

HEALTH AND
ENVIRONMENTAL
SCIENCES
DEPARTMENT

API PUBLICATION
NUMBER 4594

JANUARY
1995

A Critical Review of Toxicity Values and an Evaluation of the Persistence of Petroleum Products for Use in Natural Resource Damage Assessments



A Critical Review of Toxicity Values and an Evaluation of the Persistence of Petroleum Products for Use in Natural Resource Damage Assessments

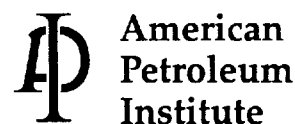
Health and Environmental Sciences Department

API PUBLICATION NUMBER 4594

PREPARED UNDER CONTRACT BY:

RALPH K. MARKARIAN, PH.D., JOSEPH P. NICOLETTE,
TIMOTHY R. BARBER, PH.D., AND LAURA H. GIESE
ENTRIX, INC.
200 BELLVUE PARKWAY, SUITE 200
WILMINGTON, DE 19809

SEPTEMBER 1994



FOREWORD

API PUBLICATIONS NECESSARILY ADDRESS PROBLEMS OF A GENERAL NATURE. WITH RESPECT TO PARTICULAR CIRCUMSTANCES, LOCAL, STATE, AND FEDERAL LAWS AND REGULATIONS SHOULD BE REVIEWED.

API IS NOT UNDERTAKING TO MEET THE DUTIES OF EMPLOYERS, MANUFACTURERS, OR SUPPLIERS TO WARN AND PROPERLY TRAIN AND EQUIP THEIR EMPLOYEES, AND OTHERS EXPOSED, CONCERNING HEALTH AND SAFETY RISKS AND PRECAUTIONS, NOR UNDERTAKING THEIR OBLIGATIONS UNDER LOCAL, STATE, OR FEDERAL LAWS.

INFORMATION CONCERNING SAFETY AND HEALTH RISKS AND PROPER PRECAUTIONS WITH RESPECT TO PARTICULAR MATERIALS AND CONDITIONS SHOULD BE OBTAINED FROM THE EMPLOYER, THE MANUFACTURER, OR SUPPLIER OF THAT MATERIAL, OR THE MATERIAL SAFETY DATA SHEET.

NOTHING CONTAINED IN ANY API PUBLICATION IS TO BE CONSTRUED AS GRANTING ANY RIGHT, BY IMPLICATION OR OTHERWISE, FOR THE MANUFACTURE, SALE, OR USE OF ANY METHOD, APPARATUS, OR PRODUCT COVERED BY LETTERS PATENT. NEITHER SHOULD ANYTHING CONTAINED IN THE PUBLICATION BE CONSTRUED AS INSURING ANYONE AGAINST LIABILITY FOR INFRINGEMENT OF LETTERS PATENT.

HARDWARE AND OPERATING SYSTEM SOFTWARE REQUIREMENTS FOR USE OF THIS SOFTWARE ARE SPECIFIED IN THIS MANUAL. THIS SOFTWARE HAS BEEN TESTED EXTENSIVELY; HOWEVER, API DOES NOT AND CANNOT CLAIM TO HAVE FORESEEN NOR ELIMINATED ALL POTENTIAL PROGRAM INTERFERENCES, HARDWARE INCOMPATIBILITIES, OR OTHER LIMITATIONS OF SOFTWARE USE.

ALTHOUGH API WELCOMES COMMENTS ON THE OPERATION AND UTILITY OF THIS SOFTWARE, API IS DISTRIBUTING THE SOFTWARE ON AN "AS-IS" BASIS AND DOES NOT PROVIDE SOFTWARE USER SUPPORT.



ACKNOWLEDGMENTS

THE FOLLOWING PEOPLE ARE RECOGNIZED FOR THEIR CONTRIBUTIONS OF TIME AND EXPERTISE DURING THIS STUDY AND IN THE PREPARATION OF THIS REPORT:

API STAFF CONTACTS

Stephanie Meadows, Health and Environmental Affairs Department
Alexis Steen, Health and Environmental Sciences Department

MEMBERS OF THE NATURAL RESOURCE DAMAGE ASSESSMENT TASK FORCE

James W. Scialabba, BP Oil Company
Jerry F. Hall, Ph.D., Texaco Research
Lawrence A. Reitsema, Ph.D., Marathon Oil Company
John Monarch, Chevron*
Stephen H. Bard, Texaco Inc.
Thacher W. White, Mobil Oil Corporation
Robert E. Abbott, Ph. D., Conoco Inc.
Janis M. Farmer, BP America Research and Development
Marion Fischel, Shell Oil Company*

ENTRIX PROJECT TEAM

Ralph K. Markarian, Ph.D.
Joseph P. Nicolette
Timothy R. Barber, Ph.D.
Laura H. Giese

*no longer with this organization

PREFACE

This study addresses the quality and reliability of values used for denoting the acute toxicity of crudes and oil products in aquatic environments. The scientific literature was reviewed and acute toxicity data were selected using strict quality control and quality assurance criteria. The goal of this effort was to compile, analyze, and present an overview of these data by oil product* and taxonomic group. There were a variety of data gaps and problems in comparing conventional LC50 values between studies. Methodological differences between data sets were an important consideration, and special care must be used in predicting biological impacts using these acute toxicity data. Very little published data exists for gasoline, jet fuel, and lube oil product groups. Additionally, acute toxicity data were sparse for the algal taxonomic group. Majority of data were available for the diesel, crude and bunker oil groups. Only oil product toxicity data were utilized in this study and not oil product component data (e.g., naphthalenes, benzene, etc.). Statistical comparisons were performed at a conservative level in order to determine significance. In all cases, the number of data points available in each comparison should be considered when reviewing the statistical results. Additionally, oil products were ranked based upon their median toxicity values, and a relative ranking scale is provided. Relative product toxicity rankings are based on comparisons of median toxicity values and differences shown may or may not be statistically significant.

A limited level of effort was applied for providing a relative persistence scale for oil products released into the environment. It should be emphasized that this analysis has a number of qualifications. This treatment is not compound specific. Crudes and oil products are characterized with a broad range of physiochemical data. An equilibrium-based model was used to estimate relative persistence and differentiate between classes of petroleum products (independent of site- and spill-specific information). The scope of this effort did not allow specific consideration of several important environmental parameters that influence the fate of spilled petroleum products (e.g., wind speed, wave energy, currents, water depth, and habitat).

*Note: The term "oil product(s)" is used in this report to include crude oil and oil products.

TABLE OF CONTENTS

EXECUTIVE SUMMARY	ES-1
1.0 CHAPTER 1: REVIEW OF TOXICITY VALUES	1-1
1.1 INTRODUCTION.....	1-1
1.2 METHODOLOGY:	
ANALYSIS AND RANKING OF TOXICITY VALUES	1-4
1.2.1 Literature Search and Collection	1-4
1.2.1.1 <i>Searching and Screening of Literature Citations</i>	1-4
1.2.1.2 <i>On-line Databases</i>	1-4
1.2.1.2.1 Dialog Information Services.....	1-5
1.2.1.2.2 Chemical Information Services.....	1-6
1.2.1.3 <i>Other Sources of Data</i>	1-7
1.2.1.4 <i>Selection and Collection of Pertinent Literature</i>	1-8
1.2.2 Database Development	1-9
1.2.2.1 <i>Key Study Parameters</i>	1-9
1.2.2.1.1 Oil Product.....	1-9
1.2.2.1.2 Study Purpose Endpoint.....	1-13
1.2.2.1.3 Agitation Duration During Preparation	1-13
1.2.2.1.4 Free Product Present or Absent.....	1-13
1.2.2.1.5 Analytically Measured Exposures.....	1-13
1.2.2.1.6 Test Chamber	1-14
1.2.2.1.7 Single Ratio/Multiple Ratio Test Designs ..	1-14
1.2.2.1.8 Reliability Code	1-16
1.2.2.2 <i>Data Entry</i>	1-17
1.2.2.3 <i>Data QA/QC</i>	1-18
1.2.3 Analysis and Ranking of Toxicity Values	1-18

1.2.3.1	<i>Statistical Analyses</i>	1-18
1.2.3.2	<i>Approach</i>	1-24
1.2.3.2.1	Oil Product Toxicity Values	1-24
1.2.3.2.2	Ranking of Oil Product Toxicity	1-26
1.2.3.2.3	The LL50 Concept	1-26
1.3	RESULTS:	
	ANALYSIS AND RANKING OF TOXICITY VALUES	1-29
1.3.1	Literature Search and Collection of Toxicity Data	1-29
1.3.1.1	<i>Characterization of Petroleum Toxicity Literature</i> ...	1-29
1.3.1.2	<i>Characterization of Extracted Data</i>	1-30
1.3.2	Analysis and Ranking of Oil Product Toxicity	1-33
1.3.2.1	<i>Invertebrates: Free Product Absent</i>	1-34
1.3.2.1.1	Median Toxicity Values	1-34
	Oil Product Group Comparisons	1-34
	Lifestage Comparisons	1-39
	Methodological Procedure Comparisons....	1-41
1.3.2.1.2	Ranking of Oil Product Toxicity	1-45
1.3.2.2	<i>Invertebrates: Free Product Present</i>	1-47
1.3.2.2.1	Median Toxicity Values	1-47
	Oil Product Group Comparisons	1-47
	Lifestage Comparisons	1-49
	Methodological Procedure Comparisons....	1-54
1.3.2.2.2	Ranking of Oil Product Toxicity	1-56
1.3.2.3	<i>Fish: Free Product Absent</i>	1-57
1.3.2.3.1	Median Toxicity Values	1-57
	Oil Product Group Comparisons	1-57
	Lifestage Comparisons	1-59
	Methodological Procedure Comparisons....	1-64
1.3.2.3.2	Ranking of Oil Product Toxicity	1-67
1.3.2.4	<i>Fish: Free Product Present</i>	1-68
1.3.2.4.1	Median Toxicity Values	1-68
	Oil Product Group Comparisons	1-68

Lifestage Comparisons	1-72
Methodological Procedure Comparisons....	1-75
1.3.2.4.2 Ranking of Oil Product Toxicity.....	1-78
1.3.2.5 <i>Algae: Free Product Absent</i>	1-80
1.3.2.5.1 Median Toxicity Values	1-80
Oil Product Group Comparisons.....	1-80
Methodological Procedure Comparisons....	1-80
1.3.2.5.2 Ranking of Oil Product Toxicity.....	1-85
1.3.2.6 <i>Algae: Free Product Present</i>	1-85
1.3.2.6.1 Median Toxicity Values	1-85
Oil Product Group Comparisons.....	1-85
Methodological Procedure Comparisons....	1-90
1.3.2.6.2 Ranking of Oil Product Toxicity.....	1-92
1.3.3 LL50 Value Calculations.....	1-92
1.4. DISCUSSION AND SUMMARY:	
ANALYSIS AND RANKING OF TOXICITY VALUES	1-94
1.4.1 Literature Search and Collection.....	1-94
1.4.2 Analysis and Ranking of Oil Product Toxicity.....	1-95
2.0 CHAPTER 2: ANALYSIS AND RANKING OF OIL PRODUCT	
PERSISTENCE.....	2-1
2.1 INTRODUCTION.....	2-1
2.2 METHODOLOGY	2-3
2.2.1 Physical Properties.....	2-3
2.2.1.1 <i>Molecular Weight</i>	2-4
2.2.1.2 <i>Water Solubility</i>	2-4
2.2.1.3 <i>Vapor Pressure</i>	2-4
2.2.1.4 <i>Octanol/Water Partition Coefficient</i>	2-6
2.2.2 Equilibrium Partitioning Model.....	2-6
2.2.3 Ranking of Oil Product Persistence.....	2-7
2.3 RESULTS AND DISCUSSION.....	2-8
2.3.1 Model Input Parameters.....	2-8

