# CHAPTER 4.0
# EVALUATION OF FECAL INDICATOR AND PATHOGEN LOADS

The goal in this chapter is to quantify loads of fecal indicators such as total coliforms from various sources in the Central Valley where such estimates can be made. In general, such quantification is only possible for the coliform indicator organisms because the data are mostly available for such indicators and the pathogen data are very sparse or show a high frequency of non-detects. Furthermore the orders-of-magnitude changes that occur in indicator concentrations in time and space, and the limited frequency of data collection, suggests that only approximate quantification is possible. This chapter presents an overview of microorganism die off in the ambient environment, a key process controlling observed concentrations, and identifies the relative importance of different sources in the Central Valley where adequate flow and concentration data exist.

## 4.1 DIE OFF OF FECAL INDICATORS AND PATHOGENS

Because pathogens and indicators are living organisms, and typically have human or animal hosts, they exhibit die off in the ambient environment at varying rates depending on the water temperature, exposure to sunlight, and the nature of the organism. Organisms may survive in sediments, and under warmer temperatures, may actually be able to colonize and grow in sediments. It is not known whether conditions appropriate for coliform regrowth exist in some locations in the Central Valley. However, as an illustration, the range of die off rates reported in the literature is shown in Table 4-1. The effect of die off on select fecal indicator and pathogen concentrations is shown for illustration in Figure 4-1. When transport time frames of more than a few days are involved, die off makes linkage of sources and concentrations very difficult. Alternatively, microorganism concentrations, more specifically fecal indicator concentrations, are more closely related to what happens in the proximity of a sampling station, rather than what happens in a large watershed where significant travel time and concomitant pathogen die off can occur.

**Table 4-1 Ranges of die-off rate constants (Source: USEPA, 2001)**

| Organism | First Order Die-Off Rate Constant (1/day) | Medium |
|---|---|---|
| Total Coliform | 1-5.5 | Freshwater, 20 °C |
| | 0.7-3.0 | Seawater, 20 °C |
| | 0.42 | River water, temperature not specified |
| Fecal Coliform | 37-110 | Seawater, in sunlight |
| | 0.51 | River water, temperature not specified |
| | 0.043-0.146 | Sand, 4 to 35 °C |
| | 0.043-0.156 | Loam, 4-35 °C |
| | 0.025-0.083 | Clay, 4-35 °C |
| *E. Coli* | 0.53 | River water, 37 °C |
| | 0.102 | Surface water, 5 °C |
| *Cryptosporidium* | 0.01 | Surface water, 5 °C |
| | 0.025 | Surface water, 15 °C |

