Abstract number 299546

2014 INTERNATIONAL OIL SPILL CONFERENCE

DEVELOPMENT AND APPLICATION OF DTox: A QUANTITATIVE DATABASE OF THE TOXICITY OF DISPERSANTS AND CHEMICALLY DISPERSED OIL

Adriana C. Bejarano[a], Valerie Chu[b], Jeff Dahlin[a], Jim Farr[b]

[a] Research Planning, Inc., 1121 Park Street, Columbia, SC 29201, USA

[b] National Oceanic and Atmospheric Administration, 7600 Sand Point Way NE Seattle, WA 98115

ABSTRACT

The *Deepwater Horizon* oil spill revived discussions on the use of dispersants as an oil spill countermeasure. One of the greatest concerns regarding the use of dispersants deals with potential exposure of water column organisms to high concentrations of oil. While toxicity data on dispersants and physically and chemically dispersed oil have been generated for decades under controlled laboratory conditions, the practical use of this information has been limited by the lack of a centralized data repository. As a result, the Dispersant and Chemically Dispersed Oil Toxicity Database (DTox) was created to address that shared need of unrestricted and rapid access to toxicity data. DTox is a quantitative database that gathers existing toxicity data through a careful review and compilation of data extracted from the peer-review and gray literature. Through a rigorously evaluation of the quality of each data source, this database contains pertinent information including species scientific name, life stage tested, dispersant name, exposure type, oil weathering stage, exposure duration, etc. More importantly, this database contains effects concentrations reported on measured or nominal basis. Within the database, each data source is assigned an applicability score based on their relevance to oil spills. Key criteria in the determination of source applicability include exposure type, reported effects concentrations, and reported analytical chemistry. Information in DTox has been further integrated into a user-friendly tool that allows for on-the-fly data searches and data plotting in the form of Species Sensitivity Distributions. To date, +400 papers have been evaluated for potential inclusion into the database, and data extracted from +170 sources. Despite inherent limitations, existing toxicity data are of great value to the oil spill scientific community. Although toxicity data will never be enough to answer all toxicity questions regarding the use of dispersants, this centralized data repository can help inform decisions on dispersant use and can help identify data needs and gaps. The ultimate goal of this tool is its contribution to a better understanding of the biological effects of dispersants and oil in the aquatic environment.

The toxicity of dispersants and chemically (i.e., chemically enhanced water accommodated fraction, CEWAF) and physically (i.e., water accommodated fraction, WAF) dispersed oil has been studied for several decades by many researchers (Clark et al., 2001; George-Ares and Clark, 2000; NRC, 1989, 2005; and others) and improvements have been recommended to address the need of performing laboratory tests under conditions representative of oil spill field conditions (e.g., Aurand and Coelho, 2005; Singer et al., 1995). Despite decades of research in the field of oil spill toxicology, lessons from the *Deepwater Horizon* oil spill indicated that additional research is needed to improve our understanding of the biological, toxicological, and ecological effects of dispersants and chemically dispersed oil to a wide range of aquatic species (CRRC et al., 2012). Despite current data limitations, dispersant and oil aquatic toxicity data can provide valuable information to the oil spill response community (e.g., NRC, 2005). However, given the lack of a centralized data repository, assessing the potential effects of dispersants, and physically and chemically dispersed oil is often challenging. Albeit efforts have been made over the years to catalogue published literature and peer-review research on the effects of dispersants and oil into reference databases, similar efforts have not been undertaken to rigorously compile biological and toxicological effects data into a quantitative database with immediate use to decision making.

As evident during the *Deepwater Horizon* oil spill, the ability to assess the toxicity of physically and chemically dispersed oils is a goal shared by government agencies, industry, consultants, and academic groups involved in oil spill response, where a common requirement is unrestricted and rapid access to available toxicity data. However, prior to the *Deepwater Horizon* oil spill there were no centralized data repositories gathering decades of existing acute toxicity data from aqueous exposures. As a result, the Dispersant and Chemically Dispersed Oil Toxicity Database (DTox) was created to address that shared need of the oil spill community. The ultimate goal of this effort was the synthesis of information in a meaningful way to improve scientific decision-making as well as to provide rapid access to centralized toxicity data. Data in DTox may facilitate a better understanding of the biological effects of dispersants and chemically dispersed oil, and may help assist with the development of risk estimates related to oil spills by allowing the selection of data that most closely match the needs of an end-user.