2.3.2	Crude Oil and Oil Product Persistence.....	2-8
2.3.3	Numerical Persistence Scale.....	2-16
2.4	SUMMARY	2-17
3.0	CHAPTER 3: OIL PRODUCT TOXICITY AND PERSISTENCE: A PERSPECTIVE	3-1
3.1	INTRODUCTION.....	3-1
3.2	TOXICITY OF OIL.....	3-1
3.3	PERSISTENCE AND OIL PRODUCTS	3-3
3.3.1	Persistence: Toxicity Based Concerns.....	3-3
3.3.2	Persistence: Habitat Based Concerns	3-5
3.4	SUMMARY	3-5
4.0	REFERENCES	4-1
	APPENDIX A: ARTICLES COLLECTED AND REVIEWED.....	A-1
	ARTICLES UTILIZED IN THE TOXICITY DATABASE.....	A-14
	APPENDIX B: RESULTS OF STATISTICAL COMPARISONS OF OIL PRODUCT AND TESTING METHODOLOGIES	B-1
	APPENDIX C: DATA CLASSIFIED AS LOW RELIABILITY.....	C-1
	APPENDIX D: OILTOX DATABASE SYSTEM VERSION 1.0 USER'S GUIDE.....	D-1



EXECUTIVE SUMMARY

State agencies (e.g., Alaska, Washington and Florida) have initiated tabular methods and formulas for assessing natural resource damages associated with oil product or crude oil spills within their jurisdictions. An important aspect in each of the state initiatives deals with the toxicity and persistence of the spilled hydrocarbons. A central aspect of toxicity evaluations are the LC50 values used to denote acute toxicity of oil products. How reliable are LC50s for ranking oil product toxicity? The quality and reliability of the values used for denoting oil product toxicity are the main topics of this investigation. In addition, a more limited effort was made to compare the relative persistence of oil products released into the environment. Finally, a discussion regarding the relative roles of product toxicity and persistence in predicting biological injury is presented. The results of this effort are presented in 3 chapters as follows:

CHAPTER 1: REVIEW AND RANKING OF TOXICITY VALUES

CHAPTER 2: ANALYSIS AND RANKING OF OIL PRODUCT PERSISTENCE

CHAPTER 3: OIL PRODUCT TOXICITY AND PERSISTENCE: A PERSPECTIVE

Chapter 1: Approximately 8,000 references on the fate and effects of oil products in aquatic systems were screened. The majority of the selected articles were published in the mid to late 1970's. While there was an adequate number of high quality articles, comparability between papers was limited due to variability in test methodologies. In order to determine the relative impact of the methodological differences on LC50 values, key method parameters were selected and added to a computerized database. This allowed investigators to sort on key methodological differences between studies and evaluate if and how laboratory methods impacted the actual LC50 values. The final database contained 748 toxicity values.

The majority of the data was on crude oils (55%) and diesel (31%). Gasoline, jet fuel, and lube oil comprised less than 7% of the total number of toxicity values in the database. Invertebrate data comprised 65.4% of the data in the database. Fish comprised 26.6% of the data, while algae comprised only 8% of the data.

As the basic data on methods and results were analyzed it became apparent that one of the major factors in influencing LC50 values was the presence or absence of free product in the test chambers. Since the presence or absence of free product in the test chamber was found to have the largest impact on reported LC50 values, it was maintained as the major sorting factor throughout this study.

In many cases, methodological procedures had an effect on the resulting LC50 values. Reported LC50 values for the same oil product often differed significantly based on: whether the test chambers were open or closed, if the test was conducted in freshwater or saltwater, and how long oil water solutions were mixed prior to adding test organisms. Finally it was found that LC50s

calculated and reported for similar products were very different based on which "concentration" values were used in the final calculations. Some were based on dissolved hydrocarbons from single oil:water ratio test solutions, others were based on multiple ratio test solutions, others were based on tests that used measured concentration data from individual test chambers, and finally some were based on nominal concentrations. The importance of methods was expected and investigators planned from the outset to utilize a database that was designed to allow comparisons of toxicity values developed and based on similar methods.

The database was developed into a computer program referred to as "OILTOX". With the help of this program, the user can find, review, sort, and print out individual LC50 values used in this study. The user is also able to query the database and ask for data on select test species, products, and toxicity test method characteristics. The program also allows the user to link the individual LC50 value to a specific reference. The program does not include every possible LC50 value available since certain quality criteria were used prior to deciding whether data should be included in this study. The database was provided to API as a separate diskette along with a brief users manual.

Median toxicity values were computed for each oil product and taxonomic group once the data were sorted by the absence and presence of free product in the test solutions. In all cases for a given product type, tests conducted with "free product absent" solutions reported lower LC50 values when compared to respective "free product present" LC50 values. Approximately 75% of the data records were for "free product absent" studies while 25% of the data records were for "free product present" studies. Suitable algal data sets were not found for the bunker, gasoline and lube oil groups. Gasoline data (12 values) were available only for the invertebrate "free product absent" data set. Only twelve data values were available for the jet fuel data set.

Median effect concentrations calculated for saltwater and freshwater "free product absent" tests did make a difference in the overall product ranking. It appears that, for invertebrates, the toxicity of crude is higher in freshwater when compared to bunker and diesel, but that under saltwater conditions, bunker and diesel appear much more toxic than crude.

Median effect concentrations calculated for saltwater and freshwater "free product absent" tests with fish did make a difference in the overall ranking. The toxicity of crude appeared higher to fish in freshwater when compared to bunker and diesel, but under saltwater conditions, bunker and diesel appear more toxic than crude.

Median effect concentrations calculated for saltwater and freshwater studies with "free product present" in tests did make a difference in the overall ranking. It appears that the toxicity values of crude to fish, when free product is present, are lower when compared to all other oil product groups. The median acute effect concentrations for crude in freshwater was 1525 mg/l, while across the other oil product groups the median effect concentration ranged from 12.70 mg/l to 560 mg/l. In saltwater the median effect concentration of crude was 1365 mg/l, while across the other product groups the median effect concentration ranged from 55.00 mg/l to 70.50 mg/l.

The present review indicates that the more toxic oil product groups are the diesel and bunker oils. Lube oil shows some very low LC50 values but the data set is very limited and should not be equated to those products with significantly more information. Furthermore, lube oil toxicity can be affected significantly by special additives which can vary considerably based on the manufacturer. The least toxic oil product groups were the crude, jet fuel and gasoline groups. Published data were sparse for the jet fuel, gasoline and lube oil groups.

Based on the interpretational difficulty associated with a single LC50 value, existing data were sorted using a new criteria and notation. This term, the Lethal Loading factor or LL50, expresses the results in what could prove to be a more appropriate context for oil product rankings. In short, the Lethal Loading concept attempts to quantitate the toxicity of a product in terms of the amount of whole product added to water to cause a 50% mortality of test organisms (LL50). Another limiter to the data set is that all LL50 test results must be based on multiple ratio test solutions. This means that test solutions were developed using different oil:water ratios (i.e., loadings), and the resultant water soluble components were not diluted prior to adding organisms. The importance of this selection to ranking oil products becomes evident when the data are sorted and compared using the three resultant criteria, (i.e., the LL50, the LC50-free product present, and the LC50-free product absent). This recognizes that there is no single "concentration" of any one compound in oil product toxicity test solutions, and the LC50 nomenclature is not appropriate for whole oil product toxicity tests.

Regardless of the criteria used to rank toxicity, crude oil was consistently the least toxic product. The value of the LL50, however, was that it demonstrated that the relative amounts of various products that are needed in water to cause a given effect (i.e., 50% mortality) varies considerably. This relative loading factor is transparent with typical LC50 results. The significance of the LL50 factor can be seen in Table ES-1 below.

Table ES-1. Pairwise comparisons of median effect concentration values by LL50 and the absence of free product, taxon and oil product group. Critical value = 0.05. Bold indicates significance.					
<u>Oil Product</u>	<u>Taxon</u>	<u>Median Free Product Absent LC50 (mg/l)</u>	<u>Median LL50 (mg/l)</u>	<u>Ratio LL50 to Free Product Absent</u>	<u>Significance Level</u>
Crude	Invertebrates	6.31	475	(75x)	(p < 0.001)
Crude	Fish	3.12	3200	(1,026x)	(p < 0.001)
Diesel	Invertebrates	3.36	9.4	(3x)	(p < 0.002)
Diesel	Fish	3.50	162.5	(46x)	(p < 0.001)

The differences in relative toxicity of both crude and diesel are lost when compared via the reported LC50 values. The median values represent many data points and the differences between fish and invertebrates or diesel and crude are shown only as a factor of 2 (6 / 3). The impact of sorting literature test results based on LL50 criteria is quite dramatic. Using conventional comparisons or LC50 values, it would appear that only 2X as much crude is required to have the same impact as diesel on invertebrates. Based on the amount of product actually required in water (the basis of the LL50 calculation), however, over 50X more crude than diesel is required to produce the same lethal impact on invertebrates. This distinction is lost using conventional LC50 values and thus can easily mislead efforts comparing relative toxicities of products. LC50 values developed via this study are basically reporting dissolved component levels which further mask actual product levels needed to create the acute effect. Thus by lumping all the reported literature values together with a single designation (LC50), regardless of methodology, the key *whole product* differences are lost. This in effect masks the relative differences in potential impact between various products. The grams per liter basis of the LL50 calculation helps relate the relative toxicities to a whole product basis and environmental loading levels. The LL50 value should prove to be a more realistic and useful predictor of actual acute impacts in the event of a product spill.

Chapter 2: Besides the toxicity of an oil product, the persistence, or length of exposure of an oil product, is also an important parameter for assessing the effects of an oil spill. The primary processes determining the fate of crude oils and oil products after a spill are spreading, evaporation, emulsification, dispersion, dissolution, reaction, and sedimentation. These processes are influenced by the spill characteristics, environmental conditions, and the nature of the spilled material.

An equilibrium-partitioning model was used for assessing the relative persistence of oil and oil products in aquatic environments. The ultimate fate of the petroleum products is based solely on their physiochemical properties (i.e., molecular weight, solubility, vapor pressure, and octanol/water partition coefficient). Because of the many confounding effects influencing the fate of oil in the sea (e.g., physical conditions involving wind speed and direction, surface currents, water depth, and habitat), a model based on physiochemical data will only provide a relative scale as to which oil and oil products will persist in aquatic environments.

Oil products consist of many individual components; therefore, a broad range of physio-chemical data was used to characterize the individual crude oil or oil product. Two model runs for each substance were conducted to provide both a conservative (worst case) and non-conservative (best case) prediction of product persistence.

A numerical scale was developed for crude oils and oil products based on their persistence in aquatic environments. Persistence is defined as the fraction remaining in the water, soil, and sediment. The relative persistence is estimated at the midpoint of the best case and worst case scenarios. Generally, it can be concluded that gasoline, jet fuel, and fuel oil #2 are relatively nonpersistent in the marine environment. Lube oils are slightly persistent, Bunker C (fuel oil #6) is relatively

persistent, and asphaltenes are highly persistent. Crude oils, on average, are considered persistent; however, some components are nonpersistent and others are highly persistent.