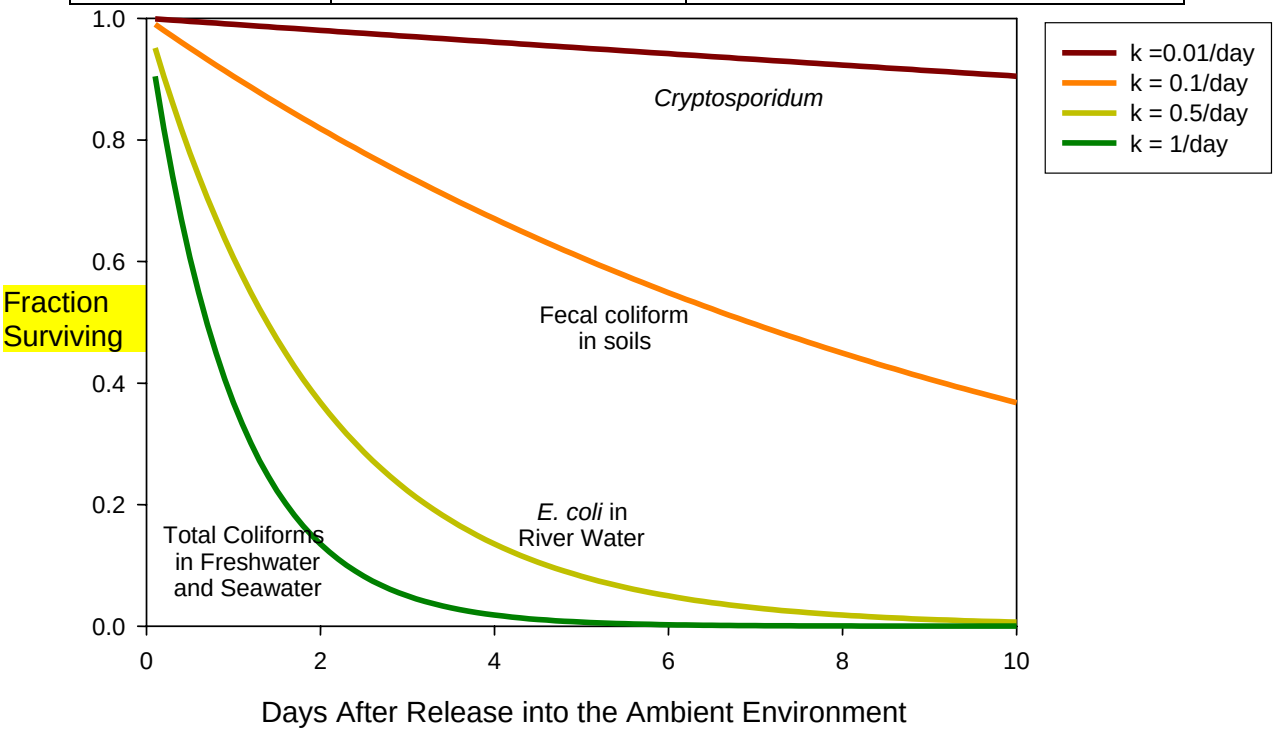


**Figure 4-1. Die off of selected bacteria and pathogens in the ambient environment using ranges of rate constants shown in Table 4-1.**

## 4.2 SACRAMENTO RIVER LOADS

Loads were computed at locations sampled by the Sacramento Water Treatment Plant at a point on Sacramento River downstream of the confluence with the American River. Flow data on the Sacramento River at Freeport was used for these calculations. Total coliform counts ranged from 80 to 16,000 MPN/100 ml, and data reporting was capped at the higher number. Daily coliform loads were computed using flow and

concentration data for the same day. Note that flow and coliform concentrations were not correlated (Figure 4-2), although a closer review of the data show that the highest concentrations correspond to the wet months of the year. The concentrations, flows, and estimated daily loads are shown in Figure 4-3. The calculated loads range from less than $10^{12}$ organisms per day to approximately $10^{15}$ organisms per day. It is recognized that the higher end values may be underestimated because of the concentration data reporting. This number is a useful basis for comparison against loads that originate from various point and nonpoint sources in the Central Valley and Delta as discussed below. The number is also useful to compare against the excretion rates of coliforms by individual organisms as shown in Table 4-2.