METHODS

Data Collection

The identification of peer-review and gray literature documenting the toxicological effects of dispersants and physically and chemically dispersed oil was conducted via online searches, visits to web sites of government agencies, industry, and research institutions, and direct contact with leading researchers in the field. Hard copies or electronic versions of each data source were obtained and cataloged prior to their review (EndNote®). Each data source was rigorously evaluated for their potential for inclusion in DTox. While the quality of data is known to vary considerably across studies, selection of data included in this searchable database followed a strict set of rules aimed at selecting the best available data. Examples of data criteria are shown in Table 1. Papers initially considered for inclusion met the following minimum criteria: 1) articles published in English, except when information in another language could be accurately translated; 2) full scientific articles, numbered government reports, and independent consultant and industry reports (abstracts were not considered); 3) data should be from original scientific publications and peer review literature (primary source) rather than from reviews or unverifiable sources; a few exceptions were made, but data sources were penalized via their applicability score (see below); 4) species' common and/or scientific name, oil source, and dispersant type should be clearly stated; 5) biological test methods should be described, or reference made to an appropriate published method; 6) effects endpoints of treatment tests should be acceptable relative to control tests; studies that do not discuss or mention the use of controls where included on a case by case basis; and 7) methods for chemical analysis used to quantify exposure concentrations associated with toxicological effects should be described or referenced. Much of the toxicity data previously compiled (NRC, 2005) were also included in DTox.

Table 1. Examples of some of the criteria used to evaluate each data source for their potential inclusion in DTox. Studies not satisfying these requirements were not included.

Criteria	Requirement/Inclusions	Limitations/Exclusions
Dispersant	Documented commercial name	Not documented
Oil	Documented fresh/weathered oil name or source	Not documented
Species	Aquatic invertebrates, vertebrates, marine bacteria	Mammals, reptiles
	Documented taxonomic information verifiable against standard taxonomic sources	Species or common name not documented
Effect/ Response	Biological effect on live, whole organisms	Dead organisms; in-vitro studies
	Adverse acute or chronic effects	Beneficial, nutritional effects
Exposure conditions	Water only exposures	Sediment, other exposures
	Acceptable control survival (at least 70%), or acceptable control endpoints	Poor control survival or unacceptable control endpoints
	Documented experimental conditions (e.g., flow through, static)	Not documented
	Documented exposure durations conditions associated with biological effects (e.g., flow through, static)	Unverifiable duration, not reported
	Documented effects concentrations	Not documented

Data were entered into a carefully designed database template in a systematic and consistent fashion. Examples of such database fields (data elements) (Table 2) included: 1) species attributes: taxonomic group, common name, scientific name, life stage, species distribution; 2) experimental conditions and settings: study type, water type, oil name and weathering condition, dispersant name, dispersant to oil ratio, exposure type, exposure duration; 3) endpoints: acute, subacute, effects concentration, effects concentration units, analyte name, analytical methods; and 4) data source: author name, publication year, article title. Many of the entries were standardized facilitating further sorting and treatment of the existing data. An additional field was created to score the applicability of each data source (e.g., High, Moderate, Low) to spill response. Source applicability was based on the relevance of each data source to oil spill response, and not on the overall scientific merit of individual sources. Data sources with High applicability generally consisted of papers reporting effects concentrations based on measured analytes, dispersant to oil ratios consistent with typical recommended formulations, and exposure conditions following existing recommendations for oil spill research (e.g., spiked, flow-through exposures). Moderate applicability criteria were similar to the criteria for High applicability papers, except that these included papers with exposure conditions following standard toxicity testing laboratory practices (e.g., 96-hour constant static tests). In some instances, Moderate applicability also included dispersant toxicity data reported on a nominal basis. Low applicability generally consisted of papers reporting effects concentrations on a nominal basis (particularly for oil), those not clearly stating if the reported effects concentration were nominal or measured, papers with missing critical information, papers reporting only NOEC, LOEC effects concentrations, and papers with toxicity data from unverifiable sources.

Data entered into the database template went through several Quality Assurance/Quality Control (QA/QC) evaluations to ensure that each record accurately represented the information and data of the original data source. This QA/QC process involved the evaluation of at least 15% of all records entered into the database. Critical steps of the QA/QC process included the review of currently accepted scientific species names, standardization of column content and applicability criteria, and identification of duplicate data (e.g., several papers by the same author(s) reported in several report/manuscripts). The revised dataset was then migrated into a database program (FileMaker® Pro 12) with expanded capabilities, allowing the development of an interactive, searchable and user-friendly tool that allows for visual exploration and examination of data (Bejarano and Dahlin, 2013). This tool was designed to allow the end-user to navigate through a series of screens where data selection can be made, narrowing the type and amount of information used in data plotting. Examples of navigation windows of the first version of DTox are shown in Figure 1.

Table 2. List of database attributes.

