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DEVELOPMENT OF THE HEALTH CRITERIA INDEX AS A TOOL TO REDUCE VARIABILITY IN SEDIMENT TOXICITY TESTS USING *LEPTOCHEIRUS PLUMULOSUS*

NGUYEN, K., M-I SWACO
MCMENNAMY, G., M-I SWACO
FRASER, L., M-I SWACO

Abstract

The sediment toxicity test was developed for use as part of U.S. EPA Effluent Limitation Guidelines for offshore drilling discharge of synthetic-based fluids. An industry-wide Synthetic-Based Mud Toxicity Work Group has been working towards the objective of reducing the intra- and inter-lab variability seen in LC_{50} results. Issues addressed have included design of tools and standardization of protocols to achieve more consistency in the test outcomes. A Health Criteria Index (HCI) is being developed by one group of experimenters as a potential aid in this endeavor. The objective in developing such an index is to use the scores generated as a tool to manage cultures so as to produce more consistent test animals and reduce test variability. By these means it would be possible to eliminate tests which have a high probability of yielding unusable data, largely as a result of very high or low LC_{50} scores for the reference fluids.

An intimate understanding of the physiology of the *Leptocheirus plumulosus* was developed through microscopic techniques with specific areas of the organism targeted along with weight measurement. The regions targeted included exoskeleton, appendages and the organism's motion, as well as the digestive, circulatory and respiratory systems. Preliminary results presented in this paper show promising trends between the health index and LC_{50} results for reference fluids.

Introduction

Many factors have been determined by the Synthetic-Based Mud (SBM) Sediment Toxicity Work Group to play crucial roles in the SBM sediment toxicity tests using *Leptocheirus plumulosus*. Some of the variables acknowledged by the group to have impacts on the reference LC_{50} values include: formulation and preparation of the standard reference mud, standard operating procedures of testing methods, consistency and quality of culturing programs in laboratories to achieve animal health, uniformity in animal sizes within a test, standardization of field sampling and handling techniques to ensure homogeneity of field mud samples, and testing laboratory personnel competency.

Previous attempts by the SBM Toxicity Workgroup to standardize laboratory testing procedures and culturing methods and efforts to reduce inter- and intra-variability among laboratories have resulted in lowering the coefficients of variation among the laboratories. The health of the organism via culturing plays an important role in the outcome of the test and therefore is the main focus of this research. Previous internal studies conducted examined whether adsorption and/or absorption via direct ingestion and secondarily through the respiratory tract at microscopic level play key roles in the overall toxicity effects of the amphipod. However, because of the uncertainty surrounding the organism's health at the time of test initiation, there was no measurable means to quantify how fit, or healthy, the amphipod was at the time of test setup. Therefore, a method to determine the health of the amphipod was developed.

This paper examines the overall health of *L. plumulosus* through the design of a Health Criteria Index (HCI) targeting pre-determined specific morphological areas of the amphipod in a given population. A percentage-based system is used to grade the health of the amphipod through specific morphological targets via microscopic techniques. Through the HCI and its grading system, a trend line may be established between the health of the organisms and the LC_{50} results on a 4-day Sediment Toxicity Test using *L. plumulosus*. Evidence captured weekly by microscopic techniques will ensure the feasibility and practicality of HCI and its suitability for amphipod testing. Microscopic techniques included close-up visualization of the organism, its internal organs and structures, especially as these are related to the understanding of the organism's health.

Study Plan

In order to develop the HCI, observations via a Keyence Digital Microscope (VHX-500K) were conducted to study physiological behavior of the organism. The microscope's capabilities include magnification from 100 to 1000X and a fiber optic light source positioned either above or below to provide a clear, enhanced digital image (Figure 1). This enhancement was crucial in gathering data needed for HCI determination. The HCI consists of specified physiological targeted areas which are graded on a percentage-based system.

L. plumulosus are cultured and maintained on a daily basis. The cultures are set up and allowed to mature for eight weeks. Amphipods from the mature cultures were sieved out and restarted with fresh natural sediment. Test-sized organisms, as retained on the 710 to 850-μm screen, were set aside for the 96-hour sediment toxicity test. A weekly sample of ten organisms from the randomization pool was arbitrarily selected for microscopic analysis. Organisms were examined and graded using the HCI on the same day the test was set up.

Figure 2 illustrates the percentage-based grading system of the HCI. The HCI spreadsheet consisted of a circulatory, digestive, and respiratory system review with specific organs being targeted. Regions of the exoskeleton, appendages and motion activities were also examined. The percentage HCl scores were derived from an academic-like grading system which utilized an A(100%), B(75%), C(50%), D(25%) and F(0%) scale. As specific regions are being examined and graded, the scores were subsequently tabulated and recorded on the HCl spreadsheet. The areas targeted in the circulatory system included the heart, ostia (heart valves) and anterior aorta. Observations were made to determine the strength of the animal's heart contractions and corpuscular flow through its arteries. In the digestive system, the stomach, midgut, hindgut and urosome regions were examined to see the extent of the food/sediment they contained. The gill area of the respiratory system is located in the coxae regions right behind the coxal plates. Evidence of a healthy gill included the appearance of straight gill hairs versus an unhealthy appearance of split or curled hairs with the presence of gel-like substances in the gill vicinity.

Before each test a representative sample of ten *L. plumulosus* were removed from the culture to determine the HCl. Once the full examination of each individual amphipod was completed, the total scores were then tabulated along with each organism's weight. The average percentage scores of the circulatory, digestive, respiratory, exoskeleton, appendages, motion and weight where tabulated and used to determine the overall health of the culture. The ten scores were then averaged to determine the HCl of the *L. plumulosus* culture being tested. Each targeted area was graphed against the LC_{50} results to look at a weekly health trend relationship.