Chapter 3: Two operating definitions associated with persistence were developed. The first deals with a toxicity-based connotation (i.e., persistent compounds or products are undesirable because they cause chemical toxicity in terms of biomagnification, bioaccumulation, or chronic toxicity). The second definition deals with habitat impacts associated with residual or persistent components of oil products or crudes.

With these two aspects of persistence in mind, a better understanding of the appropriate use of the term "persistence" can be developed. After the initial phases of a spill, when most of the active dissolution and volatilization has been accomplished, oil spill residuals from heavier products and crudes become less bioavailable with time. Thus, from the toxicity based persistence perspective, these residuals represent less of an acute threat. Thus when estimating an acute toxicity concerns in oil spills, it is not appropriate to utilize a direct proportion for estimating acute injury (i.e., multiplication of two numerical factors one representing acute toxicity and another persistence). However, based on the habitat aspects of the term, the use of injury estimators (numerical values), developed through direct proportions between appropriate persistence and toxicity ratings is somewhat more defensible. If realistic persistence values are utilized along with appropriate chronic toxicity functions, some estimate of long term habitat and chronic injury is possible. This estimate could be viewed as an overall estimator of both a habitat based concerns and, if appropriately developed, also serve as a substitute for the general lack of chronic toxicity considerations in the injury formulas.

Summary: LC50 values were evaluated regarding their usefulness in ranking oil product acute aquatic toxicity. This study clearly demonstrates that methodological differences in conducting oil product toxicity tests have a significant impact on the actual LC50 calculated for an oil product. Using selected criteria and a new notation referred to as the Lethal Loading factor, actual differences in whole product toxicities are shown to be quite pronounced although these differences have in the past been difficult to assess using the generic LC50 designation. LC50 values for the same product class can vary over three orders of magnitude. Rankings based on common methodological groupings demonstrate that, for example, diesel can be 29 times more toxic than crudes using the LL50 grouping but only 1.5 times more toxic using the "free product absent" test solution grouping. This disparity in reported toxicity values demonstrates the need for standardization of the methods used to test, calculate and compare oil product toxicities. The LL50 method is suggested as the more relevant and useful method to assess relative acute values for oil products. The method, based on multiple oil:water ratios, also yields more robust empirical information regarding the true differences that whole oil products have in their ability to cause acute aquatic injuries in the environment.

1.0 CHAPTER 1: REVIEW OF TOXICITY VALUES

1.1 INTRODUCTION

The overall objectives of the toxicity value review were to:

- Review and select toxicological information appropriate for analysis,
- Evaluate toxicity values for selected oil products, and
- Rank oil products based upon their toxicity values.

A database of toxicological information was developed from literature sources. A key feature of the organization of the database was the ability to describe the methodological parameters associated with the development of toxicity values. This is crucial in evaluating LC50 values since methods used in the aquatic testing procedures have a dramatic impact on the results.

The database allowed for a comparison of the key methodological aspects of the toxicity tests reviewed including a determination of which methodological procedures had a significant impact on the results. Areas where data are lacking or where there are low confidence levels in developed toxicity values are also discussed.

Toxicity values and ranges were determined from literature sources for the following major taxonomic groups:

- Fish,
- Invertebrates, and
- Algae.

The major categories of oil products for which toxicity was evaluated include:

- Bunker,
- Crude,
- Diesel,
- Gasoline,
- Jet Fuel, and
- Lube Oil.

A key factor in the analysis of the data was the consideration of the basis upon which each LC50 (lethal concentration needed to cause 50% mortality of test organisms) had been calculated. This is important to toxicity value development since LC50s are the basis of the Department of the Interior (DOI) Type A toxicity factors and probably have some influence on state directions. With complex mixtures such as oil products, LC50 calculations and their results can cause confusion due to the various methods used in calculating the "concentration" term. The results from these types of tests and calculations can be very different for the same oil product.

Our efforts attempted to identify the confounding factors in study design such that toxicity values could be compared in a "method normalized" manner. As the report describes and demonstrates, this approach proved to be an essential step in evaluating and developing the most accurate and representative set of toxicity values for oil products. If oil products are to be ranked from most to least toxic based on literature data, method normalization is important. The normalization had impacts not only on the relative ranking of one product to another but also demonstrated that reported LC50 values on the same product class can vary over three orders of magnitude depending on the methods used in conducting the test. As the basic data on methods and results were analyzed it became apparent that one of the major factors in influencing LC50 values was the presence or absence of free product in the test chambers.

Since the presence or absence of free product in the test chamber was found to have a sizable impact on reported LC50 values, it was maintained as the initial sorting mechanism throughout this Chapter. In addition to assessing the relative importance of other methodological factors (e.g. open or closed test chambers, duration of oil agitation), the methods and conventions used in expressing the results of oil product aquatic toxicity tests were also evaluated.

Traditional LC50 notations for oil products, as used in any number of literature reviews and rankings on oil products (e.g. DOI assessments), do not denote the basis of the LC50 value. Actually, the term LC (lethal concentration) is basically inappropriate for oil products since there is no single concentration of any one compound within toxicity test solutions derived from oil products. The term is most appropriate for a single compound dissolved in a test solution tested at a variety of concentrations allowing a true LC50 to be developed. Furthermore, the term LC50 as applied to oil products is quite misleading to a reviewer of oil product data since one immediately assumes that the number associated with the concept (e.g.,

an LC50 of 1000 mg/l) represents the dissolved fraction which was the "effective concentration".

In oil product testing, the "concentration" tested is developed by either mixing:

- A single oil to water ratio (i.e., 1 gram/litre) and diluting the resulting solution to create a series of test solutions, or
- A series of oil to water ratios (i.e., 10, 5, 2.5, 1.0, and 0.5 grams/litre of product) whereby the resulting solutions are tested without further dilution.

Although the two methods above are extremely different in approach to test solution development, current notation allows both results to be expressed simply as an LC50 result. In addition to the fact that the actual method of test solution development is totally masked by using the LC50 notation, the term "concentration" can take on many meanings that without a detailed review of the study it is difficult, if not impossible, to interpret the significance of the LC50 result. The lethal "concentration" has been found, for example, to be based on :

- Total hydrocarbons (assessed using a variety of methods all of which vary in their ability to measure different components in the test solutions),
- Aromatic hydrocarbons,
- Nominal hydrocarbons, i.e. concentrations estimated from a single measured or unmeasured stock solution which was then diluted to create multiple test concentrations for exposure, and
- Nominal concentrations estimated from multiple ratio test systems. These concentrations of hydrocarbon are developed by mixing four to five different oil to water ratios in separate test chambers thus creating individual test solutions.

Based on the above sources of confusion when viewing a single LC50 value, existing data was reviewed and sorted using a different notation. This term, the Lethal Loading (LL50), expresses the results in what could prove to be a more useful context for oil product rankings. In short, the Lethal Loading concept attempts to quantitate the toxicity of a product in terms of the amount of whole product added to water to cause a 50% mortality of test organisms

(LL50) (Girling et al. 1992). The LL50 concept is described in detail in the Methods Section and is further applied in the Results and the Discussion and Summary Section of this Chapter.

1.2 METHODOLOGY: ANALYSIS AND RANKING OF TOXICITY VALUES

1.2.1 Literature Search and Collection

1.2.1.1 Searching and Screening of Literature Citations

The objective of the literature search was to use only data collected from the original source. This was a necessity since methodological parameters were needed if the toxicity data were to be appropriately reviewed and evaluated. Therefore, the emphasis was placed on obtaining the primary literature. Review articles and secondary literature were also screened for their value in providing primary literature citations.

A vast quantity of oil product literature covering a wide range of subjects is available. It was necessary to focus on the key literature pertinent to this study. The main criteria used to select pertinent literature were that each reference:

- Was post-1970,
- Reported acute toxicity (mortality) data, and
- Reported numeric data.

1.2.1.2 On-line Databases

Informational databases available from Dialog Information Services, Inc. (Dialog) and the Chemical information Service, Inc. (CIS) were searched via computer for references of interest. Keywords were used to select references. Single keywords and keyword strings were developed for our search using words such as:

- Petroleum,
- Oil,
- Toxic,
- Effect,
- Acute,
- Spill,

- Not coal,
- Fuel,
- Crude,
- Hydrocarbons,
- Bioassay, and
- Others.

1.2.1.2.1 Dialog Information Services

The databases searched via Dialog are provided below including a brief description of each database.

Energy Science and Technology

The Energy Science and Technology database of the U.S. Department of Energy includes environmental topics covered in journal articles, report literature, conference papers, books, etc.

Aquatic Sciences and Fisheries Abstracts

Aquatic Sciences and Fisheries Abstracts provide a comprehensive database of abstracts on the science, technology, and management of marine and freshwater environments provided by the U.S. National Oceanic and Atmospheric Administration (Part 1 - Biological Sciences and Living Resources; Part 2 - Ocean Technology, Policy, and Non-living Resources; Part 3 - Aquatic Pollution and Environmental Quality).

National Technical Information Service (NTIS)

The NTIS database includes government sponsored research, development, and engineering as well as analyses prepared by federal agencies, their contractors, or grantees (and some state and local agencies).

Oceanic Abstracts

Oceanic abstracts are an organized index of technical literature published worldwide on marine related subjects.

Pollution Abstracts

Pollution Abstracts are a leading resource for references to environment-related literature on pollution, pollution sources, and pollution control.

Enviroline

Enviroline is a comprehensive environmental bibliography (fields include: management, technology, planning, law, political science, economics, geology, biology, and chemistry as they relate to environmental issues).

Biosis Previews

Biosis Previews are comprised of the following relevant subfiles: Biological Abstracts, Biological Abstracts/RPM (reports, reviews, meetings), and BioResearch Index.

Compendex Plus

Compendex Plus provides abstract information from the world's significant literature of engineering and technology (civil, energy, environmental, geological, and biological engineering and technology).

Toxline

Toxline is comprised of the following relevant subfiles: Toxicity Bibliography, Toxicology Document and Data Depository File, Federal Research in Progress, and Hazardous Materials Technical Center File.

1.2.1.2.2 Chemical Information Services

The databases searched via CIS including a brief description of each database are provided below. These four databases were accessed using Chemical Abstract Service (CAS) numbers and compound names. These are EPA-sponsored databases and contain numeric data (toxicological, physical/chemical properties) for the compound(s) of interest and include reference citations.

AQUIRE

AQUIRE is the Aquatic Information Retrieval database. AQUIRE contains information on acute, chronic, bioaccumulative, and sublethal effects data from experiments performed on freshwater and saltwater organisms.

OHMTADS

OHMTADS is the Oil and Hazardous Materials/Technical Assistance Data System. OHMTADS provides up to 126 different fields of information including physical, chemical, biological, toxicological, and commercial data on over 1,402 materials, with emphasis on their environmental effects and emergency response.

ISHOW

ISHOW is the Information System for Hazardous Organics in Water. Contains melting point, boiling point, partition coefficient, acid dissociation constant, water solubility, and vapor pressure for more than 5,400 chemical substances.

ENVIROFATE

ENVIROFATE is the Environmental Fate Database. ENVIROFATE includes data on environmental transformation rates and on physical-chemical properties for over 800 substances.

1.2.1.3 Other Sources of Data

Pertinent information was also obtained through searches of the following sources:

ENTRIX, Inc. Resource Libraries

ENTRIX possesses a great deal of literature on topics related to petroleum products and the environment. Journal articles, reports, books, and other references, including API document catalogues and spill conference proceedings, were reviewed for pertinent toxicological data on petroleum products to the aquatic organisms of interest.