Field Type	Field Name	Examples
Species	Common name	Inland silverside
	Scientific name	*Menidia beryllina*
	Taxonomic group	Crustacean, fish, mollusks, coral, other
	Life stage	Adult, juvenile, larvae, egg
	Species distribution	Tropical, subtropical, temperate, frigid, pandemic
	Species habitat[1]	Benthic, water column, epibenthic
	U.S. standard test species	Yes, No
Experimental conditions and settings	Study type	Laboratory, field, mesocosm, wave tank
	Water type	Freshwater, seawater
	Dispersant/Oil Treatment	Dispersant only, Dispersant and Oil, Oil only
	Oil name	Alaska North Slope, South Louisiana
	Oil class (based on API gravity)	Light, Medium, Heavy, Extra Heavy
	Dispersant name	Corexit 9500, Dispersit
	Exposure type	Continuous, spiked, flow through, field
	Exposure duration	24 hour, 96 hour
Endpoints	Endpoint description	Mortality, growth/development, behavior
	Acute endpoint[2]	LC50, EC50, LOEC, NOEC
	Effects concentration (mg/L)	200
	Analyte[3]	Total PAHs, THC
	Reported effects concentration	Measured, nominal
	Analytical methods	Fluorometry, GC-MS
Data Source	Complete citation	Author, Year, Title, Journal/Agency
Other	Paper applicability	Low, Moderate, High, Rejected
	Notes	[It includes other important information not captured within other data fields]

[1] Specific to the life stage of the tested species. For example, adult oysters would be categorized as inhabiting the epibenthos, while their larvae would be more likely found in the water column; [2] LC50: median lethal concentration, EC50: median effects concentration, NOEC: No Observed Effect Concentration, LOEC: Lowest Observed Effect Concentration; [3] PAH: polycyclic aromatic hydrocarbons; THC: total hydrocarbons content (containing between C6 and C44 carbon chains).

Following data selection, the remaining data are plotted in separate windows in the form of Species Sensitivity Distributions (SSDs) (Figure 2). SSDs are probabilistic models that describe the relative sensitivity of species to a particular compound or compound mixture (Posthuma et al., 2002), where species are ranked, based on their relative sensitivity, from the least to the most sensitive. Each data point in a SSD represents a unique species, specifically, the geometric mean of reported toxicity values.

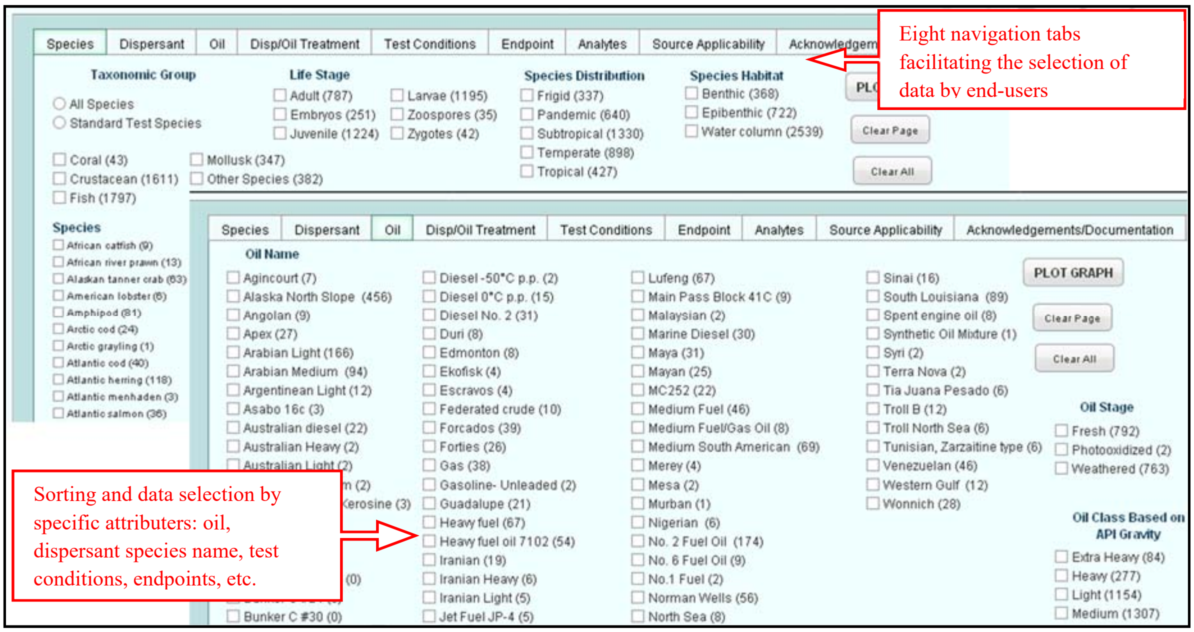

Figure 1. Examples of navigation tabs where the end-user can query the available data. A total of nine navigation tabs are currently available in DTox (Version 1).

In DTox, SSDs are generated for datasets with a minimum of 5 species by fitting the empirical toxicity data to a logistic function defined by $F(x)=\frac{e^{\left(\frac{x-\mu}{\sigma}\right)}}{\sigma\left(1+e^{\left(\frac{x-\mu}{\sigma}\right)}\right)^{2}}$, x∈ R, with parameters μ(location) and σ (scale). SSDs are advantageous in that these allow for comparison of the sensitivity of different species to the same type of exposure (e.g., dispersant only, oil only, chemically dispersed oil), and can be used to derive benchmarks or hazard concentrations (HC) (e.g., Barron et al., 2013; Bejarano et al., 2013; de Hoop et al., 2011). Some of the most commonly used HCs are the HC1 and HC5 equivalent to the concentrations at which 1% and 5%, respectively, of the species in the SSD may not be protected. Alternatively, these benchmarks represent concentrations assumed to be protective of 99% and 95%, respectively, of the species in the SSD. The current version of DTox allows for up to two SSDs per display window (Dispersant Only, Dispersant and Oil, Oil Only) based on the following data fields: exposure duration, dispersant name or oil name. When only one curve is plotted, common names are displayed to facilitate data interpretation. However, in all cases SSD background calculations are based on scientific names.