Results

Using the HCI spreadsheet to tabulate the percentage scores of each system, a graph of the targeted areas versus LC_{50} results (Figure 3) over a period of nine months was generated. While the overall relationship trend does appear to correlate between these systems and LC_{50} results, more in-depth analysis of each individual system and targeted regions versus LC_{50} data was performed to elucidate the trend relationship. Since the objective of this study was to develop HCI as a predictive tool of organism's health and its possible relationship to LC_{50} results, trends may be established to show the connections. One method to examine trends is to look at moving averages which are formed by computing the average (mean) percentages over a specified number of periods. The moving average is calculated to smooth out short-term fluctuations and identify long-term trends. When the moving average of total scores from the health index is matched against the moving average of LC_{50} data, individual data points are smoothed out indicating a relationship between the heath index trends and those of the LC_{50} trends (Figure 4).

As stated in the previous section, when graphs of each system versus LC_{50} results were generated to determine which system indicator best fit the predictability model of HCI (Figures 5-11), the moving average trends of circulatory, respiratory, digestive systems along with exoskeleton and appendage regions (although not statistically significant) appeared to track the moving average trend of LC_{50} data though the trends did not seem to hold for weight and motion (Figures 6-7) when graphed against LC_{50} data.

Moreover, while the trend tends to track between certain systems and LC_{50} results, a few data points within each graph (Figures 3-11) did not appear to follow the trend. An example can be seen in Figure 4 where a couple of data points of moving average of system totals (average of all systems) versus LC_{50} data from December 2008 and January 2009 appear to contradict one another with peaks heading either in opposite directions or intersecting one other. In this case, HCI's predictability comes into question with regards to the accuracy and dependability of the model and whether the health of the organism can be used to look at LC_{50} trends.

Discussion

There is no doubt that having healthy test organisms has improved the consistency of the LC_{50} test results and eliminated potentially bad data caused by unhealthy organisms at the beginning of the LC_{50} test. The HCI has demonstrated the ability to quantify the health of the test organisms.

Use of the HCI has enabled subjective criteria to be monitored to better manage the culturing of *L. plumulosus*. The weekly tabulated scores from the HCI allow laboratory personnel to identify and separate good from bad culturing batches and provide a way to rectify the problem immediately instead of allowing the cultures to go to maturity.

There are several possible explanations as to why discrepancies and lack of trending exist. Since organisms are submitted for microscopic analysis on the same day of test initiation, other issues in the test may continue to change over the duration of the experimental cycle that cannot be completely controlled nor would necessarily be reflected in the HCI values. These issues may include but not be limited to reference mud samples, formulated sediment, environmental changes in test jars including changes in salinity, pH, dissolved oxygen, temperature or light cycle in the test chamber, the lab technician's test initiation and termination techniques, and organism avoidance behavior observed in test sediment. Changes resulting from these issues and other sources of variability, which drive the LC_{50} values on any one week, may affect the outcome of the sediment toxicity test. Additional HCI screening at the end of the LC_{50} test could potentially strengthen the LC_{50} data by reducing or eliminating the variables which contribute to the discrepancies and lack of consistent trending shown in the results.

The HCI research has improved the understanding of some of the many variables that influence the organisms, overall culture health and toxicity test outcome. The authors intend to continue working on improvements to the HCI and better tracking to LC_{50} test results.

Conclusions

The data presented in this paper established the following:

- Data accumulated from specified physiological systems in HCI may be used as an indicator to predict the health of *L. plumulosus.*
- Data tabulated from HCI may be utilized to manage culturing of *L. plumulosus.*
- Moving average trends show a relationship between the health index and LC_{50} values observed in circulatory, respiratory, digestive, appendages and exoskeleton regions.
- Additional analysis of organism's data at the beginning of test initiation, along with data at test termination, are needed to strengthen and further validate the predictability model of HCI.

Acknowledgments

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Additional Material

Bousfield, E. L. *Shallow-Water Gammaridean Amphipoda of New England.* Ithaca, New York: Cornell University Press, 1973.

Dorn, P.B., Rabke, S., Glickman, A., Louallen, J., Nguyen, K., MacGregor, R., Candler, J., Wong, D.C.L., Hood, C., Hall, J., and Purcell, T. "Development, Verification and Improvement of a Sediment Toxicity Test for Regulatory Compliance." SPE 94269, SPE/EPA/DOE Exploration and Production Environmental Conference, Galveston, Texas, March 7-9, 2005.

Grosse, D.J., Pauley, G.B. and Moran, D. "Species Profiles: Life Histories and Environmental Requirements of Coastal Fishes and Invertebrates (Pacific Northwest): Amphipods." US Fish and Wildlife Service Biological Report 82 (11.69); US Army Corp of Engineers Report TR EL-82-4 (August 1986). Available for purchase from www.ntis.gov as report ADA181591.

Malachosky, E., Hood, C., Kuc, W., and McMahon, J. "The Impact of Contamination on the Toxicity of Synthetic Base Drilling Fluids." AADE-07-NTCE-62, AADE National Technical Conference and Exhibition, Houston, April 10-12, 2007.

"Moving Average." http://en.wikipedia.org/wiki/Moving_average last accessed March 10, 2009.

Nguyen, K.D., Candler, J.E. and Rabke, S.P. "Impact of Organism Size on the Synthetic-Based Drilling Fluid Sediment Toxicity Bioassay using Leptocheirus plumulosus," Poster, SETAC 25th Annual Conference, Portland, Oregon, Nov 14-18, 2004.

