined by the moving average method of Thompson and Weil (Thompson, W.R., and Weil, C.S., "On the Construction of Tables for Moving Average Interpolation," Biometrics 8, 51-54 [1952]). All animals will be observed for gross behavioral signs to allow estimates of lethality and lack of side effects. The onset and duration of 10 percent methemoglobin formation will be measured at prescribed intervals for 48 hours. A score of four points will be awarded to those compounds causing no behavioral changes. Compounds causing a death will get a score of zero. Intermediate responses will be scored between 3-1 as follows:

Duration of 10% Formation	Points
48 hours	3
>4 - 24 hours	2
0 - 4 hours	1

Onset of 10% Methemoglobin Formation	Points
0 - .5 hours	3
>0.5 - 4 hours	2
>4 - 48 hours	1

All points are additive so compounds could receive up to 10 points maximum.

Reference: USAMRICD SOP# SGRD-UV-DB-2-87.

10. Acute Toxicity in Mice of Non-Methemoglobin Forming Compounds

Purpose/Rationale: To obtain an initial toxicity assessment of non-methemoglobin forming compounds that will be used as treatment drugs.

Description: An intramuscular LD50 will be determined using a 24-hour end point. A dose of 600 mg/kg will be chosen as the highest dose to be tested, based both on practical use considerations and a desire to conserve sample. An LD50 value will be computed by the moving average method of Thompson and Weil (Thompson, W.R., and Weil, C.S., "On the Construction of Tables for Moving Average Interpolation," Biometrics 8, 51-54 [1952]). Various clinical signs will be recorded to determine estimates of lethality and lack of side effects. Compound scores will range between 4 - 0 with 4 being awarded to compounds showing no side-effects and 0 to compounds causing death.

Reference: USAMRICD SOP# SGRD-UV-DB-2-87.

11. Pretreatment Efficacy in Mice

Purpose/Rationale: All methemoglobin forming candidate compounds will be tested in the pretreatment mode to determine the maximum effective dose for anticyanide efficacy, motor incapacitation and to measure the extent and duration of methemoglobin formation in a mouse model.

Description: Candidate compounds will be administered intramuscularly in a dose that will be 1/16, 1/8, and 1/4 or 600 mg/kg of the LD50 determined in Module 9. Preliminary information will be obtained on the extent and duration of methemoglobin formation. Pretreatment will be followed in either 15 or 60 minutes by a intravenous cyanide challenge of 2 x LD50. Pretreatment efficacy will be determined by observing a simple behavioral component, such as measuring loss of righting reflex. Point values will be assigned as follows:

Pretreatment Dose	Points (for successful outcome)
1/16	5
1/8	4
1/4	3
fails test	1

The scores are additive with Module 12 below and would range from 20 - 1.

Reference: SOP in preparation.

12. Treatment Efficacy in Mice

Purpose/Rationale: All candidate compounds will be tested in the treatment mode to determine the maximum effective dose for anticyanide efficacy.

Description: To determine efficacy of treatment compounds the same dose ranges will be used as in Module 11 above, but the time of administration will be altered so that the candidate compounds are given after the cyanide has been administered. The test animals will be observed during recovery for behavioral signs such as loss of righting reflex. Point values will be assigned as follows:

Treatment Dose	Points (for successful outcome)
1/16	4
1/8	3
1/4	2
fails test	1

The scores are additive with Module 11 above and will range from 20 - 1.

Reference: SOP in preparation.

13. Decision Point 1

Candidate compounds will be rank ordered based on the results from Modules 11 and 12. Modules 3 through 10 will be used to provide data for future use in predicting the relationship between the *in vitro* tests and efficacy tests and to discriminate between pretreatment and treatment compounds. Those compounds receiving a "0" (positive result) on the Ames test will be flagged. Depending on rank all promising compounds will be tested further. All data generated on candidate compounds up to this point will be forwarded to the source so that the greatest use can be made of QSAR techniques for development of successive new drugs.