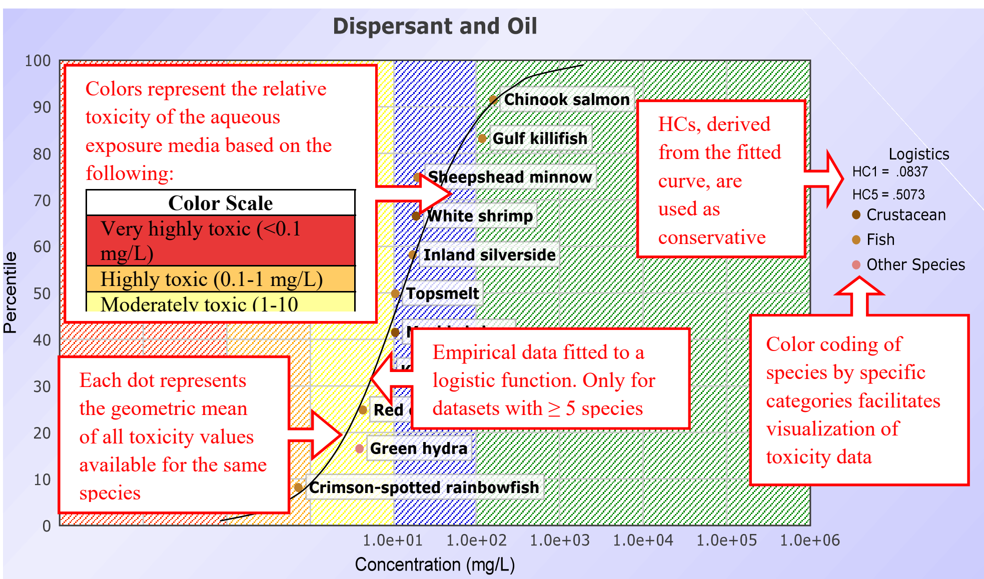

Figure 2. Example of a SSD generated following data queries, where the dots represent the available empirical data. HC1 and HC5 represent the benchmark concentrations assumed to be protective of 99% and 95%, respectively, of the species in the SSD.

As part of the end-user selection interface, plots generated in DTox display SSDs over a colored background representing a common scale used to rank the relative toxicity of contaminants[1]. When enough data are available for curve fitting (5 species minimum required),

[1] Source: http://www.epa.gov/oppefed1/ecorisk_ders/toera_analysis_eco.htm#Ecotox

the plot also shows the estimated HC1 and HC5 values from the logistic curve. For better visualization and interpretation of data, plots also display the queried data based on color-coding selections (e.g., fish vs. crustaceans). Both plots and queried data can also be exported for further applications by the end-user.

RESULTS AND DISCUSSION

Data Collection Summary

Over 400 peer-review and gray literature papers were evaluated for potential inclusion in DTox[2]. Of those, +170 data sources were deemed to contain information of relevance to the objectives of this project. To date, this database contains toxicity data for +100 oils, +120 dispersants, and +190 unique aquatic species, amounting to +3,500 toxicity records. The large majority of records are for species with subtropical distributions (+1,200 records), followed by temperate species (+900 records). Cold-water species (i.e., frigid areas) and tropical species are the most underrepresented group by geography (+800 records combined). Most available toxicological records are for species (and life stages) found in the water column (+2,400 records), while a considerably lower number of records are available for species from the other two general habitats (benthos and epibethos) (+1,500 records combined). When looking at the number of records by taxonomic group, most records are for fish (+1,500 records) followed by crustaceans (+1,400 records), while other groups especially corals, are underrepresented in the database. At least one third of all records were for 13 U.S. standard test species (+1,300 records), with data for mysid shrimp (*Americamysis bahia*) and inland silverside (*Menidia beryllina*)

[2] A copy of the complete list of citations, catalogued in EndNote®, can be obtained through the author; a runtime version of DTox-Version 1 was made available to selected end-users, but additional copies be obtained through the author. Future updated versions may be available through NOAA/ERD.

comprising nearly 50% of all standard test species records. Based on the currently available information, it is clear that there was a surge of toxicity studies on dispersants and oil in the mid 1970s, declining drastically through the 1980s. Since the late 1980s, there have been a variable number of studies, as reflected in the number of records in DTox. Nearly one fifth of all records (+700 records) has been generated since 2010, likely driven by the *Deepwater Horizon* oil spill[3]. The most studied dispersants are the Corexits (+1,500 records), while the most studied oils are Alaska North Slope, Prudhoe Bay, and Kuwait oil (+700 records combined). The large majority of oil records (+2,000) were for light and medium oils, while heavy and extra heavy oils were underrepresented in the database (+200 records combined). A large percentage of records (70%) were derived from 48- and 96-hour exposures (+2,500 records), and via static exposures (+2,400 records).