Ruppert E.E., Fox, R.S. and Barnes, R.D. *Invertebrate Zoology: A Functional Evolutionary Approach*, 7th ed.; Brooks/Cole, 2004, 650-658.

Synthetic Based Mud Joint Industry Research Project and American Petroleum Institute. "Procedure for Conducting 96-Hour Sediment Toxicity Tests of Synthetic Based Drilling Muds with Leptocheirus plumulosus." 2006.

US EPA. "Method for Conducting a Sediment Toxicity Test with Leptocheirus plumulosus and Non-aqueous Fluids or Synthetic Based Drilling Muds" in the Final NPDES General Permit for New and Existing Sources and New Dischargers in the Offshore Subcategory of the Oil and Gas Extraction Category for the Western Portion of the Outer Continental Shelf of the Gulf of Mexico (GMG290000)." *Code of Federal Regulations*, Title 40, Part 435; Subpart A; Appendix A, (October 7, 2004).

US EPA. "Notice of Final NPDES General Permit for New and Existing Sources and New Dischargers in the Offshore Subcategory of the Oil and Gas Extraction Category for the Western Portion of the Outer Continental Shelf of the Gulf of Mexico (GMG290000)." Federal Register v.69, No. 194, (October 7, 2004) 60150-60151.

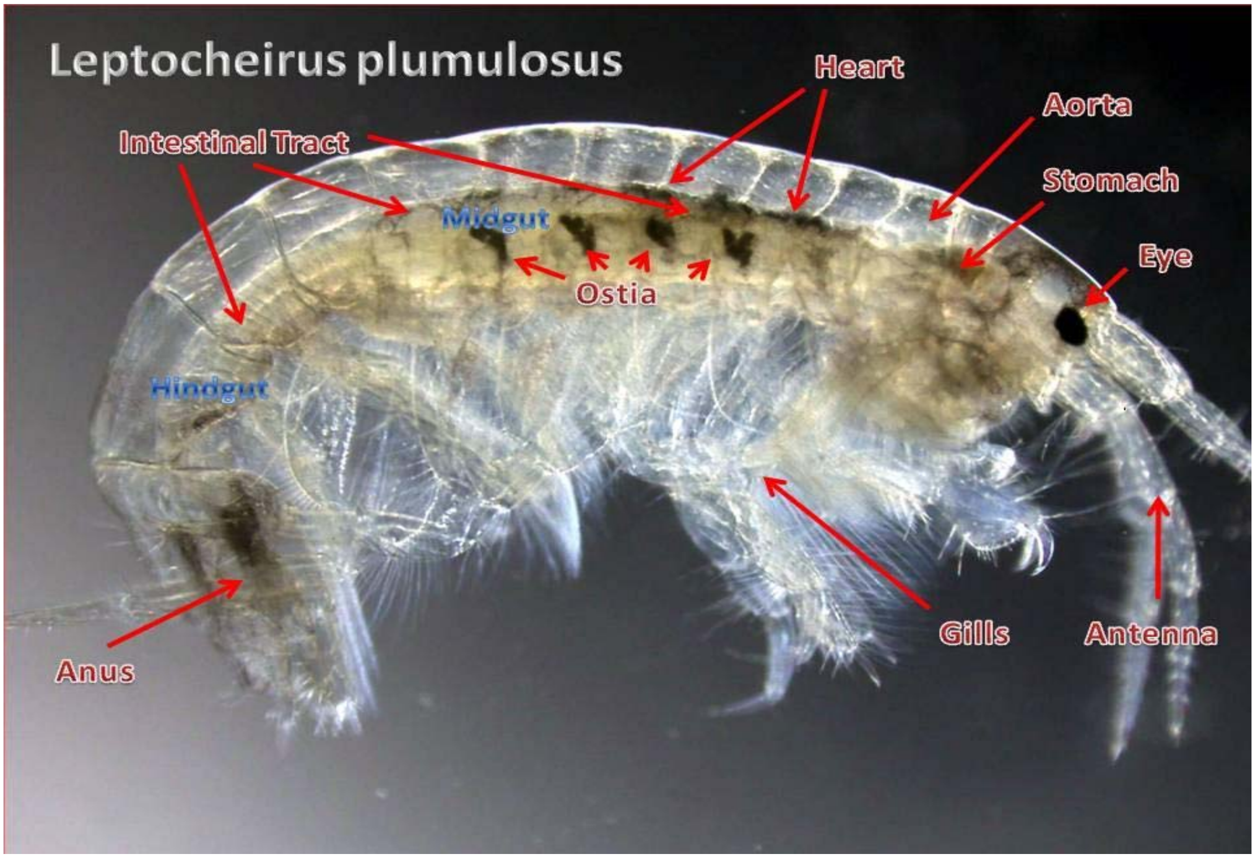
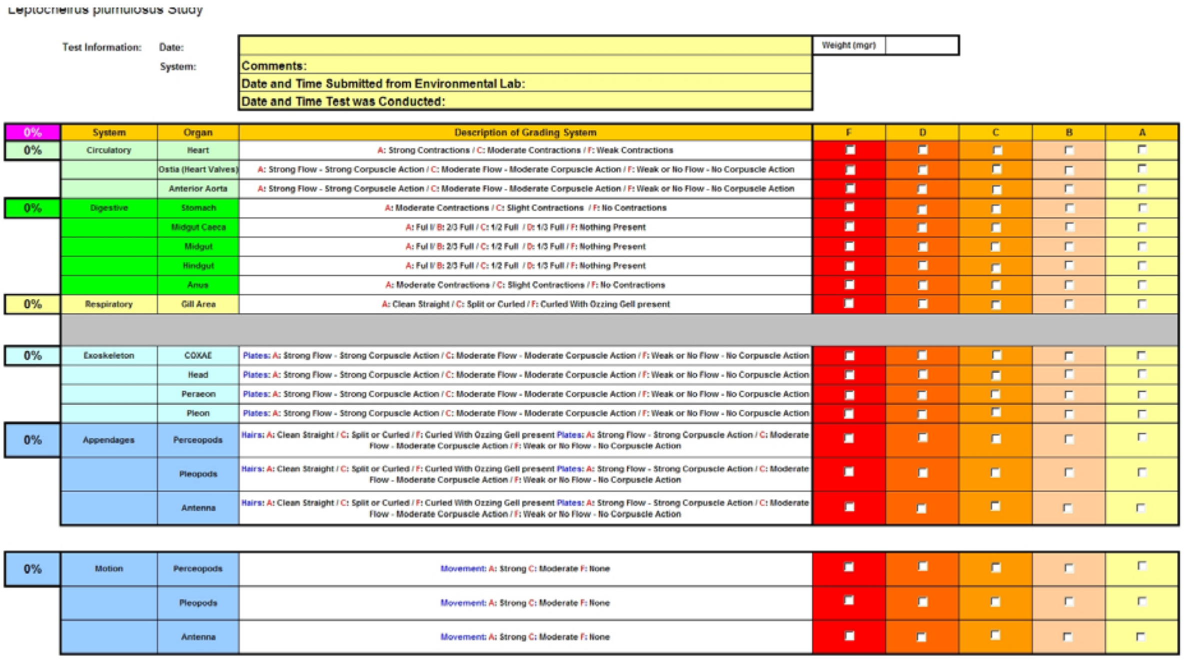