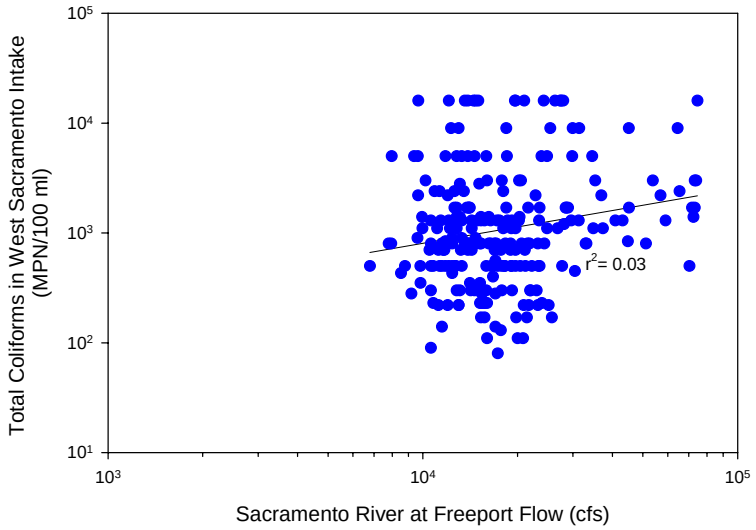


**Figure 4-2. Flow and total coliform concentrations in the Sacramento River downstream of American River.**

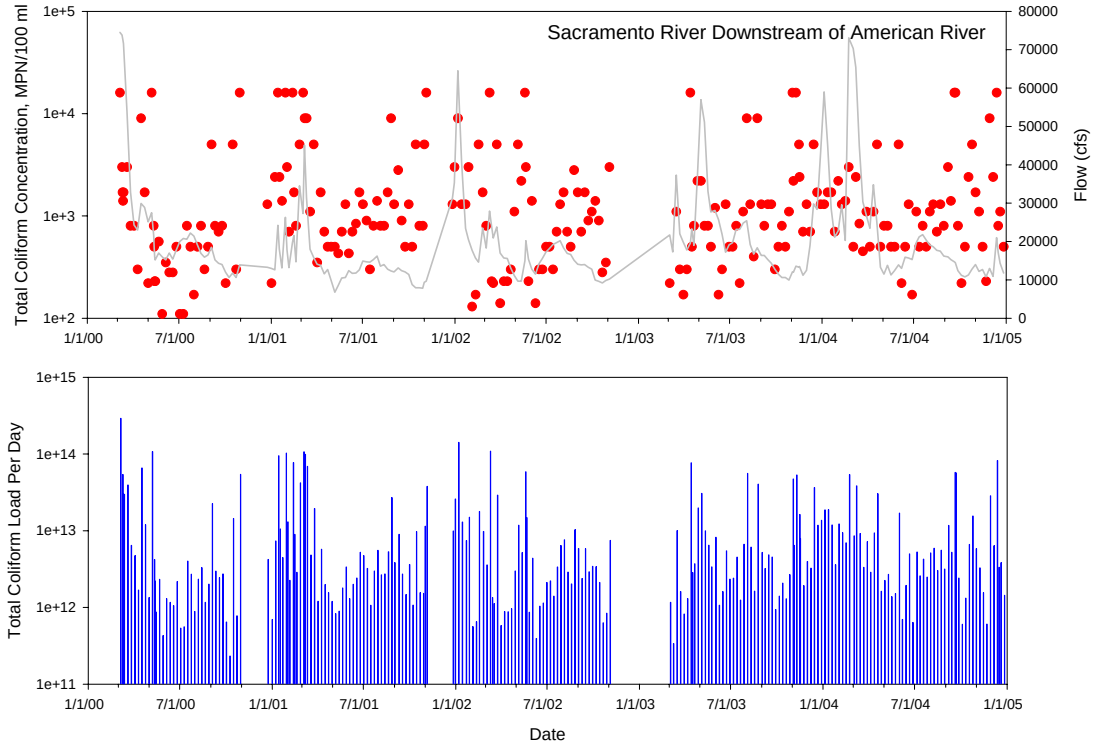


**Figure 4-3. Flow (line), total coliform concentration (circles) and total coliform load (lower plot) in the Sacramento River downstream of American River.**

**Table 4-2. Fecal coliform excretion by different animals (USEPA, 2000).**

| Animal | Fecal Coliform (count/animal/day) |
|---|---|
| Dairy cow | 1.01E+11 |
| Beef cow | 1.04E+11 |
| Hog | 1.08E+10 |
| Sheep | 1.20E+10 |
| Horse | 4.20E+08 |
| Chicken | 1.36E+08 |
| Turkey | 9.30E+07 |
| Duck | 2.43E+09 |
| Goose | 4.90E+10 |
| Deer | 5.00E+08 |
| Beaver | 2.50E+08 |
| Raccoon | 1.25E+08 |
| Dog | 4.09E+09 |

## 4.3 EVALUATION OF URBAN LOADS

Robust data to evaluate coliform loads exist for the Natomas East Main Drainage Canal (NEMDC) (MWQI, 2005; Zanoli, personal communication) with frequent measurement of flow and pathogen counts. Although the watershed of the NEMDC is not fully urban land, the watershed is rapidly urbanizing and remains the best available dataset in the region for estimating the impact of urban land on pathogen runoff. The estimated loads of total coliforms at the NEMDC are shown in Figure 4-4. These data show the extremely variable nature of the coliform source, with concentrations sometimes exceeding values of 500,000 MPN/100 ml. The variability is greater than seen for the Sacramento River in Figure 4-3. Using the flow at this sampling location, it was found that actual coliform loads can range from a little over $10^9$ coliforms/day to higher than $10^{15}$ coliforms/day. At the high end, these numbers are comparable to some of the highest loading rates estimated for the Sacramento River. Although the limited data at the high end in the Sacramento River represent an uncertainty in this calculation, for some days of the year, it is possible for nearly the entire coliform load of the Sacramento River to be of the same magnitude as the load from NEMDC. This calculation highlights the importance of urban runoff as a source of coliforms to the Sacramento River. A further observation from the NEMDC data is that the fecal coliform concentrations are almost equal to the total coliform load, i.e., in this urbanized region, most of the coliform load is fecal in origin, as opposed to originating from other environmental sources (Figure 4-5).