University of Delaware Library

The University of Delaware's library system includes a main reference/resource library and a marine studies library. The University has an on-line bibliographic database (DEL CAT) which is keyword searchable and accesses all library holdings. In addition, the library also offers on-line reference searching of Biological Abstracts and Aquatic Sciences and Fisheries Abstracts. The University's holdings were searched using keyword strings as described in Section 2.1.2.

Bibliographies

Two bibliographies on the fate and effects of aquatic oil pollution and the biological effects of oil pollution in the marine environment were screened and relevant references were selected for acquisition. These were:

- Seakem. 1987. Bibliography on the Fate and Effects of Aquatic Oil Pollution: A Survey of International Oil Pollution Literature to 1987, and
- Filion-Myklebust, C. and K. Johannessen. 1980. Biological Effects of Oil Pollution in the Marine Environment: A Bibliography. International Council for the Exploration of the Sea (ICES), Marine Environmental Quality Committee, C.M. 1980/E:31.

In addition, reference lists provided in review papers, books, and applicable journal articles on the toxicological effects of oil were screened and selected as appropriate.

1.2.1.4 Selection and Collection of Pertinent Literature

References were selected for acquisition if they contained or were thought to contain numeric information on at least one of the oil product categories of interest, a taxonomic group of interest, and a toxicity endpoint of interest (i.e., acute lethality). References with information relating toxicity and persistence of oil products were also selected.

The wide range of information created limitations in acquiring some references. Nearly all English language journals and books with pertinent information were located at the University of Delaware. References which were difficult to obtain included foreign language material,

Ph.D. theses and/or specialized foreign or domestic research group reports, seminars, or conference proceedings.

Upon acquisition of literature, it was necessary for us to review each article, extract the appropriate information and transfer this information into a usable format for management and analysis. With this in mind, a computerized database was developed to manage the specific study result (i.e. LC50) and study methodological data contained in the literature reviewed.

1.2.2 Database Development

A computerized data management system (OILTOX) was developed, programmed and compiled using the ARAGO dBXL-Quicksilver software. The developed database includes functions for LC50 data value sorting (e.g., by methodological parameter, species, product), exporting and reporting.

1.2.2.1 Key Study Parameters

The first step in the development of the database was to identify which methodological parameters most influenced the actual LC50 value developed in a study, the interpretation of results, and the comparability of data between studies. A preliminary review of a subset of references was initiated to help identify key study parameters. Based upon this review and ENTRIX experience, twenty one parameters were selected for entry into the database. The database was designed such that these parameters were entered as individual fields for ease of sorting and study grouping. A sample data entry form displaying the key fields selected is presented in Figure 1-1. The fields selected for inclusion in the database are detailed below.

1.2.2.1.1 Oil Product

Each oil product category was assigned an alphabetic code. Oil products within one category were individually identified by numeric code. For instance, all crude oils were assigned the letter "C", with Kuwait crude as C01, Cook Inlet crude as C02, Southern Louisiana crude as C03, etc. In this way, oils could be evaluated either individually or as a group. The following categories of oil products were developed for toxicity evaluations:

- B=Bunker C Fuel (No. 6 Fuel Oil),
- C=Crude Oil,

API TOXICITY DATABASE ENTRY FORM: 712400

Oil Product:

B = Bunker C (No. 6)
 C = Crude Oil
 D = Diesel (No. 2)
 G = Gasoline
 J = Jet Fuel (No. 1)
 K = Kerosene
 L = Lube Oil

Study Purpose Endpoint: Primary:

ENDPOINTS: (LC50 / LC100 / EC50 / EC100 / NOEC / LOEC)

Persistence Data Reported? (Y=yes/N=no): ☐ If Yes, Qualitative (1) or Quantitative(2)? ☐Chronic Data Reported? (Y=yes/N=no): ☐Agitation Duration During Preparation (hrs)? ☐

(0=0/1=0-1/2=1-12/3=12-24/4=>24/5=not reported)

Test Solution Development: (1=single ratio/2=multiple ratio/3=not reported): ☐Test Chamber: (1=open/2=closed/3=not reported): ☐Free Product Present: (Y=present/N=absent/U=unknown): ☐Test Duration (hours): Species Identification Code: Age/Life-stage Code: (0=egg/1=larvae/2=juvenile/3=adult): ☐Study Type (1=lab/2=field(deliberate spill)/3=field(accidental spill): ☐Exposure Method(ST=static, non-renewed/SR= static, renewed/FT= flow-through): Test Condition (FW=freshwater/SW=saltwater): Effect Concentration (mg/l): Primary: Measured/Unmeasured/Stock (M=measured/U=unmeasured/S=stock): ☐Reliability Code (L=low/M=medium/H=high): ☐

L = Study does not meet criteria for reliability (low)

M = Study meets some criteria for reliability (medium)

H = Study meets all criteria for reliability (high)

Peer Reviewed? (Y=yes/N=no/U=unknown): ☐Reference Number: Year Published:

Remarks: _____

Additional Notes: (Not for Computer Entry): _____

Figure 1-1. Example of the database entry form.

- D=Diesel (No. 2 Fuel Oil),
- G=Gasoline,
- J=Jet Fuel (No. 1 Fuel Oil and Kerosene), and
- L=Lube Oil.

Closely related oils were grouped according to similarity of boiling ranges, percent by volume of certain hydrocarbon types, and other components (carbon number, etc.). Individual oil products identified in this study, by oil product group, are provided below.

Bunker Fuels:

- B01 Bunker "C" (unspecified),
- B02 Venezuelan Bunker C,
- B03 Fuel Oil No. 6,
- B04 Bunker C light,
- B05 Heavy Fuel Oil No. 4, and
- B06 Navy Special (reported to be between fuels nos. 4 and 5).

Crude Oils:

- C01 Kuwait (light) crude,
- C02 Cook Inlet crude,
- C03 Southern Louisiana crude,
- C04 Florida Jay crude,
- C05 Prudhoe Bay crude,
- C06 Venezuelan crude (incl. BCF-22),
- C07 Western sweet blend crude,
- C08 Transmountain crude,
- C09 Norman Wells crude,
- C10 Hibernia crude,
- C11 Amauligak crude,
- C12 Tarsuit crude,
- C13 Lago Medio crude,
- C14 Atkinson crude,
- C15 Bent Horn crude,
- C16 Ramashkin crude,

- C17 West Texas crude,
- C18 Dubai crude,
- C19 Nigerian crude,
- C20 Pembina crude, and
- C21 Alaskan crude (ARCO, unspecified).

Diesel Fuels/Heating Fuels (No. 2):

- D01 Diesel,
- D02 Fuel Oil No. 2,
- D03 Fuel Oil No. 2 - furnace fuel,
- D04 Light diesel fuel,
- D05 Heavy diesel fuel,
- D06 Navy distillate fuel, and
- D07 Marine diesel.

Gasolines:

- G01 Leaded gasoline,
- G02 Unleaded gasoline, and
- G03 Low leaded gasoline.

Kerosene/Jet Fuels (incl. Fuel Oil No. 1):

- J01 Jet fuel - JP8,
- J02 Light Fuel Oil No. 1,
- J03 Jet fuel - JP9, and
- J04 Jet fuel - JP4.

Lubricating Oils:

- L01 Auto lube/lubricating oil (unspecified),
- L02 Heavy marine lube, and
- L03 9250 lube oil.

1.2.2.1.2 Study Purpose Endpoint

We anticipated that study endpoints would vary from study to study. Some studies report thresholds that kill 100% of test organisms (LC100) rather than 50% (LC50) while others use immobility rather than mortality as an endpoint. This field was created to select and group endpoint-specific values to ensure legitimate comparisons of data. *Acute toxicity values were the focus of this study.*

1.2.2.1.3 Test Solution Development: Agitation Duration During Preparation

The toxicity of oil products is generally attributed to the portion that dissolves into the water column, in other words, the concentration of dissolved hydrocarbons that results from an oil/water mixture. The amount of dissolved hydrocarbon present in a test solution is somewhat dependent on the amount of time an oil/water mixture is agitated prior to organism exposure. In some cases, oil is added directly to the water with little, if any, agitation. In others, an oil/water solution is mixed for 20 hours, allowed to settle, and the water soluble fraction (WSF) drawn from the bottom of the vessel (containing no neat product, only dissolved hydrocarbons) is used as a toxicant stock solution. The amount of dissolved hydrocarbon to which test organisms are actually exposed could differ significantly in these two situations. Each design produces different test solutions although equal amounts of neat product were used. It was therefore prudent to have the capability to identify agitation duration and subsequently evaluate studies based on this factor.

1.2.2.1.4 Test Solution Development: Free Product Present or Absent

A significant number of oil product tests actually have free product present in the test chambers. Many studies mix oil and water and then decant the water soluble component (after some period of settling) and use only the oil free phase in the tests. In order to assess the importance or impact of this factor on toxicity values, this category was developed in the database. As mentioned earlier, the presence or absence of free product was utilized as the initial sorting mechanism in the analysis of toxicity values.

1.2.2.1.5 Test Solution Development: Analytically Measured or Unmeasured Exposures

Measured/Unmeasured/Stock refers to whether total dissolved hydrocarbons were measured in the test solutions. Endpoint values (LC50s) can be calculated in a variety of ways including:

1) based on actual measured dissolved hydrocarbon concentrations in test chambers (measured), 2) based on nominal neat product levels added to test chambers (unmeasured), or 3) based on measured dissolved hydrocarbon concentrations in a single stock solution and extrapolating this measurement to test chamber concentrations (stock). The final result of the toxicity test will depend on which "concentration measurement" is used in the LC50 calculation. Since oil product tests have been known to have results expressed using all of the above concentration expressions, this parameter requires evaluation regarding its impact on toxicity values.