Figure 1. Amphipoda – Microscopic View of External and Internal Structures



Leptocheirus plumulosus Study

Test Information: Date:
System:
Comments:
Date and Time Submitted from Environmental Lab:
Date and Time Test was Conducted:
Weight (mgr)

0%	System	Organ	Description of Grading System	F	D	C	B	A
0%	Circulatory	Heart	A: Strong Contractions / C: Moderate Contractions / F: Weak Contractions	☐	☐	☐	☐	☐
		Ostia (Heart Valves)	A: Strong Flow - Strong Corpuscle Action / C: Moderate Flow - Moderate Corpuscle Action / F: Weak or No Flow - No Corpuscle Action	☐	☐	☐	☐	☐
		Anterior Aorta	A: Strong Flow - Strong Corpuscle Action / C: Moderate Flow - Moderate Corpuscle Action / F: Weak or No Flow - No Corpuscle Action	☐	☐	☐	☐	☐
0%	Digestive	Stomach	A: Moderate Contractions / C: Slight Contractions / F: No Contractions	☐	☐	☐	☐	☐
		Midgut Caeca	A: Ful l/ B: 2/3 Full / C: 1/2 Full / D: 1/3 Full / F: Nothing Present	☐	☐	☐	☐	☐
		Midgut	A: Ful l/ B: 2/3 Full / C: 1/2 Full / D: 1/3 Full / F: Nothing Present	☐	☐	☐	☐	☐
		Hindgut	A: Ful l/ B: 2/3 Full / C: 1/2 Full / D: 1/3 Full / F: Nothing Present	☐	☐	☐	☐	☐
		Anus	A: Moderate Contractions / C: Slight Contractions / F: No Contractions	☐	☐	☐	☐	☐
0%	Respiratory	Gill Area	A: Clean Straight / C: Split or Curled / F: Curled With Ozzing Gell present	☐	☐	☐	☐	☐
0%	Exoskeleton	COXAE	Plates: A: Strong Flow - Strong Corpuscle Action / C: Moderate Flow - Moderate Corpuscle Action / F: Weak or No Flow - No Corpuscle Action	☐	☐	☐	☐	☐
		Head	Plates: A: Strong Flow - Strong Corpuscle Action / C: Moderate Flow - Moderate Corpuscle Action / F: Weak or No Flow - No Corpuscle Action	☐	☐	☐	☐	☐
		Peraeon	Plates: A: Strong Flow - Strong Corpuscle Action / C: Moderate Flow - Moderate Corpuscle Action / F: Weak or No Flow - No Corpuscle Action	☐	☐	☐	☐	☐
		Pleon	Plates: A: Strong Flow - Strong Corpuscle Action / C: Moderate Flow - Moderate Corpuscle Action / F: Weak or No Flow - No Corpuscle Action	☐	☐	☐	☐	☐
0%	Appendages	Perceopods	Hairs: A: Clean Straight / C: Split or Curled / F: Curled With Ozzing Gell present Plates: A: Strong Flow - Strong Corpuscle Action / C: Moderate Flow - Moderate Corpuscle Action / F: Weak or No Flow - No Corpuscle Action	☐	☐	☐	☐	☐
		Pleopods	Hairs: A: Clean Straight / C: Split or Curled / F: Curled With Ozzing Gell present Plates: A: Strong Flow - Strong Corpuscle Action / C: Moderate Flow - Moderate Corpuscle Action / F: Weak or No Flow - No Corpuscle Action	☐	☐	☐	☐	☐
		Antenna	Hairs: A: Clean Straight / C: Split or Curled / F: Curled With Ozzing Gell present Plates: A: Strong Flow - Strong Corpuscle Action / C: Moderate Flow - Moderate Corpuscle Action / F: Weak or No Flow - No Corpuscle Action	☐	☐	☐	☐	☐