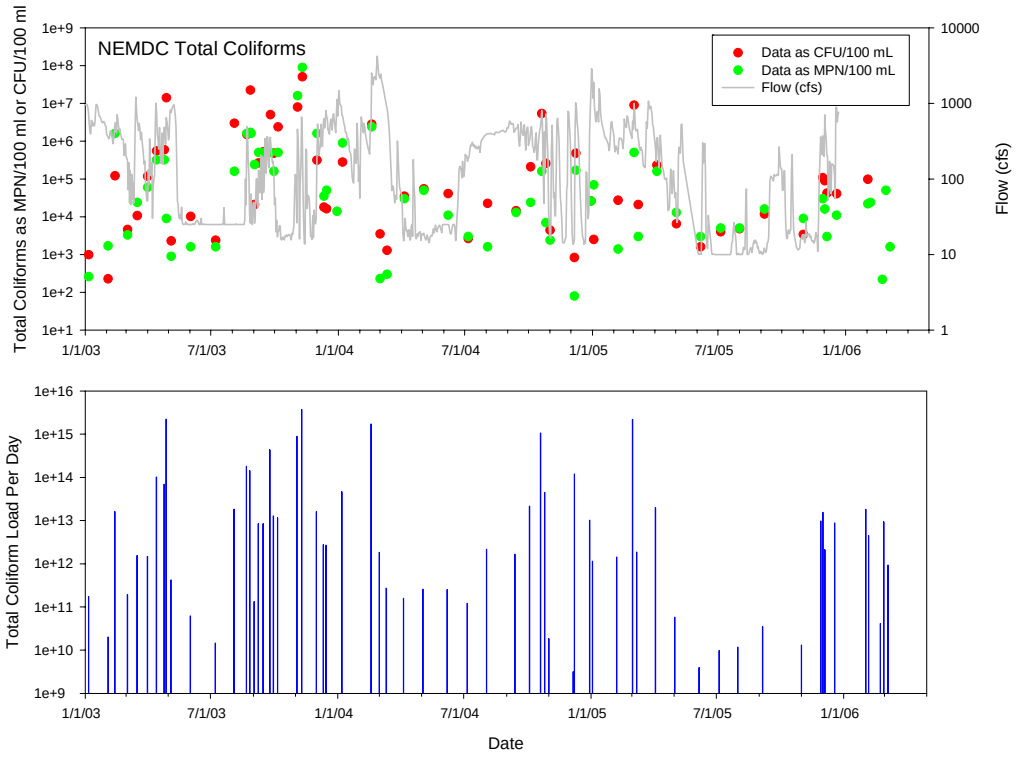


**Figure 4-4. Flow, total coliform concentration and load in the Natomas East Main Drainage Canal (NEMDC).**

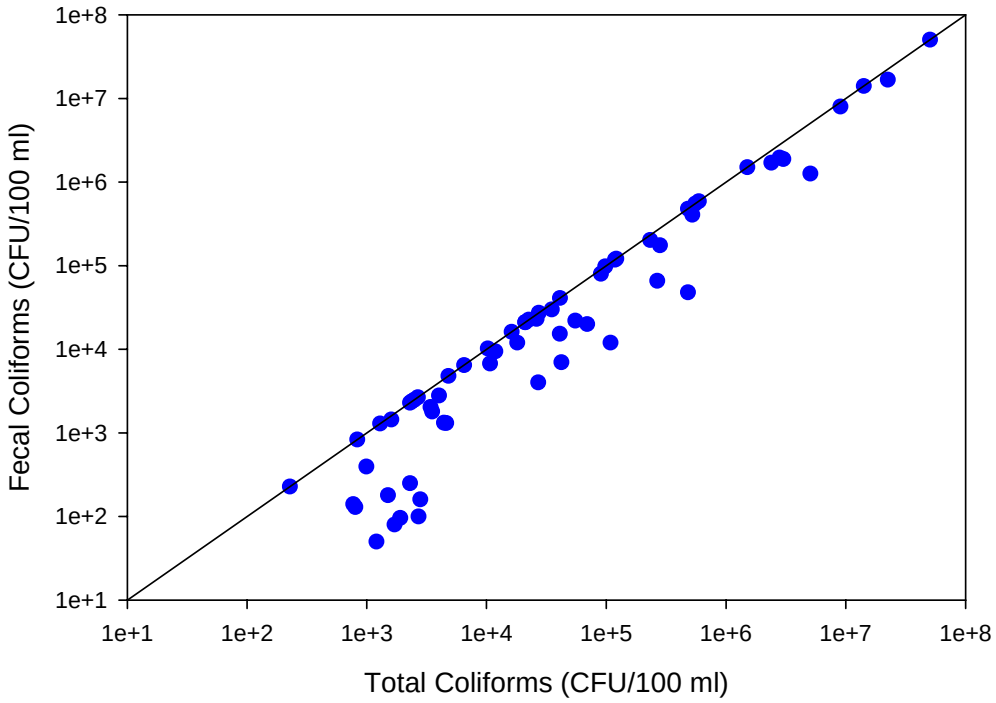


**Figure 4-5. Paired samples of total coliform and fecal coliform concentration from the Natomas East Main Drainage Canal (NEMDC).**

## 4.4 EVALUATION OF WASTEWATER LOADS

Substantial data on a wastewater source (including daily flow data) was available for the Sacramento Regional Wastewater Treatment Plant. Using total coliform concentrations and reported effluent discharge volumes from the plant, the estimated coliform loads range from $10^8$ to nearly $10^{11}$ organisms per day (Figure 4-6). Even at the highest level, these loads are orders of magnitude lower than the high values for urban runoff from the Natomas East Main Drainage Canal. This is true even though this wastewater plant serves a population of more than 1 million people and is the largest in the Central Valley.