ADVANCED SCREEN

Advanced rodent efficacy is evaluated at the beginning of the Advanced Screen. After a behavioral evaluation the protective ratio of candidate compounds is determined in Modules 15A and B. The first test, 15A uses intravenous cyanide to challenge the candidate compounds and 15B uses cyanide by inhalation, the most probable threat route of cyanide exposure. Research to develop the inhalation route of administration is now ongoing, and until the inhalation procedure is developed, validated, and the results correlated, the intravenous route for cyanide will be used. A protective ratio will allow prioritization of compounds based on their maximal efficacy.

This is followed by an assessment of physical properties, a preliminary stability study and a feasibility analysis of large lot synthesis. Toxicity and efficacy studies for both pretreatment and treatment candidates are then repeated in a higher species before the formal DATEC review.

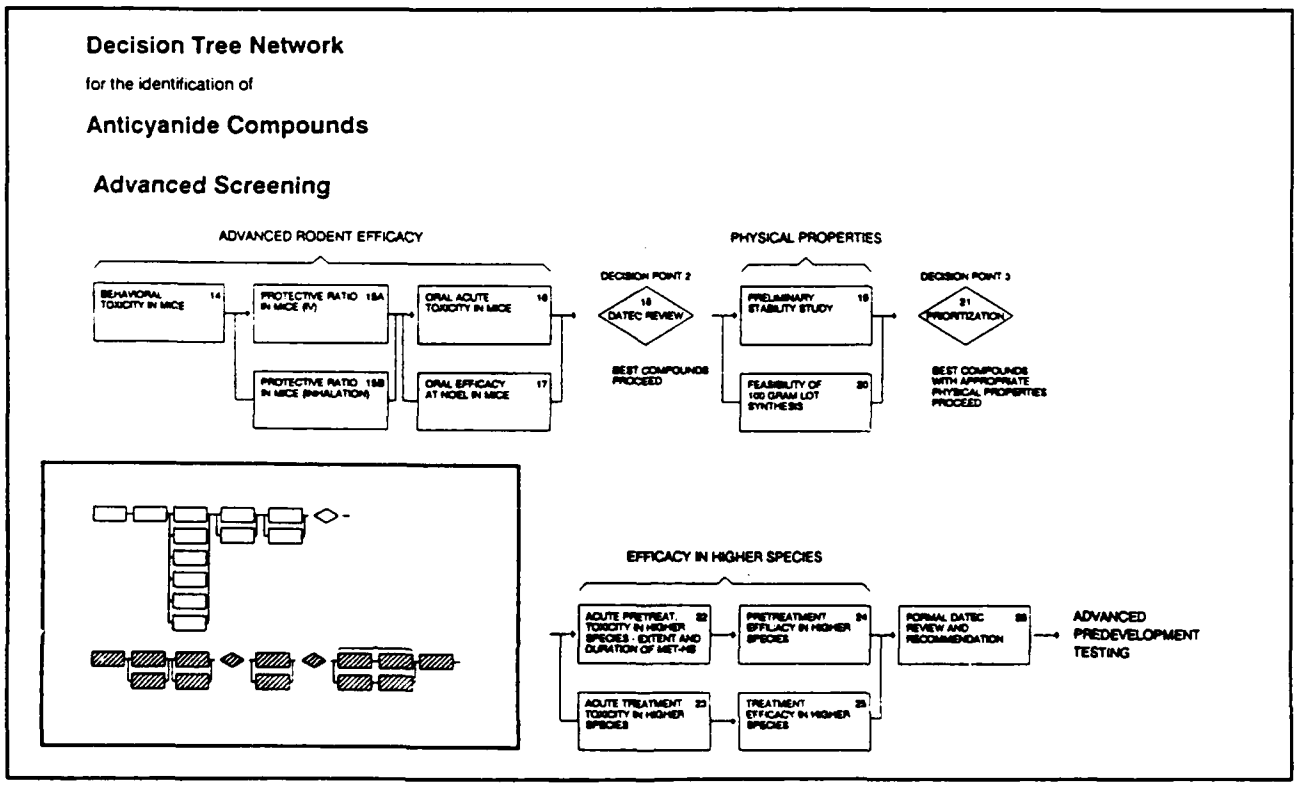

Figure 3

14. Behavioral Toxicity in Mice

Purpose/Rationale: To determine the behavioral side effects and behavioral no observable effect level (NOEL) of candidate compounds.