Database Query Demonstration

A series of queries were performed to demonstrate the usability of this tool. A first query focused on comparing the toxicity of two dispersants currently on the Subpart J of the National Contingency Plan (NCP) Product Schedule[4]: Corexit 9500 and Corexit 9527. A comparison of the acute toxicity of these two dispersants (Figure 3 top) was based on the following query criteria: Endpoint– LC50, EC50; Concentration– Measured, Nominal (for dispersants only); Analyte– Dispersant; Exposure duration– 48- and 96-hours; Applicability– Moderate, High. This comparison shows that the HC5 of Corexit 9500 is slightly greater than that of Corexit 9527, while their HC1s are relatively close. In both cases, HC1 and HC5 values are within the same

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[3] Data generated through to support NRDA from the *Deepwater Horizon* oil spill are not currently included in the database.

[4] EPA Emergency Management, NCP Product Schedule – Subpart J: http://www.epa.gov/emergencies/content/ncp/product_schedule.htm

order of magnitude, suggesting that the toxicity of these two dispersants to aquatic organisms is likely similar.

A second query focused on all oils chemically dispersed with Corexit 9500 (Figure 3 middle) and was based on the following query criteria: Dispersant– Corexit 9500; Concentration– Measured; Endpoint– LC50, EC50; Exposure duration– 96-hours; Analyte– all options containing Total Hydrocarbon Content (THC); Applicability– Moderate, High. This query shows that both HC1 and HC5 values were at or below 0.80 mg THC/L, with hamoor-orange-spotted grouper (*Epinephelus coicoides*) being the most sensitive species (96h-LC50 0.5 mg THC/L). Based on this query, this species may be protected by the HC1 benchmark.