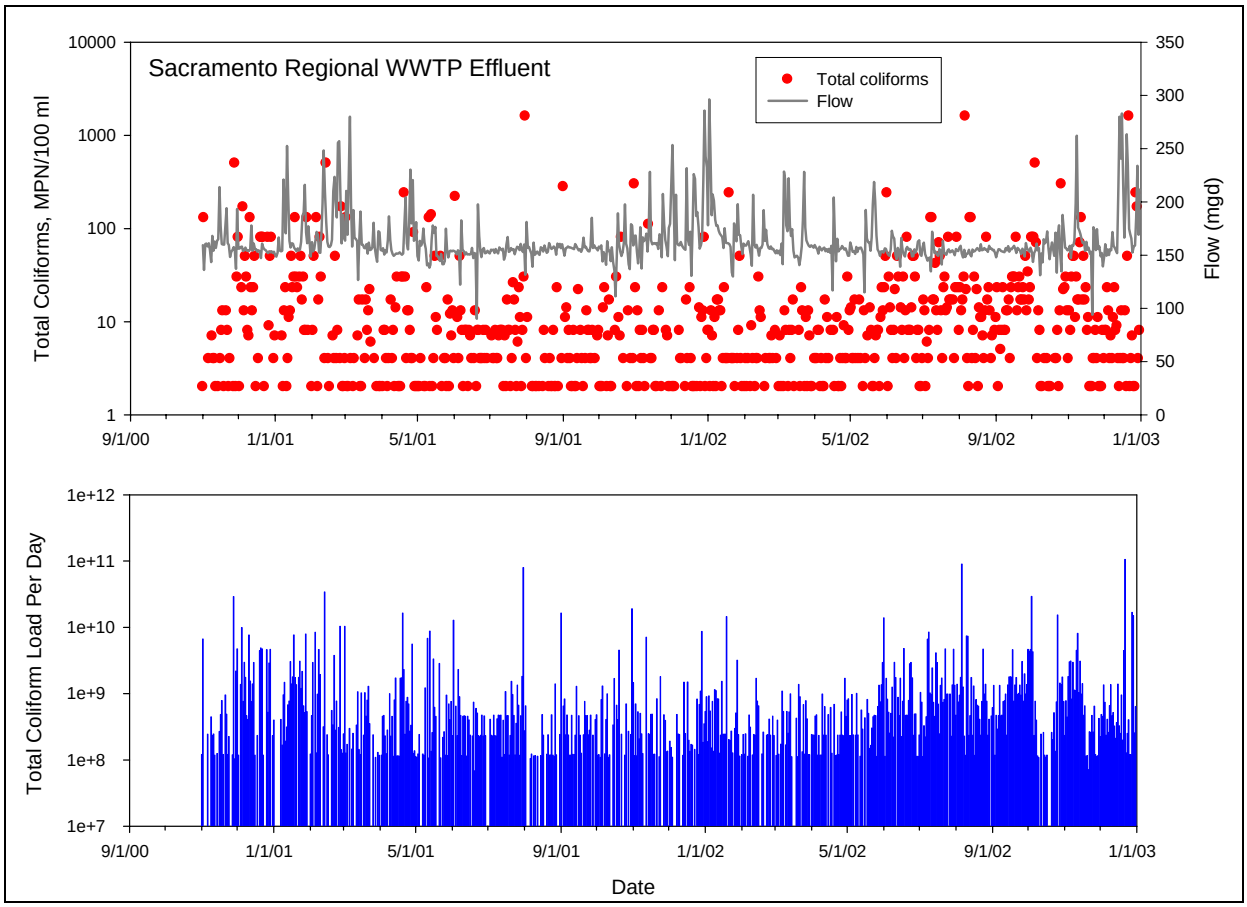


**Figure 4-6. Flow and total coliform concentration in Sacramento Regional WWTP effluent.**

## 4.5 LAND-BASED EVALUATION OF COLIFORM LOADS USING LOADING RATES

A simplified approach for estimating the loads from different land uses is to estimate coliform areal loading rates as developed by Horner (1992), and summarized in Table 4-3. Although these rates are gross approximations, and have not been estimated for the climate and conditions of the Central Valley, a rough calculation was performed for lands in the Sacramento River watershed as described below.

The incremental watershed area draining into the Sacramento River between Colusa and Freeport corresponds to an area of 1,590 square miles (1.02 million acres). Assuming that the contribution of upstream watersheds is minimal because of runoff, and assuming a midpoint fecal loading rate of ~$5 \times 10^9$ fecal coliforms per acre per year from Table 4-2, an estimated load of $1.3 \times 10^{13}$ fecal coliforms per day is calculated. This load is higher than what is actually seen in the Sacramento River (Figure 4-2) and is likely explained by die off during transport that was not considered.