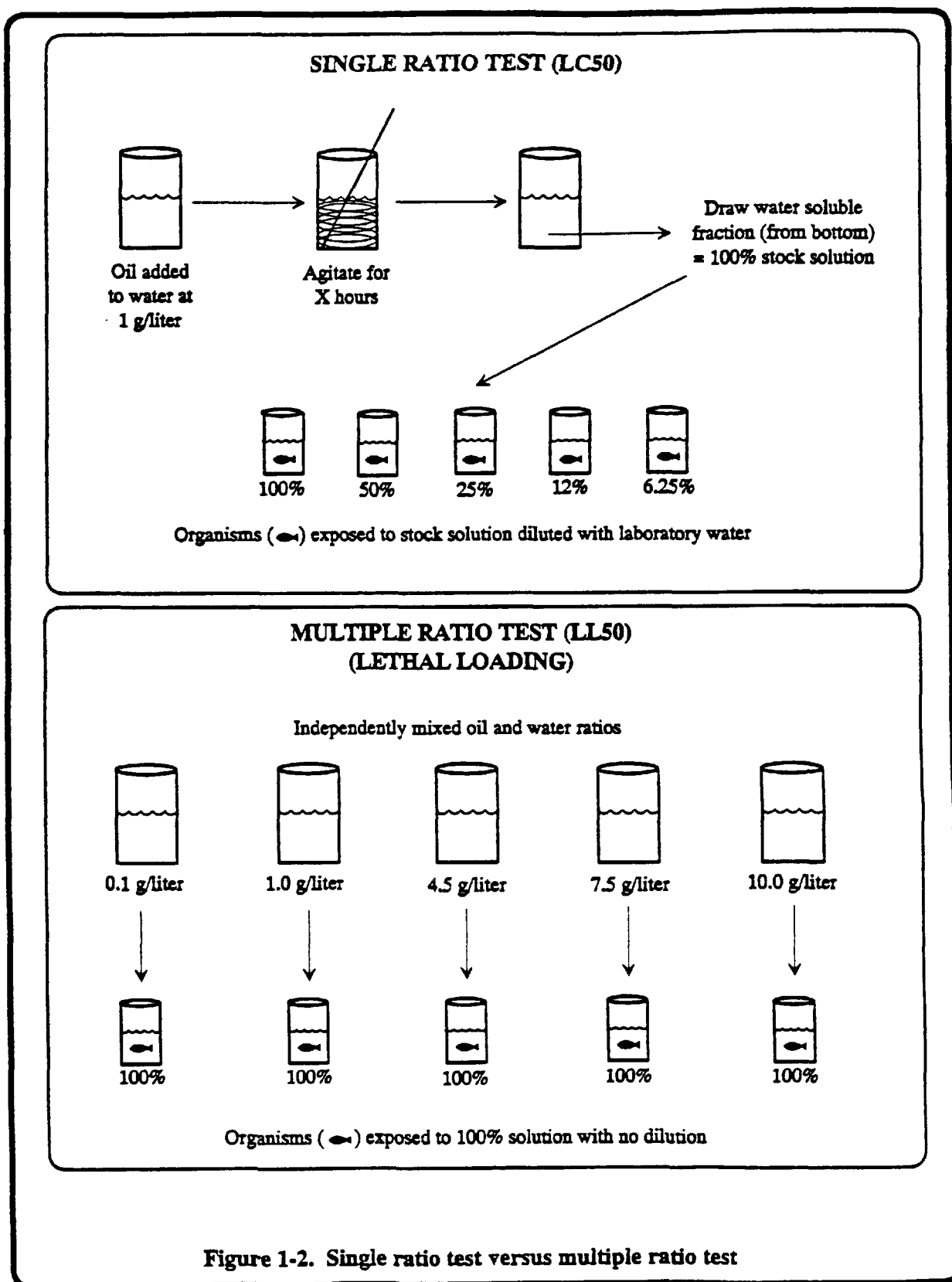
Even when test solutions are measured regarding the hydrocarbon levels or types, the information is of little value since the measurement is a generic type of analytical term i.e. "total hydrocarbon" or "total organic carbon". Unlike a specific compound LC50 where the concentration of a component can be related to an effect, total hydrocarbon measurements encompass from a few to over 100 components, all of which have varying individual toxicities. This aspect combined with the variability in other methodological procedures gives one cause to question the utility of even measured values in ranking and comparing oil product toxicity values.

1.2.2.1.6 Test Chamber

Due to the volatile nature of many hydrocarbons, it was necessary to try and differentiate between those studies using open test chambers versus those using closed test chambers. Those studies utilizing open test chambers will experience a loss of toxicant during the course of experimentation. Open chamber test systems, using volatile products, could result in higher LC50 values than equivalent tests using closed chambers.

1.2.2.1.7 Test Solution Development: Single Ratio and Multiple Ratio Test Designs

As noted in the above discussions, a number factors can influence the actual concentrations of hydrocarbons found in solutions produced for aquatic toxicity testing. Aside from agitation duration, open or closed test chambers, and the presence or absence of free product, the impact of "single" versus "multiple" ratio test solution development on product toxicity values was evaluated. Hydrocarbon concentrations in the test solutions will vary according to whether the test solutions were derived from a single ratio (oil:water) mix or from a multiple ratio (oil:water) mix (Figure 1-2). Oil:water ratios will have a significant impact on the toxicity results since the ratio of oil to water impacts both the quantitative, and to an extent,



the qualitative characteristics of the test solution being developed for testing (Shiu, et al, 1988). Oil:water ratios need critical assessment as to their role in influencing toxicity value development.

Studies using multiple versus single ratio tests were identified during the data review. Multiple and single ratio test systems are presented in Figure 1-2. The relative amounts of individual hydrocarbon components found in stock solutions using the single ratio design is limited due to the single oil to water mixture. When the single stock solution is diluted to create toxicity test solutions, the ratio of components remains the same and only the amount or concentration of dissolved components change. Toxicity tests conducted with test solutions derived from single oil:water ratio mixtures would be expected to show predictable dose response relationships since solution strength is primarily dependent on levels of dilution.

This is contrasted to the multiple ratio study design. When multiple oil:water test ratios are developed, a number of significant changes occur. First the component concentrations and ratios vary based on the oil:water mixture used. Given a specific percentage by weight of water soluble materials for any given oil product, the absolute amounts of water soluble materials in a 10 g/l versus a 1 g/l oil:water test mixtures will be quite different. One might expect that the total amount of potentially soluble hydrocarbon levels can be estimated by assuming that all soluble materials have an equal tendency to dissolve in water regardless of the oil:water ratio. This is not necessarily the case since surface area considerations become a factor when large amounts of oil are added to water. The multiple ratio approach provides the researcher with an empirical estimate of the importance of oil:water ratios in determining toxicity. Results derived from a multiple ratio experiment provide a more useful predictive tool when applied to real world situations. Since oil:water ratios are site and spill specific, a single ratio test provides a result of questionable utility in predicting oil product impact during a spill.

1.2.2.1.8 Reliability Code

Data extracted from references were evaluated for quality by analyzing the methodologies through which they were generated. Data were assigned to a category of high, medium, or low quality as described below:

Data assigned to the "high" quality category met most or all of the following criteria for quality:

- a. the test methodology was judged satisfactory, with appropriate test design and acceptable procedures including:
 - controls utilized,
 - acceptable survival in controls (80% minimum),
 - minimum of 10 organisms per treatment,
 - evidence of test organism acclimation,
 - minimum of 5 toxicant concentrations used, and
 - evidence of quality assurance.
- b. the test material was well characterized and dissolved chemical measurements were made in test chambers.

If a paper exhibited a deficiency in one of the above areas, it was not automatically placed in the next lower quality category. Rather, the deficiency was weighed against the remaining strengths of the paper and judged as to its significance to overall data results. If the majority of the remaining information presented in the paper was superior, a single deficiency in the above criteria did not warrant a lower quality ranking.

Data assigned to the "medium" quality category met some of the high quality criteria, but lacked 2 or more items listed in a) above. Data were also assigned to the "medium" quality category if criteria b) above was not satisfied.

Data assigned to the "low" quality category did not meet the criteria for quality. A low quality study may be characterized as not meeting any of the criteria above and may not have sufficient methodological detail to judge the quality of experimental design.

1.2.2.2 Data Entry

The key study parameter data as identified in Section 1.2.2.1 was extracted from each article and transcribed onto the database entry form. Every individual toxicity value within a reference was recorded on a separate data form, i.e., became an individual record. Information on these forms was then entered into the database using the designed database software.

1.2.2.3 *Data QA/QC*

Data QA/QC is essential in protecting the integrity of the database. A strict QA/QC transmittal sequence was developed to monitor data from initial transcription to final storage into the database. Data transmittal forms (Figure 1-3) accompanied completed data forms as they proceeded through verification and validation checks. The data transmittal form was used to track data to ascertain the immediate status of all data.

The major steps of the data transmittal process were as follows:

- Reference data were transcribed onto a database entry form,
- The date completed and transcriber's initials were entered onto the transmittal form,
- Completed forms were submitted to the data entry personnel who would then enter the data into the computer,
- The data entry personnel would then produce a QA/QC verification table to be checked against the original hard copies,
- Any necessary revisions to the database were made and re-checked against the hard copies, and
- Backup copies of the database were made.

Completion of each of the above transmittal steps were recorded on the data transmittal form by date completed and initialized.

1.2.3 Analysis and Ranking of Toxicity Values

1.2.3.1 *Statistical Analyses*

Statistical inference testing of effect concentration (LC50 data) values presented in this Chapter was performed using nonparametric test statistics. The use of nonparametric statistics was more appropriate than parametric statistics since the effect concentration data by major taxonomic group for free product presence and absence were not normally distributed as indicated in Figures 1-4 and 1-5. Also, calculated LC50 values for free product present and free product absent data have differing variances. These factors make analysis using nonparametric statistics (i.e., median values) more appropriate than using parametric statistics (i.e., mean values).

DATA TRANSMITTAL FORM		AMERICAN PETROLEUM INSTITUTE TOXICITY VALUE DATABASE	
BATCH NUMBER: _____	# OF PAGES SUBMITTED: _____		
STUDY TYPE(S) INCLUDED: _____			
FILE NAME:(IF COMPUTERIZED): _____			
		DATE COMPLETED:	INITIALS:
PROJECT MANAGER/TECHNICIAN STAFF:		_____	_____
DATA FORM CHECKED:		_____	_____
TECHNICIAN STAFF/COMPUTER APP. TECH:		_____	_____
DATA KEYPUNCHED :		_____	_____
BACKUPS COPIES MADE:		_____	_____
DATA FILE REFORMATTED:		_____	_____
COMPUTER APP. TECH/TECHNICIAN STAFF:		_____	_____
QA/QC PROCEDURES COMPLETED:		_____	_____
TECHNICIAN STAFF/COMPUTER APP. TECH:		_____	_____
DATA FILES UPDATED:		_____	_____
BACKUP COPIES MADE:		_____	_____
COMPUTER APP. TECH/PROJECT MANAGER:		_____	_____
DATA LOADED INTO RAW DATABASE:		_____	_____
QA/QC TABLES PRODUCED:		_____	_____
PROJECT MANAGER/COMPUTER APP. TECH:		_____	_____
QA/QC TABLES VERIFIED:		_____	_____
DATA FILES UPDATED:		_____	_____
BACKUP COPIES MADE:		_____	_____
COMPUTER APP. TECH/PROJECT MANAGER:		_____	_____
DATA FILES LOADED INTO FINAL DATABASE:		_____	_____
BACKUP COPIES MADE:		_____	_____
NOTES: _____			

Figure 1-3. Example data transmittal form.