0%	Motion	Perceopods	Movement: A: Strong C: Moderate F: None	☐	☐	☐	☐	☐
		Pleopods	Movement: A: Strong C: Moderate F: None	☐	☐	☐	☐	☐
		Antenna	Movement: A: Strong C: Moderate F: None	☐	☐	☐	☐	☐

Figure 2. Health Criteria Index (Spreadsheet)

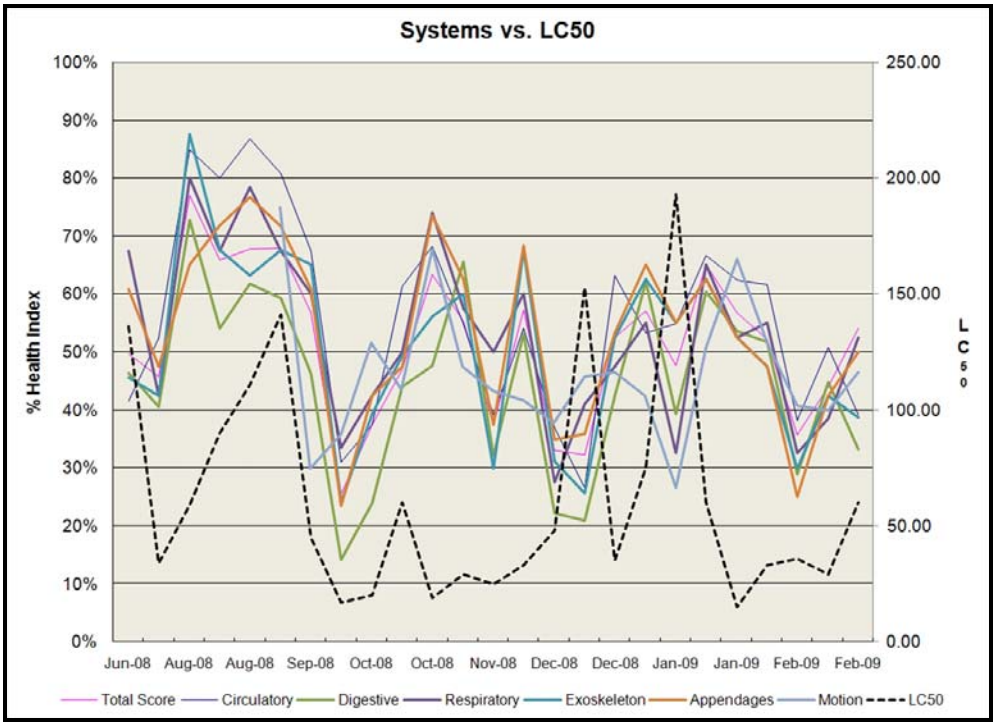
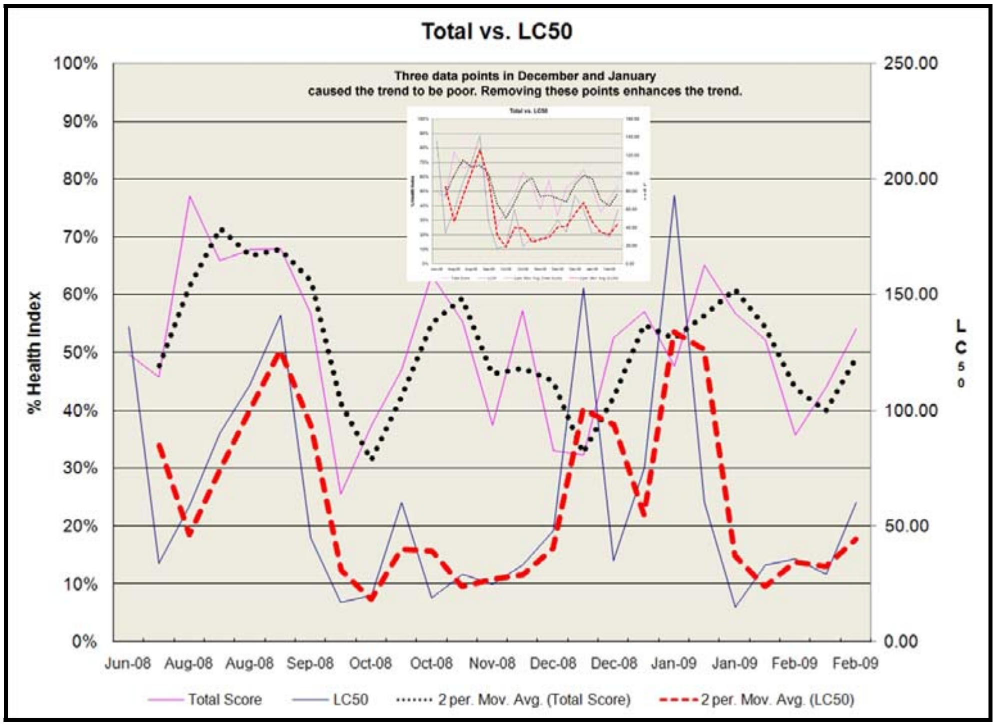


Figure 3. Health Index Percent for Systems vs. LC_{50} Data (July 2008 – February 2009)



Three data points in December and January caused the trend to be poor. Removing these points enhances the trend.