A similar calculation can be performed for the NEMDC watershed, an area covering approximately 180 square miles. Using the same loading rates as above, a daily estimated load of $1.6 \times 10^{12}$ fecal coliforms/day is computed. This falls in the middle

range of values shown for the NEMDC in Figure 4-4. The agreement appears to be better than for the Sacramento River because die off is less of a confounding effect for a smaller watershed with shorter travel times.

**Table 4-3 Land based loading of fecal coliforms (Source: Horner, 1992)**

| Land Use | Fecal Coliform Loading (number/acre/year) |
|---|---|
| Road | 7.3E+07 |
| Commercial | 2.3E+09 |
| Single Family Residential, Low Density | 3.8E+09 |
| Single Family Residential, High Density | 6.1E+09 |
| Multifamily Residential | 8.5E+09 |
| Forest | 1.6E+09 |
| Grass | 6.5E+09 |
| Pasture | 6.5E+09 |

## 4.6 EVALUATION OF *CRYPTOSPORIDIUM* LOADS

There are direct measurements of *Cryptosporidium* loads from various mammals in California, as shown in Table 4-4, based on the work of Atwill et al. (2003). These data show the tremendous contribution of calves to Cryptosporidium production, and the likely importance of grazing and confined animal facilities.

Assuming an average effluent flow rate of 165 mgd (averaged over 1998-2002) at the SRWTP (Chapter 3), and median *Cryptosporidium* concentrations of 1.9 oocysts/liter, results in a median daily load of $1.2x10^9$ oocysts/day.

Data on *Cryptosporidium* exists at different location on Sacramento River and the American River as previously shown in Figure 3-6. The highest average *Cryptosporidium* oocyst counts of 0.12/liter were observed at Sacramento River at Mile 44. This can be translated to an estimated average load of $6x10^9$ cysts per day at this location (using an average flow in the Sacramento River of 20,400 cfs, based on data shown in Figure 4-3).

Both the wastewater and Sacramento River *Cryptosporidium* loads may be compared with the numbers of oocyts shed by a single calf ($3x10^9$ oocysts per day) as shown in Table 4-4. In other words, the *Cryptosporidium* loads flowing through Sacramento River or discharged from SRWTP are of the same order of magnitude as the excretion of a single infected animal. Although wastewater loads are significant, this comparison highlights the importance of animals in the landscape as sources of pathogens. The relatively low concentrations that are observed in the Sacramento Rivers could be caused by the presence of natural or artificial barriers/processes that limit pathogen transport to water, by the significant die off of oocysts that do reach the water, as well as limitations in the analytical detection of *Cryptosporidium* oocysts in natural waters.

**Table 4-4 Mean Daily *Cryptosporidium parvum* excretion rates in certain domestic and wildlife species in California (Source: Atwill et al., 2003)**

| Animal | Species | Daily oocyst excretion rates *(oocysts/day/animal)* | Mean oocyst concentration in feces *(oocysts/kg)* | Total daily fecal production *(kg)* |
|---|---|---|---|---|
| San Joaquin dairy cattle | Cows | 4,000 | 67 | 60 |
| | Calves | 3,000,000,000 | 3,000,000,000 | 1 |
| California beef cattle | Cows | 6,000 | 150 | 40 |
| | Calves | 600,000 | 150,000 | 4 |
| California horses | Adults | *Similar to adult beef and dairy cattle* | | |
| | Foals and weanlings | *Not done adequately* | | |
| Striped skunk | Adults | 140,000 | 2,800,000 | 0.05 |
| | Juveniles | 88,000 | 4,400,000 | 0.02 |
| California ground squirrels | Adults | 78,000 | 6,500,000 | 0.012 |
| | Juveniles | 412,000 | 10,300,000 | 0.004 |
| Coyotes | Adults | 41,000 | 205,000 | 0.2 |
| | Juveniles | 35,000 | 505,000 | 0.07 |
| Yellow-bellied marmot | Adults | 208,000 | 10,400,000 | 0.02 |
| | Juveniles | *Not done* | | |

## 4.7 SUMMARY

The variable nature of pathogen and indicator concentrations in surface waters, and the rapid die off of many of these organisms in the ambient environment, makes it very difficult to quantify the importance of different sources on a scale as large as the Central Valley, especially for coliforms that are widely present in water. A single source in close proximity to the sampling location can dominate the coliform concentrations observed at a location downstream of several thousand square miles of watershed. In the Sacramento River, loads of pathogens, especially an animal-derived pathogen such as *Cryptosporidium*, are transported at levels at that are orders of magnitude lower that their excretion in the watershed. Similar data were not available for the San Joaquin River.