Description: A NOEL test will be run using mice. The candidate compound will be administered initially at the highest efficacious dose and then at 1/8, 1/16, and 1/64 of the highest efficacious dose as determined in Modules 11 and 12. If NOEL is reached before the lowest proposed dose, the test will be terminated. Signs of behavioral changes will be tested using the escape-avoidance test, a rotarod test or the recording of animals in an activity cage. Animals will be observed for 24 hours for treatment compounds and for one week in the case of pretreatment compounds.

Reference: USAMRICD SOP# SGRD-UV-DA-1-86.

15A. Protective Ratio in Mice (IV)

Purpose/Rationale: To determine the protective ratio for candidate compounds given at the NOEL level when challenged with intravenous cyanide.

Description: The protective ratio will be determined for both pretreatment and treatment candidate compounds in a mouse model when challenged by intravenous cyanide. The candidate compounds will be administered at the NOEL level determined in Module 14 and the results compared to the toxicity found with untreated animals. Upon completion candidate compounds would be scored by multiplying the protective ratio by the sum of scores from Modules 3 through 12.

Reference: SOP in preparation.

15B. Protective Ratio in Mice (Inhalation)

Purpose/Rationale: To determine the protective ratio for candidate compounds given at the NOEL level when challenged with cyanide given by inhalation.

Description: The protective ratio will be determined for both pretreatment and treatment candidate compounds in a mouse model when challenged with cyanide given by inhalation. Inhalation is the most probable threat route of cyanide exposure. Toxicity will be measured using a plethysmograph. The candidate compounds will be administered at the NOEL level determined in Module 14 and the results compared to the toxicity found with untreated animals. Upon completion candidate compounds will be scored by multiplying the protective ratio by the sum of scores from Modules 3 through 12.

Reference: SOP in preparation

16. Oral Acute Toxicity in Mice

Purpose/Rationale: To determine oral toxicity of candidate compounds shown to have pretreatment efficacy.

Description: The oral toxicity (LD50) will be carried out using a maximum dose of 600 mg/kg with log dosing intervals using two animals per dose. The method of Thompson and Weil will be used to calculate the LD50.

Reference: SOP in preparation.

17. Oral Efficacy at NOEL in Mice

Purpose/Rationale: To determine the oral NOEL and oral dose response (protective ratio) of candidate compounds that have demonstrated pretreatment efficacy. Those compounds which show increased protective ratio by both oral and intravenous routes of administration would then be used as pretreatment compounds in future studies in higher species. Those compounds which gave increased protective ratio values after intravenous but not oral administration would be used as treatment compounds in future studies.

Description: A determination of the NOEL oral dose (carried out in a manner analogous to that used in Module 15) would be followed by a protective ratio estimation, using 2 mice per dose, as determined by the method of Thompson and Weil. The candidate compounds will be rank ordered after the oral protective ratio determination as follows: the scores from Modules 3 through 12 will be summed and multiplied by the oral protective ratio.

Reference: SOP in preparation.

18. Decision Point 2 DATEC REVIEW

Candidate compounds are rank ordered on basis of results from Modules 11 through 17, which included oral efficacy against cyanide and efficacy against inhaled or intravenous cyanide. In addition the results of the Ames test (Module 8) will be considered at this point. Pretreatment and treatment compounds will be prioritized separately. The ten highest scoring compounds in the treatment and pretreatment categories will then advance to the next Module. All the data collected after the oral dosing experiments will be compiled and sent to the synthetic contractors.