Figure 4. Moving Average – System Totals (Average of All Systems) vs. LC_{50} Data

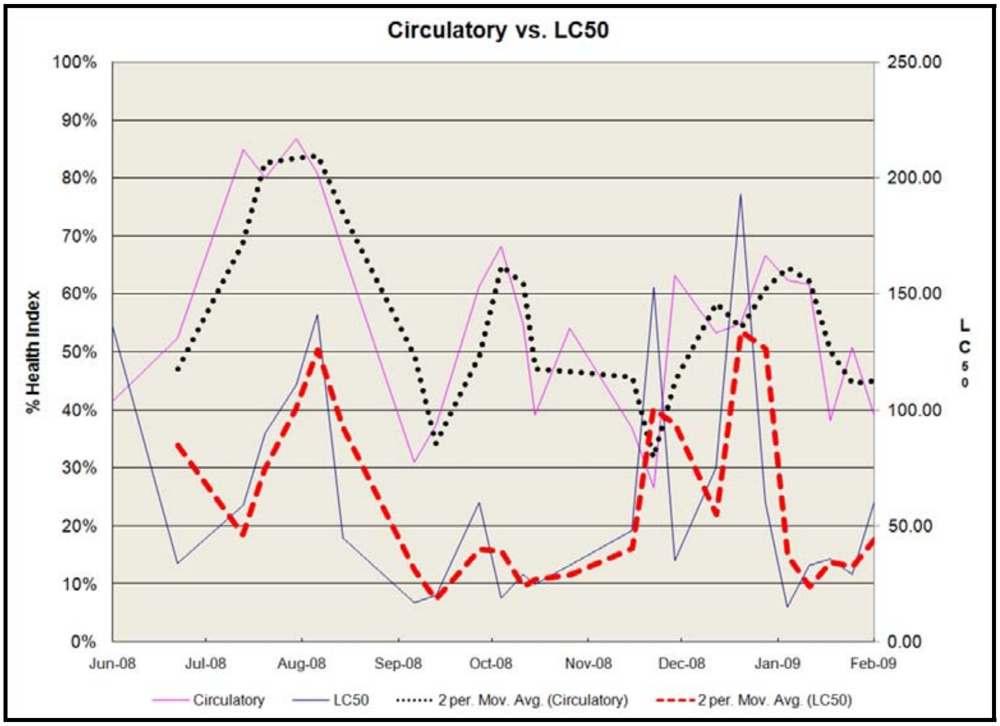


Figure 5. Moving Average – Circulatory System vs. LC_{50} Data

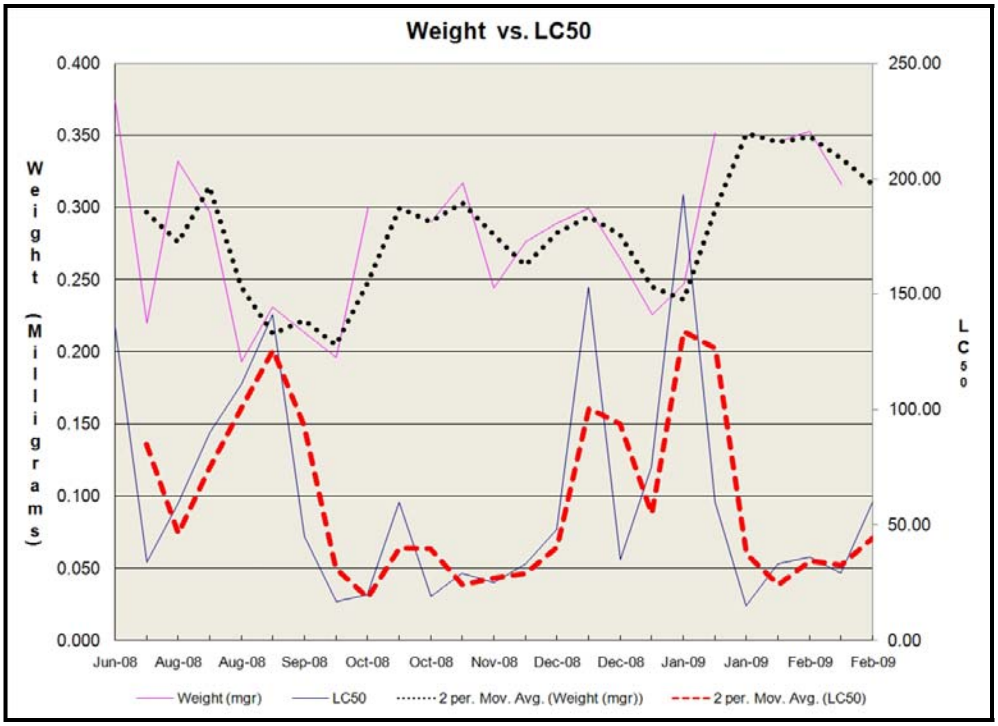