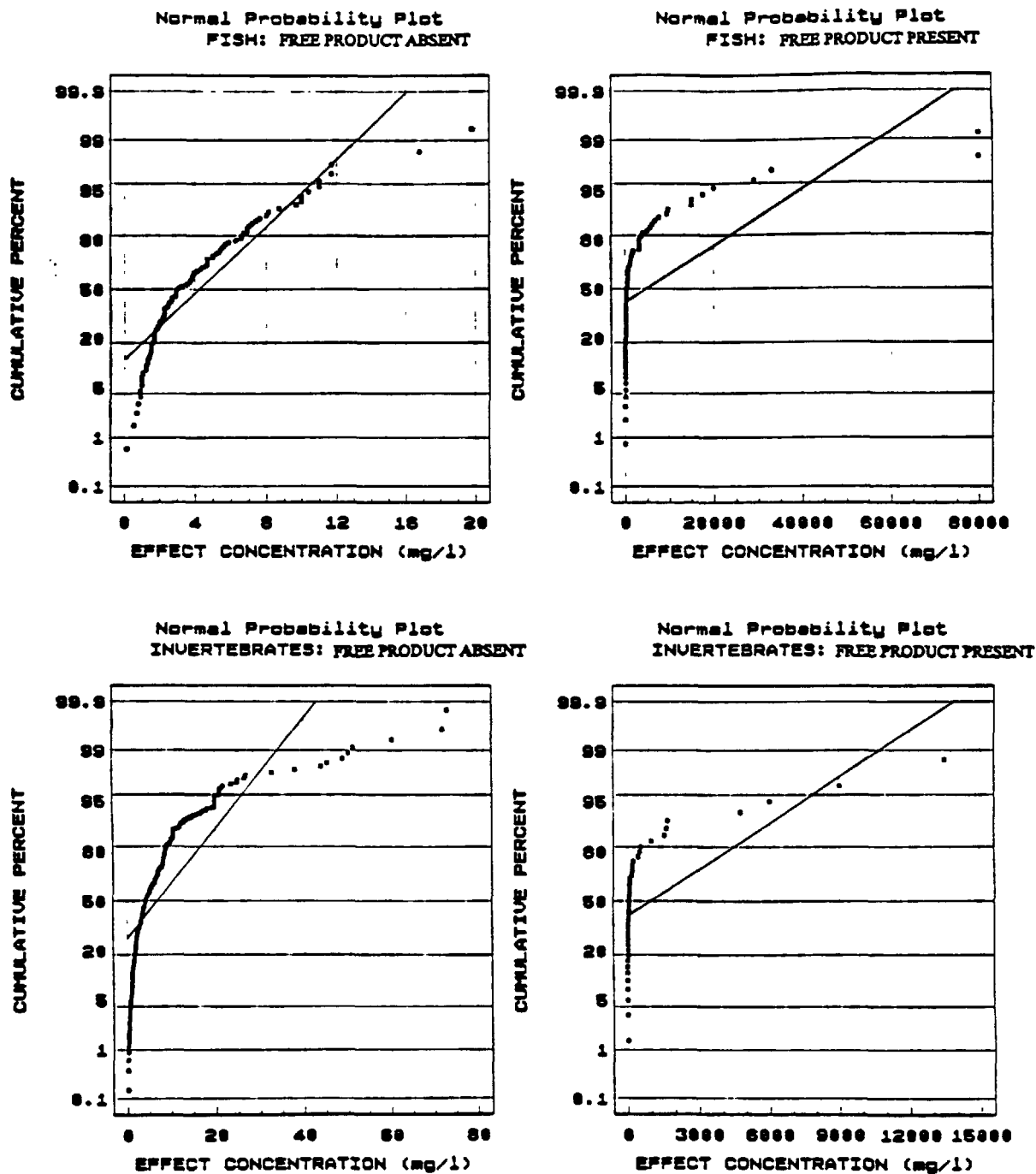


Figure 1-4. Normal probability plots of effect concentration (mg/l) values by taxonomic group.

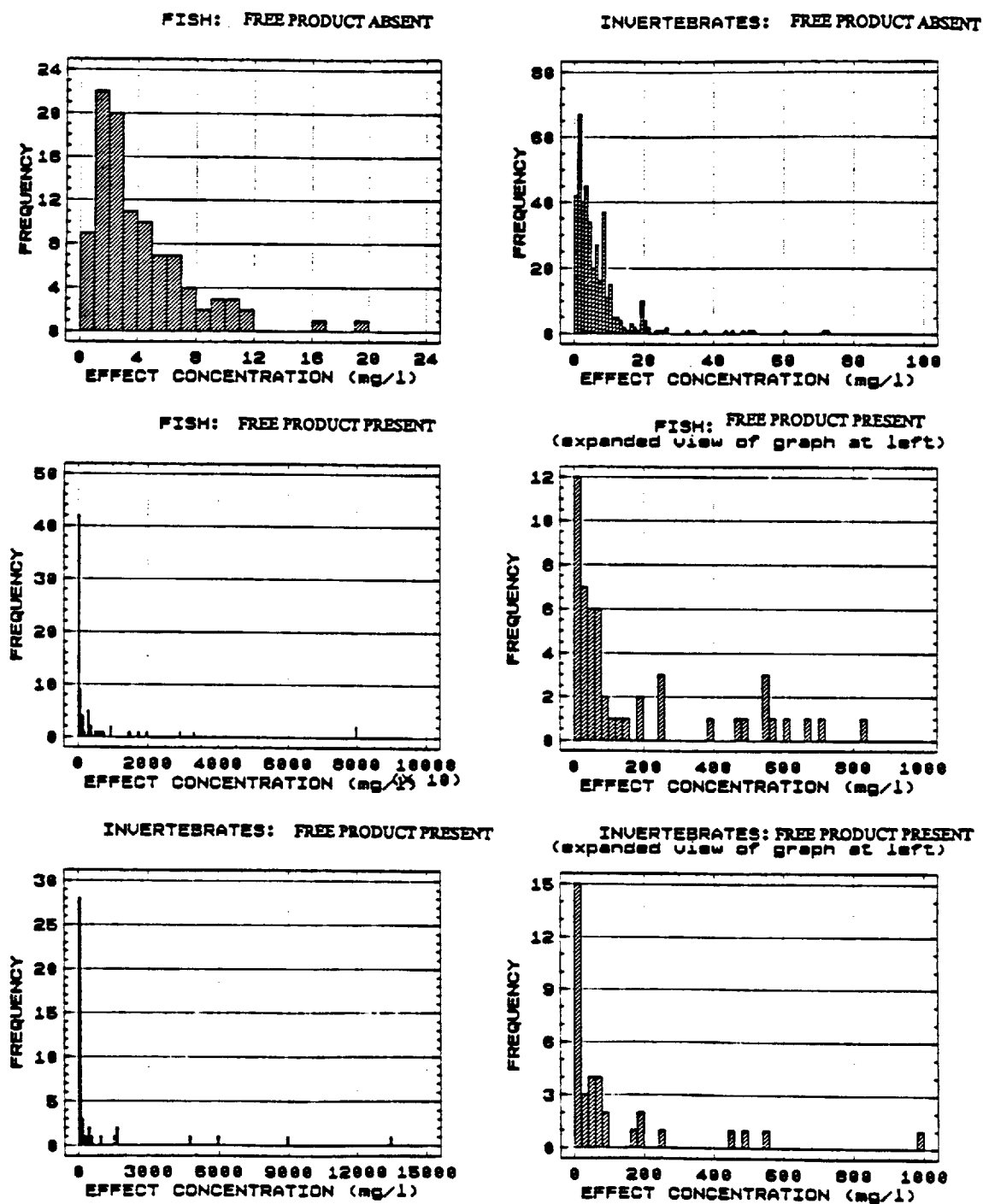


Figure 1-5. Frequency histograms of LC50 values by taxonomic group.

The Mann Whitney test (Zar, 1974) was used to determine whether two independent samples (e.g. bunker versus crude) had the same median. The level of significance used for all analyses refer to significance at the five percent level ($\alpha=0.05$, 95% confidence level).

The Kruskal-Wallis test (Snedecor and Cochran, 1979) was used when more than two classes were compared in a pairwise manner. If the Kruskal-Wallis test produced a significant difference, the Mann-Whitney test was used for all pairs in the comparison with critical values being adjusted by Bonferroni's method (Johnson and Wichern, 1982). Bonferroni's method allows for examination of all possible pairwise combinations without increasing the probability of making a Type I error. A Type I error occurs when a significant difference is found but does not actually exist. The use of Bonferroni's method provided a conservative estimate of significance. In all cases the significance levels are presented as two-tailed p-values. Adjusted statistical critical levels based on Bonferroni's method are presented in Table 1-1.

Table 1-1. Adjusted Critical Values based on Bonferroni's Method (0.05/# of comparisons).

<u># of Classes</u>	<u># of Comparisons</u>	<u>Critical Value</u>
2	1	.050
3	3	.017
4	6	.008
5	10	.005
n	$[n(n-1)]/2$	

Notched box-and-whisker plots are provided for several comparisons tested in this report. *In some cases, more than one plot is provided for the same data set.* In these cases, the additional plots allow an expanded view of the data for easier comparison of the data. Box-and-whisker plots are useful in comparing parallel batches of data. The plot divides the data into four areas of equal frequency. The box encloses the middle 50 percent. The median is drawn as a horizontal line inside of the box. The width of the box is proportional to the square root of the number of observations in the group. A notch is added to each box at the median. The length of the notch represents an approximate 95 percent confidence interval for the median. Comparisons of median values can be made at the 95 percent confidence level by examining whether two notches overlap. If two notches overlap, the medians are not significantly different. An example box-and-whisker plot is presented in Figure 1-6. The letters along the X axis refer to product types (B-Bunker, C-Crude, D-Diesel, J-Jet Fuel, L-Lube Oil).

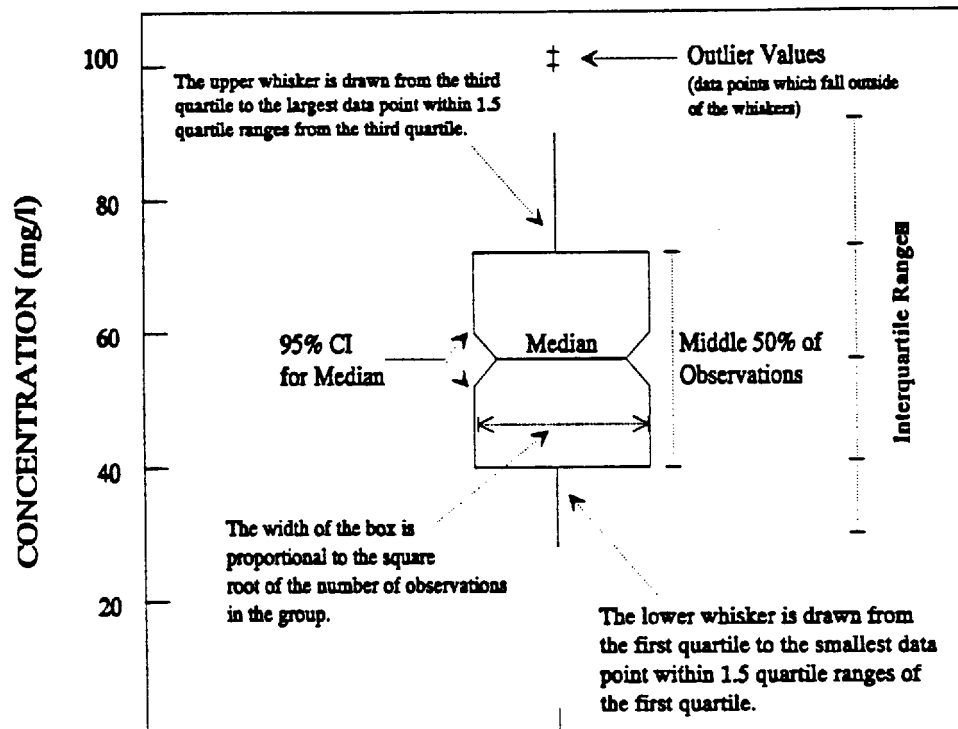


Figure 1-6. Example of a box-and-whisker plot.

All statistics used in the analysis of toxicity values were generated using the Statgraphics Statistical Software, Version 5.0.

1.2.3.2 Approach

1.2.3.2.1 Oil Product Toxicity Values

A main goal of our analysis was to normalize the data set in order to produce the most useful and meaningful toxicity values possible. The first step of our normalization was to exclude, from the analysis, data records classified as "low reliability" (Appendix Table C-1). Data classified as "low reliability" were considered to have insufficient methodological detail to judge the quality of the study or were judged to be deficient in study design or procedures.

As noted earlier, with complex mixtures such as oil products, LC50 data are problematic because the meaning of the term "concentration" can vary extensively depending upon the methodological procedures used. A key methodological parameter which is important in the calculation of a toxicity value for a given oil product is the presence or absence of free product during the study. A preliminary analysis of the free product present data versus free product absent data, by two major taxon groups, indicated that free product presence was significant in calculations of toxicity values (Table 1-2).

In all cases, median effect concentrations for free product present studies were significantly higher when compared to the median effect concentrations of studies with free product absent. With this in mind, free product presence and absence data were analyzed separately. The majority (99.2%) of free product absent LC50 values were derived from single ratio tests while the majority (80%) of free product present LC50 values were derived from multiple ratio tests.