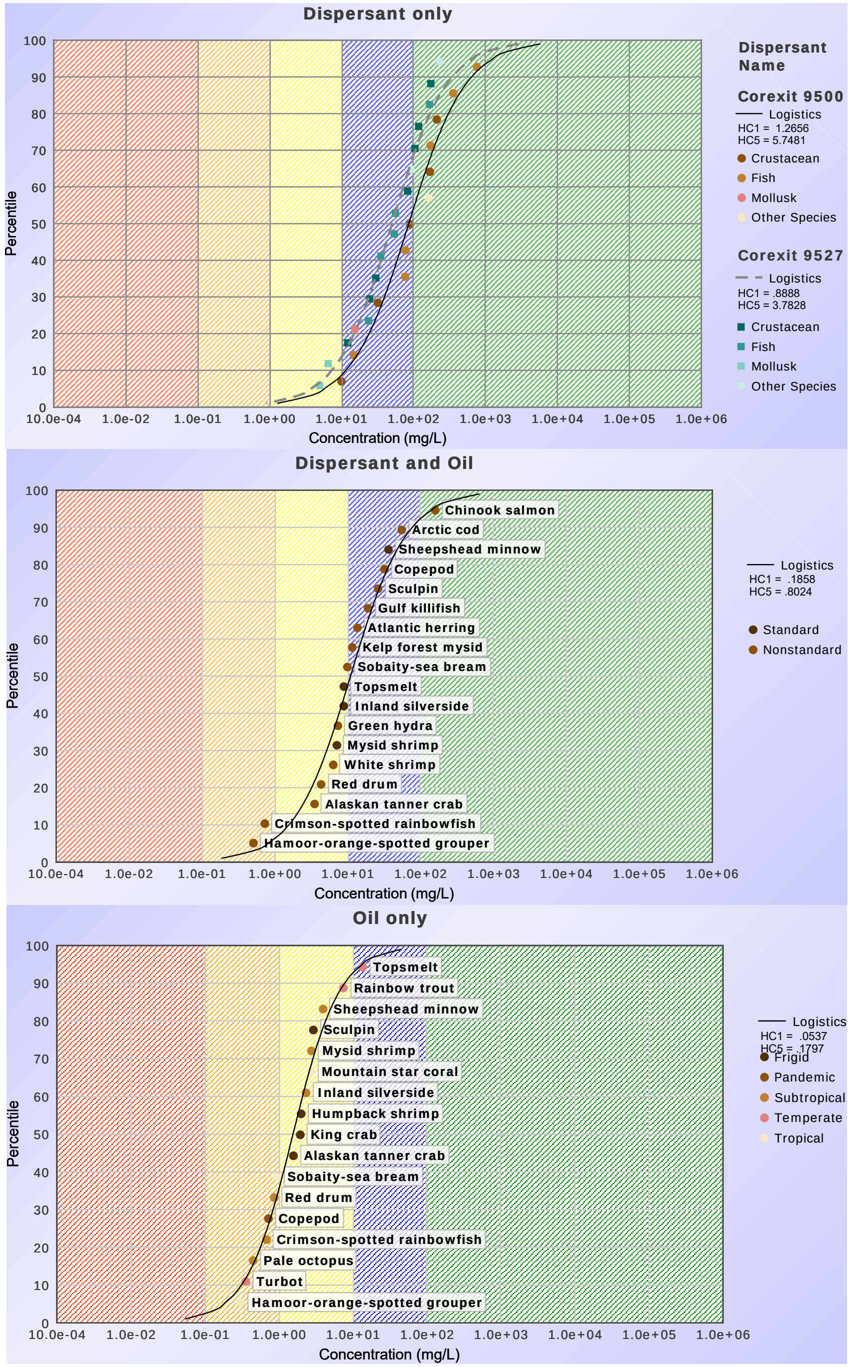

Figure 3. SSDs generated through data queries. Top: Comparison of the acute toxicity of Corexit 9500 and Corexit 9527 (mg Dispersant/L), with data visualization by taxonomic group; Middle: acute toxicity data of oil chemically dispersed with Corexit 9500 (mg THC/L), with data visualization by standard/nonstandard test species; Bottom: acute toxicity data of physically dispersed oil (mg THC/L) with data from early life stages only, and data visualization by geographic distribution.

A third query focused on physically dispersed oil (Figure 3 bottom) and was based on the following query criteria: Species-life stage– Larvae, Embryos; Concentration– Measured; Exposure duration– 48- and 96-hours; Analyte– all options containing Total Hydrocarbon Content; Applicability– Moderate, High. This query shows that both HC1 and HC5 values are at or below 0.18 mg THC/L, with early life stages of hamoor-orange-spotted grouper being the most sensitive species. Based on this query, early life stages of this species may also be protected by both benchmarks, and compared to the second query, HC benchmarks are lower consistent with the general assumption that early life stages are more sensitive than adults of aquatic species.

One limitation important to note is that because the platform used to develop DTox does not allow for inclusion of goodness of fit tests, the end-user should use common sense and critically evaluating the fit of the logistic curve before conclusions can be drawn from the output plots. Furthermore, while HC values may be used as benchmarks concentrations assumed to be protective of a range of species, the practical use HC values requires an evaluation of uncertainties and data availability (e.g., quality, species tested) before the implementation of benchmark values in decision making processes.

Database Application

Data from DTox can be exported and analyzed to satisfy specific end-user needs. For example, a comparison can be made to assess the relative toxicity of chemically (CEWAF) vs. physically dispersed oil (WAF) for a combined number of petroleum hydrocarbon products. Toxicity data (96-hour LC50 and EC50) for all fish and crustacean species (regardless of life stage) from constant static aqueous exposures and papers with Moderate and High applicability reporting toxicity on the basis of measured THC in the exposure media were used to generate Figure 4.

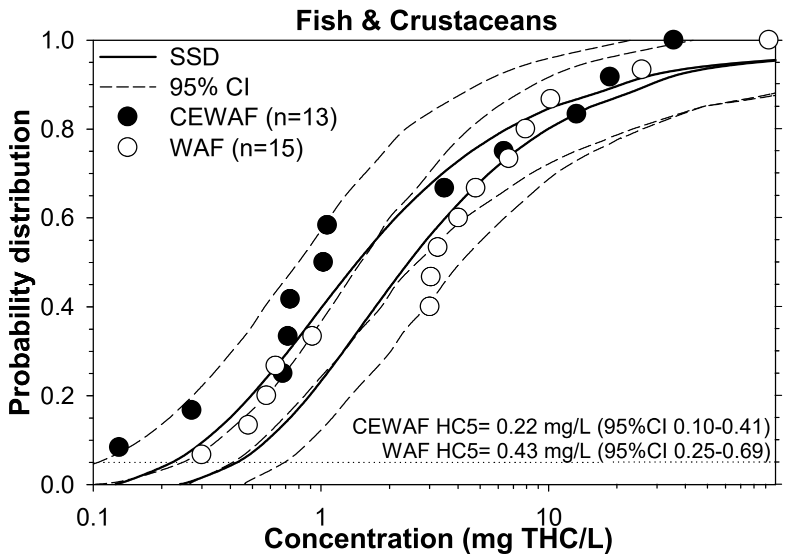

Figure 4. Comparison of the relative toxicity of chemically and physically dispersed oil (CEWAF and WAF, respectively), using constant static 96-hour LC50|EC50 toxicity data for fish and crustacean from studies reporting toxicity on the basis of measured THC.

The mean response of the SSD and its associated 95% confidence interval (obtained following the methodology in Bejarano and Farr, 2013) between WAF and CEWAF SSDs were statistically compared via log-likelihood functions, which use the chi-square statistic (Piegorsch and Bailer, 1997). While the estimated HC5 values from CEWAF were smaller than those from WAF, the analysis above showed that these SSDs were not statistically significantly different from each other ($p>0.05$). These results are consistent with a previous conclusion (NRC, 1989, 2005) indicating that there is no strong scientific evidence that the toxicity of CEWAF (dispersed with current generation dispersants) is substantially greater than that of WAF.