Figure 6. Moving Average – Weight vs. LC$_{50}$ Data

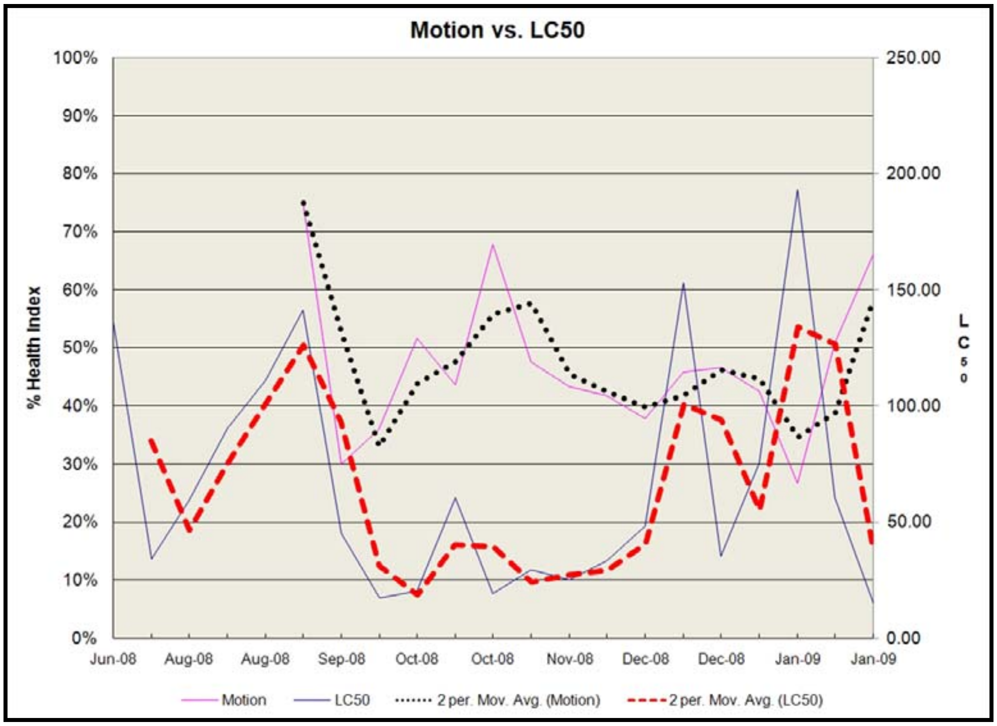


Figure 7. Moving Average – Motion vs. LC$_5$ Data

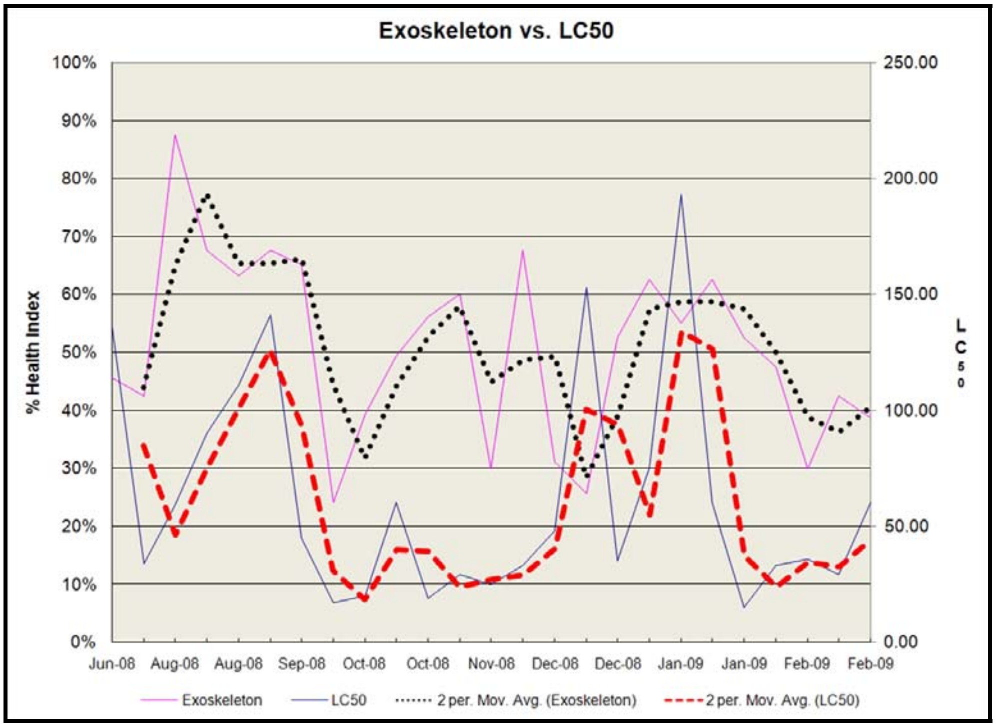


Figure 8. Moving Average – Exoskeleton vs. LC_{50} Data

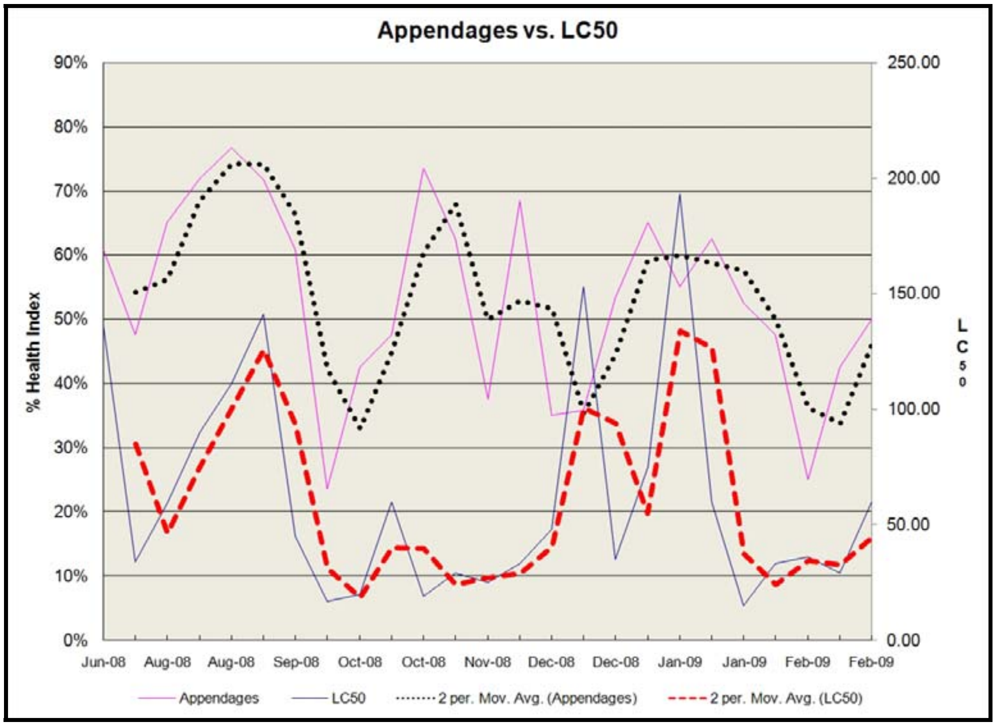