Table 1-2. Pairwise comparisons of median effect concentration values by the presence of free product, taxon and oil product group. Critical value = 0.05. Bold indicates significance.

<u>Oil Product</u>	<u>Taxon</u>	FPA Median LC50 Free Product <u>Absent (mg/l)</u>	FPP Median (LC50) Free Product <u>Present (mg/l)</u>	Ratio of FPP to <u>FPA</u>	Significance <u>Level</u>
Bunker	Invertebrates	3.00	55.85	(19x)	(p=0.022)
Bunker	Fish	3.60	55.70	(15x)	(p<0.004)
Crude	Invertebrates	6.31	225.00	(36x)	(p<0.001)
Crude	Fish	3.12	1365.00	(438x)	(p<0.001)
Diesel	Invertebrates	3.36	9.40	(3x)	(p<0.002)
Diesel	Fish	3.50	45.10	(13x)	(p<0.001)
Lube Oil	Invertebrates	1.58	55.50	(35x)	(p=0.032)
Lube Oil	Fish	2.25	68.00	(30x)	(p=0.0497)

Since study methods can have substantial impacts on calculated toxicity values, the effects of selected methodological procedures on calculated toxicity values were examined by oil product group, major taxonomic group, and free product presence or absence. The methodological procedures examined include:

- Agitation During Preparation,
- Test Solution Development,
- Test Chamber,
- Test Duration,
- Lifestage Tested,
- Exposure Method
- Test Condition, and
- Measured/Stock/Unmeasured.

The above methodological procedures are described in Section 1.2.2.1. The lack of algal toxicity data precluded an in-depth analysis of the effect of methodological procedures on calculated toxicity values for this taxonomic group.

Median toxicity values were calculated by oil product group, specific oil product, free product presence/absence, and taxonomic group. Methodological parameters found to have a significant effect on toxicity values are described. Toxicity values were calculated for each

lifestage of each taxonomic group by oil product where data were available. *When interpreting the effect concentration data, note that the higher the effect concentration value, the lower the toxicity of the product, and the lower the effect concentration value, the higher the toxicity of the product.*

1.2.3.2.2 Ranking of Oil Product Toxicity

A relative ranking of oil product toxicity was developed as part of this study. The major oil product groups (i.e. bunker, crude, diesel, gasoline, jet fuel and lube oil) were ranked, relative to one another, based upon calculated median effect concentrations. Rankings were also developed for each taxonomic group for both free product absent and free product present data. Rankings were also developed for each life stage by taxonomic group. An oil product group which had no data for a particular ranking was excluded from the ranking. Any methodological procedures which may affect the rankings are presented.

Pairwise statistical comparisons of the median effect concentration values of the oil products in each major ranking are presented.

For each ranking presented, an approximate scale is provided for comparison of the relative median concentration of the oil product as compared to the median concentration of the least toxic oil product. Each scale value was derived by dividing the median value of the least toxic group by the median value of the oil product group being compared. The result of the division was rounded to the nearest integer. *Since the oil product rankings are derived from the median effect concentration values, rankings with different scale values may or may not be significantly different.* Tables are provided where statistical differences can be ascertained.

A summary of the types of toxicity value comparisons and rankings performed is presented schematically in Figure 1-7. The figure also provides guidance as to where the toxicity value or ranking results are found within the report.

1.2.3.2.3 The LL50 Concept

Based on all the factors that can influence and confound LC50 values when applied to oil product toxicity data, it is apparent that a different method for expressing toxicity data derived from whole product aquatic tests is needed. Although hydrocarbon toxicity can be investigated using LC50 principles, for the reasons described above, complex solutions from whole product

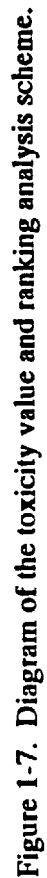


Figure 1-7. Diagram of the toxicity value and ranking analysis scheme.

toxicity tests do not lend themselves well to the LC50 notation. There is no straightforward way to compare relative product toxicity when results are expressed as a function of selected dissolved components. Since API is interested in developing accurate rankings of acute toxicity on oil products, any method which helps place products on a normalized scale should be assessed.

The lethal loading concept (LL50) was developed (Girling, et al, 1992) as a method of expressing actual whole oil product toxicity to aquatic organisms. The method relies on selected toxicity data sets which use whole oil products at multiple oil:water ratios as the method for test solution development. A further restriction inherent in the expression is that oil to water solutions are not diluted further and that all test solutions are independently created using a series of oil:water ratios. The method does not require any measured concentrations in order to be used. Observed mortality based on various oil:water mixtures (as presented in Figure 1-2) is the basis of the calculation resulting in a "lethal loading" level which causes an effect.

The LL50 concept has been developed further through numerous discussions within CONCAWE's Ecology group as the latter assessed the industry needs regarding oil product ecotoxicity data. As the group reviewed oil product data and tried to compare relative toxicities of products, the weakness of the LC50 term as a tool for comparing oil products became apparent (CONCAWE, 1988). Methodological differences between toxicity tests were abundant in the literature although the importance or impacts of the varying methods on toxicity values were not evaluated by CONCAWE.

The database developed in this study allowed for sorting of information by multiple versus single ratio test solution procedure. Since the LL50 approach does not rely on analytical measurements, the comparisons do not suffer from the uncertainties associated with comparing a host of analytical methods within the literature. An LL50 data set was created by selecting literature test results which satisfied the LL50 criteria (i.e., multiple:ration solution and based no dilution). This subset of LL50 values were then used to calculate median toxicity values and rank oil products by major taxon and oil product group. The product ranking results from the LL50 approach were compared to median toxicity values and rankings based on the "free product present" and "free product absent" data sets.

1.3 RESULTS: ANALYSIS AND RANKING OF TOXICITY VALUES

1.3.1 Literature Search and Collection of Toxicity Data

Several databases, bibliographies, and the ENTRIX library were searched for toxicity values. Database searches utilizing the Dialog Information Service yielded approximately 325 relevant references. Titles were screened and abstracts were obtained for approximately 200 of these references. Upon screening the abstracts for content and availability, 115 references were selected for acquisition.

Database searches utilizing the Chemical Information System were not very productive. References selected in OHMTADS were pre-1970 and thus did not meet our search requirements. The search of the AQUIRE, ENVIROFATE and ISHOW databases produced no references meeting our selection criteria.

Through database searches, combined with bibliographies and ENTRIX library resources, approximately 8,000 references on the fate and effects of oil products in aquatic systems were screened. Of these, approximately 250 were selected for acquisition and 140 were actually obtained and reviewed. Of the 140 articles reviewed (Appendix A), 46 references reported data in a usable format for entry into the toxicity database, representing 748 individual data records. The data presented in this report are representative of the 140 articles reviewed.

1.3.1.1 Characterization of Petroleum Toxicity Literature

It became evident through screening the literature references on oil products, that only a small portion (0.6%) of the references were useful to our study. Much of the literature was secondary in nature, discussing laboratory or field observations without generating new data. Many times hydrocarbon toxicity data were developed using only specific components of oil products (e.g., naphthalene) and the toxicity of the whole product was not evaluated. Also, a great number of papers dealt with aspects of petroleum toxicity which are not addressed by this study. These papers included studies on bioaccumulation and chronic, histopathologic, and behavioral effects. As a result, the 8,000 titles originally screened were reduced to approximately 250 pertinent references.

In many cases the titles of references were misleading. Papers frequently did not report mortality data or LC50 values. In some cases results were reported in percent (%) of test

solution, which had no meaning independent of that study. In addition, certain references were from sources which were difficult to locate and collect. Consequently, 140 titles were actually collected and reviewed for this study, 46 of which contained usable data. These were papers that computed the median lethal concentration (LC50 or TLM) for a given oil to a test species, or papers whose data allowed the calculation of an LC50 or LL50 value. Generally, the literature is reflective of a component orientation to toxicity and not the whole product approach helpful in ranking products themselves.

The majority of the selected articles were published in the mid to late 1970's. While there was an adequate proportion of high quality articles reviewed, comparability between papers was limited due to variability in test methodologies. The importance of test methodological procedures to calculated toxicity values is presented in Section 1.2.2.1.

1.3.1.2 Characterization of Extracted Data

The final developed database contained 748 data records. Of these 748 data records, LC50 values were reported for 741 records. Seven records contained effect concentration data denoted as "NOEC" (No Observed Effect Concentration).

The "low reliability" data comprised approximately 7% (52 records) of the entire database (Figure 1-8). A statistical evaluation of the data indicated that the median effect concentration of the "low reliability" data records (47.50 mg/l) was significantly higher than the median effect concentration of either the "high reliability" data records (5.04 mg/l) or the "medium reliability" records (6.97 mg/l, $p < 0.001$). The "high reliability" and "medium reliability" data sets did not have significantly different median effect concentrations ($p = 0.07$).

The data set used in our analysis excluded the 52 "low reliability" records and the 7 "NOEC" data records. Our final dataset was comprised of 689 data records. The number of toxicity values collected by oil product, taxonomic group and free product presence is provided in Table 1-3.

The majority of the data was on crude oils (55%) and diesel (31%). Gasoline, jet fuel, and lube oil combined comprised less than 7% of the total number of data records in the database. Invertebrate data comprised 65.4% of the data records in the database. Fish comprised 26.6% of the data while algae comprised only 8% of the data. Appropriate data for aquatic macrophytes were not found.

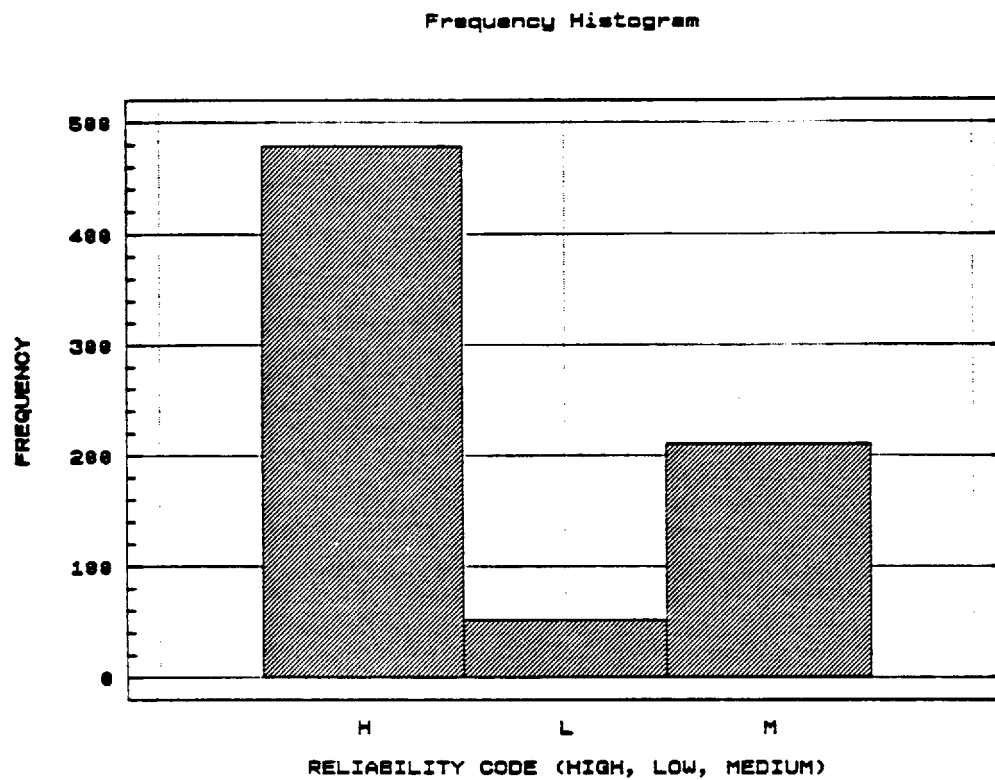


Figure 1-8. Frequency of data records by reliability code.