19. Preliminary Stability Study

Purpose/Rationale: To provide an early assessment of potential stability problems.

Description: Stability of the test compound is evaluated by measuring rate of degradation in environmentally stressed samples.

Reference: WRAIR SOP # SGRD-UWM-MC-1-87

20. Feasibility of 100-Gram Lot Synthesis

Purpose/Rationale: To assess any problems involved in producing sufficient compound needed for advanced testing.

Description: An investigation will be conducted and a report made to determine the availability of starting chemicals and economic feasibility of producing a 100-gram lot of test compound.

Reference: WRAIR SOP # SGRD-UWM-MC-87

21. Decision Point 3

The candidate compounds will be evaluated on the basis of both stability and availability.

22. Acute Pretreatment Toxicity in Higher Species - Extent and Duration of Methemoglobin Formation

Purpose/Rationale: To determine the toxicity (LD50) of pretreatment compounds in a higher species.

Description: For those compounds identified as pretreatment candidates, the Thompson and Weil design will be used to determine the LD50 with the maximum dose being 600 mg of compound. Blood samples will be collected and the methemoglobin formation monitored for 48 hours. All animals will be observed for gross behavioral signs, those with none getting a score of 4 and those dying getting a score of 0. Intermediate responses will be scored between 3-1. The duration and onset of 10% methhemoglobin formation will be followed for 48 hours with the following point assignment:

Duration of 10% Formation	Points
48 hours	3
> 4 - 24 hours	2
0.5 - 4 hours	1

Onset of 10% MetHb Formation	Points
0 - 0.5 hours	3
>0.5 - 4 hours	2
>4 - 48 hours	1

All points are additive so candidate compounds could receive up to 10 points maximum and 2 points minimum.

Reference: SOP in preparation.

23. Acute Treatment Toxicity in Higher Species

Purpose/Rationale: To determine the toxicity (LD50) of treatment compounds in a higher species.

Description: For those compounds identified as treatment candidates the Thompson and Weil design will be used to determine the LD50 with the maximum dose being 600 mg of compound. Blood samples will not be taken as in the previous Module, but behavioral point scores will be assigned between 4 and 0.

Reference: SOP in preparation.

24. Pretreatment Efficacy in Higher Species

Purpose/Rationale: All candidate compounds will be tested in the pretreatment mode to determine the maximum effective dose for anticyanide efficacy, motor incapacitation and to measure the extent and duration of methemoglobin formation in a higher animal model.

Description: Those compounds identified as pretreatment candidates will be run in both pretreatment and treatment modes. Pretreatment compounds will be administered intravenously 15 or 60 minutes before cyanide challenge. The dose level of the pretreatment compound will be

1/4, 1/8, and 1/16 of the LD50 or the 600 mg dose as determined in Module 22. The standard for comparison will be sodium nitrite. The assignment of point values is as follows:

Pretreatment Dose	Points (for successful outcome)
1/16	5
1/8	4
1/4	3
Fails test	1

The scores are additive and, combined with Module 25 below, will have a range from 21 - 1.

Reference: SOP in preparation.

25. Treatment Efficacy in Higher Species

Purpose/Rationale: All candidate compounds will be tested in the treatment mode to determine the maximum effective dose for anticyanide efficacy in a higher animal model.

Description: Treatment candidate compounds will be given one minute post-cyanide at the dose level of 1/4, 1/8, and 1/16 of the LD50 or the 600 mg dose as determined in Module 23. The standard for comparison will be sodium nitrite. The assignment of point values is as follows:

Treatment Dose	Points (for successful outcome)
1/16	4
1/8	3
1/4	2
Fails test	1

The scores are additive and combined with Module 24 above will have a range from 21 - 1.

Reference: SOP in preparation.