# CHAPTER 5.0
# SUMMARY AND RECOMMENDATIONS FOR FUTURE WORK

The development of the pathogen conceptual model involved the synthesis of a large amount of previously collected data and information from published reports. The information presented in this document can be used to direct future investigations to improve understanding of sources, impacts, and management of fecal indicators and pathogens in the Central Valley and Delta.

## 5.1 SUMMARY

Evaluation included mapping and plotting of available data by location and source type across the Central Valley and Delta. Unlike other constituents prioritized for evaluation as part of ongoing work toward development of the Central Valley Drinking Water Policy, such as organic carbon and nutrients, that do not have formal numeric criteria, specific numeric criteria do apply to pathogens and fecal indicators as they relate to recreational use and municipal water supply. Levels of fecal indicators and pathogens in source waters directly affect the degree of treatment required for drinking water treatment plants. In the Central Valley and Delta, the recreational standards are routinely exceeded in the San Joaquin River Basin, although they are generally within limits in the Sacramento River Basin.

Although a large quantity of data was available for this analysis, the size of the Central Valley watershed, and complexity of many pathogens and fecal indicator response, especially rapid dieoff, prevented a detailed quantitative analysis of pathogen or indicator loads in the manner performed in prior work for organic carbon and nutrients (Tetra Tech, 2006a, 2006b). Sources considered in this evaluation include wastewater, storm runoff from urban land, and terrestrial wildlife. Aquatic wildlife, although known to a significant contributor of coliforms and possibly pathogens, were not quantified as a source in this analysis.

Substantially more data were available to evaluate fecal indicator organisms, such as coliform bacteria, than true pathogens, such as *Cryptosporidium* and *Giardia*. Of the known sources of coliforms into the waters of the Central Valley, it was found that wastewater total coliform concentrations for most plants were fairly low (<1000 MPN/100 ml). Coliform loads from the largest wastewater treatment plant in the Central Valley were substantially lower than from a canal draining a rapidly urbanizing watershed (NEMDC). In general, along the Sacramento River, the highest total coliform concentrations in water (>10,000 MPN/100 ml) were observed near sample locations influenced by urban areas. Similar total coliform concentration data were not available for the San Joaquin Valley (the highest values were capped at ~2400 MPN/100 ml). However, *E. coli* data were not similarly capped, and for this parameter, comparably high concentrations were observed for waters affected by urban environments and intensive agriculture in the San Joaquin Valley. Finally, sites in the vicinity of the Delta that were close to urban stormwater discharges, had elevated concentrations of coliforms.

Coliform data showed minimal relationships with flow rates, although most of the high concentrations were observed during the wet months of the years, possibly indicating the contribution of stormwater runoff.

Data on pathogens was available primarily for *Cryptosporidium* and *Giardia* along the Sacramento River. Where monitored, these pathogens were often not detected, and when detected, the concentrations were generally very low, typically less than one organism per liter. Given the flows of the Sacramento River and estimates of *Cryptosporidium* oocyst excretion by mammals, typical loads flowing into the Delta from the Sacramento River are of the same order of magnitude as the number of organisms excreted by a single calf (one of the most prolific sources of *Cryptosporidium*). This result could be caused by the presence of natural or artificial barriers/processes that limit transport to water, by the significant die off of oocysts that do reach the water, as well as limitations in the analytical detection of *Cryptosporidium* oocysts in natural waters.

There were limited data on pathogens from one wastewater plant in the Central Valley (Sacramento Regional Wastewater Treatment Plant). These data showed average and median effluent concentrations of both *Cryptosporidium* and *Giardia* well in excess of concentrations in the Sacramento River (*Giardia* >100 times Sacramento River levels; *Cryptosporidium* >50 times Sacramento River levels). Also, the fraction of samples where either of these pathogens was detected in the effluent was high: 100% for *Giardia* and 80% for *Cryptosporidium*. However, the viability of these organisms at the point of discharge is not known.

## 5.2 RECOMMENDATIONS FOR FUTURE WORK

Coliforms are recognized to not be ideal indicators for pathogens because of their lower survivability compared to some pathogens. A wide variety of new indicators are under development although their applicability, generality, and cost remain concerns (NAS, 2004). For the foreseeable future, it appears that despite all of the limitations of coliform measurements, these will remain the *de facto* standard for identifying the presence of pathogens. It is recommended that the CVDWPWG

continue to support collection of data on coliforms for consistency with historical data, but also continually evaluate new analysis techniques recommended by NAS (2004), described below, for application across the entire Central Valley.