Table 1-3. Number of toxicity values (data records) by oil product group, taxonomic group, and Free Product Presence/Absence.

	INVERTEBRATES		FISH		ALGAE		TOTAL # 's	TOTAL (%)
	Free Product Absent	Free Product Present	Free Product Absent	Free Product Present	Free Product Absent	Free Product Present		
BUNKER	28	2	13	4	-	-	47	6.8%
CRUDE	224	22	54	46	5	28	379	55.0%
DIESEL	129	17	31	20	4	15	216	31.3%
GASOLINE	12	-	-	-	-	-	12	1.7%
JET FUEL	1	-	-	8	1	2	12	1.7%
LUBE OIL	14	2	4	3	-	-	23	3.3%
TOTAL #'s	408	43	102	81	10	45	689*	
(%)	59.2%	6.2%	14.8%	11.8%	1.5%	6.5%		
% BY TAXON	65.4 %		26.6%		8.0%			

This data set excludes 52 data records classified as "low reliability" and 7 records whose primary endpoints were designated as "NOEC - no observed effect concentration". The database contains a total of 748 records.

Toxicity studies utilizing algae and plants showed extreme variability in test methodologies and toxic endpoints. Endpoints included effects on photosynthetic rates, growth rates, chlorophyll content, mortality rates, and community structure. Only limited mortality data were available for algal species. In our database, effect threshold concentrations included reductions in growth rates and mortality.

The majority of the studies were saltwater tests (84%), using adult organisms (62%) under static conditions (91%). In addition, the majority of studies utilized open test chambers (72%), with all of the fish data generated under open chamber conditions.

Approximately 75% of the data records were for "free product absent" studies while 25% of the data records were for "free product present" studies. Over 90% of the invertebrate data was comprised of "free product absent" data while approximately 56% of the fish data was "free product absent" data. Only 18% of the algal data was "free product absent" LC50 data.

Toxicity values for "free product absent" studies were typically generated (99.2%) by "single-ratio" tests. This indicates that a single stock solution (water soluble fraction, WSF) was developed from the test oil and diluted to the various test concentrations. Of the data generated by the WSF studies, approximately half were based on measured total dissolved hydrocarbons present in the stock solution. The other half were actual measured concentrations in the test chambers after serial dilution of the stock.

Toxicity values for "free product present" studies were typically generated (80%) from "multiple-ratio" tests. In these studies different oil:water ratios were mixed and each WSF was mixed and tested independently. Endpoint values were reported in the amount of neat oil added to water, i.e., "lethal loading". Only one reference reported LC50 values both in terms of amount of neat product as well as the total dissolved hydrocarbons that resulted from that loading. These two methods represent very different approaches and the concentrations of water soluble components will vary significantly between methods for the same product. A detailed discussion of these phenomena is available in Shiu, et al. (1988).

1.3.2 Analysis and Ranking of Oil Product Toxicity

The results of our analysis of oil product toxicity values and ranking is divided into 6 main areas. These are discussed, in turn, as follows:

- 1.3.2.1 **Invertebrates: Free Product Absent**
- 1.3.2.2 **Invertebrates: Free Product Present**

- 1.3.2.3 **Fish: Free Product Absent**
- 1.3.2.4 **Fish: Free Product Present**

- 1.3.2.5 **Algae: Free Product Absent**
- 1.3.2.6 **Algae: Free Product Present**

1.3.2.1 Invertebrates: Free Product Absent

A schematic of the results included in this Section is presented in bold print in Figure 1-9.

1.3.2.1.1 Median Toxicity Values

Oil Product Group Comparisons

Gasoline exhibited the highest (least toxic) median effect LC50 concentration (20.25 mg/l) while lube oil exhibited the lowest (most toxic) median effect LC50 concentration (1.58 mg/l). Median effect concentration values for bunker, diesel and jet fuel were not significantly different. Crude oil had a significantly higher median effect concentration (6.31 mg/l) when compared to the median effect concentrations of both bunker (3.00 mg/l) and diesel (3.36 mg/l).

The median effect LC50 concentration values for invertebrates by oil product group are presented in Table 1-4 and Figure 1-10. The distribution of invertebrate effect concentration values are presented in Figure 1-11. Only 1 data value for jet fuel was available in this category. Figure 1-9, Table 1-4, Figure 1-10 and Figure 1-11 are presented on the following 4 pages.

Median effect LC50 concentrations for invertebrates were significantly different between several oil product groups ($p < 0.001$). Pairwise comparisons of median LC50 values by oil product group are presented in Table 1-5.

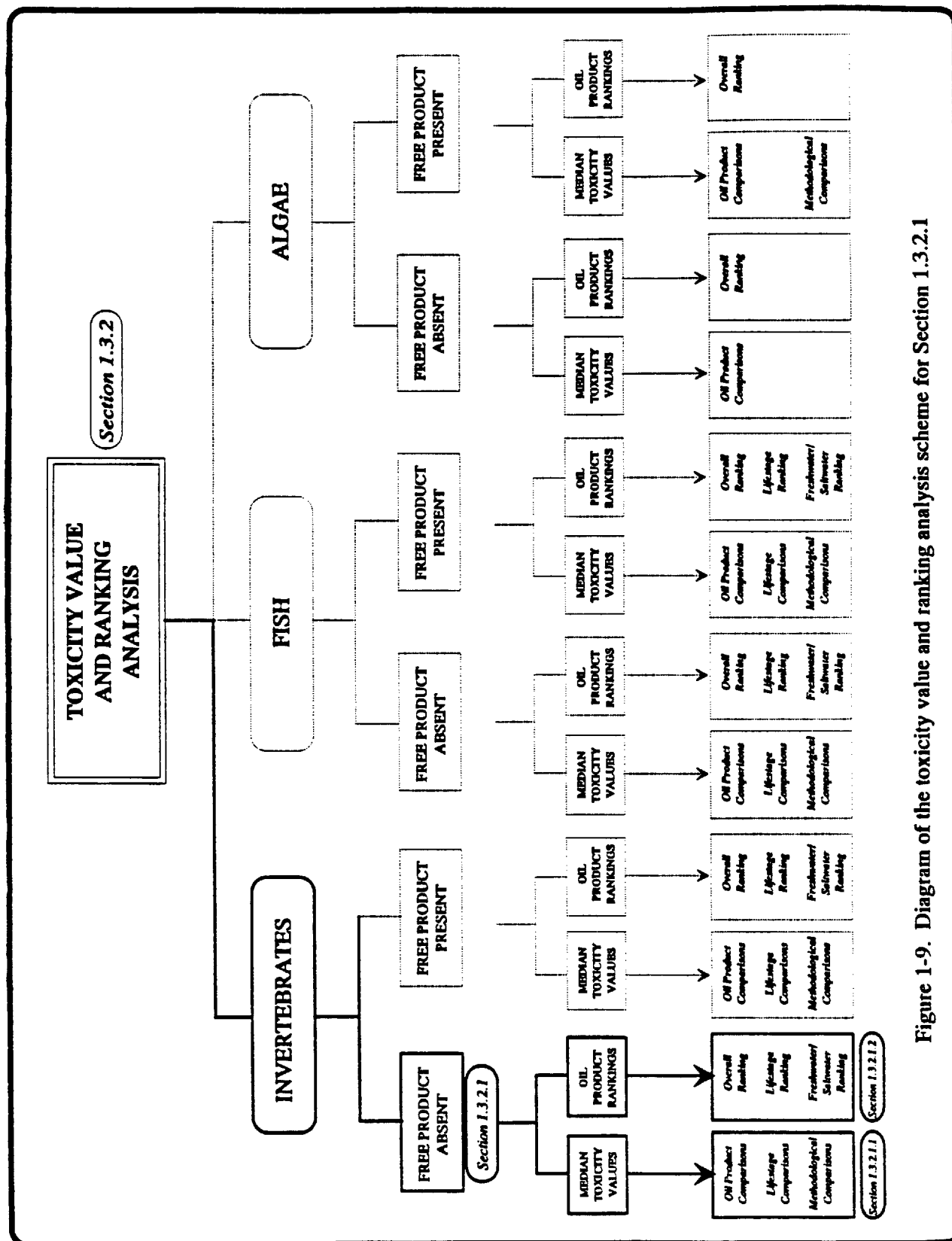


Figure 1-9. Diagram of the toxicity value and ranking analysis scheme for Section 1.3.2.1

**Table 1-4. Median effect concentrations (mg/l) for invertebrates by oil product.
(Free Product Absent)**

Oil Product	Invertebrates			
	Free Product Absent			
	N	Median	Min	Max
Bunker	28	3.00	0.90	6.30
Bunker C (unspecified)(B01)	8	3.10	0.90	6.30
Venezuelan Bunker C (B02)	12	2.70	0.90	6.30
Bunker C Light (B04)	6	3.22	2.29	4.50
Navy Special (B06)	2	3.85	3.70	4.00
Crude	224	6.31	0.04	73.00
Kuwait (light) Crude (C01)	17	10.40	0.77	25.00
Cook Inlet Crude (C02)	97	5.16	0.04	20.97
Southern Louisiana Crude (C03)	27	16.20	2.10	19.80
Florida Jay Crude (C04)	4	0.17	0.05	0.25
Prudhoe Bay Crude (C05)	16	2.12	0.69	73.00
Venezuelan Crude (C06)	6	4.06	1.72	8.06
Western Sweet Blend Crude (C07)	6	7.23	1.12	10.60
Transmountain Crude (C08)	6	3.94	1.10	5.56
Norman Wells Crude (C09)	7	5.53	0.60	6.84
Hibernia Crude (C10)	6	5.92	1.08	10.60
Amauligak Crude (C11)	6	6.46	1.66	6.73
Tarsuit Crude (C12)	6	7.08	3.47	7.20
Lago Medio Crude (C13)	6	9.24	3.22	12.10
Atkinson Crude (C14)	6	2.02	0.58	2.27
Bent Horn Crude (C15)	6	2.57	1.07	5.30
Ramashkin Crude (C16)				
West Texas Crude (C17)	2	5.00	5.00	5.00
Diesel	129	3.36	0.21	71.90
Diesel (D01)	10	22.70	4.07	71.90
Fuel Oil No. 2 (D02)	99	3.20	0.21	10.00
Fuel Oil No. 2, Furnace Oil (D03)	16	3.50	1.00	20.00
Light Diesel Fuel (D04)				
Heavy Diesel Fuel (D05)				
Navy Distillate (D06)	2	5.65	4.60	6.70
Marine Diesel (D07)	2	3.50	3.50	3.50
Gasoline	12	20.25	4.90	51.40
Leaded Gasoline (G01)	6	16.35	5.40	27.00
Unleaded Gasoline (G02)	6	28.95	4.90	51.40
Jet Fuel	1	3.50	3.50	3.50
Jet Fuel - JP8 (J01)	1	3.50	3.50	3.50
Jet Fuel - JP9 (J03)				
Jet Fuel - JP4 (J04)				
Lube Oil	14	1.58	0.08	2.40
Auto Lube (L01)	6	0.41	0.08	1.50
Heavy Marine Lube (L02)	6	1.73	0.92	2.40
9250 Lube Oil (L03)	2	2.00	2.00	2.00

• Note: Low reliability data excluded