Figure 9. Moving Average – Appendages vs. LC_{50} Data

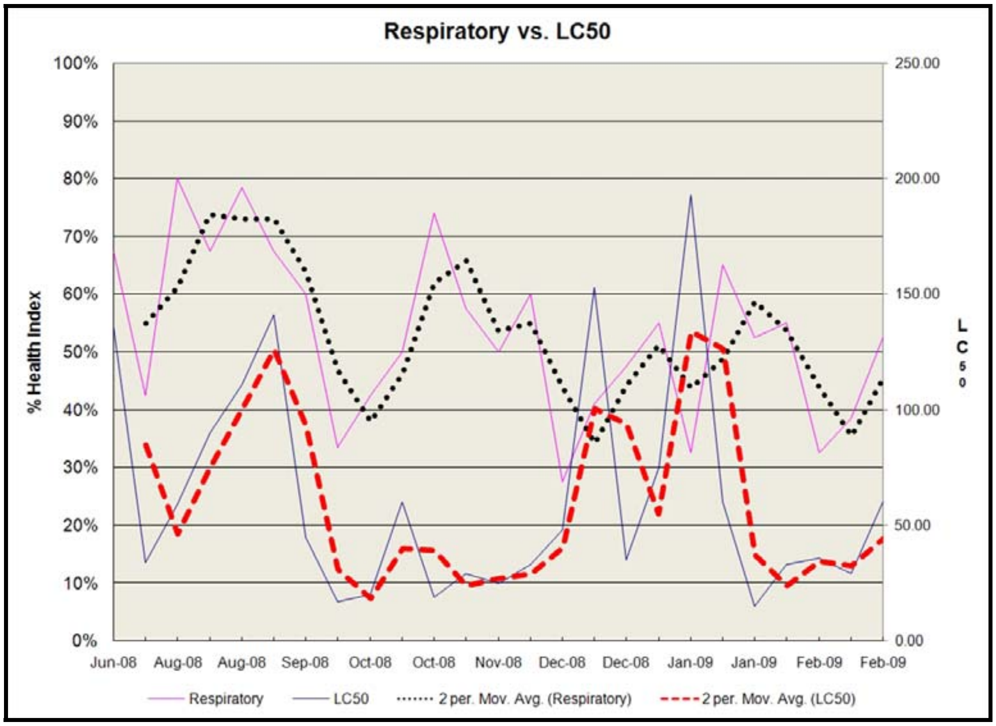


Figure 10. Moving Average – Respiratory vs. LC_{50} Data

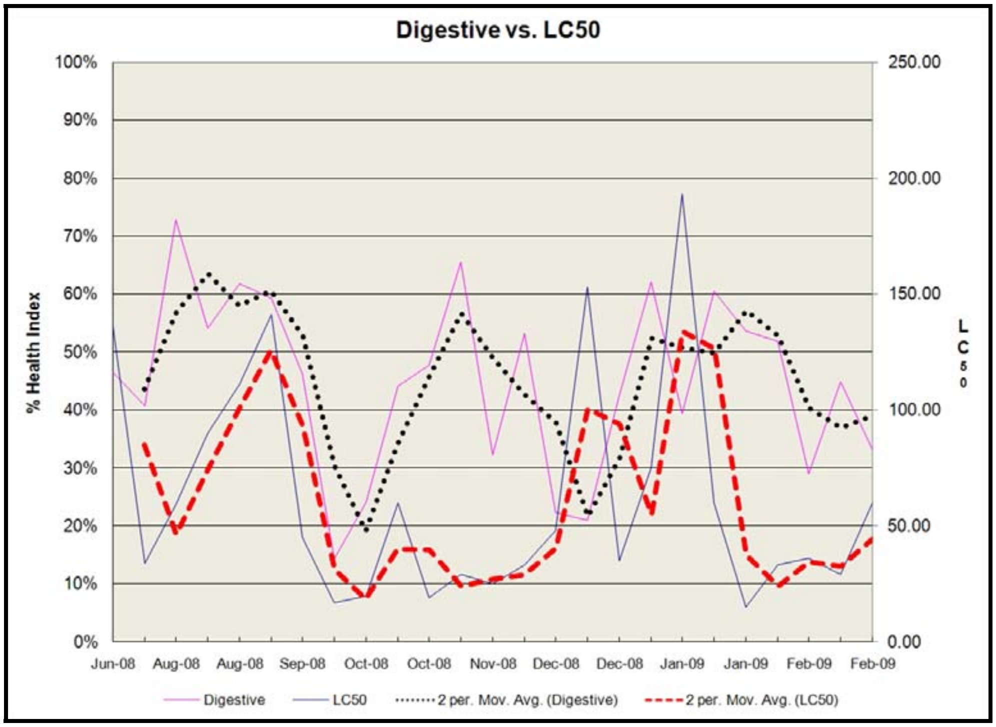


Figure 11. Moving Average – Digestive vs. LC_{50} Data