Recent advancement in microbiology, molecular biology and analytical chemistry provides opportunities for more accurate, timely and direct detection and measurements of pathogens (NAS, 2004). Traditional methods for bacterial indicators often are based on measuring organisms by culture or infectivity and often require a prolonged incubation period. Some newer molecular-based or immuno-based techniques are based on measuring cell constituents or components that are unique to the target organisms such as nucleic acids, surface proteins, carbohydrates, some specific enzyme activities, ATP levels or some specific toxins. A combination of the traditional and newer methods has also been used in measuring waterborne pathogens, particularly for the detection of protozoa in water.

Generally there are three groups of newer methods other than the traditional culture methods:

1) Molecular based method of nucleic acids analysis. Nucleic acids analysis generally involves measuring DNA or RNA that are unique to a particular microorganism. DNA from a sample is typically amplified through PCR (polymerase chain reaction) and then analyzed through sequencing or by hybridization to a gene probe or array containing the complementary genetic sequence (microarray method). Detection of PCR products can be performed using electrophoresis or fluorescence technologies. Pulsed-field gel electrophoresis (PFGE), ribotyping and other technologies used to label and measure DNA fragments.

2) Immunological methods for detecting surface proteins of bacteria, protozoa and viruses unique to the microbes. Immno-based methods are based on detection of specific antigens such as soluble proteins and whole microorganisms through antibodies. The most common immunological method is the enzyme-linked immunosorbent assay (ELISA), in which two antibodies are used to bind the antibody of the microorganisms.

3) Measuring of other cell components such as ATP levels or specific toxins of the organisms.

For example, a variety of methods have been used in detection of infectious *Cryptosporidium* oocysts in water samples. Cell culture methods are now being used in measuring *Cryptosporidium* infectivity. Molecular based methods using PCR or RT-PCR techniques that target the nucleic acid components as well as methods combine PCR and cell culture or real-time PCR are now being used. Immuno-based assays using antibodies specific to *Cryptosporidium* parvum and a second antibody conjugated to a fluorescent dye have been also been used.

Compared to traditional culture methods, the molecular and immunological methods generally offer higher precision and higher specificity to the desired target organisms, require less time and smaller sampling volume (NAS, 2004). Traditional culture

methods offer moderate quantification capability compared to low to moderate quantifying capabilities by nucleic acid analysis.

Unlike chemical constituents analyzed as part of other conceptual models developed for the CVDWPWG, coliform indicators vary by orders of magnitudes over small distances and short time-scales. Accurate quantification of such parameters requires substantial data, which are often not available. A key observation of the source evaluation presented in this report is that coliform indicator levels are most responsive to sources and events in close proximity to the monitoring location, and that large scale modeling, with consideration of transport over many days, may be of limited benefit. While the large-watershed modeling approach, i.e., on the scale of the Central Valley, is appropriate for somewhat stable constituents such as total dissolved solids and organic carbon, a fundamentally different approach is recommended for modeling fecal indicator loading, with an emphasis on relatively small watershed and surface water areas. Within these smaller areas of interest, individual sources, specifically wild and domestic animals, and aquatic species, can be characterized with greater precision. US EPA's FecalTool model (US EPA, 2000) is a useful approach for computing coliform loads for such situations.

Given the strength of the stormwater source, more detailed evaluation needs to be performed of the linkage between rainfall and coliform loads, with a view to develop management practices for minimizing the loading from stormwater.

Computer tools can be used to make more detailed estimates of bacterial loads in surface waters, and have the benefit of being developed for use in a predictive mode such that the public or water supply agencies can get advance notice of elevated bacterial levels under specific weather conditions or other forcing events. However, the additional effort and data collection needed to make such predictions meaningful has to be weighed against the collection of data on true pathogens.

Substantially greater data collection, particularly in the San Joaquin Valley, is recommended for *Cryptosporidium* and *Giardia* given their longer survival times in water relative to indicator organisms, and given the numbers of domesticated animals in the watershed. In general, sampling of San Joaquin and Sacramento River source waters as well as potential sources such as urban stormwater drainage/runoff for a wide range of potential pathogens including bacteria and viruses identified in Chapter 2, even on a limited scale and frequency, will provide valuable information on the health of this extremely vital resource. Sampling of pathogens and indicators at delta pump locations is also recommended for direct evaluation of source water quality for export to other parts of the state.

Besides sampling surface water, sampling of other discharges such as wastewater and urban stormwater for pathogens is also strongly desired. The limited pathogen data on wastewater effluent that is currently available indicates that pathogen levels may be much higher than in surface waters, and reflects the survival of these organisms following chlorination. There is no similar data on pathogens for stormwater discharges, although coliform data in stormwater indicate a highly significant microbial source. Given the general proximity of major wastewater and urban stormwater discharges to the Delta, and its significance as a drinking water source,

better understanding of the loads, fate, and transport of pathogens in and around the Delta is of vital importance.

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