26. Formal DATEC Review and Recommendation

At this point the compounds will be rank ordered again based on Modules 22 through 25 and all the data assembled for review by DATEC. The DATEC will conduct a formal review and make written recommendations to the Commander, USAMRICD as to which compound(s) should be advanced to the predevelopment stage for the anticyanide compounds.

SUMMARY

This decision tree will provide data on the utility of candidates as either pretreatment and/or treatment compounds in two species, rodent and a higher species. In all cases emphasis has been placed on pretreatment, but the possibility of finding a useful treatment compound has not been ignored. Data to allow for a correlation of *in vitro* tests with *in vivo* results will be available. Preliminary data on efficacy against cyanide inhalation and oral toxicity and efficacy data will also be available. Some *in vivo* pharmacokinetic data regarding methhemoglobin formation in two animal species will have been determined and the extent of protection at a NOEL dose level in two animal models will be known. There will be information on gross behavioral side effects, and some data on the mutagenicity of candidate compounds will be known. There will be data on the stability and feasibility of synthesizing compounds. *In vitro* and *in vivo* data will be available to send back to the synthesis contractor for QSAR purposes. The Anticyanide Decision Tree Network will provide sufficient data on which to base a recommendation for advancing a compound to predevelopment. The rank ordering of all compounds tested provides for continuous selection of the "best of the best" compounds for further evaluation.

Rationale for Animal Model Selection

The mouse serves as the Initial Screening *in vivo* model. Pigs or dogs are used in the later part of the Advanced Screening Phase.

Mice were selected for the initial studies in this DTN for the following reasons:

a. Genetically pure strains are available in the large numbers required.

b. Mice are easily housed and handled.

c. Their small size enables testing of sufficient animals to allow statistical validity with minimum of test compound.

d. Mice provide a rigorous screen for eliminating all but the most effective compounds because they do not respond well to therapy.

e. A large historical data base of screening results for a variety of chemicals has previously been generated using mice.

Pigs are proposed as the second species:

a. Pigs serve as a good pulmonary model for humans.

b. Kinetics may differ because the pig is very slow to convert its methhemoglobin back to hemoglobin.

c. Comparability data on the efficacy of methemoglobin formers in the dog, pig, and rat indicated no difference between the three species although the time course of methemoglobin formation and efficacy differ appreciably.

The dog has traditionally been the species of choice because

a. of its similarity to humans in pulmonary function.

b. of its use as a model for testing the cardiac toxicity of cyanide.

c. the kinetics of methemoglobin formation is more similar to humans than that of other species.

d. studies have shown the dog to be the most sensitive species for cyanide toxicity.

This has resulted in the creation of a large data base using the dog as a test animal for evaluating cyanide poisoning. If not used in advanced screening the dog can be used in the advanced predevelopment phase.

Definitions

Candidate Compound: A drug or formulation selected for drug assessment.

Cyanide (CN): A highly toxic threat agent that inhibits cellular respiration.

Decision Tree Networks (DTNs): A defined sequence of testing modules and selection criteria which identifies active compounds and, through prioritization, advances the best candidates.

Drug Assessment: The quantitative evaluation of drugs and formulated products for efficacy and safety (*in vitro* and animal models) through the application of screening protocols.

Drug Assessment Technical Evaluation Committee (DATEC): A select panel of U.S. Army Medical Research and Development Command scientists who provide to the Commander, USAMRICD technical evaluation and recommendations regarding the Medical Chemical Defense Tech Base Drug Development Program.

Methemoglobin (metHb): Formed by the oxidization of hemoglobin, competes for cyanide in the red blood cell to form the nontoxic complex cyanmethemoglobin.

Pretreatment Agent: A drug intended to mitigate the effects of cyanide threat agents by being taken in advance of the threat challenge.

Screening: The systematic testing of candidate compounds for efficacy and safety in the prevention and treatment of chemical threats.

Testing Module: An experimental procedure, defined in a written protocol or Standing Operating Procedure, which is designed to evaluate specific characteristics of the compound being screened.

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