Similarly, queried data can also be used to visually determine where chemically dispersed aqueous toxicity data fall relative to data from physically dispersed oils (Figure 5). The following data were used in this example: 96-hour toxicity data (LC50 and EC50) for fish and crustacean species (regardless of life stage), generated through flow-through aqueous exposures using fresh oil only, and from papers with Moderate and High applicability reporting toxicity on the basis of measured THC in the exposure media. For the purpose of this example, WAF and CEWAF data for the same species were treated as if these were different species. As shown in Figure 5, CEWAF data do not always fall within the lower end of the SSD (e.g., greater toxicity), but rather, these data fall across the entire range of the SSD visually confirming the previous discussion on the relative toxicity of WAF vs. CEWAF. A comparison of SSDs across oils via the log-likelihood function showed that the SSD for Venezuelan crude oil was significantly different from SSDs for Alaska North Slope (p=0.03) and Prudhoe Bay (p=0.006), but the SSDs from these last two oils were not different from each other (p>0.05). As a result, the HC5 for Venezuelan crude oil was much lower (greater toxicity) than that of the two Alaskan oils. One caveat of these comparisons is that the number of species tested varies substantially among oils, with relatively little acute toxicity data available for Venezuelan crude oil. Nevertheless, a greater toxicity of Venezuelan crude oil may be the result of a relatively greater content of volatile hydrocarbons (Aurand and Coelho, 2005).

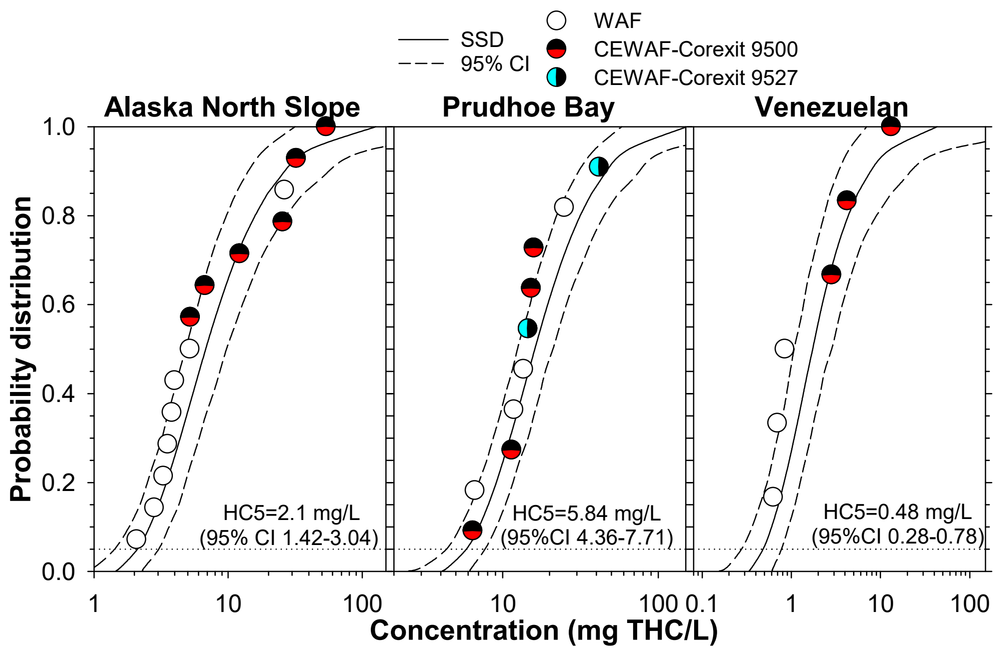

Figure 5. Comparison of the relative aqueous toxicity of various oils, and relative position of CEWAF within oil type. SSDs used 96-hour LC50|EC50 data for fish and crustacean from flow-through exposures, and studies reporting toxicity on the basis of measured THC.

Similarly, queried data can also be used to compare SSDs based on: 1) sub-lethal endpoints (EC50) versus those derived using mortality data (LC50); and 2) data from species with specific global distributions (e.g., cold climates vs. other climates) (Figure 6). Data used in this example included: 96-hour toxicity data (LC50 and/or EC50) for fish and crustacean species (regardless of life stage), generated through flow-through and constant exposures combined using medium oils only (API >22.3- <31.1), and from papers with Moderate and High applicability reporting toxicity on the basis of measured THC in the exposure media. Comparison of medium oil SSDs via the log-likelihood function showed that SSDs based on LC50 and EC50 values were not significantly different from each other (p>0.05), nor were SSDs from cold climates species and species from other climates (p>0.05). The former analyses indicate that when LC50 data are limited for specific end-user queries, EC50 data, which in many instances may be more conservative (e.g., behavioral responses found at lower concentrations than mortality), could provide additional sources of information without adding substantial bias to the development of SSDs. The later findings are also consistent with recent work showing that polar species have similar sensitivities to petroleum crude oil and related compounds (naphthalene, and methyl-naphthalene) as temperate species (de Hoop et al., 2011; Gardiner et al., 2013; McFarlin et al., 2011). Consequently, temperate species may be suitable surrogates for species from colder climates.

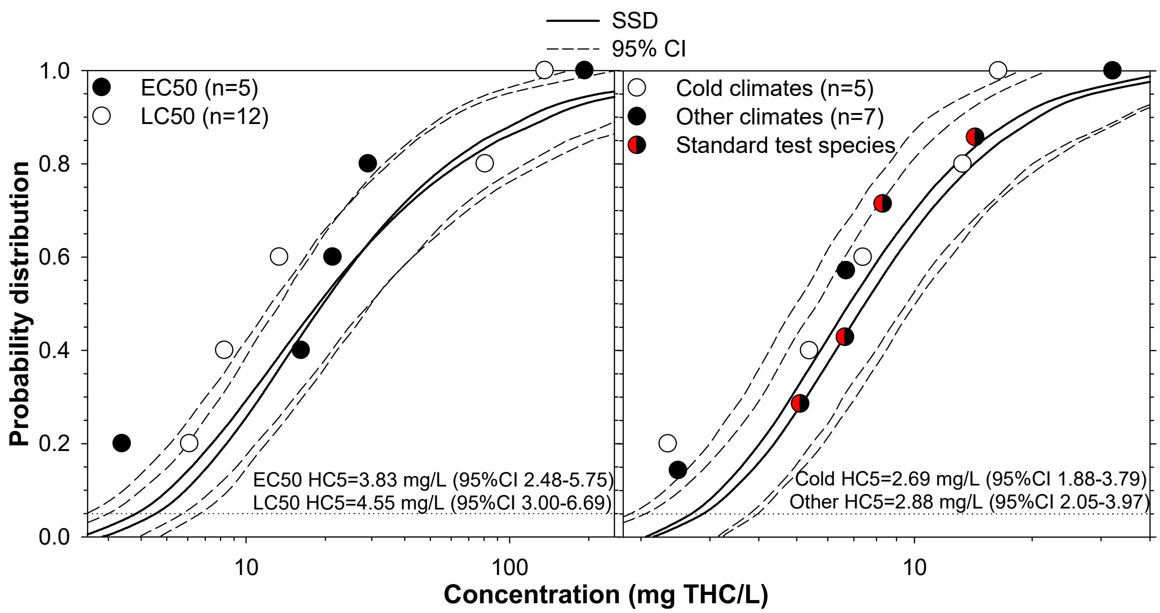

Figure 6. Comparison of SSDs for medium oils using: EC50 or LC50 endpoints (left), and aqueous toxicity data (EC50 and LC50) from species with cold climate distributions or other climates (right). SSDs used 96-hour LC50|EC50 data for fish and crustacean from flow-through and constant static exposures combined, and from studies reporting toxicity on the basis of measured THC.

DTox provides useful information to spill response and assessment efforts, as demonstrated through the examples presented here. Acute toxicity data synthesized from a number of studies, centralized into a single user-friendly repository, and summarized in the form of SSDs can facilitate discussions on the environmental implications of the offshore use of dispersant. For example, SSDs can be used to make comparisons of the relative toxicity of measured environmental concentrations of physically and chemically dispersed oil from monitoring data (as shown in Bejarano et al., 2013). Of great importance is the fact that toxic benchmarks generated from SSDs (as shown here and also in Barron et al., 2013; Bejarano et al., 2013; de Hoop et al., 2011) can also be used to support environmental assessments. Interestingly, benchmarks estimated through various DTox queries are within the range of toxicity threshold values commonly used in ecological risk assessment discussions on the potential effects of chemically dispersed oil to water column organisms (NRC, 2005). A practical application of DTox to spill scenarios and drills could be achieved by integrating query outputs from DTox with modeled environmental concentrations. These data integration could help provide larger scale assessments of the potential fraction of aquatic species at risk of adverse effects from exposures to oil. As with any database, DTox is expected to undergo multiple updates as data from ongoing studies become available in the peer review literature.

CONCLUSIONS

Until now, there were no centralized data repositories gathering decades of existing acute toxicity data on dispersants and chemically dispersed oil. DTox contains quantitative toxicity data collected through a careful review and rigorous evaluation of the quality of each data source, facilitating unrestricted and rapid access to toxicity data. DTox can be used to query data which are synthesized in the form of SSDs. Both SSDs and their estimated HC benchmarks can help inform decisions on the use of dispersants as a response tool for offshore oil spills. Finally, DTox can provide useful information to both, environmental assessments and decision making efforts.

ACKNOWLEDGMENT

We are thankful to our collaborators HRD|Ecosystem Management for their contributions to this project. This research was made possible by a grant from NOAA and the University of New Hampshire's Coastal Response Research Center (Contract No. 13-034) to Research Planning, Inc. None of these results have been reviewed by CRRC and no endorsement should be inferred.

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